Impacts of Tree Diversity and Drought on Forest Resilience to Pathogens

Elsa Field

Department of Plant Sciences, University of Oxford

Linacre College











Thesis submitted for the degree of Doctor of Philosophy

Michaelmas Term, 2020

I am so grateful to many people for their assistance over the course of my thesis. First and foremost, thank you to my DPhil supervisors: Andy Hector, Melanie Gibbs, Karsten Schönrogge, and Nadia Barsoum. You were the dream supervisory team and I am so grateful to all of you for assisting me with your knowledge and expertise.

Several other researchers were particularly involved over the course of my thesis and are co-authors on papers. Thank you to Hervé Jactel and Bastien Castagneyrol at INRA Bordeaux for their warm welcome and for all of the assistance along the way. I have loved working with you. Thank you to Julia Koricheva for her support with the meta-analysis, and to all the other researchers at TreeDivNet who contributed data. I am very inspired by the work that you all do.

Thank you to the amazing Plant Ecology lab group in Plant Sciences for the brilliant hive mind that came in handy many times over the course of my thesis! And many thanks to Gabriel, Paul and especially Jen at the Sylva Foundation for offering me an amazing opportunity to work with you for a few months during my thesis, to gain practical experience and some much-needed perspective.

With great thanks and love to the inspiring community that I live in, in Glastonbury, Somerset, for all of their support and vision that has assisted me in finding the motivation to finish this thesis. Thanks to my family for their love and belief in me throughout this DPhil. And thank you to my Beloved, Jerome, for walking this Divine path with me with the Nature spirits and with Goddess.

This thesis was funded by: NERC and a CASE Studentship from Forest Research

Copyright (©) Field 2020. All rights reserved

ELSAFIELDUK@GMAIL.COM

TABLE OF CONTENTS

Thesis Abstract *5*

List of Figures and Tables *6*

Chapter 1: **General Introduction** *16*

Chapter 2: **Individual tree traits shape insect and disease damage on oak in a climate-matching tree diversity experiment** *38*

Chapter 3: **Associational resistance to both insect and pathogen damage in mixed forests is modulated by tree neighbour identity and drought** *80*

Chapter 4: **Effects of water stress on growth characteristics, phenology and oak powdery mildew infection of *Quercus robur*** *112*

Chapter 5: **What are the impacts of mixed forest stands on forest pathogens? A systematic review and meta-analysis** *147*

Chapter 6: **General Discussion** *189*

References *204*

Appendix 1: **Supplementary material for Chapter 2** *243*

Appendix 2: **Supplementary material for Chapter 3** *259*

Appendix 3: **Supplementary analysis document for Chapter 3** *270*

Appendix 4: **Supplementary material for Chapter 4** *282*

Appendix 5: **Supplementary material for Chapter 5** *283*

Appendix 6: **Additional resources on Tree Health for Educators** *300*

Elsa Field
Linacre College

Doctor of Philosophy
Michaelmas Term 2020

Impacts of tree diversity and drought on forest resilience to pathogens

Thesis Abstract

The area of planted forest around the globe is increasing. Most new plantations are planted as monocultures, but evidence shows that mixed species forests can provide higher levels of multiple ecosystem functions without compromising productivity. In addition, mixed species forests are often purported to be more resilient to natural disturbances such as pest and pathogen outbreaks. In European forests, widespread species such as oak *(Quercus robur)* are currently under threat from multiple interacting abiotic and biotic threats, including pathogen damage and drought, both of which could increase under conditions of climate change.

In this thesis, I used planted experiments in which tree species and genetic diversity had been varied and studied the impacts on a specific, widespread forest pathogen (oak powdery mildew, *Erysiphe* spp.), and how it interacted with the insect herbivore community on oak. I found that the effects of tree diversity were strongly dependent on environmental context. In my UK field study, results were variable between years and study sites. I also found no evidence for local adaptation of the insect and pathogen community on oak to local hosts. In my field study from SW France, in which both tree species diversity and drought were experimentally varied, I did find evidence for lower pathogen damage at higher diversity levels, as well as effects of tree neighbour identity. Drought did not alter pathogen damage, but it did alter the strength of the diversity effects on insect herbivory. I also found evidence for tripartite interactions between host, pathogen and insects, showing that interactions between multiple damage agents can play an important role in determining tree health.

Lastly, I synthesised evidence of the effects of mixed species forests on pathogen damage using meta-analysis and systematic review of previous studies. I found a negative effect of tree diversity on pathogens that was only significant in temperate forests. Synthesis of both quantitative and qualitative evidence again showed the importance of tree neighbour identity rather than host abundance in determining diversity effects.

Overall, I show that tree diversity can lower forest pathogen damage, but that consideration of specific interacting abiotic and biotic variables is also crucial to provide evidence-based recommendations to practitioners.

List of Figures and tables

Figures

Figure 1: Primary and Lammas Shoots on a pedunculate oak (*Quercus robur*). 30

Figure 2: Location of UK Climate Match experiment sites, Hucking in Kent (black circle) and Hartshorne in Derbyshire (black square), and sources of "climate matched" oak provenances. 47

Figure 3: Design of the treatments planted at the Climate Match experiment. 48

Figure 4: The impact of provenance identity on powdery mildew, gallers, leaf miners, and leaf manipulators. 63

Figure 5: Partial residual plots showing the modelled impacts of lammas shoot length, tree height and tree apparency on powdery mildew infection at Hucking and Hartshorne in 2016 and 2017. 64

Figure 6: Partial residual plot showing the conditional impact of leaf miner abundance on powdery mildew infection at Hucking (2016), when all other variables are held constant in the model. 65

Figure 7: The effect of tree species richness and irrigation (A) and irrigation and birch presence (B) on leaf miner abundance. 100

Figure 8: The effect of tree species richness and birch presence (A) and the effect of irrigation and birch presence (B) on leaf chewer damage. 101

Figure 9: Partial residual plot with the regression lines showing the strength of the relationship between tree species richness and mildew infection when all other variables are held constant in the model. 102

Figure 10: The relationship between leaf miner abundance and oak powdery mildew infection. 103

Figure 11: Experimental set up of oak saplings on pallets, showing the plastic skirt coverings (July 2018). 122

Figure 12: Weight monitoring of pots in different experimental treatments during the experimental period. 124

Figure 13: Differences in pot weight (g) and volumetric soil moisture content (%) between treatments at the end of the experimental period. 131

Figure 14: Total July rainfall (A) and maximum July temperature (B) recorded at the CEH Met Office Weather Station, 1980 - 2018. 132

Figure 15: Differences between oak height (A), stem diameter (B) and the change in height (C) and change in stem diameter (D) over the period assessed. 133

Figure 16: Differences in oak sapling shoot biomass, root biomass, gravimetric water content of shoots, and shoot:root biomass ratio between treatments. 134

Figure 17: Differences in the number of bud bursts per sapling (A) number of primary flush shoots (B), secondary flush shoots (C), third flush shoots (D), fourth flush shoots (E) and the total number of lammas (second-fourth flush shoots, F) between treatments. 135

Figure 18: Correlations between initial bud burst stage of saplings and secondary bud flushes. 136

Figure 19: PRISMA record workflow of studies included in the systematic review. 155

Figure 20: Geographical distribution of countries for which quantitative or qualitative data was extracted. 165

Figure 21: Pie charts showing the distribution of studies by damage organ, pathogen type and host type. 167

Figure 22: Caterpillar plot showing effect sizes and associated 95% confidence intervals, in order of effect size for all 74 studies included in the meta-analysis. 169

Figure 23: Effect of biome on effect size (Hedges' *d* – mean effect size and 95% confidence intervals for each biome; tropical, temperate or boreal). 170

Figure 24: Effect of pathogen specialism on effect size (Hedges' *d*). Shown is the mean effect size and 95% confidence intervals for pathogen specialists and generalists. 171

Figure 25: Effect of presence of alternative hosts (Y/N) on effect size (Hedges' *d*). Shown is the mean effect size and 95% confidence intervals for each group. 171

Figure 26: Effect of pathogen life history on effect size (Hedges' *d*). Shown is the mean effect size and 95% confidence intervals for necrotrophs and biotrophs. 172

Figure 27: The effect of risk of bias on effect size. Shown is the mean effect size and 95% confidence intervals for low and high risk studies. 173

Figure 28: Summary of factors to consider in the outcome of mixed stands on forest pathogens, arranged around the classical disease triangle of pathogen, host and environment. 183

Figure 29: Partial residual plots from Chapter 2 (left-hand plot) and Chapter 3 (right-hand plot), presented side by side. 194

Tables

Table 1: Three major theories describing the effects of plant diversification on pest organisms. 21

Table 2: An overview GLMs of oak powdery mildew infection and insect herbivore abundance, showing all explanatory variables included in starting models. 55

Table 3: Summary of results of GLMs of oak powdery mildew. 68

Table 4: Summary of results of GLMs of oak insect herbivores. 69

Table 5: Results of GLMMs testing the effects of plot composition and tree traits on oak powdery mildew and insect herbivory at shoot and tree level. 98

Table 6: Treatments used in the water stress experiment. 121

Table 7: Results of factorial ANOVAs (F tests) conducted to test the impacts of treatment and bud burst on response variables. 137

Table 8: A breakdown of the mechanisms behind diversity-disease effects reported in qualitative papers, with citations provided for which that mechanism was reported. 175

Supplementary Figures

Figure S1: Differences in primary shoot length (A-C) and lammas shoot length (D-F) between provenances. 248

Figure S2: Differences in tree height between provenances. A = Hucking 2017, B = Hartshorne 2017. 249

Figure S3: Differences in lammas shoot availability between provenances. A = Hucking 2017, B = Hartshorne 2017. 249

Figure S4: Boxplots showing differences in tree apparency and tree height between diversity treatments in the two sites. 250

Figure S5: Partial residual plot showing the conditional effects of provenance and block on powdery mildew at Hartshorne in 2017. 253

Figure S6: Partial residual plot showing the conditional effects of provenance and treatment on gall abundance at Hucking in 2017. 254

Figure S7: Partial residual plot showing the conditional effects of provenance and treatment on gall abundance at Hartshorne in 2017, in block 1 (top panel) and block 2 (bottom panel), as these two blocks contain the mixed species plots. 255

Figure S8: Partial residual plot showing the conditional effects of treatment and block on leaf miner abundance at Hartshorne in 2017. 257

Figure S9: Partial residual plot showing the impact of tree height on powdery mildew infection for an updated model where outliers are removed, Hucking 2017. 258

Figure S10: Experimental plan of the ORPHEE tree diversity experiment in SW France (44°440 N, 00°460 W). 259

Figure S11: Boxplots showing A) oak (*Quercus robur*) height (cm), B) oak apparency (%) and C) canopy closure (%) between different plot compositions, measured July 2018. 260

Figure S12: Correlations between continuous variables used as covariates in models of oak insect herbivory and powdery mildew. 262

Figure S13: The relationship between tree species richness and resource dilution of *Q. robur* (Qr) in plots surveyed at the ORPHEE experiment. 263

Figure S14: The relationship between tree species richness and presence/absence of birch in plots surveyed at the ORPHEE experiment. 264

Figure S15: Comparison of microclimate in oak monocultures ('Oak') and oak-birch mixtures ('Oak + Birch'). Temperature (°C) and Relative Humidity (RH, %) were measured every hour from May-October, in 2016, 2017 and 2018. 265

Figure S16: The relationship between mean leaf chewer damage (at tree level per leaf, %) and mean defoliation (at tree level per leaf, %). 266

Figure S17: Oak Bud Burst stages (as assessed in March 2018). 282

Figure S18: Bar charts showing categorical moderators used in mixed effects meta-analysis models, plotted against number of studies. 295

