OIKOS

Research

Climate and host genotype jointly shape tree phenology, disease levels and insect attacks

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Oikos
129: 391–401, 2020
doi: 10.1111/oik.06707

Subject Editor: Kailen Mooney
Editor-in-Chief: Dries Bonte
Accepted 21 November 2019

One of the best known ecological consequences of climate change is the advancement of spring phenology. Yet, we lack insights into how changes in climate interact with intraspecific genetic variation in shaping spring and autumn phenology, and how such changes in phenology will translate into seasonal dynamics of tree-associated organisms. To elucidate the impact of warming and tree genotype on spring and autumn phenology, as well as the consequences for the population dynamics of a fungal pathogen *Erysiphe alphitoides* and plant-feeding insect *Tuberculatus annulatus*, we conducted an active field heating experiment using grafts of five oak genotypes *Quercus robur*. We found that experimental warming generally advanced oak bud burst in spring and delayed leaf senescence in autumn, while additional variation was explained by tree genotype and warming-by-genotype interactions. Warming or tree genotype did not affect disease levels at the beginning of the season, but shaped both disease levels and aphid density during the latter part of the season. Overall, our findings demonstrate that elevated temperature and genetic variation affect spring and autumn phenology, as well as the seasonal dynamics of higher trophic levels. Such effects may be either direct (i.e. temperature affecting tree phenology and attack independently) or indirect (as due to climate-induced changes in plant traits or the synchrony between trees and their attackers). To achieve a predictive understanding of the ecological responses and potential evolutionary changes of natural food webs in response to climate warming, we should merge the frameworks of global warming and community genetics.

Keywords: aphid–climate interactions, disease, experimental heating, herbivore, host genotype, pathogen–climate interactions, phenology, plant–climate interactions, powdery mildew, *Quercus robur*

Introduction

Variation in climate has a major impact on the timing of phenological events, with several reviews describing a general advancement of spring phenology in plants (Menzel et al. 2006, Forrest and Miller-Rushing 2010, Thackeray et al. 2016) and plant attackers (Both and Visser 2005, Dodd et al. 2008, Liu et al. 2011, Thackeray et al. 2016) during the last few decades. Differences in the response to climate may result in altered synchrony among species, and thereby affect the temporal dynamics and community

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structure of species at higher trophic levels (Van Nouhuys and Lei 2004, Both and Visser 2005, Kharouba et al. 2018). Likewise, plant genotype is known to influence both plant phenology (Elamo et al. 1999, Hoffman and Arnold 2008) and the community of associated species, with the latter studied within the framework of community genetics (Whitham et al. 2003, Tack et al. 2010, 2012, Zytynska et al. 2011, Barker et al. 2018). Hence, to predict the ecological and evolutionary responses of plant phenology and plant-based food webs to climate change, we may need to integrate studies on global warming with a community genetics perspective.

Long-term field observations and experimental warming studies have demonstrated that bud burst and the onset of growth in spring will advance with increasing temperature (Menzel and Fabian 1999, Parmesan 2007). However, the plant's response may differ among plant species and plant genotypes (Springate and Kover 2014, Evans et al. 2016). For example, Fu et al. (2015) demonstrated that while field heating generally advanced the timing of budburst in three species of temperate trees, the strength of the response differed among the tree species examined. There is less agreement in the literature on the drivers of autumn leaf senescence (Menzel et al. 2006, Gill et al. 2015), which has been shown to be affected by photoperiod, temperature, precipitation, plant genotype, spring phenology and their interactions (Liang 2016, Liu et al. 2016, Xie et al. 2018). Several studies have shown that higher summer and autumn temperatures delay autumn tree senescence (Shutova et al. 2006, Fu et al. 2018), whereas others found no effect of elevated temperature on autumn phenology of coniferous trees (Slaney et al. 2007). While spring phenology has been shown to be partly under genetic control (Forrest and Miller-Rushing 2010, Ghelardini et al. 2014, Evans et al. 2016), and plant genetic variation has been demonstrated to affect the response of plants to warming (Cooper et al. 2019), the role of genetic variation for autumn phenology remains poorly understood. As for spring phenology, autumn phenology may differ among plant genotypes, and plant genotypes may differ in their response to warming (Gallinat et al. 2015, Cooper et al. 2019). Understanding the genetic and climatic drivers of spring and autumn phenology is key to a predictive understanding of tree phenology and the length of the vegetation season, as well as the potential for evolutionary changes and adaptation of tree phenology to climate warming.

