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- **Background and Aims** In plants, high costs of reproduction during some years can induce trade-offs in resource allocation with other functions such as growth, survival and resistance against herbivores or extreme abiotic conditions, but also with subsequent reproduction. Such trade-offs might also occur following resource shortage at particular moments of the reproductive cycle. Because plants are modular organisms, resource allocation strategies to reproduction can also vary among hierarchical levels. Using a defoliation experiment, our aim was to test how allocation to reproduction was impacted by resource limitation.

- **Methods** We applied three levels of defoliation (control, moderate and intense) to branches of eight *Quercus ilex* trees shortly after fruit initiation and measured the effects of resource limitation induced by leaf removal on fruit development (survival, growth, and germination potential), and on the production of vegetative and reproductive organs the year following defoliation.

- **Key Results** We found that defoliation had little impact on fruit development. Fruit survival was not affected by the intense defoliation treatment, but reduced by moderate defoliation, and this result could not be explained by an upregulation of photosynthesis. Mature fruit mass was not affected by defoliation, nor was seed germination success. However, in the following spring, defoliated branches produced less shoots and compensated leaf loss by overproducing leaves at the expense of flowers. Therefore, resource shortage decreased resource allocation to reproduction the following season but did not affect sex ratio.

- **Conclusions** Our results support the idea of a regulation of resource allocation to reproduction beyond the shoot scale. Defoliation had larger legacy effects than immediate effects.

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INTRODUCTION

Climate change is currently affecting the reproductive success of trees (Pérez-Ramos *et al.* 2010; Sánchez-Humanes and Espelta 2011; Caignard *et al.* 2017; Bogdziewicz, Kelly, *et al.* 2020) and their allocation of resources to reproduction (Monks *et al.* 2016; Gavinet *et al.* 2019). While the frequency of reproductive failures has been increasing in some species and areas (Bogdziewicz, Kelly, *et al.* 2020), jeopardizing the natural regeneration of the forest, fruit production has been increasing in others (Caignard *et al.* 2017). Climate change effects on tree growth can be either positive due to CO_2 fertilization, nitrogen deposition (Fernández-Martínez *et al.* 2017), and the lengthening of the growing season (Menzel and Fabian 1999; Piao *et al.* 2007; Delpierre *et al.* 2009), or negative because of more stressful conditions especially in water limited ecosystems where aggravated droughts are expected (IPCC 2013; Dai 2013). These climate change effects are likely to affect both the carbon source through photosynthesis (Luyssaert *et al.* 2007; Biederman *et al.* 2016) and the carbon sink through the cambial activity (Babst *et al.* 2013; Lempereur *et al.* 2017), and to modify carbon allocation to the different organs, especially the reproductive structures (Gavinet *et al.* 2019; Bogdziewicz, Fernández-Martínez, *et al.* 2020). Understanding strategies of resource allocation into reproductive functions *versus* other functions, as well as the environmental determinants of reproductive success is therefore essential to grasp and predict how the reproductive success of trees and regeneration will be affected by future climatic conditions.

Trade-offs in resource allocation arise from the fact that when limiting resources are allocated to one function, such as reproduction, they become unavailable for others (Bazzaz *et al.* 1987; Stearns 1989; Obeso 2002), thereby creating the need for priority rules of allocation (Suzuki 2001; Wiley and Helliker 2012). Reproduction being costly for plants, it might result in trade-offs with other functions such as growth, survival, defence against pests and herbivores, and resistance to extreme abiotic conditions (Obeso 2002; Barringer *et al.* 2013), as well as with subsequent reproductive efforts. In trees, the trade-off between growth and reproduction, generally studied at the stand scale, has been the focus of most investigations and has found some support in some cases (Sánchez-Humanes *et al.*

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2011; Han *et al.* 2011; Martín *et al.* 2015; Vergotti *et al.* 2019), but not in all (Dick *et al.* 1990; Yasumura *et al.* 2006; Knops *et al.* 2007; Alla *et al.* 2012; Redmond *et al.* 2019; Vergotti *et al.* 2019).

Understanding how reproduction may be limited by other functions, or on the contrary limit them, is of particular importance in mast seeding species. Mast seeding species show years of massive and synchronous production of seeds that alternate with one or more years of negligible production. Fruit production during a mast year consumes a significant amount of resources (Janzen 1971; Kelly 1994; Kelly and Sork 2002) as fruit biomass amounting up to 79% and 52% of annual wood biomass production have been reported during a mast year in beech and sessile oak, respectively (Mund *et al.* 2010; Delpierre *et al.* 2016). Different mechanisms of allocation to reproduction have been proposed to explain mast seeding (Pearse *et al.* 2016). Among them, two have found some support in oaks. First, the resource depletion (or storage) hypothesis, proposes that the tree's reserves are depleted during mast years and that the tree needs to stock resources for several years before it can invest strongly in reproduction again (Sork *et al.* 1993; Sánchez-Humanes *et al.* 2011; Pearse *et al.* 2016). Second, the resource switching hypothesis proposes that resources are shifted from vegetative growth to reproduction in mast seeding years (Norton and Kelly 1988; Hirayama *et al.* 2008; Sánchez-Humanes *et al.* 2011). Whichever hypothesis, or their combination, is correct, the nature of the limiting resource(s) inducing the mast seeding behaviour remains unknown in many species (Han and Kabeya 2017).

Studies investigating mast seeding are usually based on correlative analyses of fructification time-series at the tree or the plot scale. Understanding the physiological mechanisms responsible for mast seeding, and more generally the allocation of resources to reproduction in trees, requires a deeper understanding of the regulation of reproduction all along the reproductive cycle from bud initiation to fruit maturation (Miyazaki 2002; Bañuelos and Obeso 2005; Ichie *et al.* 2013; Allen *et al.* 2017). The goal of this study was thus to better understand how allocation to reproduction is regulated at the different steps of the reproductive cycle, from flowering to seed germination.

A key question to understand mast seeding, and more generally the inter-annual variation in tree fecundity, is which resource is most limiting to reproduction and how this resource is allocated to

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the different functions. Phosphorus, nitrogen and non-structural carbohydrates are known to be involved in the proximate mechanisms driving mast seeding (Han *et al.* 2011; Sala *et al.* 2012; Miyazaki *et al.* 2014; Allen *et al.* 2017; Han and Kabeya 2017; Fernández-Martínez *et al.* 2019). Most of the carbon used for fruit growth, either in forest trees or in fruit trees, has been shown to derive from short-term photoassimilates mostly produced by leaves in the close vicinity of the fruit (Hasegawa *et al.* 2003; Volpe *et al.* 2008; Hoch *et al.* 2013; Ichie *et al.* 2013; Han *et al.* 2016). This suggests that foliated branches are autonomous for carbon for most of the growing season to produce the fruits, as proposed by the branch autonomy theory (Watson and Casper 1984; Sprugel *et al.* 1991). However, other studies have also shown that individual fruit-bearing branches are sometimes unable to supply all the carbon required for the development of their fruits (Newell 1991; Miyazaki *et al.* 2007; Pasqualotto *et al.* 2019), which suggests that a whole-tree regulation or a physiological integration among branches is sometimes necessary. Therefore, it seems that the scale of the regulation of carbon allocation to sexual reproduction in woody plants can range from the branch to the whole-individual (Ushimaru and Genkai-Kato 2011).

