Drivers of insect herbivory in Pedunculate oak (Quercus robur) from tree to biogeographical scale

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Par Elena VALDES CORRECHER

Déterminants de l'herbivorie des insectes chez le chêne pédonculé (*Quercus robur*) de l'échelle de l'arbre à l'échelle biogéographique

Sous la direction de : Arndt HAMPE & Bastien CASTAGNEYROL

Soutenue le **07/05/20**

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Déterminants de l'herbivorie des insectes chez le chêne pédonculé (*Quercus robur*) de l'échelle de l'arbre à l'échelle biogéographique

Résumé: L'herbivorie par les insectes est un processus écologique important qui affecte la dynamique des populations de plantes, les communautés et les écosystèmes. La distribution et l'abondance des insectes herbivores et l'activité qui en résulte sont façonnées par une multitude de facteurs, intrinsèques ou extrinsèques à la plante hôte, qui agissent à différentes échelles spatiales et souvent de concert. Une classification largement utilisée fait la distinction entre les forces ascendantes, telles que l'activité des herbivores est influencée par la distribution et la qualité des ressources (incluant les défenses), et les forces descendantes, telles que l'activité des herbivores est limitée par le contrôle exercé par les ennemis des herbivores (prédateurs, parasitoïdes). Les forces ascendantes et descendantes sont toutes deux impliquées dans les cascades trophiques qui accompagnent inévitablement les interactions plantes-herbivores dans les populations naturelles de plantes, mais leur importance relative peut varier considérablement selon le contexte particulier local, et les mécanismes biologiques sous-jacents restent mal compris.

J'ai étudié les facteurs écologiques qui façonnent les relations entre le chêne pédonculé (*Quercus robur*) et ses insectes herbivores à différentes échelles spatiales. En particulier, j'ai examiné les effets du contexte du paysage, de l'apparentement entre les arbres et du climat sur l'activité des herbivores. Un des principaux objectifs de ma thèse était d'évaluer l'importance relative des forces ascendantes et descendantes dans la structuration des relations chêne-herbivores.

La thèse est structurée en trois chapitres principaux correspondant à des manuscrits indépendants qui sont publiés (chapitre 1), en cours de révision (chapitre 2), ou en préparation (chapitre 3) au moment de la soumission du document de thèse. Dans le chapitre 1, j'ai étudié la relation entre l'herbivorie et la communauté et l'activité des oiseaux insectivores dans les chênaies qui diffèrent en taille et en connectivité. J'ai constaté que l'herbivorie, la prédation des oiseaux et les communautés d'oiseaux étaient influencées par les caractéristiques du paysage, mais que les forces descendants exercées par les oiseaux n'avaient pas d'effet significatif sur l'herbivorie. Dans le chapitre 2, j'ai étudié la relation entre le génotype du chêne, les défenses chimiques des feuilles et l'herbivorie dans les mêmes peuplements. J'ai constaté que l'herbivorie des insectes et les défenses chimiques étaient non seulement influencées par les caractéristiques du paysage, mais aussi par le génotype de l'arbre, et que l'herbivorie des insectes diminuait avec la concentration des défenses foliaires. Enfin, au chapitre 3, j'ai étudié l'effet de la variabilité climatique à grande échelle sur les interactions entre les plantes, l'herbivorie et la prédation par les oiseaux dans les chênes selon un gradient latitudinal. J'ai découvert que les facteurs climatiques influençaient l'herbivorie des insectes ainsi que les caractéristiques nutritionnelles des feuilles, alors qu'ils n'influençaient pas les défenses foliaires et la prédation des oiseaux. De plus, l'herbivorie des insectes n'était influencée que par des forces ascendantes dont l'importance variait selon les guildes d'insectes.

Dans l'ensemble, ces résultats aident à améliorer notre compréhension des différentes forces écologiques qui façonnent l'herbivorie par les insectes et de leur variabilité dans les populations naturelles d'arbres. Les études futures sur les interactions plantes-herbivores-prédateurs devraient tenir compte du fait que celles-ci sont influencées simultanément par le génotype de la plante hôte, les caractéristiques du paysage et le climat. Enfin, la thèse illustre également la valeur des approches de science citoyenne qui peuvent combiner la recherche scientifique avec une éducation scientifique et à l'environnement bien nécessaires.

Mots clés : Herbivorie; Chêne pédonculé; Prédation; Traits foliaires; L'apparentement génétique; Paysage; Climat

Drivers of insect herbivory in Pedunculate oak (*Quercus robur*) from tree to biogeographical scale

Abstract: Insect herbivory is an important ecological process that affects plant populations, communities and ecosystems. The distribution and abundance of insect herbivores and their resulting activity are shaped by a multitude of drivers, intrinsic or extrinsic to the host plant, that act at different spatial scales and often in concert. A widely used classification distinguishes between bottom-up forces where herbivore activity is influenced by the distribution and dynamics of the resource stock (including the defenses), and top-down forces where herbivore activity is constrained by drivers of mortality (e.g. predators, pests). Both bottom-up and top-down forces are involved in the trophic cascades that inevitably accompany plant-herbivore interactions in natural plant populations, yet their relative importance can vary greatly depending on the particular study context, and the underlying biological mechanisms remain poorly understood.

I investigated the ecological drivers shaping the relationships between Pedunculate oak (*Quercus robur*) and its insect herbivores across different spatial scales. In particular, I examined the effects of the ecological neighbourhood, the landscape context, tree genetic relatedness and climate on herbivore activity. A major aim of my thesis was to evaluate the relative importance of bottom-up and top-down forces in shaping oak-herbivore relationships.

The thesis is structured in three main chapters corresponding to independent manuscripts that are either published (chapter 1), under review (chapter 2) or under preparation (chapter 3) at the moment of submitting the thesis document. In chapter 1 I investigated the relationship between herbivory and the community and activity of insectivorous birds in oak stands that differed in size and connectivity. I found that herbivory, bird predation and bird communities were influenced by landscape characteristics, but neither predation on herbivores nor bird communities had a significant effect on herbivory. In chapter 2 I investigated the relationship between oak genotype, leaf defenses and herbivory in the same stands. I found that insect herbivory and leaf defenses were not only influenced by landscape characteristics but also by the genotype of the tree, and that insect herbivory decreased with increasing concentration of leaf defenses. Finally, in chapter 3 I investigated the effect of large-scale climate variability on the interactions between plants, herbivory and bird predation in oak trees along a latitudinal gradient. I found that climatic factors influenced insect herbivory as well as leaf nutritional traits, while they did not influence leaf defenses and bird predation. Furthermore, insect herbivory was only influenced by bottom-up forces (e.g. leaf nutritional traits and leaf defenses) and these effects on herbivory varied among herbivore feeding guilds, while neither other traits nor top-down forces affected insect herbivory.

Overall, these results help improve our understanding of the different ecological forces shaping insect herbivory and their bottom-up and top-down drivers in natural tree populations. Future studies of plants-herbivores-predator interactions should take into account that these are simultaneously influenced by host plant genotype, landscape characteristics and climate. Finally, the thesis also illustrates the value of citizen science approaches that can combine scientific research with much-needed environmental education.

Keywords: Insect herbivory; Pedunculate oak; Predation; Leaf traits; Genetic relatedness; Landscape; Climate

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1. Introduction

1.1. Herbivory

Insect herbivory is an important ecological process that affects primary productivity (McNaughton et al., 1989) by altering the functioning, recruitment, mortality and growth of plants (Kim et al., 2013; Maron and Crone, 2006; Visakorpi et al., 2019, 2018). It may exert a strong selection pressure on plants at all stages of development and in many forms (Atsatt and O'Dowd, 1976). Depending on the way they feed on leaves, defoliators (from now on called insect herbivores) can be grouped in different feeding guilds such as ectophagous (including chewers and skeletonizers) and endophagous species (including leaf miners and gall forming insects) (Fig. 1.1). Leaf-chewers represent the great majority of herbivore species and feed on the whole spectrum of leaf tissues including veins and leaf cuttings (Novotny et al., 2010) (Fig. 1A), while skeletonizers only feed on the epidermis and the parenchyma, leaving the veins intact (Fig. 1.1B). Both leaf miners and gall makers feed and live completely enclosed within the host plant tissue. Many leaf miners belong to the orders Coleoptera or Microlepidoptera that live between two epidermis of the leaf and feed on the parenchyma (Fig. 1.1C), while leaf galls are a deformity in a plant tissue caused in response to another organism (mostly Hymenoptera) and include a large number of species (Harris and Pitzschke, 2019) (Fig. 1.1D).

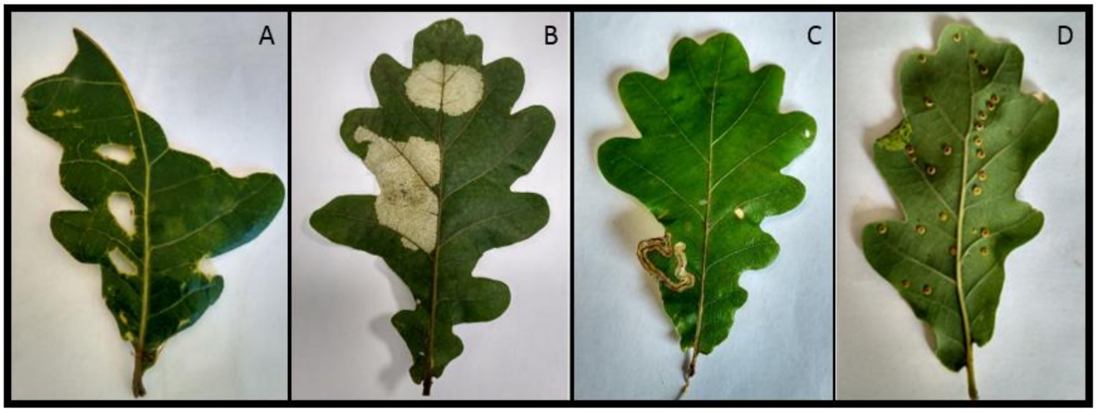

Figure 1.1. Damage made by insect herbivores belonging to different feeding guilds associated to Pedunculate oak leaves. A - Leaf-chewers. B - Skeletonizers. Here, the epidermis and the parenchyma has been eaten by Hymenoptera larvae of the genus *Caliroa*. C – Leaf miners. Here, the larvae correspond with a microlepidopter from the genus *Stigmella*. D – Leaf galls. Here, it correspond with an endophyte larvae from the genus *Neuroterus*.

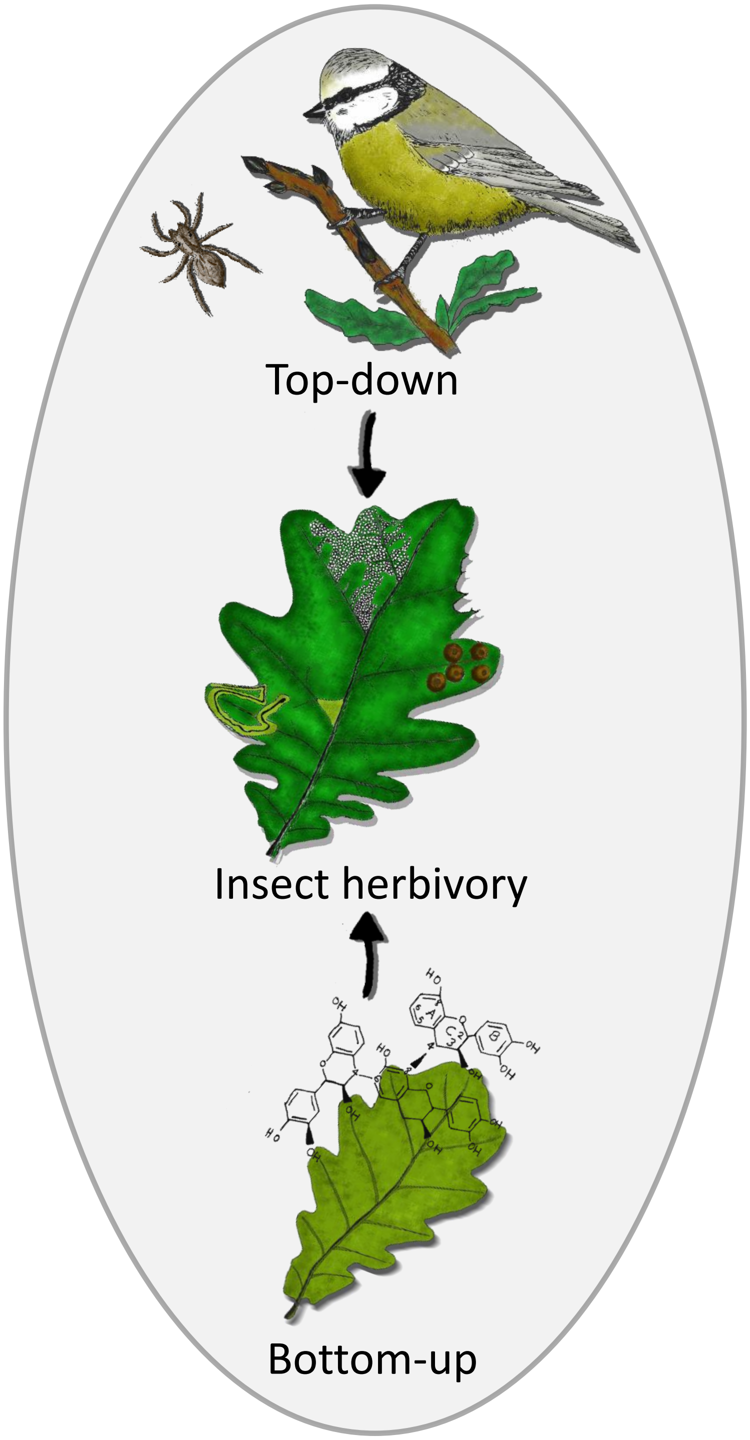

Figure 1.2. Bottom-up (e.g. leaf defenses, leaf nutrients) and top-down (e.g. predators, parasitism) forces driving insect herbivory. © E. Valdés Correcher.

1.1.1. Top-down and bottom-up forces controlling insect herbivory

Insect herbivory is influenced by biotic and abiotic forces. Biotic drivers are subdivided into bottom-up and top-down forces (Fig. 1.2). A bottom-up force refers to how the plant resource (e.g. individual plant nutrients and defences, but also plant abundance at population and community level) influence higher trophic levels (e.g. insect herbivores) whereas a top-down force focuses on interactions at top-level consumers (e.g. predators and parasitism) and its influence on lower trophic forms (Fig. 1.2).

Insect herbivores are influenced negatively and positively by plant characteristics such as chemical defenses and nutritional quality, respectively (Fig. 1.2) (Schoonhoven, 2005). For instance, the variation in leaf traits can directly affect the abundance and composition of the associated herbivore community and the consequent amount of damage by influencing the ability of insect herbivores to find, colonize and/or exploit specific plant individuals (Finch et al., 2003). Several leaf chemical compounds such as alkaloids or phenolic compounds are toxic to insect herbivores (Mithöfer and Boland, 2012; Salminen and Karonen, 2011) and act as chemical defenses that reduce leaf herbivory (Abdala-Roberts et al., 2016b; Moreira et al., 2018b). On the other hand, low concentrations of other leaf traits such as nutrients in plants also reduce herbivore performance (Wetzel et al., 2016). For instance, insect herbivores require nitrogen in high amounts, which is a limited nutrient of plants (Mattson, 1980). At the same time, plants also respond to insect herbivores and are able to induce the production of leaf defenses as a response against herbivory (Arimura et al., 2001).

At the same time, predators negatively influence insect herbivores as they can reduce insect herbivory by regulating herbivorous insect populations (Fig. 1.2) (Maguire et al., 2015; Sanz, 2001). A decrease in the abundance of predators may result in trophic cascading effects releasing insect herbivores and thus increasing the negative effects on the primary producers (Boege and Marquis, 2006; Böhm et al., 2011; Genua et al., 2017). Thus, a decrease in the abundance of predators may benefit insect herbivores indirectly. However, this is not always the case since an increase in predator diversity may also result in an increase in intra-guild predation (i.e. predation of predators among them), resulting in a relaxation of predation on herbivores (Finke and Denno, 2005). Thus, it is unclear how strong the effect of predators is on insect herbivores in different ecological contexts.

Plant-herbivore interactions are controlled by a plethora of drivers extrinsic and intrinsic to the host plant. These act often simultaneously and interact with each other. This thesis focuses on three of them: the landscape matrix (chapter 1), the genotype of the host plant (chapter 2), and the local climate (chapter 3) (see Fig. 1.3). Each of these drivers involves different spatial scales and biological mechanisms. Together, they illustrate the great complexity and context-dependence of plant-herbivore interactions in real-world contexts.

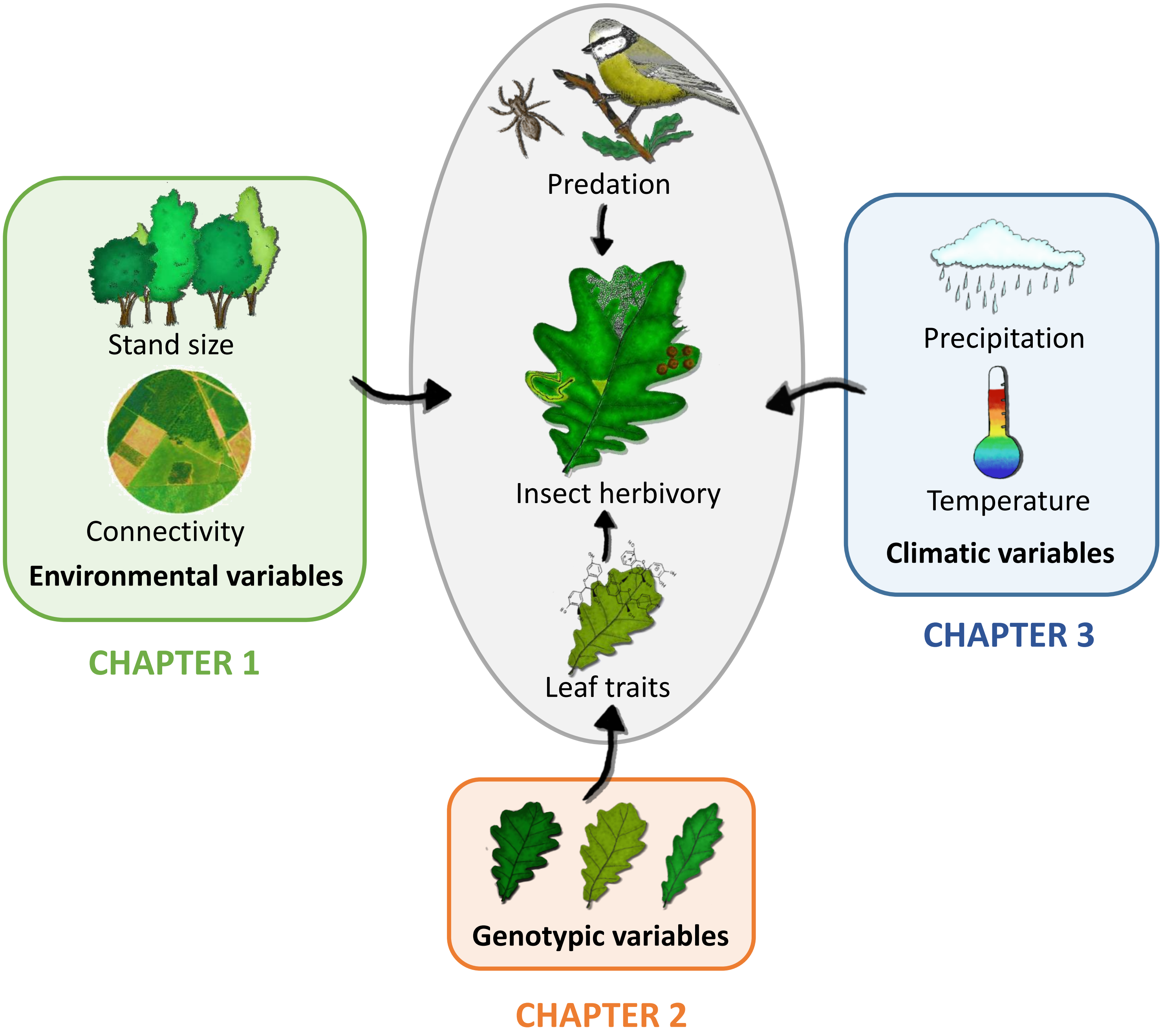

Figure 1.3. Summary scheme of the ecological forces shaping insect herbivory at different scales. © E. Valdés Correcher.

1.1.2. The effects of landscape context on plant-herbivore interactions

Forest fragmentation can alter ecosystem functioning and complex relationships between organisms (Fahrig, 2017; Lindenmayer and Fisher, 2013). Some studies have shown that both the size and the connectivity of the forest influence the trophic cascade differently depending on the relative importance of the bottom-up and top-down effects involved (De La Vega et al., 2012; Rossetti et al., 2014). In isolated and small forest stands the availability and quality of resources for insect herbivores is reduced (Chávez-Pesqueira et al., 2015) and insect dispersion increases in order to reach resources, which has high mortality risk and energetic and fitness costs (O'Rourke and Petersen, 2017) and consequently insect movements are magnified, increasing their risk of extinction (Rossetti et al., 2017). It results in lower insect herbivore abundance in small and isolated forests (De La Vega et al., 2012). At the same time, a small stand is likely to impose stronger constraints on predators than on their prey, especially in the case of insectivorous vertebrates (Barbaro et al., 2014; Bereczki et al., 2014; Genua et al., 2017; Maguire et al., 2015; Tscharntke et al., 2002), while isolated forest impose lower constrains since predators typically are more mobile than their herbivorous prey. Hence, predatory vertebrates are more likely to colonize more distant but larger stands (Barbaro et al., 2014; Bereczki et al., 2014; Cooper et al., 2012; Maguire et al., 2015). At the same time that forest fragmentation takes place, in other places forest cover is expanding as a consequence of cropland abandonment and natural transition to woodlands (Fuchs et al., 2015, 2012), creating a network of more or less connected woody habitats. Although forest expansion is taking place as well as forest fragmentation, trophic interactions in new forest stands has been little studied and what we know about the functioning of new forest expansion comes mainly from studies of fragmentation.

1.1.3. The effect of genetic relatedness on plant-herbivore interactions

Many plant traits involved in plant-herbivore relationships, including morphological features as well as levels of nutrients or chemical defenses, are known to have a genetic basis and hence to be heritable (Alonso and Herrera, 2001; Barker et al., 2019; Tomas et al., 2011). Insect herbivores are able to distinguish among different plant genotypes and tend to actively select the phenotypes they forage on (Donaldson and Lindroth, 2007; Fritz and Price, 1988; Wimp et al., 2005). Thus, arthropod communities associated to particular plants can be seen as their 'extended phenotypes'

(Whitham et al., 2006), which implies that their structure is partially shaped by plant genotype and that genetic similarities between plant individuals correlate positively with ecological similarities in plant associated arthropod communities (Bangert et al., 2006; Kagiya et al., 2018). It has been proposed that plant secondary metabolites represent the mechanistic link between plant genes and plant associated arthropods (Bangert et al., 2006; Kagiya et al., 2018; Wimp et al., 2005). As a consequence, there is ample evidence that plant intraspecific genetic diversity drives arthropod communities (Koricheva and Hayes, 2018). However, the relative importance of plant genotype as a driver of plant associated arthropod communities assembly – and resulting insect herbivory – is still controversial, in particular because biotic and abiotic factors also play a great role in determining plant traits, especially in long living trees. In addition, intriguingly, the abundant evidence from greenhouse experiments contrasts with weak evidence from field studies (Tack et al., 2012), bringing up the question how important the phenomenon actually is in real-world situations. One possible explanation for the apparent contradiction is that experiments may underestimate the extensive variation that characterizes natural populations. For instance, a large proportion of trait variation occurs within individual plants (Herrera, 2017). This within-individual variation has, however, rarely been considered in studies of genotype-herbivory relationships.

Intra-individual variation in herbivory and leaf traits can be the result of differences in the microclimate (e.g. temperature, irradiation) along tree vertical gradients. For instance, upper canopy leaves are thicker, tougher, smaller, drier and have higher levels of leaf defenses than lower canopy leaves (Castagneyrol et al., 2019a; De Casas et al., 2011; Le Corff and Marquis, 1999; Murakami et al., 2005). Differences in microclimate across the tree canopy may influence the expression of genes triggering the production of leaf defenses (Lämke and Unsicker, 2018). As a consequence, herbivory is ultimately shaped by the complex interplay between differences in leaf traits, insect herbivore communities and predators along the tree canopy (Aikens et al., 2013; Ulyshen, 2011).

1.1.4. Biogeography of plant-herbivore interactions and the effects of climate

Bottom-up and top-down drivers of insect herbivory are also influenced by abiotic factors such as the climate. For example, variation in sunlight and climatic factors (e.g. temperature and precipitation) may affect directly plant-herbivore interactions by influencing plant and herbivore traits, and indirectly by altering the biotic community (e.g. predators). There are different approaches to investigate the effect of climate on biotic interactions. One of them consist on experiments in warming facilities such as greenhouses where we can control light availability, temperature and precipitation (Wolkovich et al., 2012). However, this approach has some limitations in the case we want to work with mature trees as their growth is slow and they need a lot of space. Another approach that is commonly used is the use of *natural laboratories* through monitoring the response of biotic interactions to temporal or spatial temperature variation such as across latitudes, altitudes or years (Moreira et al., 2018a). Studies along latitudinal gradients have long recognized that insect herbivory increases towards lower latitudes as a result of higher temperatures and longer growing seasons (Marquis et al., 2012; Pennings et al., 2009; Schemske et al., 2009), thus leading to a parallel increase in plant defenses (Coley and Kursar, 2014; Moreira et al., 2014; Rasmann and Agrawal, 2011; Roslin et al., 2017). However, a meta-analysis by Moles et al. (2011) has shown that commonly claimed relationships are not well supported by the literature since many studies show an increase, a decrease or no variation of herbivory and plant defenses with latitude (Adams et al., 2009; Adams and Zhang, 2009; Anstett et al., 2016; Del-Val and Armesto, 2010; Gaston et al., 2004; Moreira et al., 2018b). Thus, it is needed to identify the mechanisms underlying the variation of herbivory and their drivers across latitudinal and climatic gradients.

Plant traits such as chemical defenses and nutritional quality also vary along climatic gradients. Regarding plant defenses, two contrasting hypothesis have been formulated. The *resource availability hypothesis* (Endara and Coley, 2011) states that plants produce more defenses at high latitudes because the cost of losing leaves in these environments might be higher than at lower latitudes where productivity is higher. Hence, plants may invest more on defenses to support less herbivory (Endara and Coley, 2011; Martz et al., 2009; Moreira et al., 2017). In sharp contrast, the *latitudinal herbivory defense hypothesis* poses that plants have higher level of defenses at lower latitudes because species interactions are stronger in these environments, requiring plants to produce more defenses (Coley and Barone, 1996; Wieski and Pennings, 2014). Empirical evidence for both hypotheses is mixed. One possible reason could be that plant defenses often show no clear association with plant susceptibility to herbivores (Carmona et al., 2011). On the other hand, herbivory could also be modified by predator activity (i.e., top-down forces) that also tend to vary along latitudinal gradients (Lövei and Ferrante, 2017; Roslin et al., 2017; Zvereva et al., 2019). It has been described that arthropod predator activity decreases towards higher latitudes (Roslin et al., 2017; Zvereva et al., 2019; but also see Lövei and Ferrante, 2017), whereas bird predators increases or does not vary across latitude (Roslin et al., 2017; Zvereva et al., 2019). However, only a few studies have addressed the effect of latitudinal gradients on predators (Björkman et al., 2011). Yet, latitudinal variation in bottom-up and top-down drivers of insect herbivory has rarely been studied concomitantly, making previous attempts to understand large scale variation in insect herbivory unsuccessful.

1.1.5. Main objectives and hypotheses of this thesis

The overarching objective of this thesis is to elucidate the top-down and bottom-up forces driving insect herbivores on Pedunculate oak (*Quercus robur*) at individual (tree), local (stand) and European (climatic) scales.

The specific aims are:

(1) To investigate the effect of forest **stand size** and **connectivity** at landscape scale on insect herbivory, herbivore predation and the abundance and diversity of insectivorous birds (Chapter 1; Fig. 1.3). I predict that oak stands differing in size and connectivity would influence differently insect herbivory and insectivorous bird predators, and that the latter would influence negatively insect herbivory.

(2) To investigate the relationships between tree **genetic relatedness**, leaf trait variation and insect herbivory in oaks (Chapter 2; Fig. 1.3). I predict that insect herbivory and leaf defenses would vary among stands, among trees within stands and between canopy layers within trees, and also that both insect herbivory and leaf defenses would be influenced by tree genetic relatedness.

(3) To study the effect of **climate** on leaf traits (plant nutritional quality and chemical defenses), insect herbivory, and herbivore predation on oak trees along a latitudinal gradients spanning much of Europe (Chapter 3; Fig. 1.3). I predict that climate driven variability in insect herbivory would be determined by joint variation in bottom-up (plant defenses and nutritional quality) and top-down (bird attack rates) forces acting upon herbivores.

1.2. Study system

1.2.1. Natural history

Pedunculate oak is a long-lived deciduous tree that belongs to the family Fagaceae. It is an allogamous and monoecious species with an anemophilic pollination. Leaves are approximately 8–12 cm long with four to seven pairs of lobes, and have almost no petiole. The acorns are 2–2.5 cm long, pedunculate (having a peduncle or acorn-stalk, 3–7 cm long) with one to four acorns on each peduncle. Acorn dispersal is primarily performed by scatter-hoarding rodents and birds (Bossema, 1979; Ouden et al., 2005).

The species is widely distributed across Europe (Fig. 1.4) in a substantial range of climatic conditions (Eaton et al., 2016) and frequently found on moist and nutrient-rich soils (Rushton, 1979). In France it occupies 12,37% of the forest area dedicated to wood production (IFN, 2018). The timing of leaf burst and leaf fall varies along its distribution range due to climatic variation. In southern Europe, leaf burst and fall occurs in April and in November respectively, whereas in northern Europe leaf burst occurs later while fall occurs earlier. In its native range, this species shelters a large community of specialist and generalist herbivorous insects, especially leaf chewers, skeletonizers, leaf miners and galls (Southwood et al., 2005).

The management of European oak forests include traditional silvicultural practices like coppices and wood pastures (Altman et al., 2013). The wood of Pedunculate oak has a high economic value and quality being employed for firewood and furniture (Liziniewicz et al., 2016), and its ecological and economical role in forest management is likely to increase (Schelhaas et al., 2015). Oaks also have high historical, cultural, aesthetic, and spiritual values (Carvalho, 2012).

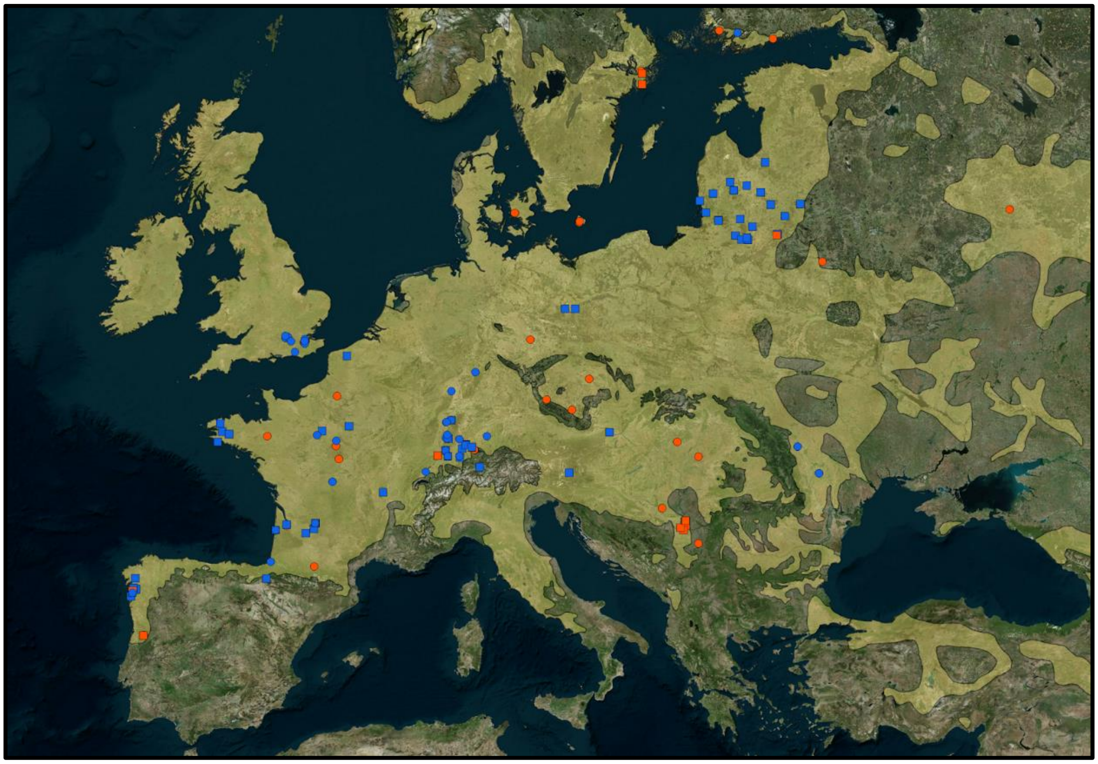

Figure 1.4. Distribution range of Pedunculate oak in Europe (shaded in yellow) (EUFORGEN, 2008) and location of trees sampled by professional scientists (orange symbols) and school children (blue symbols) in 2018 (circle symbols) and 2019 (square symbols).

1.2.2. Study sites

1.2.2.1 Studies for chapter 1 and 2

The first two studies were carried out in the Landes de Gascogne region (south-western France) about 40 km southwest of Bordeaux (44°41'N, 00°51'W). The region is characterized by an oceanic climate with mean annual temperature of 12.8°C and annual precipitation of 873 mm over the last 20 years, and a low elevation of *c.* 50 m a.s.l.. The soil consists of sandy substrate and the area is covered by extensive plantations of maritime pine (*Pinus pinaster* Ait.) that cover *ca* 10,000 km^2. There are also small stands of broadleaved forests along the rivers and within the pine plantations that are dominated by Pedunculate oak (*Quercus robur* L.), Pyrenean oak (*Quercus pyrenaica* Willd.), silver birch (*Betula pendula* L.) and contain other tree species such as alders and willows in minor abundance. The management of these small stands is very extensive and they are primarily used for firewood, hunting or mushroom collecting. These small stands have different origins.

Some surround ponds that were colonized by broadleaved trees, and others have colonized isolated settlements or spots that are not suitable for pine plantations (e.g. unevenness, flood zone). Open areas are mainly pine clear-cuts and agricultural fields. Agricultural fields consist mainly on irrigated corn or vegetables and occupy 15% of the area (Mora et al., 2012).

The silvicultural cycle of maritime pine has a profound influence on the structure of the landscape, constraining the forest natural regeneration and development of deciduous species such as oak. However, many of the broadleaf stands are actively expanding (Gerzabek et al., 2017), favoured by a recent change in silvicultural management that tends to conserve broadleaved trees within pine plantations as a mean of conservation biological control (Castagneyrol et al., 2014; Dulaurent et al., 2011).

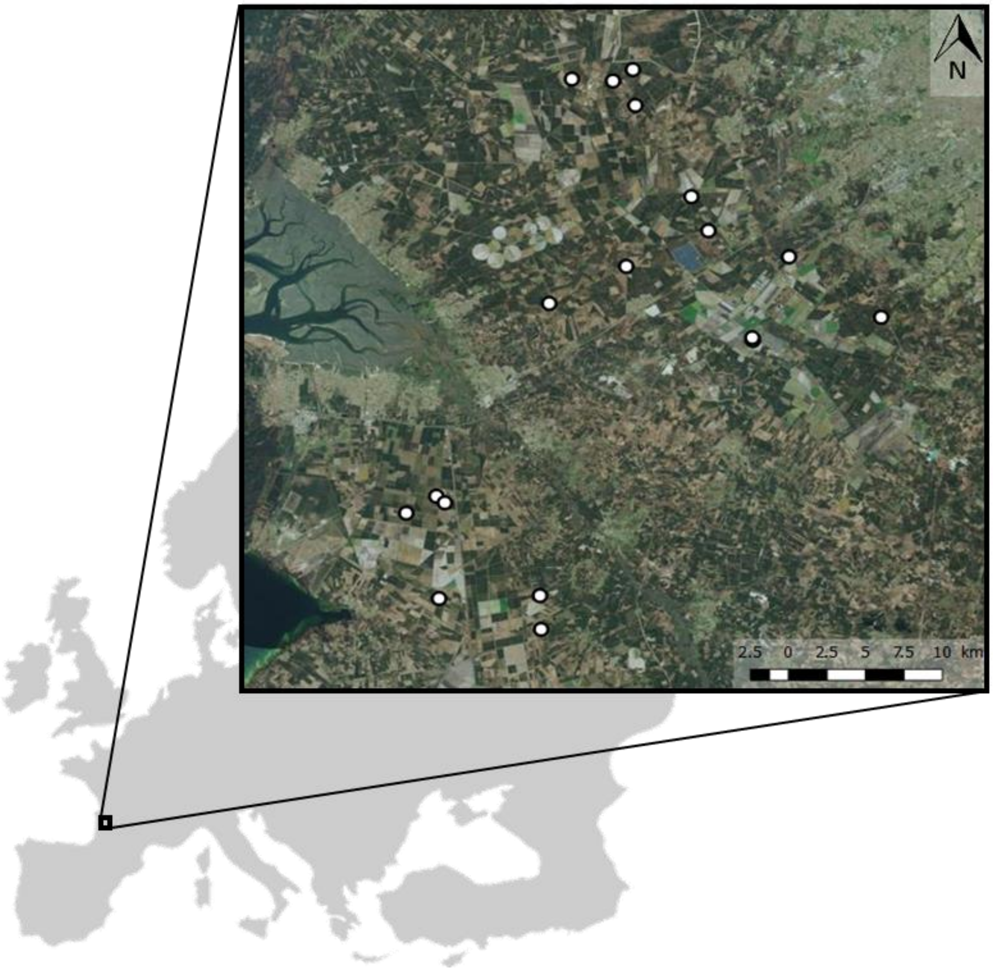

Figure 1.5. Map of the study area in the Aquitaine region, south-western France, showing the 18 oak stands.

The primary objective of this thesis was to investigate the effect of recent forest expansion on biodiversity and biotic interactions (chapter 1). To that end, I carefully selected a total of 18 new oak forest stands along gradients of stand size and connectivity (Fig. 1.5). To ensure that forest stands were of recent origin, I confirmed on aerial photographs from the 1950s that only very few trees were present at that time (Fig. 1.6). The second objective was to investigate the effect of genetic relatedness on biotic interactions (chapter 2). To that end, I selected 15 stands from the original 18 selected stands.

Figure 1.6. Pictures of one of the stands in different years.

1.2.2.2. Study for chapter 3

For this study, a total of 261 mature Pedonculate oak trees were sampled by professional scientists (*n* = 115) and school children (*n* = 146) in 2018 (*n* = 149) and 2019 (*n* = 113) from Portugal to Sweden, and from the UK to Serbia, thus covering most of the species' geographic and climatic range (Fig. 1.4). The research consisted in part in an international citizen science project that involved the participation of 30 scientists and 82 school teachers (and their pupils) from 17 European countries (Fig. 1.4).

2. Chapter 1: What is the effect of stand size and connectivity on insect herbivory, herbivore predation and the abundance and diversity of insectivorous birds?

Elena Valdés-Correcher, Inge van Halder, Luc Barbaro, Bastien Castagneyrol & Arndt Hampe (2019) – **Insect herbivory and avian insectivory in novel native oak forests: divergent effects of stand size and connectivity** – *Forest Ecology and Management* 445: 146-153.

Abstract

The value of novel native broadleaf woodlands for biodiversity conservation is important to consider for adequate forest management in rural landscapes. Passive reforestation has been proposed as a cost-efficient tool for creating networks of novel native forest stands that would help restoring biodiversity and associated ecosystem services. Yet to date the ecological functioning of such stands remains strongly understudied compared to forest remnants resulting from longer-term fragmentation. We assessed how the size and connectivity of newly established Pedunculate oak (*Quercus robur* L.) stands in rural landscapes of SW France affect rates of herbivory by different insect guilds as well as rates of avian insectivory and the abundance and richness of insectivorous birds. Comparing 18 novel forest stands along a gradient of size (0.04-1.15 ha) and cover of broadleaf forests in the surroundings (0-30% within a 500m radius), we found that even the smallest stands are colonised by leaf miners and chewers/skeletonizers, and that rates of herbivory are globally comparable to those reported from older and larger oak forests. The size of stands had a relatively minor effect on herbivory, whereas it increased the abundance of insectivorous bird. It also determined rates of avian insectivory as estimated by an experiment with plasticine caterpillars. These rates were however rather low and unrelated with the extent of herbivory in the stand. Overall, our study indicates that insect herbivores tend to react more rapidly to the establishment of novel native forests than their avian predators as the latter may depend on the development of larger stands of suitable habitat in the surrounding landscape. To favour a rapid build-up of diverse, and hence stable, trophic networks involving insect herbivores and their predators, woodland creation schemes should therefore primarily focus on habitat size and quality.

Keywords: Herbivory, Avian predation, Bird communities, Native oak forest, Connectivity, Afforestation

2.1. Introduction

Forest fragmentation is well-known to alter patterns of species distribution and abundance, relationships between organisms and resulting ecosystem processes (Ewers and Didham, 2006; Fahrig, 2017; Haddad et al., 2015; Lindenmayer and Fisher, 2013). Among others, it exerts strong effects on trophic cascades such as plant-herbivore-predator interactions, eventually affecting rates of tree damage and health (Bagchi et al., 2018; Chávez-Pesqueira et al., 2015; Rossetti et al., 2017). While forest fragmentation continues to occur in many regions of the world, forest cover is increasing in many others as a consequence of active planting and passive afforestation following rural abandonment (Fuchs et al., 2015; Hansen et al., 2013). For instance, Europe has experienced a steady increase of forested surfaces by 0.8 million ha per year since 1990 (Forest Europe, 2015), a trend that is expected to continue in the coming decades (Fuchs et al., 2015; Schröter et al., 2005). Habitat defragmentation through passive afforestation has been proposed as an effective tool to reinforce biodiversity and ecosystem functioning in rural and urban landscapes where forest stands were formerly sparse and isolated (Fischer et al., 2006; Rey Benayas et al., 2008; Rey Benayas and Bullock, 2012). Yet little ecological research has to date focused on newly established native forest stands and we largely ignore whether trophic interactions in such stands underlie similar mechanisms as in remnants of similar sizes but resulting from forest fragmentation.

Novel native forest stands establish from a few founder trees that colonize an available habitat patch within an unsuitable matrix through long-distance dispersal and fill their neighbourhood with their offsprings (Gerzabek et al., 2017; Sezen et al., 2005). Such stands share certain characteristics that set them apart from those created by fragmentation: (i) they typically are quite small-sized – even smaller than the smallest fragments of remnant forest; (ii) they are dominated by young trees, resulting in a reduced amount and range of habitats available to forest-dwelling species (Franklin, 1988; Fuller et al., 2018); and (iii) all their species necessarily originate from colonization events over a limited period of time, implying that these systems are triggered by immigration credit instead of extinction debt (Jackson and Sax, 2010). Recent studies on insect and bird species richness along chronosequences of novel native forest development have shown that these are rapidly colonized by woodland generalists whereas specialists can still remain absent even 150 years after forest establishment (Fuentes-Montemayor et al., 2015; Fuller et al., 2018; Whytock et al., 2018). These studies also revealed that local stand characteristics are relatively more important than landscape characteristics for successful colonization by insects and birds. Similar findings have been reported for planted forests (reviewed in Burton et al., 2018). However, their consequences for trophic relationships between plants, insect herbivores and insectivores remain unknown.

Despite the differences between novel native forest stands and remnant forest fragments, the ecological mechanisms underlying trophic cascades involving trees, insect herbivores and birds can to some extent be inferred from fragmentation studies. These have documented that the size and connectivity of forest stands can shape trophic cascades very differently depending on the relative importance of the bottom-up and top-down effects involved (De La Vega et al., 2012; Rossetti et al., 2014). Thus, small and isolated forest stands provide less and possibly lower-quality resources to herbivores (Chávez-Pesqueira et al., 2015) and their colonization requires longer-distance movements that increase energetic and fitness costs (O'Rourke and Petersen, 2017), eventually resulting in lower herbivore abundance (De La Vega et al., 2012; Simonetti et al., 2007). However, small stands also experience greater edge effects which typically go along with increased herbivory (Bagchi et al., 2018; De Carvalho Guimarães et al., 2014). On the other hand, insect herbivores are more likely to colonize small but closer novel forest stands while their predatory vertebrates are more likely to colonize more distant but larger ones (Barbaro et al., 2014; Bereczki et al., 2014; Cooper et al., 2012; Maguire et al., 2015).

There is broad consensus that, generally, predators can notably reduce insect herbivory by regulating herbivore populations (Böhm et al., 2011; Letourneau et al., 2009; Maguire et al., 2015; Rosenheim, 1998). However, their actual relevance in novel native forest stands depends strongly on how both prey and predators respond to stand size and connectivity (Gripenberg and Roslin, 2007). This study investigated how levels of insect herbivory, avian predation and the abundance and diversity of insectivorous birds in recently established native Pedunculate oak (*Quercus robur*) forest stands are influenced by their size and the cover of broadleaf forest in the surrounding landscape. Specifically, we addressed the following questions: (i) Does herbivory increase or decrease along gradients of increasing stand size and connectivity? (ii) Does avian predation increase or decrease along the same gradients? (iii) Are the observed trends related with the local abundance and diversity of insectivorous birds? We contrast our findings with those reported from studies of forest fragmentation and discuss implications in a context of increasing forest connectivity following ongoing changes in landscape use and management (Burton et al., 2018; Rey Benayas and Bullock, 2012).