Figure S19: Effect of diversity metric used in studies on effect size (Hedges' *d*). Shown is the mean effect size +/- 95% confidence intervals for each group. 296

Figure S20: Effect of study type (experimental versus observational) on effect size (Hedges' *d*). 297

Figure S21: Effect of study level (single tree level versus plot level estimates) on effect size (Hedges' *d*). 297

Figure S22: Comparison of directly calculated effect sizes and those converted from other metrics of effect size. 298

Figure S23: Funnel plots showing observed outcome (study effect sizes) against study standard error, variance, and inverse standard error/inverse variance. 299

Supplementary Tables

Table S1: Explanation of the post hoc tests and/or analyses completed for each model. 243

Table S2: F tests for one-way ANOVAs testing the effect of provenance on tree phenotypic traits. 247

Table S3: The effects of diversity treatment on powdery mildew infection and insect herbivore abundance at each site and year. 252

Table S4: Results of GLMMs testing the effects of plot composition and tree traits on oak powdery mildew and insect herbivory at shoot level. 267

Table S5: List of search strings used in bibliographic databases and their relationship to each component of the primary question. 285

Table S6: List of search strings used in each major database queried during the systematic review, with number of hits returned and date initially searched. 286

CHAPTER 1: GENERAL INTRODUCTION

Forests cover 30.8% of land area on the globe, supporting not only the majority of terrestrial animal and plant species, but also providing crucial eco-system functions and services for life on Earth (Boyd et al. 2013; FAO 2020). Significant anthropogenic deforestation mostly driven by agricultural expansion threatens these eco-systems, with a reduction in net forested area of 2.7% between 1990 and 2020 (Keenan et al. 2015; FAO 2020). Primary forests globally are being replaced, in part, with plantation forests, which increased in area from 168 Mha to 294 Mha between 1990 and 2020 (Keenan et al. 2015; FAO, 2020). The increase in planted forests is also driving the trend towards net forest gain over the last decade in some continents, such as Asia and Europe (FAO, 2020).

Over 99.9% of new plantations are planted as monocultures rather than tree species mixtures, contributing to global biodiversity loss (Nichols, Bristow, & Vanclay, 2006). Moreover, much evidence suggests a strong link between biodiversity and ecosystem functioning in forests (Forrester & Bauhus, 2016; Gamfeldt et al., 2013). Thus, the conversion of biodiverse natural forest to monoculture plantations will likely result in depauperate provision of ecosystem services that are crucial for human well-being (Cardinale et al., 2012; van der Plas, 2019).

Biodiversity and Ecosystem Functioning Experiments

Over the last two decades, researchers have developed experimental approaches to understand the relationship between biodiversity and ecosystem functioning. Early research utilised short-lived, fast growing grassland ecosystems, where species diversity was experimentally varied (Hector et al. 1999; Tilman, Wedin, and Knops 1996). These studies have shown that there is often a positive, saturating relationship between biodiversity and several ecosystem functions (Cardinale et al., 2006). Two mechanisms drive the relationship between biodiversity and ecosystem functioning: the selection effect, and complementarity, which are not mutually exclusive (Hector 1998; Loreau and Hector 2001). The selection effect describes a null hypothesis whereby functions are higher in mixtures due to the increased chance of sampling an especially functional species as the species pool increases. Alternatively, the complementarity hypothesis suggests that functional trait complementarity between species can lead to higher provision of services, over and above the additive effects of individual species (Loreau & Hector, 2001).

Forest Diversity and Ecosystem Functioning Experiments

In the last few years, research on the relationship between biodiversity and ecosystem functioning has moved towards longer term, larger scale studies in forests (Verheyen et al., 2016). Consistent with grasslands, there is a positive relationship between forest species diversity and productivity (Benneter, Forrester, Bouriaud, Dormann, & Bauhus, 2018; Fichtner et al., 2018), ecological stability (Morin, Fahse, de Mazancourt, Scherer-Lorenzen, & Bugmann, 2014; Plas

et al., 2016), and resistance to natural disturbances (Jactel et al. 2017) and drought stress, among other ecosystem functions (Ampoorter et al., 2019; Forrester, 2015). Several networks have been established to synthesise research on biodiversity and ecosystem functioning in forests, including observational forest platforms in European forest, FunDivEurope and EuMixfor (Baeten et al., 2013; Bravo-Oviedo, Barreiro, Strelcova, & Pretzsch, 2014) and a global network of tree diversity experiments, TreeDivNet (Paquette et al., 2018; Verheyen et al., 2016). Tree diversity experiments provide an important intermediary platform between purely observational studies in the forest environment and highly controlled experiments in greenhouses or climate chambers. Experimental designs within TreeDivNet vary from investigating forest species to genetic and functional diversity impacts on ecosystem functioning, and have been established in temperate, tropical and boreal systems (Verheyen et al., 2016).

Tree Diversity Impacts on Insect Pests

Tree diversity experiments are providing evidence to forest policy makers and managers, incentivising an increase in the planting of mixed forests (Grossman et al., 2018; Paquette et al., 2018; Pretzsch, Forrester, & Bauhus, 2017). However, a recent survey of European forest managers revealed that many knowledge gaps remain surrounding mixed forests (Coll et al., 2018). Notably, the top two questions identified by foresters concerned the resilience of mixed forests to natural disturbances and to climate change. Foresters had questions both around the overall relative resilience of mixed forests compared to monocultures, and

about which mixtures of species provide highest resilience (Coll et al., 2018).

Planting stands of multiple species is often promoted as a method of economic "bet-hedging" for foresters, reducing the risk associated with large scale disturbances such as the impact of emerging pests and pathogens (Jactel et al. 2009). If one species is removed due to the impact of a pest organism, the economic value of the stand is not totally lost. In addition to economic resilience, it is often assumed that ecological mechanisms lead to mixed forests being more resistant to pest and pathogen damage compared to monocultures, as can be the case in agricultural systems (Mundt, 2002) and experimental grassland communities (Mitchell, Tilman, and Groth 2002). A more species rich planting may reduce pathogen damage via the dilution effect, which describes a reduction in susceptible host density leading to reduced colonisation of pathogens by hosts (Keesing et al., 2010). However, the lack of clear empirical evidence for the dilution effect on forest pathogens has been noted by previous authors (Koricheva et al. 2006; Pautasso, Holdenrieder, and Stenlid 2005). Meta-analysis describes the quantitative synthesis of results from multiple studies on the same topic (Koricheva, Gurevitch, & Mengersen, 2013). A recent broad scale meta-analysis finding evidence of the dilution effect across several study systems included just one article from forests (Civitello et al., 2015). Another meta-analysis focused on plant diversity effects on infectious disease found non-significant effects of tree diversity on pathogens, but this included only six papers on forests, all of which were from higher latitudes (Liu et al, 2020).

There are innate difficulties involved in carrying out experiments on long-lived,

large woody organisms compared to short-lived agricultural crops and grassland species. However, these same difficulties may result in differences in the response of mixed forest stands to pathogens compared to other ecosystems. In particular, Mundt (2002) notes that the larger host:pathogen size ratio of woody species compared to crop species can lead to increased frequency of autoinfection, reducing the effectiveness of using mixtures to lower pathogen damage, especially over the long life span of a forest plantation.

In contrast to pathogens, the effects of forest stand diversity on insect pests have been a topic of greater study. The three major theories concerning the effects of plant diversification on insect herbivory are shown in Table 1 (reviewed in Moreira et al. 2016). Recently, Jactel et al (2017) proposed expanding the use of the terms associational resistance (a reduction in pest damage in more diverse stands) and associational susceptibility (an increase in pest damage in more diverse stands) to all biotic agents of damage in plant communities, including both pests and pathogens. Meta-analysis has proved a useful tool in the study of mixed forests and insect outbreaks. Several meta-analyses have shown that associational resistance, rather than associational susceptibility, is the most common response for specialist insect herbivores (Castagneyrol et al. 2014; Jactel and Brockerhoff 2007; Vehviläinen, Koricheva, and Ruohomäki 2007). In a study of seven long term forest experiments, Vehvilainen et al (Vehviläinen et al., 2007) found that damage by specialist herbivores (leaf miners) was consistently reduced in more diverse stands. Jactel and Brockerhoeff (2007) in a meta-analysis of literature from 1966-2006, also found that damage by specialist herbivores was consistently reduced in mixed stands, while the response of generalist insects was more

Theory	Description	Representative references
Associational Resistance (Tahvanainen & Root, 1972)	Increased plant diversity reduces pest damage, various suggested mechanisms	Forests: (Jactel & Brockerhoff, 2007) Grassland: (Hambäck, Ågren, & Ericson, 2000)
Associational Susceptibility (Brown & Ewel, 1987)	Increased plant diversity increases pest damage, various suggested mechanisms	Forests: (Castagneyrol et al., 2012) Grassland: (Utsumi et al., 2011)
Natural Enemies Hypothesis (Elton, 1958)	More diverse plant assemblages have greater diversity and abundance of natural enemies of pests, leading to reduced pest damage overall	Forests: (Sobek et al., 2009) Grassland: (Johnson, Lajeunesse, & Agrawal, 2006)

Table 1: Three major theories describing the effects of plant diversification on pest organisms.

variable. Castagneyrol et al. (2014) showed that while specialist herbivores were consistently reduced in mixed stands, generalist herbivores were only reduced in the presence of highly unrelated hosts (in mixtures of broadleaved and coniferous species). Because the most damaging herbivores are normally specialist species,

this may explain the overall tendency towards associational resistance in mixed stands, rather than associational susceptibility (though see Aakala et al. 2016; Plath et al. 2012; Schuldt et al. 2010 for examples of associational susceptibility). Associational resistance in mixed stands may therefore be largely driven by a dilution effect (lower concentration of susceptible hosts in mixed stands), preventing insect populations from reaching outbreak levels (Castagneyrol, Jactel, & Moreira, 2018; Moreira et al., 2016).

In addition to lower density of susceptible hosts in mixed stands, other mechanisms have been proposed that could explain associational resistance to insect pests in mixed forests. Associational resistance may be driven by a reduction in "plant apparency" in mixtures (Castagneyrol, Giffard, Péré, & Jactel, 2013). The Plant Apparency hypothesis (Feeny, 1976), predicts that larger plants may be "bound to be found" by herbivores, due to higher apparency in the landscape (height relative to their surroundings), and that this may lead to lower investment in quantitative defences against herbivores. Although limited support has been found for differences in defence compound allocation that can be linked to apparency (Smilanich, Fincher, & Dyer, 2016), recent studies have shown that more apparent trees can receive higher herbivore loads (Castagneyrol et al., 2013; Damien et al., 2016), regardless of any differences in defence allocation. As well as providing physical barriers to colonisation, heterospecific neighbours may interfere with pest ability to locate hosts through the release of volatile compounds that interfere with olfactory cues (Jactel et al. 2011). There is also evidence that non-host neighbour tree species modify functional traits of focal hosts (so-called "bottom up" effects of tree diversity), for instance nutrition or

plant defence allocation, that could impact upon herbivores (Castagneyrol et al., 2017). However, results are inconsistent between studies, with some authors reporting higher allocation to anti-herbivore defences with increased plant diversity (Moreira, Abdala-Roberts, Parra-Tabla, & Mooney, 2014), and others reporting the opposite (Castagneyrol, Jactel, et al., 2018; Glassmire et al., 2016).

Unlike the literature surrounding insect pests, to date there has not been any systematic synthesis of the literature concerning mixed stands and forest pathogens, nor of the underlying mechanisms behind any effects. For instance, while pest specialisation clearly plays an important role for insects, the same has not been shown for forest pathogens. Evidence for the dilution effect occurring has been shown for specialist pathogens such as oak powdery mildew (Hantsch et al., 2014) and for root rot pathogens (spread by root-to-root contact) in the presence of non-host neighbouring species (Gerlach, Reich, Puettmann, & Baker, 1997; Korhonen, Delatour, Greig, & Schonhar, 1998). However, studies of the invasive generalist forest pathogen *Phytophthora ramorum* in the USA have found that increasing host species diversity of native oak forests still led to reduced disease outbreaks (Dillon and Meentemeyer 2019; Haas et al. 2016; Haas et al. 2011). This suggests that mixing host species can still lower pathogen outbreaks even for generalist pathogens.