Temperature and plant genotype may also affect the population dynamics of plant-feeding insects and fungal diseases (Roy et al. 2004, Johnson and Agrawal 2005). Temperature can affect pathogen and insect growth either directly or indirectly through changes in the phenology of, and synchrony with, the host plant. For example, the highest infection levels of oak powdery mildew were detected when spore release coincided with the early stages of leaf development in spring (Marçais et al. 2009, Desprez-Loustau et al. 2010), and the abundance of oak herbivores may differ between early and late-flushing trees (Crawley and Akhteruzzaman 1988). Plant genotype is an additional strong driver of disease and insect dynamics in wild and agricultural systems (Flor 1955, Whitham et al. 2008, Laine 2011, Busby et al. 2014, Burdon and Laine 2019). As one mechanism, plant genotypes can vary greatly in chemical defense compounds and, subsequently, in their susceptibility to different herbivores (Service 1984, Donaldson and Lindroth 2007, Johnson 2008, Barker et al. 2019). Overall, we still lack insights in the joint impact of climate and plant genetics on the seasonal dynamics of plant-attackers (e.g. insect herbivores and fungal pathogens) within natural food webs.

In this study, we investigate the joint impact of global warming, plant genetics and their interaction on the spring and autumn phenology of the pedunculate oak *Quercus robur*, as well as the consequences for the seasonal dynamics of higher trophic levels. For this, we used an experimental approach, subjecting a set of grafted oak trees to either elevated or ambient temperatures for a full season under field conditions. More specifically, we targeted the following questions:

1) What is the relative importance of warming, tree genotype and their interaction for the timing of bud burst, leaf senescence and leaf longevity?
2) What is the relative importance of warming, tree genotype and their interaction for fungal disease levels and insect density?

We note that our study explicitly aims at establishing, quantifying and comparing the impact of temperature and tree genotype on tree phenology and plant attackers, whereas exposing the exact mechanisms involved will call for further targeted experiments informed by the current work.

Material and methods

Study system

The pedunculate oak *Quercus robur* is a long-lived deciduous tree, which is widely distributed across Europe and reaches its northernmost limit in Sweden (Lindbladh and Foster 2010). The oak provides habitat for a wide range of generalist and specialist fungal pathogens and plant-feeding insects (Tack et al. 2010, Marçais and Desprez-Loustau 2014). Among its fungal pathogens, powdery mildews are the most common. In particular, oak trees in Sweden are frequently attacked by the specialist powdery mildew pathogen *Erysiphe alphitoides*. This pathogen is easily detected in the field as the mycelium and conidial spores growing on the leaf surface, with only feeding organs (haustoria) penetrating epidermal cells (Bushnell 1972). Infection of oaks in spring is most likely through sexual spores (ascospores) that are released by overwintering fruiting bodies (chasmothecia) (Desprez-Loustau et al. 2010). During the growing season, the pathogen produces asexual spores (conidia), which results in multiple generations during the growing season. Infection by *Erysiphe alphitoides* may induce leaf necrosis and, in

extreme cases, cause leaf shedding in both natural populations and nurseries of young oak seedlings (Hajji et al. 2009, Marçais and Desprez-Loustau 2014). Among the sap-sucking insects, the aphid *Tuberculatus annulatus* is one of the most common species on *Q. robur* in Sweden, where it feeds on the abaxial leaf surface (Heie 1980, Avila et al. 2014). The aphid *T. annulatus* has multiple asexual generations during the growing season, allowing for rapid population growth. Peak aphid density is generally reported in mid-July, after which aphid reproduction slows down and densities decline (Silva-Bohorquez 1987). Aphids overwinter as eggs in either tree buds, tree bark or leaf litter (Leather 1980).

Experimental setup

To investigate the impact of warming and tree genotype on phenology, powdery mildew disease levels and aphid density, we conducted a heating experiment under field conditions. In the experiment, we used small (ca 1.2 m in height) oak trees that were grafted from five large mother trees, and will henceforth be referred to as genotypes. The mother trees were randomly selected from a 5 km^2 island in southwestern Finland (Supplementary material Appendix 1 Fig. A1). We have previously used 15 nuclear microsatellite loci to characterize genetic variation in the study landscape, including the five mother trees included in the current study. These studies revealed substantial genetic variation within the study landscape, with support for two genetic clusters (Pohjanmies et al. 2015, 2016). While the five genotypes are selected from, and reflective of this landscape scale, we do note that the number of genotypes selected are too few to thoroughly represent the genetic variation at the landscape scale.

The small oak trees used here were produced by grafting twigs from the five mother trees in 2011–2013. One twig was grafted onto a randomly selected rootstock as grown from acorns. Any foliage and branches sprouting from the rootstock were successively pruned, resulting in a crown composed only of the grafted genotype. The grafts were grown for several years, until large enough (ca 1.2 m) to be used in the experiment. As such, our experiment identifies the genotypic effect through the scion (i.e. the grafted twig of the mother tree). However, additional genetic variation, which ends up as among-tree variation within genotypes in our analysis, may be explained by the genotype of the rootstock (Tworkoski and Miller 2007, Kumari et al. 2015, Gautier et al. 2019).