2.2. Material and methods

2.2.1. Study area and selection of study sites

The study was carried out in the Landes de Gascogne region (south-western France) about 40 km southwest of Bordeaux (44°41'N, 00°51'W). The region is characterized by an oceanic climate with mean annual temperature of 12.8°C and annual precipitation of 873 mm over the last 20 years. The area is covered by extensive plantations of maritime pine (*Pinus pinaster* Ait.) interspersed with small stands of broadleaved forests that are dominated by Pedunculate oak (*Quercus robur* L.) and contain Pyrenean oak (*Quercus pyrenaica* Willd.), birch (*Betula pendula* L.) and other tree species in minor abundance. Such stands are largely exempt from forest management. Many are actively expanding (Gerzabek et al., 2017), favoured by a recent change in silvicultural management that tends to conserve broadleaved trees recruiting within adjacent pine plantations as a mean of conservation biological control (Castagneyrol et al., 2014; Dulaurent et al., 2011).

We carefully selected a total of 18 novel oak forest stands along gradients of stand size and connectivity (Fig. A2.1). To ensure that forest stands were of recent origin, we confirmed on aerial photographs from the 1950s that only very few trees were present at that time. We measured the stand area (henceforth referred to as stand size) as the minimum polygon including all oak trees with a stem diameter at breast height of ≥3cm (range: 0.04-1.15 ha; Table A2.1). The basal area of the stand was also measured and was highly correlated with stand size so we decided to include only stand size in the analysis (Pearson $r = 0.92$, $P < 0.05$). We quantified the spatial connectivity of stands to more ancient forests by calculating the cover of broadleaf forests in a circular buffer of 500m radius around each stand (range: 0-30%). The size of the buffer (78.5 ha) has previously been shown to be well-suited for studying plant-herbivore-predator interactions (Barbaro et al., 2014; Chaplin-Kramer et al., 2011). Preliminary analyses revealed that the results were qualitatively the same with buffers of 250, 750 and 1000m radius. Habitat mapping was based on aerial photos using QGIS version 2.18.13 (Quantum GIS Development Team, 2017). Stand size and connectivity were not correlated (Fig. A2.1; Pearson $r = 0.39$, $P = 0.11$).

2.2.2. Leaf insect herbivory

In early June 2017, we haphazardly selected four adult oak trees in each forest stand for assessing herbivory and avian predation. On each tree, we haphazardly cut two south facing and two north facing branches, respectively, at 4 and 8 m height and haphazardly sampled 20 fully developed leaves from each branch (summing 80 leaves per tree and 320 per stand). Leaves were taken to the laboratory for counting the number of leaf mines and galls per leaf and for estimating the percentage of leaf surface consumed or scratched by chewing and skeletonizing herbivores. A previous study (Giffard et al., 2012) had shown that the most common chewers and skeletonizers in the study area are Lepidoptera and Hymenoptera (sawfly) larvae. We distinguished eight levels of surface damage (0%, 1-5%, 6-10%, 11-15%, 16-25%, 26-50%, 51-75%, and >76%). The gall records were finally discarded from the study because they were too infrequent for independent analyses. In the following, we will refer to ‘herbivory’ as the tree level average leaf area removed by chewing or skeletonizing invertebrates, and to ‘number of mines’ as the average number of mines per leaf. We used the number of mines instead of the proportion of leaves with mines as 9 % of leaves had more than one mine.

2.2.3. Avian predation

We used dummy caterpillars made of plasticine (Staedler, Noris Club 8421, green[5]) to estimate predation on insect herbivores. Although not representative of absolute predation rates in the wild, this method allows to compare relative avian predation across stands (González-Gómez et al., 2006; Gunnarsson et al., 2018; Lövei and Ferrante, 2017). Plasticine caterpillars were 30 × 3 mm and light green to mimic late-instar larvae of caterpillars commonly found on oak in the field (Barbaro et al., 2014). We secured 10 plasticine caterpillars at 1.5-2 m height in the canopy of each of our four experimental trees per stand using 0.5 mm metal wires. Predation on plasticine caterpillars was surveyed every six to eight days from 15th May to 15th June (Low et al., 2014). Previous studies have shown that this time period matches the peak activity of insectivorous birds in the study area and is therefore relevant to quantify variation in avian predation (Barbaro et al., 2014; Bereczki et al., 2014; Castagneyrol et al., 2017). All caterpillars with beak marks left by insectivorous birds were recorded and replaced with undamaged ones during each survey. We decided to discard marks putatively left by insectivorous arthropods because we did not assess insectivorous arthropod communities of the stands (see below for birds). Previous to statistical analysis, we standardized our observation by calculating the mean daily predator activity per tree.

2.2.4. Bird communities

We surveyed the insectivorous bird community in each forest stand using 10-min point counts. Censuses were performed by a trained observer between 6:00 and 9:00 a.m. from the centre of the stand. Each stand was censused twice, once between 26th May and 2nd June and a second time between 21th and 29th June during the exposure period of plasticine caterpillars. All birds within the stand were recorded. Further analysis considered only those species that have a predominantly insectivorous diet during the breeding season. We used the highest count of a given species during any of the censuses as estimate of its abundance within the stand.

2.2.5. Data analysis

We built three types of models for our different response variables. First, we used linear mixed-effect models (LMM) to model either insect herbivory or the number of mines as a function of stand size ('Size'), stand connectivity in the surrounding landscape ('Connectivity') and their interaction ('Size × Connectivity'). Size, Connectivity and Size × Connectivity were included as fixed effects and the identity of the stand as a random factor. With these predictors three different models were built, each with one further fixed effect, to assess the influence of insectivorous birds on herbivory. These additional fixed effects were either predation on plasticine caterpillars (measured experimentally) or the abundance or species richness of insectivorous birds in the stand (recorded during point counts). We analysed these effects separately because of their non-independence. Second, we modelled predation on plasticine caterpillars as a function of stand size, stand connectivity and their interaction. All were included as fixed effects and stand identity as random effect. Adopting the same approach as for herbivory and the number of mines, we built three models with either herbivory or the abundance or species richness of insectivorous birds per stand as additional fixed effect. Third, we built a generalised linear model (GLM) with stands as replicates to assess the effect of stand size, connectivity and their interaction on the abundance and richness of insectivorous birds. We used Quasi-poisson and Poisson error distributions to model bird abundance and bird species richness, respectively.

All continuous predictor variables were scaled and centred prior to modelling to make their coefficients comparable (Schielzeth, 2010). We first built a full model including all fixed effects, interactions and random factors. Then we applied model simplification by sequentially removing non-significant fixed effects, starting with the least significant interaction. We stopped model simplification with the minimum adequate model when all non-significant terms were taken out. Hereafter, we only report statistics for the simplified models. We estimated and compared model fit by calculating marginal and conditional R^2 (respectively R_m^2 and R_c^2) in order to estimate the proportion of variance explained by fixed (R_m^2) and fixed plus random factors (R_c^2) (Nakagawa and Schielzeth, 2013).

All analyses were done in R version 3.4.1 (2018), using the following packages: *car*, *doBy*, *forecast*, *lmerTest*, *MuMIn* and *vegan* (Barton, 2018; Fox and Weisberg, 2011; Højsgaard and Halekoh, 2018; Hyndman et al., 2018; Kuznetsova et al., 2017; Oksanen et al., 2018).

2.3. Results

Insect herbivory was on average (± se, n = 72) 8.02 ± 4.51 % (Table A1). The effect of stand size on herbivory depended on the connectivity of the stand (significant Size × Connectivity interaction, Table 2.1): herbivory tended to increase with stand size in landscapes with a low stand connectivity whereas it decreased in landscape where broadleaf forests where more abundant (Fig. 2.1). Neither avian predation on plasticine caterpillars nor bird abundance or richness had a significant effect on herbivory. The number of mines per leaf was on average 0.07 ± 0.05 (Table A1) and decreased in stands that were more connected. Leaf miners were not affected by stand size (Table 2.1).

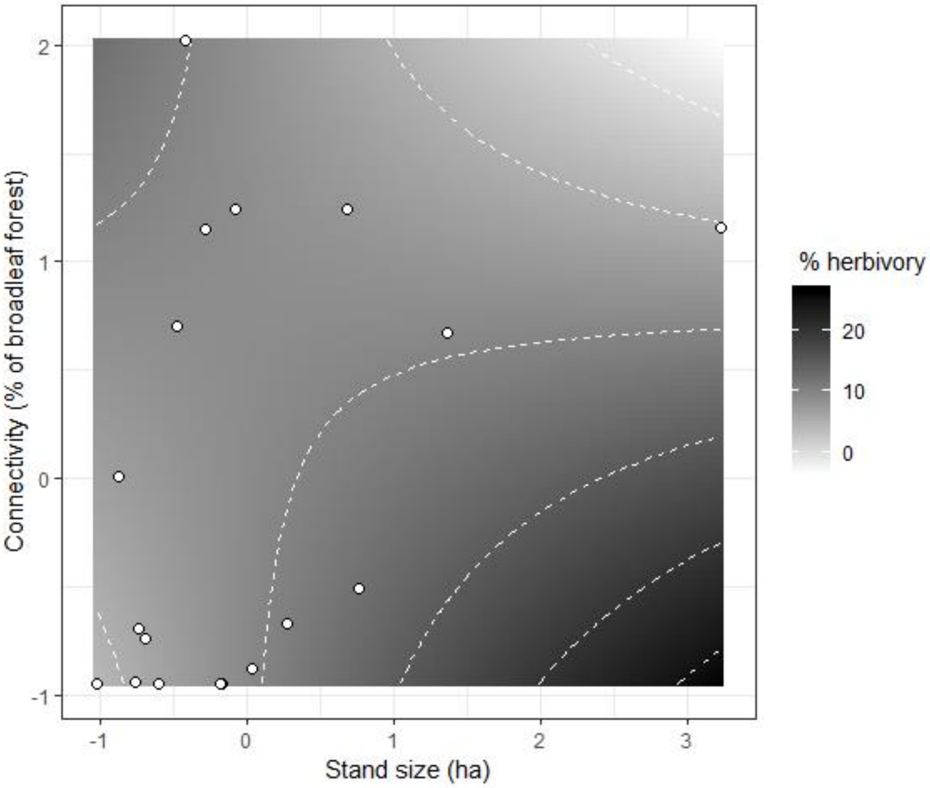

Figure 2.1. Interactive effect of stand size and connectivity on herbivory. White to black colour scale and isolines show the predicted percentage of herbivory along standardized gradients of stand size (measured as the stand area) and stand connectivity (measured as the cover of broadleaf forest within a buffer of 500m radius). White dots show the distribution of the original data.

A total of 18 caterpillars out of the 720 exposed (2.5 %) presented marks of bird attacks. Avian predation slightly increased with stand size while it did not vary with stand connectivity or the abundance or richness of insectivorous birds in the stand (Table 2.1).

Table 2.1. Summary of LMM testing the effect of stand size, connectivity, their interaction and either Avian predation, abundance or richness on insect herbivory. For avian predation the effect of stand size, connectivity, their interaction and either herbivory, bird abundance or richness were tested. Significant variables are indicated in bold. Only predictors retained after model simplification are shown. Predictors were scaled and centred. R^2m and R^2c correspond to the variance explained by fixed and fixed plus random factors, respectively.

Response	**Predictors**	x^2	**Df**	**Coef. ± SE**	P	R^2_m (R^2_c)
Herbivory	Size	0.06	1	2.010 ± 1.061	0.807	0.20 (0.43)
	Connectivity	0.87	1	-0.041 ± 0.784	0.351	
	Size × Connectivity	8.35	1	-2.933 ± 1.015	**0.004**	
No. of mines	Connectivity	4.53	1	-0.016 ± 0.007	**0.033**	0.10 (0.31)
Avian predation	Size	3.94	1	0.135 ± 0.068	**0.047**	0.06 (0.13)

We detected a total of 17 bird species within the studied oak stands. The mean (± se, n = 18) abundance was 4.22 ± 2.59 individuals (range: 1 - 9) and the mean species richness was 3.22 ± 1.66 (range: 1 - 6). The most abundant bird species were blue tit (*Cyanistes caeruleus*), common chaffinch (*Fringilla coelebs*) and chiffchaff (*Phylloscopus collybita*) (Fig. A2.2). These three species accounted for 38.2 % of all records. Total bird abundance increased with stand size (Fig. 2.2a, Table 2.2) and decreased with stand connectivity (Fig. 2.2b, Table 2.2). The strength of stand size and connectivity effects was comparable although their effects were opposite. Species richness did not vary with stand size nor with stand connectivity.

Table 2.2. Summary of the GLM on insectivorous bird abundance and species richness as a function of stand size and connectivity. Only predictors remaining after model simplification are shown. Stand size and connectivity were previously standardized. LR: Likelihood Ratio.

Response	**Predictors**	χ^2	**Df**	**Coef. ± SE**	***P***	R^2_m (R^2_c)
Bird abundance	Size	8.569	1	0.373 ± 0.124	**0.003**	0.42 (0.43)
	Connectivity	6.554	1	-0.391 ± 0.164	**0.010**	

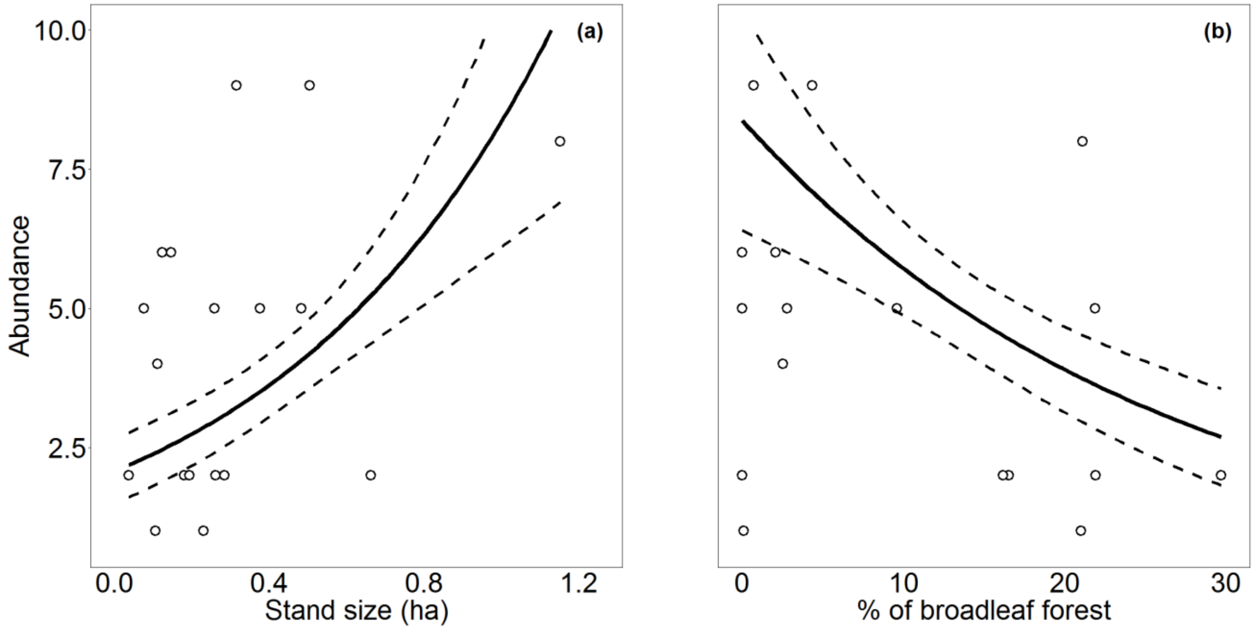

Figure 2.2. Effects of stand size and connectivity on bird abundance (a, b). Dots represent the individual stands. Solid lines and dashed lines represent model predictions and corresponding standard errors, respectively.

2.4. Discussion

Our study revealed that the size and connectivity of novel native forest stands affect herbivorous insects and insectivorous birds in different ways. While the abundance of leaf miners depended on stand connectivity alone, herbivory by chewers and skeletonizers was influenced by an interplay between stand size and connectivity, and bird abundance (but not species richness) showed consistent independent and opposite responses to stand size and connectivity. This divergence of relationships is likely to arise from differences in the spatial grain of habitat perception and use by the different trophic guilds. It illustrates the complex nature of trophic cascades involving trees, insect herbivores and insectivorous birds in novel native forest stands (Gripenberg and Roslin, 2007).

2.4.1. Insect herbivores

The observed decrease in the abundance of leaf mining insects with increasing stand connectivity contrasts with previous detailed studies of leaf miners on *Quercus robur* (Gripenberg et al., 2008; Tack et al., 2010) that reported the opposite trend. Importantly, however, these studies focused on a finer spatial grain since they compared individual oak trees with different small-scale ecological neighbourhoods, not with entire forest stands. While the context of their study implies limited movement ranges of leaf mining insects, our results suggest that low abundance of source populations in the surroundings does not limit the ability of this guild to colonise and persist in small novel forest stands. The observed trend could instead be triggered by a resource dilution effect (Otway et al., 2005) whereby herbivore concentrate on the fewer available host individuals (Bañuelos and Kollmann, 2011). Dietary specialists such as many leaf miners should be particularly concerned by resource dilution (Elzinga et al., 2005).

Herbivory by chewing and skeletonizing insects was triggered by stand size in areas where oaks were generally sparse. Positive relationships between stand size and herbivory have also been reported by several studies conducted in considerably larger forest fragments (De La Vega et al., 2012; Simonetti et al., 2007 but see Maguire et al., 2015; Silva and Simonetti, 2009). They could arise from a higher density and/or diversity of insect herbivores in larger stands (Chávez-Pesqueira et al., 2015), as predicted by the resource concentration hypothesis (Hambäck and Englund, 2005; Root, 1973). This hypothesis states that the intensity of physical and chemical cues makes these stands more likely to be found and colonised and less likely to be left by herbivores. The resource concentration hypothesis should be particularly relevant in small habitat patches, such as those of our study system. However, we found that leaf herbivory ceased to increase with stand size and started instead to decline when broadleaf forest became more abundant in the surroundings. We have two possible, non-exclusive explanations for this phenomenon: (i) colonization rates of chewers and skeletonizers could generally be so high in our study system that even the smallest forest stands will be effectively reached (and, if necessary, re-colonized) when a certain threshold abundance of suitable habitats and associated herbivore source populations exist in the landscape (Fahrig, 2013). This hypothesis is supported by the fact that novel established forest stands are very rapidly colonised by woodland generalist species (Fuentes-Montemayor et al., 2015; Fuller et al., 2018). Second, (ii) insect herbivory tends to be favoured by edge effects (De Carvalho Guimarães et al., 2014), especially when it involves generalist species (Bagchi et al., 2018). Edge effects decrease in larger stands, which would counteract other positive effects of stand size on herbivory. Both explanations together suggest that the patterns of leaf herbivory that we observed are likely to be primarily driven by a relatively limited set of mobile generalist species. These species generated however leaf consumption rates that were low but comparable to those recorded in many older and larger oak forests (Gunnarsson et al., 2018; Moreira et al., 2018; Sanz, 2001), and they enabled a quick build-up of trophic cascades even in the smallest and youngest stands of our study system (Hagen et al., 2012).

2.4.2. Avian insectivores and insectivory

Overall bird abundance and species richness were rather low as well as the size of the stands compared to previous works conducted in the same area (Barbaro et al., 2005; Giffard et al. 2012), and so was also the rate of avian predation (Castagneyrol et al., 2017). Previous studies by Genua et al. (2017), Peter et al. (2015) and Ruiz-Guerra et al. (2012) also found an increase in bird abundance with an increase in continuous forest in the landscape. These forests were however larger than the stands of our study, supporting the idea that avian predation rate and bird abundance (but not species richness) increased with stand size. Overall, these findings suggest that the activity of insectivorous birds in our study system is constrained by the carrying capacity of their wooded habitats. Typical breeding season territories of the most frequently recorded bird species actually exceed the size of our smallest stands (Hinsley et al., 1995) and only the largest stands could regularly sustain more than one territory of the same species. These large stands should also provide the broadest range of tree ages and vegetation structures to different species, although it certainly is still inferior to that of mature forests (Fuentes-Montemayor et al., 2015). Habitat diversity and quality might then also be behind our rather surprising finding that bird abundance (although not species richness) tended to decrease with increasing stand connectivity (Fig. 2.2). Around the least connected stands, the broadleaf forest cover typically consisted of small, early-successional woodland patches, whereas several of the most connected stands were close to more continuous, older forests, expected to host a large functional diversity of insectivorous birds. The habitat quality of our focal stands should hence equal or exceed that of their surroundings in the former case but be inferior in the latter. The lower use of stands located near larger forests could then be interpreted as a resource dilution effect (see also Berg, 1997; Brotons et al., 2003). That we failed to see this landscape-scale effect reflected in our predation experiment could then simply be due to the low overall number of caterpillar attacks that we recorded and/or other potential limitations of the experimental approach (Muchula et al., 2019). It is however consistent with previous studies that fail to correlate herbivory with predation on plasticine caterpillars (Bereczki et al., 2014; Castagneyrol et al., 2017; but see Gunnarsson et al. 2018).

2.4.3. Tree-herbivore-insectivore interactions and the management of novel native forests

To date most studies on the ecological impacts of active or passive afforestation in fragmented landscapes have focused on patterns of biodiversity (reviewed in Burton et al., 2018), whereas functional ecological aspects have received far less attention (but see Rey Benayas and Bullock, 2012). Our study on bird-insect relationships in novel established native forest stands adds a novel perspective to this field. Taken together, our results indicate that novel forest stands can be very effectively colonised by different guilds of insect herbivores. Although this process is likely to involve primarily a subset of mobile generalist species, these alone can generate levels of herbivory that are quite comparable to those at later stages of forest succession and in areas with higher forest cover. In turn, the build-up of insectivorous bird communities tends to occur more slowly because these depend more than their prey on the development of suitable habitat patches of a certain minimum size (Genua et al., 2017). Birds, as long-lived mobile vertebrate insectivores, typically need to find enough substitutable or non-substitutable resources in the surrounding habitat patches to fulfil entirely their life cycles, namely landscape supplementation and complementation processes (Brotons et al., 2005; Dunning et al., 1992; Fahrig, 2017; Tubelis et al., 2004). Globally, we failed to detect any evidence of top-down control of herbivory by predators. As a consequence, trophic networks in our study system are likely to underlie strong stochasticity, resulting in extensive among-stand heterogeneity and variation through time, which is also typical of forest ecosystems having experienced long-term fragmentation processes (Hagen et al., 2012; Bregman et al., 2015; Fahrig, 2017).

The value of native broadleaf woodlands for biodiversity conservation is important to consider for sustainable forest management in rural landscapes. Landscape defragmentation through networks of novel native forest stands represents a cost-efficient tool for restoring biodiversity and numerous associated ecosystem services (Rey Benayas and Bullock, 2012). Yet the dynamics and ecological functioning of novel native forest stands remain much less well understood than those of forest remnants resulting from fragmentation. Our study underpins that different trophic guilds respond very differently to these novel habitats depending on the spatial grain at which they perceive and exploit them (Gripenberg and Roslin, 2007). To favour a rapid build-up of diverse, and hence stable, trophic networks involving insect herbivores and their predators, woodland creation schemes should focus on habitat size and quality rather than connectivity, including a management that facilitates a diverse tree and understorey vegetation structure (see also Burton et al., 2018; Fuller et al., 2018).

2.5. Appendix A2

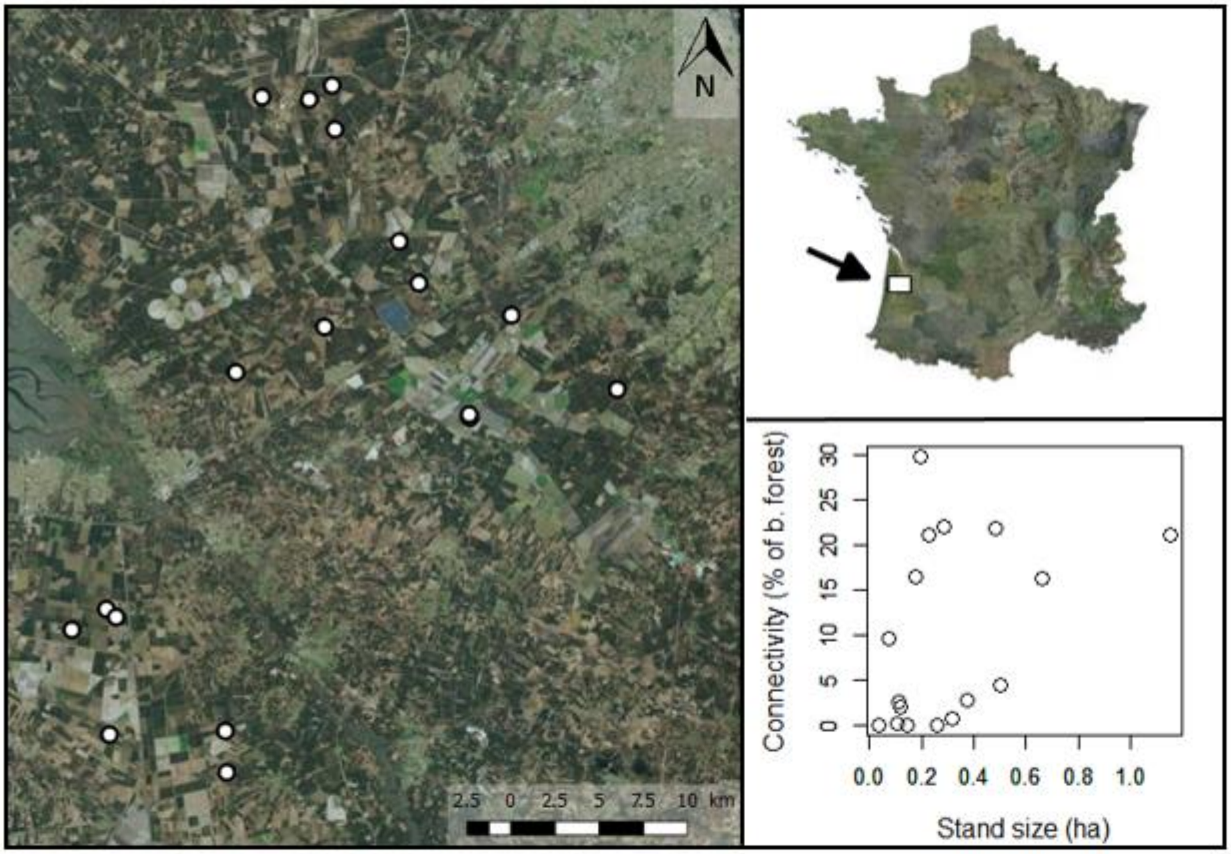

Figure A2.1. Location map of the study area in the Aquitaine region, south-western France, showing the 18 oak stands at the top right and left of the figure, and figure showing stand size (ha) and connectivity of each stand at the bottom right of the figure.

Table A2.1. Information about the location and size of the oak stands included in the study and summary of the results of herbivory (% leaf damage and Number of mines), predation on plasticine caterpillars and bird abundance and species richness within each stand.

Stand	**Latitude**	**Longitude**	**Stand size (ha)**	**No. of Oaks**	**Herbivory**	**No. of mines / leaf**	**Avian predation on caterpillars**	**Bird abundance**	**Bird species richness**
1	44.743	-0.800	0.375	110	13.88	0.147	0.589	5	4
2	44.729	-0.733	0.123	28	4.68	0.078	0.089	6	4
3	44.764	-0.816	0.179	35	13.72	0.056	0.000	2	1
4	44.568	-1.011	0.315	50	4.41	0.069	0.268	9	6
5	44.564	-1.004	0.111	32	6.58	0.050	0.000	4	3
6	44.556	-0.035	0.106	30	6.50	0.059	0.268	1	1
7	44.834	-0.919	0.504	33	13.77	0.025	0.324	9	6
8	44.834	-0.885	0.229	71	5.81	0.044	0.893	1	1
9	44.842	-0.869	0.663	132	6.54	0.034	0.491	2	2
10	44.819	-0.865	0.483	150	4.92	0.056	0.000	5	4
11	44.677	-0.760	0.261	55	7.31	0.141	0.263	2	2
12	44.675	-0.759	0.036	17	5.58	0.103	0.781	2	2
13	44.693	-0.655	0.146	64	3.97	0.072	0.179	6	4
14	44.504	-0.004	0.193	43	10.55	0.022	0.179	2	2
15	44.692	-0.928	1.151	156	6.35	0.088	0.964	8	6
16	44.719	-0.869	0.283	29	11.65	0.066	0.536	2	2
17	44.509	-0.922	0.075	16	8.78	0.072	0.089	5	4
18	44.487	-0.920	0.258	38	9.40	0.075	0.655	5	4

3. Chapter 2: Is there a relationship between tree genetic relatedness, leaf trait variation and insect herbivory in oaks?

Elena Valdés-Correcher, Audrey Bourdin, Santiago C. González-Martínez, Xoaquín Moreira, Andrea Galmán, Bastien Castagneyrol & Arndt Hampe – **Leaf chemical defences and insect herbivory in oak: accounting for canopy position unravels marked genetic relatedness effects** – *Annals of Botany* – Under review – 07/01/2020

Abstract:

Background and Aims

Highly controlled experiments revealed that plant genetic diversity and relatedness can shape herbivore communities and patterns of herbivory. Evidence from the field is scarce and inconsistent. We assessed whether a genetic signal underlying herbivory can be detected in oak forest stands when accounting for variation at smaller (within-tree) and larger (among-stand) scales.

Methods

We tested relationships between tree genetic relatedness, leaf chemical defences and insect herbivory at different canopy layers in 240 trees from 15 Pedunculate oak (*Quercus robur*) forest stands and partitioned sources of variability in herbivory and defences among stands, individuals, and branches.

Key Results

Leaf defences, insect herbivory, and their relationship differed systematically between the upper and the lower tree canopy. When accounting for this canopy effect, the variation explained by tree genetic relatedness rose from 2.8 to 34.1 % for herbivory and from 7.1 to 13.8 % for leaf defences. The effect was driven by markedly stronger relationships in the upper canopy.

Conclusions

Our findings illustrate that properly accounting for other sources of variation acting at different scales can reveal potentially relevant effects of the host plant genotype on patterns of leaf chemical defences and associated insect herbivory in natural tree populations.

Keywords: Genetic relatedness, chemical defenses, herbivory, *Quercus robur*, plant-insect interactions

3.1. Introduction

A rapidly growing number of studies have shown over the last decade that plant genetic diversity and genetic relatedness can influence herbivore communities and associated patterns of herbivory (Crutsinger et al., 2006; Kagiya et al., 2018; McArt and Thaler, 2013). It has been proposed that the composition and activity of herbivore communities are heritable traits of the host plant that are partly driven by the heritability of its anti-herbivore chemical defences (Bangert et al., 2006; Barker et al., 2019; Bustos-Segura et al., 2017; Wimp et al., 2005). Plant families vary indeed considerably in their edibility and resulting herbivore damage (Barker et al., 2018; Damestoy et al., 2019; Donaldson and Lindroth, 2007; Fernandez-Conradi et al., 2017). However, most previous research has been performed on highly-controlled experiments (e.g. common gardens), often with juvenile plants and minimized spatial and environmental effects, settings that could lead to overemphasize the putative role of genetics in nature (Lämke and Unsicker, 2018; Tack et al., 2012). Accordingly, more research in natural plant populations is needed for understanding to which extent genetically-based variation in plant chemical defences determines insect herbivory (Carmona et al., 2011; Wimp et al., 2005).

Diverse biological mechanisms can contribute to blur links between plant genotype, plant chemical defences and herbivory patterns in mature trees in the wild. Many secondary metabolites exhibit low heritability because their production is controlled by multiple genes and their interactions (Büchel et al., 2016; Külheim et al., 2011). Different plant parts experience different microclimates (e.g., irradiation, temperature, humidity) that can trigger extensive within-individual variation in leaf morphology and chemistry, especially along tree vertical gradients. Upper canopy leaves are typically thicker, tougher, smaller, drier and contain higher levels of chemical leaf defences than lower canopy leaves (Castagneyrol et al., 2019a; De Casas et al., 2011; Le Corff and Marquis, 1999; Murakami et al., 2005; Murakami and Wada, 1997; Ruhnke et al., 2009). More specifically, differences in microclimate should directly affect the expression of genes that code the production of leaf chemical defences (reviewed in Lämke and Unsicker, 2018). In turn, tree vertical gradients in insect herbivory can result from differences in herbivore dispersal (e.g., flying insects concentrated in the upper canopy; Ulyshen, 2011) or herbivore exposition to predators (e.g., lower predation rates in the upper canopy; Aikens et al., 2013) that are not driven by leaf chemistry. Genotype-phenotype-herbivory associations can also be obscured at larger spatial scales owing to the non-random distribution of host plant genotypes (i.e., spatial or population genetic structure) that is widespread within and among natural plant populations as a consequence of limited effective gene flow and/or genetic drift (Hoban et al., 2016; Rellstab et al., 2015; see also Tack et al., 2012). Finally, landscape-scale patterns of herbivore abundance and diversity are well-known to be strongly influenced by resource availability and by herbivores' spatial grain of habitat perception and use (Bagchi et al., 2018; O'Rourke and Petersen, 2017; Tack et al., 2010; Valdés-Correcher et al., 2019). The plethora of potential confounding factors underpins that careful study designs including multiple-scale sampling are needed to thoroughly assess effects of genetically-based variation in leaf chemical defences on herbivory in natural plant populations.

This study investigated the relationships between tree genetic relatedness, leaf defences and herbivory in natural forest stands of Pedunculate oak (*Quercus robur*). For this, we genotyped 703 trees from 15 stands and quantified the concentration of leaf phenolic compounds and herbivory by leaf-chewing insects at the intermediate and upper canopy layer for a subset of 235 trees. Specifically, we addressed the following questions: (i) To what extent do leaf phenolics and insect leaf herbivory vary among stands, among trees within stands and between canopy layers within trees? (ii) Do leaf phenolics and herbivory show a genetic signal when accounting for their scale-dependent variation? (iii) To what extent does variation in leaf phenolics explain patterns of leaf herbivory? By addressing these questions, we aim at combining a thorough description of cross-scale patterns typical of natural systems with insights into the biological mechanisms that underlie plant-insect herbivore relationships in non-experimental contexts.

3.2. Material and Methods

3.2.1. Study system

We performed this study in the Landes de Gascogne region (SW France) about 40 km South from Bordeaux (44°41'N, 00°51'W). The area is dominated by extensive maritime pine (*Pinus pinaster* Ait.) plantations with scattered small stands of broadleaf forest. These are usually dominated by Pedunculate oak and contain other tree species like birch (*Betula pendula* L.), Pyrenean oak (*Quercus pyrenaica* Willd.), holly (*Ilex aquifolium* L.) or willows (*Salix* spp.) in minor abundance. Such stands are not subjected to intensive forest management and many are actively expanding

(Gerzabek et al., 2017), favoured by a recent change in silvicultural management that tends to conserve oaks recruiting within adjacent pine plantations in order to increase biological pest management (Dulaurent et al., 2012). Pedunculate oak supports a large community of specialist and generalist herbivore insects in these stands (Giffard et al., 2012). Leaf chewers, skeletonizers, miners and gallers are the principal guilds responsible for background herbivory (damage imposed by a community of herbivores whose populations are at normal low densities) that amounts to values around 17.8 % (Giffard et al., 2012).

3.2.2. Forest stands, sampling and herbivory measurements

We selected 15 forest stands of variable size and connectivity within the landscape. All stands were second-growth forests that have established since the 1950s through natural tree regeneration (Valdés-Correcher et al., 2019). They were strongly dominated by Pedunculate oak and contained a variable but often rather sparse woody understory vegetation. The number of established oak trees ranged from 16 to 124 individuals and their surface (as derived from the minimum polygon including all trees) from 0.04 to 0.5 ha. Further information can be found in Table A3.1 of the Supplementary Material (see also Valdés-Correcher et al., 2019). Within each stand, we mapped and tagged every oak tree with a diameter at breast height (dbh) >3 cm and collected leaf material that was stored in silica gel until DNA isolation for the genotyping. This exhaustive sampling included a total of 703 individuals (see Table A3.1).

In September 2018, we randomly selected 16 individuals with a dbh >6 cm within each stand (total *n* = 235). On each tree, we haphazardly choose and cut two south-facing branches situated at 4 and 8 m above ground level, respectively, which corresponds to the intermediate (shaded) and upper (sun-exposed) tree canopies in most of our trees (see also Castagneyrol et al., 2019a). Three of the 235 sampled trees did not reach 8 m, so we shifted the position of the intermediate and upper tree canopy layers 2 m downward (i.e., 2 and 6 m, respectively). Operators unaware of the study design systematically picked the 30 most apical leaves from each branch, resulting in a total of 60 leaves per tree. Samples were stored at -18°C until insect herbivory measurement (see below). For each leaf, we visually estimated the percent leaf area removed by chewing insects using the following scale: 0 = 0%, A = 1-5%, B = 6-15%, C = 16-25%, D = 26-50%, E = 51-75%, F= >75%). We used pre-established templates mimicking known levels of insect herbivory on oak leaves to increase reliability and repeatability of herbivory measurements. Herbivory levels were always estimated by the same observer (A. Bourdin) blind to leaf origin to maximise consistency of the estimates and reduce unconscious bias. We averaged values across all leaves to obtain a mean value per branch, and then used the median of each percentage class for statistical analyses (Castagneyrol et al., 2019a).

We also collected 10 fully expanded leaves with no signs of herbivory or pathogen infection from each branch for quantification of phenolic compounds. We immediately oven-dried these leaves for 48-72 h at 45°C and grounded them to a thin powder before further chemical analyses (see below).

3.2.3. Molecular analyses

Genomic DNA was isolated from the leaves using the Invisorb® DNA Plant HTS 96 kit/C and the standard protocol. All trees were genotyped using 141 single nucleotide polymorphism (SNP) markers from the sets described in Gerzabek (2017) and Guichoux (2013). The list of loci is provided in Guichoux (2013). For genotyping, SNP loci were multiplexed using an iPLEX Gold kit on a MassARRAY Typer Analyzer 4.0.26.75 (Agena Biosciences) at the Genomic and Sequencing Facility of Bordeaux (France), as described in Gerzabek et al. (2017). High-quality data with a low proportion of missing calls were obtained for all markers and individuals.

3.2.4. Chemical analyses

We extracted phenolic compounds from 20 mg of dry leaf tissue with 1 mL of 70% methanol in an ultrasonic bath for 20 min, followed by centrifugation (Moreira et al., 2014). Samples were centrifuged at 3500 rpm and transferred to chromatographic vials. We performed the chromatographic analyses in an Ultra-High-Performance Liquid-Chromatograph (UHPLC Nexera LC-30AD; Shimadzu Corporation, Kyoto, Japan) equipped with a Nexera SIL-30AC injector and a SPD-M20A UV/VIS photodiode array detector.

For the compound separation, we used a Kinetex™ 2.6 µm C18 82-102 Å, LC Column 100 × 4.6 mm (Phenomenex, Torrance, CA, USA), protected with a C18 guard cartridge. The flow rate was established at 0.4 mL min^{-1} and the oven temperature was set at 25 ºC. The mobile phase consisted of two solvents: water-formic acid (0.05%) (A) and acetonitrile-formic acid (0.05%) (B), starting with 5% B and using a gradient to obtain 30% B at 4 min, 60% B at 10 min, 80% B at 13 min and 100 % B at 15 min. The injection volume was 15 µL. We recorded chromatograms at 330 nm and processed data with the LabSolutions software (Shimadzu Corporation, Kyoto, Japan). For phenolic compound identification, we used an ultra-performance liquid chromatography coupled with electrospray ionization quadrupole (Thermo Dionex Ultimate 3000 LC; Thermo Fisher Scientific, Waltham, MA, USA) time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS; Bruker Compact™, Bruker Corporation, Billerica, MA, USA). We identified four groups of phenolic compounds: flavonoids, ellagitannins and gallic acid derivatives ("hydrolysable tannins" hereafter), proanthocyanidins ("condensed tannins" hereafter), and hydroxycinnamic acid precursors to lignins ("lignins" hereafter). We quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents, hydrolysable tannins as gallic acid equivalents, and lignins as ferulic acid equivalents (Moreira et al., 2018; Galmán et al., 2018). We achieved the quantification of these phenolic compounds by external calibration using calibration curves at 0.25, 0.5, 1, 2 and 5 µg mL^{-1}. We calculated total phenolics for each branch as the sum of flavonoids, lignins, condensed tannins and hydrolysable tannins, and expressed concentrations of each phenolic group in mg g^{-1} tissue on a dry weight basis.

3.2.5. Statistical analyses

Prior to the analysis of genetic relatedness, we first examined the landscape-scale genetic structure of our oak stands by calculating pairwise F_{st} between stands according to Weir and C. Cockerham (1984). Overall low values (mean F_{st} = 0.041; range = 0.006-0.111) (Table A3.2), confirmed that the 15 stands can be considered a single gene pool and confounding effects due to population genetic structure are negligible. Then, we quantified the level of genetic relatedness between each pair of trees relative to the full sample (n = 703). For this, we computed a kinship matrix using Nason's kinship coefficient (Loiselle et al., 1995) with the software SPAGeDi version 1.2 (Hardy, Olivier J.; Vekemans, 2002). We extracted the values for our 16 target trees per stand from the global matrix and used this information as a quantitative estimate of their genetic relatedness in the subsequent analyses (Van Horn et al., 2008). Note that kinship-based estimates of relatedness, while commonly used in population genetics (Pemberton, 2008), are not directly comparable to those obtained through direct pedigree analyses.

We modelled patterns of insect leaf herbivory and leaf phenolics at the whole-tree and at the branch level by means of linear mixed-effect models (LMM). At the tree level, we built two independent LMM with stand ID and the kinship values of the target trees as random factors in order to estimate the variance and the percentage of the overall variance explained by the local environment (stand ID) and by the genetic relatedness among trees (the kinship matrix). The first model was an intercept only model with (tree-level mean) concentration of leaf phenolics as response variable (Eq. 1). The second model included (tree-level mean) insect herbivory as response variable and leaf phenolics as an additional explanatory variable (Eq. 2).

Model 1: $Phenolics_{i,j} = \beta_0 + S_i + T_j + \varepsilon_{i,j}$ (1)

Model 2: $Herbivory_{i,j} = \beta_0 + \beta_1 \times Phenolics_{i,j} + S_i + T_j + \varepsilon_{i,j}$ (2)

where β_0 is the model intercept, β_1 the fixed effects of leaf phenolics, S_i the random effect of stand i, T_j the random effect of tree genetic similarity j (entered as the kinship matrix) and $\varepsilon_{i,j}$ the error, with $S_i \in N(0, \sigma_S^2)$, $T_j \in N(0, \sigma_T^2)$ and $\varepsilon_{i,j} \in N(0, \sigma_E^2)$. For each model, we computed the variance of the fixed effects (if any, σ_F^2) and calculated the percentage of variance explained by each random factor (*e.g.*, $100 \times \sigma_T^2 / (\sigma_S^2 + \sigma_T^2 + \sigma_E^2)$ for the random effect of tree genetic similarity in model 2).

The second group of models, performed at canopy level, adopted the same approach with two independent LMMs modelling the response of total leaf phenolics and herbivory, respectively (Eqs. 3 and 4):

Model 3: $Phenolics_{i,j,k} = \beta_0 + \beta_1 \times Canopy\ layer_{i,j,k} + \mathrm{S}_i + T_j + \varepsilon_{i,j,k}$ (3)

$$\text{Model 4:} \qquad Herbivory_{i,j,k} = \beta_0 + \beta_1 \times Phenolics_{i,j,k} + \beta_2 \times Canopy\ layer_{i,j,k} + \mathrm{S}_i + T_j + \varepsilon_{i,j,k} \qquad (4)$$

where k indicates the branch, β_0 the intercept, β_1 and β_2 the coefficient parameters of the fixed effects and S_i, T_j and $\varepsilon_{i,j,k}$ as above. Again, we computed the variance of the fixed effects (if any, σ_F^2) and calculated the percentage of variance explained by each random factor.

All analyses were done in R version 3.5.2 (R Core Team 2018). LMMs including a kinship matrix as random factor were fit with the function *lmekin* in package *coxme* (Terry M. Therneau, 2018).

3.3. Results

3.3.1. Leaf phenolics and insect herbivory at tree level

Leaf phenolic concentration was on average (± se) 14.69 ± 0.39 $\mathrm{mg \cdot g^{-1}}$ (Fig. 3.1). The random factors collectively explained 26.9 % of the overall variation, with stand ID accounting for 19.7 % and tree genetic relatedness for 7.1 %. Insect leaf herbivory was on average 12.27 ± 0.29 % and decreased significantly with increasing leaf phenolic concentration (model coefficient parameter estimate: -0.12 ± 0.05, $z = -2.48$, $P = 0.013$). The effect size was however small (2.0 %). Stand ID explained 38.1 % of the overall variation in herbivory and genetic similarity among trees only accounted for another 2.9 %.

3.3.2. Leaf phenolics and insect herbivory at canopy level

Leaf phenolic concentration was significantly lower in the intermediate than in the upper canopy layer (mean ± SE: 13.63 ± 0.50 *vs* 15.79 ± 0.59 $\mathrm{mg \cdot g^{-1}}$) (Fig. 3.1, Table 3.1). Stand ID accounted for 13.7 % and tree genetic relatedness for 13.9 % of the overall variation (Table 3.1). Insect leaf herbivory did not differ significantly between tree canopies (12.53 ± 0.39 % *vs* 11.99 ± 0.43 %) and was independent of leaf phenolic concentration (Table 3.1). Stand ID and tree genetic relatedness accounted for 32.0 and 34.7 % of the overall variability in insect herbivory, respectively (Table 3.1).