Part of the challenge in disentangling the mechanisms underpinning diversity-pathogen relationships in forests may be that studies have often considered pathogens in conjunction with insect pests, quantifying overall damage by pest organisms (e.g. Guyot et al. 2016; Setiawan et al. 2014). This assumes that the same

mechanisms operate between both groups. Also, studying the total pathogen community often involves a trade off in terms of statistical power: attempting to measure all fungal pathogens on all tree species may result in a limited sample size for each individual tree species or pathogen under study (e.g. Hantsch et al. 2014).

Interactions between Damage Agents

Previous studies have generally considered the effects of tree diversity on either insect pest or pathogen damage. However, interactions between damage agents can occur, adding an extra layer of complexity to understanding the mechanisms that underpin associational resistance or susceptibility to a given pest or pathogen. Many tree health syndromes can have multiple interacting biotic causes, which have been called "primary" and "secondary" damaging agents (Marçais and Bréda 2006). In another analogy, the spiral model of tree health decline (Manion 1991; Manion and Lachance 1992) adapts conventional ideas about plant pathology to suit the unique traits of forest trees, by distinguishing pre-disposing, inciting and contributing variables that contribute to tree health decline. Rather than being attributable to a specific pest or pathogen, tree diseases could thus be better described as declines, complexes or syndromes, involving multiple biotic and abiotic agents.

For instance, fungal infection of plants can lead to modification of plant functional traits that subsequently impact herbivores, such as phenology (Desprez-Loustau 2014), plant nutritional quality (Cardoza et al., 2003) and plant defence (Cardoza,

Alborn, & Tumlinson, 2002). Recently, a meta-analysis across 205 study systems showed that initial colonisation by biotrophic fungi reduced the preference and performance of insects on host plants, whereas when trees were infected with necrotrophic pathogens, insect preference, but not performance, was negatively impacted (Fernandez-Conradi et al., 2018). The authors considered that biotrophic pathogens, by feeding on living tissues, may generate competition for plant resources with chewing insects, while necrotrophic pathogens, by releasing soluble sugars through tissue breakdown, may have a positive impact on feeding insects (Fernandez-Conradi et al., 2018). This suggests that fungi can be important mediators of plant-insect interactions. A second meta-analysis linked interactions between stress organisms to cross-talk between plant defence pathways (Moreira, Abdala-Roberts, & Castagneyrol, 2018a). The authors found that attackers utilising the jasmonic acid (JA) pathway (such as leaf chewers, miners and necrotrophic pathogens) had a negative impact (antagonistic effect) on subsequent herbivores using either the JA or salicylic acid (SA) pathways. In contrast, initial attackers using the SA pathway (sucking herbivores and biotrophic pathogens) had a nonsignificant effect on both JA and SA attackers (Moreira et al., 2018a). If cross-talk occurs between defence pathways when multiple damage agents attack trees, then the efficacy of using mixed stands to control pest outbreaks may be difficult to predict (Schuldt et al., 2017). Therefore, whether the aim is to control a particular forest pathogen, or to lower overall pest and disease damage to a host tree population, considering the impact of tree diversity on multiple feeding guilds is an important consideration.

Impacts of Abiotic Factors

As well as considering interactions between multiple damage agents, abiotic factors will also impact tree diversity effects on both hosts and pathogens (Ratcliffe et al., 2017; van der Plas, 2019). This will be particularly relevant to forest management if, as predicted, climate change leads to a reduction in tree growth due to increasing temperatures coupled with increased drought frequency and intensity (Broadmeadow, 2005). The Plant Stress hypothesis predicts that insect outbreaks increase under conditions of plant water stress (White, 1974). Similarly, for pathogens, the 'predisposition concept' (Schoenweiss, 1986) argues that water stress can predispose plants to attack by opportunistic plant pathogens. In contrast, the Plant Vigour hypothesis predicts that conditions of plant stress will reduce insect fecundity and performance (Cornelissen, Wilson Fernandes, & Vasconcellos-Neto, 2008; Price, 1991).

As argued by White (2009), there is growing evidence that plant stress and plant vigour are not mutually exclusive hypotheses. Evidence from meta-analysis predicts that responses of pest organisms to plant stress depend on feeding guild (insects) or damage organ (pathogens), the degree of drought stress (moderate vs severe, short burst vs prolonged) and whether attackers are primary or secondary agents. Drought stress may lead a reduction in photosynthate production, protein hydrolysis and reduction in allocation to plant defence, leading to an increase in soluble nitrogen and lower osmotic potential of tissues. All of these factors can have positive or negative impacts on feeding insects and plant pathogens (Jactel et al. 2012). Koricheva and Larsson (1998) in a meta-analysis of experimentally stressed woody plants, found that plant stress increased the performance of sap suckers and borers but decreased the performance of chewers and gallers. In

contrast, a meta-analysis by Huberty and Denno (2004) found that drought negatively impacted sap feeders but had a non-significant impact on leaf chewers. Leaf gallers have been consistently shown to respond negatively to drought in meta-analyses (Huberty and Denno 2004; Jactel et al. 2012), in line with work finding that leaf gallers positively respond to plant module size as a metric of plant vigour (Flaherty & Quiring, 2008; Kopelke, Amendt, & Schönrogge, 2003; Larsson, 1989). Overall, it seems that primary foliar feeders (chewers, gallers and biotrophic pathogens) may respond positively to moderate, but not severe or prolonged, plant stress impacting plant growth, as they are dependent on vigorous hosts for survival. Secondary agents (necrotrophic pathogens, bark beetles), meanwhile, may respond positively to plant stress regardless of stress severity (Jactel et al. 2012; Oliva, Stenlid, and Martínez-Vilalta 2014).

Thus, both plant stress/vigour, and neighbourhood tree diversity, can separately modify the insect pest and pathogen communities on individual trees. The two bodies of literature have so far largely been considered separately from each other. However, several studies have now considered the potential for interactive impacts of abiotic factors on associational resistance/susceptibility, for instance, through modifications to plant functional traits such as defensive compounds (Castagneyrol, Jactel, et al., 2018; Castagneyrol, Moreira, & Jactel, 2018; Walter et al., 2012).

In my thesis, I argue that in order to provide informative and specific recommendations to foresters about the efficacy of using mixed stands to control pest and pathogen outbreaks, several new approaches to research are needed:

(1) the same efforts to systematically review the literature that have been applied to insect pests should also be extended to forest pathogens (the aim of Chapter 5 of this thesis);

(2) as shown for insect herbivores, understanding the mechanisms driving associational resistance to specific pathogens (for instance, plant apparency), will be important in order to predict whether mixed forests increase forest resilience to pathogens (explored in Chapters 2-3);

(3) interactions between attacking organisms from different feeding guilds (e.g. fungi and insect herbivores) need to be considered in order to understand the mechanisms underpinning associational resistance (explored in Chapters 2-3);

(4) interactions with abiotic factors need to be considered in the context of climate change, such as whether impacts of tree diversity on pathogen damage are robust to conditions of drought.

By considering a specific model system, the foliar herbivore and pathogen community on *Quercus* (oak) species in European broadleaved forests, this thesis combines all of these research approaches in the study of mixed forests. In addition to providing management recommendations to foresters managing oak woodland, this thesis contributes to ecological theory surrounding mixed forests and pest/pathogen outbreaks.

A Model Pathosystem for Mixed Forests: Oak Powdery Mildew

Oaks are key species in many European forests and are an important forest crop (Thomas, Blank, & Hartmann, 2002). For several decades, oaks have been acknowledged as supporting a highly diverse assemblage of invertebrate and vertebrate life (Southwood, 1961). A recent study in the UK found that oaks (*Q. robur)* support over 2300 species (Mitchell et al. 2019). Oaks in Europe are currently under threat from an emerging episode of oak decline, thought to be caused by multiple interacting abiotic and biotic factors (Camy et al. 2003; Marçais and Bréda 2006; Quine et al. 2019). One biotic factor implicated in oak declines is oak powdery mildew (*Erysiphe* spp.), the most common foliar fungal pathogen of oak in Europe, which can significantly reduce tree growth (Bert et al., 2016). The pathogen is thought to most significantly affect the growth of young trees and is a well-known nuisance in nurseries, with several fungicides being developed to control the pathogen (Glavaš, 2011). Recent work from UK forests also showed that oak powdery mildew DNA was present even in asymptomatic leaves, suggesting that the pathogen can also live endophytically (Roy, 2019).

Oak powdery mildew was first observed in Europe in the early 20th century (Desprez-Loustau, Hamelin, & Marçais, 2019). Genomic sequencing has shown there are four cryptic species of oak powdery mildew in Europe, at least one of which may be endemic (Mougou, Dutech, & Desprez-Loustau, 2008). From the 20th century epidemic onwards, it is thought that three introduced species have been the major cause of the powdery mildew on oak: *Erysiphe alphitoides, Erysiphe quercicola* and *Erysiphe hypophylla* (Marçais & Desprez-Loustau, 2014). Distinct phenological niches may exist for two powdery mildew species: *E. quercicola* is

primarily found in flag shoots (infected shoots that fail to fully emerge in the spring due to mildew infection), while *E. alphitoides* affects fully emerged shoots from secondary bud flushes (so-called "lammas" growth, see Fig. 1 and Feau et al. 2012; Kleinschmit 1993). *Erysiphe alphitoides* is the more common species in Europe, though this ongoing 'two-pronged attack' by two species may be one reason why oak powdery mildew is believed to be increasing in prevalence in Europe (Lonsdale, 2015). Oak powdery mildew can easily be identified to genus level as being caused by *Erysiphe* spp. in the UK, but as of yet, no study has confirmed the proportions of different *Erysiphe* spp. present on *Quercus* in the UK (Roy, 2019).

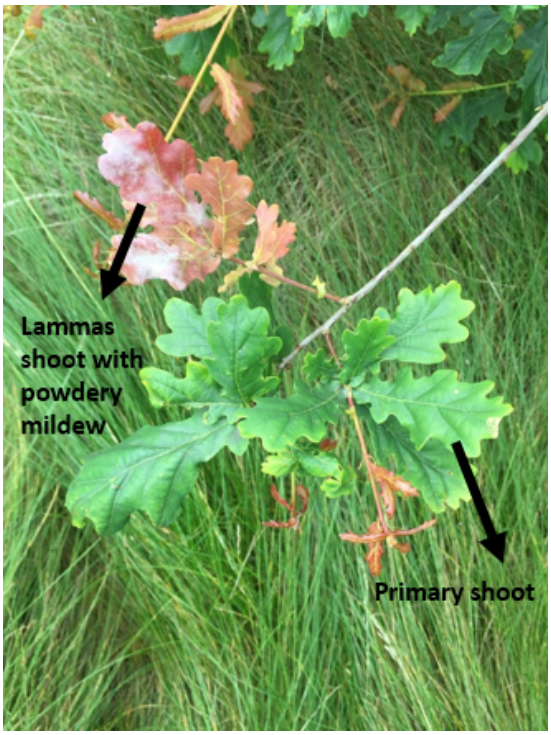


Figure 1: Primary and Lammas Shoots on a pedunculate oak (*Quercus robur*). Photograph by the author.