The degree of physiological autonomy of shoots for reproduction varies among species and situations (Henriksson 2000; Hasegawa *et al.* 2003; Díaz *et al.* 2004; Sánchez-Humanes *et al.* 2011). Therefore, costs of reproduction may vary in contradictory ways if studied at the tree, the branch or the shoot level (Obeso 1997), but few studies have examined reproduction costs and allocation trade-offs between reproduction and other functions at multiple hierarchical levels (Sánchez-Humanes *et al.* 2011; Alla *et al.* 2012; Barringer *et al.* 2013; Hossain *et al.* 2017). Furthermore, very few of these studies are explicitly related to mast seeding (Miyazaki 2013). Here we aimed at bringing new insights to the elucidation of the mechanisms of mast seeding by investigating the investment in reproduction at different physiological scales. More precisely, we aimed at investigating the allocation to reproduction at the branch and the shoot levels from the fruit initiation to the fruit initiation of the next season in a mast-seeding species.

One method to study the allocation relationships between different sinks is to manipulate sink-source relationships (Iqbal *et al.* 2012; Bogdziewicz, Ascoli, *et al.* 2020). Manipulation of sink-source

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relationships by defoliation can help determining whether branches or individual plants are able to compensate the loss of photosynthetic capacity and nutrient storage to achieve their reproduction. Compensation may happen by either changing allocation from other functions to reproductive organs (growth, survival, storage, future reproduction) (Sprugel *et al.* 1991; Obeso 1998; Hoch 2005) or by increasing the photosynthetic activity of the remaining leaves in the case of carbon (Iqbal *et al.* 2012).

Most defoliation and herbivore exclusion experiments on temperate forest trees and fruit trees have shown a negative effect of defoliation on fruit set (Mehouachi *et al.* 1995; Obeso 1998; Iglesias *et al.* 2003; Frioni *et al.* 2018) and on fruit size (Obeso 1998; Hoch 2005; Matsumoto *et al.* 2017). In oaks, most studies concerned natural herbivory and showed also negative impact on fruit set and total fruit production (Crawley 1985; May and Killingbeck 1995; Hochwender *et al.* 2003; Pearse *et al.* 2015; Nakajima 2015; Canelo *et al.* 2018). However, other studies have found no effect of herbivory on fruit set, fruit size or yield in other species (Obeso and Grubb 1993; Mehouachi *et al.* 1995; Tamura and Hiura 1998; Ezzahouani and Williams 2003; Bañuelos and Obeso 2005; Frioni *et al.* 2018; Pasqualotto *et al.* 2019). In some cases, an overcompensation, i.e. a positive effect of herbivory or leaf removal (Agrawal 2000; Iqbal *et al.* 2012), has been observed on fruit size ((Ezzahouani and Williams 2003) in *Vitis vinifera*) and on flower production ((Díaz *et al.* 2004) in *Quercus ilex*). However, it is difficult to draw general conclusions from these studies because they differ in many respects: defoliation intensity, defoliation extent (branch or whole tree), moment of the reproductive cycle, targeted variables (fruit number, fruit set, total seed biomass, yield, etc…). Moreover, most studies have looked at the immediate effect of defoliation on the ongoing reproduction, while few of them have explored the legacy effect of defoliation on resource allocation to different functions the subsequent seasons (Noyce *et al.* 2016; Wiley *et al.* 2017). At last, the majority of defoliation studies on forest trees has focused on deciduous species, which sustain lower leaf construction cost than evergreen species (Villar and Merino 2001) and might be more tolerant to defoliation (Piper and Fajardo 2014), but have also a shorter photosynthetic activity period. It is thus not straightforward which impact defoliation has on resource allocation in evergreen species.

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Here, we studied the regulation of allocation to reproduction relatively to other functions in the monoecious evergreen tree *Q. ilex*, which is the most widespread forest tree species in the Mediterranean Basin, using experimental defoliation to create a situation of resource limitation. The originality of our study is to cover the main steps of the reproductive cycle, at different scales within the tree and with a large range of defoliation intensities, from 0 to over 80% of defoliation, and to quantify its impact not only on the allocation to reproduction of the same year but also on allocation to vegetative and reproductive organs the following year. More specifically, we aimed to answer the following questions:

1) Are branches and shoots able to compensate resource limitation to maintain fruit production (fruit survival, growth, and germination potential) and production of vegetative and reproductive organs the following year?

2) Are there trade-offs of allocation at the shoot scale between reproductive and vegetative organs, between subsequent years, and between male and female reproductive organs, and how are these trade-offs affected by resource limitation?

3) Are there some steps of the reproductive cycle that are more sensible to resource limitation than others?

We applied three defoliation treatments (0% i.e. control, 50% i.e. moderate, 85% i.e. intense leaf removal) to six branches of eight trees and monitored the impact it had on allocation to reproduction at different steps of the reproductive cycle as presented in Fig. 1. First, we expected that shoots would be less able to compensate leaf loss for fruit production in the intense defoliation treatment compared to the moderate defoliation treatment. Second, we expected a reduced germination success for seeds produced by defoliated branches due to a reduced amount of reserves. Third, we expected a strongly reduced, or even suppressed production of flowers the following year on the defoliated branches because of priority investment in leaves to compensate defoliation as observed by Wiley *et al.* (2017) in *Q. velutina*. Finally, since sex allocation theory predicts that female function is more expensive (Charlesworth and Morgan 1991), we expected a shift toward maleness the following year on the defoliated branches.

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MATERIALS AND METHODS

Study species and area

Quercus ilex L. is an evergreen wind-pollinated monoecious tree that usually flowers in May in the study area. The male inflorescences, called catkins, bear around 20-25 staminate flowers ((Yacine and Bouras 1997; Gómez-Casero *et al.* 2004), Fig. 1). Catkins develop in the axils of lower leaves of the current-year shoot or in separate buds bearing only catkins. Female pistillate flowers mature a few days after staminate flowers and are located on an inflorescence at the upper part of the current-year shoot. Female inflorescence can bear one to six pistillate flowers (Fig. 1). Fertilization occurs in late June early July, leading to fruit initiation. Fruits (acorns) achieve their maturation in November-December (Yacine and Bouras 1997).

The experimental plot was located in Montpellier, France (latitude: 43.64°N, longitude: 3.86°E, altitude: 76m). The climate is Mediterranean with an annual rainfall of 629 mm and mean annual temperature of 15.2°C. The soil of the field site is a rendzina-like silty clay soil, with a pH of 8 and a depth varying from 150 to 200 cm. The trees used for the experiment were planted in 1998 from fruits collected from nearby natural populations. Trees were on average ($\pm$ SD) 4.3 ± 0.3 meters tall with a mean basal area of 124 ± 39 cm² during the experiment.

Experimental setting

In 2018, eight trees bearing fruits were selected for the experiment. On each tree, six branches bearing at least 10-15 initiated fruits (i.e. fertilized pistillate flowers) were selected in different and distant parts of the tree crown. The shoot is defined here as the growth unit of the current year (or the spring and summer growth units in cases when two growth flushes happened due to polycyclism). The branch refers to a ramified structure consisting of several shoots and including all the ramifications above the lowest one carrying at least one fruit (see Fig. 2). The number of 2018 shoots per branch varied from 18 to 192 among all trees, and the number of fruit-bearing 2018 shoots per branch varied from 4 to 17 among all trees.

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For each tree, branches were randomly assigned to either of the 3 following treatments (2 replicates per tree): no defoliation (control), moderate defoliation and intense defoliation. Defoliation consisted in half of the leaves removed in the moderate treatment, and to all leaves removed except the uppermost leaf of each shoot, which lead to 85 ± 3 % of the leaves removed in the intense defoliation treatment. For this last treatment, one leaf per shoot was left to allow minimal transpiration flow in the shoot (see Figs 1 and 2).