In the intermediate canopy layer, stand ID and genetic relatedness accounted for 13.5 % and 0.03 % of the overall variation in leaf phenolics, respectively. Leaf phenolics had no significant effect on herbivory (Fig. 3.2). Stand ID explained 40.5 % of the overall variation in herbivory, while tree genetic relatedness accounted for less than 0.02 %.

In the upper canopy layer, stand ID and genetic relatedness explained 17.4 % and 24.8 % of the overall variation in leaf phenolics, respectively. There was a significant, albeit weak, negative effect of leaf phenolic concentration on herbivory (coefficient parameter estimate ± SE: -0.12 ± 0.05, $z = -2.63$, $P = 0.009$) (Fig. 3.2). Leaf phenolics accounted 2.8 % of the overall variation in herbivory while stand ID and tree genetic relatedness accounted for 25.3 and 14.5 %, respectively.

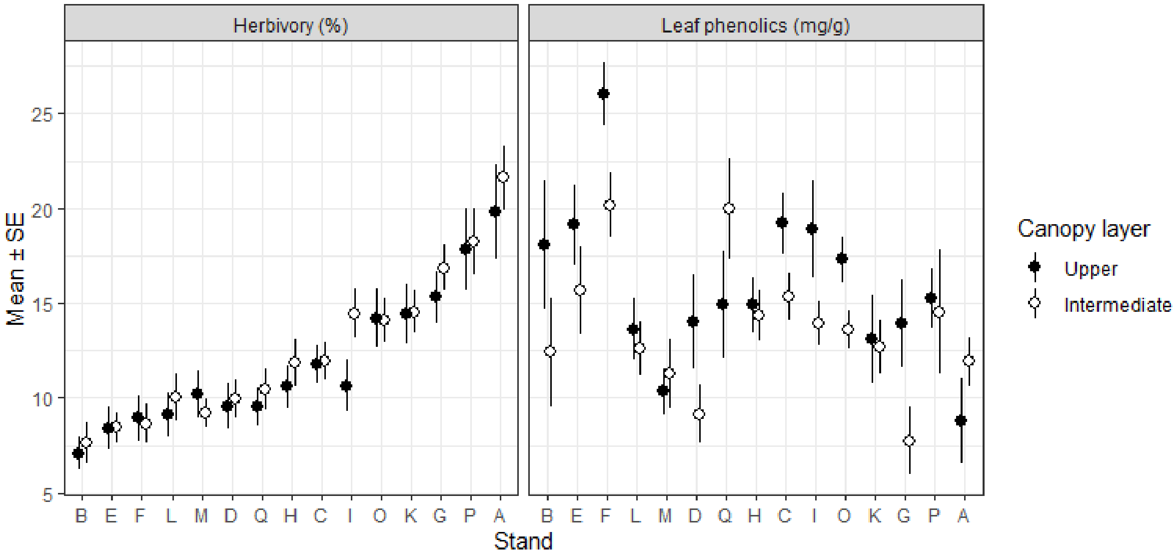

Figure 3.1. Percentage of insect herbivory and concentration of total leaf phenolics (mg/g) in the intermediate (white dots) and upper (black dots) tree canopy. Dots and error bars represent means ± SE aggregated at the level of oak stands (A-Q). Note that stands were ordered according to mean insect herbivory and the same order was used to display stand-level variability in leaf phenolics.

Table 3.1. Summary of LMM testing the effect of canopy layer (upper vs. intermediate) on either leaf phenolics or insect herbivory. For insect herbivory, the effect of leaf phenolics was also included in the model. Significant variables are indicated in bold. σ^2 and % correspond to the

variance and the percentage of variance explained by the random factors: stand ID, genetic similarity introduced as a kinship matrix, and the residuals. R^2m and R^2c correspond to the variance explained by fixed and fixed plus random factors, respectively.

Response	Predictors	Coef. ± SE	*z*-value	*P*-value	σ² (%)			R^2m (R^2c)
					Stand ID σ_S^2	Genetic relatedness σ_T^2	Residuals σ_E^2	
Phenolics	Canopy layer	2.15 ± 0.66	3.22	**0.001**	9.27 (19.7)	9.64 (13.8)	50.22 (70.9)	1.63 (29.1)
Herbivory	Phenolics	-0.458 ± 0.24	-1.9	0.057	13.44 (32.0)	14.57 (34.7)	13.95 (33.3)	0.79 (66.6)
	Canopy layer	-0.585 ± 0.36	-1.62	0.1				

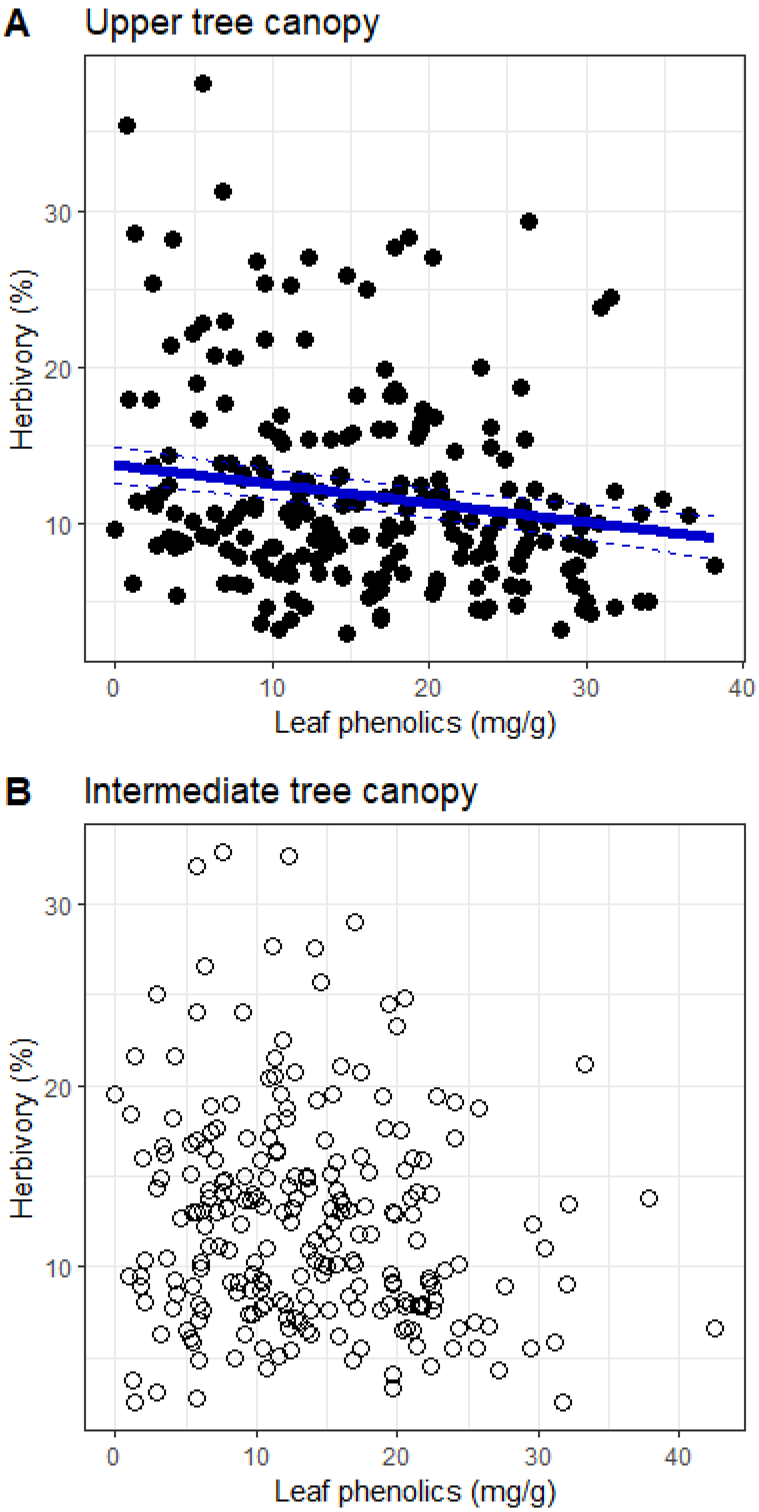

Figure 3.2. Effects of total leaf phenolics on insect herbivory in the upper (A) and intermediate (B) canopy layer. Dots represent individual trees. The thick solid line and the thin dashed lines in graph A represent model predictions and corresponding standard errors, respectively.

3.5. Discussion

Tree genetic relatedness explained a noteworthy part of the overall variation in leaf phenolics and associated insect leaf herbivory. However, this genetic effect was only evident in the upper tree canopy where concentrations of leaf phenolics were consistently higher. To our knowledge, our work represents one of the first evidence of genotype-phenotype-herbivory links in natural tree populations and argues for increased consideration of canopy effects to improve our understanding of ecological and evolutionary factors driving plant-herbivore interactions on long-lived plants.

Oak trees lost between 7 and 22% of their leaf area to insect herbivores, a range of insect herbivores similar to previous estimates (Castagneyrol et al., 2019a; Giffard et al., 2012; Valdés-Correcher et al., 2019). Our analysis at the whole-tree level attributed most of the overall variation in leaf herbivory to differences among forest stands whereas the contributions of tree genetic relatedness and leaf phenolics were very weak. This result might suggest that insect leaf herbivory in our system would be basically driven by the nature of the forest stand, which encapsulates diverse environmental drivers acting at the local (e.g. stand size, tree density and species composition, vegetation structure; Fuller et al., 2018; Maguire et al., 2016; van Schrojenstein Lantman et al., 2018) to landscape (e.g. stand connectivity, nature of matrix habitats; Morante-Filho et al., 2016) scale. Valdés-Correcher et al. (2019) actually reported for the same study stands that their size and connectivity affected patterns of herbivory by different insect guilds. Limiting our analyses to the whole-tree level would hence have led to the conclusion that insect herbivory is primarily determined by extrinsic drivers and shaped by the ecological neighbourhood of the focal tree.

While tree genetic relatedness had little effect on herbivory (2.9%), it was somewhat more influential in the case of leaf phenolics (7.1%) (Fig. 3.3). Together with the likewise weak but statistically significant negative association between leaf phenolics and herbivory, one might argue that our results mirror - albeit extremely faintly - experimental studies that have consistently identified plant chemistry as the phenotypic link between the host plant genotype and the structure of associated arthropod communities (Bangert et al., 2006; Barbour et al., 2015, 2009) or patterns of herbivory (Andrew et al., 2007; Bailey et al., 2006; Donaldson and Lindroth, 2007). But consistent empirical support for this linkage from natural populations remains very scarce. In one of the few available studies, Kagiya et al. (2018) found that genetic relatedness of alder (*Alnus hirsuta*) trees largely determined associated arthropod communities, yet the effect was stronger for herbivore enemies (i.e., predators) than for herbivores. Maldonado-López et al. (2015) observed that tree genetic relatedness of *Q. castanea* trees was significantly associated with chemical defences but not with insect herbivory. In turn, Tack et al. (2012) and Gossner et al. (2015) failed to detect relationships between tree genetic relatedness and herbivory in *Q. robur* populations and concluded that genetic effects tend to be overwhelmed by environmental and spatial factors.

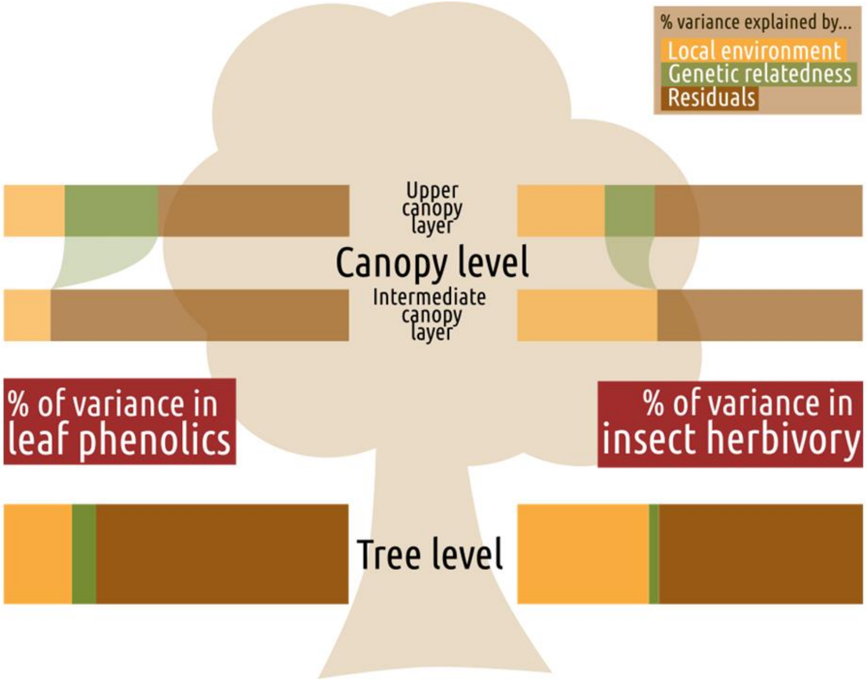

Figure 3.3. Summary of the variance partitioning among random effects and model residuals for leaf phenolics (left) and insect herbivory (right). Box length is proportional to the percentage of variance explained by each component.

In line with the predominant trend reported in the literature (e.g., Lämke and Unsicker, 2018; Poorter et al., 2006; Yamasaki and Kikuzawa, 2003; but see Roslin et al., 2006), we observed that upper canopy leaves systematically contained higher concentrations of leaf phenolics than those from the intermediate canopy. Extensive within-individual variation in leaf morphological and chemical traits is an inherent feature of plants (Herrera, 2017; Niklas et al., 2009). For leaf phenolics, the phenomenon has been primarily explained as an ecophysiological, enzymatic and transcriptomic consequence of the higher irradiance that upper-canopy leaves receive, given that diverse phenolic compounds are involved in the protection from UV-B damage (reviewed in Jenkins and Brown, 2018); see also Lämke and Unsicker, 2018). This vertical gradient in leaf phenolics could have important consequences for plant-insect herbivore interactions. Herbivores tend to forage preferentially on upper-canopy leaves owing to their higher nutritive value (Fortin and Mauffette, 2002; Oishi et al., 2006), yet field surveys typically report higher levels of leaf removal in lower canopy layers (e.g. Castagneyrol et al., 2019a; Stiegel et al., 2017; Yamasaki and Kikuzawa, 2003), which is in line with a higher abundance and diversity of herbivores in these layers (reviewed in Ulyshen, 2011). Numerous studies have assessed within-individual variation in leaf traits and associated herbivory over the past twenty years (Lämke and Unsicker, 2018), giving rise to the hypothesis that variance in nutritional quality itself could act as a defence mechanism that reduces insect herbivore performance by forcing herbivores to actively forage for suitable food (e.g. Wetzel et al., 2016; Wetzel and Meek, 2019). Yet few if any studies have addressed the implications of this within-individual variability for genotype-phenotype-herbivory relationships.

The effect of tree genetic relatedness on insect herbivory and leaf phenolics was contingent on the canopy layer. Effects were considerable in the upper canopy but negligible in the lower canopy. Sun leaves are far more productive in terms of carbon fixation than shade leaves (Poorter et al., 2006) and their defence against herbivores is therefore disproportionately important for overall tree performance. Our finding that tree genotypes with high phenolic compound contents in the upper canopy systematically experience lower herbivory hence suggests that such genotypes could have a non-negligible fitness advantage. On the other hand, the extent of intra-individual variability in phenolic compounds can also be heritable (Herrera, 2017) and might act as an indirect defensive trait (Wetzel et al., 2016). If this were the case in our study system, we would expect that trees with large differences in defence allocation between upper and lower canopy leaves would tend to experience reduced herbivory. Our data did however not confirm such a trend (results not shown), suggesting that the strength of within-individual variation in leaf defences either lacks a genetic basis or has no effect on (tree-level) herbivore activity. Finally, the genetic signal in leaf herbivory that we detected suggests that leaf defences may differentially drive herbivory community heritability across different parts of the canopy. The phenomenon has been thoroughly documented at the whole-plant level in common garden experiments (e.g., Andrew et al., 2007; Robinson et al., 2012), whereas studies in natural populations have reported lower or non-significant levels of genetic variation and heritability. One important reason may be that most previous studies investigating the role of tree genetics on defences and associated herbivory have not explicitly addressed the role of the canopy layer (but instead pooled leaf samples from different heights; e.g. Gossner et al., 2015; Kagiya et al., 2018; Maldonado-López et al., 2015). Our study shows, however, that not taking within-individual variability in herbivory and defences properly into account can easily mask effect of genetic signals. Based on our findings, we recommend that future studies adopt hierarchical sampling designs and properly consider within-individual variability in both plant traits and insect herbivory when exploring their genetic basis in real-world contexts. Finally, we also recommend that further studies include other defence traits (e.g. physical defences such as trichomes and toughness or indirect defences such as volatile organic compounds) and strategies (e.g. induced defences or tolerance). Distinguishing between all these traits or strategies would allow to fully characterize multivariate defensive phenotypes (i.e. syndromes) and to better understand within and among-individual variation in genotype-phenotype-herbivory relationships.

3.6. Appendix A3

Table A3.1. Information about the location and size of the oak stands included in the study.

Stand	Latitude	Longitude	Area (ha)	No. of Oaks
A	44.743	-0.800	0.375	105
B	44.729	-0.733	0.123	29
C	44.764	-0.816	0.179	34
D	44.568	-1.011	0.315	48
E	44.564	-1.004	0.111	31
F	44.556	-0.035	0.106	31
G	44.834	-0.919	0.504	33
H	44.834	-0.885	0.229	60
I	44.842	-0.869	0.663	124
k	44.677	-0.760	0.261	52
L	44.675	-0.759	0.036	17
M	44.693	-0.655	0.146	64
O	44.719	-0.869	0.283	29
P	44.509	-0.922	0.075	16
Q	44.487	-0.920	0.258	30

Table A3.2. Pair-wise F_{st} between the 15 stands.

Stand	A	B	C	D	E	F	G	H	I	K	L	M	O	P	Q
A															
B	0.015														
C	0.016	0.024													
D	0.044	0.053	0.054												
E	0.014	0.020	0.023	0.060											
F	0.006	0.016	0.013	0.042	0.008										
G	0.015	0.025	0.013	0.046	0.021	0.018									
H	0.016	0.023	0.021	0.053	0.024	0.012	0.021								
I	0.007	0.011	0.017	0.048	0.017	0.010	0.019	0.018							
K	0.038	0.051	0.062	0.067	0.057	0.042	0.057	0.049	0.041						
L	0.068	0.077	0.073	0.111	0.088	0.065	0.078	0.071	0.071	0.107					
M	0.014	0.022	0.029	0.054	0.026	0.017	0.022	0.018	0.014	0.046	0.072				
O	0.034	0.036	0.044	0.057	0.045	0.033	0.046	0.040	0.037	0.079	0.098	0.033			
P	0.044	0.054	0.062	0.072	0.051	0.046	0.058	0.048	0.045	0.071	0.122	0.047	0.082		
Q	0.012	0.019	0.025	0.062	0.017	0.013	0.020	0.020	0.013	0.052	0.083	0.019	0.044	0.048	

4. Chapter 3: Is there an effect of climate on insect herbivory, leaf traits and herbivore predation on oaks along a latitudinal gradient in Europe?

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Abstract

A long-held view in ecology holds that the strength of species interactions become stronger toward the equator. However, recent work has reported opposite or inconsistent latitudinal trends in the bottom-up and top-down forces driving insect herbivory. This could be because bottom-up and top-down forces that determine herbivory have rarely been studied concomitantly, making previous attempts to understand the effect of large scale climatic variation on insect herbivory unsuccessful. We used citizen science to test for latitudinal variation in plant-herbivore-enemy (i.e. tritrophic) interactions simultaneously and to investigated the underlying climatic factors associated with variation in herbivory, leaf traits and predation in *Q. robur* across its complete latitudinal range in Europe. To that aim, we quantified insect herbivory and the occurrence of specialist herbivores as well as leaf traits and attack rates on artificial caterpillars on 261 oak trees. We observed that climatic factors rather than latitude per se were the best predictors of the large-scale variation in the abundance of leaf galls and miners as well as in variation in leaf nutritional quality to herbivores. However, we found that plant direct (leaf phenolics) and indirect (bird attack rate) defences were not influenced by latitude or climatic factors. The proportion of leaves with mines was positively related to the concentration of hydrolysable tannins, but neither other traits nor bird attack rates affected insect herbivory. Our study shows that although insect herbivory on oak leaves, leaf traits and bird attack rates were all highly variable across Europe, they were weakly influenced by climate variation and were not related to each other. These findings urge for further examination of the drivers of insect herbivory on trees.

Key words: leaf chemical traits, plant defenses, avian insectivory, climate, artificial prey

4.1. Introduction

A long-held view in ecology holds that, due to warmer temperatures, longer growing seasons, and greater species abundance and diversity at lower latitudes, the strength of species interactions become stronger toward the equator (Dobzhansky, 1950; Janzen, 1970; Schemske et al., 2009). Within a framework of plant-herbivore interactions, plant species at lower latitudes commonly experience increased rates of herbivory (Coley and Barone, 1996; Lim et al., 2015; Moreira et al., 2018b; Pennings et al., 2009; Salazar and Marquis, 2012; Schemske et al., 2009) and thus evolve higher levels of anti-herbivore defenses (Abdala-Roberts et al., 2016b; Johnson and Rasmann, 2011; Moreira et al., 2014; Pearse and Hipp, 2012). Whereas early experimental studies reported patterns that matched these predictions (Coley and Barone, 1996; Coley and Aide, 1991; Dyer and Coley, 2009), several studies over the last decade have also reported no evidence for a latitudinal gradient in herbivory and plant defenses (Moles et al., 2011; Moles and Westoby, 2003) or greater levels of herbivory and defenses at higher latitudes (Moreira et al., 2020, 2018b; Pennings et al., 2009; Woods et al., 2012). Under this confusing scenario, it is needed that upcoming studies will identify the reasons behind the substantial variation detected in herbivory and plant defenses across latitudes world-wide.

Recent work has identified several potential sources of variation in the sign and strength of latitudinal gradients in herbivory and plant defenses (Anstett et al., 2016; Johnson and Rasmann, 2011). First, theory on latitudinal gradients in herbivory and plant defense has been typically described at a plant-centric equilibrium in which plants at low latitudes have adapted to higher herbivory by evolving higher levels of defenses. However, most studies have commonly measured herbivory patterns or plant defenses (but not both but see Abdala-Roberts et al., 2016b; Anstett et al., 2015; Moreira et al., 2018b), leading to an incomplete understanding of latitudinal clines on plant-herbivore interactions. Second, few attention has been paid to latitudinal variation in tri-trophic dynamics (Roslin et al., 2017). In the few available examples, authors have found no variation of parasitism (Dyer and Coley, 2002; Moreira et al., 2015; Stireman et al., 2005), lower predation by ants (Roslin et al., 2017), and higher (Zvereva et al., 2019) or no variation in predation by birds (Roslin et al., 2017) with increasing latitude. Considering tri-trophic interactions is crucial for a complete understanding of latitudinal clines in plant-herbivore interactions because herbivore enemies can drastically modify such interactions by suppressing herbivore populations or reducing herbivore feeding (Böhm et al., 2011; Maguire et al., 2015; Rosenheim, 1998).

Latitudinal gradients are broadly used as ‘natural laboratories’ to study the relationship between climate and plant-herbivore interactions (Abdala-Roberts et al., 2016b; De Frenne et al., 2013; Kozlov et al., 2015; Lim et al., 2015; Moreira et al., 2018b). In the Northern extratropical hemisphere, mean annual temperature drops by 0.73 °C and mean annual precipitation by 4.04 mm per degree of latitude northward (De Frenne et al., 2013). Latitudinal variation in plant-herbivore interactions is therefore generally associated with large-scale variability in climatic conditions (Abdala-Roberts et al., 2016b; Moreira et al., 2018b, 2014) and numerous studies have demonstrated an effect of temperature and precipitation on plant traits (Chen et al., 2013; Gely et al., 2019; Holopainen et al., 2018; Wu et al., 2012) and herbivory (Gely et al., 2019; Jamieson et al., 2015). However, many regions deviate from the global decrease in temperature and precipitation toward higher latitudes due to their proximity to oceans or the presence of mountains (De Frenne et al., 2013), which can markedly contribute to modify the relationship between latitude and plant-herbivore-predator interactions (Loughnan and Williams, 2019; Moreira et al., 2019; Roslin et al., 2017). Thus, further studies should not simply rely on latitudinal clines in plant defenses or herbivory to infer the effect of climate on plant-herbivore interactions, but also should stretch latitudinal gradients longitudinally to better capture the diversity of climatic conditions in which plant-herbivore interactions are embedded (Anstett et al., 2016).

The study of the effect of latitude and climate on plant-herbivore interactions at large scale poses several technical constraints. In particular, it is necessary the match between plant and herbivore phenology across sites (Anstett et al., 2016). Yet, this is practically challenging, even for large networks of researchers. Citizen science, defined as the volunteer participation of the general public in scientific activities (Haklay, 2015), is a powerful tool to meet this challenge, as it allows scientists to synchronize data collection at large geographical scales (McKinley et al., 2017). Although some researchers have been concerned about the accuracy of data acquired by non-professional scientists (Burgess et al., 2017), recent evaluations have confirmed that, providing appropriate methodology and data quality checks, data generated by citizen science programs can satisfyingly contribute to the study of the drivers and consequences of climate change on biotic interactions (e.g., Bison et al., 2019; Castagneyrol et al., 2019b; Ekholm et al., 2019; Miczajka et al., 2015; Saunders et al., 2018).

In a previous large-scale field study, we found strong but contrasting latitudinal clines for insect leaf herbivory and leaf defences for the Pedunculate oak *Quercus robur* (Fagaceae), whereby populations found at lower latitudes had higher levels of herbivory but had lower concentrations of chemical defences (Moreira et al., 2018b). Furthermore, we also found that abiotic factors (e.g. temperature and soil porosity) influenced leaf defences and, in doing so, indirectly influenced herbivory (Moreira et al., 2018b). Here we go a step forward and concomitantly tested for latitudinal variation in plant-herbivore-enemy (i.e. tritrophic) interactions in *Q. robur*, as well as the underlying climatic factors associated with variation in herbivory and defences. In particular, we asked the following questions: (1) Are there latitudinal clines in herbivory, leaf chemical traits and herbivore predation rates? (2) Is latitudinal variation in leaf chemical traits (bottom-up effects) and/or herbivore predation (top-down effects) associated with concomitant latitudinal variation in herbivory? (3) Are climatic correlates of latitude associated with latitudinal variation in herbivory, leaf chemical traits and herbivore predation? For this, we used data collected by professional scientists and school children from 17 European countries across most geographical distribution ranges of *Q. robur*. In particular, we quantified insect leaf damage, leaf chemical traits (phenolics, soluble sugars and nutrients) and dummy caterpillar predation in mature oak trees. Overall, our study challenges common beliefs on latitudinal patterns in plant-herbivore interactions and help refine our understanding of bottom-up and top-down mechanisms that may, or may not, drive geographical variation in plant-herbivore interactions while engaging citizens in such research activities.

4.2. Material and methods

4.2.1. Study design and partner network

The present study represents a citizen science project that involved both professional scientists and school children (together with their teachers). Between June and October 2017 and 2018, we invited European scientists with expertise in tree-herbivore or herbivore-predator interactions to participate in the project. In parallel, we also invited teachers of primary and secondary schools through mailing lists, social networks and press releases disseminated by the communication services of institutional partners (France: INRA, Switzerland: WSL, Germany: University of Freiburg, England: Royal Holloway). The press release specified the objectives of the project, and referred to the project website. The detailed protocol was uploaded to the webpages, so that at the time of registration, the work that would be requested was clearly known to potential participants. The project involved 30 professional scientists from 14 countries and 82 school teachers from 10 countries between 2018 and 2019, giving a total of 112 partners from 17 countries in Europe, covering thus most geographic range of the Pedunculate oak (Fig. 4.1). Only project partners who provided data that could be used in the present article were considered.

4.2.2. Target species

The Pedunculate oak (*Quercus robur* L.), is one of the dominant deciduous tree species in western European forests with high economic and symbolic value (Eaton et al., 2016; Leroy et al., 2019). Its distribution ranges from Central Spain (39°N) to southern Fennoscandia (62°N), thus experiencing variable climatic conditions (Petit et al., 2002). This species supports a large community of specialist and generalist herbivore insects; especially suckers, chewers, skeletonizers, miners and gallers that are mainly active between the time of leaf burst and fall (Brändle and Brandl, 2001; Giffard et al., 2012; Moreira et al., 2018b; Southwood et al., 2005), as well as xylophagous insect herbivores (Marković and Stojanović, 2011). The wide distribution of Pedunculate oak and the high abundance of associated herbivorous insects makes it a suitable model species for research on the effect of climate on biotic interactions.

Professional scientists and school children received similar instructions, with the following exceptions. School children were requested to select mature oak trees (*i.e.,* producing acorns) with low branches easily accessible (2-3 m above ground level). We did not impose any other restrictions on oak selection by partner schools to be as inclusive as possible at this stage, meaning that school children may have selected oaks in different environments, from isolated trees in urban or rural parks, in woods or hedgerows. In contrast, professional scientists were instructed to select oaks in > 1 ha woods. In total, the study consisted of 261 oak trees surveyed by professional scientists (n = 115) and school children (n = 146) in 2018 (n = 149) and 2019 (n = 113) (Fig. 4.1). However, not every partner measured or provided material allowing measuring herbivory, bird attack rate and leaf traits simultaneously on every tree (Fig. A4.1, S A4.2, A4.3, A4.4, supplementary material).

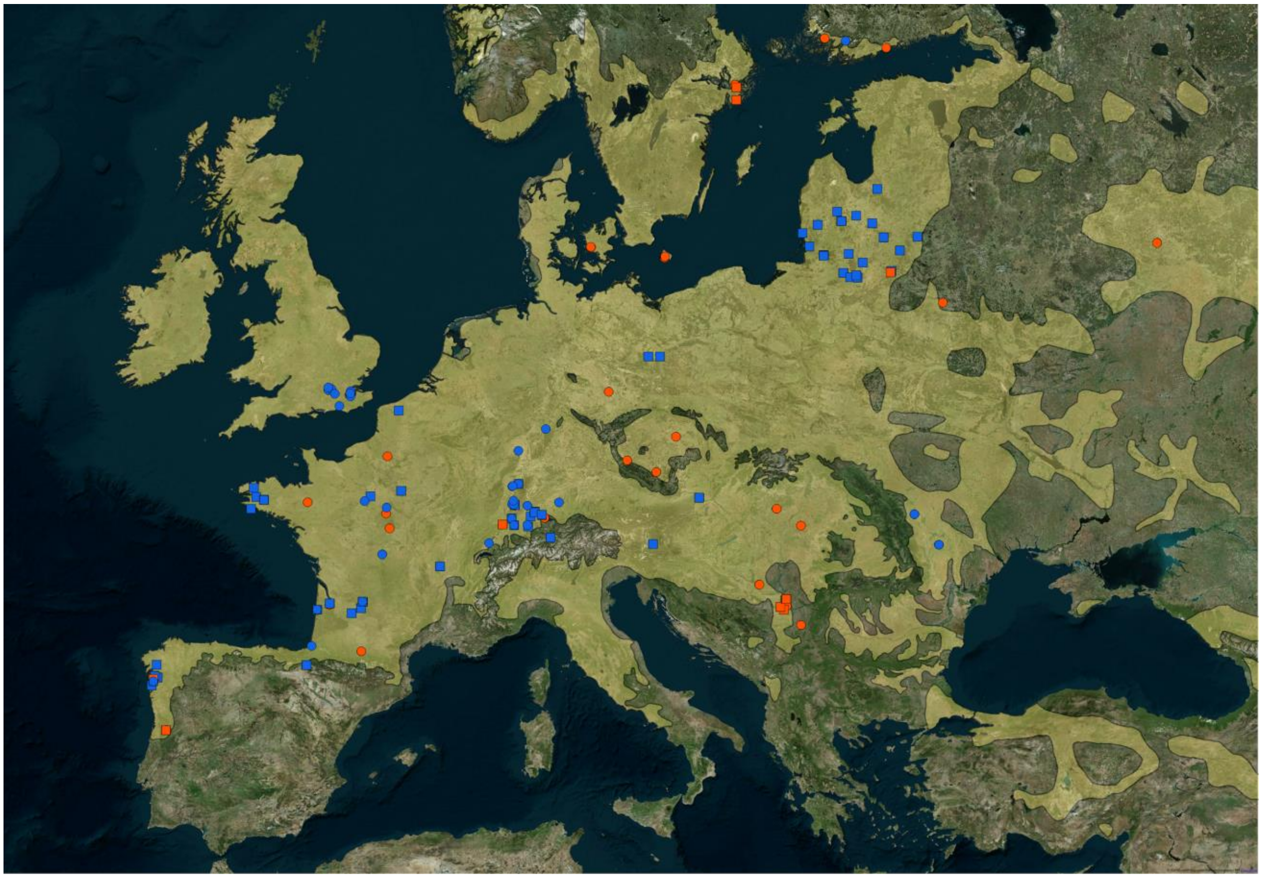

Figure 4.1. Distribution range of *Quercus robur* L. (shaded in yellow) and location of trees sampled by professional scientists (orange symbols) and school children (blue symbols) in 2018 (circle symbols) and 2019 (square symbols). An interactive version of this map, as well as additional maps showing oak trees used for herbivory, attack rate and trait analyses, are provided in supplementary material.

4.2.3. Attack rate on artificial caterpillars

To control for latitudinal variation in environmental conditions, we matched the start of the experiment in each locality to the local phenology of the oak trees. Six weeks after oak budburst, partners installed 20 dummy caterpillars per tree, *i.e.*, five caterpillars on each of four branches (facing north, south, east and west) and a minimum distance of 15 cm between caterpillars. We also verified that the starting date of budburst and the latitude were positively correlated (Pearson $r = 0.45$, $P < 0.05$).

The project coordinators (EVC and BC) provided the same green plasticine (Staedler, Noris Club 8421, green[5]) to all partners to make the caterpillars. In order to standardize caterpillar size among partners, we made caterpillars from a ball of plasticine of 1 cm diameter, and gently pressed/rolled onto the middle of a 12 cm long metallic wire until a 3 cm long caterpillar was obtained. Partners were instructed to attach the caterpillars to branches using wire and leave caterpillars on trees for 15 days before recording predation marks. A second survey using the same procedure immediately followed the first one.

Every partner received a field 'bite guide' containing a collection of photos with attack marks left by different types of predators as well as "false positive" marks on plasticine surfaces by leaves, buds or finger nails. The different predator guilds that can be easily identified from their typical marks left on plasticine include passerine birds, rodents, snakes, lizards and insects, mainly beetles and bush-crickets (Lövei and Ferrante, 2017). The 'bite guide' was also available online and accessible to all partners through a hyperlink from the protocol (Castagneyrol et al., 2019c). Teachers were invited to contact the scientific coordinator or local scientific partners in case of doubt. Although we provided them with a predator guide that included different types of predators, only bird marks were further considered, as more reliable than other marks (generally more frequent and easier to check with the lowest risk of misidentification).

In 2018, school children photographed every caterpillar with the suspected attack marks from any potential predatory taxon. To minimize the probability of false negative results, we also advised the school children to send photographs of marks that were not clearly recognized as attack marks. Photos were taken from three different angles to show the observed damage and were labeled in such a way that the file name indicated both tree and caterpillar identities. Professional scientists were asked to gently remove all caterpillars from the trees and sent them back to the project coordinators. In 2019, both school children and professional scientists were instructed to send caterpillars back to the project coordinators. Photos and dummy caterpillars were used by the first author (EVC) to double-check and standardize attack rate assessment made by individual partners.

Data and biological material were collected by both school children and professional scientists during the same time period (from May till July). At the end of the project, all partners filled in the predation recording form and sent it to the project coordinators together with the photos of the caterpillars (2018) or with actual caterpillars (2019). However, we did not consider raw data provided by project partners, because preliminary analyses revealed that school children overestimated attack rate (Castagneyrol et al., 2019b). In order to be consistent, we only used data recorded by EVC from photos or caterpillars. It must be acknowledged that "false positives" were more likely to be identified from the photos than "false negatives". False positives are caterpillars classified by project partners as having been attacked, whereas they were not (e.g. fingernails, marks left by buds). School children were instructed to take photos of caterpillars with suspected attack marks, even marks they could not attribute to any predator type. It is therefore possible that caterpillars that were photographed because they considered that marks left by buds, leaf or fingernails also had real attacks that they did not notice. Such cases would represent "false negatives". The probability of detecting false negative was not an issue when project partners returned caterpillars to the project coordinators. Our comparison of 2018 and 2019 data confirmed that false negatives were rare in 2018 (Castagneyrol et al., 2019b).

For each oak tree and survey period, we assessed attack rate as the proportion of artificial caterpillars with at least one attack mark. Although we asked partners to record attack rate marks left by different types of predators (in particular birds and arthropods), this level of precision for arthropod predators could not be reached on photos because of low resolution. In addition, the relevance of marks left by arthropods on plasticine model prey has recently been questioned, in particular after mandibular marks were observed on lizards or frog models (Rößler et al., 2018). For these reasons, we decided to discard arthropod attack rate from the study and focused on marks that were unambiguously attributed to birds, *i.e.*, conic holes or V-shaped beak marks. We did not include neither attach rate marks left by reptile or rodent as only a few caterpillars where attacked. Most bird marks were directed towards the head or the body center of the artificial caterpillars, which is typical to bird attacks and indicates prey recognition (Rößler et al., 2018). We therefore refer to the proportion of artificial caterpillars with such marks as bird attack rate.

Between 2018 and 2019, 137 partners installed twice 6,380 artificial caterpillars on 319 oak trees (Total number of caterpillars installed: 12,760). Despite clear instructions regarding caterpillar installation, removal and conditioning prior to shipping, the material sent by 22 school partners was of poor quality (with no particular geographic bias) such that only caterpillars returned by 115 partners (*i.e.*, 78.4%, corresponding to 254 oaks) were screened for attack marks and included in subsequent analyses (Table A4.1; Fig. 4.1).

4.2.4. Insect herbivory

Professional scientists and school children were instructed to collect oak leaves after the second bird attack rate survey, *i.e.*, roughly 10 weeks after oak budburst, on the same branches where artificial caterpillars were installed. They haphazardly collected 30 leaves per branch, totalling 120 leaves from which they blindly drew 60 leaves. Professional scientists oven-dried leaves for a minimum of 48 h at 45°C immediately after collection, and leaves collected by school children were oven dried upon reception back to project coordinators, to ensure optimal conservation prior herbivory assessment.

For each leaf, we visually assessed insect herbivory as the percentage of leaf area removed by leaf chewers and skeletonizers following eight levels of defoliation (0%, 0.1-5%, 5.1-10%, 10.1-15%, 15.1-25%, 25.1-50%, 50.1-75%, and >75.1%). It was always estimated by the same blind observers (YK and YM) to maximise consistency of the estimates and reduce unconscious bias. We then averaged herbivory at tree level using the midpoint of each percentage class to obtain a mean value per tree. This measurement also included the surface covered by leaf mines, but we did not consider punctures made by sap feeders. Additionally, we also scored the presence of mines and insect galls at leaf level and calculated mine and gall incidence as the proportion of leaves with mines or galls.

4.2.5. Leaf chemical traits

We used leaves sent by professional scientists and school children in 2018 to quantify several leaf chemical traits typically recognized as deterrents against insect herbivores for several oak species. In particular, we quantified leaf phenolics as toxic defensive metabolites (Damestoy et al., 2019; Forkner et al., 2004; Moreira et al., 2018b), and C:N ratio, N:P ratio and soluble sugars as proxies for leaf nutritional quality to herbivores (Castagneyrol et al., 2017; Moreira et al., 2019).

***Leaf phenolics* -** We quantified leaf phenolics only on leaves collected by professional scientists in 2018. For each tree, we selected 10 mature leaves with no evidence of insect damage and we grounded them to thin powder. Then, we extracted phenolic compounds from 20 mg of dry plant tissue with 1 mL of 70% methanol in an ultrasonic bath for 15 min. We centrifuged and subsequently transferred them to chromatographic vials. To perform the chromatographic analyses we used Ultra-High-Performance Liquid-Chromatograph (UHPLC Nexera LC-30AD; Shimadzu) equipped with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS photodiode array detector. The compound separation was carried out on a Kinetex™ 2.6 µm C18 82-102 Å, LC Column 100 × 4.6 mm, protected with a C18 guard cartridge. The flow rate was 0.4 mL min-1 and the oven temperature was set at 25 °C. The mobile phase consisted of two solvents: water-formic acid (0.05%) (A) and acetonitrile-formic acid (0.05%) (B), starting with 5% B and using a gradient to obtain 30% B at 4 min, 60% B at 10 min, 80% B at 13 min and 100 % B at 15 min. The injection volume was between 15-30 µL (from a total of 24 samples we injected 30 µL because the concentration of secondary metabolites was quite low).

We identified four groups of phenolic compounds: flavonoids, ellagitannins and gallic acid derivates ("hydrolysable tannins" hereafter), proanthocyanidins ("condensed tannins" hereafter) and hydroxycinnamic acid precursors to lignins ("lignins" hereafter). We quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents, hydrolysable tannins as gallic acid equivalents, and lignins as ferulic acid equivalents (Moreira et al., 2018a). We obtained the quantification of these phenolic compounds by external calibration using calibration curves at 0.25, 0.5, 1, 2 and 5 µg mL-1. Phenolic compound concentrations were expressed in mg·g^{-1} tissue on a dry weight basis.

Nutritional traits **-** We quantified plant nutritional quality on leaves collected by both professional scientists and school children. We grounded the 60 oven dried leaves on which we scored herbivory to thin powder such that leaf nutritional traits reflected the content of leaves with different amount of herbivore damage.

We quantified macroelements (C, N, P) after wet mineralisation (H_2SO_4+H_2O_2). Phosphorus and nitrogen were quantified colorimetrically with an AutoAnalyser 3 High Resolution colorimeter (SEAL), using ammonium molybdate (for P) and sodium salicylate (for N) as reagents. We also quantified leaf C:N ratio with a gas chromatography in an automatic elemental analyser (FlashEA 1112; Thermo Fisher Scientific Inc.) using 6 µg of dried leaf powder.

We purified between 0.1 and 0.5 g of dried leaf powder to holocellulose using the Jayme–Wise method (Leavitt and Danzer, 1993). Leaf powder was placed in a Teflon bag and sequentially treated in a Soxhlet extractor with 2:1 toluene:ethanol, then 100% ethanol, to remove extractables. The samples were then boiled in water to remove soluble carbohydrates, and bleached at a temperature of 70°C in 4 mL of acetic acid solution with 21 g of sodium chlorite to decompose the lignin. The samples were weighed and this weight corresponded with the content on cellulose.

We extracted soluble sugars from 50 mg of dried leaf powder. The dry material was transferred to a tube (tube A) with 1 mL of ethanol in a water bath for 30 min at 80°C. We centrifuged and subsequently transferred the liquid to an Eppendorf (tube B). We added 1 mL of 50% ethanol in the tube A and placed it in water bath for 30 min at 80°C. We centrifuged again and subsequently transferred the liquid to the tube B. We added 1 mL of 20% ethanol in the tube A and placed it in water bath for 30 min at 80°C. We centrifuged and subsequently transferred the liquid to the tube B. We added 1 mL of NaOH 0.02N in the tube A and placed it in water bath for 30 min at 90°C. We centrifuged and subsequently transferred the liquid from the tube B to the tube A. Both tubes were placed in a speed vac for complete evaporation. Then, 50 µL aliquots of the diluted solutions were injected into 2.5mL of anthrone reagent which allows colorimetric analysis of the total sugar content (all monosaccharides, disaccharides and polysaccharides in their hydrolyzed or non-hydrolyzed forms). The preparation of the anthrone reagent was adapted from Bachelier and Gavinelli (1966): 0.5 g of anthrone was directly dissolved in 250mL of sulphuric acid at 95–98%. The colorimetric reaction was accelerated by heating at 80°C for 30 min and the total sugar content was then determined by measuring the absorbance at 560nm with a spectrophotometer (Biochrom Libra S22, Biochrom, Cambridge, UK). The sugar concentration was determined from calibration curves established using standard sucrose solutions with a range of known concentrations.

4.2.6. Statistical analysis

We were primarily interested in testing the interactive effects of climate and leaf traits on herbivory and bird attack rate. Thus, we primarily focused on temperature and precipitation that we obtained from the WorldClim database (Hijmans et al., 2005) based on oak coordinates as retrieved on Google maps by project partners, so that the sampled geographic gradients was taken as a proxy for climatic gradients. Specifically, we extracted the mean temperature and precipitation from April to June, which roughly corresponds to the period when caterpillars were present in trees, irrespective of latitudinal cline in moth phenology. Yet, because latitude can have interactive effects on temperature and precipitation, we tested the effect of geography and climate separately.

We were interested in whether bird attack rate or leaf traits mediated the effect of climate on insect herbivory. Yet, leaf traits were only measured on a subset of trees such that we could not link herbivory with its top-down and bottom-up drivers using the complete dataset. Therefore, we built three types of Linear Mixed-effects Models (LMM): (i) a geographic model analysing the effect of latitude on herbivory, leaf traits and bird attack rate, (ii) a climatic model in which we substituted latitude with climatic data (temperature and precipitation) and (iii) an abiotic and biotic model analysing the effects of leaf traits and bird attack rate together with temperature and precipitation or latitude (both linear and quadratic) on herbivory.