On 9 May 2017, when buds were still dormant, we placed 140 grafted oak trees in six cages. Due to winter mortality, we lost eight trees, resulting in 132 trees in the experiment. The six cages were placed on a pasture belonging to the Swedish Livestock Research Centre at the Swedish University of Agricultural Sciences (SLU) at Lövsta, Uppsala (59°50′14.19″N, 17°48′77.82″E). To prevent grazers entering the experimental site, an electric fence was installed around the cages. For the experiment, cages (5 × 5 × 2.2 m) were built using wooden frames, and covered with a 0.20 × 0.40 mm mesh net to exclude insect predators during the course of the experiment (Supplementary material Appendix 1 Fig. A2). To achieve similar initial densities of powdery mildew infection and aphid infestation, we added 0.70 m^3 of homogenized leaf litter to the bottom of each cage. Within each of the six cages, 20–23 trees belonging to five different genotypes were randomly placed in a regular grid, with inter-pot distances of ca 30 cm. The design was slightly unbalanced, due to variation among the number of replicates per tree genotype (25–30 replicates per tree genotype, resulting in 3–6 replicates per genotype per cage). The position of the trees, within each cage, was randomized every second week. To keep soil moisture similar in both treatments and through time, trees were watered ad libitum. Following Kimball (2005), the temperature in three of the cages was increased by ca 2°C above ambient temperature from 9 May to 20 October, using three ceramic heaters (2000 W, 240 V) placed at 120-degree angles to each other. A thermostat maintained the temperature difference between control and heated cages. We explicitly aimed to test for the impact of warming during the growing season, ranging from the weeks before bud burst in spring till the end of bud formation in autumn. As such, our experiment does not shed light on the potential impact of warming during the preceding autumn and winter on spring phenology (Fu et al. 2012, Hänninen 2016).

Measurements

Spring phenology

To score spring phenology, we marked five random shoots per tree before the start of the growing season. For each shoot, median leaf development stage was scored following a categorical scale, where 1 = small dormant buds; 2 = large, slightly elongated buds; 3 = larger, loosened greenish brown buds; 4 = elongated buds with leaves starting to erupt (i.e. bud burst); 5 = leaves emerging but still tight; 6 = leaves loosening and extending outwards; and 7 = leaves are fully expanded and adopt their mature, dark green coloration (Hinks et al. 2015). Bud burst was then defined as the day when one of the five marked shoots on the tree had elongated buds with erupting leaves (i.e. leaf development stage 4). Leaf development stage was assessed every third day from mid-May, when buds were still dormant, until the beginning of June, when leaves were fully expanded.

Autumn phenology

Autumn phenology was scored using three complementary measures: 1) chlorophyll concentration, 2) leaf discoloration and 3) leaf abscission. Measurements were taken every fourteen days from the end of August until mid-October. We note that while our method allowed to assess the relative importance of warming and tree genotype on autumn phenology, a finer temporal resolution of measurements (e.g. once or twice per week) would be preferable to assess how individual drivers and their interactions affect the precise shape of the leaf-senescence curve.

To assess photosynthetic activity, we measured leaf chlorophyll concentration of ten randomly selected leaves with the chlorophyll content meter SPAD-502. On the same set of leaves, we scored leaf coloration using the following categories: 1 = leaf is entirely green; 2 = leaf with some discoloration, but more than half of the leaf is still green; 3 = more than half of the leaf with discoloration, but less than half of the leaf is brown; and 4 = more than half of the leaf is brown. We visually assessed the percentage of leaves dropped for each tree. Leaf senescence was defined as the day when 50% of the leaves had turned brown or had fallen, as interpolated from the measures of leaf senescence.

Leaf longevity

To detect the impact of warming and host genotype on leaf longevity, we calculated – for each individual tree – the difference between the date of bud burst and leaf senescence.

Disease levels

To detect the impact of warming and host genotype on fungal disease levels, we permanently marked ten randomly selected leaves per grafted oak tree at the beginning of June. During the two surveys in summer (on 14 July and 8 August), we scored the total number of leaves (out of ten) showing the disease symptoms and the percentage of leaf surface covered with the powdery mildew.

Insect density

To investigate the impact of elevated temperatures and host genotype on insect density, we counted the number of aphids on up to ten leaves on each of three shoots (the leading shoots of the main stem and two upper branches). These measurements were taken twice during the latter part of the season (on 1–2 August and 5–7 September) and used to calculate aphid density on a tree level.

Oak growth

To assess the impact of elevated temperature and tree genotype on tree growth, we measured two growth traits: average shoot length and bud size. Shoot length and bud size reflect the allocation of oak resources to growth processes (Saxe et al. 2002, Sanz-Pérez and Castro-Díez 2010). The length and diameter of ten randomly selected apical buds per tree were measured on 25 October 2017, using a digital caliper (accuracy 0.01 mm). These measurements were used to calculate volumetric bud size using the formula bud size = (bud diameter)^2 × bud length (Rutledge et al. 2008).

Statistical analyses

For the analyses, we used the framework of (generalized) linear mixed-effects models. Mixed-effects models were fitted using the lmer function in the package lme4 (Bates et al. 2015), and generalized mixed-effects models were fitted using the function glmer and function glmmPQL in the R-package MASS (Venables and Ripley 2013). The function Anova in the car package (Fox and Weisberg 2019) was used to assess the significance of the fixed effects.