Branches were defoliated between June 21st and June 27th 2018, shortly after fruit initiation and once the leaves of the spring flush were mature. On 28 out of the 48 monitored branches, a second flush of leaves (called "summer flush" hereafter) happened in early July. These shoots underwent the same defoliation treatment than their respective spring shoot at mid-July.

In order to estimate the mean individual leaf area for each tree, we randomly selected and scanned around 200 leaves per tree using the image analysis software ImageJ ©, and then used that to calculate the total leaf area of every shoot.

Fruit growth monitoring and germination the year of defoliation

Fruit growth and survival between initiation and maturity was followed on a total of 930 acorns that were individually tagged and monitored. Up to six fruits could grow at the axil of the same leaf and this information was recorded for each fruit as "single" or "two or more fruits growing at the axil of the same leaf". From July 2018 until November 2018, fruit survival and size was monitored 6 times (July 1^{rst} (day 0), August 23th (day 53), September 14th (day 75), October 5th (day 96), October 19th (day 110) and November 8th (day 130)). Fruit size was measured with an electronic calliper. During the early stage of fruit development, we measured the fruit diameter because its shape is round (the embryo is invisible, inside the acorn cup). As soon as the shell protecting the embryo protrudes from the cup, we measured fruit length from the basis of the cup to the top of the fruit excluding the remains of the style. Fruit mass at maturity was strongly correlated to fruit size (Supplementary data Fig. S1B), so we assume that seed growth in mass and volume occurred simultaneously. Shoots that had died or were broken between July and November (3 %) were removed from the dataset so that

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fruit survival could be calculated without interference of shoot mortality (Canelo *et al.* 2018). After the last fruit size measurement in November, we monitored fruit maturation (dessication) twice a week. Once they started turning brown, fruits were collected and weighed.

In order to determine their capacity to germinate, all collected fruits were placed in closed Magenta© boxes filled with 18 g of vermiculite imbibed with 50 mL of distilled water and kept at 25°C in the dark in germination stoves (LMS[TM]). Fruits infested by insects (19 out of 247) were discarded, and germination success was recorded after 4 weeks if a radicle had emerged. Holm oak acorns are recalcitrant seed, meaning that they are extremely sensitive to dehydration (Joët *et al.* 2013). For this reason, they have no dormancy and have to germinate rapidly as germination probability decreases with desiccation (Joët *et al.* 2016).

The number of leaves and the basal diameter of all the monitored 2018 spring shoots were counted and measured in December 2018-January 2019, i.e. after fruit maturity and before 2019 spring flush. In addition, on each of the 48 manipulated branches, we tagged five 2018 shoots that had not borne a single fruit in 2018, on which we performed the same leaf and basal area measurements. Although we did not count the total number of leaves that 2018 shoots carried before defoliation, we checked that the shoot basal area and the number of spring leaves were very strongly correlated in the control treatment (Supplementary data Fig. S1A).

Mean leaf area per fruit for each 2018 shoot was obtained by multiplying the number of leaves per shoot by the mean leaf area for that particular tree, and dividing by the number of fruits that were initiated by the shoot in July.

Photosynthesis and predawn leaf water potential the year of defoliation

In order to check treatment effect on photosynthesis, we measured leaf gas-exchange on current-year leaves adjacent to the fruits. We measured gas-exchanges on one leaf per treatment (i.e. 3 leaves per tree) of 6 trees, except the moderate defoliation treatment on the 6th tree due to accessibility constraints (17 leaves in total). Leaf gas exchange was measured on July 4th, 2018, 1-2 weeks after defoliation and after the spring leaves had reached maturity. Measurements were carried out with two

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portable photosynthesis systems (Li-6400, Li-Cor, Lincoln, NE, USA) equipped with a light source (6200-02B LED, Li-Cor). Leaves were first acclimated in the chamber for more than 20 min at ambient temperature, ambient CO_2 concentration (400 ppm) and a saturating photosynthetic photon flux density (PPFD) of 1500 µmol m^{-2} s^{-1}.

Tree water stress at the end of summer was determined by the predawn leaf water potential measured on September 5th and October 4th, 2018 with a pressure chamber (PMS 1000, PMS Instruments, Corvallis, OR, USA). Leafy shoots were collected before sunrise and stored in airtight bags in a fridge until measurements two hours later. All trees were sampled, including two leafy shoots per tree, and the difference between the two shoots never exceeded 0.2 MPa.

Shoots, leaves, flowers and initiated fruits production the year following defoliation

On each of the 48 branches that had been manipulated in 2018, we randomly selected five 2018 shoots that had borne at least one fruit and on which at least one bud was starting to break. On each of these 2018 fruit-bearing shoots and on each of the tagged 2018 non-fruit-bearing shoots (produced either during the spring flush or the summer flush), we counted: the number of 2019 spring shoots, the number of leaves per 2019 spring shoot, the number of catkins per 2019 spring shoot and the number of female flowers per 2019 spring shoot. Note that 2019 spring shoots could be composed exclusively by catkins (in this case, there is no twig), by both leaves and flowers (catkin or female inflorescence), or exclusively by leaves.

For each 2018 shoot of 7 of the trees (out of 8), we collected two catkins (if existing) out of all the catkins produced by 2019 shoots. On these catkins, we counted the total number of staminate flowers that they bore and we selected one staminate flower in the middle of each to count the number of stamens per flower.

The sex ratio of the shoots was calculated as the proportion of female flowers produced by the shoots out of the total number of inflorescences of the shoot (female flowers plus catkins).

The number of female flowers that had developed into fruits (fruit set) was counted on July 17th, 2019. We could not monitor fruit growth and maturation in summer-autumn 2019 as an early extreme heat wave damaged significantly the leaves of the tagged shoots in June 28th, 2019.

Statistical analyses

All statistical analyses and visual representations were conducted using the software R version 3.6.1 (2019) and the ggplot2 package (Wickham 2009). We used the following packages for data analysis: lme4, car, multcomp and MuMIn (Hothorn *et al.* 2008; Fox *et al.* 2015; Bates *et al.* 2015; Bartoń 2019).

We studied the effect of defoliation and additional covariates on reproductive allocation with twelve generalized linear mixed models (Table 1, Fig. 1). Four of these models are dedicated to allocation to reproduction in the year of defoliation (2018), and eight are dedicated to allocation to reproduction the following year (Table 1, Fig. 1). All continuous covariables were standardized prior to analysis to compare models estimates between variables. The analyses (5), (11) and (12) (of the number of 2019 shoots, the 2019 sex ratio and the 2019 fruit set, respectively) took into account the number of 2018 shoots with an offset. The analyses (6), (7) and (10) (of the number of leaves, the number of catkins and the number of female flowers produced in 2019, respectively) took into account the number of 2019 shoots with an offset. We only included the interactions between defoliation and the other covariates in the complete models. For each response variable, we then applied a simplification of the model by sequentially removing the insignificant interaction terms, starting with the weakest and least significant interaction. We considered our level of significance as p-value < 0.05.

We used the tree, branch and shoot as random factors. For measurements at the shoot scale (e.g. fruit survival), we considered the tree, branch and shoot together as nested random factors. Thus, the model estimates the variability of the data due to the differences between trees, between the branches of a tree and between the shoots of a branch. For variables analysed at the branch scale (e.g. the number of leaves per shoot in 2019), we have nested the branches within tree as random factors.