In every LMM, we used Partner ID as a random factor to account for the fact that some partners surveyed multiple trees. For instance, the geographic models were of the form:

$$Y = \beta_0 + \beta_1 \times \text{Year}_{2019} + \beta_2 \times \text{Partner}_{\text{Schoolchildren}} + \beta_3 \times \text{Latitude} + \beta_4 \times \text{Latitude}^2 + \gamma + \varepsilon$$

where Y was the response variable, β_i model coefficient parameter estimates, $\text{Partner}_{\text{Schoolchildren}}$ was the effect of partner type (the estimate for school children being compared with the estimate for professional scientists that was included in the intercept), Year_{2019} was the effect of each year (2018 contrasted with 2019), Latitude (and their quadratic terms) the geographic conditions around sampled oak trees, $\sigma^2_{\text{Partner ID}}$ the random effect of Partner ID (assuming that $\gamma \in N(0, \sigma^2_{\text{Partner ID}})$) and ε the residuals (assuming $\varepsilon \in N(0, \sigma^2_e)$). When Y was bird attack rate, we added the survey (first vs. second) as a fixed effect and Tree ID as a random effect nested within Partner ID to account for repeated measurements on the same tree individuals. Partner type was added to adjust for differences between the two partner types. When needed, we used arcsine square-root (bird attack rate) or logarithm (insect herbivory, soluble sugars, N:P ratio and leaf defences) transformations of response variable to satisfy model assumptions.

We ran geographic and climatic models on the complete dataset including 2018 and 2019 data collected by both professional scientists and school children. Note that because not every partner provided reliable data on both bird attack rate and herbivory, the sample sizes differed between models using bird attack rate or herbivory as response variables (Fig. 4.1, Fig. A4.1). We ran the geographic and climatic models on leaf phenolics as well as the biotic model on 2018 data collected by scientific partners only, as we did not quantify leaf defences on leaves collected and sent by school children.

The tree-level response variables for each year and survey period (Y) were either herbivory (% leaf area removed by herbivores), mine or gall incidence (proportions), mean bird attack rate (ratio of % attacked caterpillars on exposition period) or leaf chemical traits (C:N ratio, N:P ratio, soluble sugar content [g L^{-1}], cellulose content (g), concentrations of condensed or hydrolysable tannins, flavonoids or lignins [mg g^{-1} d.w.]). We scaled and centred every continuous predictor prior to modeling to facilitate comparisons of their effect sizes. We used LMM with Gaussian error distribution, with the exceptions of geographic, climatic and process-based models with mine or gall incidence as response variables. In these cases, we used Generalized LMM with binomial error distribution.

We analyzed the data within information theory framework (Burnham and Anderson, 2002). We first built a set of models including geographic and climatic models as well as nested models for each response variable separately. Biotic models were ran on the subset of samples where all data were measured simultaneously. We then applied a procedure of model selection based on AIC criteria corrected for small sample size (AICc). In the first step, we ranked the models according to the difference in AICc between a given model and the model with the lowest AICc (ΔAICc). Models within 2 ΔAICc units of the best model (*i.e.*, the model with the lowest AICc) are generally considered as equally likely. We also computed AIC weight (w_i) that is the probability a given model to be the best model, as well as the relative variable importance RVI as the sum of w_i of every model including this variable. When several models compete with the best model (*i.e.*, when multiple models are such that their ΔAICc < 2), we applied a procedure of multimodel inference building a consensus model including the variables in the set of best models. We then averaged their effect size across all the models in the set of best models, using variable w_i as a weighting parameter (*i.e.*, model averaging). We considered that a given predictor had a statistically significant effect on the response variable when its confidence interval excluded zero.

In the results section, we report model AICc, ΔAICc and w_i for every model, as well as averaged coefficient parameter estimates and variable importance for all variables present in the set of competing models. When appropriate, we plotted the relationship between raw data and explanatory variables together with the predictions of simplified models, holding undisplayed predictors constant. All analyses were run in R language (R Core Team, 2018) with packages MuMIn (Barton, 2018) and lme4 (Bates et al., 2018).

4.3. Results

4.3.1. Latitudinal and climatic gradients in insect herbivory, plant traits and herbivore predation rates

Insect herbivory was on average (± se) 8.7 ± 0.4 % (n = 182, see Table A4.1 for details). Model simplification identified the null model as the best model given the model set, indicating that none of predictors had a consistent effect on insect herbivory (Table A4.2). Insect galls were present on 7.1 ± 0.6 % of inspected leaves (n = 182, Table A4.1). In the set of best models (Table A4.2), galler incidence increased non-linearly with increasing spring temperature, with a steeper slope at higher temperatures (Fig. 4.2A). The incidence of insect galls peaked at intermediate levels of spring precipitation (Fig. 4.2B) and was on average higher in 2018 than in 2019 (Fig. A4.5). Other predictors had no significant effects on galler incidence. Leaf mines were present on 18.2 ± 1.3 % of inspected leaves (Table A4.1). In the set of best models (Table A4.2), miner incidence peaked at intermediate mean spring temperatures (Fig. 4.2C). The incidence of leaf miners decreased non-linearly with increasing spring precipitation, with a steeper slope at lower precipitations (Fig. 4.2D). Miner incidence was significantly higher in 2018 than in 2019 (Fig. A4.5) and higher in leaves sampled by professional scientists than those sampled by school children.

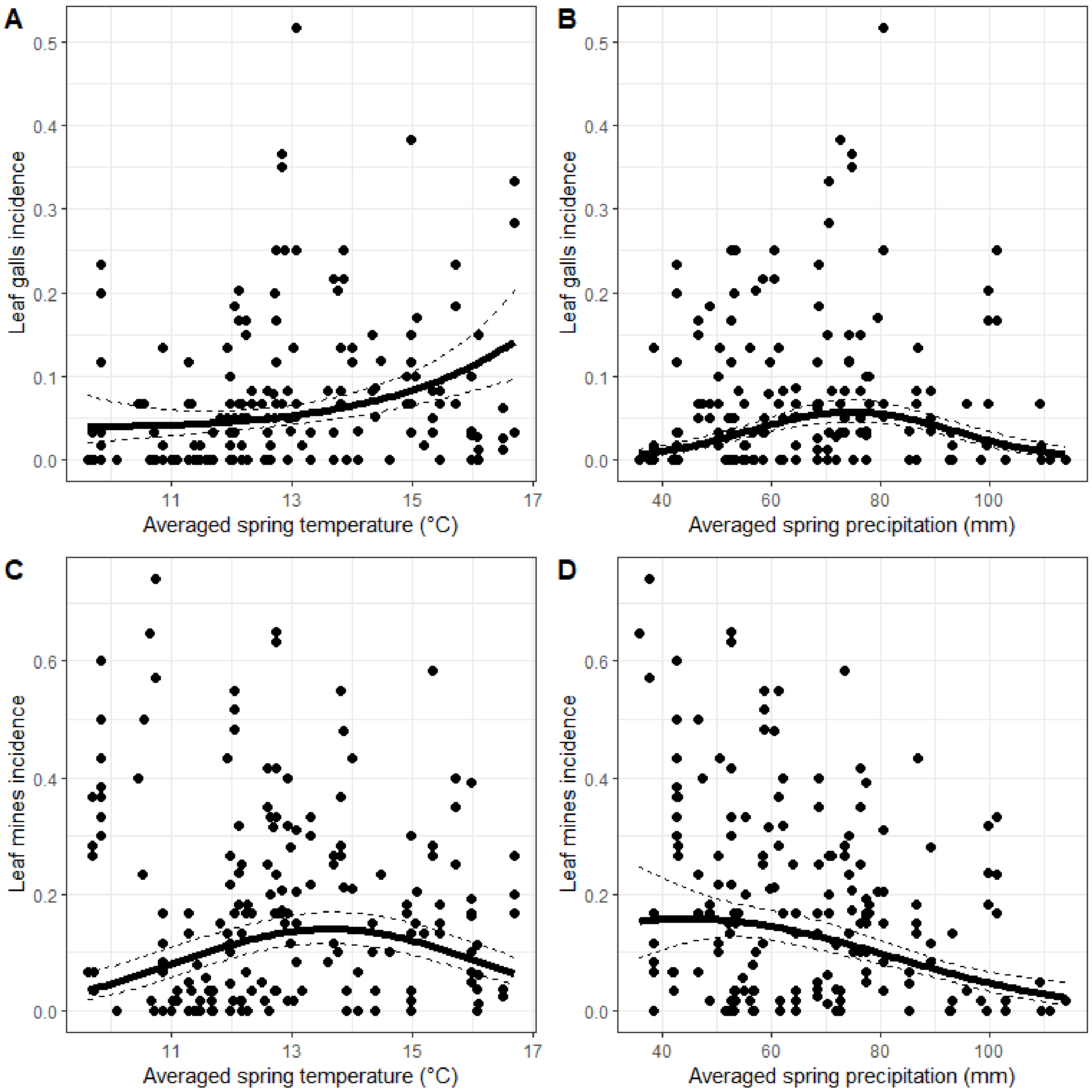

Figure 4.2. Effects of temperature and precipitation on the proportion of oak leaves with insect galls (A, B) and mines (C, D). Dots represent raw data averaged at the tree level. Solid and dashed lines represent model predictions (and corresponding standard error) for temperature and precipitation calculated after other significant variables (see Table A4.5) were set to their mean value. Only statistically significant relationships are shown. Regression line equations are as follows: **A**, $y = -2.28 + 0.34 \cdot x + 0.05 \cdot x^2$; **B**, $y = -2.28 + 0.39 \cdot x - 0.35 \cdot x^2$; **C**, $y = -1.36 + 0.23 \cdot x - 0.29 \cdot x^2$.

We found that climate and latitude significantly affected some nutritional traits, but not phenolic compounds (Table A4.1). Specifically, leaf soluble sugar content (mean ± se: 3.7 ± 0.2 g·L^{-1}, n = 114, Table A4.1) decreased with increasing precipitation (Fig. 4.3A). Leaf C:N rate (18.6 ± 0.2 , n = 114, Table A4.1) increased non-linearly with latitude (with a steeper slope as latitude decreased, Fig. 4.3B) and was on average lower in leaves collected by professional scientists than those collected by school children. None of the predictors had a significant effect on N:P or cellulose content (Table A4.1).

From a total of 10,000 caterpillars, 2,390 had bird marks (*i.e.*, 23.9%). Model selection identified the null model as the best model, with no other competing model within two units of ΔAICc of the best model. This indicates that none of the predictors had a significant effect on bird attack rate on oaks at European scale.

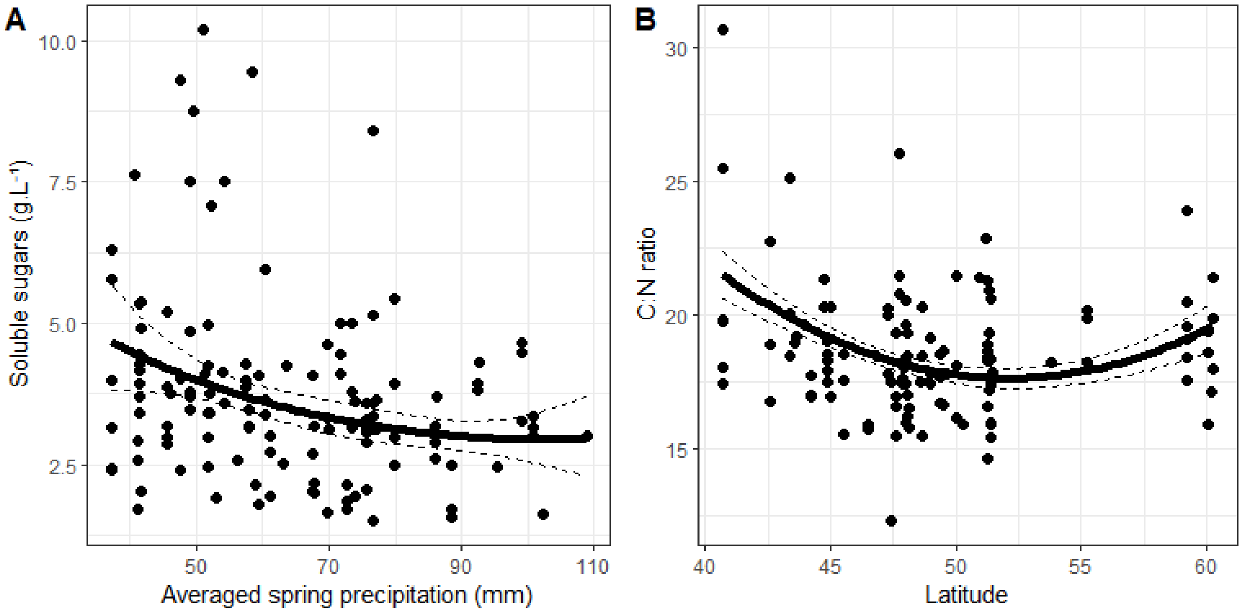

Figure 4.3. Effect of precipitation and latitude on soluble sugar (A) and C:N ratio (B) on leaves, respectively. Dots represent raw data averaged at tree level. Solid and dashed lines represent model predictions (and corresponding standard error) for temperature and precipitation calculated after other significant variables (see Table A4.6) were set to their mean value. Only significant relationships are shown. Regression line equations are as follows: **A**, $y = 1.51 - 0.12 \cdot x + 0.03 \cdot x^2$; **B**, $y = 1.52 - 0.03 \cdot x + 0.03 \cdot x^2$.

4.3.2. Mechanisms underlying latitudinal and climatic variation in herbivory

Model selection based on this data subset identified the null model as the best model, indicating that none of the predictors had a significant effect on insect herbivory (Table A4.3).

When leaf traits were included in the model, galler incidence increased with increasing soluble sugar concentration and decreased with increasing C:N (Fig. 4.4). The positive relationship between temperature and galler incidence remained significant, suggesting independent effects of C:N ratio and temperature on galler incidence. Galler incidence also increased with increasing latitude. However, the relative importance of leaf trait predictors (RVI > 0.8) was much higher than that of latitude or temperature (RVI < 0.4, Fig. A4.8).

Leaf miner incidence increased with increasing concentration of hydrolyzable tannins and decreased with increasing concentration of condensed tannins. Other predictors had no significant effects on leaf miner incidence (Fig. 4.4; Table A4.3).

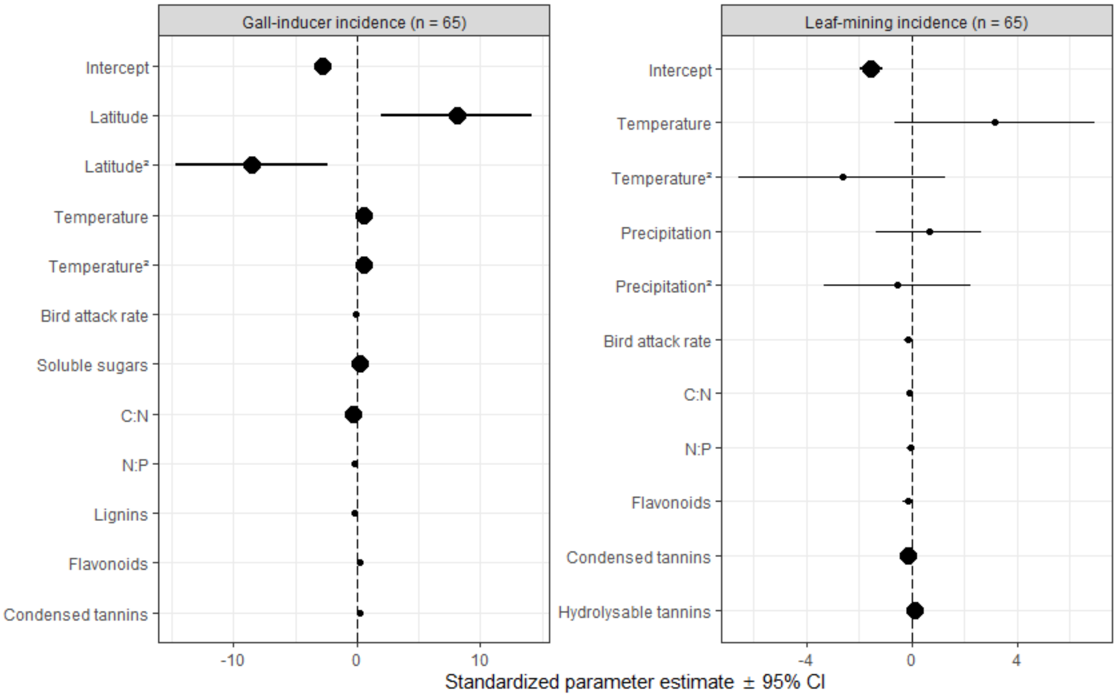

Figure 4.4. Effects of latitude, temperature, precipitation and leaf traits on leaf gall (left) and mine (right) incidences. Circles and error bars represent standardized parameter estimates and corresponding 95% CI. The vertical dashed line centered on zero represents the null hypothesis. Big and small circles represent significant and non-significant effect sizes, respectively.

4.4. Discussion

Latitudinal and climatic gradients in insect herbivory, plant traits and predation rates - Our results showed that variation in insect herbivory was associated with variation in temperature and precipitation, rather than with latitude *per se* (Anstett et al., 2018; Loughnan and Williams, 2019; Moreira et al., 2018b). Climatic effects on herbivory were, however, contingent on herbivore feeding guild, whereby significant effects of climatic conditions were only detected in leaf gallers and miners, but not in leaf chewers and skeletonizers. In particular, the incidence of leaf gallers and miners both increased non-linearly with increasing temperature, but the shape of this relationship was accelerating (*i.e.*, concave up) in gallers and decelerating (*i.e.*, concave down) in mines (Fig. 4.5). Similarly, in a study in northern Europe, Kozlov et al. (2013) found that diversity of leaf miners in birch trees increased linearly toward lower latitudes and that it was most likely associated with the direct impact of temperature on leaf miners, especially during cold years. In our study, the incidence of leaf gallers peaked at intermediate levels of precipitation (Blanche and Ludwig, 2001; Leckey et al., 2014) whereas leaf miners exhibited the opposite pattern. It has been hypothesized that feeding behaviour of leaf gallers and miners have evolved, among other causes, in response to abiotic factors such as UV radiation and desiccation (Connor et al., 1997; Danks, 2002; Fernandes and Price, 1992). If so, herbivores inducing galling and mining leaves may have been favoured in the warmest and driest parts of Pedunculate oak range and at low latitudes where light intensity is markedly higher (Cuevas-Reyes et al., 2004; Fernandes and Price, 1992; Lara and Fernandesrs, 1996; Price et al., 1998). However, even within the leaf galling and mining groups, relationships to climate were reported to be highly variable among species and years (Blanche, 2000; Kozlov et al., 2013; Sinclair and Hughes, 2010), thus suggesting that other factors could have driven observed variation in the incidence of galling and mining herbivores. Overall, because each herbivore guild responds differently to climatic clines, pooling different types of herbivores may prevent the detection of latitudinal and climatic variation in herbivory (Anstett et al., 2016, 2014; Pennings et al., 2009).

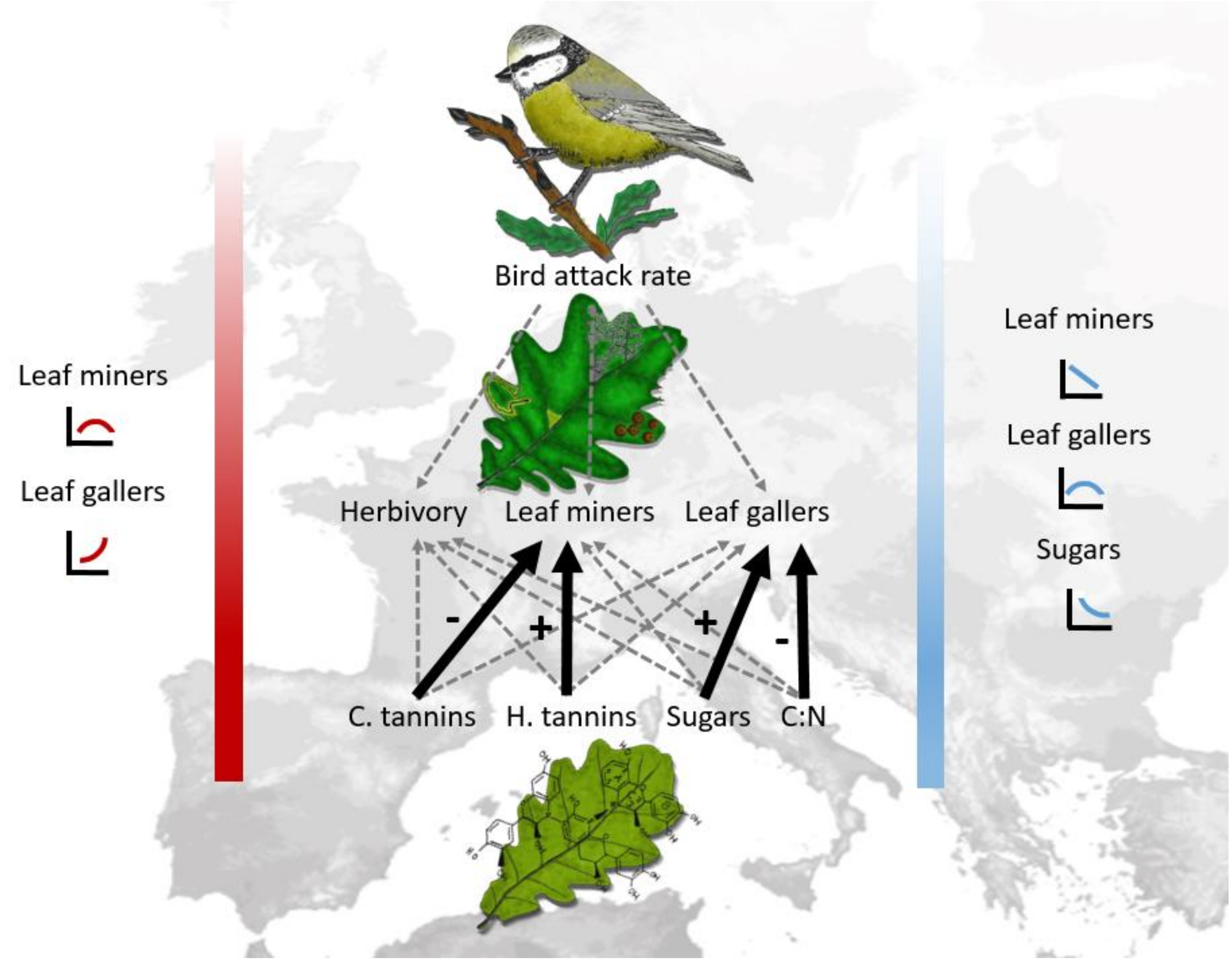

Figure 4.5. Summary illustrating plant-herbivore-predator relationships along a latitudinal gradient in Europe. The red and blue bands denote the variation in mean spring temperature and precipitation. The two figures on the left size represent the correlation between the mean spring temperature and the incidence of leaf miners and gallers. The tree figures on the right side correspond with the correlation between mean spring precipitation and the incidence of leaf miners and gallers and the concentration in sugar on leaves. Solid black arrows represent significant positive (+) and negative (-) relationships while dashed grey lines represent non-significant relationships.

Our results also showed that there were no detectable latitudinal and climatic gradients in plant defenses, and that only leaf C:N and sugar varied along latitudinal and climatic gradients, respectively. Leaf C:N ratio increased significantly with latitude (Reich and Oleksyn, 2004) and it may be due to temperature-related plant physiological stoichiometry and biogeographical gradients in soil substrate age (limitation of soil N at higher latitudes). Leaf soluble sugar content decreased with increasing precipitation (Cao et al., 2018; Rodriguez-Calcerrada et al., 2011). Soluble sugars, especially glucose and fructose, accumulate together with other osmolytes during drought (Nio et al., 2011), resulting in high concentration in areas where precipitation is low. The lack of variation of leaf defences contradict the Latitudinal Herbivory Defense Hypothesis which predicts that plant species at lower latitudes frequently experience higher rates of herbivory than their temperate counterparts (Coley and Barone, 1996; Lim et al., 2015; Pennings et al., 2009; Salazar and Marquis, 2012; Schemske et al., 2009) and, for this reason, should have evolved higher levels of anti-herbivore defences (Abdala-Roberts et al., 2016b; Moreira et al., 2014; Pearse and Hipp, 2012; Rasmann and Agrawal, 2011). However, the generality of this hypothesis is currently under debate (Moles and Ollerton, 2016), as an increasing number of studies have found either no evidence for a latitudinal gradient in herbivory and plant defences (Moles et al., 2011; Moles and Westoby, 2003), greater levels of herbivory and defences at higher latitudes (Salgado and Pennings, 2005; Woods et al., 2012), or mixed evidence when comparing different herbivore species or plant defensive traits (Abdala-Roberts et al., 2016b; Anstett et al., 2015, 2014; Moreira et al., 2018b, 2015; Pennings et al., 2009). A plausible explanation for the lack of latitudinal gradients in oak defences could be that we sampled leaves at mid growing season rather than at the end. This is an insightful point because oak leaves may have differentially accumulate phenolics in response to herbivory (*i.e.*, induced defenses) or supported marked differences in light intensity toward the end of the growing season (Karolewski et al., 2013). Therefore, further studies should include measurements at multiple time points during the growing season and to distinguish between different types of defenses, including physical vs. chemical defenses (Wang et al., 2018) and constitutive vs. induced defenses (Anstett et al., 2018) in order to address latitudinal gradients in plant defence more comprehensively.

We found no latitudinal or climatic gradients in bird attack rates on artificial larvae (Fig. 4.5). These results agree with a previous large-scale study by Roslin et al. (2017) who found an increase of the activity of predatory arthropods in several plant species toward the equator, but no significant trend in avian predation. Several factors may explain the lack of response of avian predation to latitudinal or climatic gradients. First, bird species are distributed through migration and are able to move long distances in response to local food availability (Alerstam et al., 2003), resulting in a constant predation rate across climatic and geographical clines. Contrarily, other predators with lower mobility such as arthropods (e.g. ants, ladybirds) are much more abundant at lower latitudes, resulting in a higher selection pressure toward the equator (Roslin et al., 2017). Second, birds may tolerate higher and lower temperatures in comparison to arthropods, and thus be present in the whole climatic gradient. For instance, Whitfield et al. study (2015) showed that several southern African arid-zone passerines were able to thermoregulate in the heat and maintain body temperature below lethal levels. Finally, it is also possible that birds were more active but had more food (e.g. fruits, predatory arthropods) alternatives in warm areas, which may have reduced attack rates on artificial larvae. Such a dilution effect would result in constant predation rate on artificial larvae along the climatic gradient.

Mechanisms underlying latitudinal and climatic variation in herbivory - The incidence of leaf-gallers and leaf-miners was partially explained by variability in some leaf chemical traits. For instance, the incidence of leaf-gallers increased with increasing leaf soluble sugars and N concentrations, which is consistent with galls being metabolic sinks (Huang et al., 2014). However, the effects of temperature and precipitation on leaf miners were likely indirectly mediated by climatic variation in defences, as such effects became non-significant once condensed tannins and hydrolysable tannins were included in the model. These results agree with previous studies reporting indirect effects (via leaf defences) of climate on herbivory (Anstett et al., 2018; Moreira et al., 2018b). For instance, Moreira et al. (2018b) found significant indirect effects of precipitation and soil porosity on insect leaf herbivory in *Q. robur*, which were mediated by leaf condensed tannins. Similarly, Anstett et al. (2018) found indirect effects of climate on insect herbivory in 80 species of evening primroses, which were mediated by leaf chemicals (total phenolics and oenothein A). Contrarily, the effects of temperature and precipitation on leaf gallers were not indirectly mediated by climatic variation in defences, as in this case such effects remained significant after chemical traits were accounted for in the models. In this sense, it is possible that other unmeasured defensive traits (e.g. physical defences) or strategies (e.g. induced defences, tolerance) would have accounted for the observed climatic variation in leaf galler incidence.

Our results showed that the effects of temperature and precipitation on herbivory were not indirectly mediated by climatic variation in predation, as such effects remained significant after including bird attack rates in the models. In this sense, previous research has been inconsistent, whereby some authors have observed positive effects of birds on herbivores (Gunnarsson et al., 2018; Mäntylä et al., 2014, 2008; Sam et al., 2015), while others have reported neutral (Castagneyrol et al., 2017; Moreira et al., 2019; Valdés-Correcher et al., 2019) or strong negative effects (Kozlov et al., 2017; Maguire et al., 2015). Previous studies have observed that arthropod predators (*e.g.* ants, ladybirds) play an important role on herbivore populations and may respond to large-scale variation in climatic conditions at greater extent than vertebrate predators (Roslin et al., 2017; Zvereva et al., 2019). For example, a meta-analysis conducted by Rodríguez-Castañeda (2013)found that ant predation on herbivores significantly increased at higher temperatures and precipitations, indicating that plants growing under warmer and wetter conditions exhibited consequently lower insect herbivory. Unfortunately, we were not able to quantify predation rates by such arthropods, weakening our conclusions about the potential role of predators across climatic gradients.

Conclusion - By simultaneously investigating bottom-up and top-down forces driving insect herbivory along latitudinal and climatic clines in a widespread tree species in Europe, this study brings new insights into the vivid debate about latitudinal variation in the sign and strength of biological interactions (Anstett et al., 2016; Moles et al., 2013; Roslin et al., 2017; Schemske et al., 2009). We found that climatic factors rather than latitude *per se* were the best predictors of the large-scale variation in the abundance of mining and galling herbivores as well as in variation in leaf nutritional quality to herbivores. In sharp contrast, we found no evidence that plant direct (leaf phenolics) and indirect (bird attack rate) defences were influenced by latitude or climatic factors, which conflicts with the dominant view in ecology (Moles and Ollerton, 2016; Roslin et al., 2017; Zvereva et al., 2019). Because unravelling causes of latitudinal variation in the strength of biological interactions is one of the common approaches for the prediction of biotic interactions under global warming (Verheyen et al., 2019), it is crucial that future studies will simultaneously test for effects of latitude *per se* and climate on insect herbivory by different feeding guilds (Kozlov et al., 2017), as well as investigate the mechanisms underlying such effects.

4.5. Appendix A4

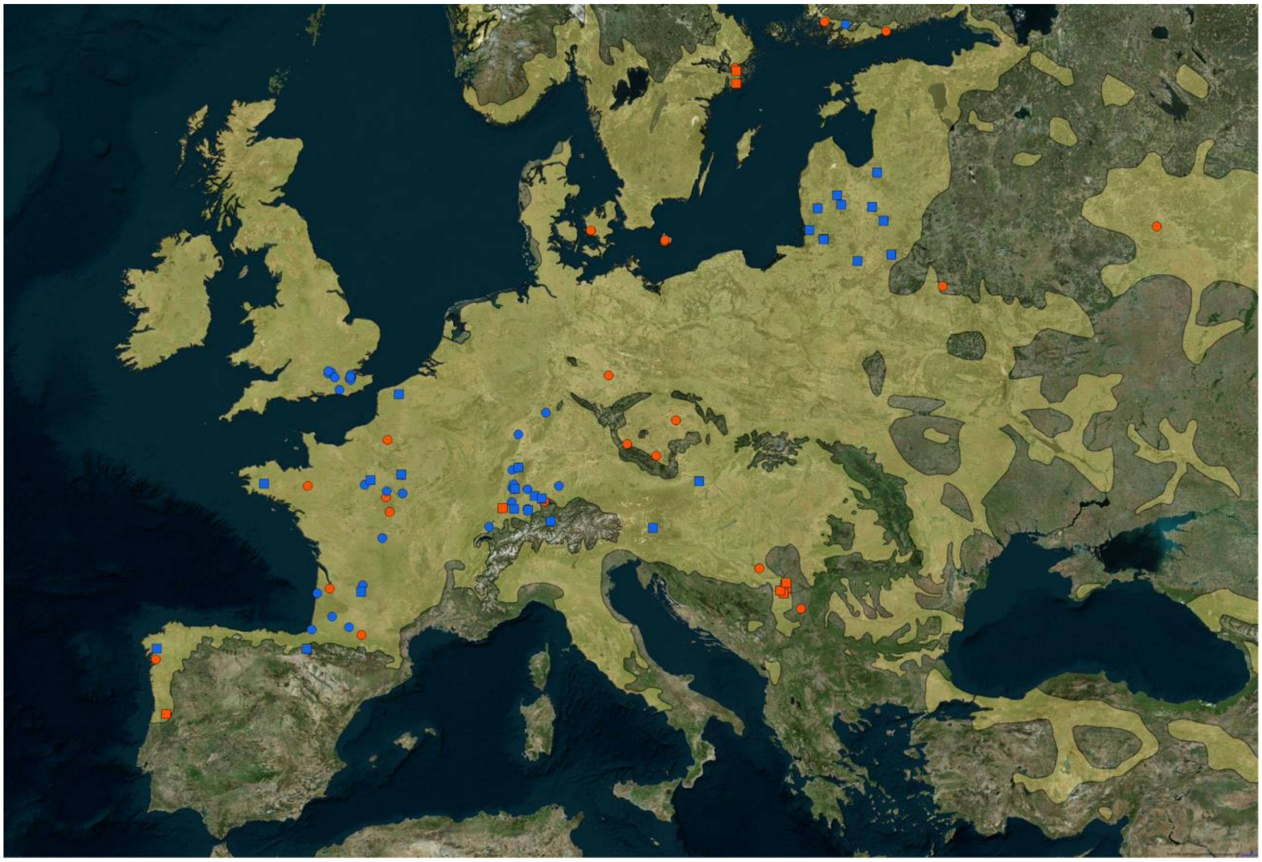

Figure A4.1. Distribution range of *Quercus robur* L. (shaded in yellow) and location of trees sampled by professional scientists (orange symbols) and school children (blue symbols) in 2018 (circle symbols) and 2019 (square symbols) for the assessment of herbivory.

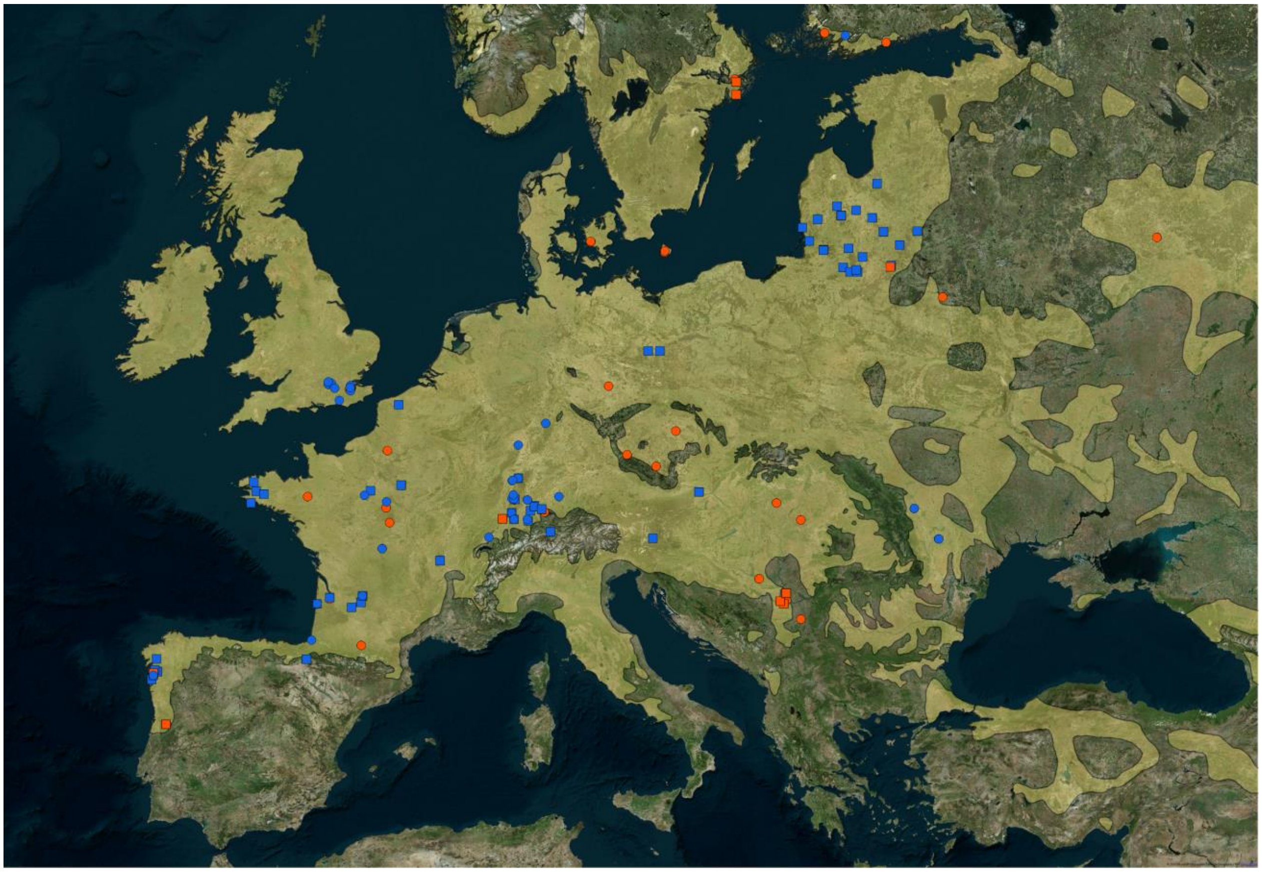

Figure A4.2. Distribution range of *Quercus robur* L. (shaded in yellow) and location of trees sampled by professional scientists (orange symbols) and school children (blue symbols) in 2018 for the assessment of leaf nutrients.

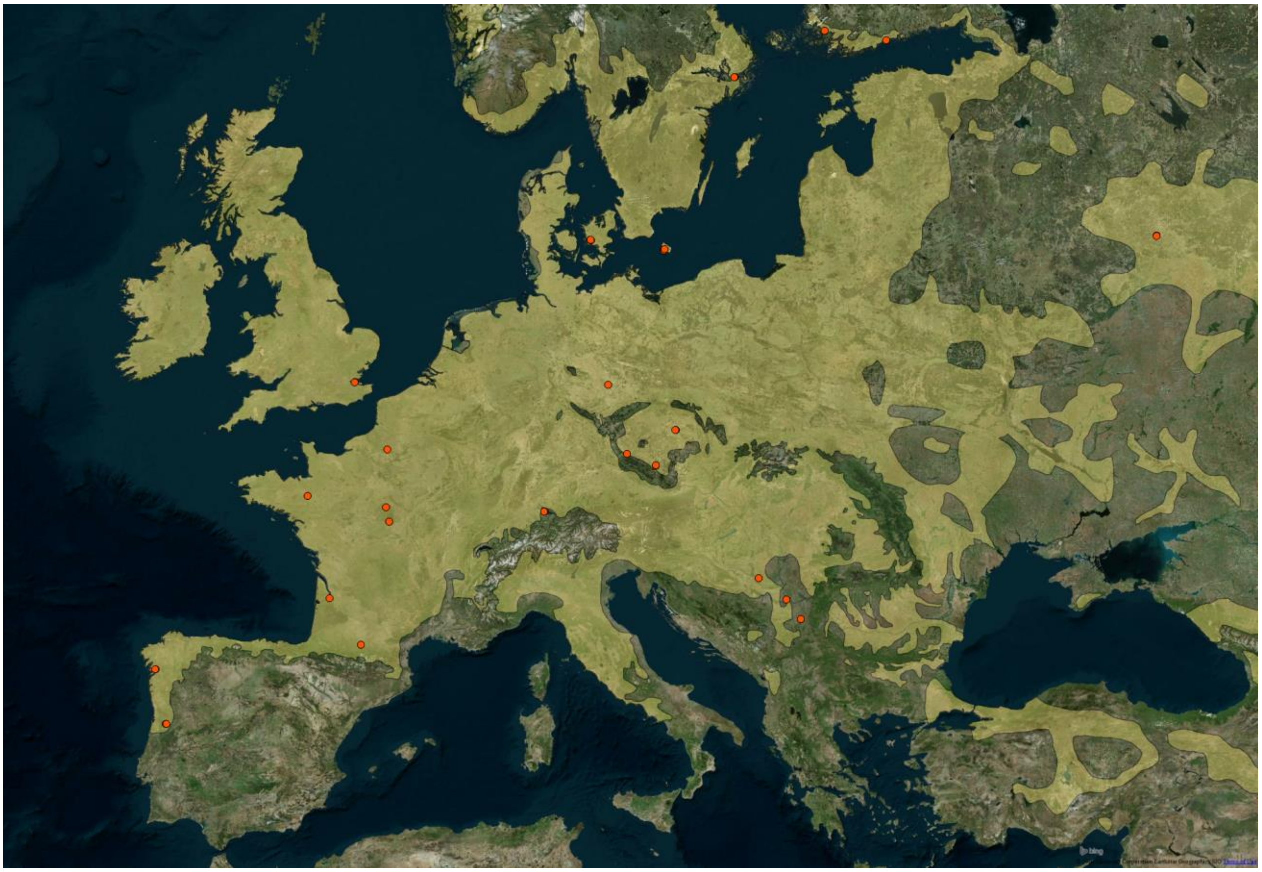

Figure A4.3. Distribution range of *Quercus robur* L. (shaded in yellow) and location of trees sampled by professional scientists in 2018 for the assessment of leaf defences.

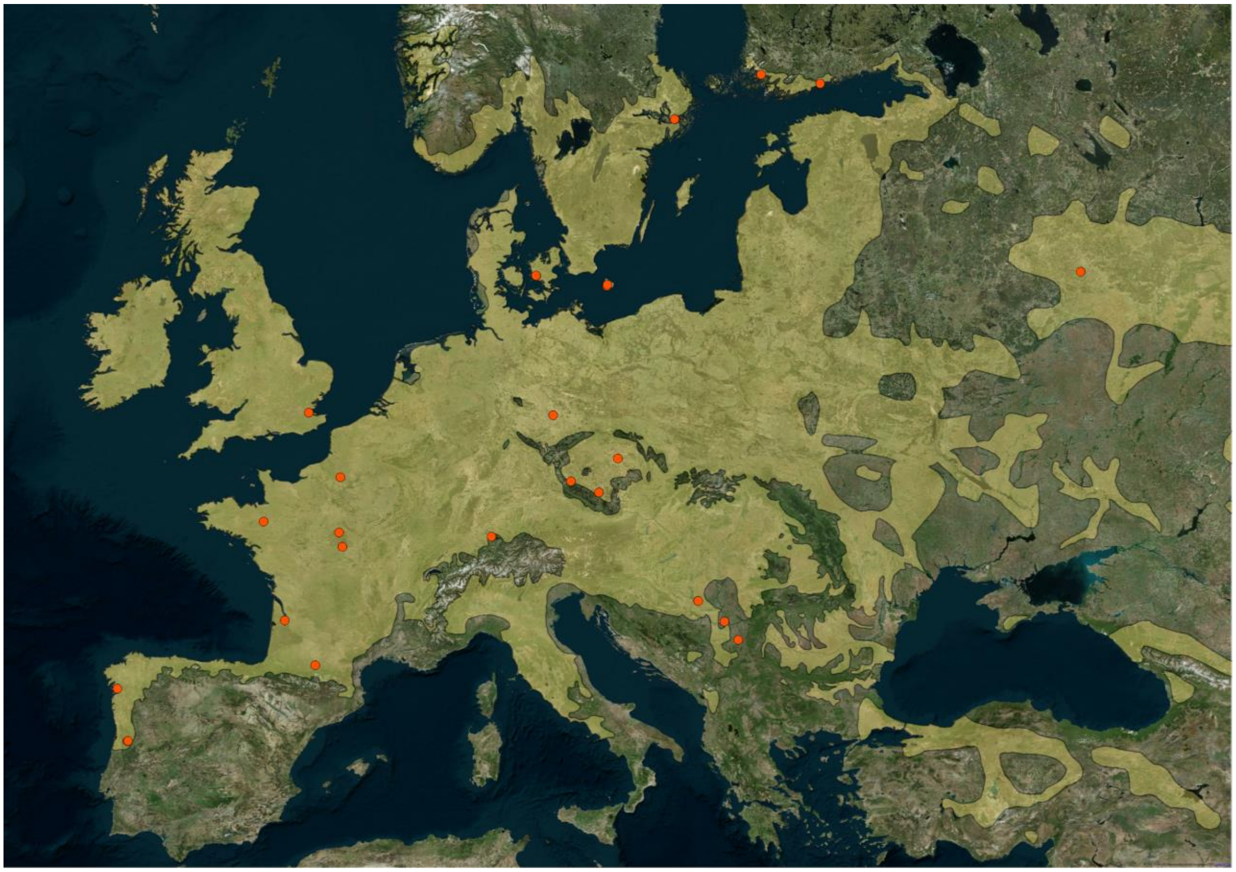

Figure A4.4. Distribution range of *Quercus robur* L. (shaded in yellow) and location of trees sampled by professional scientists in 2018 for the assessment of herbivory, bird attack rate, leaf nutrients and leaf defences.