To explore the impact of warming and oak genotype on the response variables of interest (leaf development in spring and leaf chlorophyll content, leaf discoloration and leaf drop in autumn, leaf longevity, disease levels, aphid density, shoot length and bud size) on specific dates, we modelled the response variables as a function of the fixed effects 'Treatment', 'Tree genotype' and their interaction. As we focused on the response of a relatively small number of tree genotypes (n = 5) to warming, we modelled 'Tree genotype' as a fixed effect. To account for variation among cages, we included 'Cage' as random effect. To account for measures taken on the same trees and shoots, we included the random effects 'Tree' and 'Shoot'. Additionally, to investigate the impact of warming and oak genotype on responses of interest across the season (from May to October), we modelled spring and autumn phenology, disease levels and aphid density as a function of the fixed effects 'Treatment', 'Tree genotype', 'Date' and their interaction. Here, 'Date' was treated as a factor, with levels reflecting the scoring events with different time intervals for each of the six responses: 1) for leaf development stage, 'Date' was used as a factor with ten levels, reflecting the scoring events with three day intervals; 2) for leaf chlorophyll content, leaf discoloration and leaf drop, 'Date' was used as a factor with three levels, reflecting measurements taken every fourteen days; 3) for fungal disease levels, factor 'Date' had two levels, with measurements taken on 14 July and 8 August; and 4) for aphid density, factor 'Date' had two levels, with measurements taken on 1–2 August and 15–16 September. A more detailed description of the statistical analysis can be found in Supplementary material Appendix 1. All model structures, their responses and functions are summarized in Supplementary material Appendix 1 Table A1.

Results

Spring phenology, autumn phenology and leaf longevity

Spring phenology was strongly affected by warming and tree genotype (Fig. 1, Table 1, Supplementary material Appendix 1 Table A2), with heating consistently advancing leaf development. The direction and strength of the response to warming differed among tree genotypes on some of the dates (Fig. 1, Table 1, Supplementary material Appendix 1 Table A2).

While warming accelerated spring phenology, it had the opposite effect in autumn: both leaf discoloration and leaf abscission were delayed in the heated cages (Fig. 2, Table 2, Supplementary material Appendix 1 Table A3). Chlorophyll content of leaves varied among tree genotypes during the early and middle part of autumn, whereas warming did not affect chlorophyll content at any time during the autumn (Fig. 2a–c, Table 2, Supplementary material Appendix 1 Table A3). While tree genotype affected the onset of autumn coloration, warming strongly delayed autumn coloration in late autumn (Fig. 2d–f, Table 2, Supplementary material Appendix 1 Table A3). Likewise, tree genotype and warming affected leaf abscission during the early autumn, whereas warming played a more decisive role at the end of the season

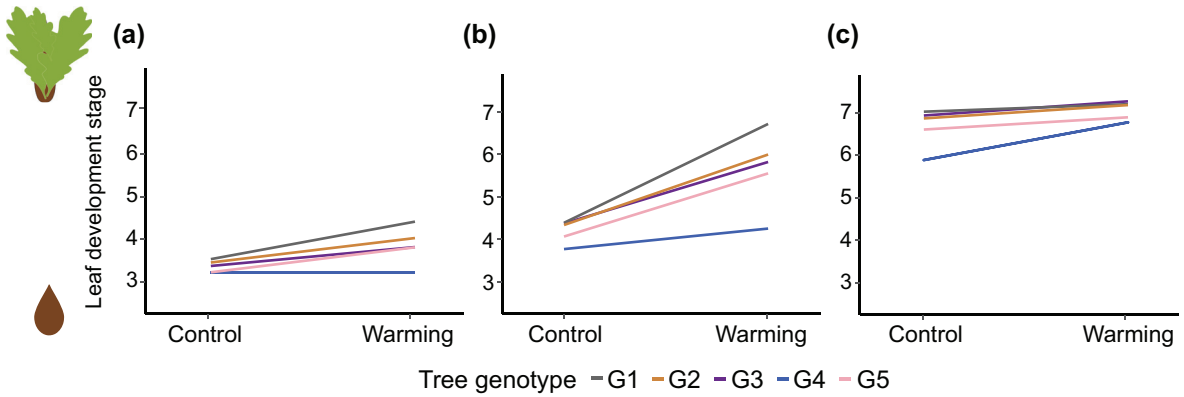

Figure 1. Interaction plot showing the impact of warming and tree genotype on spring phenology of *Quercus robur* on (a) 19 May, (b) 26 May and (c) 31 May. The lines connect the mean values for the control and warming treatment, with a separate line for each tree genotype. A similar figure, but including standard deviation for each treatment–genotype combination, is provided in Supplementary material Appendix 1 Fig. A3.

(Fig. 2g–i, Table 2, Supplementary material Appendix 1 Table A3). Leaf longevity was generally higher in the heated cages (Supplementary material Appendix 1 Fig. A5, Table A4).