For random sampling at the tree scale (for example, the rate of photosynthesis), we used trees as a random factor to take into account the between-trees heterogeneity (Table 1).

We fitted GLMMs with a Gaussian distribution for the response variables presenting a normal error-structure, with a binomial distribution and its logit link function for dichotomous response variables and with a Poisson distribution and its log link function for count data (Table 1). The significance of the studied effects was determined through an ANCOVA of type II using a Wald χ^2 test followed by a Tuckey posthoc test with Bonferroni-Holm correction to perform pair-wise comparisons.

We completed our analyses by characterizing fruit length and survival rate at each measurement date over the course of the year for the different treatments of defoliation with GLMMs (Gaussian and binomial distribution respectively, treatment as the fixed effect and Tree / Branch / Shoot as a random effect).

We checked for dataset homogeneity (Supplementary data Table S1) and evaluated collinearities between variables by calculating the variance inflated factor (Dormann *et al.* 2013). This index is calculated as the inverse of the proportion of variance specific to each explanatory variable. It was calculated as $1/(1 - R^2)$ with R^2 the coefficient of determination of the linear regression between a given explanatory variable and the remaining variables. Marginal (R^2_m) and conditional (R^2_c) were calculated with the package MuMIn according to Nakagawa's method (Nakagawa and Schielzeth 2013) to estimate the variance explained by fixed effects and fixed plus random effects, respectively.

RESULTS

Fruit growth showed a linear progression in 2018, although it slowed down strongly from early September to early October when drought stress was maximum (measured predawn water potential of -3.1 ± 0.2 MPa on the 5th of September and -3.8 ± 0.3 MPa on the 4th of October). Growth resumed after the first heavy rains of autumn which occurred on October 6 and 7th (Fig. 3A and C). Less than half of the fruits reached maturity whatever the treatment (Fig. 3B). Like almost all the studied variables, mature fruit mass and fruit survival varied strongly between trees, branches (Supplementary data Fig. S2), and shoots, which also explains the fact that marginal R² (only fixed effects) is much lower than conditional R² (fixed + random effects, here shoot nested in branch nested in tree) of the models (1) and (3) (0.07 and 0.33 for model (1), 0.07 and 0.73 for model (3), Table 2). Besides, fruit-bearing branches were usually larger than non-fruit-bearing branches (Supplementary data Fig. S3).

Effect of defoliation on fruit growth, fruit survival, seed germination and photosynthetic activity

The effect of experimental defoliation on fruit survival depended on defoliation intensity: the moderate defoliation (MD) treatment had a negative effect on fruit survival compared to the control (coefficient parameter estimate ± standard error (SE): -0.67 ± 0.3), but not the intense defoliation (ID) treatment (Table 2 Analysis (1)), Supplementary data Table S2). Fruit survival decreased with second summer flush (Table 2 Analysis (1), -0.94 ± 0.3, Supplementary data Table S2) but increased with the number of fruits growing at the axil of the same leaf (Table 2 Analysis (1), 0.37 ± 0.2, Supplementary data Table S2). Neither the 2018 shoot basal area, nor the mean leaf area per fruit, nor the minimum predawn water potential of the tree affected the probability of fruit survival (Table 2 Analysis (1)). Defoliation did not affect the photosynthetic rate measured between one and two weeks after defoliation (Table 2 Analysis (2)).

The interaction of defoliation with the number of acorns per leaf was significant (Table 2 Analysis (3)), Supplementary data Table S2). Mature fruit mass increased with both moderate and

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intense defoliation only in case of more than one fruit per leaf (0.22 ± 0.09 and 0.21 ± 0.09 respectively). Neither the 2018 shoot basal area nor the mean leaf area per fruit, nor summer flush, nor the minimum predawn water potential of the tree affected the mature fruit mass (Table 2 Analysis (3), Supplementary data Table S2).

Mean germination rate was low in the three treatments (52 %, 52 % and 56 % in the control, MD and ID treatments respectively) and strongly positively correlated to fruit mass (Table 2 Analysis (4), coefficient parameter estimate ± SE: 0.84 ± 0.3). Defoliation did not affect the seed germination rate (Table 2 Analysis (4), Supplementary data Table S2).

Effect of defoliation on the production of vegetative and reproductive organs the following year

Shoots and leaves The number of shoots produced per 2018 shoot in spring 2019 was higher on branches that had re-flushed during the summer 2018 (9.7 shoots on average on reflushed branches compared to 4.0 on branches with no 2018 reflush, Table 3 Analysis (5), Fig. 4A). It was also positively correlated with the 2018 shoot basal area whatever the treatment (Table 3 Analysis (5)), Supplementary data Table S3), although defoliation affected this relationship (Table 3 Analysis (5), Supplementary data Table S3). For an equivalent 2018 basal area increment, less shoots were produced during spring 2019 when the branch was intensely defoliated compared to control (-0.39 ± 0.08). The effect was in a similar direction in moderately defoliated shoots although it was only marginally significant (-0.18 ± 0.09). Overall, the number of 2019 spring shoots per 2018 shoot was lower in the ID treatment (3.9 shoots on average compared to 5.3 shoots in the control treatment, Fig. 4A), while the number of leaves supported by 2019 spring shoots increased both with defoliation (Table 3 Analysis (6), Fig. 4B), Supplementary data Table S3) and with the basal area of 2018 shoots (Table 3 Analysis (6), 0.08 ± 0.02).

Male flowers The number of catkins per 2019 spring shoot was lower in the MD treatment than in the control, and even more so in the ID treatment (Table 4 Analysis (7), Fig. 5A), Supplementary data Table S4). Thus defoliation reduced the number of catkins and it reduced also the number of staminate flowers per catkin (Table 4 Analysis (8), -2.8 ± 1.2 for MD, -3.7 ± 1.2 for ID) but it had no effect on the number of stamens per staminate flower (Table 4 Analysis (9), Supplementary data Table S4). 2019 spring shoots carried by large 2018 shoots produced less catkins (Table 4 Analysis (7), coefficient parameter estimate ± SE: -0.05 ± 0.02). Catkin number was negatively related to the leaf number in the MD and ID but not in the control treatment (-0.33 ± 0.05 for MD, -0.35 ± 0.07 for ID, Supplementary data Table S4).

Female flowers The number of female flowers per 2019 spring shoot was reduced in both the MD and the ID treatments (Table 4 Analysis (10), Fig. 5B). The number of female flowers was positively correlated to the number of leaves in all treatments (Table 4 Analysis (10), coefficient parameter estimate ± SE: 0.66 ± 0.07). The number of female flowers was also positively related to the 2018 shoot basal area in the control and ID treatments (0.25 ± 0.11 and 0.09 ± 0.04 respectively, Table 4 Analysis (10)). The number of female flowers was negatively related to the number of fruits produced the year before both in the MD and the ID treatments (-0.46 ± 0.11 and -0.47 ± 0.12 respectively, Table 4 Analysis (10), Supplementary data Table S4), but not in the control treatment.