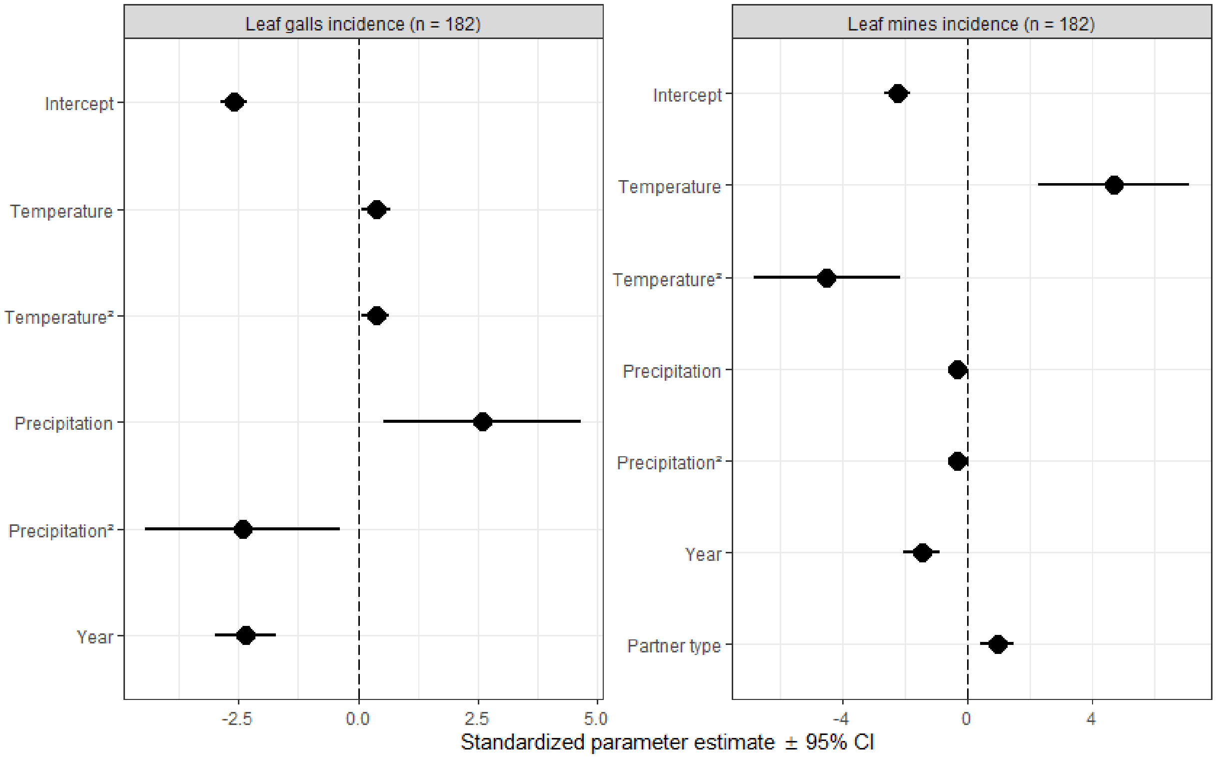

Figure A4.5. Effects of partner type, year, temperature and precipitation on leaf mine and gall incidences. Circles and error bars represent standardized parameter estimates and corresponding 95% CI. The vertical dashed line centered on zero represents the null hypothesis. Big and small circles indicate significant and non-significant effect sizes, respectively.

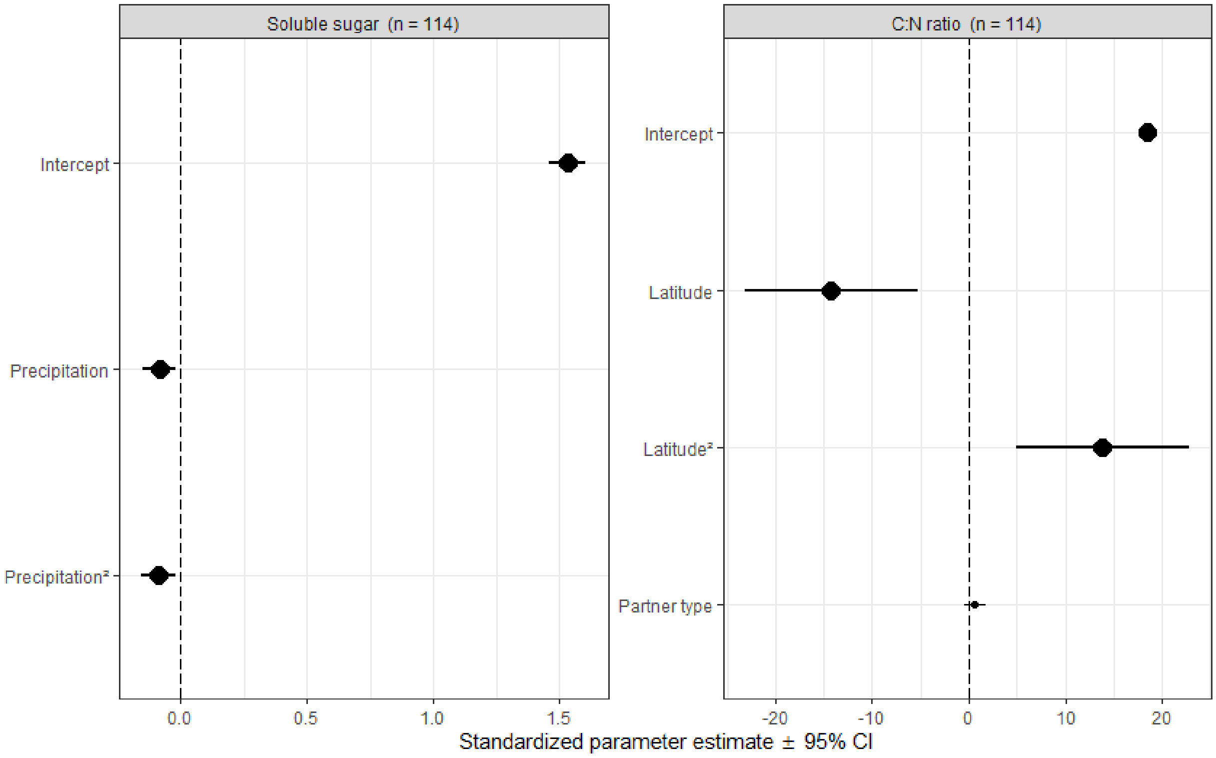

Figure A4.6. Effects of partner type, year, latitude, longitude, temperature and precipitation on leaf C:N ratio and leaf soluble sugar (g L^{-1}). Circles and error bars represent standardized parameter estimates and corresponding 95% CI. The vertical dashed line centered on zero represents the null hypothesis. Big and small circles indicate significant and non-significant effect sizes, respectively.

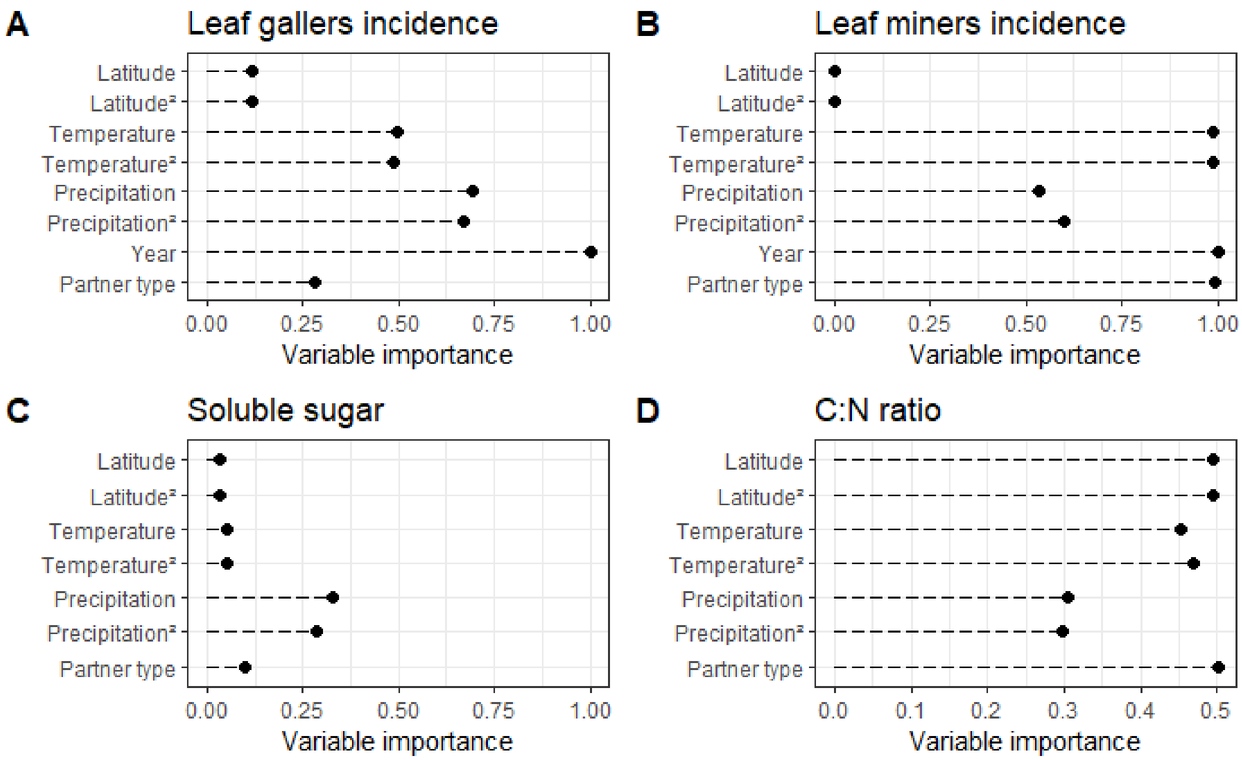

Figure A4.7. Variable importance of every variable included in the geographic and climatic models that considered the effect of longitude, latitude, temperature and precipitation on herbivory (the proportion of leaves with galls and mines) and on leaf traits.

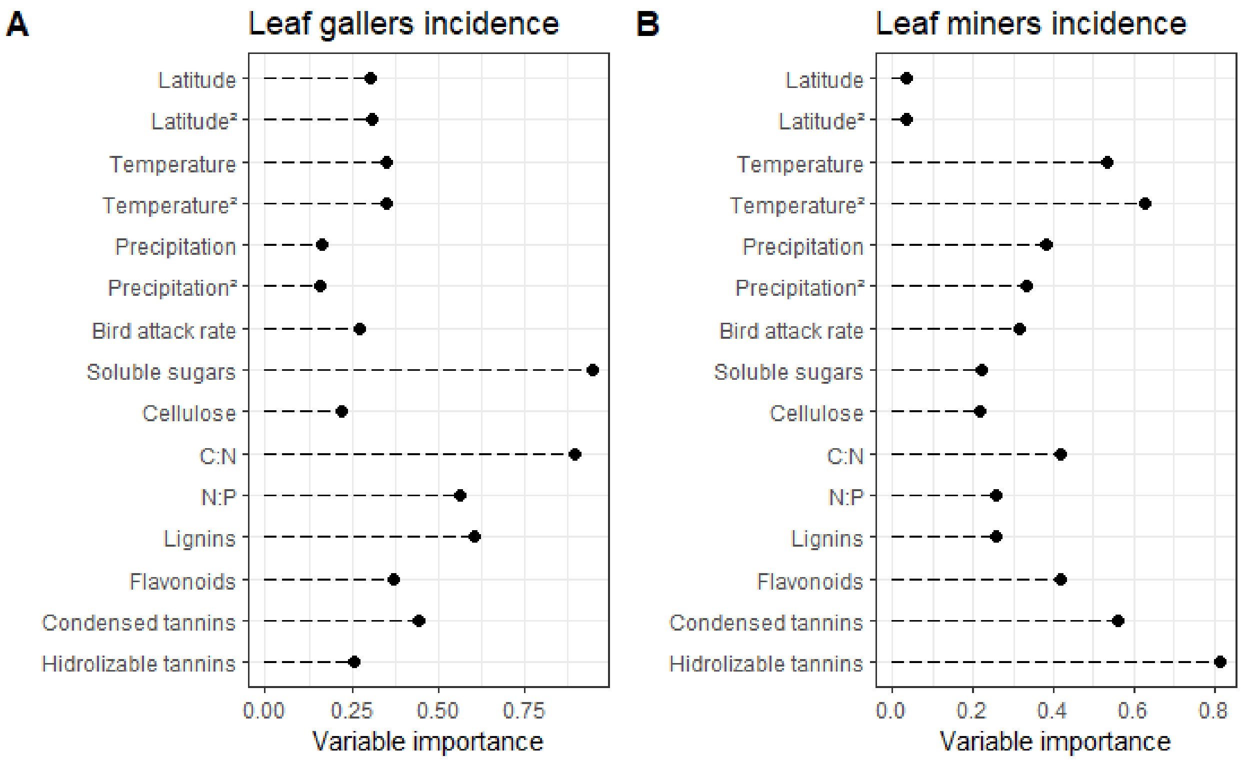

Figure A4.8. Importance of every variable included in the biotic model that considered the effect of leaf traits, bird attack rate, climatic variables on the proportion of leaves with galls and mines.

Table A4.1. Summary of the different variables measured.

Variables	Mean (n, sd)	
	Scientific partner	School partner
Tree height (m)	14.75 (97, 7.06)	13.01 (126, 7.45)
Tree circumference (cm)	121.35 (97, 79.81)	103.94 (126, 93.71)
Herbivory (%)	9.55 (104, 6.64)	7.46 (78, 4.33)
Leaf galls incidence	0.08 (104, 0.09)	0.05 (78, 0.09)
Leaf mines incidence	0.24 (104, 0.19)	0.10 (78, 0.11)
Bird attack rate	0.02 (115, 0.01)	0.01 (137, 0.01)
Soluble sugar (g L^{-1})	3.51 (72, 1.49)	4.09 (42, 2.09)
Cellulose (g)	0.09 (72, 0.04)	0.12 (42, 0.05)
C:N ratio	19.0 (72, 2.56)	18.04 (42, 2.17)
N:P ratio	17.22 (72, 5.55)	14.82 (42, 2.88)
Lignin (mg g^{-1})	1.05 (78, 1.23)	
Hydrolyzable tannins (mg g^{-1})	0.47 (78, 0.54)	
Condensed tannins (mg g^{-1})	1.25 (78, 1.08)	
Flavonoids (mg g^{-1})	2.12 (78, 2.07)	
Total defences (mg g^{-1})	4.89 (78, 4.30)	

Table A4.2 (https://drive.google.com/file/d/1DfGxUnK19cwcjKulU0_-YiCtS0IzHhVm/view?usp=sharing).

We included model parameters loglink, AICc, delta and weight of the different climatic models.

Table A4.3 (https://drive.google.com/file/d/1BMGNom9pjKpW-rdJO83__RHN0ecR-J_z/view?usp=sharing).

We included model parameters loglink, AICc, delta and weight of the different biotic models.

5. General discussion

Tree-insect herbivore interactions in natural tree populations are shaped by a plethora of drivers, some intrinsic and others extrinsic to the host plants. Their respective importance varies greatly depending on the context and scale of observation, and different drivers typically interact with each other. Experimental approaches can be used to examine some drivers in isolation, yet the relevance of experimental results for real-world situations is often difficult to gauge. This thesis examined three types of ecological drivers of tree-herbivore interactions in Pedunculate oak under natural conditions: the landscape context (chapter 1); the character of individual trees (chapter 2) and the climate under which trees develop (chapter 3). While the three chapters illustrate the complexity and context-dependence of this type of ecological interactions, they also provide a series of interesting and sometimes surprising insights and have broader implications for future research and application.

5.1. Effect of landscape variables on predator-plant-insect herbivore interactions

The trophic interactions that take place in small forest stands resulting from forest fragmentation are well known and have been largely studied whereas little is known about the functioning of small forest stands resulting from recent forest establishment and expansion (*a.k.a.,* new forest stands). Overall, the results of chapter 1 indicate that although new forest stands are smaller and have a different origin than forest fragments, some biological processes that take place in both forest types are comparable as both insect herbivores and birds responded as reported in studies of fragmentation. For instance, I found that there was an increase in insect herbivory and in the abundance of birds with an increase in stand size as also shown in studies of fragmentation (De La Vega et al., 2012; Peter et al., 2015; Ruiz-Guerra et al., 2012; Simonetti et al., 2007; but see Maguire et al., 2015; Silva and Simonetti, 2009). However, even if birds were present, their abundance was rather low in comparison with studies of fragmentation, and they decreased with an increase of the percentage of forest around the studied stands.

I have found that leaf insect herbivory in new forest stands was influenced by the characteristics of the forest stands, which encapsulates diverse environmental drivers acting at the local (e.g. stand size, tree density and species composition, vegetation structure; Fuller et al., 2018; Maguire et al., 2016; van Schrojenstein Lantman et al., 2018) and at the landscape (e.g. stand connectivity, nature of matrix habitats; Morante-Filho et al., 2016) scale (chapter 1). In particular, I found that leaf chewer herbivory increased with increasing stand size and isolation, whereas the number of mines decreased in stands that were more connected, but was not affected by stand size (Valdés-Correcher et al., 2019). Similarly, Morante-Filho et al., (2016) study found that insect herbivory increased with increasing isolation in understory plants in the fragmented Brazilian Atlantic rainforest. Herrault et al. (2016) study showed that the species richness of forest specialist hoverflies was explained by the combination of several factors that act at different scales including stand area and isolation in a fragmented landscape in Southwest France.

5.2. Conservation of new forest expansion

My results have implications for the management of forest stands at the landscape level. The maintenance and conservation of forested areas is an important scientific and socio-economic issue as they have important ecosystem services, including the provisioning of habitat for biodiversity, climate regulation, carbon storage, and water supplies (Foley et al., 2005). Nowadays, forest expansion is taking place in many parts of Europe though novel native broadleaf woodlands and is expected to continue (Fuchs et al., 2015; Hansen et al., 2013; Schröter et al., 2005). For instance, in the maritime pine forests of the Landes de Gascogne region in Southwest France, oaks are currently increasing in abundance and spatial extent by means of spontaneous reforestation (Gerzabek et al., 2017). It is also the case of the expansion of oaks in Madrid in central Spain (Cruz-Alonso et al., 2019), and the expansion of English and Scottish broadleaved woodland stands in the UK (Fuller et al., 2018). However, the dynamics and ecological functions of new forest stands remain much less well understood than those of forest remains resulting of forest fragmentation.

New forest stands are rapidly colonized by woodland generalist whereas specialists can still remain absent even 150 years after forest establishments (Fuentes-Montemayor et al., 2015; Whytock et al., 2018). More specifically, the study performed in the first chapter has shown that they can be colonized by birds and by different guilds of insect herbivores. Even the smallest stand was colonized by insect herbivores and birds, although birds were reduced in number in comparison with forest stands originated from fragmentation. These results highlights the importance of conservation of even small stands as they can have significant beneficial effects on the health of surrounding pine plantations (Castagneyrol et al., 2014; Dulaurent et al., 2012; van Halder et al., 2015). Even background insect herbivory can reduce tree growth (Zvereva et al., 2012), but can also be seen as providing an overall benefit to ecosystem services such as timber production, the aesthetic value of forests, soil quality, and carbon sequestration (Maguire et al., 2015). In particular, because herbivores contribute to the acceleration of the recycling of organic matter (Chapman et al., 2003) and serve as a trophic resource for predators (Maguire et al., 2015), they are therefore necessary to maintain the biodiversity. Thus, the conservation of new forests may be crucial to keep the positive functions that insect herbivores and birds have in forest ecosystems.

5.3. Effect of genetic relatedness on predator-plant-insect herbivore interactions

Field-based research has primarily focused on landscape drivers on herbivory patterns as I have investigated in the first chapter; at the same time, experimental research has shown the importance of genotype-phenotype-herbivory relationships but the relevance of this finding in natural populations remains elusive (Lämke and Unsicker, 2018; Tack et al., 2012). In chapter 2, I wanted to go one step further and investigated the effect of tree genetic relatedness on plant-herbivore interactions in the same oak stands were I studied the effect of the landscape matrix. I showed that tree genetic relatedness influenced insect herbivory and leaf defenses. Importantly, this effect became only evident after accounting for within-crown variation. Overall, this chapter nicely illustrates to which extent the angle of observation may influence the detection of patterns (and underlying biological mechanisms).

More specifically, in chapter 2 I detected that herbivory was mainly influenced by landscape characteristics rather than by the tree genetic relatedness when data was analysed at tree level. Gossner et al. (2015) performed a (quite) comparable study in adult oaks across the German federal state of Bavaria and also found that the community composition of arthropods was mainly influenced by landscape and also by climatic variables, whereas it was not influenced by the tree genotype. Likewise Tack et al. (2010) study did not find neither an effect of oak genotype on insect communities, and found that insects were chiefly influenced by local factors and landscape variables. However, these findings partly conflicts with previous studies that have reported clear and sometimes strong effects of plant genotype and plant genetic diversity on herbivores (Bailey et al., 2006; Johnson and Agrawal, 2005; Kagiya et al., 2018; Silfver et al., 2014) and on leaf phenolic defenses (Agrawal et al., 2002; Maldonado-López et al., 2015).

Unexpectedly, taking into account intra-individual variability (upper versus intermediate canopy layers) allowed me to perceive that insect herbivory, as well as leaf defenses, were influenced by tree genetic relatedness. I also found that the influence of tree genetic relatedness was higher in upper canopy layers where leaves are exposed to higher amounts of light than in intermediate and lower canopy layers (chapter 2). These results suggest that there are different mechanisms that prevent the detection of a link between plant genotype, leaf defenses and herbivory when data is analysed at tree level. For instance, this result is fully in line with that of a parallel study to which I contributed (Castagneyrol et al., 2019a; Appendix A5.1), in which we show that the identity of mature oak had different effects on insect herbivores at upper, intermediate and lower canopy layers. It indicates that different microclimates along the canopy may result in differences in insect herbivory (Stiegel et al., 2017). Microclimate is affected by light availability, increasing temperature and decreasing air humidity from lower to upper canopies (Tal et al., 2008). The microclimate influences indirectly herbivory through changes on leaf traits (Muiruri and Koricheva, 2017; Stiegel and Mantilla-Contreras, 2018), uneven distribution of arthropod communities (Tal et al., 2008; Ulyshen, 2011) and the activity of predators (Aikens et al., 2013). These differences along the canopy may be reflected in the genotype of the tree as it is an important driver of the expression of chemical defenses (Barbour et al., 2016; Bernhardsson et al., 2013; Donaldson and Lindroth, 2007; Whitham et al., 2006).

Leaves that are located in higher levels of the canopy are more exposed to the light and thus have higher UVB radiation. A higher UVB radiation can influence the production of leaf defenses and volatiles. For instance, a study showed that in shade-intolerant species, solar UVB radiation is a positive modulator of plant defenses, and thus light helps plants concentrate defenses in photosynthetically valuable leaves (Ballaré, 2014). Kegge et al. (2013) also found that constitutive, methyl-jasmonate-induced green leaf volatiles and terpenoids were partially suppressed under severe shading conditions in Arabidopsis. UVB radiation can also influence plant diseases. For instance, the incidence of blister blight disease of tea caused by *Exobasidium vexans* also decreased under higher fluxes of UVB radiation (Gunasekera et al., 1997), and also UVB radiation increased plant resistance against pathogen infection of *Botrytis cinerea* by controlling the expression of the sinapate biosynthetic pathway in *Arabidopsis*, a precursor for soluble secondary metabolites (Demkura and Ballaré, 2012). Thus, the higher production of defenses in leaves exposed to higher levels of UVB radiation reported in these studies may be a response of the plant to reduce herbivory in these valuable leaves. Sun leaves are far more productive in terms of carbon fixation than shade leaves and their loss to herbivores hence is more costly for the plant (Poorter et al., 2006), and the photosynthetic activity of these leaves may be influenced by herbivory. For instance, a manipulative experiment in *Quercus robur* has shown that even a moderate intensity of insect herbivory (6 %) reduces 48 % of the potential photosynthesis (Visakorpi et al., 2018). Thus, these results support the idea that the production of leaf defenses to cope with herbivory may be higher in sun exposed leaves, and consequently the expression of genes that code the production of leaf defenses may vary along the canopy layer (Lämke and Unsicker, 2018).

Most previous studies investigating the role of tree genetics on herbivory and defenses have sampled at one or several canopy layers that are finally considered as replicates to get rid of within tree variability (Gossner et al., 2015; Kagiya et al., 2018; Maldonado-López et al., 2015). However, these results calls for some revision of how to study and what to expect from tree genotype-phenotype-insect herbivory interactions. Regarding ecological sampling designs, the differences in the variance of leaf defenses and insect herbivory explained by tree genetic relatedness among canopy layers implies that the commonly used method of inferring overall plant quality from the quality of a few collected leaves is unlikely to provide very accurate results. Previous studies have also suggested to take into account the variation in leaf defenses and insect herbivory when sampling (Gripenberg and Roslin, 2005; Roslin et al., 2006) but this is the first study that has observed that it is also important to take it into account when incorporating and exploring their genetic basis in real-world contexts. To overcome a part of this problem in studies trying to link tree genotype, phenotype and insect herbivory, not only the canopy layer but also leaves from a single shoot should be used to reduce variation among replicates. The same conservative approach should perhaps be extended to other tree species as well.

5.4. Variation of predator-plant-insect herbivore interaction along latitudinal gradients

Whereas in chapter 1 and 2 I focused on the effect of different drivers on plant-herbivores interactions, in chapter 3 I increased the zoom and investigated the effect of climate on plant-herbivore interactions at a geographical scale. There are several studies that have investigated the effect of latitudinal gradients on biotic interactions, but none of them have taken into account both forces simultaneously (e.g. Moreira et al., 2018; Roslin et al., 2017). However, both bottom-up and top-down drivers should be taken into account simultaneously because they jointly drive insect herbivory (Maguire et al., 2015; Sanz, 2001; Schoonhoven, 2005), and also because it has been shown that they also vary along climatic gradients (Moreira et al., 2018b; Roslin et al., 2017; Zvereva et al., 2019). In chapter 3, I investigated simultaneously bottom-up and top-down forces driving insect herbivory at a geographical scale along Europe. I found that climatic variables influenced insect herbivory and leaf nutritional traits while they did not influence leaf defenses nor bird predation. Furthermore, insect herbivory was only influenced by bottom-up forces (e.g. leaf nutritional traits and leaf defenses) and these effects on herbivory varied among herbivore feeding guilds, while top-down forces did not have an effect (chapter 3). The results obtained brings new insights into the vivid debate about latitudinal variation in the direction and strength of biological interactions.

There are a large number of studies that have supported the idea that biotic interactions are stronger at lower latitudes than at higher latitudes (Dobzhansky, 1950; Janzen, 1970; Schemske et al., 2009). However, this idea is still under debate as several studies and meta-analyses published over the last decade (Baskett and Schemske, 2018; Moles et al., 2011; Moles and Ollerton, 2016; Zvereva et al., 2019), as well as my third chapter, do not support the idea that interactions are generally stronger or more specialized in lower latitudes. Specifically, I have found that predator-herbivory-plant interactions did not vary along a latitudinal gradient and that only some insect herbivore guilds and leaf traits varied along climatic gradients reinforcing the idea that this assumption has to be considered with caution.

Investigating the effect of climatic gradients on biotic relationships along Europe (chapter 3) allowed us to examine the consequences of climatic warming on biotic interactions. Especially, these *natural laboratories* are used because they allow using space for the substitution of time.

However, it is also necessary to take into account that it has some drawbacks since in this way we cannot take into account the correlation between temperature and other factors that will not be affected by climate change such as day length and irradiation. Our planet is warming at a steady pace and the global mean annual temperature was 1.0°C higher during the last decades (2000s) than at the beginning of the twentieth century and is projected to continue increasing 1 - 4 °C in the next 50 - 100 years (Guilyardi et al., 2018; Hansen et al., 2010). Temperature is a determinant of the physiology, fitness and distribution of organisms (Woodward, 1987), and climate warming is expected to have profound consequences on biodiversity and ecosystem functioning (Lovejoy and Hannah, 2005). For instance, effects of climate change have already been documented as shifts in species geographical distribution (Chen et al., 2009), and the frequency and severity of outbreaks by some forest insects is predicted to intensify with climate change (Tobin, Park et al., 2014). Temperature may also influence foliar quality for herbivores through changes in primary and secondary metabolism (Zvereva and Kozlov, 2006). In order to better understand the magnitude of the effect and its biological feedbacks, it is important to understand how changes in temperature and precipitation will affect the biology of organisms. This information will allow us to anticipate and have a more sensitive understanding of where we should look at in the future.

5.5. The power of citizen science

To investigate the effect of latitude and climate on the mentioned biotic interactions across Europe requires to work simultaneously across large spatial scales, which can be quite challenging. Citizen science, the volunteer participation of the general public in scientific research through the collection of data, is a powerful tool to meet these challenges. This practice has increased worldwide in the last years (Roy et al., 2012). It allows scientists to simultaneously perform an experiment at a large scale (McKinley et al., 2017) and it is also *expected* to benefit citizens by increasing their interest, knowledge and skills related to scientific objects and the process of science (Bela et al., 2016; Bonney et al., 2016). This ideal view has however been challenged by recent research suggesting that knowledge gained from the volunteer participation in citizen science programs may have been overstated (Brossard et al., 2005; Scheuch et al., 2018). Working directly with schoolchildren and their teachers may, however, move citizen science toward win-win fruitful interactions between scientists and the general public by enhancing the long-term educational and social goals of citizen science programs (Makuch and Aczel, 2018) for several reasons. It likely improves understanding and retention of scientific concepts (Gormally et al., 2009; Minner et al., 2010). School pupils exposed to outdoor nature during childhood also increase their knowledge about nature, motivation and relationship with the environment (Ganzevoort and Van Den Born, 2019).

In our case, the participation of scientists and schoolchildren (under the supervision of their teachers) though citizen science in the chapter 3 has enabled us to investigate the effect of climate on biotic interactions, while engaging schoolchildren with the process of science. They performed the experiment and sent me all the material allowing me also to measure herbivory, leaf traits and predation rate in the laboratory. Schoolchildren and scientific partners also estimated predation rate and herbivory, and thanks to that I also contributed to a study were we could evaluate the precision and accuracy of the estimation of herbivory and predation performed by schoolchildren vs. professional scientists. We found that schoolchildren can support ecological research (Castagneyrol et al., 2019b; Appendix A5.2). However, their contribution needs to be considered with caution and requires several quality checks as kids' estimates proved to be biased, but this was also the case of the data acquired by different professional scientists. From a more personal perspective, leading this citizen science project also allowed me to work directly with schoolchildren and divulgate different aspects of ecology to a different public, something that I really recommend (Appendix A5.3). This practice has been a really positive experience, not only for me but also – I heard – for schoolchildren. It was quite gratifying to work with them and to see their motivation and their involvement in the project.

5.6. Limitations and future perspectives

5.6.1. Did I look at the appropriate leaf traits?

The fact that the leaf defenses measured did not vary along climatic gradients (chapter 3) and that they did not highly influence herbivory (chapter 2 and 3) may indicate that maybe I did not measure the appropriate leaf traits. Although it has been previously shown that phenolic compounds, especially condensed tannins, are recognised as defensive traits that influence negatively insect herbivory in *Quercus* and other species (Abdala-Roberts et al., 2016a; Roslin and Salminen, 2008), we have to take into account that these phenolics do not only act as defensive traits against insect herbivory but have also other functions as protect against UV radiation and temperature changes (Edreva et al., 2008; Janská et al., 2010). Furthermore, it has been debated several times if phenolics commonly measured are the adequate defensive traits (Anstett et al., 2016; Carmona et al., 2011; Damestoy et al., 2019; Pearse, 2011). For instances, it has been shown that there are other polyphenols that can have a higher influence on insect herbivores such as ellagitannins (Salminen and Karonen, 2011) and total phenolic oxidized leaf defenses (Appel, 1993). In the future, the rapid development of analytical methods will permit the identification of each individual phenolic compound, instead of simply quantifying the total amount of phenolics which will, no doubt, help characterise oak-herbivore interactions in a much finer way.

In addition, physical traits such as leaf toughness are also considered effective leaf defensive traits that influence herbivore performance, which I did not quantify. For instance, Carmona et al. (2011) study compared both physical and chemical leaf defenses and showed that physical leaf traits have larger effects on the preference and performance on herbivores than polyphenols. Furthermore, it could have been also interesting to distinguish between constitutive (plant defensive traits that are always expressed) and induced (plant defensive traits induced following herbivore attack) defenses. Both defenses are differently modulated by the abiotic environment, and may respond differently to environmental variables (Moreira et al., 2014; Sampedro et al., 2011). For instance, Moreira et al. (2014) showed that inducible defenses decreased while constitutive defenses increased towards higher elevations, and that it was strongly driven by variation in temperature in pine seedlings. In addition, Sampedro et al. (2011) showed the existence of genetic variation not only in constitutive defenses but also in inducible defenses in pine trees, indicating that induced defenses are genetically determined, and could have evolutionary consequences. Thus, future studies should include the measurement of other leaf defensive traits, and also distinguish between constitutive and induced leaf defenses.

5.6.2. Does tree genetic relatedness influence different herbivore guilds and predators?

Like in most of previous studies having addressed plant-herbivore interactions from an ecological perspective, I defined herbivory as the amount of leaf area removed or impacted by insect herbivores. I described "symptoms" with no clue on the identity of the causal agents. Yet, these were likely made by a large array of insect herbivore species. How do plant genetics and local environment shape herbivore communities has been well documented (Donaldson and Lindroth, 2007; Gossner et al., 2015; Maldonado-López et al., 2015). However, the relationship between the composition of herbivore community and the actual damage on host plants is much less straightforward. In my study system, it could be also expected that the link between insect herbivory and tree genetic relatedness may vary among herbivore guilds. For instance, the link between tree genetic relatedness and specialized insect herbivores may be stronger than between generalized insect herbivores, as specialized herbivores cannot switch onto other host species (Barantal et al., 2019; but see Barton et al., 2015). Thus, similarly as investigated in chapter 1, it could have been interesting to further investigate if different herbivore guilds (leaf miners and gallers) vary among canopy layers and if they are also differently influenced by tree genetic relatedness. For instance in other tree species, the leaf galls of *Mikiola fagi* are concentrated in upper canopy where light intensity is higher (Kamplicher and Teschner, 2002), while leaf mines of *Cameraria hamadryadella* and *C. ohridella* are concentrated in lower canopy (Brown et al., 1997; Nardini et al., 2004). However, previous studies investigating the effect of tree genetic relatedness on insect herbivore species are quite scare.

The effect of genetic relatedness (chapter 2) could also influence herbivory though an effect on predators. Because predators depend on herbivores and the reduction of insect herbivores by predators consequently favour plants, predation could also be influenced by plant genotype (Koricheva and Hayes, 2018). In accordance with this assumption, Bailey et al. (2006) study found that the effect of tree genotype on predators was even higher than on insect herbivores in cottonwood trees. Kagiya et al. (2018) study also found that the effect of tree genotype on predators was stronger than on herbivores in alder trees. The results of both studies suggest that the evolution of plant traits can influence and alter higher trophic levels and community composition, and that plant genetic effects on predators might be driven by mechanisms independent of herbivores (Koricheva and Hayes, 2018). When plants are attacked by herbivores, they are able to emit volatile organic compounds (VOC) to attract predators and parasitoids (Holopainen, 2004; Vet and Dicke, 1992). For instance, birds and parasitoid wasps are well known to be attracted by VOC emitted by plants (Gouinguené et al., 2001; Mäntylä et al., 2008). Thus, this effect of tree genotype on predators may be mediated by the expression of VOC by the plant as it has been shown that plant genotypes differ widely in their production of VOC (Heil, 2008; Wason and Hunter, 2014). Thus, in order to better understand the forces driving plant-herbivore interactions that take place in my study system, it could be interesting to investigate the effect of genetic relatedness on VOC and predation. This may have allowed investigating if there was a link between genetic relatedness and predators thought the emission of VOC, even if predation did not influence insect herbivory.

5.6.3. May predation rate reflect predator abundance?

I did not detect any effect of predation rate on herbivory in any of our case studies (chapter 1 and 3) and neither a correlation between the abundance of birds and bird predator activity (chapter 1). However, it does not mean that predators do not influence herbivory as their effect could by masked. Predators such as bird are considered intraguild predators that not only eat insect herbivores but also arthropod predators (Gunnarsson, 2007), and intraguild predation may weaken herbivore suppression (Finke and Denno, 2005; Polis and Strong, 1996). For instance, Finke and Denno (2005) study showed that increasing the number of intraguild predator species resulted in a high density of herbivores, which then led to a decrease on plant productivity in a saltmarsh food web. Furthermore, it is also important to take into account that the use of dummy caterpillars may underestimates the importance of top-down forces, as it does not capture the effect of other natural enemies such as spiders or parasitoids. Thus, to investigate the effect of bird predators on herbivory should also include arthropod and intraguild predation.

Furthermore, it is surprising that I did not detect variation in bird predation along a geographic gradient (chapter 3) as previous studies have shown that biotic interactions are stronger in lower latitudes (Coley and Kursar, 2014; Schemske et al., 2009). The absence of variation detected may have several explanations. First, the overall abundance and type of prey may vary along the gradient. For instance, even if the abundance of insect herbivores is higher in warmer areas than in colder areas (Coley and Barone, 1996; Moreira et al., 2018b; Pennings et al., 2009), it is also the case for the amount and abundance of alternative food for birds (such as fruits and nonherbivore arthropods; Cardoso et al., 2011), and it may result in lower predation rate on insect herbivores in warmer areas as birds rely also on other resources. Second, in colder areas, even if the abundance of birds is lower (Rabenold, 1978), they may feed more on insect herbivores such as caterpillars as the availability of resources is reduced (Marquis et al., 2012; Pennings et al., 2009; Schemske et al., 2009). Thus, it may prevent the detection of variation in predation rate along climatic gradients.

Another explanation may be related with the use of plasticine caterpillars to measure predation rate. This technique is widely used because it is suitable for comparative studies and allows measuring the activity of predators easily (Howe et al., 2009). However, we must keep in mind that it also has some limitations. For instance, Lövei and Ferrante (2017) review compared predation rate measured with plasticine caterpillars and with live sentinel prey, and found that predation rate using plasticine caterpillars was generally lower than live sentinel prey, suggesting that this technique may possibly underestimate predation intensity. Thus, in areas where the density of prey is low as could be the case in higher latitudes, predation activity may be inflated because they are the only prey available. It highlights the need to consider with caution the correlation between bird predation and bird abundance (chapter 1) and to better characterize the relationship between the composition of bird communities and the actual predation rate of herbivores.

5.7. Conclusion

This thesis shows that the landscape context, tree genetic relatedness and climate are drivers of tree-herbivore interactions that act simultaneously in Pedunculate oak under natural conditions. It illustrated the complexity and context-dependence of these types of ecological interactions. This study also provided a series of interesting insights and with broader implications for future research and application.

I found that plant-herbivore interactions in new forests were influenced by both forest size and connectivity, and that their effect were similar to previous studied of the well-known forest that comes from fragmentation. Thus, these results highlights the importance of conservation of new broadleaf stands as they are commonly found in the nature and support important ecosystem services.

The results of this thesis also highlights the importance of taking into account intra-individual variability not only when investigating the relationships between insect herbivory and leaf defenses but also when including the effect of genetic relatedness on the mentioned relationships. Thus, these results calls for the improvement of future ecological sampling designs, as these results shows that the commonly used method is unlikely to provide very accurate results when investigating the effect of different drivers on plant-herbivore interactions.

In addition, I also found that the effect of climate on plant-herbivore interactions was not stronger in lower latitudes as it has been widely described previously and need to be considered with caution. The results of the study of the effect of climate on plant-herbivore interactions help us to better understand how changes in temperature and precipitation due to climate change will affect the biology of organisms. This information will allow us to anticipate and have a more sensitive understanding of where we should look at in the future. Furthermore, the incorporation of the use of citizen science for data acquisition in this thesis encourages future studies to make use of it since it allows not only the acquisition of data and carrying out large-scale experiments but also working with a different audience.

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Tree diversity effects on leaf insect damage on pedunculate oak: The role of landscape context and forest stratum

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A B S T R A C T

Tree diversity has long been recognized as a major driver of insect herbivory in forest ecosystems. However, predicting the strength and direction of tree diversity effects in real-world situations has proven elusive. One likely reason is that most studies have focused on within-stand dynamics and insufficiently captured other ecological drivers of insect herbivory that can act at broader (i.e., landscape) and finer (i.e., individual trees) scales. We measured herbivory as leaf area consumed by insect herbivores in pedunculate oaks (*Quercus robur*) growing in mixed and pure forest stands in southwestern France. We assessed the effects of oak spatial isolation within the landscape, tree stand diversity, forest canopy stratification as well as the influence of leaf traits on insect herbivory. Insect herbivory increased with stand isolation regardless of tree diversity. Diversity effects were contingent upon the canopy stratum as insect herbivory in mixed stands exceeded that of pure stands only in the upper stratum. Leaf traits varied between pure and mixed stands and among canopy strata. Insect herbivory was negatively correlated with LDMC and positively with SLA. However, the observed effects of tree diversity, canopy stratum and stand isolation on insect herbivory were only partially driven by variability in oak leaf traits. Our findings illustrate that, in real-world contexts, insect herbivory can be driven by a complex interplay of multiple, scale-dependent drivers. They help step forward towards a more profound understanding of the complex forces drive insect herbivory in managed forest ecosystems.

1. Introduction

A long held view in forest ecology is that tree diversity strongly influences insect herbivory. Extensive research has demonstrated that trees are generally more prone to suffering damage when grown in monospecific stands than when associated with other tree species (Castagneyrol et al., 2014; Vehviläinen et al., 2007), although neutral (Rosado-Sánchez et al., 2018) or even opposite patterns have also been reported (Schuldt et al., 2010). The underlying phenomenon, termed associational resistance, appears to be widespread in forests (Castagneyrol et al., 2014; but see Haase et al., 2015; Schuldt et al., 2015). However, to date most empirical evidence on associational effects in forests stems from case studies of outbreaks of particular pest species and from highly controlled experiments (Castagneyrol et al., 2013; Damien et al., 2016; Schuldt et al., 2015; Vehviläinen et al., 2007). Despite their evident strengths (Grossman et al., 2018; Paquette et al., 2018; Verheyen et al., 2016), such experiments fail to properly consider the diversity and complexity of drivers that tend to affect background insect herbivory under natural, non-outbreak conditions (Guyot et al., 2016; Moreira et al., 2017; Kozlov and Zvereva 2017). This lack strongly constrains our understanding of the actual ecological relevance of associational effects on insect herbivory in real-world situations.

Patterns of insect herbivory are moulded by a variety of factors controlling plant accessibility at different scales. At the landscape scale, herbivore density tends to be highest in those habitat patches where their resource is most abundant, because the intensity of physical and chemical cues makes these patches more likely to be found and colonized (Andersson et al., 2013; Hambäck and Englund, 2005; Root, 1973). Within patches, herbivory on individual plants is influenced by the identity and diversity of their neighbours which alter the focal plant's physical and chemical apparency and its colonization (Castagneyrol et al., 2013; Finch and Collier, 2000; Moreira et al., 2016). Finally, herbivory is controlled by individual plant traits including nutritional quality and anti-herbivore defences (Castagneyrol et al., 2018b; Finch and Collier, 2000; Schoonhoven, 2005). Insects rely

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on a complex system of decision cues for selecting the plants they forage on. These can be used hierarchically or sequentially, and their role can vary depending on the animal's spatial scale of perception or nutritional status (Andersson et al., 2013; Schoonhoven, 2005). For instance, herbivores can be attracted by large patches from the distance but their final decision to feed on a given plant depends on its traits and its neighbours (Finch and Collier, 2000, 2012; Hambäck et al., 2014). While great advances have been made in our understanding of specific drivers of insect herbivory, the scale-dependent interplay between different drivers remains poorly understood.

Some such interactions have recently been hypothesized. It has for instance been suggested that resource isolation at the landscape level may cause herbivores to dedicate more time to foraging within resource patches, thus increasing damage in isolated stands as a result of reinforced small-scale effects of tree diversity on feeding decisions (Hambäck et al., 2014; Stutz et al., 2015; Underwood et al., 2014). However, observational studies demonstrating such an interaction are lacking. Within habitat patches, herbivory tends to vary along vertical gradients in forest canopy as a result of parallel changes in microclimate (in particular, higher temperatures in upper strata, Stiegel et al., 2017), leaf traits (Dudt and Shure, 1994; Stiegel et al., 2017; Thomas et al., 2010), or the diversity and activity of herbivores' enemies (Aikens et al., 2013). For instance, Stiegel et al. (2017) showed that the decrease in insect herbivory from lower to upper stratum was accounted for by the parallel increase of temperatures and decrease in nitrogen content of more sun-exposed leaves. However, the effect of stratification on herbivory has proven to vary among insect feeding guilds and forest management (Gossner et al., 2014). Tree diversity is known to alter the vertical stratification of forest canopies (Forrester, 2017; Vanhellemont et al., 2018). In turn, forest stratification will likely alter the effects of tree diversity on herbivores. For instance, both the position in the canopy (Stiegel et al., 2017) as well as the density and diversity of neighbouring trees can trigger the surface or the C:N ratio of plant leaves, two traits that are commonly related with patterns of herbivory (Castagneyrol et al., 2017; Moreira et al., 2017; Loranger et al., 2013; Pearse, 2011; Schoonhoven, 2005). However, quantifying the relative contribution of trait-dependent and trait-independent effects of plant density and diversity on insect herbivory remains challenging and the few existing studies have yielded conflicting results.

Here, we address how tree diversity effects on leaf damage caused by defoliating insect herbivores in pedunculate oak (*Quercus robur*) are shaped by ecological drivers independently and interactively acting at the individual (i.e., leaf traits), habitat (i.e., forest stratum) and landscape (i.e., forest stand isolation) scales. For this purpose, we measured leaf herbivory in oaks in the lower, intermediate and upper stratum of pure and mixed oak forest stands along a gradient of forest isolation at the landscape level across the season. We hypothesised that (i) insect herbivory would be lower in mixed oak-pine stands than in pure oak stands, and (ii) the difference would be strongest in isolated stands. We further predicted that (iii) leaf traits and insect herbivory would vary among forest strata and that (v) differences in herbivory among forest strata would be larger in pure than in mixed stands. By addressing tree diversity effects on insect herbivory at different scales, both within and between stands, our study pursues a better understanding of the hierarchical mechanisms that drive tree-herbivore interactions in real-world landscapes.