Climate Change Impacts on Oak Powdery Mildew

As well as being an emerging health threat in European forests, oak powdery mildew may increase in severity under conditions of climate change. Unlike most fungal pathogens of plants, powdery mildews are xerophilic pathogens that do not require free water for germination (Aust & Hoyningen-Huene, 1986). Moreover, genetic sequencing has identified a high level of sequence homology between *E. alphitoides* and a sub-tropical/tropical species of powdery mildew, *Oidium mangiferae,* occurring on mango, while *E. quercicola* was found to be homologous to *Oidium heveae,* a pathogen of rubber (Mougou et al., 2008). Thus, an increase in dry, hot conditions under climate change may increase the severity of oak powdery mildew species through direct impacts on the growth and survival of the pathogen, although powdery mildew outbreaks do not seem to be specifically related to dry years (Basova, 1987). However, oak powdery mildew is also a specialist biotrophic pathogen, and thus relies on vigorous hosts for survival (Jactel et al. 2012). Under conditions of moderate drought stress, favourable microclimatic conditions may therefore lead to increased oak powdery mildew severity, but under severe drought stress, negative impacts on host growth may outweigh this (Jactel et al. 2012). Moreover, a recent study showed that mycophagous ladybirds preferred to graze on powdery mildew from drought stressed oak saplings (Dillen et al., 2017). Thus, drought stress could also lead to an increase in biocontrol of powdery mildew via ladybird grazing.

Climate change also has the potential to alter host-pathogen dynamics by modifying the phenology of both host and parasite. Oak leaves are most susceptible to powdery mildew shortly after bud burst (Edwards & Ayres, 1982), which is thought to explain why later flushing species of oak, such as *Q. pyrenaica*, are particularly susceptible to mildew (Desprez-Loustau, Hamelin, & Marçais, 2019; Kleinschmit, 1993). The timing of bud burst of several tree species, including *Q. robur,* is thought to be advancing in the UK due to an increase in the frequency of mild, wet winters (Hinks et al., 2015). One study showed that along latitudinal gradients, oak powdery mildew populations became increasingly out of sync with the phenology of oak hosts (Desprez-Loustau et al. 2010). Thus, depending on the rate of environmental change and the speed of adaptation of both host and pathogen, oak powdery mildew may increase or decrease in prevalence as the climate changes.

Interactions Between Oak Powdery Mildew and Insect Herbivores

In addition to being altered by climate change, mildew infection is also thought to be altered by interactions with insect herbivores. One hypothesis for the role of mildew in oak decline is that it follows early season defoliation by insect herbivores, which facilitates a second bud flush that is then susceptible to mildew (Marçais and Bréda 2006; Marçais and Desprez-Loustau 2014). Moreover, powdery mildew may be able to directly manipulate the phenology of its host to promote infection. One study showed infected trees had a greater tendency to produce multiple bud flushes (polycyclism) over the growing season (Desprez-

Loustau 2014). Oak powdery mildew therefore makes an ideal model pathogen for studying interactions between pathogens and other plant damage agents.

Tree Diversity Impacts on Oak Powdery Mildew

As a wind-dispersed pathogen, we predicted that oak powdery mildew would be strongly impacted by factors such as host dilution and reduced physical apparency of trees in species mixtures. Moreover, an altered microclimate in mixed stands compared to monocultures may impact pathogen growth and development, as well as interactions with herbivory (Chen et al., 1999). Previous studies of the effects of tree diversity on powdery mildew have not shown consistent trends. Hantsch et al (2014) found a weak but significant reduction in powdery mildew infection intensity in mixed species stands compared to pure stands of sessile oak (*Quercus petraea)*. In contrast, Dillen, Verheyen, and Smit (2016) found no significant reduction of powdery mildew infection on *Quercus robur* saplings placed in mixed species stands. Studies of mildew populations on *Quercus robur* on the Finnish island of Wattkast showed that both the size and spatial connectivity of oak trees within the wider landscape influenced probability of occupancy by mildew, suggesting that mildew may act as a dynamic metapopulation (Ekholm, Roslin, Pulkkinen, & Tack, 2017; Tack, Ovaskainen, Pulkkinen, & Roslin, 2010). However, Roslin, Laine and Gripenberg (2007) found that the infection intensity of neighbouring oak trees was a poor predictor of infection intensity on focal oak trees. For wind-dispersed pathogens, spatial connectivity of hosts within the landscape, as well as individual host quality, may

both be important determinants of parasite occupancy, and this may explain differences in results from previous studies.

Due to the potentially strong impacts of climate change on this pathosystem, as well as its interaction with other feeding guilds (insect herbivores), oak powdery mildew is an ideal model pathogen species for exploring the use of mixtures in forest management, as well as the resilience of such a strategy under long-term conditions of increasing plant stress.

Thesis structure

Aim of the thesis

This thesis aims to inform future planting recommendations for tree species in Europe, by considering both the impacts of stand diversity and drought (a likely impact of climate change) on the outbreak of emerging forest diseases. This has been achieved through a variety of experimental and theoretical approaches. The thesis consists of six chapters: a general introduction, four data chapters, and a general discussion. Two of the four data chapters have been published in peer reviewed journals and the remaining two chapters are presented in manuscript form. The overall research objectives of the thesis are given below, along with overviews of each chapter.

Research Objective 1 – ***How does tree genetic and species diversity influence oak powdery mildew infection on Quercus robur? (Chapters 2-3)***

Research Objective 2 – ***How are diversity effects on oak powdery mildew and oak insect herbivores moderated by environmental variation? (Chapters 2-3)***

I used the model pathosystem of *Quercus robur* – *Erysiphe* spp. to investigate how tree diversity influenced pathogen damage in two separate tree diversity experiments, and how this damage was impacted by environmental variation.

Chapter 2 considers the impacts of both species and genetic (provenance) diversity on oak powdery mildew and foliar oak insect herbivory, in a climate-matching tree diversity experiment in the UK. Using data measured over multiple sites and years, I could investigate spatial and temporal effects of tree diversity on the oak foliar community. This chapter was published in July 2019 in the journal *Ecology and Evolution.*

Chapter 3 considers the impact of both tree species diversity and drought stress on oak powdery mildew and foliar oak insect herbivory. This study was undertaken in a tree diversity experiment in SW France where an irrigation treatment as well as a diversity treatment has been imposed. This chapter was published in March 2020 by *Journal of Ecology.*

Research Objective 3 – ***How does water stress impact oak powdery mildew infection on Quercus robur? (Chapters 3 & 4)***

Chapter 4 presents the results of a greenhouse experiment which aimed to experimentally test the effects of different water stress levels on oak growth parameters and powdery mildew. This aimed to complement the results from **Chapter 3**, in which drought effects on oak powdery mildew were testing in a field setting. The overall aim of including water stress was to investigate the likely impacts of climate change on this pathogen and on diversity-disease interactions.

Research Objective 4 – ***What are the overall effects of planting mixed species stands on forest resistance to pathogens? (Chapter 5)***

Finally, in **Chapter 5** I have undertaken a systematic review of the previous literature on tree species diversity effects on forest pathogens, utilising previous published studies as well as additional data collected from the tree diversity experiment network, TreeDivNet. This chapter provides a global review of the evidence base on mixed species stands and forest pathogens, and identifies knowledge gaps. The aim is to provide a knowledge base for practitioners and researchers.

Complementary Educational Resources: Tree Pests and Diseases

As well as improving knowledge provisional to foresters, there is an identified need to improve knowledge of plant health threats among educators, especially those working with trees such as Forest School leaders (Hemery, Hurst, &

Petrokofsky, 2019). As a complementary research internship to my thesis, I produced a collection of resources aiming to increase the knowledge of common pests and diseases of native broadleaved trees, designed specifically for educators. This was in collaboration with the Sylva Foundation, an environmental charity based in Oxfordshire, and the Forestry Commission Plant Health Forestry Team. The Sylva Foundation currently disseminate the resources via their website and via the Forest School Association. The resources are available to view in Appendix 5 to the main thesis.

Chapter prefaces

Several authors (including my DPhil supervisors and other researchers) contributed to different aspects of this thesis. Each chapter is prefaced by a short statement with details of author contributions and data accessibility.

CHAPTER 2: INDIVIDUAL TREE TRAITS SHAPE INSECT AND DISEASE DAMAGE ON OAK IN A CLIMATE-MATCHING TREE DIVERSITY EXPERIMENT

Elsa Field[1], Karsten Schönrogge[2], Nadia Barsoum[3], Andrew Hector[1] & Melanie Gibbs[2]

[1] Department of Plant Sciences, South Parks Road, University of Oxford, Oxford, OX1 3RB, United Kingdom

[2] Centre for Ecology & Hydrology, MacLean Building, Crowmarsh Gifford, Wallingford, OX10 8BB, United Kingdom

[3] Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10, 4LH, United Kingdom

Article Status

This chapter was published in *Ecology and Evolution* in July 2019.

Citation information:

Field, E., Schönrogge, K., Barsoum, N., Hector, A., & Gibbs, M. (2019). Individual tree traits shape insect and disease damage on oak in a climate-matching tree diversity experiment. *Ecology and Evolution* 9 (15): 8524–8540. https://doi.org/10.1002/ece3.5357

Data accessibility

Data for this study is available for download from the NERC Environmental Information Data Centre (DOI: https://doi.org/10.5285/cbccb101-c877-4e43-ac70-e8a852b51f07).

Author contributions

MG, KS, NB and EF devised the study. EF carried out the field assessments with other trained observers. EF analysed the data with input and advice from AH, MG and KS. EF wrote the manuscript. All authors commented on previous and final versions of the manuscript.

Abstract

Diversifying planted forests by increasing genetic and species diversity is often promoted as a method to improve forest resilience to climate change and reduce pest and pathogen damage. In this study we used a young tree diversity experiment replicated at two sites in the UK to study the impacts of tree diversity and tree provenance (geographic origin) on the oak *(Quercus robur)* insect herbivore community and a specialist biotrophic pathogen, oak powdery mildew. Local UK, French and Italian provenances were planted in monocultures, provenance mixtures and species mixes, allowing us to test whether: 1) local and non-local provenances differ in their insect herbivore and pathogen communities, and 2) admixing trees leads to associational effects on insect herbivore and pathogen damage. Tree diversity had variable impacts on foliar organisms across sites and years, suggesting that diversity effects can be highly dependent on

environmental context. Provenance identity impacted upon both herbivores and powdery mildew, but we did not find consistent support for the local adaptation hypothesis for any group of organisms studied. Independent of provenance, we found tree vigour traits (shoot length, tree height) and tree apparency (the height of focal trees relative to their surroundings) were consistent positive predictors of powdery mildew and insect herbivory. *Synthesis.* Our results have implications for understanding the complex interplay between tree identity and diversity in determining pest damage, and show that tree traits, partially influenced by tree genotype, can be important drivers of tree pest and pathogen loads.

Introduction

Much ecological research in recent decades has shown that plant species richness has substantial effects on different aspects of ecosystem functioning (Isbell et al., 2017). For instance, increasing plant species richness has been shown to positively benefit productivity (Benneter et al., 2018; Pretzsch et al., 2013; Tilman et al., 1996), temporal stability (Morin et al., 2014) and resistance to natural disturbances and climate change (Isbell et al., 2015; Jactel et al., 2017). Diversifying production forests using both species and genetic diversity can result in an economic insurance effect, such that if one crop fails due to adverse environmental changes, forest managers have alternatives in the same plantation to ensure profit (Jactel et al. 2017). In addition, mixed species forests may also be intrinsically more resistant to pest and disease outbreaks. Meta-analyses have shown a trend towards reduced insect damage in tree species mixtures compared to monocultures (Jactel

and Brockerhoff 2007; Castagneyrol et al. 2014), and the same has been reported for forest pathogens (Hantsch et al., 2014; Pautasso et al., 2005).