Disease levels and aphid density

Warming and genotype did not affect the infection levels at the start of the growing season (when infection was low), but the infection level of the tree genotypes differed among the heated and control cages during the peak of the epidemic (Fig. 3a–b, Table 3, Supplementary material Appendix 1 Table A5). Warming, but not tree genotype, affected aphid density in early August (Fig. 3c–d, Table 3, Supplementary material Appendix 1 Table A6). Moreover, aphid density was strongly affected by both warming and tree genotype in early September (Fig. 3c–d, Table 3).

Oak growth

There was no detectable effect of warming on oak growth, as measured by either shoot length or bud size (Supplementary material Appendix 1 Table A7). Both shoot length and bud size differed strongly among tree genotypes (Supplementary material Appendix 1 Table A7).

Discussion

The advancement of spring phenology with climate change has been well documented, but we lack insights in how genetic variation and temperature drive phenomena during the remainder of the season (autumn phenology, length of the growing season), and how they affect interactions of organisms with higher trophic levels. In this study, we found that warming advanced spring phenology and delayed autumn phenology, with substantial variation among five tree genotypes in the timing of phenology and their response to warming. Trees exposed to elevated temperatures had similar disease pressure during the onset of the growing season, but disease levels depended on the interactive effect of temperature and tree genotype during the peak of the epidemic season. Herbivore density was higher in the heated cages, and differed among trees genotypes, during the end of the season. Overall, we show that the independent effects of warming

Table 1. The impact of warming treatment (T) and tree genotype (G) on the spring phenology of *Quercus robur* at ten dates during the growing season. Shown are the results of linear mixed models described in the text. Significant estimates ($p < 0.05$) are shown in bold.

	Treatment (T)			Genotype (G)			T × G		
Date	df	χ^2	p	df	χ^2	p	df	χ^2	p
Leaf development stage									
15 May	1	1.03	0.311	4	9.79	**0.044**	4	5.66	0.226
17 May	1	0.062	0.803	4	2.06	0.724	4	3.62	0.461
19 May	1	4.56	**0.033**	4	2.09	0.720	4	4.90	0.300
22 May	1	17.92	**<0.001**	4	13.76	**0.008**	4	7.12	0.129
24 May	1	31.16	**<0.001**	4	29.64	**<0.001**	4	5.93	0.205
26 May	1	53.59	**<0.001**	4	40.98	**<0.001**	4	16.13	**0.003**
29 May	1	20.73	**<0.001**	4	65.02	**<0.001**	4	5.53	0.340
31 May	1	6.31	**0.012**	4	31.18	**<0.001**	4	8.28	0.082
2 June	1	0.87	0.350	4	23.87	**<0.001**	4	10.18	**0.037**
5 June	1	0.31	0.580	4	12.39	**0.015**	4	14.52	**0.006**