2. Materials and methods

2.1. Study site and stand selection

The study was carried out in the Landes de Gascogne region (southwestern France), about 40 km southwest of Bordeaux (44°41′N, 00°51′W). This region harbours the largest plantation forest in Europe with a monoculture of maritime pine (*Pinus pinaster*) covering approximately 10 000 km^2. The remaining landscape is characterized by agricultural lands and woodlands whose most common tree species are oaks (*Quercus robur*, *Q. pyrenaica*) and birch (*Betula pendula*). These deciduous tree species are also present in variable abundance in the pine plantations.

Table 1
Summary of stand characteristics.

Stand	Coordinates	Stand type
Berganton	44° 45′40.85″N, 0° 49′ 37.58″W	Pure
France	44° 44′ 44.10″N, 0° 50′ 50.82″W	Pure
St Alban	44° 43′ 18.78″N, 0° 45′ 3.25″W	Pure
Barlan	44° 44′ 57.00″N, 0° 49′ 53.12″W	Pure
Castéra	44° 44′ 0.1″N, 0° 52′ 42.29″W	Pure
Croix d'Hins	44° 43′ 21.69″N, 0° 49′ 32.31″W	Pure
Hermitage	44° 44′ 50.69″N, 0° 46′ 10.78″W	Mixed
Renardière	44° 43′ 42.33″N, 0° 50′ 8.78″W	Mixed
H5	44° 43′ 8.11″N, 0° 49′ 59.83″W	Mixed
H6	44° 43′ 30.63″N, 0° 51′ 10.72″W	Mixed
H8	44° 43′ 10.51″N, 0° 50′ 36.85″W	Mixed
H20	44° 42′ 56.23″N, 0° 51′ 48.50″W	Mixed

In early 2009, we selected 12 forest stands for study: six pure stands in which *Quercus robur* was the main species, and six mixed stands consisting of pine-oak mixtures where oaks were abundant (Table 1). For each stand, we mapped the main habitat types in circular buffers of 500 m radius (*ca.* 78.5 ha). We distinguished pine plantations, deciduous forests and open habitats. Open habitats included roads, forest tracks, firebreaks, clearcuts, field and field margins, and young pine plantations (Barbaro et al., 2005). The buffer of 500 m radius provided the largest gradient of habitat variability, avoided spatial overlapping between nearby buffers, and was previously found to be suitable to study plant-herbivore-predator interactions in different landscape contexts (Barbaro et al., 2005; Chaplin-Kramer et al., 2011). Habitat mapping was based on satellite images read and analysed with QGIS version 2.18.13 (QGIS Development Team, 2017).

2.2. Leaf sampling and measurements

In each stand, we randomly selected individual oak trees and assigned them to one of three forest strata. All leaves collected below 1 m were assigned to the lower stratum, which may have included hanging low branches of adult trees as well as leaves of younger individuals. The canopies were further divided in an intermediate stratum (lower and mid-height branches) and an upper stratum (upper branches). We chose to define these strata in relative instead of absolute terms because tree height varied among the different stands.

In 2009, we sampled twice a total of 300 oak leaves per stratum in each stand, in early (July) and late (September) season. The two sampling dates were considered as temporal replicates. Although leaf herbivory is a cumulative process such that early season damage remains visible in late season, oaks produce three and up to four generations of leaves in the study area. Thus, the first and second campaigns represent independent assessments of leaf insect herbivory.

Leaves were collected on a sample of six individuals per stratum and per season, that were haphazardly selected each time to ensure statistical independence of the temporal replicates. The number of sampled leaves was held constant between pure and mixed stands and between temporal replicates, whereas the sampling methodology was adapted to the forest stratum because of technical constraints. Leaves were collected using a pruning shear for the lower stratum, and with a 10 m pole pruner for the intermediate stratum of all stands and the upper stratum of mixed stands. Rifle shooting was used to cut branches down in the upper stratum of pure stands. For the lower stratum, we assembled a 300 leaf sample by haphazardly collecting 35 leaves on one hanging branches of six different individuals, plus an additional pool of leaves from younger individuals. For intermediate and upper strata, we assembled the 300 leaf sample by collecting 30–50 leaves on six to ten

Table 2

Summary of LMM testing the effects of season, forest type, stratum and isolation on herbivory and leaf traits. P-values are indicated within brackets and significant effects are shown in bold. Marginal (R^2_m) and conditional (R^2_c) R^2 are reported for the simplified model.

Predictors	df	Total herbivory χ^2-value	Leaf surface χ^2-value	SLA χ^2-value	LDMC χ^2-value
Season	1	**121.06** (< 0.001)	0.66 (0.416)	**4.01** (0.045)	**122.03** (< 0.001)
Forest type	1	3.2 (0.072)	**4.74** (0.029)	**11.08** (< 0.001)	**13.55** (< 0.001)
Stratum	2	**129.79** (< 0.001)	**1090.22** (< 0.001)	**1240.75** (< 0.001)	**102.15** (< 0.001)
Isolation	1	**8.27** (0.004)	**3.95** (0.047)	1.00 (0.318)	0.05 (0.819)
Forest type × Stratum	2	**71.71** (< 0.001)	**26.69** (< 0.001)	**68.70** (< 0.001)	**15.45** (< 0.001)
Forest type × Isolation	1	0.86 (0.352)	0.02 (0.885)	1.59 (0.207)	2.37 (0.123)
Stratum × Isolation	2	1.08 (0.583)	2.89 (0.216)	1.26 (0.533)	2.58 (0.275)
Forest type × Stratum × Isolation	2	0.67 (0.716)	2.21 (0.331)	0.59 (0.744)	0.32 (0.854)
R^2m (R^2c)		0.36 (0.45)	0.65 (0.66)	0.68 (0.72)	0.36 (0.45)

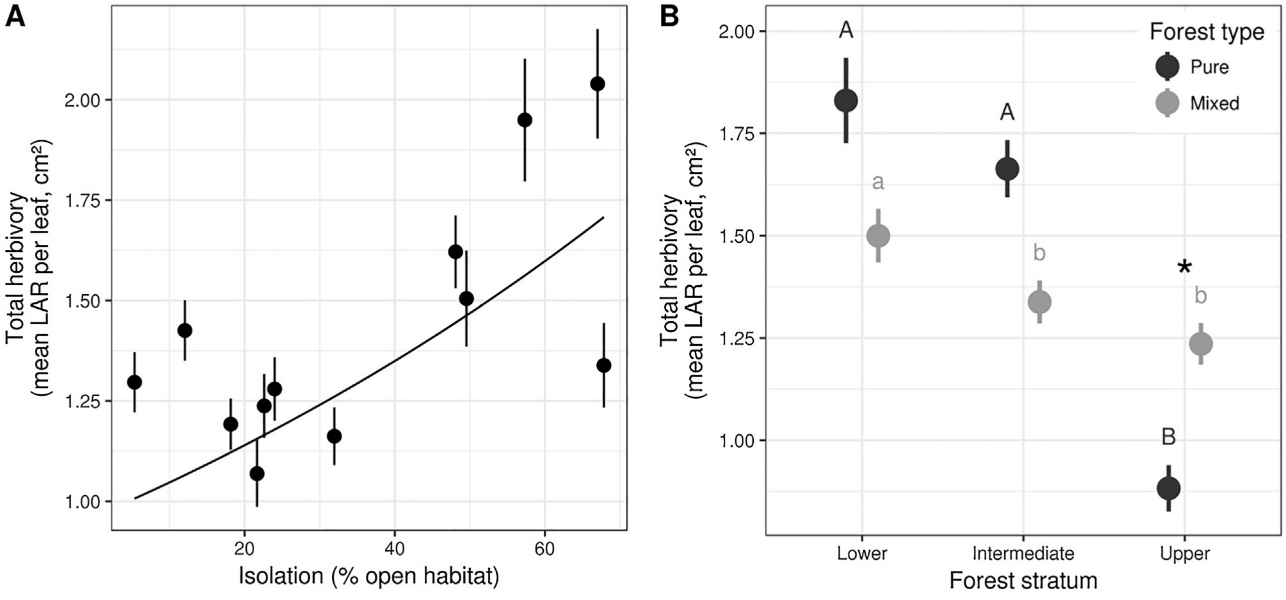

Fig. 1. Effects of stand isolation, forest type and stratum on leaf herbivory. (A) Effects of stand isolation. Each point represents leaf herbivory averaged (± SE) per stratum and season. (B) Interactive effects of forest type and stratum on leaf herbivory. Each point represents leaf herbivory averaged per season across all forest stands (± SE, n = 6 per forest type). Letters above bars indicate statistical differences between forest types.

branches of 6 different trees (1 or 2 branches per tree). In each sample, we took every tenth leaf until reaching the scheduled sample size.

Herbivory was estimated by two measurers aware of sample origin. To reduce variability among observers, we used a grid of 0.25 cm^2 (0.5 × 0.5 cm) printed on a transparent plastic sheet and overlaid on leaves. We calculated the total leaf area removed or affected by insect herbivores divided by the number of leaves analysed. We initially disentangled damage caused by different feeding guilds (chewers, skeletonizers, leaf-rollers, leaf-miners). However, some of these guilds caused too scant damages to allow separate analyses and we therefore pooled all types of damages.

We measured three leaf traits: leaf surface, Specific Leaf Area (SLA) and Leaf Dry Matter Content (LDMC). These traits were measured on 10 leaves per stratum, stand and sampling date following Cornelissen et al. (2003) after confirming that this sample size satisfyingly captures variability among strata. We only used undamaged, mature, and fully expanded leaves. Leaf surface and water-saturated fresh mass were measured with a planimeter (WinFolia Pro 2007b, Regent Instruments, Canada Inc.) and a balance (Ohaus EP114 Explorer Pro Analytical Balance). Leaf surface, SLA and LDMC were first calculated at the level of individual leaves and then averaged per replicate, stratum, stand and season.

2.3. Statistical analyses

We calculated the percentage of open area in buffers of 500 m radius centered on selected stands as a proxy for stand isolation at the landscape level. We preferred to use this variable instead of deciduous forest cover because pedunculate oaks commonly grow below the canopy of pine plantations without being detectable on satellite images (Gerzabek et al., 2017). The cover of deciduous forest alone therefore underestimates oak abundance in the landscape. We used linear mixed-effect models (LMM) to analyse the effects of landscape, tree diversity and canopy stratification on leaf traits and insect herbivory. Fixed effects were season (early *vs.* late), forest type (pure *vs.* mixed stand), stratum (lower, intermediate and upper) and isolation (percentage of open areas in the landscape). Season was considered as a temporal replicate. We tested all two- and three-way interactions between forest type, stratum and isolation. We declared stand identity as a random factor to account for the non-independence of samples from the same stand. For each response variable (herbivory, leaf surface, SLA and LDMC), we first built the full model and then applied model simplification by sequentially removing non-significant terms, starting with the highest-order interaction term. We made no attempt to simplify the random factor as it was imposed by the sampling design. Significant interactions between stratum and forest type were treated by estimating contrasts among strata for each forest type separately and contrasts between pure and mixed stands for each stratum independently. Finally, we estimated

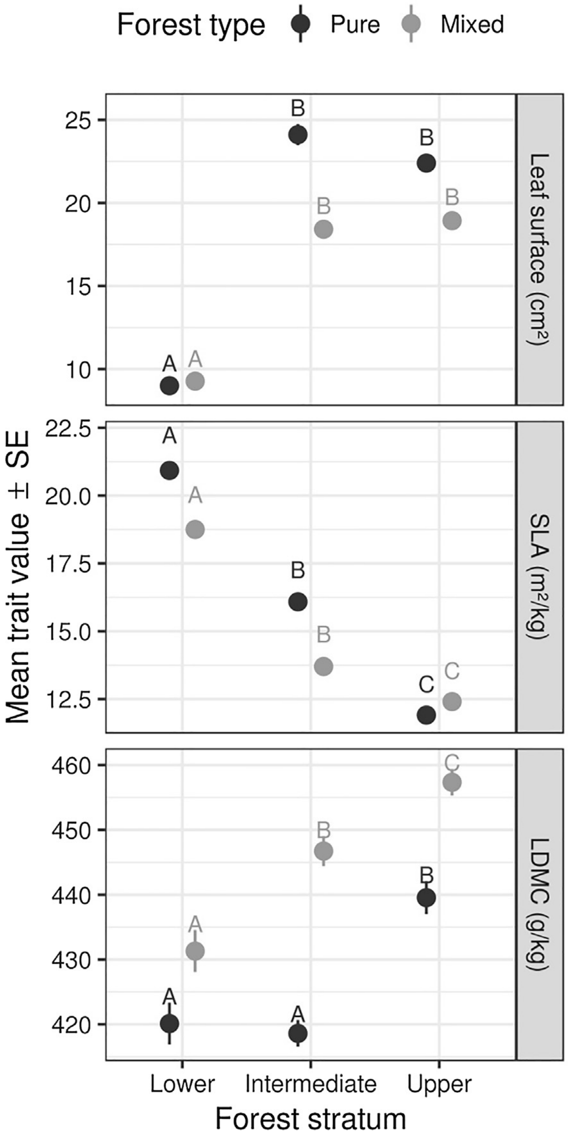

Fig. 2. Interactive effects of stratum and forest type on leaf traits. Dots and error bars represent means (± SE) across seasons and forest types. Letters above bars indicate statistical differences between strata. Contrasts between strata are shown for each forest type separately (indicated by different grey shades).

model coefficients of the simplified model and calculated R^2 for fixed effects ($R_m{}^2$) and fixed plus random effects ($R_c{}^2$). Response variables were log-transformed to improve the distribution of model residuals. We back-transformed model predictions with an exponential function to plot them on figures.

We were interested in disentangling the effects of isolation, forest type, stratum and leaf traits on herbivory. Yet, leaf traits were influenced by both forest type and stratum (see Results). In order to avoid issues arising from collinearity, we also tested the effect of leaf traits on herbivory in a separate model where we replaced the factors forest type and stratum by SLA and LDMC. We then applied the same modelling procedure as described above. SLA, LDMC and isolation were scaled and centered to allow comparing coefficient parameter estimates, although we present raw data in the figures.

Finally, we used a structural equation modelling (SEM) to confirm the indirect trait-mediated effect of tree diversity and forest stratum on herbivory. We first built a theoretical model in which herbivory was only explained by SLA and LDMC. SLA and LDMC were endogeneous variables. Tree diversity and forest stratum were exogeneous variables that only influenced SLA and LDMC. We used the piecewiseSEM package (Lefcheck, 2016) and Shipley's test of direct separation to evaluate the probability that none of the paths missing from the hypothesised network contain useful information (in particular direct paths linking forest stratum and tree diversity to herbivory). This hypothesis was considered rejected if χ^2-test of Fisher's *C* statistic fell below the significance level ($P < 0.05$).

All analyses were done in R version 3.3.1 (R Development Core Team, 2016) using the packages *lmer4*, *car*, *multcomp*, *MuMIn* and *piecewiseSEM* (Bartoń, 2016; Fox and Weisberg 2011; Hothorn et al., 2008; Kuznetsova et al., 2016, Lefcheck, 2016).

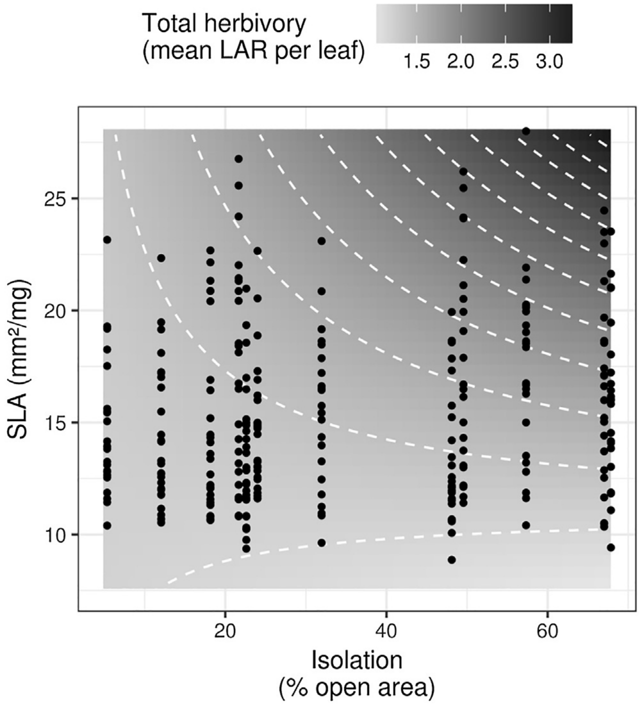

Fig. 3. Interactive effect of Specific Leaf Area (SLA) and stand isolation on leaf herbivory. Grey shades and isolines show predictions from mixed-effects models for early-season data and for an average value of LDMC. Dots show the original data.

3. Results

3.1. Effects of forest type, stratum, isolation and season on herbivory

Herbivory was on average (± SE) 1.42 ± 0.03 cm^2 per leaf (corresponding to *ca.* 8% leaf area), which corresponds to background insect herbivory in the study area. Herbivory was 37% higher in the late season than in the early season (Table 2). It increased with stand isolation (Fig. 1A) and decreased from the lower to the upper stratum (Fig. 1B). Herbivory did not differ between mixed and pure stands, but we observed a significant forest type × stratum interaction (Table 2) as differences between pure and mixed stands were only significant in the upper stratum (coefficient parameter estimate ± SE: 0.60 ± 0.13, Fig. 1B). In pure stands, leaf herbivory was significantly lower in the upper stratum (Fig. 1B) whereas it did not differ between the intermediate and the lower stratum. In mixed stand, leaf herbivory was higher in the lower stratum than in the intermediate and upper stratum, whereas the latter two did not differ (Fig. 1B).

3.2. Effects of forest type, stratum and isolation on leaf traits

Leaf traits varied consistently among strata and between forest types

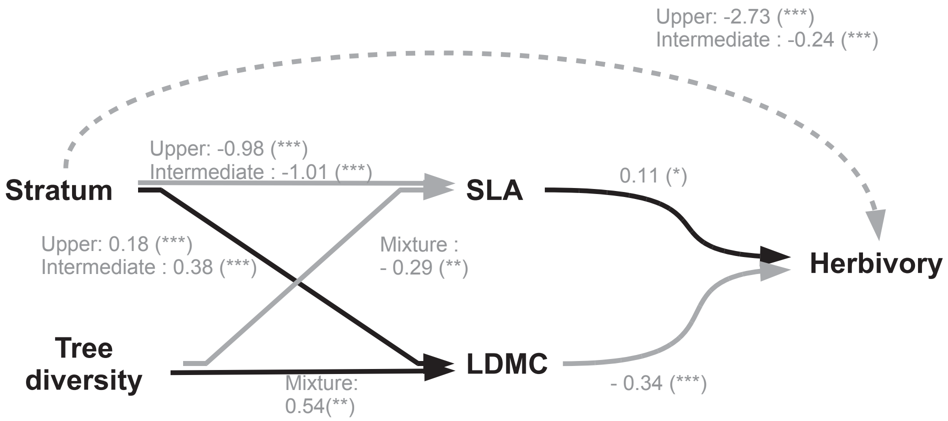

Fig. 4. Path diagrams showing the results of the piecewise SEM. Solid and dashed lines represent significant direct and indirect relationships among variables, respectively. Black and grey lines represent negative and positive correlations, respectively. Standardized coefficients and significance thresholds are shown along paths.

(Fig. 2) with a significant forest type × stratum interaction for all traits (Table 2). Leaf surface was on average (± SE) $17.5 \pm 0.3\,\text{cm}^2$. It was twice as large in the intermediate and upper stratum than in the lower stratum, both across forest types and seasons, while there were no differences between the intermediate and the upper stratum. Differences between the lower and the intermediate and upper stratum were larger in pure stands than in mixed stands (Table 2). Oaks had larger leaves in pure stands than in mixed stands, but only in the upper and intermediate strata. SLA was on average $15.40 \pm 0.15\,\text{mm}^2 \cdot \text{mg}^{-1}$. It consistently decreased from lower to upper stratum in both forest types and seasons. All contrasts between strata were significant, but differences were larger in pure than in mixed stands. SLA tended to be higher in pure stands than in mixed stands, but this difference was only significant in the lower and the intermediate stratum. LDMC was on average $436.0 \pm 1.2\,\text{mg} \cdot \text{g}^{-1}$. It consistently increased from lower to upper stratum in both seasons and forest types. However, differences among strata were contingent on forest type (Table 2). In pure stands, LDMC was greater in the upper stratum than in the intermediate and lower stratum, while the latter two did not differ. In mixed stands, all contrasts between strata were significant. LDMC was greater in mixed stands than in pure stands, but this difference was only significant in the intermediate and the upper stratum.

4. Leaf traits associated with the effects of forest type and stratum on herbivory

Replacing the factors forest type and stratum in the LMM by the leaf traits SLA and LDMC, we found that differences in herbivory between forest types and among strata could be accounted for by LDMC and SLA. Herbivory decreased with LDMC ($[82.4 \pm 24.8] \times 10^{-3}$, df = 1, $\chi^2 = 11.00$, $P = 0.001$) and increased with SLA ($[6.0 \pm 37.6] \times 10^{-3}$, df = 1, $\chi^2 = 34.90$, $P < 0.001$, Fig. 3). The effect of SLA was however contingent on stand isolation (significant SLA × isolation interaction: df = 1, $\chi^2 = 14.46$, $P < 0.001$). The positive coefficient parameter estimate (± SE) for the interaction (0.06 ± 0.02) indicated that the effect of SLA on herbivory was slightly stronger in more isolated stands and that the effect of stand isolation was in turn stronger for leaves with greater SLA. These results are consistent with our observation that SLA and herbivory both decreased from the lower to the upper stratum. Model R^2 was however lower when the factors forest type and stratum were replaced by SLA and LDMC ($R^2_m = 0.29$ and $R^2_c = 0.42$ *vs.* $R^2_m = 0.36$ and $R^2_c = 0.44$, Table 2), suggesting that the measured leaf traits account largely but not completely for differences in herbivory arising from differences between forest types and among strata.

In line with these results, the SEM analysis (Fig. 4) confirmed that there were missing paths in our *a priori* network ($C = 9.69$, df = 4, $P = 0.046$), indicating that the effects of tree diversity and forest stratum on insect herbivory were only partially mediated by their effect on the measured leaf traits. In particular, there was a significant missing path between forest stratum and herbivory, indicating an additional, trait-independent, effect of forest stratum on insect damage.

5. Discussion

The factorial and hierarchical sampling design of this single year-study enabled us to get a detailed insight into the complex interplay of environmental drivers that determine patterns of background insect herbivory across spatial scales (Fig. 5). We did not detect a global difference in herbivory between mixed and pure stands, yet a finer analysis revealed that such an effect of tree diversity did exist but only in certain canopy strata in pure stands. We also observed that both stand isolation and stratum influenced herbivory. Finally, both stand isolation and stratification effects were partly explained by the concomitant variation in the leaf traits SLA and LDMC. This set of interrelationships clearly illustrates the complex nature of the multiple, scale-dependent drivers of insect herbivory in real-world contexts. It calls for caution when interpreting ecological studies that address limited sets of putative drivers of insect herbivory in simplified environments.

Leaf insect herbivory was not globally reduced in mixed stands but in certain strata. We found that tree diversity effects on insect herbivory were not consistent across canopy strata. While mixed stands experienced lower herbivory than pure stands in the lower and intermediate stratum, the opposite occurred in the upper stratum. Previous studies on effects of tree diversity on insect herbivory have acknowledged potential stratum effects but, instead of quantifying them, seeked to reduce them by averaging herbivory at the level of individual trees (Castagneyrol et al., 2013; Muiruri et al., 2015; Vehviläinen et al., 2006). Our study pinpoints the pitfalls of such an approach by demonstrating that the canopy stratum can exert a significant influence on the (non-)detection of global effects of tree diversity. The pattern that we observed might be explained by the fact that insects coming from other forest stands are likely to arrive in the upper stratum. Reduced herbivory in the upper stratum of pure stands could then reflect a dilution of the recently arrived herbivores among a larger number of host trees (Bañuelos and Kollmann, 2011; Damien et al., 2016; Otway et al., 2005).

Leaf insect herbivory increased from the upper to the lower stratum. In accordance with previous studies (e.g. Reynolds and Crossley, 1997; Stiegel et al., 2017), we found that insect herbivores caused most damage in the lower stratum. This trend has several possible, non-exclusive explanations. First, vertical stratification in herbivory can be a direct consequence of stratification in leaf traits. SLA increased and LDMC decreased towards the lower stratum, suggesting that its leaves were most palatable and hence most attractive for herbivores (Le Corff and Marquis, 1999; Murakami and Wada, 1997; Stiegel et al., 2017). This assumption was fully confirmed by the distinct effects that both parameters exerted on levels of herbivory. Although we did not measure microclimate in our stands, it is likely that

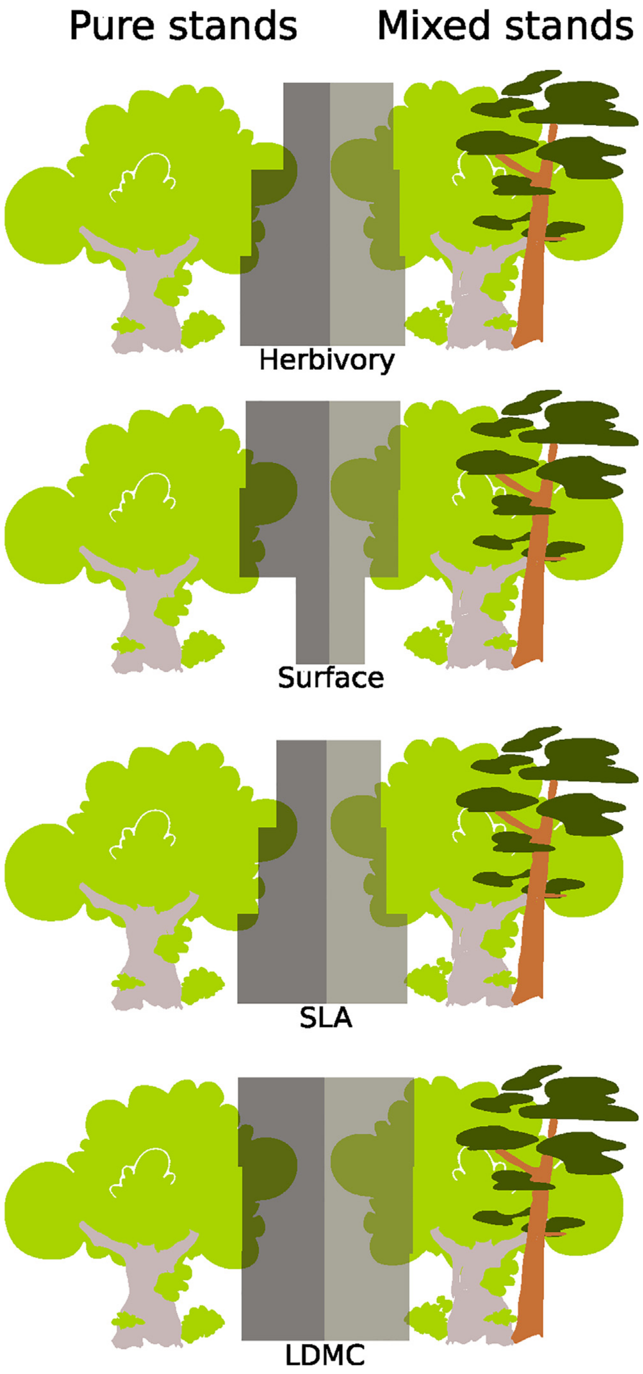

Fig. 5. Summary of herbivory and leaf trait response to forest type and stratification. Grey squares represent herbivory and traits for different forest types and strata. Differences in square size are proportional to observed changes in herbivory or traits. The figure therefore illustrates the extent of differences between forest types and among strata.

light and temperature were the primary drivers of the observed SLA and LDMC trends (Yamasaki and Kikuzawa, 2003, Stiegel et al., 2017). The elevated herbivory in the lower stratum might also be triggered to some extent by reduced levels of chemical defences in the youngest individuals (Boege and Marquis, 2005; Moreira et al., 2017). Yet this effect does not explain the observed differences between the intermediate and the upper stratum. Second, stratification of herbivory could result from stratification of herbivore communities. Several studies have reported higher abundance or richness of herbivores in lower canopy strata and linked this trend with greater leaf quality (Le Corff and Marquis, 1999; Murakami et al., 2005) or a vertical transfer of herbivores from the canopy to the understory (Murakami and Wada, 1997; White and Whitham, 2000). However, relationships between herbivore abundance or diversity and herbivore damage are not straightforward (Basset et al., 1992; Rhainds and English-Loeb, 2003; Rossetti et al., 2017); hence the relevance of this explanation is difficult to gauge in our case. Third, top-down control of herbivores by their enemies could generate lower herbivory in upper strata, if predation pressure is higher in this part of the canopy (Sobek et al., 2009; Aikens et al., 2013). Unfortunately, we possess no empirical data yet on predator abundance or activity that would enable us to test the validity of this explanation.

Effects of forest type and stratum on leaf insect herbivory were partly explained by leaf traits. On the one hand, we detected an interaction between canopy stratum and tree diversity on leaf traits whereby differences in leaf traits among strata were more pronounced in pure stands. Recent studies reported that tree diversity may alter abiotic factors in individual canopies (and in particular light environment), and hence insect herbivory (Castagneyrol et al., 2018a, 2017; Muiruri and Koricheva, 2016; Rosado-Sánchez et al., 2017). Given the different growth form of oaks and pines, it is likely that the vertical stratification of abiotic factors was far more heterogeneous in mixed than in pure stands (Forrester, 2017), resulting in neater vertical gradients of leaf traits in pure stands. On the other hand, we found significant relationships between leaf traits and herbivory. In particular, leaf insect herbivory increased with increasing SLA and decreased with increasing LDMC. However, despite this direct effect of leaf traits on herbivory, we still detected a direct effect of forest stratum on leaf insect herbivory, while the effect of tree diversity on herbivory seemed to be primarily driven by an effect of tree diversity on leaf traits. To the best of our knowledge, to date no study addressing leaf trait-mediated effects of tree diversity on leaf insect herbivory has systematically compared leaves from different strata. Yet our results indicate that controlling for this effect will be critical for future studies of tree-herbivore interactions in mixed forests.

Leaf insect herbivory increased with oak isolation at the landscape scale. A long held view in ecology is that herbivory increases with the density of resources (resource concentration hypothesis, Root, 1973). Yet, we found the opposite. Whereas studies on forest fragmentation often find that herbivore abundance and richness decrease with patch size and isolation (Rossetti et al., 2017), results are less consistent for herbivory itself (Maguire et al., 2016; Rossetti et al., 2017; Simonetti et al., 2007). Our results suggest that the variation in outcomes of previous studies may to a considerable extent be caused by tree cues at the within-patch and individual tree scales that dilute among-patch trends in herbivory. For instance, virtually no studies we are aware of have to date accounted for stratification effects. Yet such effects could be far more determinant for patterns of herbivory than for herbivore richness or abundance if they affect the quality of the plant diet – which was the case in our system. On the other hand, we cannot exclude that the trend we observed was to some extent also mediated by differential top-down control of herbivores by predators, as has been reported by some authors (Gonzalez-Gomez et al., 2006; Maguire et al., 2015; Rossetti et al., 2014). Likewise, we present here the results of a single year-study that was conducted in 12 mixed and pure forest stands in southwestern France. We cannot exclude that our results may reflect particular abiotic conditions and the management applied to forest stands in this region.

6. Conclusions

Multiple independent approaches have been used so far to address the effect of tree diversity on insect herbivory. Studies on the influence of habitat diversity at the landscape level and tree diversity within forest patches mainly addressed insect movements and population dynamics at large scales. Studies focusing on a much smaller scale addressed tree-tree interactions and their effects on herbivores through changes in leaf traits. Here, we linked knowledge on tree-herbivore interactions from these different perspectives by integrating landscape-, forest type- and individual-levels effects on insect herbivores within the same study. By demonstrating that insect herbivory in mixed stands exceeded that of pure stands only in the upper stratum, we unravel the importance of considering small-scale variability of biotic and abiotic factors when addressing insect herbivory on forest trees. Our findings therefore help step forward towards a more profound understanding of the complex forces that drive insect herbivory in forests.

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Author contributions

BG conceived the study and acquired the data. BC, BG and EVC analyzed the data. BC and BG drafted the first version of the manuscript. All authors wrote the final version of the manuscript.

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RESEARCH PAPER

Can School Children Support Ecological Research? Lessons from the *Oak Bodyguard* Citizen Science Project

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Scientific knowledge in the field of ecology is increasingly enriched by data acquired by the general public participating in citizen science (CS) programs. Yet, doubts remain about the reliability of such data, in particular when acquired by schoolchildren. We built upon an ongoing CS program, *Oak Bodyguards,* to assess the ability of schoolchildren to accurately estimate the strength of biotic interactions in terrestrial ecosystems. We used standardized protocols to estimate attack rates on artificial caterpillars and insect herbivory on oak leaves. We compared estimates made by schoolchildren with estimates made by professional scientists who had been trained in predation and herbivory assessments (henceforth, trained scientists), and trained scientists' estimates with those made by professional scientists with or without expertise (untrained) in predation or herbivory assessment. Compared with trained scientists, both schoolchildren and untrained professional scientists overestimated attack rates, but assessments made by the latter were more consistent. Schoolchildren tended to overestimate insect herbivory, as did untrained professional scientists. Raw data acquired by schoolchildren participating in CS programs therefore require several quality checks by trained professional scientists before being used. However, such data are of no less value than data collected by untrained professional scientists. CS with schoolchildren can be a valuable tool for carrying out ecological research, provided that the data itself is acquired by professional scientists from material collected by citizens.

Keywords: artificial prey; citizen science; data quality; insect herbivory; measurement bias; predation; schoolchildren

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Introduction

Scientific knowledge is more accessible than ever before, particularly owing to an increase in open access publications and the outreach activities of scientists worldwide. Still, many topics in life and environmental sciences that are considered settled by scientists are misunderstood by the general public, even among individuals with substantial science literacy and education (Drummond and Fischhoff 2017; Fiske and Dupree 2014; Kahan et al. 2012). Citizen science (CS) programs rely on participation of the

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general public in scientific research in collaboration with or under the direction of professional scientists (European Commission 2013; Haklay 2015). The rapid development of these programs, in addition to vastly increasing available data, offers an unprecedented opportunity to bridge gaps between science and society by engaging the general public with the process of science and increasing motivation for inquiry and interest in scientific topics.

CS programs in the field of ecology can benefit both science and society (Wals et al. 2014). For professional scientists, involving the general public enables the collection of data on broader spatial and temporal scales than would otherwise be possible (i.e., crowdsourcing). This practice has been recognized as a highly effective way to track various biological phenomena (Dickinson et al. 2012; Schwartz, Betancourt, and Weltzin 2012). Typical CS studies in ecology address the effect of environmental factors on biodiversity (e.g., Lucky et al. 2014; Miczajka, Klein and Pufal 2015; Saunders et al. 2018) or climate change impact on plant or animal phenology (Ekholm et al. 2019; Hurlbert et al. 2019; Schwartz, Betancourt, and Weltzin 2012). In turn, volunteers engaged in CS programs can gain recognition for their skills and develop a deeper understanding of scientific concepts and the scientific process (Trumbull et al. 2000). This may positively contribute to both science and environmental education (Wals et al. 2014) and raise awareness of environmental issues. As a result, CS programs are now promoted by major funding agencies in Europe and North America (e.g., European Commission 2013; McLaughlin, Benforado, and Liu 2019).

Engaging schoolchildren and their teachers can enhance the long-term educational and social goals of CS programs for several reasons (Makuch and Aczel 2018). First, school pupils are guided by their instructors when learning about the scientific question raised by the CS program, and about the nature of science and its social aspects (Jenkins 2011; Koomen et al. 2018). Second, exposure to outdoor nature during childhood provides a long-lasting positive relationship with the environment while increasing people's interest and knowledge about nature (Ganzevoort and van den Born 2019; Wells and Lekies 2012). Third, CS programs that involve self-selecting volunteers may underrepresent many social groups—although strategies exist to increase engagement (Pandya 2012)—whereas CS programs that target schoolchildren for CS projects have the potential to engage a wider cross-section of society in science (Jordan et al. 2011).

Nonetheless, the enthusiastic views of win-win interactions through CS programs have been questioned by social scientists and ecologists (Jordan et al. 2011). The former point out that the educational and social impact may be overstated (Brossard, Lewenstein, and Bonney 2005; Kelemen-Finan, Scheuch, and Winter 2018; Riesch and Potter 2014; Scheuch et al. 2018; Trumbull et al. 2000), while the latter are concerned about the accuracy of data collected by the general public (Burgess et al. 2016), especially when schoolchildren are involved. The main reason for these concerns is that CS data are arguably of lower quality than those collected by professional scientists (Burgess et al. 2016; Makuch and Aczel 2018; Riesch and Potter 2014). In response, it has been proposed that data collected by schoolchildren involved in CS programs can contribute to environmental research, provided that research methods are kept simple and require skills that the children already have or are able to gain when mentored by adults (Makuch and Aczel 2018; Miczajka, Klein, and Pufal 2015; Saunders et al. 2018), and the participants receive training, even remotely (Ratnieks et al. 2016). However, only a few studies have directly compared the quality of data acquired by professional scientists versus schoolchildren (Miczajka, Klein, and Pufal 2015; Pocock and Evans 2014; Saunders et al. 2018; Steinke et al. 2017). Evidence that CS programs can generate reliable scientific productions are needed to engage scientists with CS.

Here, we report on the preliminary results of the *Oak Bodyguards* CS program which has so far involved schoolchildren and professional scientists from 16 European countries. The project aims to assess the effects of climate on two key biotic interactions occurring widely in natural and anthropogenic ecosystems, i.e., the top-down and bottom-up forces controlling insect herbivory on leaves of the pedunculate oak, *Quercus robur*. This species is one of the most common and emblematic forest trees in Europe (Leroy, Plomion, and Kremer 2019), with a geographic range spanning more than 19 degrees of latitude. Furthermore, it is also widespread in natural, rural, suburban, and urban environments. In this project, schoolchildren and professional scientists placed dummy plasticine caterpillars in oak trees to estimate attack rates (Lövei and Ferrante 2017; Mäntylä et al. 2008; Roslin et al. 2017). We

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assessed the accuracy of CS data by comparing attack rate and insect herbivory estimates by three types of observers: professional scientists with previous experience in the project methodology (henceforth called trained professional scientists), professional scientists with no previous experience in the project methodology (untrained professional scientists), and schoolchildren. We first compared caterpillar attack rate estimates by schoolchildren or untrained professional scientists with those of a single professional scientist (Elena Valdés Correcher, henceforth known as EVC) trained to identify predation marks on artificial larvae. Second, in a separate experiment, schoolchildren and trained and untrained professional scientists estimated leaf insect herbivory from the percentage of leaf area removed or damaged by insect herbivores (Johnson, Bertrand, and Turcotte 2016), and we compared their herbivory estimatesto determine whether schoolchildren were able to conduct an ecological experiment and acquire scientific data of a quality comparable to that acquired by professional scientists. We use the results to discuss risks and opportunities for the future of CS programs with schoolchildren.

Materials and Methods

Oak selection

We designed a simple protocol that was applied by both schoolchildren and trained and untrained professional scientists. The protocol was written by scientists in collaboration with science instructors and communication officers. It was available in French, English, German, Spanish, and Portuguese (Castagneyrol et al. 2019).

In early 2018, 58 teachers with their students and 27 scientists from 16 European countries participated in the project. Each school and scientist selected a minimum of 1 and maximum of 18 mature pedunculate oak trees with lower branches accessible from the ground (schoolchildren: 1 to 8 oak trees, median = 2; scientists: 1 to 18 oak trees, median = 6). We imposed no restrictions on oak tree location, age, or size, but professional scientists were asked to choose oaks in woods larger than 1 ha. All partners measured oak tree circumference at 1.30 m from the ground and recorded oak coordinates with the GPS function of their smartphones.

All partners installed dummy caterpillars on lower branches of their selected oak trees to estimate attack rate, and haphazardly collected fresh leaves from the same trees to estimate insect herbivory. Although most of the schools estimated attack rates, none assessed herbivory. We also set up a complementary experiment to evaluate precision and accuracy of estimating insect herbivory by schoolchildren and professional scientists (see section entitled *Insect herbivory* below).

Attack rate

To control for latitudinal variation in environmental conditions, we matched the start of the experiment to the local phenology of the oak trees. Six weeks after oak budburst, partners installed 20 dummy caterpillars per tree, i.e., five caterpillars on each of four branches (facing north, south, east, and west) with a minimum distance of 15 cm between caterpillars. Caterpillars were made of the same green plasticine (Staedler, Noris Club 8421, green[5]) provided to all partners by the project coordinators (B. Castagneyrol, EVC). To standardize caterpillar size among partners, caterpillars were made from a ball of plasticine of 1 cm diameter, and gently pressed/rolled onto the middle of a 12 cm-long metallic wire until a 3 cm-long caterpillar was obtained. Partners were instructed to attach the caterpillars to branches using wire, and leave the caterpillars on trees for 15 days prior to recording predation marks. Schoolchildren counted predation marks and attributed them to birds, mammals, arthropods, or reptiles. In 2018, they tagged and photographed every caterpillar with the suspected predation marks from any potential predator taxa. To minimise the probability of false negative results, we also advised the schoolchildren to send photographs of marks that were not clearly recognized as predation marks. Photos were taken from three different angles to show the observed damage and were labeled in such a way that the file name indicated both tree and caterpillar ID. Professional scientists were asked to gently remove all caterpillars from the trees and send them back to the project coordinators. One school also returned caterpillars, although this was not requested. A second survey using the same procedure immediately followed the first one. In 2019, both schoolchildren and professional scientists were instructed to send caterpillars back to the project coordinators. Photos and actual caterpillars were used by EVC to double-check and to standardize the predation assessment made by individual partners.

Every partner received a field bite guide containing a collection of photos illustrating predation marks left by different types of predators as well as false positive marks on plasticine surfaces that were made by leaves, buds, or finger nails. The different predator guilds that can be easily identified from their typical marks left on plasticine include passerine birds, rodents, snakes, lizards, and insects—mainly beetles and bush-crickets (Lövei and Ferrante 2017). The bite guide was available online and accessible to all partners through a hyperlink from the protocol (Castagneyrol et al. 2019), and teachers were invited to contact the scientific coordinator or local scientific partners in cases of uncertainty regarding the marks.

All partners were required to record their observations in the same standardized recording form. Partners indicated (*a*) the total number of caterpillars installed; (*b*) the number of caterpillars with any type of predation marks, (*c*) the number of caterpillars without predation marks; and (*d*) the number of caterpillars with predation marks left by birds (typically V-shaped beak marks and holes), arthropods (mandible marks), mammals (parallel teeth marks), or lizards (ellipse-shaped line of small teeth marks). Therefore, the same attacked caterpillar made a minimum of two entries in the recording form. We intentionally asked for redundant information to limit the risk of error in data reporting.

Data and biological material were collected by both schoolchildren and professional scientists during the same time period (from May through July). Project partners filled in the recording form and sent it to the project coordinators with the photos or the caterpillars. A single observer (EVC) with expertise in identifying predation marks on model caterpillars (Valdés-Correcher et al. 2019) screened every photo or caterpillar to verify observations reported by partners. It must be noted that false positives were more likely to be identified from the photos than false negatives. False positives are caterpillars classified by project partners as having been attacked when they were not. Because of previous reports (Low et al. 2014) and our own experience with undergraduate students trained to identify predation marks on artificial prey, we anticipated that schoolchildren and their teachers would be overly enthusiastic, making false positives more likely than false negatives. Schoolchildren were instructed to take photos of caterpillars with suspected predation marks, even marks they could not attribute to any predator type. It is therefore possible that they did not notice real predation marks on caterpillars that were photographed because they had marks left by buds, leaves, or finger nails. Such cases would represent false negatives. The probability of detecting false negative was not an issue when project partners returned caterpillars to the project coordinators.

For each oak tree and survey period, we assessed attack rate as the proportion of dummy caterpillars with at least one predation mark. Although we asked partners to record predation marks left by different types of predators (in particular birds and arthropods), this level of precision could not be reached on photos because of low resolution. Therefore, we quantified overall attack rate, regardless of predator type.

We estimated the precision and accuracy of attack-rate assessments by schoolchildren and untrained professional scientists by running two separate linear mixed-effect models with attack rate estimated by schoolchildren or professional scientists as a dependent variable, attack rate estimated by a single trained professional scientist and year (as factor) as independent variables, and Partner ID and Tree ID nested within Partner ID as random factors (Johnson, Bertrand, and Turcotte 2016). From each regression, we quantified the bias (a deviation between attack rate estimated by partners and a single trained observer) as the intercept (β_0). Positive deviation from $\beta_0 = 0$ indicates an overestimation of attack rate by partners. We quantified accuracy as the regression slope (β_1), where $\beta_1 = 1$ indicates high accuracy and $\beta_1 \neq 1$ indicates that accuracy in attack-rate assessment varied with actual attack rate. We used parametric bootstrapping with 1,000 simulations to compute 95% confidence interval (CI) around β_0 and β_1 and estimate how they deviated from 0 and 1, respectively. The null hypotheses were that $\beta_0 = 0$ and $\beta_1 = 1$. We considered that the null hypothesis was rejected if the 95% CI did not bracket zero or one. The significance of the fixed effect of year was tested based on the *F*-distribution and estimating degrees of freedom with Kenward-Roger methods (Kuznetsova, Brockhoff, and Christensen 2017).