Sex ratio

The sex ratio (defined here as the proportion of female flowers produced by the shoot out of the total number of inflorescences of the shoot, i.e. female flowers plus catkins) was clearly biased towards male on all trees (13%, 21% and 21% of female flowers in the control, MD and ID treatments respectively, Supplementary data Fig. S4A). In average the sex ratio was not affected by defoliation because both female and male flower production was decreased on defoliated branches (Table 4 Analysis (11)), Supplementary data Table S4). The sex ratio was positively related to the 2018 shoot basal area (coefficient parameter estimate ± SE: 0.28 ± 0.05) and negatively related to the number of mature fruits produced in 2018 (-0.27 ± 0.07, Table 4, Analysis (11)). The interaction term between

treatment and a second flush in summer 2018 was significant (Table 4, Analysis (11), Supplementary data Table S4), with an effect of ID significant and positive only in absence of summer flush.

There was no relationship between female flower production and catkin production per 2019 spring shoot in the control and ID treatments, but a negative relationship appeared in the MD treatment (Supplementary data Fig. S5).

Fruit set

Fruit set in 2019 (proportion of female flowers that succeeded to initiate a fruit) was relatively low (33%, 48% and 30% in the control, MD and ID treatments respectively, Supplementary data Fig. S4B). Fruit set did not differ between treatments (Table 4 Analysis (12)) and was not affected by the 2018 shoot basal area (Table 4 Analysis (12)). However, fruit set was positively related to the number of leaves produced in 2019 (coefficient parameter estimate $\pm$ SE: 0.40 ± 0.18, Table 4, Analysis (11)).

Interactive effect of defoliation and reproductive status at defoliation

Shoots on defoliated branches that did not bear a single fruit in 2018 were similarly affected by defoliation than shoots that bore a fruit in terms of shoot production and catkins production the following year (Supplementary data Table S5 and Supplementary data Figs S6A and C). Leaf production per 2019 shoot was higher for fruit-bearing shoots compared to non-fruit-bearing shoots in control and MD treatment, but not in ID treatment (Supplementary data Table S5 and Supplementary data Fig. S6B). Female flower production in 2019 was higher on 2018 fruit-bearing shoots compared to non-fruit-bearing shoots in the control treatment, but this difference disappeared in the defoliated treatments (Supplementary data Table S5 and Supplementary data Fig. S6D).

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DISCUSSION

In this study, we investigated the impact of resource limitation induced by defoliation on the main steps of the reproductive cycle, from flower bud development to seed germination (Fig. 1). We found that defoliation applied shortly after fruit set had limited effects on fruit development, no effect on fruit final mass and germination success, and did not upregulate photosynthesis. In the following spring, we found that defoliated branches produced less shoots, less flowers per shoot and more leaves per shoot. We also found negative relationships between staminate flower and leaf productions in defoliated treatments, as well as between fruit production and subsequent flower production. Finally, we found that defoliation did not affect the sex ratio the following year.

Branches upregulate leaf production relative to flowers following defoliation

As we expected, defoliation reduced the number of catkins per shoot, the number of staminate flowers per catkin and the number of female flowers per shoot in the following year, while it increased the number of leaves. This allocation shift did not impact the number of stamen per flower, which might be more developmentally constrained. We observed a reduction, but not an interruption of female flowers production the year following defoliation. This contrasts with findings by Wiley *et al.* (2017) after whole tree defoliation in *Quercus velutina*, probably because, in our case, defoliated branches could rely on resources from non-defoliated branches further away.

The intense defoliation induced a reduction in the number of spring shoots the following year, consistently with the usually observed growth reduction after experimental defoliation in *Q. ilex* and other species (Vanderklein and Reich 1999; Piper and Fajardo 2014; Wiley *et al.* 2017; Schmid *et al.* 2017), although an increase in shoot production after defoliation has sometimes been observed (Cherbuy *et al.* 2001). *Q. ilex* has preformed buds (Montserrat-Martí *et al.* 2009), which were already formed at the time of defoliation. Thus, our experimental defoliation could not affect the bud set, but might have affected the allocation of resources to buds during their development from the summer that followed defoliation to the next spring. 2019 spring shoots bore on average more leaves in the defoliation treatments compared to control, consistently with previous studies on *Q. ilex* (Cherbuy *et*

al. 2001; Schmid *et al.* 2017) and other species (Iqbal *et al.* 2012; Nakajima 2015). *Q. ilex* is an evergreen species in which two or three cohorts of leaves usually coexist, and which has been shown to compensate for leaf loss in the following year (Cherbuy *et al.* 2001; Limousin *et al.* 2012). Therefore, a larger allocation toward vegetative organs was expected to compensate for the previous leaf loss.

We observed that branches favoured the completion of fruit development during the year of defoliation, but favoured the production of leaves relatively to flowers the following year. Our results at the branch level are consistent with the few studies which investigated the impact of resource manipulation on the different reproductive steps. In *Q. velutina*, Wiley *et al.* (2017) observed that production of second-year acorns was not significantly reduced after whole tree defoliation but that production of flowers was suppressed the following year on the defoliated branches because of priority investment in defoliation recovery. We could hypothesize that the regulation of reproduction in case of resource limitation preferentially takes place before flower initiation and/ or development than after a significant amount of resources, and especially nutrients, has already been invested in flowers. As both carbohydrates and nitrogen availabilities have been identified as potentially involved in the initiation of flowers (Miyazaki 2013; Miyazaki *et al.* 2014), such a regulation might be linked to the carbohydrate and nitrogen content of the branch.

To sum up, defoliated branches compensated for leaf loss the year following defoliation by increasing the number of leaves per shoot consistently with the defoliation intensity. The production of shoots, staminate flowers and female flowers was, however, reduced compared to those of the control treatment. The loss of resources induced by defoliation was compensated primarily for achieving the fruit development during the year of defoliation, and for recovering the leaf area in the following year.

Compensation of resource limitation and branch autonomy

Branch defoliation had contrasted effects on fruit and seed development during the months that followed (Fig. 1). First of all, defoliation, either moderate or intense, did not decrease the mass of the mature fruits, contrary to our expectations. Second, fruit abortion increased with moderate defoliation but not with intense defoliation. Third, defoliation had no effect on seed germination success, which was only positively related to the fruit mass, as previously observed in other oak species (Bonfil 1998; Huerta-Paniagua and Rodríguez-Trejo 2011; Sánchez-Montes de Oca *et al.* 2018; Shi *et al.* 2019). Results in the moderate defoliation treatment are consistent with the generally negative impact of natural herbivory on oak fruit production, even though natural herbivory intensity is usually, but not always, lower than a removal of half of the leaves (Crawley 1985; May and Killingbeck 1995; Pearse *et al.* 2015; Nakajima 2015; Canelo *et al.* 2018). The absence of the effect of intense defoliation on fruit production is, however, more surprising but is consistent with the recent studies of Pasqualetto *et al.* (2019) and Wiley *et al.* (2017) on hazelnut development, and oak acorns production (initiated before defoliation) in *Q. velutina*, respectively. However, our results contrast with those of the few experiments that tested how different defoliation intensities impacted fruit production, and either found an increasingly negative effect with increasing defoliation intensity (Mehouachi *et al.* 1995; Kaitaniemi *et al.* 1999; Hoch 2005) or no effect of defoliation at all (Tuomi *et al.* 1988; Obeso 1998; Tamura and Hiura 1998). Our results suggest that at the level of the branch, the compensation of resource loss in order to maintain fruit growth might differ depending on defoliation intensity.