The reduction of pest and disease damage in mixtures may be due to a pure dilution effect, where lower host abundance in a given area reduces pest abundance on susceptible hosts, as pest species are unable to build up to epidemic levels (Civitello et al., 2015). Additionally there may be associational effects, relating to the impact of planting non-host neighbour trees around a focal tree (Barbosa et al., 2009). In associational resistance, a focal host experiences lower pest damage in mixtures compared to monocultures, while associational susceptibility describes the opposite (Barbosa et al., 2009). Mechanisms proposed to underpin associational effects are diverse. Direct effects of non-host neighbour trees may cause associational resistance by influencing the microclimate of plots with knock-on impacts on insects (Muiruri and Koricheva 2017), or by interfering with olfactory cues used by pests to find hosts (Jactel et al. 2011). Tree species diversity may also modify plant apparency, which can be defined the height of focal trees relative to their surroundings, and has been shown to be an important predictor of insect herbivore loads on individual trees (Castagneyrol et al., 2013; Régolini et al., 2014). Moreover, complex indirect effects of plant neighbourhood identity on insect herbivory and pathogens may be mediated via modifications to plant traits, including foliar nutritional quality and defences against herbivory (Castagneyrol et al., 2017; Forey et al., 2016a)

Genetic diversification of forests may also be encouraged as a climate change

adaptation strategy (Bucharova, Bossdorf, Hölzel, Kollmann, & Prasse, 2018). In forestry, tree provenance is defined as the geographic location of the original stand from which seed or cuttings were taken (Hubert & Cottrell, 2007). The local adaptation hypothesis predicts that local provenance trees will be optimally adapted for local environmental conditions (Bucharova et al., 2017). Assisted migration of provenances from areas predicted to meet the future climate of a region ("climate matching") provides an alternative to planting local material, that may be favourable if climate change outpaces the speed of adaptation of local provenances (Broadmeadow, 2005; Ennos et al., 2019; Marris, 2009). Climate matched provenances may also experience growth benefits if they escape some damage by local pest and disease populations, which can be co-adapted to local hosts through tight phenological synchrony (Egan & Ott, 2007; van Asch & Visser, 2007) and co-adaptation to functional traits such as plant defences (Pearse & Hipp, 2009). However, non-native provenances may also experience stressful growing conditions under current local climates, leading to reduced tree vigour. Plant stress generally has a negative effect on the performance of primary foliar insect herbivores and biotrophic pathogens, while plant vigour can have the opposite effect (Jactel et al. 2012; Huberty and Denno 2004). Despite the potential importance of introduced provenances as a future-proofing strategy against climate change, few experimental studies exist comparing the growth characteristics and pest resistance of local versus climate matched provenances under current climatic conditions (Barsoum, 2015; Broadmeadow, 2005).

While provenance identity and tree diversity can both shape associated pest communities (Dickson & Whitham, 1996; Wimp et al., 2005), the simultaneous impacts of diversifying both the genetic and species composition of forest stands have rarely been tested. Moreover, tree diversity effects on pathogens have been less frequently investigated than for insect pests (Hantsch et al., 2014; Jactel & Brockerhoff, 2007; Pearse & Hipp, 2009). Even fewer studies have considered diversity effects on both pests and pathogens together, despite the potential for multitrophic interactions which could impact upon associational effects (Schuldt et al., 2017). For instance, initial damage to a plant by insect herbivores or pathogens can have both a reciprocal and antagonistic effect on subsequent plant attackers, via cross-talk between plant defence signalling pathways that are induced following attack (Moreira et al., 2018a).

Here, we tested the impacts of varying both tree species and genetic diversity on oak (*Q. robur)* foliar insect herbivores, oak powdery mildew and the interaction between the two groups. We used a tree diversity experiment replicated at two sites in the UK, in which local and climate-matched oak provenances are planted as monocultures or in mixtures of provenances and species. There is increasing evidence that tree diversity effects on pest organisms are context dependent, and can be modulated by abiotic conditions such as temperature and water availability impacting upon plant growth and functional traits (Castagneyrol et al., 2018; Kambach et al. , 2016; Walter et al., 2012). By studying replicate trials between years (two years of data from one site) and between trials, we looked for consistent impacts of provenance identity and tree diversity across space and

time. By measuring herbivory by three insect guilds differing in their life history strategies and powdery mildew infection, on the same trees, we were able to look for interactions between insects and pathogens interacting with tree diversity. We suspected the presence of interactions between the two groups as oak powdery mildew has been reported to benefit from insect herbivory, where early season insect defoliation promotes an increase in secondary bud flushes ("lammas shoots"), providing more susceptible leaf material for mildew infection (Marçais and Desprez-Loustau 2014).

We tested the following hypotheses:

(1) Local and non-local provenances would differ in their tree phenotypic traits. Phenotypic traits measured were those with a known impact on insect herbivores and foliar pathogens: plant vigour (tree height and shoot length, both commonly used proxys of vigour (Flaherty & Quiring, 2008; Gripenberg, Ovaskainen, Morriën, & Roslin, 2008)) and the number of lammas shoots produced (as a predictor of powdery mildew infection).

(2) Insect herbivore abundance and powdery mildew infection would be highest on local provenances (local adaptation hypothesis).

(3) Associational resistance to both insect herbivores and powdery mildew would occur in mixed species plots compared to single species plots.

(4) Differences in provenance susceptibility to insect herbivores and powdery mildew could lead to associational effects in mixed provenance plots, compared to provenance monocultures.

(5) Both tree vigour, and tree apparency, would be significant positive drivers of insect herbivory and powdery mildew infection.
(6) Insect herbivory and oak powdery mildew infection would be positively associated on the same trees.

Our results emphasise the importance of considering tree identity as well as diversity setting in the design of mixed forests, and illustrate how environmental context can affect plant growth, with impacts on forest pests and pathogens.

Materials and Methods

Experimental Field Sites

The study took place at the Climate Match tree diversity experiment planted in 2011 at two sites in the UK. One site was established in South East England at Hucking, Kent (51°17'47.5"N 0°37'58.2"E), the other in the East Midlands at Hartshorne, Derbyshire (52°47'41.1"N, 1°30'58.8"W).

Four broadleaved species of varying provenance were planted: wild cherry (*Prunus avium),* sweet chestnut (*Castanea sativa),* ash (*Fraxinus excelsior)* and pedunculate oak (*Q. robur).* UK provenances of the four tree species were sourced from areas delineated as native seed zones (Herbert, Samuel, & Patterson, 1999) while French and Italian provenances were sourced from regions "climate-matched" to predictions of the UK climate in 2050 and 2080, respectively (Barsoum, 2015; Fig. 2). Due to the trial sites being located in different geographic

regions of the UK, local and climate-matched provenances differed between sites for oak, with only the French provenance in common between the two sites (Fig. 2).

The two trials follow a similar planting design (Fig. 3). There are three experimental blocks with treatments replicated once per block for each tree species. We focused only on treatments containing oak: monocultures of Local, French and Italian provenances, 50:50 provenance mixtures (Local:Italian and Local:French), 75:25 provenance mixtures (Local:Italian and Local:French), 33:33:33 provenance mixtures (Local: French: Italian) and mixed species plots (containing Local and Italian provenances of each of the four species). Within mixtures, trees are planted in a regular pattern ensuring different provenances (or species) are mixed with no two trees of the same type next to each other (Fig. 3). Provenance monocultures and mixed provenance plots are 12 m x 12 m, with 36 trees per plot planted at 4 m^2 spacing giving 6 rows of trees of 6 trees each. Mixed species, mixed provenance plots are 36 x 32 m, containing 288 trees in 16 rows of 18 trees each. Trials were planted using two-year-old saplings. For more information on the trial design and establishment, see Barsoum (2015). The experiment is part of TreeDivNet, a global network of tree diversity experiments (http://www.treedivnet.ugent.be).

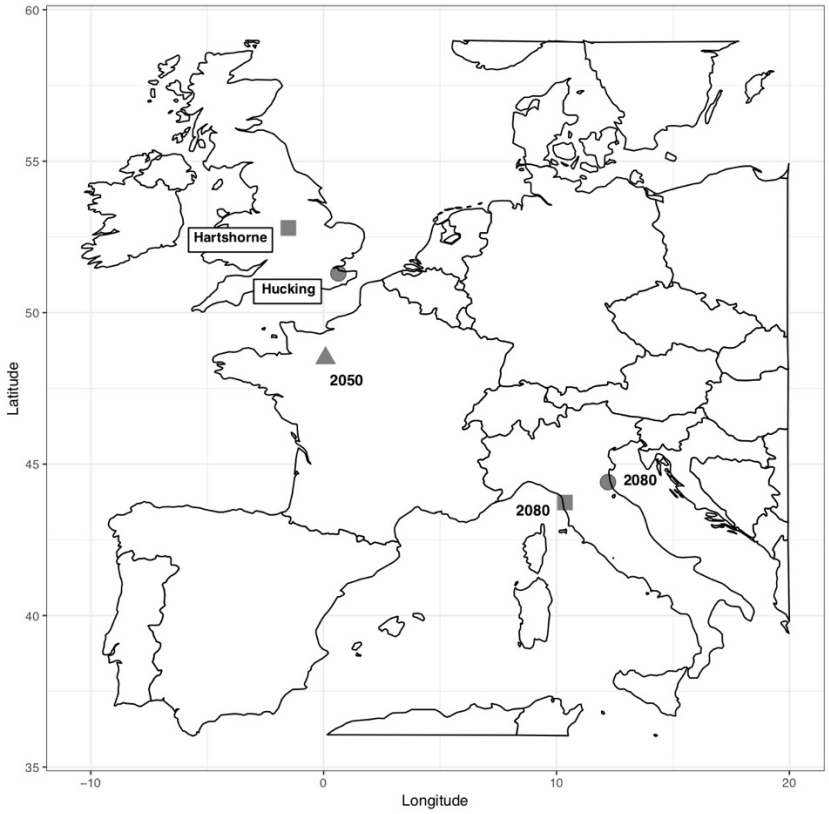


Figure 2: Location of UK Climate Match experiment sites, Hucking in Kent (black circle) and Hartshorne in Derbyshire (black square), and sources of "climate matched" oak provenances. At Hucking, local provenance material was from Kingsnorth, Kent, at Hartshorne it was UK 404 provenance. The same provenance (QR0100 North-West) was planted from France at both sites to represent a 2050 climate match (black triangle). At Hucking, the 2080 climate matched provenance planted was from the Ravenna region of Italy (black circle), while at Hartshorne it was from San Rossore region of Italy, Tuscany (black square).