Insect herbivory

To compare insect herbivory estimated by schoolchildren versus trained and untrained professional scientists, we set up a complementary survey (administered by AB). In April 2019, we prepared 12 sets of 5 oak leaves randomly drawn from a large sample of oak leaves collected in September 2018 on 162 oak trees around Bordeaux city (SW France) and stored in paper bags at –18°C. For each set of leaves, five trained professional scientists with previous experience in scoring insect herbivory on oak leaves (BC, EVC, AB, TD, and YK [see acknowledgements]) estimated insect herbivory as the percentage of leaf area removed or impacted by insect herbivores by giving each individual leaf a damage score: (0: 0%, A: 1–5%, B: 6–15%, C: 16–25%, D: 26–0%, E: 51–75%, F: > 75%; Castagneyrol et al. 2013). To reduce variability in estimates of herbivory due to observers, we created digital model leaves with given amounts of simulated herbivore damage that were used as examples for the seven damage classes (Castagneyrol et al. 2019). Leaf chewers were the main source of insect herbivory on oak leaves, but because leaves were drawn at random from a large pool of leaves, some were attacked by leaf miners, although none had galls. We asked participants to score total insect herbivory, regardless of damaging agents. As a result, the damage score incorporated leaf area removed by chewers as well as covered by leaf mines.

We invited schoolchildren 11 to16 years old (and their teachers) from six local secondary schools (equivalent US grades 6–10) to visit the first author's research facilities (INRA research station of Pierroton, Bordeaux, France). Five groups of 10 to 12 students were introduced to the study of insect herbivory by the survey administrator, who challenged them to score insect herbivory as accurately as professional scientists would do. Students worked in groups of 2 or 3, with a total of 24 student groups. Each group was given 3 sets of 5 leaves, selected at random from the pool of 12 leaf sets. All students scored damage using the same digital model leaves as a template. In total, each of the 12 leaf sets was processed by six independent groups of students.

The same day (or the day after), we invited INRA permanent and non-permanent staff members to participate in the survey. The volunteers were researchers, engineers, technicians, and Master of Science students. They were considered untrained professional scientists). They received the same information from the survey administrator as secondary school students and used the same templates to score herbivory. Each of the nine volunteers processed every set of five leaves.

We did not keep records of individual leaves and we therefore averaged herbivory estimates across leaves for each set. We first tested whether individuals with a different background differed in their estimation of insect herbivory by running Linear Mixed-effects Models (LMM) with (log-transformed) insect herbivory as a response variable, observer type (*Observer*) as a fixed-effect factor, and leaf-set identity and observer identity as random effect factors. Because repeated handling of the same leaves may have caused some breakage, leading to a progressively increased estimation of herbivory, we added *Time* (number of hours since the first assessment) and *Time* × *Observer* interactions as additional fixed effects in the model. The model equation was

$$Herbivory_{ijk} = \beta_0 + \beta_1 \times Observer_{Trained} + \beta_2 \times Observer_{Untrained} + \beta_3 \times Time + \beta_4 \times Time \times Observer_{Trained} + \beta_5 \times Time \times Observer_{Untrained} + \gamma_j + \delta_k + \varepsilon_{ijk}$$

where β_0 was the model intercept (i.e., $Observer_{schoolchildren}$), β_1 and β_2 were the coefficients of the fixed effects of the treatment for trained ($Observer_{Trained}$) and untrained professional scientists ($Observer_{Untrained}$), β_3 was the effect of *Time*, β_4 and β_5 were the effects of the *Time* × *Observer* interaction, γ_j and δ_k were the random intercepts for the observer and leaf-set identities, and ε_{ijk} were the residuals. For γ_j, δ_k and ε_{ijk}, we assumed a normal distribution with zero mean and variance σ^2_γ, σ^2_δ, and σ^2_ε, respectively. σ^2_ε contained variation among observers in scoring different leaf sets, i.e., the *Observer* × *Leaf set* interaction, but also all other noise. The significance of fixed effects was tested based on the *F*-distribution and estimating degrees of freedom with Kenward-Roger methods (Kuznetsova, Brockhoff, and Christensen 2017).

Second, we used σ^2_δ to quantify consistency among observers in rating herbivory. To do so, we ran an intercept only LMM for each group separately (i.e., for students and for trained and untrained professional scientists) and calculated intraclass correlation (ICC) for the *Leaf set* random factor ($\sigma^2_\delta/(\sigma^2_\delta + \sigma^2_\gamma + \sigma^2_\varepsilon)$). ICC represents the proportion of the total variance that is explained by *Leaf set* identity. It is a metric commonly used to estimate repeatability (Nakagawa and Schielzeth 2010). The greater the ICC, the greater rating consistency among observers scoring the same leaf set. We used parametric bootstrap with 1,000 random draws to estimate ICC 95% CI.

All analyses were done in R (R Core Team 2018) using packages *lmerTest* and *car* (Fox et al. 2016; Kuznetsova, Brockhoff, and Christensen 2015).

Results

Attack rate

In total, 7,338 dummy caterpillars were installed on 195 oak trees by 58 schools and 27 scientists. Schools and scientists' data came from from 8 and 14 countries throughout Europe, respectively (**Figure 1**). Schoolchildren installed and returned 3,289 dummy caterpillars. They counted 1,802 of them as attacked by predators (i.e., 55%), whereas EVC counted only 868 caterpillars with predation marks (26%). Professional scientists installed 4,045 caterpillars, 1,629 of which they identified as attacked by predators (40%); EVC counted 1,338 of these caterpillars as attacked by predators (33%).

Attack-rate estimates by schoolchildren were more biased (intercept estimate ± 95% bootstrap CI: $\beta_0 = 40.63 \pm [22.45, 59.27]$) than those by professional scientists ($\beta_0 = 23.41 \pm [13.11, 33.17]$). Detailed examination of pairwise comparisons at the tree level reveals that 81.5% of assessments made by schoolchildren were above the 1:1 line (**Figure 2**), thus indicating overestimation of attack rate as compared with assessments made by a single trained observer.

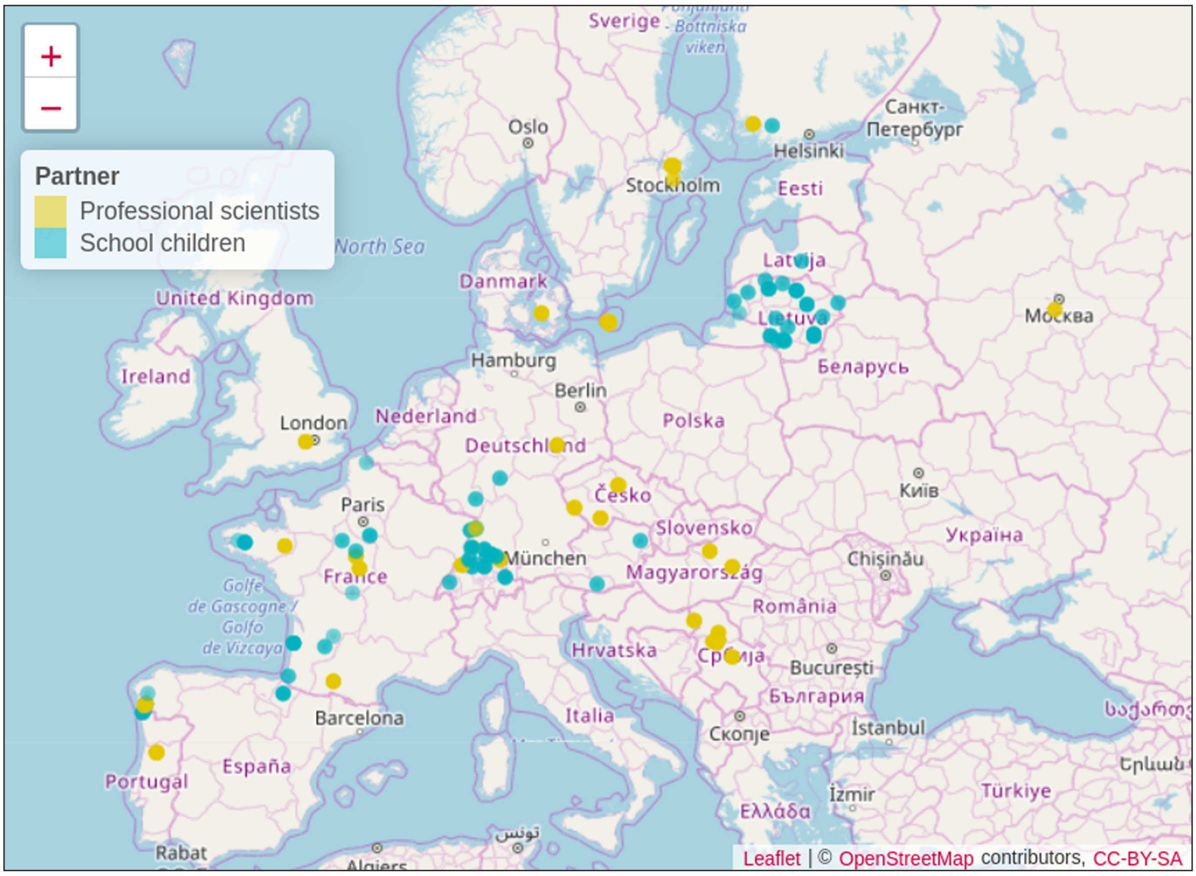

Figure 1: Location of oak trees included in the study. An interactive version of this map can be found in the Supplemental File as Figure 1.

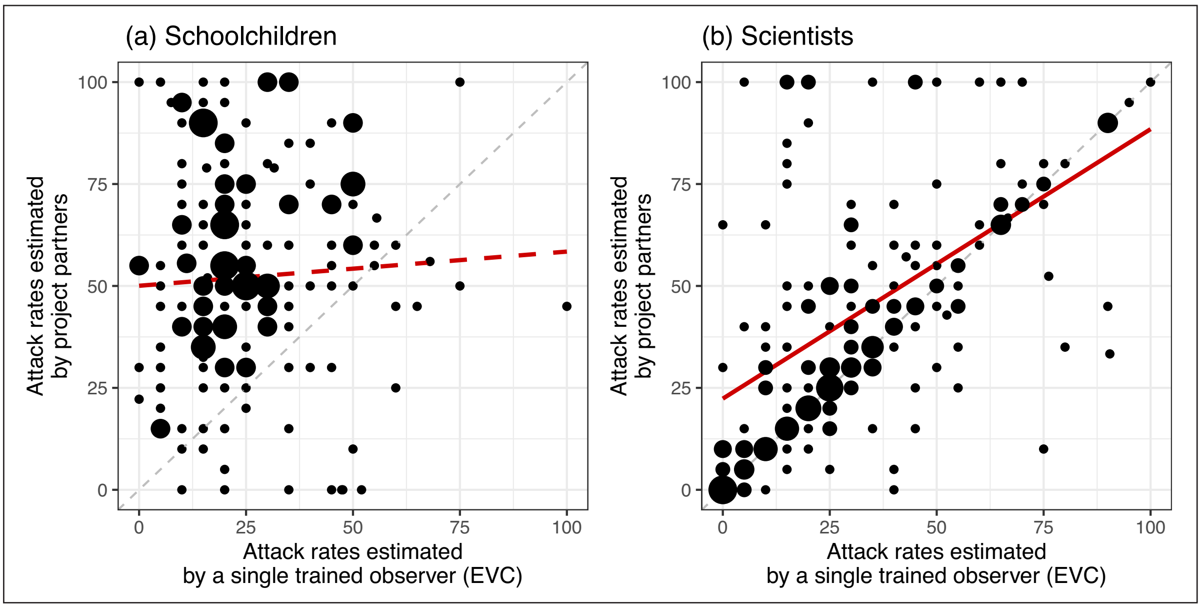

Figure 2: Precision and accuracy of school children (a) and professional scientists (b) in assessing attack rate (% artificial larvae with predation marks). Dots represent attack rate aggregated at the level of oak trees for each survey separately. Dot size is proportional to the number of overlapping dots. Dashed lines indicate a 1:1 relation. In Panel *a*, the thick dashed red line represents the non-significant regression line ($y = 0.08 \cdot x + 50.32$, marginal R^2: $R_m^2 < 0.01$, conditional R^2: $R_c^2 = 0.66$). In Panel *b*, the bold red line represents the significant regression line ($y = 0.66 \cdot x + 23.41$, $R_m^2 = 0.31$, $R_c^2 = 0.78$). EVC, Elena Valdés Correcher (a single professional scientist trained to identify predation marks on artificial larvae).

There was no relationship between attack rates estimated by schoolchildren versus a single trained observer (slope estimate ± 95% bootstrap 95% CI: $\beta_1 = 0.43 \pm [-0.02, 0.90]$), whereas professional scientists made more accurate assessments ($\beta_1 = 0.66 \pm [0.54, 0.77]$, **Figure 2**). Attack rates estimated by schoolchildren and professional scientists did not differ between years ($F_{1,\ 53.9} < 0.01$, $P = 0.952$ and $F_{1,\ 23.5} = 0.22$, $P = 0.644$, respectively).

Insect herbivory

Insect herbivory estimates by trained professional scientists were the lowest (mean ± SE = 9.00% ± 0.51%, range 2.20% to 19.6%) (**Figure 3**; Figure S1 in the Supplemental File), whereas insect herbivory estimates by untrained professional scientists were the highest (14.65% ± 1.01%, range from 3.80% to 62.00%) (**Figure 3**; Figure S1 in the Supplemental File). Schoolchildren estimates of insect herbivory were intermediate (11.55% ± 0.64%, range from 2.20% to 27.40%) (**Figure 3**; Figure S1 in the Supplemental File). Both untrained professional scientists and schoolchildren consistently overestimated insect herbivory compared wiht trained professional scientists (Figure S1 in the Supplemental File), but this effect was not statistically significant at $\alpha = 0.05$ ($F_{2,31.9} = 2.79$, $P = 0.076$) (**Figure 3**). Herbivory did not vary significantly with time (*Time*: $F_{1,\ 28.5} < 0.01$, $P = 0.954$; *Time* × *Observer*: $F_{2,\ 33.0} = 0.62$, $P = 0.544$).

Interestingly, ICC revealed that the consistency of herbivory estimates was comparable between trained professional scientists (ICC ± 95% CI: 0.58 ± [0.31, 0.84]) and schoolchildren (0.54 ± [0.22, 0.76]), whereas estimates made by untrained professional scientists were less consistent (0.44 ± [0.13, 0.67]).

Discussion

Our comparison of data collected by different audiences (schoolchildren, untrained scientists, and trained scientists) allowed us to examine the quality of ecological data collected by schoolchildren, and to suggest improvements for future CS programs.

Can schoolchildren collect data of sufficient quality for ecological research?

The main strength of CS programs, from a research perspective, is the collection power achieved by volunteers (especially if the data are independently verified). However, our findings proved ambiguous with respect to whether the resulting data are of sufficient quality to yield scientifically robust results. On the one hand, we clearly show that schoolchildren overestimated attack rate compared with trained professional scientists (**Figure 2**). They also tended to overestimate insect herbivory, but this effect was not significant at the common $\alpha = 0.05$ threshold (**Figure 4**). On the other hand, professional scientists with mixed expertise in these fields also tended to overestimate attack rate and insect herbivory (**Figures 2** and **3**).

Importantly for the interpretability of the data, overestimation of attack rates was consistent across schools, as overestimation occurred in 81% of observations. Attack rates as assessed by professional scientists were, on average, slightly higher than attack rates re-estimated by a single trained observer. However, pairwise comparisons revealed that over- and underestimation of attack rates were more balanced in this group. In sharp contrast, we also found schoolchildren assessed insect herbivory in a more consistent way than untrained professional scientists did. Collectively, our results indicate that data

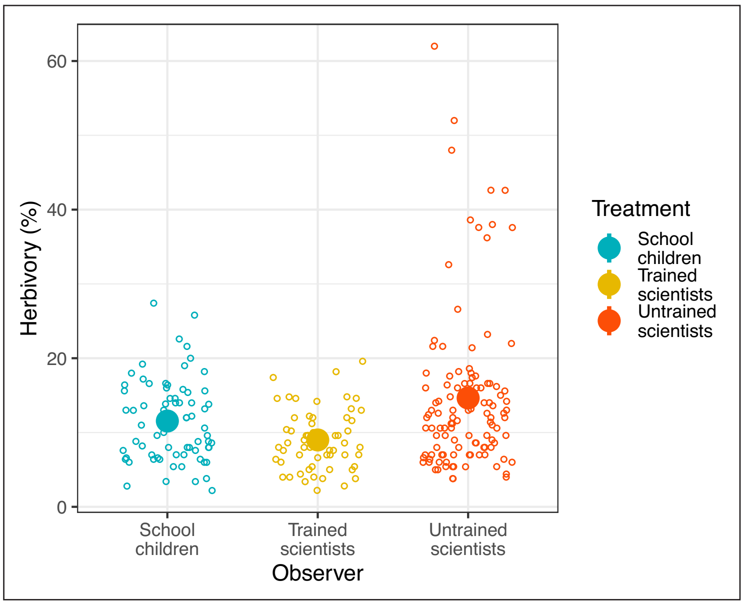

Figure 3: Comparisons between insect herbivory as estimated by school children, trained scientists, and untrained professional scientists. Empty dots represent individual observations (i.e., a single assessment on a particular leaf set). Filled circles and vertical bars represent means ± SE of the raw data.

provided by schoolchildren should be considered with caution, but the same holds true for data provided by untrained professional scientists.

Why did (so) many schools overestimate attack rate?

Overestimation principally arose from partners scoring scratch marks left by contact with buds or leaves as signs of predation (**Figure 4**). Other sources of overestimation of predation cannot be ignored. Although no teachers mentioned vandalism of experiments, researchers should be aware of this possibility, particularly when caterpillars are placed on trees in urban environments. This may lead to missing caterpillars falsely scored as attacked. In addition, schoolchildren were told by teachers that the aim of the study was to determine "who protects oaks" against herbivores. It is possible that schoolchildren (and their teachers too) felt they had to see predation marks because this is what they perceived as the aim of the experiment. However, although confirmation bias is more likely to occur in schoolchildren and their teachers, it is important to stress that this type of cognitive bias is also common among trained professional scientists who may have interpreted small cracks (for example) on the caterpillar surface as predation marks (Forstmeier, Wagenmakers, and Parker 2017; Zvereva and Kozlov 2019).

Although the protocol clearly specified how to standardize caterpillar size and shape, and emphasized the importance of standardization, we noticed that the dimensions of dummy caterpillars varied widely, both within and among schools. In other studies, the probability of detecting predation marks left by avian or arthropod predators was found to be influenced by the length and width of artificial caterpillars (Lövei and Ferrante 2017). It is unlikely that variability in the dimension of artificial caterpillars has affected the comparison of attack rate as estimated by schoolchildren versus trained observers. However, the variation found should be regarded as a potential source of bias in large-scale multi-partner studies. As a potential mitigation procedure, researchers can provide pre-made caterpillars to project partners (Roslin et al. 2017). That said, making caterpillars according to a standard protocol is also an important dimension of student training. Despite potential biases in data collection, the pedagogical aspects of citizen science programs at schools must not be neglected, and scientists must recognize trade-offs between scientific and pedagogic objectives when planning mitigation procedures. As a compromise, scientists could provide partners with a reference caterpillar made of hardened undeformable clay. 3D-printed models of caterpillars attacked by different predator types may also be included as examples. In any case, we advise that project partners be instructed to carefully pack caterpillars when sending these to lead scientists for calibration of predation assessment. We also recommend that data collected by schoolchildren are not directly used in the project—their value lays in the pedagogical outcomes—but that trained professional scientists use their own scoring on the material provided by schoolchildren.

Schoolchildren scored insect herbivory in a more consistent way than untrained professional scientists did

Johnson et al. (2016) found that bias in herbivory assessment decreased with the number of years of experience in herbivory assessment. Assuming that being trained as a scientist increases accuracy and the sense of rigor, we expected that herbivory would have been scored more accurately by untrained professional scientists than by schoolchildren. Our findings do not support this prediction. Although both schoolchildren and untrained professional scientists ranked the different leaf sets in the same order, for a given leaf set, schoolchildren always overestimated herbivory compared with trained professional scientists, with only one exception (Figure S1 in the Supplemental File), and untrained professional scientists always overestimated herbivory compared with schoolchildren, with only one exception (Figure S1 in the Supplemental File). However, this tendency was not statistically clear (**Figure 3**).

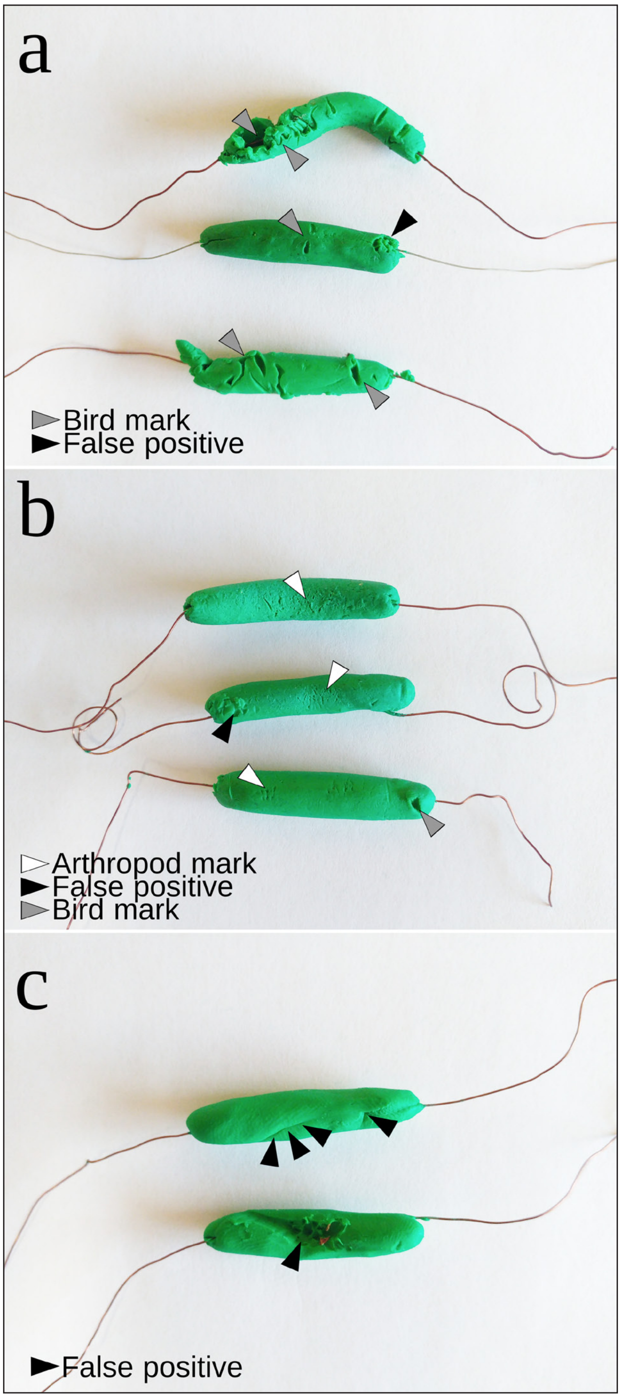

Figure 4: Examples of real and false-positive observations of predation. (a) Grey arrows point to typical bird predation marks. The black arrow points toward marks made by the wire when attaching the caterpillar on the branch and taking it off. **(b)** White and grey arrows indicate marks made by arthropod mandibles and bird beaks, respectively. **(c)** Black arrows indicate typical marks erroneously counted as predation marks by school children. The scar-like mark on the top caterpillar was made when rolling the caterpillar onto the wire. Deep marks on the bottom caterpillar are imprints of branches and buds.

Interestingly, both schoolchildren and trained professional scientists assessed herbivory in a more consistent way than untrained professional scientists did, as revealed by the greater ICC in estimates. Individuals may vary in their observational skills, but training likely reduces this variability. Schoolchildren formed groups of 2 to 3 participants, while untrained professional scientists were alone when estimating herbivory. It is possible that within-group discussion leveled out intrinsic variability in observational skills and therefore variability of estimates made by schoolchildren. An alternative explanation for this unexpected finding is that schoolchildren took the activity more seriously than untrained professional scientists did. Regardless of the cause, these results stress that schoolchildren are no less reliable than untrained professional scientists when it comes to estimating insect herbivory (on oak leaves).

How can we make data collected by schoolchildren more reliable?

CS programs can help to generate a large amount of data, but the quality has been questioned, especially when these big data are not based on standard protocols (Bayraktarov et al. 2019; Burgess et al. 2016). Few studies have evaluated the quality of data collected by schoolchildren participating in CS programs (Miczajka, Klein and Pufal 2015; Saunders et al. 2018; Steinke et al. 2017). It emerges from these studies that schoolchildren can actually provide data accurate enough to support ecological research, provided that the tasks they are requested to undertake are adapted to their skills and that they receive proper training (Miczajka, Klein and Pufal 2015; Ratnieks et al. 2016; Saunders et al. 2018). Although we could not provide face-to-face training sessions for every school partner involved in the *Oak Bodyguards* project, the project methodology was simple and based on a detailed protocol. Nonetheless, this simplicity did not suffice to guarantee unbiased data, as illustrated by the fact that schoolchildren consistently overestimated attack rates. We therefore emphasize that CS programs relying on data collected by schoolchildren should include several checks of data quality and appropriate mitigation procedures. In particular, training sessions undertaken face-to-face or at least remotely must be planned before data collection (Ratnieks et al. 2016). Finally, whenever possible, the researcher analyzing the data should recover the raw material collected by children, or at the very least access pictures that allow for the re-assessment of measurements (Ekholm et al. 2019; Steinke et al. 2017). Importantly, these recommendations also hold true for large multi-partner research programs, as we also detected bias in data collected by professional scientists (Zvereva and Kozlov 2019). Whether variability in observations made by schoolchildren is random or can be modelled using appropriate covariates is an important question deserving further attention.

Conclusion

We found that schoolchildren involved in CS programs can support ecological research, but only if their contributions are considered with caution. The acquisition of reliable data requires experimental procedures that are easy to implement, but even so, a measurement of interpretation bias seems essential. Several quality checks and curation procedures are needed prior to using data collected by schoolchildren for ecological research. Unexpectedly, we found that such checks are necessary even for data acquired by professional scientists. It must be kept in mind that thrill, motivation, and self-confidence are keys to schoolchildren engagement with science and with practical scientific activities (Ganzevoort and van den Born 2019; Ruiz-Mallen et al. 2016). Our findings that schoolchildren did no worse than untrained professional scientists in collecting ecological data (here, in estimating insect herbivory) can strengthen their confidence and help them gain motivation and a positive attitude toward science in general. Despite legitimate concerns about the quality of data acquired by schoolchildren, following a protocol, collecting and formatting data, and sharing the process with scientists are valuable parts of training schoolchildren in scientific literacy. The trade-off between positive learning outcomes and the quality of raw data cannot be ignored, but with appropriate data quality checks and curation procedures, it actually favors the implementation of CS programs at school.

Data Accessibility Statement

Raw data used in the present article are available in the Supplemental File.

Supplementary File

The supplementary file for this article can be found as follows:

- **Supplemental File.** Article Preprint with Supplemental Figures and Raw Data. DOI: https://doi.org/10.5334/cstp.267.S1

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Competing Interests

The authors have no competing interests to declare.

Author Contributions

BC conceived the study, analyzed the data, and led the writing. BC and EVC coordinated the research, with help from DH, MB, MKD, MMG, MSL and RT. EVC acquired and formatted the data. All authors contributed data, critically commented, and edited the manuscript.

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NATHALIE VESSILLIER
IN COLLABORATION WITH
INRA NOUVELLE-AQUITAINE BORDEAUX

OAK BODYGUARDS

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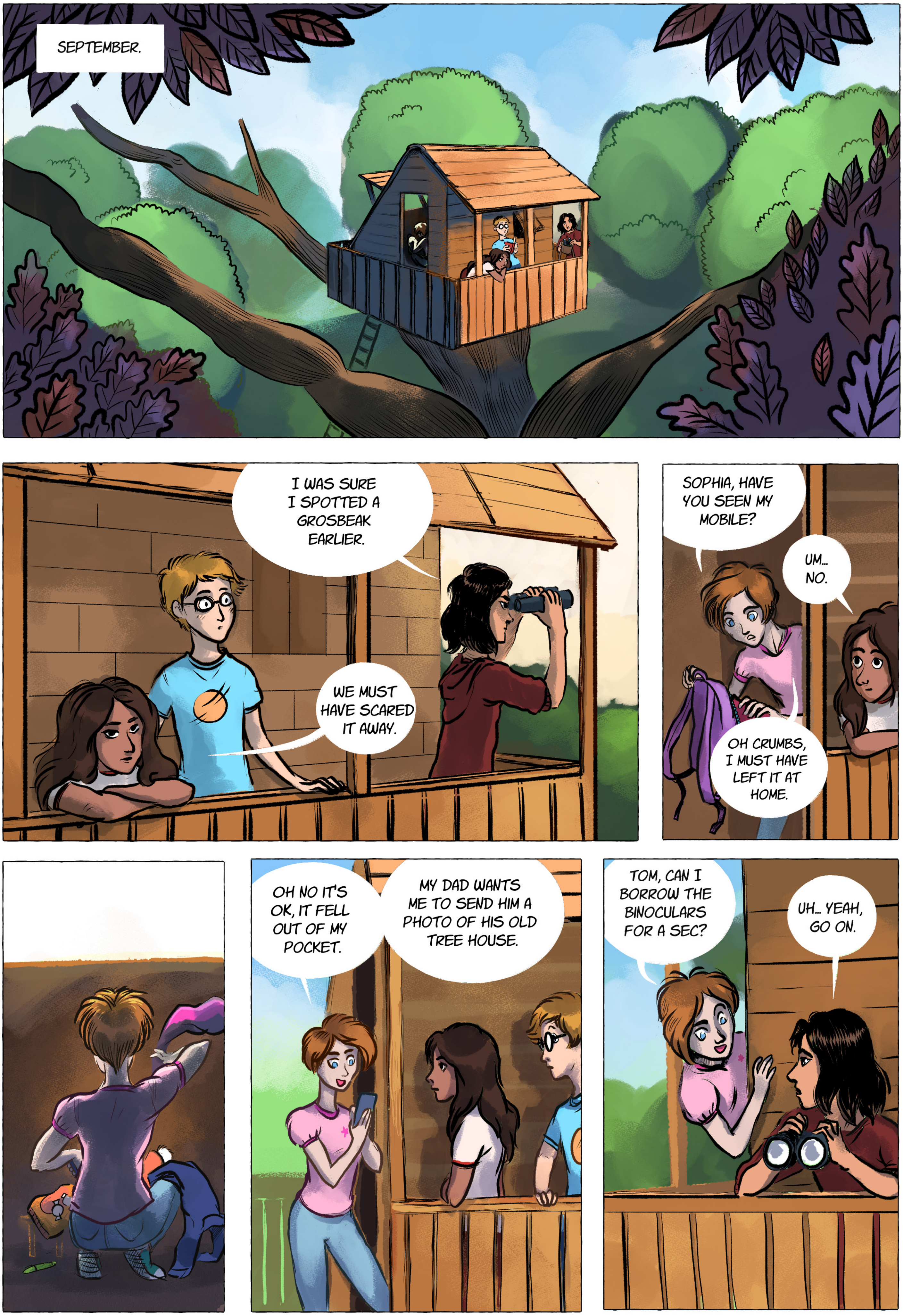
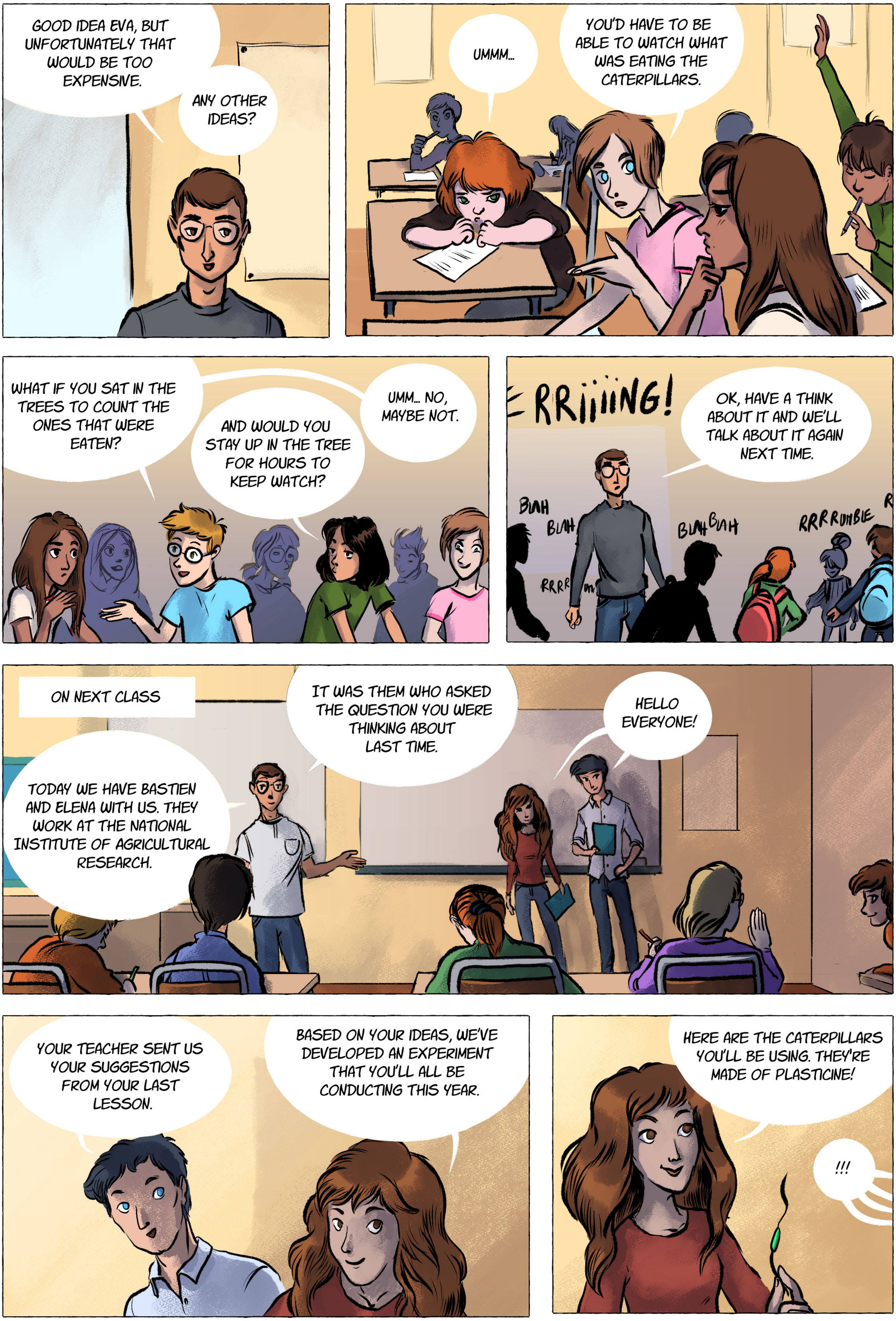

SEPTEMBER.
I WAS SURE I SPOTTED A GROSBEAK EARLIER.
WE MUST HAVE SCARED IT AWAY.
SOPHIA, HAVE YOU SEEN MY MOBILE?
UM... NO.
OH CRUMBS, I MUST HAVE LEFT IT AT HOME.
OH NO IT'S OK, IT FELL OUT OF MY POCKET.
MY DAD WANTS ME TO SEND HIM A PHOTO OF HIS OLD TREE HOUSE.
TOM, CAN I BORROW THE BINOCULARS FOR A SEC?
UH... YEAH, GO ON.


EVA! DON'T LEAN SO FAR OVER!
I'M TOO FAR AWAY, I CAN'T SEE ANYTHING!
PFFT... I CAN'T BELIEVE THE SUMMER HOLIDAYS ARE OVER ALREADY. I DON'T KNOW IF I'M READY FOR A NEW SCHOOL YEAR.
COME OOOON, IT'S GOING TO BE FINE.
OK, I'M GOING DOWN.
IT WOULD BE GOOD IF WE WERE ALL IN THE SAME CLASS THIS YEAR.
WE COULD MAKE PLANS TO WORK ON THE TREE HOUSE.
DO YOU THINK WE'LL HAVE TIME BEFORE WINTER? WHAT WITH SCHOOL STARTING AND HOMEWORK?
DON'T WORRY THEO. LET'S SEE WHAT HAPPENS.
ALRIGHT. DONE!
SEE YOU ON MONDAY!
BYE!
HAVE A NICE EVENING!

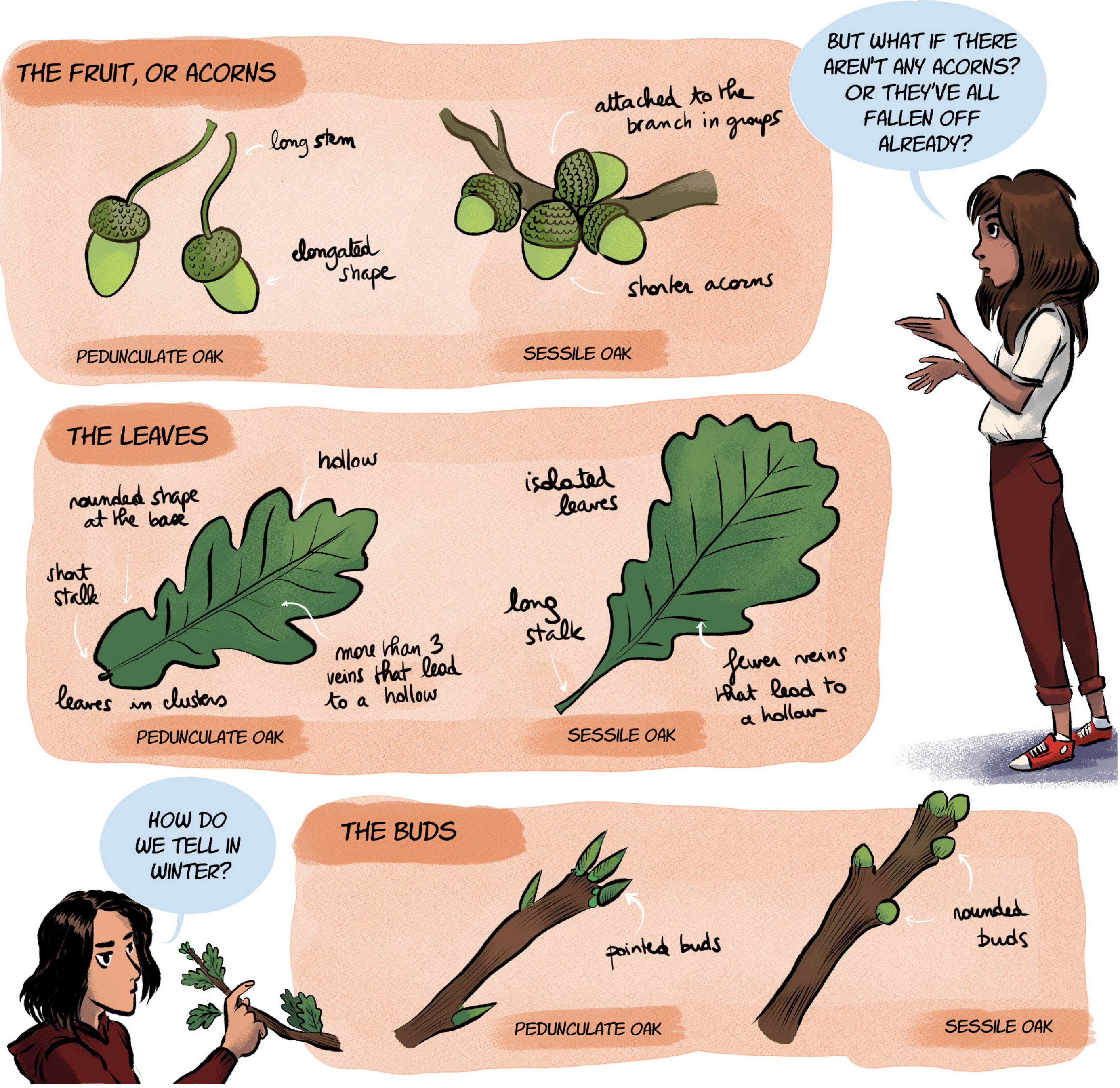

A FEW DAYS LATER
CAROLINE HERSCHEL SCHOOL
COME IN AND SIT DOWN QUICKLY AND QUIETLY.
TODAY WE'RE GOING TO LOOK AT AN ENVIRONMENTAL PROBLEM.
THIS IS AN OAK LEAF FROM THE PARK BEHIND THE SCHOOL.
WHAT DO YOU THINK AFFECTS HOW MANY TREE LEAVES ARE EATEN BY CATERPILLARS?
YES, SOPHIA?
THERE ARE MORE OR LESS CATERPILLARS DEPENDING OF WARM OR COLD THE WEATHER IS.
YES, THAT COULD BE ONE EXPLANATION.
THERE MIGHT BE LOTS OF BIRDS IN THE AREA WHICH EAT THE CATERPILLARS?
YES, THAT'S AN IDEA. HOW COULD YOU TEST YOUR THEORY?
UMMM...
YOU COULD INSTALL SURVEILLANCE CAMERAS!

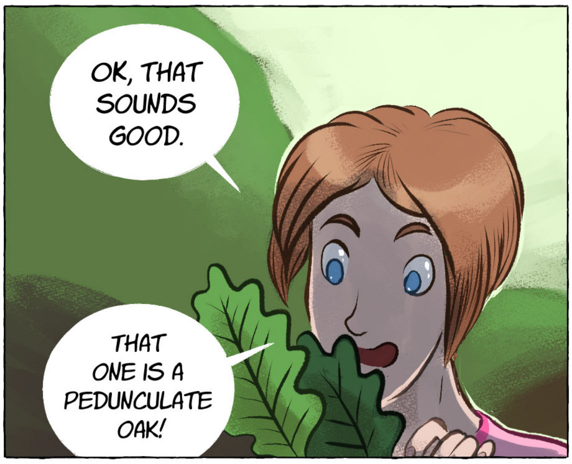

GOOD IDEA EVA, BUT UNFORTUNATELY THAT WOULD BE TOO EXPENSIVE.
ANY OTHER IDEAS?
UMMM...
YOU'D HAVE TO BE ABLE TO WATCH WHAT WAS EATING THE CATERPILLARS.
WHAT IF YOU SAT IN THE TREES TO COUNT THE ONES THAT WERE EATEN?
AND WOULD YOU STAY UP IN THE TREE FOR HOURS TO KEEP WATCH?
UMM... NO, MAYBE NOT.
RRiiiiNG!
OK, HAVE A THINK ABOUT IT AND WE'LL TALK ABOUT IT AGAIN NEXT TIME.
BLAH
BLAH
BLAH BLAH
RRRRUMBLE
ON NEXT CLASS
TODAY WE HAVE BASTIEN AND ELENA WITH US. THEY WORK AT THE NATIONAL INSTITUTE OF AGRICULTURAL RESEARCH.
IT WAS THEM WHO ASKED THE QUESTION YOU WERE THINKING ABOUT LAST TIME.
HELLO EVERYONE!
YOUR TEACHER SENT US YOUR SUGGESTIONS FROM YOUR LAST LESSON.
BASED ON YOUR IDEAS, WE'VE DEVELOPED AN EXPERIMENT THAT YOU'LL ALL BE CONDUCTING THIS YEAR.
HERE ARE THE CATERPILLARS YOU'LL BE USING. THEY'RE MADE OF PLASTICINE!
!!!

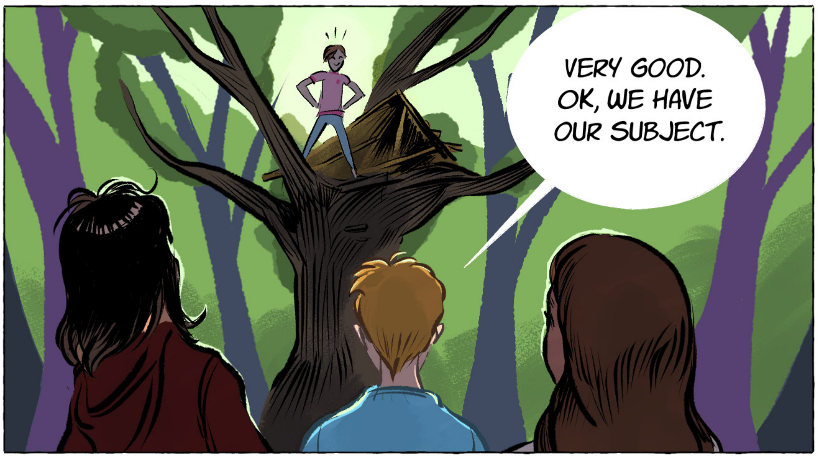

BUT THE BIRDS WON'T EAT THEM!
NO, BUT THEY'LL TRY TO. BIRDS, SPIDERS AND OTHER PREDATORS THAT EAT CATERPILLARS WILL LEAVE MARKS IN THE PLASTICINE.
WE'LL BE ABLE TO SEE IF THERE ARE LOTS OF PREDATORS IN THE AREA THANKS TO THE NUMBER OF MARKS THEY LEAVE.
YOU CAN WRITE INFORMATION DOWN IN YOUR FIELD NOTEBOOKS.
chenille 12
Traces de ma
lézard ?
1 cm
WE'LL GIVE YOU EVERYTHING YOU NEED FOR THE EXPERIMENT.
IN CLASS WE'LL LOOK AT HOW TO PUT YOUR FAKE CATERPILLARS IN THE TREES AND HOW TO RECOGNISE PREDATORS' MARKS.
TO GIVE THE CATERPILLARS AND LEAVES TIME TO GROW, WE WILL SET UP THE EXPERIMENT IN MAY. YOU'VE GOT SOME TIME.
WE'LL PUT THE CATERPILLARS IN PEDUNCULATE OAK TREES. WE'VE GIVEN YOU A GUIDE TO HELP YOU LEARN THE DIFFERENCE BETWEEN PEDUNCULATE OAKS AND SESSILE OAKS.
WE CHOSE THIS SPECIES AS IT CAN BE FOUND THROUGHOUT EUROPE, AND WE'LL BE WORKING WITH CLASSES IN A FEW DIFFERENT COUNTRIES.
THEY'LL ALL PUT THEIR CATERPILLARS IN PEDUNCULATE OAKS.
PFFFFF...
HAHA! IT CANNOT BE THAT BIG.
SHHH!
START LOOKING AT THE OAK TREES AROUND YOU NOW.