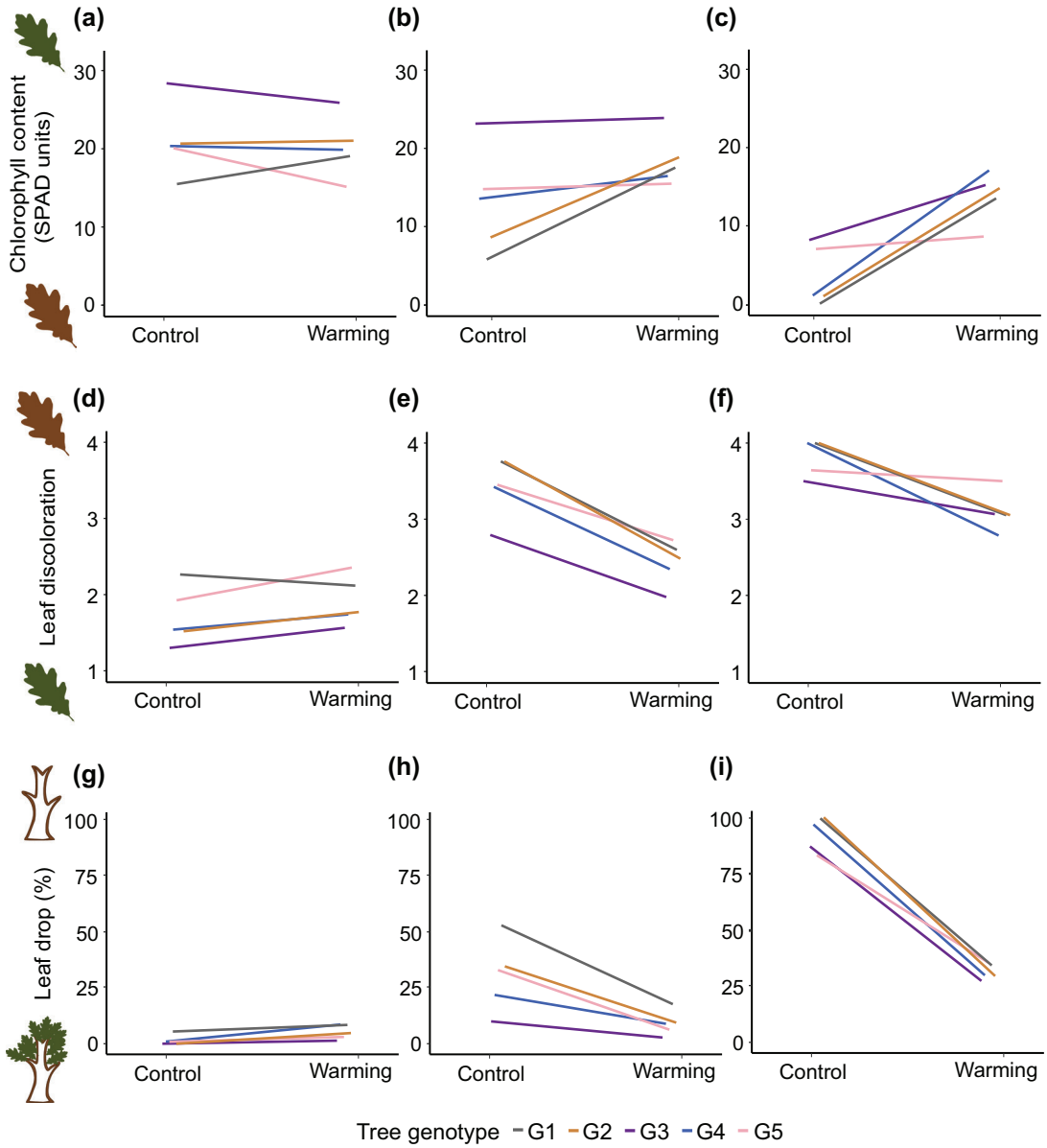

Figure 2. Interaction plot showing the impact of warming and tree genotype on autumn phenology of *Quercus robur*. The lines connect the mean values for the control and warming treatment, with a separate line for each tree genotype. Panels (a), (b) and (c) show the effect of warming and tree genotype on chlorophyll content on 24 August, 26 September and 10 October, respectively. Panels (d–f) show the effect of warming and tree genotype on autumn leaf coloration on 24 August, 26 September and 10 October, respectively. Panels (g–i) show the effect of warming and tree genotype on the percentage of leaves dropped on 24 August, 26 September and 10 October, respectively. A similar figure, but including standard deviation for each treatment–genotype combination, is provided in Supplementary material Appendix 1 Fig. A4.

and tree genotype, as well as their interaction, have an impact on tree phenology, length of the growing season and the seasonal dynamics of the associated natural attackers.

The impact of climate and host genotype on spring and autumn phenology

While many studies have demonstrated a relationship between temperature and plant phenological responses, few studies have experimentally explored its impact on both spring and autumn phenology (Morin et al. 2010, Kuster et al. 2014, Lutter et al. 2016, Sivadasan et al. 2017, Fu et al. 2018). As expected, we found that spring phenology advanced with warming (Parmesan 2007, Fu et al. 2015). Leaf senescence was delayed under elevated temperatures, a pattern that matches a delay in leaf senescence in response to warming as previously detected in both observational studies and experiments (Vitasse et al. 2009, Liu et al. 2016). However, it contrasts with experimental studies that found an advancement of autumn phenology with winter–spring warming for

Table 2. The impact of warming treatment (T) and tree genotype (G) on autumn phenology of *Quercus robur* at three dates during the growing season. Shown are the results of linear mixed models described in the text.

	Treatment (T)			Genotype (G)			T×G		
Date	df	χ^2	p	df	χ^2	p	df	χ^2	p
August 24									
Chlorophyll content	1	0.11	0.738	4	43.13	**<0.001**	4	9.60	**0.048**
Coloration	1	0.67	0.415	4	19.65	**0.001**	4	3.74	0.442
Leaf drop	1	3.54	**0.044**	4	27.62	**<0.001**	4	7.04	0.134
September 26									
Chlorophyll content	1	0.05	0.820	4	41.72	**<0.001**	4	2.66	0.620
Coloration	1	101.55	**<0.001**	4	31.31	**<0.001**	4	3.87	0.423
Leaf drop	1	17.61	**<0.001**	4	66.43	**<0.001**	4	7.11	0.130
October 10									
Chlorophyll content	1	0.11	0.742	4	4.06	0.400	4	1.54	0.673
Coloration	1	15.07	**0.001**	4	1.59	0.811	4	9.12	0.581
Leaf drop	1	15.53	**<0.001**	4	1.16	0.885	4	6.19	0.190

Significant estimates ($p < 0.05$) are shown in bold.