The branch autonomy theory suggests that foliated branches are carbon, but not nutrient, autonomous for most of the growing season (Watson and Casper 1984; Sprugel *et al.* 1991). At the shoot scale, acorn growth in oaks is thought to be mainly based on carbohydrates produced during their development by the adjacent leaves (Hoch *et al.* 2013; Ichie *et al.* 2013; Fernández-Martínez *et al.* 2015) and also by the photosynthetic cells of their own pericarp until it dehydrates (Hoch and Keel 2006). At the branch scale, shoots bearing fruits are known to obtain a part of their resources from their adjacent shoots that do not bear fruits (Miyazaki *et al.* 2007; Sánchez-Humanes *et al.* 2011; Xie

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and Guo 2015). In *Q. ilex*, Alejano et al. (2008) observed that acorns from the southern side of trees were significantly heavier than those at other positions, suggesting a local regulation of resources allocation to fruits.

Fruit growth in the intense defoliation treatment was similar to that in the control, although we did not observe any increase of the photosynthetic rate in the remaining leaves to compensate for assimilate loss, consistently with most previous studies on oak saplings (Lovett and Tobiessen 1993; Vanderklein *et al.* 2001; Wiley *et al.* 2013). Therefore, carbon used for fruit filling either originated from recent photo-assimilates produced by non-defoliated branches further away (Oitate *et al.* 2011), or from local reserves in the shoot and its vicinity, or reserves in distant storage organs such as the stem and the below-ground parts. Additional measurements of the amount of stored non-structural carbohydrates in some small branches at the time of defoliation suggest that they would not be sufficient to fill all the acorns that reached maturity (results not shown). This suggests that the carbon contained in the mature fruits of highly defoliated branches probably came from further away, although girdling experiments would be necessary to strictly assess branch autonomy for fruit filling. Our experiment shows, however, that the higher fruit abortion rate on moderately defoliated branches results from a branch allocation strategy rather than from an impossibility to sustain fruit development despite lower leaf area. 2018 was an intermediate year in terms of fruit production for *Q. ilex* in the area, i.e. neither a mast seeding year nor a year with important reproductive failure (data not shown). If the same experiment had been realised during a mast year, the effect of defoliation might have been stronger because of increased competition for carbon between fruits. More experimental work is needed to understand how fruiting intensity might interact with defoliation effect by repeating the experiment on multiple years, and by coupling them to chemical analysis of both carbohydrates and nutrients reserves in branches in order to understand the physiological basis of allocations regulations.

Interestingly, shoot basal area did not affect mature fruit mass in 2018 and fruit set in 2019, contrarily to what has been observed in some fruit trees (George *et al.* 1996). The mean leaf area per fruit did not affect fruit mass either, consistently with observations at the branch scale in hazelnut trees (Pasqualotto *et al.* 2019). Therefore, fruit development might not be as dependent of local leaf

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photosynthesis as we initially expected. The ability of the branches to rely on distant sources of carbon to sustain fruit development might explain the absence of a clear defoliation effect in our experiment.

The production of shoots and flowers the year following defoliation appeared to be resource limited. The limiting resources might have been carbon, as leaf removal prevents local production of non-structural carbohydrates. However, leaf and flower production might depend more on nutrients such as phosphorus and nitrogen that are known to be involved in the proximate mechanisms driving mast seeding (Han *et al.* 2011; Sala *et al.* 2012; Miyazaki *et al.* 2014; Allen *et al.* 2017; Han and Kabeya 2017; Fernández-Martínez *et al.* 2019). As evergreen species store part of their nitrogen and phosphorus reserves in their foliage (Chapin *et al.* 1990; Cherbuy *et al.* 2001), trees lose a substantial portion of their nutrients with defoliation (Millard *et al.* 2001; Iqbal *et al.* 2012), although *Q. ilex* stores also a non-negligible part of the nutrient reserves in the shoots (Palacio *et al.* 2018). Defoliation treatment did not affect fruit set the following year. Besides, other factors known to impact fruit set in *Q. ilex*, such as pollen limitation or spring drought (Bogdziewicz *et al.* 2017), probably had minor impacts on fruit set in 2019 because spring was neither particularly dry nor particularly rainy during pollination.

Increasing resource limitation generates allocation trade-offs

Resource allocation trade-offs in trees may exist between vegetative growth and reproduction (Obeso 2002; Barringer *et al.* 2013), between current and future reproduction as currently assumed in mast seeding species (Koenig and Knops 2000) and between male and female flowering in monoecious species (Charlesworth and Morgan 1991). In this study, we found no clear evidence for a trade-off between vegetative growth and female reproduction at the shoot scale. On the contrary, we found that shoots that had initiated a fruit in 2018 were larger than those that had not, as already observed in *Q. ilex* (Sánchez-Humanes *et al.* 2011; Alla *et al.* 2012), and that large 2018 shoots produced 2019 shoots with more leaves and more female flowers. During morphogenesis, there might be a developmentally constrained positive relationship between growth and female function at the shoot scale, probably because large shoots can provide more nutrients to fruits. However, summer flush in 2018 had a negative impact on fruit growth, which suggests that the summer shoot growth was

a competing resource sink for fruits, and that there is a trade-off between shoot growth and fruit production when the two occur simultaneously.

The resource budget model hypothesis predicts that resources would be depleted after fruiting, which would limit the number of flowers produced the following year (Crone *et al.* 2009). A negative correlation between seed production one year and the next has indeed often been observed at the tree level in oaks (Sork *et al.* 1993; Kelly and Sork 2002; Pérez-Ramos *et al.* 2010). Consistently, we found a trade-off, at the shoot scale, between mature fruit production and female flower production the following year in defoliated branches. This result thus highlights the need for evaluating the reproductive costs at both the modular (shoot and branch) and the individual level, and over multiple years throughout individuals lifespan (Genet *et al.* 2010; Sánchez-Humanes *et al.* 2011; Sala *et al.* 2012; Bogdziewicz *et al.* 2019).

Sex allocation

Factors determining sexual allocation in natural tree populations are still poorly known, especially in mast seeding species (Kazuhiko 2007; Knops and Koenig 2012; Rapp *et al.* 2013). The sex allocation theory assumes the existence of a trade-off between male and female functions, and that in case of resource shortage, monoecious plants should shift towards maleness because maintaining the male function usually requires less investment than maintaining the female function (Charlesworth and Morgan 1991). This hypothesis might however not apply to mast seeding species in which increased pollination efficiency requires synchronous investment in male and female function during mast years (Rapp *et al.* 2013). Sex allocation is very much male-biased in *Q. ilex*, but we found no correlation between the number of female flowers and the number of catkins at the shoot scale in the control treatment, as observed in previous studies on *Q. ilex* (Pulido *et al.* 2014) and other oak species (Knops and Koenig 2012). Our results provide no support for the sex allocation theory, even in a context of resource limitation by defoliation, as defoliation reduced allocation to both pistillate and staminate flowers similarly. Sex allocation shift toward maleness therefore might occur after a resource limitation imposed by a defoliation at the plant scale (e.g. (Narbona and Dirzo 2010)), but not when defoliation is only applied at the shoot or branch scale (e.g. (Wang *et al.* 2016)).

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CONCLUSION

Our results strongly suggest that the regulation of resource allocation to reproduction occurs at a larger scale than the shoot scale, and that flower production is more sensitive to resource fluctuation than fruit development and seed germination success. Most importantly, our results also reveal the complexity in resource allocation strategies to the different plant functions over two consecutive years depending on the resource availability. Climate change is currently modifying significantly water and carbon availability through altered phenology, and sub-optimal temperature and soil moisture conditions, subsequently modifying the allocation of resources to the different organs. Our results show that it is essential to explore deeper the complexity of resource allocation to flowers to propose robust projections of tree fecundity, and subsequently of forest regeneration in future climatic conditions.