THIS IS A FUNNY EXPERIMENT, IT'S DIFFERENT FROM OUR NORMAL LAB CLASSES.
YEAH, IT DOESN'T SEEM VERY SCIENTIFIC.
I FEEL LIKE I'M BACK IN PRIMARY SCHOOL WITH THIS THING.
EURÊKA!
IT'S FUNNY, I DIDN'T THINK SCIENTISTS WERE LIKE THAT.
SAME HERE. I THOUGHT THEY WOULD BE... OLDER.
WE'RE GOING TO GET REALLY GOOD MARKS FOR THIS PROJECT!
I'VE ALREADY GOT A FEW IDEAS FOR TREES TO PUT OUR CATERPILLARS IN.
DO YOU THINK OUR TREE IS PEDUNCULATE OR SESSILE?
NO IDEA.
SESSILE.
AWW, THAT WOULD'VE BEEN GREAT.
OKAY THEO, CAN YOU SHOW US YOUR HIGH-SCORING TREES?
OVER HERE!
OH YEAH, IT'S THE TREE MY DAD'S TREE HOUSE IS IN!
I REMEMBER LOOKING FOR ACORNS HERE A FEW MONTHS AGO FOR ANOTHER PIECE OF HOMEWORK.


REMIND ME HOW WE CAN TELL IF IT'S A PEDUNCULATE OAK?
HÉHÉ
I'VE GOT THE NOTE HERE. HERE'S THE LIST OF THE DIFFERENCES BETWEEN PEDUNCULATE AND SESSILE OAKS.

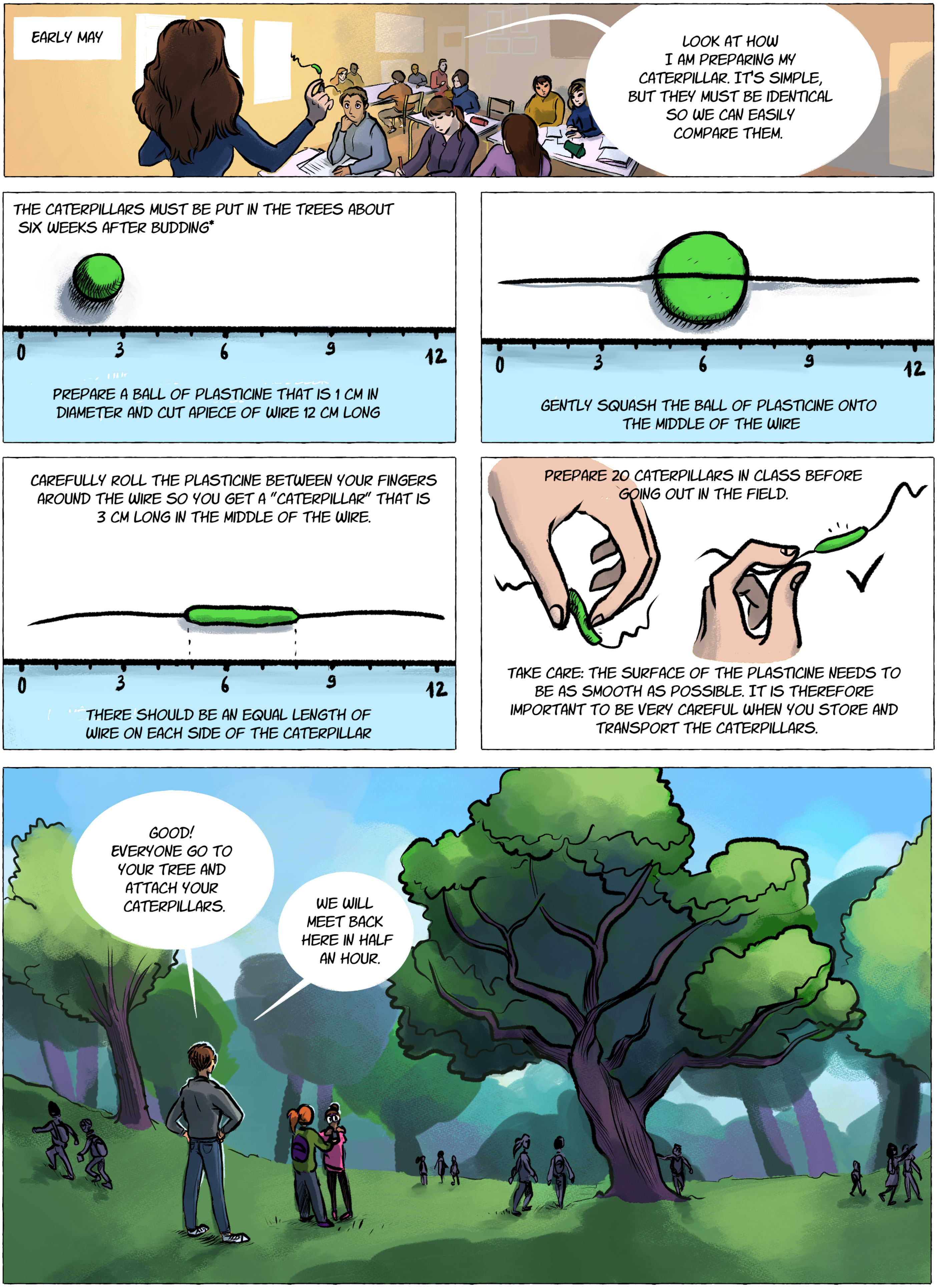

THE FRUIT, OR ACORNS
long stem
elongated shape
PEDUNCULATE OAK
attached to the branch in groups
shorter acorns
SESSILE OAK
BUT WHAT IF THERE AREN'T ANY ACORNS? OR THEY'VE ALL FALLEN OFF ALREADY?
THE LEAVES
rounded shape at the base
hollow
short stalk
more than 3 veins that lead to a hollow
leaves in clusters
PEDUNCULATE OAK
isolated leaves
long stalk
fewer veins that lead to a hollow
SESSILE OAK
HOW DO WE TELL IN WINTER?
THE BUDS
pointed buds
PEDUNCULATE OAK
rounded buds
SESSILE OAK


OK, THAT SOUNDS GOOD.
THAT ONE IS A PEDUNCULATE OAK!

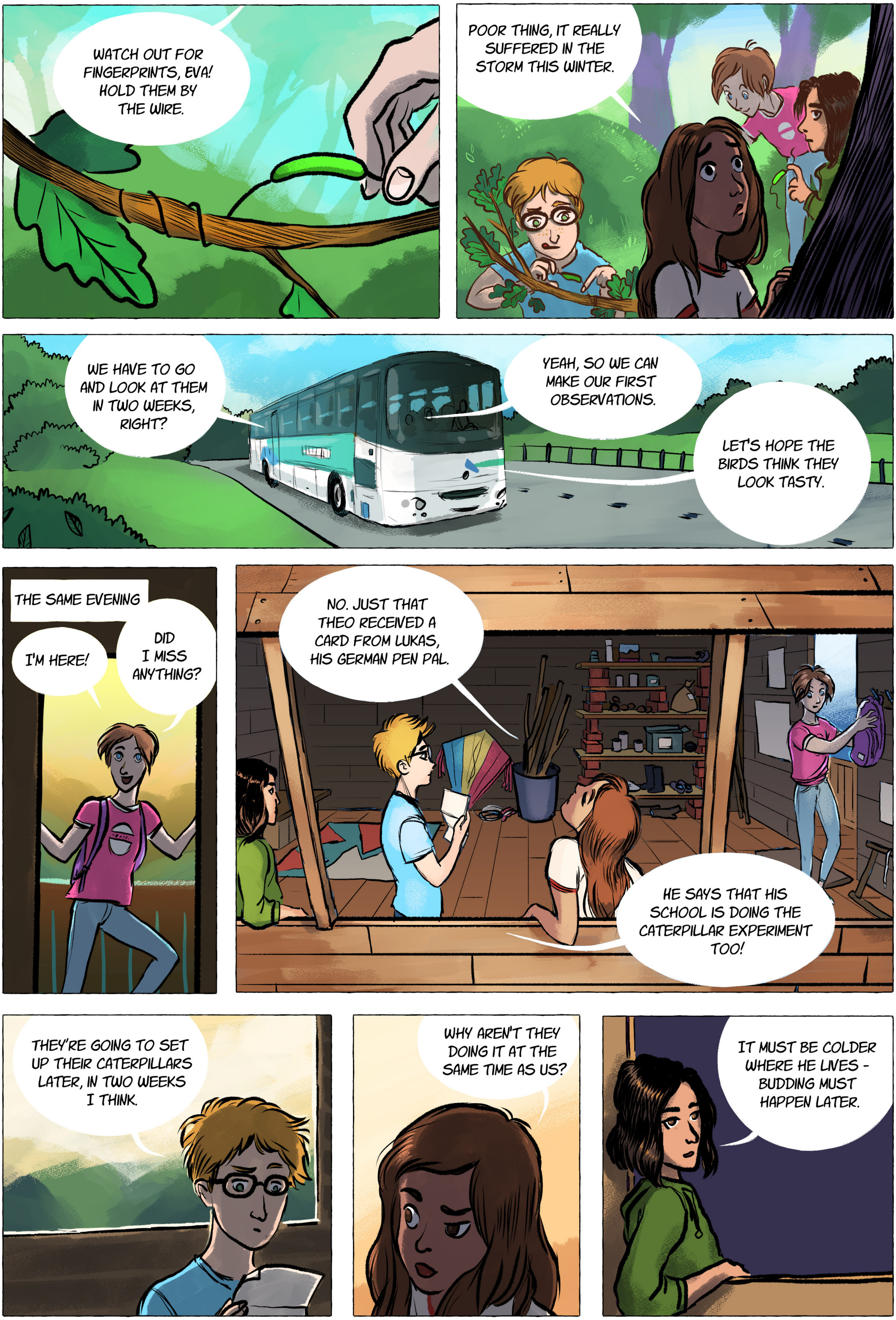

VERY GOOD. OK, WE HAVE OUR SUBJECT.


WEEKS PASS...
WOOOOSHHHH...
WOOOOOOSHHHH...
CRAC!
WOW, WHAT A STORM!
L'ECHO des BOIS
Violentes
THEY'RE SAYING THERE'S BEEN DAMAGE IN THE VILLAGES NEARBY. GUSTS OF WIND HAVE DAMAGED A LOT OF TREES.


GIVE ME THE NAILS, SOPHIA!
WAIT A SECOND WHILE I FINISH THIS.
THERE ARE SO MANY INSECTS, THEY'RE SWARMING EVERYWHERE!
!
WOW! COME AND LOOK AT THIS ONE!
FLAP!



* BUDDING, ALSO KNOWN AS BUD BREAK, ORV BUDBURST. IT IS THE TIME OF YEAR WHEN THE FLOWER AND LEAF BUDS OF THE TREES DEVELOP AND THEIR DOWNY COVER APPEARS (THE COAT AND YOUNG LEAVES AND FLOWERS HIDDEN IN THE BUDS OF MANY TREES), AND LATER THE LEAVES AND FLOWERS THEMSELVES APPEAR.


WATCH OUT FOR FINGERPRINTS, EVA! HOLD THEM BY THE WIRE.
POOR THING, IT REALLY SUFFERED IN THE STORM THIS WINTER.
WE HAVE TO GO AND LOOK AT THEM IN TWO WEEKS, RIGHT?
YEAH, SO WE CAN MAKE OUR FIRST OBSERVATIONS.
LET'S HOPE THE BIRDS THINK THEY LOOK TASTY.
THE SAME EVENING
I'M HERE!
DID I MISS ANYTHING?
NO. JUST THAT THEO RECEIVED A CARD FROM LUKAS, HIS GERMAN PEN PAL.
HE SAYS THAT HIS SCHOOL IS DOING THE CATERPILLAR EXPERIMENT TOO!
THEY'RE GOING TO SET UP THEIR CATERPILLARS LATER, IN TWO WEEKS I THINK.
WHY AREN'T THEY DOING IT AT THE SAME TIME AS US?
IT MUST BE COLDER WHERE HE LIVES - BUDDING MUST HAPPEN LATER.

Haha lol
good night 😊

ttyl 🙂😉

7:02

The oak where we set up our caterpillars is going to be cut down! Meet me there before school.

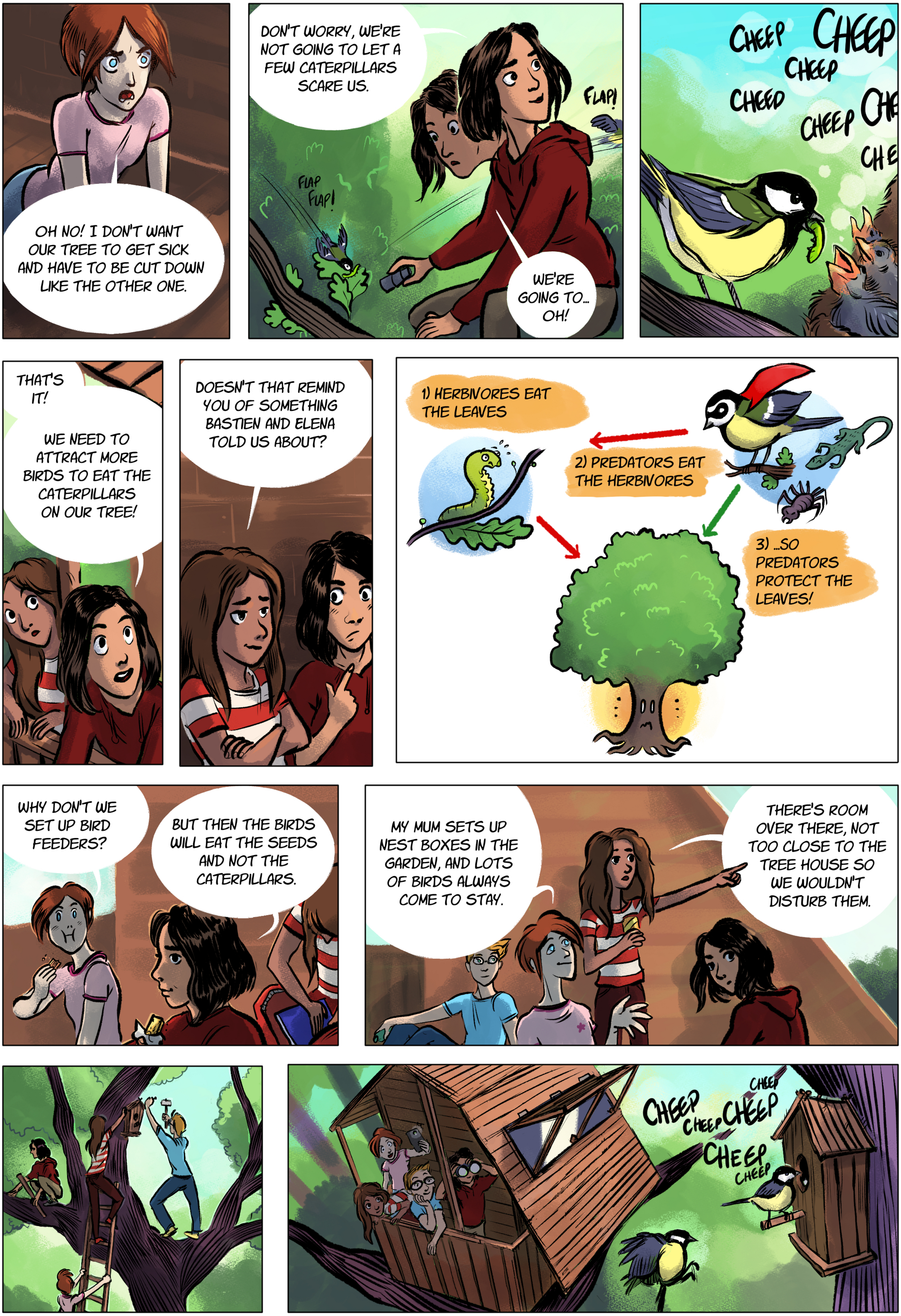
Now ● SMS

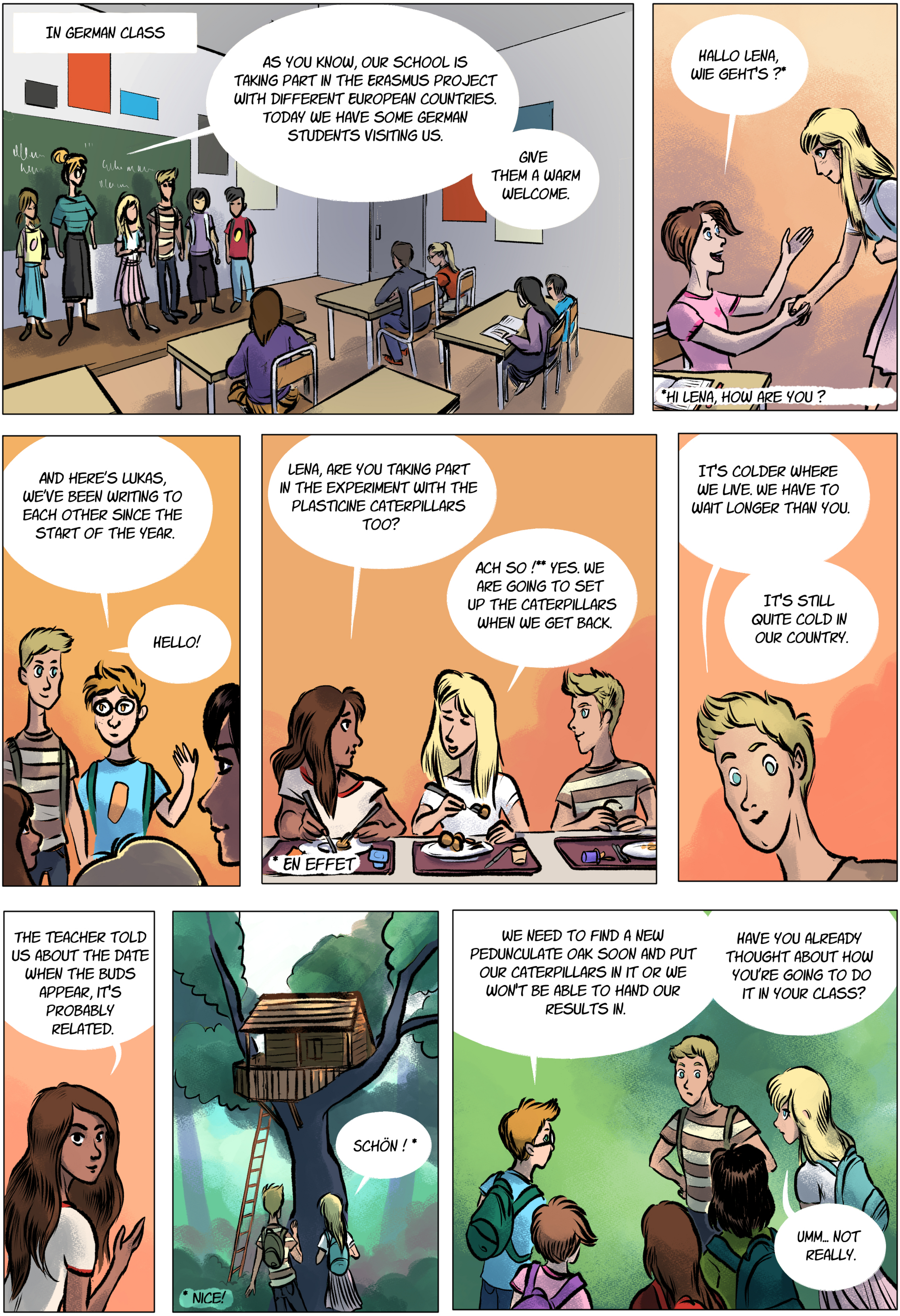

WHEN I SEE THAT OLD TREE HOUSE IT BRINGS BACK MEMORIES.
I SPENT MANY HOURS THERE WITH MY FRIENDS AS A CHILD. THAT'S WHAT MADE ME WANT TO WORK AT THE FORESTRY COMMISSION.
I'M HAPPY YOU BUILT YOURS TOO.
YES! AND WE TAKE CARE OF IT. YOU SHOULD COME AND SEE IT SOMETIME.
THEY'VE JUST CUT OFF THE BRANCHES WHERE WE PUT OUR CATERPILLARS!
PFFT...
THERE ARE SO MANY SHAVINGS AND SAWDUST, IT'S GOING TO BE DIFFICULT TO FIND THEM.
THERE! I'VE GOT ONE!
OH NO!
IT LOOKS LIKE IT WAS CHEWED UP AND SPAT OUT BY A VERY LARGE BIRD.
THERE ISN'T A SINGLE CATERPILLAR WE CAN USE. WE WON'T BE ABLE TO TAKE PART IN THE EXPERIMENT.

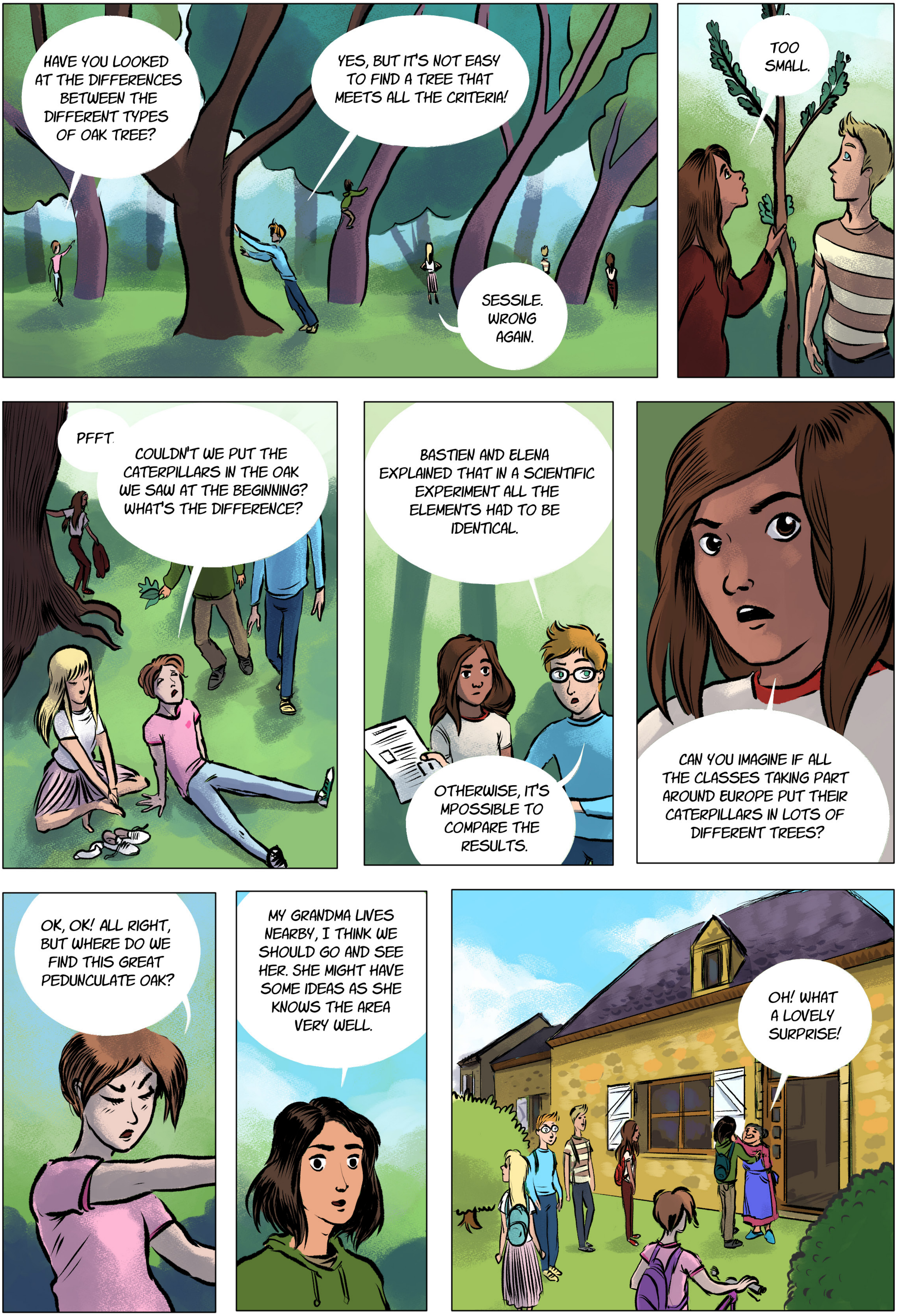

MR WILLIAMS, IS THERE REALLY NO WAY TO SAVE THIS TREE?
NO MY BOY, IT WAS ALREADY FULL OF HOLES FROM LONGHORN BEETLES, AND THE STORM THIS WINTER REALLY DAMAGED IT. I'LL CHOP IT INTO LOGS FOR NEXT WINTER AND PLANT SOMETHING ELSE IN ITS PLACE.
IT'S SAD, BUT THAT'S THE WAY IT IS, CHILDREN. TREES GET DISEASES AND THEN AFTER THE FIRST GUST OF WIND, BANG!
BUT THE OTHER TREES DON'T LOOK ILL.
WE ARE GETTING MORE AND MORE STORMS IN THE REGION. THE ONE THIS WINTER CERTAINLY WON'T BE THE LAST. REMEMBER, IT DID A LOT OF DAMAGE.
BUT ALL THIS HAS RUINED OUR EXPERIMENT!
WILL IT STILL COUNT IF WE START AGAIN A FEW DAYS LATE? WE'LL HAVE TO ASK OUR TEACHER.
WE'RE GOING TO HAVE TO FIND ANOTHER PEDUNCULATE OAK TREE.
IF NOT, OUR EXPERIMENT WILL BE COMPROMISED.


DID YOU SEE THE TWO PHOTOS OF THE SEA ICE TAKEN TEN YEARS APART? IT'S SCARY!
YEAH I SAW THAT, IT TOTALLY IS.
UM.
I SAW THAT WITH MY DAD, IT'S A FAKE.
THE PHOTOS WERE TAKEN AT THE TWO DIFFERENT POLES. AND THEY WEREN'T TAKEN TEN YEARS APART EITHER.
WHAT?!
DON'T YOU BELIEVE IN GLOBAL WARMING?
IT ISN'T ABOUT WHETHER YOU BELIEVE IN IT OR NOT. GLOBAL WARMING IS A PROVEN PHENOMENON.
BUT THIS KIND OF PHOTO EDITING, RATHER THEN HELPING PEOPLE UNDERSTAND THE PROBLEM, JUST REINFORCES PEOPLE'S SCEPTICISM INSTEAD.
BUT...
THEO, I DIDN'T MEAN TO CALL YOU A LIAR, OF COURSE.
FAKE PHOTOS ARE OFTEN SHARED ONLINE, SO IT'S IMPORTANT TO CHECK THEIR SOURCES ON SEVERAL SITES.
DON'T JUST BLINDLY BELIEVE EVERYTHING YOU READ ON THE INTERNET, AND EVERYTHING WILL BE FINE.


THE NEXT AFTERNOON
THERE ARE SO MANY CATERPILLARS, THE LEAVES ON OUR TREE HAVE BEEN REALLY EATEN AWAY.

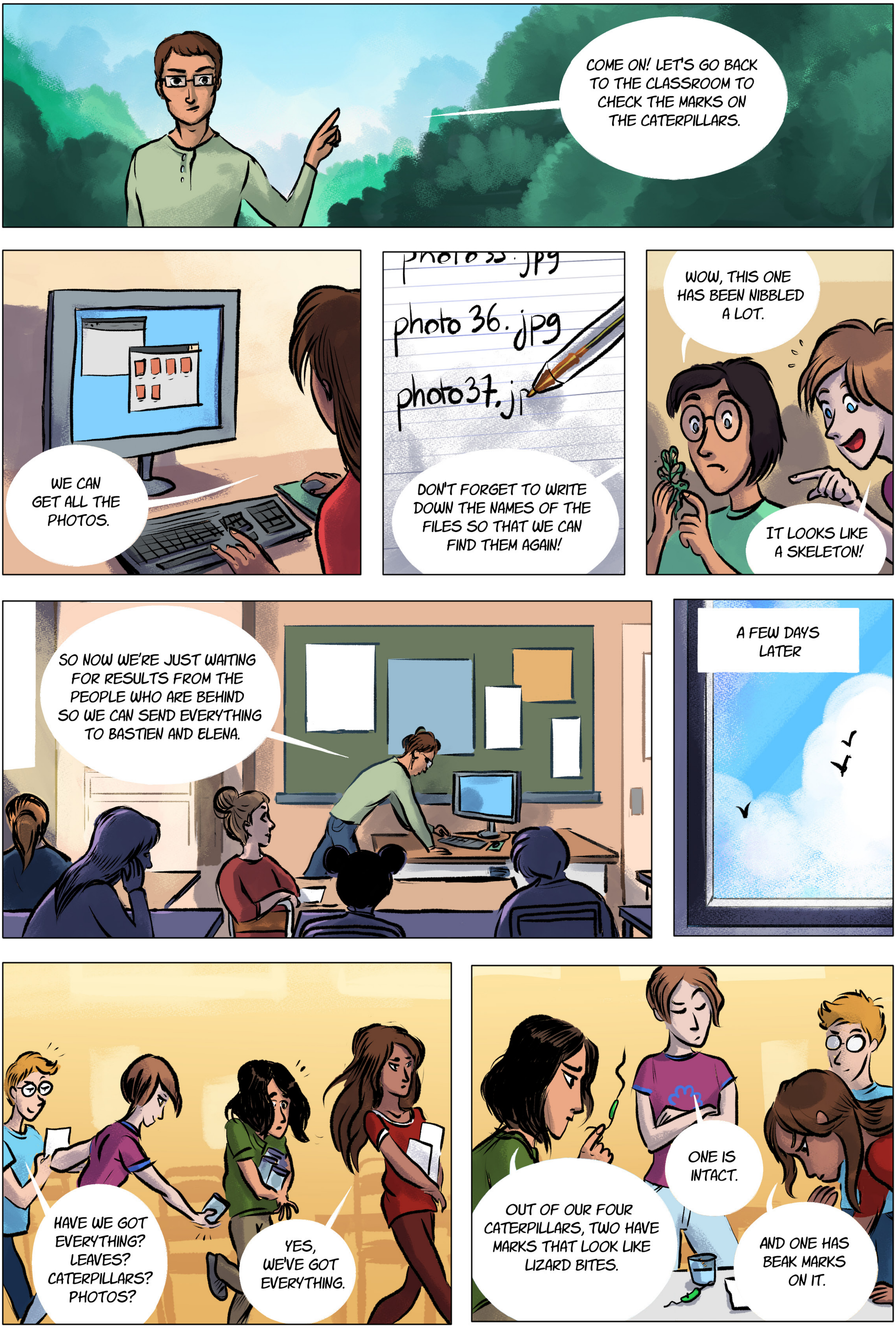

OH NO! I DON'T WANT OUR TREE TO GET SICK AND HAVE TO BE CUT DOWN LIKE THE OTHER ONE.
DON'T WORRY, WE'RE NOT GOING TO LET A FEW CATERPILLARS SCARE US.
FLAP!
FLAP FLAP!
WE'RE GOING TO... OH!
CHEEP CHEEP CHEEP CHEEP CHEEP CHE CHE
THAT'S IT!
WE NEED TO ATTRACT MORE BIRDS TO EAT THE CATERPILLARS ON OUR TREE!
DOESN'T THAT REMIND YOU OF SOMETHING BASTIEN AND ELENA TOLD US ABOUT?
1) HERBIVORES EAT THE LEAVES
2) PREDATORS EAT THE HERBIVORES
3) ...SO PREDATORS PROTECT THE LEAVES!
WHY DON'T WE SET UP BIRD FEEDERS?
BUT THEN THE BIRDS WILL EAT THE SEEDS AND NOT THE CATERPILLARS.
MY MUM SETS UP NEST BOXES IN THE GARDEN, AND LOTS OF BIRDS ALWAYS COME TO STAY.
THERE'S ROOM OVER THERE, NOT TOO CLOSE TO THE TREE HOUSE SO WE WOULDN'T DISTURB THEM.
CHEEP CHEEP CHEEP CHEEP CHEEP CHEEP

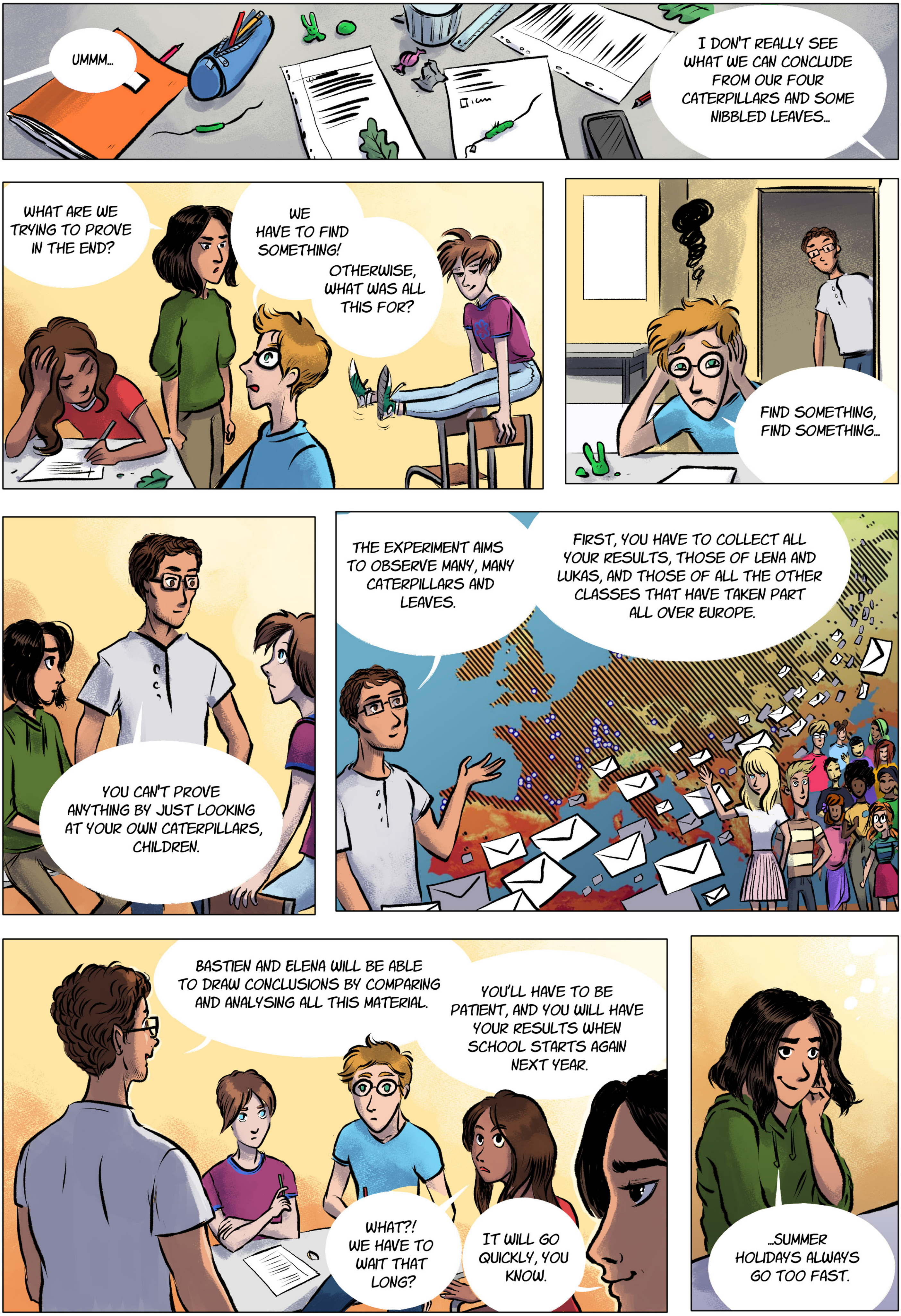

IN GERMAN CLASS
AS YOU KNOW, OUR SCHOOL IS TAKING PART IN THE ERASMUS PROJECT WITH DIFFERENT EUROPEAN COUNTRIES. TODAY WE HAVE SOME GERMAN STUDENTS VISITING US.
GIVE THEM A WARM WELCOME.
HALLO LENA, WIE GEHT'S ?*
*HI LENA, HOW ARE YOU ?
AND HERE'S LUKAS, WE'VE BEEN WRITING TO EACH OTHER SINCE THE START OF THE YEAR.
HELLO!
LENA, ARE YOU TAKING PART IN THE EXPERIMENT WITH THE PLASTICINE CATERPILLARS TOO?
ACH SO !** YES. WE ARE GOING TO SET UP THE CATERPILLARS WHEN WE GET BACK.
* EN EFFET
IT'S COLDER WHERE WE LIVE. WE HAVE TO WAIT LONGER THAN YOU.
IT'S STILL QUITE COLD IN OUR COUNTRY.
THE TEACHER TOLD US ABOUT THE DATE WHEN THE BUDS APPEAR, IT'S PROBABLY RELATED.
SCHÖN ! *
* NICE!
WE NEED TO FIND A NEW PEDUNCULATE OAK SOON AND PUT OUR CATERPILLARS IN IT OR WE WON'T BE ABLE TO HAND OUR RESULTS IN.
HAVE YOU ALREADY THOUGHT ABOUT HOW YOU'RE GOING TO DO IT IN YOUR CLASS?
UMM... NOT REALLY.


HAVE YOU LOOKED AT THE DIFFERENCES BETWEEN THE DIFFERENT TYPES OF OAK TREE?
YES, BUT IT'S NOT EASY TO FIND A TREE THAT MEETS ALL THE CRITERIA!
SESSILE. WRONG AGAIN.
TOO SMALL.
PFFT.
COULDN'T WE PUT THE CATERPILLARS IN THE OAK WE SAW AT THE BEGINNING? WHAT'S THE DIFFERENCE?
BASTIEN AND ELENA EXPLAINED THAT IN A SCIENTIFIC EXPERIMENT ALL THE ELEMENTS HAD TO BE IDENTICAL.
OTHERWISE, IT'S MPOSSIBLE TO COMPARE THE RESULTS.
CAN YOU IMAGINE IF ALL THE CLASSES TAKING PART AROUND EUROPE PUT THEIR CATERPILLARS IN LOTS OF DIFFERENT TREES?
OK, OK! ALL RIGHT, BUT WHERE DO WE FIND THIS GREAT PEDUNCULATE OAK?
MY GRANDMA LIVES NEARBY, I THINK WE SHOULD GO AND SEE HER. SHE MIGHT HAVE SOME IDEAS AS SHE KNOWS THE AREA VERY WELL.
OH! WHAT A LOVELY SURPRISE!


I'M SO HAPPY YOU CAME TO SEE ME. PARTICULARLY AS SOME OF YOU HAVE COME FROM SO FAR AWAY!
GRANDMA, DO YOU KNOW ANY OAK TREES ROUND HERE? WE'RE LOOKING FOR ONE FOR A SCHOOL PROJECT.
WELL, IF YOU ARE LOOKING FOR AN OAK, THERE IS ALWAYS THE ONE AT THE BOTTOM OF THE GARDEN.
IT WAS ALREADY THERE WHEN YOUR DAD WAS BORN...
...CHILDREN?
TELL ME IT'S A PEDUNCULATE OAK!
IT CERTAINLY LOOKS LIKE IT! THE LEAVES ARE RIGHT.
WE HAVE A WINNER!
GREAT!
THAT'S PERFECT!
UH... PERFEKT* !
*PERFECT IN GERMAN
EVERYTHING ALL RIGHT?
YES, IT'S GREAT!
WE'LL BE ABLE TO TELL THE OTHERS ABOUT IT WHEN WE GET BACK!
AND NOW, DO YOU HAVE TIME FOR A PIECE OF CAKE?
YES! JA *!
*YES IN GERMAN (PRONOUNCE « YA »)


ONE MONTH LATER
THE FIRST RESULTS OF THE EXPERIMENT ARE IN.
I'D SAY THAT'S A PECK, WHAT DO YOU THINK?
UMMM...
IT'S NOT A CLEAR RESULT...
SIR! I CAN'T FIND MY CATERPILLAR! I THINK A BIRD FLEW OFF WITH IT!
OH BLAST!
CRIC!
REMIND ME AGAIN WHY WE HAVE TO PICK LEAVES UP TOO?
TO LOOK AT THE DAMAGE CATERPILLARS HAVE DONE TO THE TREES.
NO NEED FOR CATERPILLARS, JUST LOOK AT SOPHIA WITH HER LEAVES...
FLAP!
HA HA, VERY FUNNY.


COME ON! LET'S GO BACK TO THE CLASSROOM TO CHECK THE MARKS ON THE CATERPILLARS.
WE CAN GET ALL THE PHOTOS.
photo35.jpg
photo 36.jpg
photo37.jp
DON'T FORGET TO WRITE DOWN THE NAMES OF THE FILES SO THAT WE CAN FIND THEM AGAIN!
WOW, THIS ONE HAS BEEN NIBBLED A LOT.
IT LOOKS LIKE A SKELETON!
SO NOW WE'RE JUST WAITING FOR RESULTS FROM THE PEOPLE WHO ARE BEHIND SO WE CAN SEND EVERYTHING TO BASTIEN AND ELENA.
A FEW DAYS LATER
HAVE WE GOT EVERYTHING? LEAVES? CATERPILLARS? PHOTOS?
YES, WE'VE GOT EVERYTHING.
OUT OF OUR FOUR CATERPILLARS, TWO HAVE MARKS THAT LOOK LIKE LIZARD BITES.
ONE IS INTACT.
AND ONE HAS BEAK MARKS ON IT.

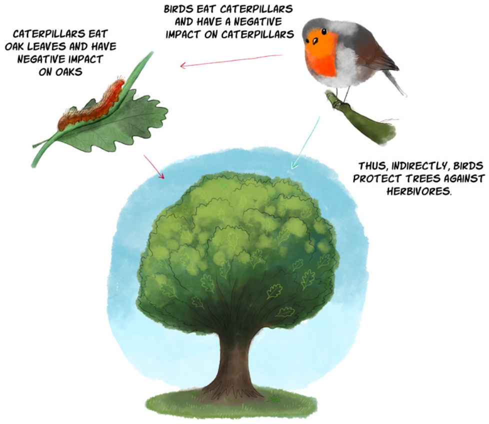

UMMM...
I DON'T REALLY SEE WHAT WE CAN CONCLUDE FROM OUR FOUR CATERPILLARS AND SOME NIBBLED LEAVES...
WHAT ARE WE TRYING TO PROVE IN THE END?
WE HAVE TO FIND SOMETHING!
OTHERWISE, WHAT WAS ALL THIS FOR?
FIND SOMETHING, FIND SOMETHING...
YOU CAN'T PROVE ANYTHING BY JUST LOOKING AT YOUR OWN CATERPILLARS, CHILDREN.
THE EXPERIMENT AIMS TO OBSERVE MANY, MANY CATERPILLARS AND LEAVES.
FIRST, YOU HAVE TO COLLECT ALL YOUR RESULTS, THOSE OF LENA AND LUKAS, AND THOSE OF ALL THE OTHER CLASSES THAT HAVE TAKEN PART ALL OVER EUROPE.
BASTIEN AND ELENA WILL BE ABLE TO DRAW CONCLUSIONS BY COMPARING AND ANALYSING ALL THIS MATERIAL.
YOU'LL HAVE TO BE PATIENT, AND YOU WILL HAVE YOUR RESULTS WHEN SCHOOL STARTS AGAIN NEXT YEAR.
WHAT?! WE HAVE TO WAIT THAT LONG?
IT WILL GO QUICKLY, YOU KNOW.
...SUMMER HOLIDAYS ALWAYS GO TOO FAST.

WHO ATE MY CATERPILLARS?

Call to schools for a participatory science project

In spring 2018, scientists from 15 European countries, with the help of French, English, Spanish, Swiss and German schoolchildren, installed hundreds of clay caterpillars in oaks. They counted traces of predator attacks and estimated the damage caused by herbivorous insects on oak leaves. In autumn 2018, they will carry out chemical analyses on the leaves to quantify their defences.

They will carry on next year and need help!



THIS IS THE TROPHIC CASCADE

Oaks are not defenceless: they produce repulsive or toxic molecules in their leaves. They also receive help from firends. Herbivorous insects are on the menu of many predators: birds, carnivorous insects, spiders and mammals. Oaks are therefore protected by the enemies of their enemies, it is what we call **a trophic cascade.**

Scientists have recently shown that herbivores cause less damage to oaks as one moves from southern to northern Europe. By studying the chemical composition of the oak leaves, they found that this could be explained by oaks being more defended in colder regions. But what about herbivores' enemies? Are they less effective in warmer regions or do they compensate for oaks lower investment into defenses? This is what we want to test in this project.

Over the last ten years, a simple, effective, fun and inexpensive method developped in the scientific community to estimate the activity of herbivore predators: the installation, on plants, of frake caterpillars made with modelling clay. Predators are tricked: they attack lures as if they were real preys and leave traces of beaks, teeth or mandibles, which are then counted.

This book was realised with the collaboration of
Bastien Castagneyrol and Elena Valdès-Correcher.
Scan the following barcode to learn more about this experiment ->

Sophia, Theo, Eva and Tom are KS3 students.

The four friends built a shack in a tree from which they can observe the forest. When one day their class was selected to participate in a citizen science project about oaks and their enemies, they joined forces to carry out the experiment to the end.

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