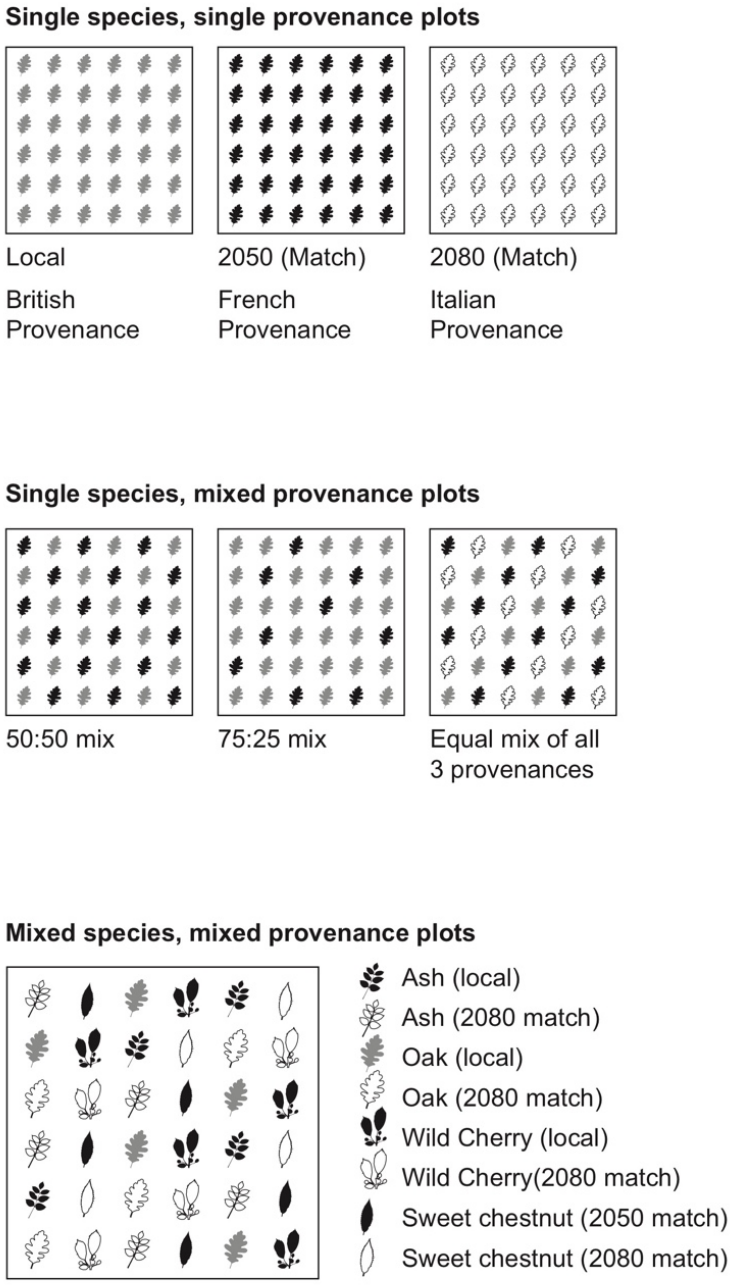


Figure 3: Design of the treatments planted at the Climate Match experiment. Each treatment is replicated at each site in three consecutive experimental blocks, apart from the mixed species plots at the Hartshorne site, where there are replicates in 2 out of 3 blocks only, due to site space restrictions.

Study System

We studied three insect guilds ("insect herbivores") on oak (*Quercus robur):* cynipid gallwasps ("gallers"), leaf miners and leaf manipulators. Gallers and leaf miners were known as specialist herbivores of the genus *Quercus* section Quercus (Hayward & Stone, 2005; Nieukerken & Johansson, 2003), whereas leaf manipulators described free-feeding herbivores that included both generalist and specialist species. The most abundant gallers were the spangle galls *Neuroterus quercusbaccarum*, *N. numismalis,* and *N. anthracinus.* The most abundant leaf miner species were *Phyllonorycter sp.,* and *Stigmella* sp.

We measured infection by the specialist biotrophic pathogen, oak powdery mildew, caused by multiple cryptic species of *Erysiphe* with different phenological niches: *E. quercicola* infects both buds and leaves, and *E. alphitoides* infects leaves (Marçais & Desprez-Loustau, 2014). Surveying of leaves in August means that we likely scored *E. alphitoides,* believed to be the most common species in the UK (Desprez-Loustau et al., 2018). However, in the absence of molecular identification we use the term "powdery mildew" to describe the pathogen.

We observed that in the UK, insect herbivore and powdery mildew damage are temporally and spatially separate. Almost all insect herbivore damage occurs on primary shoot leaves emerging during the first flush in late spring (April-May), while oak powdery mildew infection occurs on leaves on lammas shoots emerging during subsequent bud bursts in June-August, consistent with *E.*

alphitoides releasing ascospores from overwintering chasmothecia in early to mid-summer (Marçais, Kavkova, and Desprez-Loustau 2009). Oak leaf susceptibility to powdery mildew declines sharply following bud burst, so that leaves on primary shoots largely remain completely uninfected (Ayres & Edwards, 1982). Thus, we recorded herbivory on primary shoots, and powdery mildew infection on lammas shoots, which can be clearly differentiated by a ring scar separating different flushes.

Tree Selection for Surveying

At both the Hucking and Hartshorne sites, nine oak trees of each provenance per plot were selected for recording both powdery mildew and herbivory, by starting in the core of the plot and moving outwards. Powdery mildew and herbivory were scored in 2016 only at Hucking and at both Hucking and Hartshorne in 2017. This gave a total of 411 trees sampled at Hartshorne, and 420 and 416 trees sampled at the Hucking site in 2016 and 2017, respectively. The difference in tree numbers at Hucking was due to tree mortality between years.

Lammas Shoot Scoring

To establish whether provenances differed in the timing of lammas shoot production, in July 2017 we randomly selected three trees per provenance in the core of each plot (a subset of trees later scored for herbivory and mildew). On each tree, we randomly selected five primary shoots at varying heights and all aspects around the tree and recorded the presence of one or more second flush shoots, to produce an index of "lammas shoot availability" per tree (the proportion of five

shoots producing lammas shoots). This snapshot of lammas shoot growth reflects relative differences in the abundance of lammas shoots on different trees, when scored at the same time point during summer. This allowed us to test for differences between provenances in the amount of susceptible leaf material for mildew infection available at the start of the infection window (between July-August for oak powdery mildew in the UK).

Oak Powdery Mildew

In August at each site (in 2016 and 2017 for Hucking, 2017 only for Hartshorne), powdery mildew infection was recorded on 10 lammas (second flush) shoots per tree. Shoots were sampled randomly across a range of heights and from all aspects around the tree. We used a 0-3 scale of infection severity based on the % of total leaf material on the shoot infected by fungal mycelium: 0 (0% infection), 1 (<50 % infected), 2 (51-75% infected), 3 (76-100% infected). This four point scale is similar to those used by other authors scoring this pathogen (Bert et al., 2016). We also recorded lammas shoot length (mm) to use as a covariate.

Insect Herbivores

In August at each site (in 2016 and 2017 for Hucking, 2017 only for Hartshorne), insect herbivore abundance was recorded on 10 primary shoots per tree. Shoots were sampled randomly across a range of heights and from all aspects around the tree. We recorded insect herbivore abundance by counting the number of leaf mines, galls (on leaves and buds), and incidences of leaf skeletonisers, leaf rollers and leaf webbers for all leaves on a shoot (after Sinclair et al. 2015; Moore, Warrington, and Whittaker 1991). We also recorded primary shoot length (mm) to

use as a covariate.

Tree Height and Apparency

At the end of the recording period in August 2017, we recorded the height of all sampled trees, as well as 27 randomly chosen additional trees per plot, to calculate an index of tree apparency based on the index used in Castagneyrol, Jactel, and Moreira (2018). This was calculated as:

$$Plant\ Apparency = \left(\frac{\text{Height Focal tree} - \text{Average Height of Plot}}{\text{Average Height of Plot}}\right) * 100$$

Statistical Analysis

All analyses were completed in R version 3.4.3 (R Core Team, 2020).

Phenotypic traits of different provenances

To determine whether provenances differed in tree phenotypic traits we conducted one-way ANOVAs by provenance for each trait at each site and year. Tree traits analysed were: tree height, primary shoot length, lammas shoot length, and lammas shoot availability. As data on insect herbivore abundance and powdery mildew infection was analysed separately for each site and year, we also split the analysis of tree traits in the same way, to allow comparability between analyses.

Powdery mildew infection and insect herbivore abundance

We used Generalised Linear Models (GLMs) to test for differences in insect herbivore abundance and powdery mildew infection on *Q. robur* trees between provenances and diversity settings. We favoured GLMs over Mixed Effect Models as the factor describing spatial autocorrelation (Block) had only 3 levels, below the recommended 5 levels for random effects (Gelman & Hill, 2007). We analysed insect herbivory and powdery mildew at the tree level. Due to the rarity of leaf rollers and webbers, we aggregated herbivore abundance into three guilds for analysis: gallers, leaf miners, and leaf manipulators (skeletonisers, rollers, and webbers). Numbers of each herbivore group were summed over the 10 primary shoots sampled per tree to produce tree level totals. For powdery mildew, we averaged infection scores across the 10 shoots surveyed per tree.

Data from different sites and years were analysed separately for the three insect herbivore guilds and powdery mildew. We analysed the two years of data from Hucking separately as tree height was measured only in 2017, so we ran separate models for the two years with slightly different explanatory variables. Data from the two sites were analysed separately since the origin of the local and Italian provenances differed between them (with only the French provenance in common between sites).

For powdery mildew infection and for each herbivore guild we therefore produced three models: Hucking 2016, Hucking 2017, and Hartshorne 2017 (Table 1). Model explanatory variables were: tree provenance, tree diversity (diversity treatment), tree vigour (individual tree height and average shoot length), and tree

apparency. The full model for each foliar group at each site and year included diversity treatment, provenance and block as categorical predictors, and tree height and shoot length as continuous predictors (Table 2). Models of powdery mildew infection included lammas shoot length as a predictor, herbivore abundance models included primary shoot length as a predictor. Full models included all two-way interactions between provenance, treatment, and block, and two-way interactions between provenance and shoot length (Table 2). Prior to model fitting, we visually inspected continuous explanatory variables (tree height, shoot length) for linearity and normality, applying log transformations where necessary, and included quadratic terms in our models to account for non-linear relationships when required (Table 2). We inspected multi-collinearity between model variables using variance inflation factors (VIFs) calculated using the corvif() function R (Zuur et al., 2009). No VIF observed was > 5, the suggested cut off point for collinearity between variables interfering with model inference (Zuur et al., 2009).

Model Response Variable	Model Explanatory Variables	Model Type
Oak Powdery Mildew (Hucking 2016)	Provenance [F]; Diversity [F]; Block [F]; Lammas Shoot Length [C]; Galler Abundance [C]; Leaf Miner Abundance [C]; Leaf Manipulator Abundance [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Lammas Shoot Length [I]	Gaussian GLM, log link
Oak Powdery Mildew (Hucking 2017)	Provenance [F]; Diversity [F]; Block [F]; Tree Height [C]; (Tree Height)^2 [C]; Lammas Shoot Length [C]; Galler Abundance [C]; Leaf Miner Abundance [C]; Leaf Manipulator Abundance [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Lammas Shoot Length [I]	Gaussian GLM, log link
Oak Powdery Mildew (Hartshorne 2017)	Provenance [F]; Diversity [F]; Block [F]; Tree Height [C]; Lammas Shoot Length [C]; Galler Abundance [C]; Leaf Miner Abundance [C]; Leaf Manipulator Abundance [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Lammas Shoot Length [I]	Gaussian GLM, log link
Galler Abundance (Hucking 2016)	Provenance [F]; Diversity [F]; Block [F]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Negative binomial GLM, log link
Galler Abundance (Hucking 2017)	Provenance [F]; Diversity [F]; Block [F]; Tree Height [C]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Negative binomial GLM, log link
Galler Abundance (Hartshorne 2017)	Provenance [F]; Diversity [F]; Block [F]; Tree Height [C]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Negative binomial GLM, log link
Leaf Miner Abundance (Hucking 2016)	Provenance [F]; Diversity [F]; Block [F]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Negative binomial GLM, log link
Leaf Miner Abundance (Hucking 2017)	Provenance [F]; Diversity [F]; Block [F]; Tree Height [C]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Negative binomial GLM, log link

Leaf Miner Abundance (Hartshorne 2017)	Provenance [F]; Diversity [F]; Block [F]; Tree Height [C]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Poisson GLM, log link
Leaf Manipulator Abundance (Hucking 2016)	Provenance [F]; Diversity [F]; Block [F]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Negative binomial GLM, log link
Leaf Manipulator Abundance (Hucking 2017)	Provenance [F]; Diversity [F]; Block [F]; Tree Height [C]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Negative binomial GLM, log link
Leaf Manipulator Abundance (Hartshorne 2017)	Provenance [F]; Diversity [F]; Block [F]; Tree Height [C]; Primary Shoot Length [C]; Provenance:Diversity [I]; Provenance:Block [I]; Diversity:Block [I]; Provenance:Primary Shoot Length [I]	Negative binomial GLM, log link

Table 2: An overview GLMs of oak powdery mildew infection and insect herbivore abundance, showing all explanatory variables included in starting models. In the Explanatory Variables column, letters in brackets [] refer to the type of variable: F = Factor; C = Continuous; I = Interaction.