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Tables

Table 1: Detail of the structure of GLMMs used to establish the effect of defoliation on the allocation to reproduction in the year of defoliation and the following year: response variable, distribution of the model, set of covariates and random effects, number of observations and residual degrees of freedom. In addition to the effect of defoliation, the set of covariates shared by the models is given by the letters. A: 2018 shoot basal area, mean leaf area per fruit on 2018 shoot, summer flush in 2018 (yes / no), more than one fruit initiated per leaf (yes / no), minimum predawn potential of the tree (measured on October 4th); B: fruit mass ; C: 2018 shoot basal area, summer flush in 2018; D: 2018 shoot basal area, summer flush in 2018, number of mature acorns on 2018 shoot, number of leaves per 2019 shoot; E: 2018 shoot basal area, number of leaves per 2019 shoot; none: no covariable added.

Model #	Response variable	Model distribution	Set of covariables	Random effects	Nb. of obs (df)
Allocation to reproduction the year of defoliation (2018)					
(1)	Fruit survival	Binomial	A	tree|branch|shoot	918 (907)
(2)	Photosynthesis rate	Gaussian	none	tree	17 (12)
(3)	Mature fruit mass	Gaussian	A	tree|branch|shoot	247 (233)
(4)	Fruit germination success	Binomial	B	tree|branch|shoot	228 (221)
Allocation to reproduction the year following defoliation (2019)					
(5)	Nb. of 2019 spring shoots per 2018 shoot	Poisson	C	tree|branch	241 (230)
(6)	Nb. of leaves per 2019 spring shoot	Poisson	C	tree|branch	241 (232)
(7)	Nb. of catkins per 2019 spring shoot	Poisson	D	tree|branch	241 (230)
(8)	Nb. of staminate flowers per catkin	Gaussian	none	tree|branch	190 (184)
(9)	Nb. of stamens per staminate flower	Gaussian	none	tree|branch	190 (184)
(10)	Nb. of female flowers per 2019 spring shoot	Poisson	D	tree|branch	241 (228)
(11)	Sex ratio of 2019 spring shoots	Binomial	D	tree|branch	241 (228)
(12)	Fruit set on 2019 spring shoots	Binomial	E	tree|branch	140 (133)

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Table 2: Summary of GLMM testing the effect of defoliation and of other biological covariates on fruit survival, growth and germination, as well as photosynthetic rate in 2018. We report statistics, p-values, marginal (R^2_m) and conditional (R^2_c) R^2 for the reduced, final model. Characters in bold font refer to significant effects (p<0.05).

#	Response variable	Predictor	Wald χ^2 (p-value)	R^2_m (R^2_c)
(1)	Fruit survival between initiation and maturation	Defoliation treatment	8.7 (**0.01**)	0.07 (0.33)
		2018 shoot basal area	0.3 (0.6)	
		Mean leaf area per fruit on 2018 shoot	0.2 (0.7)	
		Summer flush in 2018 (yes / no)	8.1 (**0.004**)	
		More than one fruit initiated per leaf (yes / no)	4.0 (**0.04**)	
		Minimum predawn potential	0.6 (0.4)	
(2)	Photosynthesis rate	Defoliation treatment	0.3 (0.9)	0.01 (0.44)
(3)	Mature fruit mass	Defoliation treatment	4.1 (0.1)	0.07 (0.73)
		2018 shoot basal area	1.8 (0.2)	
		Mean leaf area per fruit on 2018 shoot	0.005 (0.9)	
		Summer flush in 2018	0.02 (0.9)	
		More than one fruit per leaf	0.2 (0.6)	
		Minimum predawn potential	0.9 (0.3)	
		Defoliation x More than one fruit per leaf	9.5 (**0.009**)	
(4)	Fruit germination success	Defoliation treatment	0.4 (0.8)	0.12 (0.51)
		Fruit mass	11.6 (< **0.001**)	

Table 3: Summary of GLMM testing the effect of experimental defoliation and of other biological covariates on growth parameters in 2019. For each model, interactions between treatment and all covariables were first included, and non-significant interactions were sequentially removed from the model starting with the least significant ones. We report statistics, p-values, marginal (R^2_m) and conditional (R^2_c) R^2 for the reduced, final model. Characters in bold font refer to significant effects ($p<0.05$).

#	Response variable	Predictor	Wald χ^2 (p-value)	R^2_m (R^2_c)
(5)	Number of 2019 spring shoots per 2018 shoot	Defoliation treatment	12.0 (**0.002**)	0.40 (0.63)
		2018 shoot basal area	103.3 (< **0.001**)	
		Summer flush in 2018 (yes / no)	23.7 (< **0.001**)	
		Defoliation x 2018 shoot basal area	29.0 (< **0.001**)	
		Defoliation x Summer flush in 2018	8.0 (**0.02**)	
(6)	Number of leaves per 2019 spring shoot	Defoliation treatment	22.7 (< **0.001**)	0.21 (0.90)
		2018 shoot basal area	26.9 (< **0.001**)	
		Summer flush in 2018	3.0 (0.08)	
		Defoliation x Summer flush in 2018	17.0 (< **0.001**)	

Table 4: Summary of GLMM testing the effect of defoliation and of other biological covariates on flowering and fruit set parameters in 2019. We report statistics, p-values and conditional (R^2_c) R^2 for the reduced, final model. Characters in bold font refer to significant effects (p<0.05).

#	Response variable	Predictor	Wald χ^2 (p-value)	R^2_m (R^2_c)
(7)	Number of catkins per 2019 spring shoot	Defoliation treatment	31.8 (< **0.001**)	0.43 (0.83)
		2018 shoot basal area	4.8 (**0.03**)	
		Summer flush in 2018 (yes / no)	1.6 (0.2)	
		Number of mature acorns on 2018 shoot	0.1 (0.3)	
		Number of leaves per 2019 shoot	37.9 (< **0.001**)	
		Defoliation x Leaves per 2019 shoot	17.9 (< **0.001**)	
(8)	Number of staminate flowers per catkin	Defoliation treatment	10.5 (**0.005**)	0.05 (0.62)
(9)	Number of stamens per staminate flower	Defoliation treatment	0.01 (1.0)	0.001 (0.52)
(10)	Number of female flowers per 2019 spring shoot	Defoliation treatment	12.6 (**0.002**)	0.40 (0.76)
		2018 shoot basal area	3.9 (**0.05**)	
		Summer flush in 2018	1.0 (0.3)	
		Number of mature acorns on 2018 shoot	23.4 (< **0.001**)	
		Number of leaves per 2019 shoot	89.2 (< **0.001**)	
		Defoliation x 2018 shoot basal area	7.9 (**0.02**)	
		Defoliation x Number of mature acorns	11.7 (**0.003**)	
(11)	Sex ratio	Defoliation treatment	2.5 (0.3)	0.12 (0.32)
		2018 shoot basal area	28.4 (< **0.001**)	
		Summer flush in 2018	1.0 (0.3)	
		Number of mature acorns on 2018 shoot	13.6 (< **0.001**)	
		Number of leaves per 2019 shoot	59.8 (< **0.001**)	
		Defoliation x Leaves per 2019 shoot	20.1 (< **0.001**)	
		Defoliation x Summer flush in 2018	8.9 (**0.01**)	
(12)	Fruit set in 2019	Defoliation treatment	2.3 (0.07)	0.05 (0.35)
		2018 shoot basal area	0.1 (0.5)	
		Number of leaves per 2019 shoot	5.1 (**0.02**)	