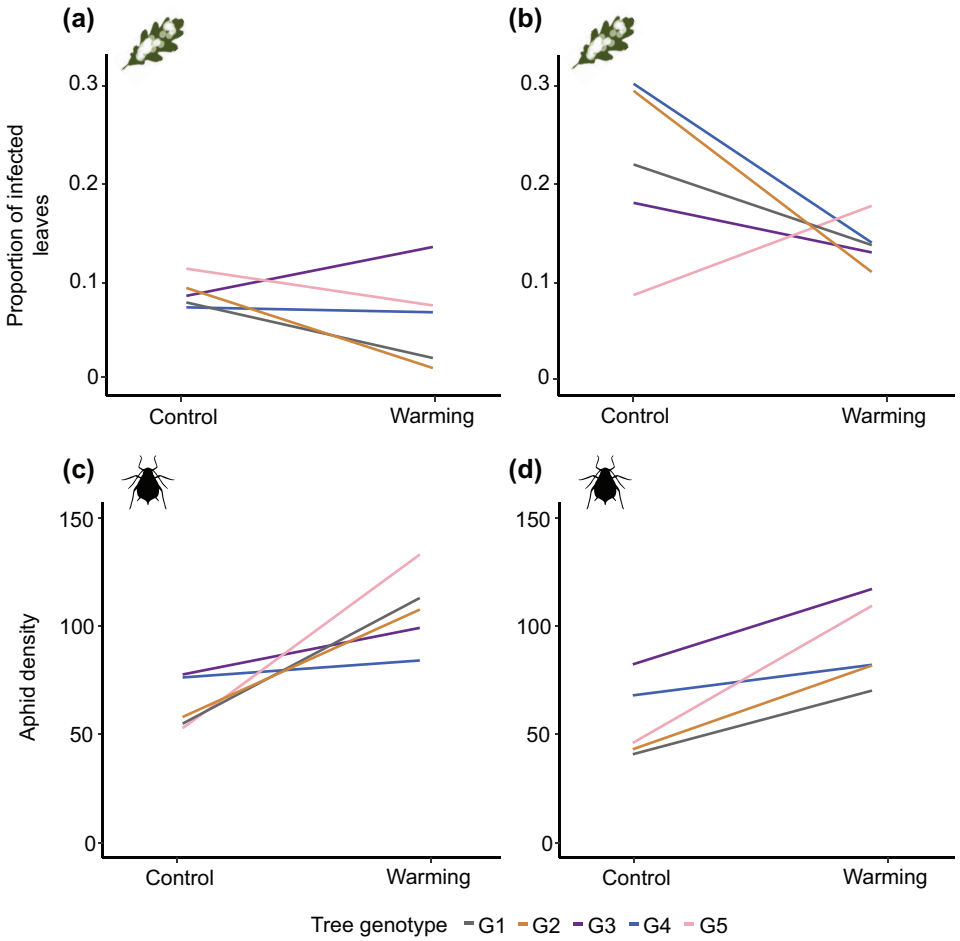

Figure 3. Interaction plot showing the impact of warming and tree genotype on powdery mildew *Erysiphe alphitoides* disease levels and aphid *Tuberculatus annulatus* density. The lines connect, for each tree genotype, the mean values for the control and warming treatment. Panel (a–b) show the effect of warming and tree genotype on the proportion of infected leaves on a tree level on 14 July and 8 August, respectively. Panels (c–d) show aphid density on 1–2 August and 5–7 September, respectively. A similar figure, but including standard deviation for each treatment–genotype combination, is provided in Supplementary material Appendix 1 Fig. A6.

Table 3. The impact of warming treatment (T) and tree genotype (G) on powdery mildew *Erysiphe alphitoides* disease levels and aphid *Tuberculatus annulatus* density. Shown are the results of linear mixed models. Significant estimates ($p < 0.05$) are shown in bold.

	Treatment (T)			Genotype (G)			T×G		
Date	df	χ^2	p	df	χ^2	p	df	χ^2	p
Fungal disease levels									
14 July	1	0.03	0.874	4	4.43	0.352	4	6.93	0.140
8 August	1	1.01	0.368	4	4.29	0.368	4	11.84	**0.020**
Aphid density									
1–2 August	1	4.14	**0.042**	4	1.29	0.862	4	6.90	0.141
15–16 September	1	6.93	**0.008**	4	28.77	**<0.001**	4	4.33	0.363

several tree species (Fu et al. 2014). Importantly, while warming advanced bud burst and delayed leaf senescence, it also affected leaf longevity. Similar findings have been reported in descriptive studies (Menzel and Fabian 1999, Penuelas et al. 2002). For example, Vitasse et al. (2009) showed that leaf longevity (and thus the growing season) of four species of deciduous trees (*Acer pseudoplatanus*, *Fagus sylvatica*, *Fraxinus excelsior* and *Q. petraea*) was extended at lower elevations as compared to higher elevations.

While several studies have separately reported effects of warming (Frei et al. 2014, Lutter et al. 2016) and plant genotype (Weih 2009) on spring or autumn phenology, the joint impact of these factors on plant phenology has rarely been studied (Vitasse et al. 2013, De Kort et al. 2016). In our study, spring phenology was advanced by warming and, at some of the dates, the response to warming differed among the tree genotypes. In autumn, the onset of leaf senescence (as measured by leaf discoloration and leaf drop) was strongly affected by tree genotype, whereas warming substantially delayed leaf senescence later in the season. Notably, the strong impact of tree genotype on the timing of both spring and autumn phenology indicates a potential for natural selection on oak phenology (including the length of the tree-specific growing season), and thereby an evolutionary response to a changing climate.