To test hypothesis 6, that high herbivory on primary growth may promote powdery mildew infection on lammas growth, for powdery mildew models we included the log-transformed abundance of gallers, leaf miners and leaf manipulators as additional predictors.

Herbivore counts in all guilds were highly overdispersed and were analysed using negative binomial GLMs with log link function, except for a poisson GLM with log link for leaf miners at Hartshorne in 2017, where data was not

overdispersed (Pearson's dispersion statistic = 1.04). For powdery mildew we used a Gaussian GLM with log link function in order to constrain the response variable as positive but retain the normality and independence of residuals.

We carried out model simplification by comparing pairs of nested models using likelihood ratio testing, starting with removal of nonsignificant interaction terms, to achieve minimal adequate models retaining only significant explanatory variables. We used F tests for GLMs with gaussian errors, and Chi-squared tests for GLMs negative binomial and poisson errors (Crawley, 2007).

To test hypothesis 5, that the response to tree height by insect herbivores and powdery mildew was driven by tree apparency rather than just tree vigour, for models of 2017 data we substituted tree apparency into minimal adequate models *post hoc* as a predictor instead of tree height. We compared the likelihood ratio test scores when either predictor was removed from the model, to assess which was the single best predictor of insect herbivory and powdery mildew. Although the VIF of neither apparency nor tree height was > 5 at Hucking the two were still strongly correlated so we did not include both in the same initial model (value of Pearson's pairwise correlation coefficient = 0.61 at Hucking, 0.26 at Hartshorne).

We also carried out *post hoc* pairwise comparisons of means to test for specific differences in insect herbivore abundance and powdery mildew infection between provenances and diversity treatments, using z tests (function glht() from the multcomp package in R). We computed comparisons between means of factor

levels only if no significant interaction terms were retained that could interfere with statistical inference (Table S1). Rather than calculating all pairwise comparisons between means, we computed comparisons only where model summaries indicated significant differences were present, as the probability of finding that at least one comparison is significant increases with each test (Gelman, Hill, & Yajima, 2012). For the model of gallers at Hucking in 2017, the minimal adequate model retained a significant interaction between provenance and treatment, so we carried out a subset analysis as visual exploration of the model predictions indicated there may be consistent differences in gall abundance between diversity treatments dependent on provenance. We ran a separate GLM for the Italian provenance only, fitting treatment, block and the interaction between treatment and block as the only predictors (Table S1).

We visualised model residuals to check model assumptions were met. To visualise final model predictions, we used partial residual plots to show the effects of significant predictors on response variables (Faraway 2005).

Results

Differences in tree phenotypic traits between provenances

Primary Shoot Length

At Hucking in 2016, Local and French trees had significantly longer primary shoots than Italian trees, but did not significantly differ from each other (Table S2,

Fig. S1, A). At both sites in 2017, Local trees had longer primary shoots than both French and Italian trees (Table S2, Fig. S1, B and C). At Hucking, non-local provenances did not differ in primary shoot length in 2017, while at Hartshorne, Italian trees had significantly shorter primary shoots than French trees (Table S2, Fig S1, B and C).

Lammas Shoot Length

At Hucking in both years, lammas shoot length was similar for all provenances (Table S2, Fig. S1, D and E). At Hartshorne, French trees had the longest lammas shoots, followed by Italian trees, with Local trees having significantly lower lammas shoot length than both non-local provenances (Table S2, Fig. S1, F).

Tree Height

The three oak provenances were of comparable tree height at Hucking (Table S2, Fig. S2). At Hartshorne tree heights were also comparable apart from French trees which were significantly taller than Italian trees (Table S2, Fig. S2).

Lammas Shoot Availability

At both sites, French and Italian trees had significantly higher lammas shoot scores than Local trees, but non-local provenances did not differ significantly from each other (Table S2, Fig. S3).

Oak insect herbivore abundance and powdery mildew infection

Below we describe impacts of provenance identity (Fig. 4), tree diversity treatment Fig. S5-S8), tree vigour (height and shoot length) and tree apparency (Fig. 5) where these variables were retained in minimal adequate models (Tables 3-4).

Impacts of provenance identity

Powdery Mildew

Italian provenance trees had higher powdery mildew infection compared to Local trees in both years at Hucking ($z = -4.01$, $p = <0.001$ in 2016; $z = -3.83$, $p = <0.001$ in 2017), while there was no difference between Local and French trees. Italian trees had higher infection than French trees in 2016 ($z = -2.64$, $p = 0.0083$), but not in 2017 ($z = -1.598$, $p = 0.11$). In contrast, at Hartshorne, we found no consistent differences in powdery mildew infection between provenances (Fig. 4C and S5).

Gallers

Gallers showed the most consistent response to provenance among insect guilds. Abundance was lowest on Italian trees across sites and years (Fig 4 D-F). At Hucking in 2016, Italian provenance trees had significantly fewer galls compared to Local ($z = 8.42$, $p <0.001$) and French ($z = 5.164$, $p <0.001$) trees, while Local and French trees did not significantly differ ($z = 1.68$, $p = 0.094$). At Hucking in 2017, Local trees had the highest gall abundance, followed by French (compared to Local, in monoculture: $z = -2.79$, $p =0.0052$) and Italian trees (compared to Local, in monoculture: $z = -12.57$, $p <0.001$). French trees also had significantly more galls than Italian trees (in monoculture: $z = -9.78$, $p <0.001$). However, the magnitude of

differences between provenances varied between treatments (significant provenance by treatment interaction, Fig S6, Table 4). At Hartshorne, gall abundance was significantly higher on French trees compared to Local trees (in monoculture: $z = 3.66$, $p < 0.001$), while Italian trees had lower gall abundance than both Local (in monoculture: $z = -7.82$, $p < 0.001$) and French trees (in monoculture: $z = -11.84$, $p < 0.001$). However, like at Hucking in 2017, the magnitude of provenance differences varied between treatments (Fig S7, Table 4).

Leaf Miners

Provenance was only a significant predictor of mine abundance at Hartshorne, where differences were small yet significant (Fig. 4 G-I). Both Local and French trees had higher mine abundance than Italian trees (Local versus Italian: $z = 2.68$, $p = 0.0075$; French versus Italian: $z = 2.36$, $p = 0.018$), but were not significantly different from each other ($z = 0.055$, $p = 0.96$).

Leaf Manipulators

Provenances did not differ in leaf manipulator abundance at Hucking in 2016, while in 2017, Local trees had a higher abundance of leaf manipulators compared to Italian trees ($z = 2.59$, $p = 0.0097$) but not French trees ($z = 0.80$, $p = 0.42$), and French and Italian trees did not significantly differ ($z = -1.44$, $p = 0.15$). There were no significant differences between provenances at Hartshorne (Fig. 4 J-L).

Impacts of tree diversity treatment

Overall patterns

The influence of tree diversity treatment on insect herbivory and powdery mildew differed between blocks within sites (significant interaction between treatment and block retained in most models, Tables 3-4). We also did not see consistent patterns when comparing between sites, or between survey years at Hucking. Below, we report differences between specific treatment levels only where they were consistent between blocks at each site.

Powdery Mildew

At Hucking treatment effects were variable between blocks in 2016, while in 2017, powdery mildew infection was significantly lower in mixed species, mixed provenance plots compared to all provenance monocultures ($z = 2.78$, $p = 0.0054$). Powdery mildew infection was also lower on all trees in 50:50 and 75:25 mixtures of Local and French provenance trees compared to provenance monocultures ($z = 1.98$, $p = 0.048$ and $z = 3.08$, $p = 0.0021$ respectively). At Hartshorne the only consistent difference between treatments was that powdery mildew infection was significantly lower across provenances in 33:33:33 mixtures compared to all other plots ($z = 2.39$, $p = 0.017$).

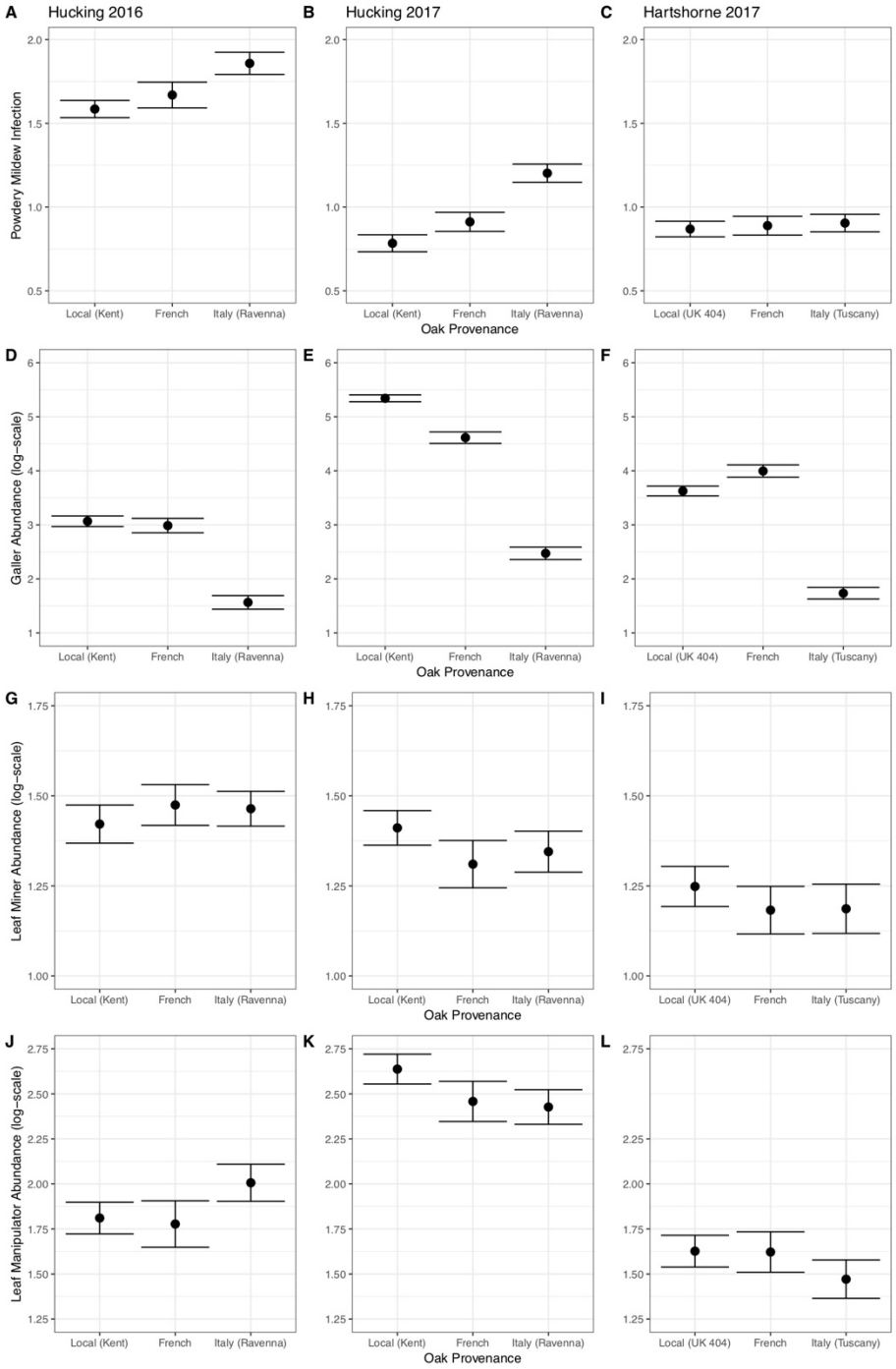


Figure 4: The impact of provenance identity on powdery mildew (A-C), gallers (D-F), leaf miners (G-I), and leaf manipulators (J-L). Plots show means of raw data (black circles) +/- standard errors (error bars). The first column shows results at Hucking in 2016, the second column Hucking in 2017, the third column Hartshorne in 2017.