Figure legends

Figure 1: Diagram summarising the main results of this study. Each box corresponds to a statistical analysis and the numbers following dependent variables correspond to the model number in Table 1, 2 and 3. Only defoliation effects and significant covariables are shown here. The sign of the relationship between dependent variables and fixed effects is expressed as follows: "NS" = no significant difference with control, "+" = positive effect and "-" = negative effect.
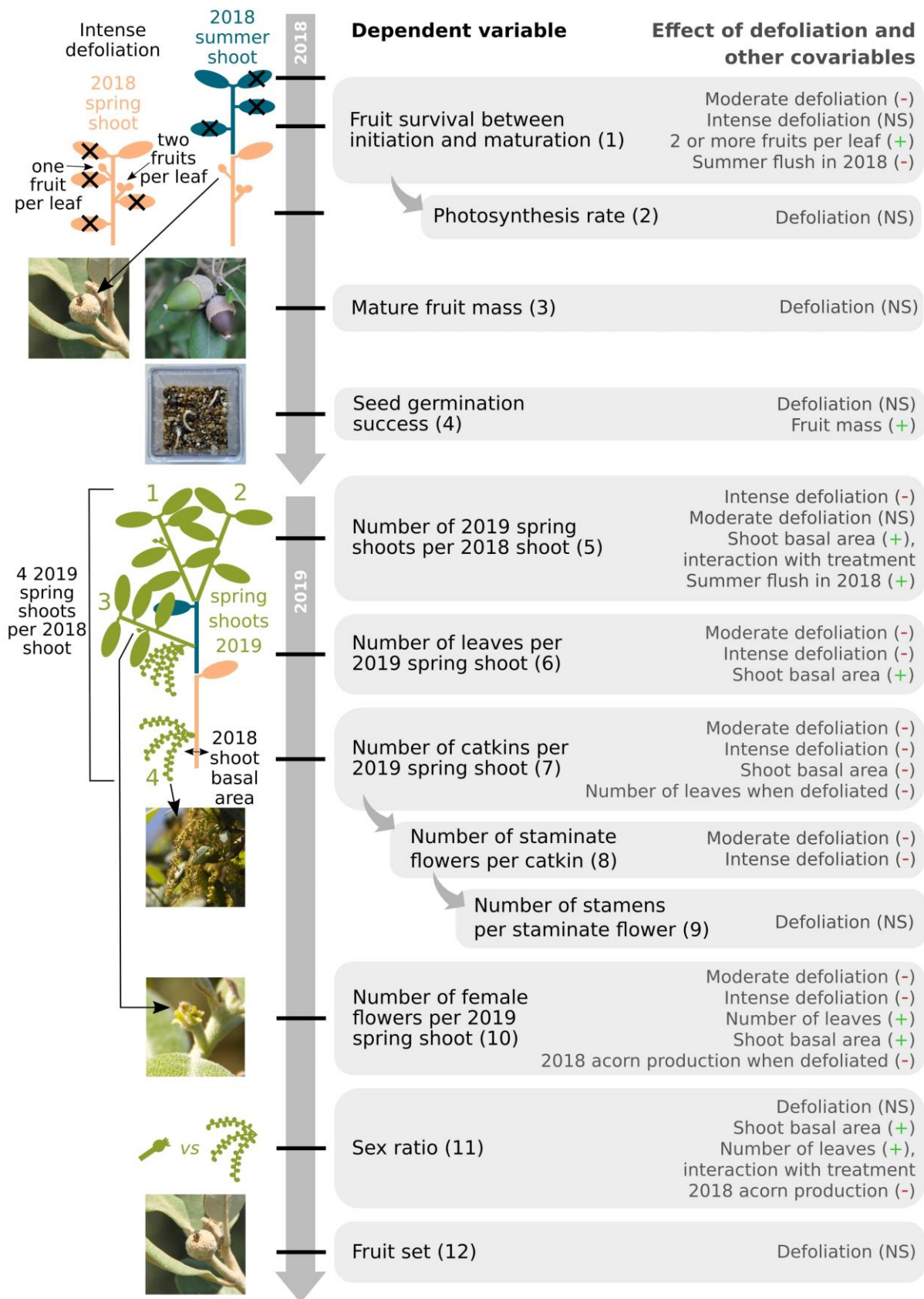
Figure 2: Schematic representation of the defoliation protocol.

Figure 3: Variation of (A) mean fruit size ($\pm$ 95% confidence interval, raw data) and (B) fruit survival rate for each treatment (all trees pooled together, raw data); and (C) daily precipitation from July 1st 2018 to Nov 7th 2018. "*" indicates a significant difference between treatments, whereas "ns" indicates no significant differences ($p < 0.05$) in the GLMM testing the effect of treatment alone as fixed effect and with Gaussian distribution for (A) and binomial distribution to test the survival of each acorn for (B).

Figure 4: Effect of the defoliation treatment and of a second growth flush during summer 2018 on (A) the number of 2019 spring shoots produced per 2018 shoot on a log10 scale, and on (B) the number of leaves per 2019 spring shoot. Full black points indicate group mean. Different letters correspond to the treatment effect in pairwise comparisons using Tukey post-hoc test on the GLMM (Table 1, Analysis (5) and (6) respectively) ($p < 0.05$). Within treatments, "*" indicates a significant effect of the summer flush in 2018, whereas "ns" indicates no significant differences (Tukey post-hoc tests with Bonferroni-Holm correction, $p < 0.05$).
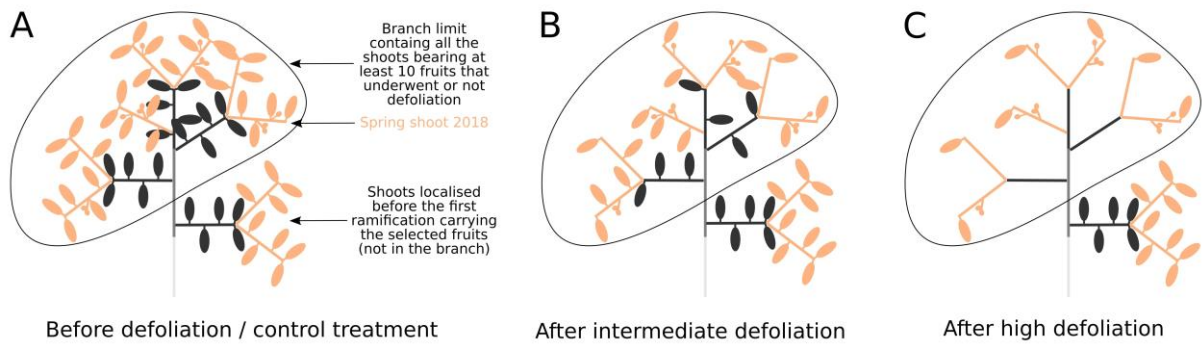
Figure 5: Effect of defoliation treatment on (A) the mean number of catkins and (B) the mean number of female flowers per 2019 spring shoot. Full black points indicate group mean. Different letters indicate significant differences between treatments (Table 1, Analysis (7) and (10) respectively). The statistical significance threshold is of 0.05.

Figure 1

Figure 2

A

Branch limit
containg all the
shoots bearing at
least 10 fruits that
underwent or not
defoliation

Spring shoot 2018

Shoots localised
before the first
ramification carrying
the selected fruits
(not in the branch)

Before defoliation / control treatment

B

After intermediate defoliation

C

After high defoliation

Figure 3

Figure 4

Figure 5