The impact of warming and host genotype on disease levels and aphid density

We found that powdery mildew disease levels were similar among genotypes and temperature treatments during the onset of the epidemic, whereas warming and tree genotype jointly influenced disease intensity during the peak of the epidemic. In contrast to our finding, previous studies have mainly found increased disease levels at higher temperatures (Garrett et al. 2006). However, these observational studies may be biased towards diseases that are known to prefer high temperatures and attack drought-stressed trees (Desprez-Loustau et al. 2007, Garbelotto et al. 2010). We further found a strong independent impact of plant genotype and warming on aphid densities, with higher densities in the heated cages during the end of the summer. Furthermore, our results add to the evidence that genetic variation of the host plant, as well as genotype-by-environment interactions, play an important role in disease dynamics and herbivore abundance (Roy et al. 2004, Tack et al. 2012, Busby et al. 2014, Barbour et al. 2015).

Several mechanisms may underlie the effect of warming on the disease levels and aphid densities in our experiment. First, the effects of warming on plant attackers can be direct, with warming affecting the development and phenology of the pathogens and herbivores. Second, the interaction may be indirect, where changes in plant traits (e.g. leaf thickness, secondary chemistry) may subsequently affect the performance of plant attackers. As the effect of temperature on the induction of defense compounds is known to differ among plant genotypes (Hartikainen et al. 2009, Shamloo et al. 2017), this may also explain the interactive effect between warming and genotype observed in the current study. Third, the effect of warming on the disease levels and insect density may be due to changes in the timing of the interactions between the plant and the plant attacker: the observed advances in bud burst and delay in leaf senescence, as well as possible changes in the phenology of the plant attackers, may result in a shift in synchrony between the plant and its attackers (Kharouba et al. 2018). Such changes in the relative timing may have pronounced impacts on oak disease levels and aphid densities. For example, oak leaves developing before or after the release of fungal pathogen inoculum in spring (i.e. the release of ascospores from the overwintering chasmothecia) may escape infection by powdery mildew (Desprez-Loustau et al. 2010). A final explanation for the higher density of aphids in the heated cages is a shortening of the generation time. The increased number of generations during the season may both accelerate population growth and adaptation of the aphids to the changing climate and plant genotypes. Overall, to better understand the mechanisms of how warming will effect host interactions with higher trophic levels, we need to focus on both direct effects of temperature on plant-attackers, but also indirect effects, through induced changes in plant traits and timing of the interaction.

Consequences for tree growth

While host genotype had a strong impact on shoot length and bud size, warming did not affect these measures of growth. The absence of an effect of warming in our experiment may be due to the temporal scale of the study (i.e. a focus on a single growing season). Indeed, previous multi-year studies have found that the effect of experimental heating on tree growth traits may take several years to become apparent (Arend et al. 2011, Kuster et al. 2014).

Conclusions

Overall, our study provides insights in the impact of climate change and genetic variation beyond spring phenology and is among the first to experimentally quantify the relative importance of, and interaction between, warming and plant genetic variation for autumn phenology, the duration of the tree-specific growing season and the consequences for interactions with higher trophic levels. Our findings highlight that to predict how climate change will affect spring and autumn phenology, the duration of active tree growth and the dynamics of diseases and herbivores at higher trophic levels, we should merge the perspectives of global change with that of community genetics. Overall, we hope that our experimental and comprehensive approach will stimulate the following avenues for future research and experiments. First, similar studies, in a range of study systems, will allow (or disprove) generalization of our findings of a joint impact of warming and tree genotype on spring phenology, autumn phenology and plant attackers. Second, evidence of the importance of warming and plant genotype (and their interaction) opens up the possibility to study the underlying mechanisms, and pinpoint the causal relationship between plant phenology and plant attackers. Third, similar studies across the distributional range of a single plant species (e.g. *Quercus robur*) will allow the assessment of latitudinal and altitudinal variation in the response of a single plant species to climate change. And fourth, our demonstration of the role of genetic variation, and of genotype-by-environment interactions, paves the way for future studies of selection gradients and micro-evolutionary adaptation of oak trees (and their natural attackers) to a changing climate.

Data availability statement

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d2547d7zv> (Faticov et al. 2019).

Acknowledgements – The authors thank Richard Childs for his assistance in setting up the experiment. We also thank Johan Ehrlén for the valuable comments on the manuscript.
Funding – This work was supported by the Bolin Centre for Climate Research, the Swedish Research Council (2015-03993 to AJMT) and financial support from SLU to the Unit of Insect Ecology (to TR).
Author contributions – MF, AJMT, AE and TR conceived and designed the experiment. MF and AE conducted the empirical work. MF analyzed the data. MF wrote the first draft, and all authors contributed to the final manuscript.

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Supplementary material (available online as Appendix oik-06707 at <www.oikosjournal.org/appendix/oik-06707>). Appendix 1.

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