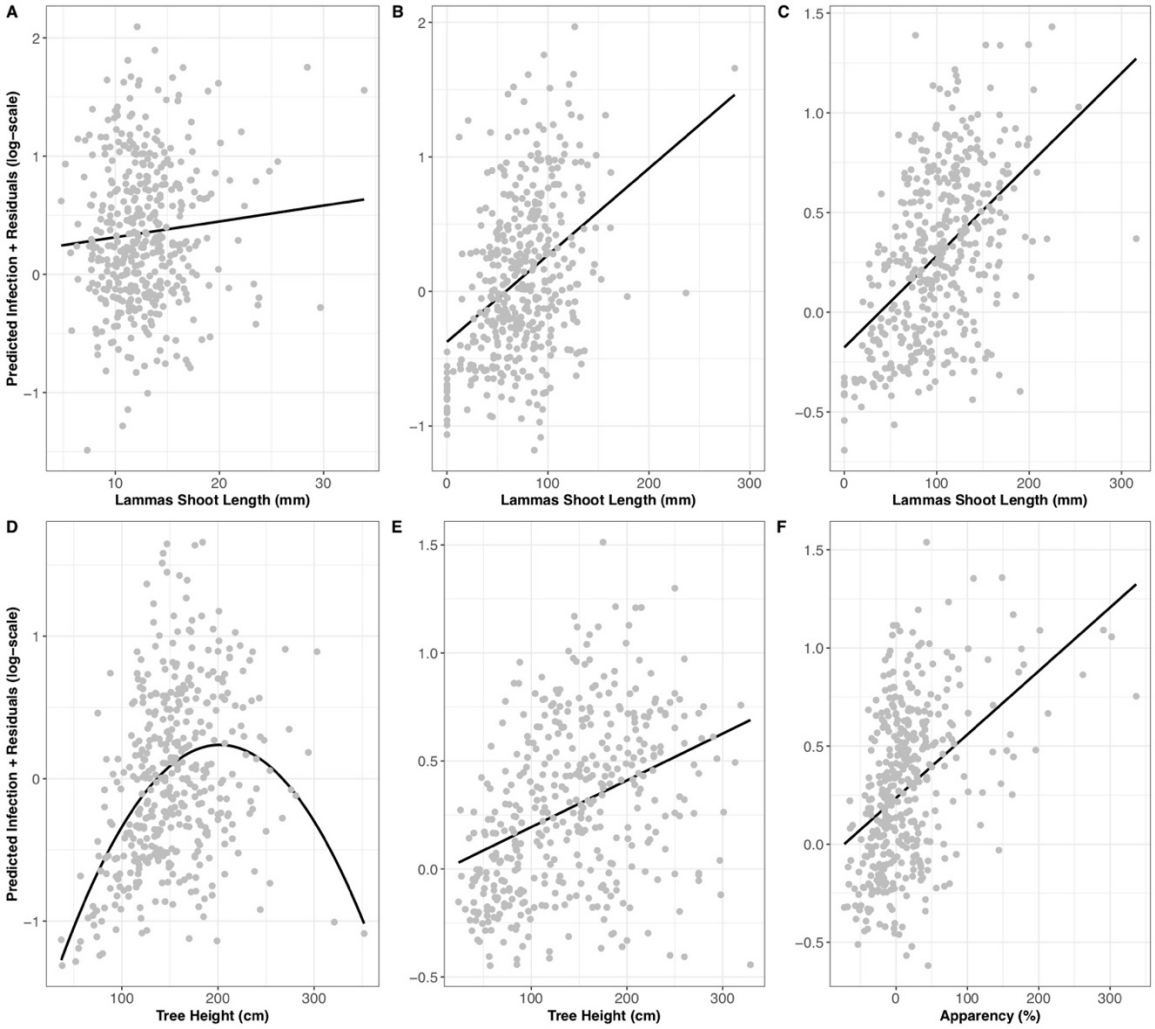


Figure 5: Partial residual plots showing the modelled impacts of lammas shoot length, tree height and tree apparency on powdery mildew infection at Hucking and Hartshorne in 2016 and 2017. Model predictions are calculated by holding all other variables constant in the model. Grey dots show the predicted infection intensity of powdery mildew to which model residuals are added. The black regression line shows either a linear relationship (A-C, E-F) or quadratic (D) depending on which terms were significant in the final model. A) Hucking in 2016; B) Hucking in 2017; C) Hartshorne in 2017; D) Hucking in 2017; E) Hartshorne in 2017; F) Hartshorne in 2017.

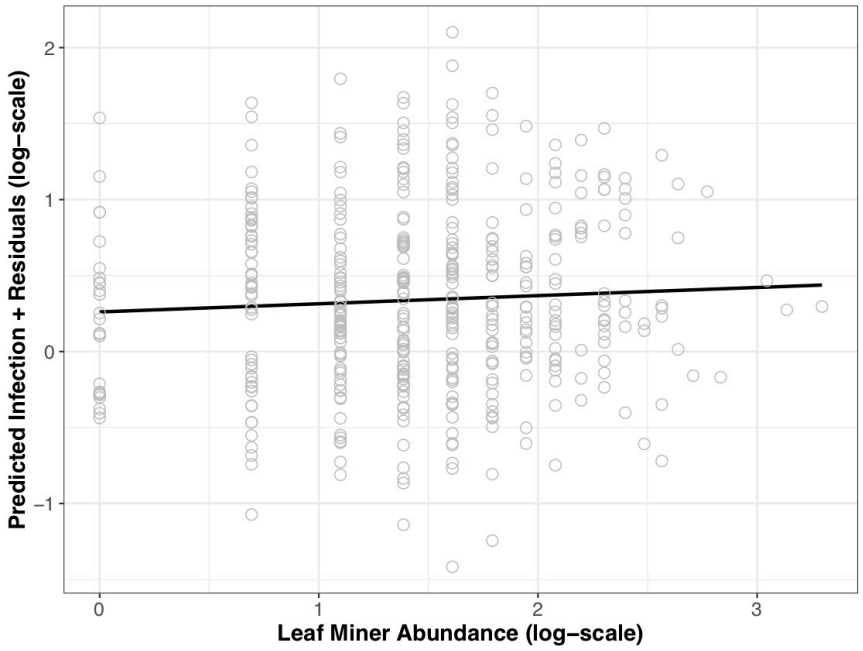


Figure 6: Partial residual plot showing the conditional impact of leaf miner abundance on powdery mildew infection at Hucking (2016), when all other variables are held constant in the model. Model predictions are calculated by holding all other variables constant in the model. Grey dots show the predicted infection intensity of powdery mildew to which model residuals are added, plus black regression line. Leaf miner abundance was a significant positive predictor of powdery mildew infection ($F_{(1,395)} = 5.05$, $p = 0.0252$).

Gallers

At Hucking in 2016, response to treatments was variable between blocks (Table 4). At Hucking in 2017, response varied according to provenance within treatments (Fig. S6). Italian trees had higher gall abundance when mixed with Local trees, compared to monocultures of Italian trees (Fig. S6). This was confirmed by a subset analysis of Italian trees only; Italian trees had higher gall abundance in both 50:50 mixes with Local trees compared to Italian monocultures ($z = 2.36$, $p =$

0.019) and in 33:33:33 provenance mixtures compared to Italian monocultures (z = 3.89, p = <0.001). However, this associational susceptibility when mixed with a more susceptible provenance was only observed in 2017 at Hucking. Despite the same patterns of provenance susceptibility occurring in 2016, Italian trees were not more susceptible in mixes with Local trees. At Hartshorne, there were no consistent treatment differences across blocks, apart from a lower abundance of gallers on both provenances in mixed species, mixed provenance plots compared to others (Table 4 and Fig. S7).

Leaf Miners

At Hucking in 2016, abundance of mines was significantly lower on both Local and Italian provenances in mixed species, mixed provenance plots compared to all other plots, (z = 2.80, p = 0.0051). There were no other consistent treatment effects in 2016, and in 2017, the response to treatment differed between blocks at Hucking. At Hartshorne, the only consistent difference between blocks was lower abundance in mixed species, mixed provenance plots compared to provenance monocultures (Fig. S8).

Leaf Manipulators

There was no significant treatment effect on the abundance of leaf manipulators at Hucking in 2016 and no consistent response to treatment across blocks in 2017. At Hartshorne leaf manipulator abundance was significantly higher in 75:25 mixtures of Local and Italian trees compared to provenance monocultures (z = -2.40, p =

0.016). Leaf manipulator abundance was also significantly lower in 33:33:33 plots, compared to provenance monocultures ($z = 2.00$, $p = 0.046$).

Impacts of plant vigour

Both tree height and shoot length were significant positive predictors in all models, apart from leaf manipulators at Hartshorne, where only primary shoot length, not tree height, was a significant predictor (Table 4). At Hucking in 2017, the quadratic term for tree height was retained as a significant predictor (Fig. 5D) suggesting a non-linear relationship between tree height and powdery mildew infection with a "peak" infection level followed by a decrease at taller tree heights. This term remained significant even when outliers were removed (Fig. S9). There were significant interactions between provenance and lammas shoot length on powdery mildew at both Hucking and Hartshorne in 2017 (Table 3). The strength of the relationship between powdery mildew infection intensity and lammas shoot length differed between provenances, though remained positive across provenances.

Model Response Variable	Year + Site	Explanatory Variable	DF	F value	P value	Effect size and direction (if continuous)
Oak Powdery Mildew	2016 Hucking	Provenance	2	8.05	<0.001	***
		Diversity	6	/	/	/
		Block	2	/	/	/
		Lammas Shoot Length	1	6.57	0.011	+*
		Leaf Miner Abundance	1	5.05	0.025	+*
		Diversity: Block	12	8.74	<0.001	***
	2017 Hucking	Provenance	2	/	/	/
		Diversity	6	3.71	0.0014	**
		Block	2	4.26	0.015	*
		Lammas Shoot Length	1	/	/	/
		Tree Height	1	34.00	<0.001	+***
		Tree Height ^ 2	1	24.52	<0.001	-***
		Provenance : Lammas Shoot Length	2	3.74	0.025	*
	2017 Hartshorne	Provenance	2	/	/	/
		Diversity	6	4.65	<0.001	***
		Block	2	/	/	/
		Lammas Shoot Length	1	/	/	/
		Tree Height	1	5.09	0.025	+*
		Apparency	1	12.20	<0.001	+***
		Provenance : Block	4	4.91	<0.001	***
		Provenance : Lammas Shoot Length	2	3.92	0.021	*

Table 3: Summary of results of GLMs of oak powdery mildew. Results shown are likelihood ratio tests (F tests) for each variable retained in minimal adequate models. Stars denote level of significance in F tests (* = p < 0.05; ** = p < 0.01, *** = p < 0.001) with +/- indicating the direction of effect (positive or negative) for continuous variables. Note that for models with interaction effects, likelihood ratio tests cannot be calculated on the main effect without removing the interaction term from the model, so are not reported here.

Model Response Variable	**Year + Site**	**Explanatory Variable**	**DF**	**χ2 value**	**P value**	**Effect size and direction (if continuous)**
Gallers	2016 Hucking	Provenance	2	64.80	<0.001	***
		Diversity	1	/	/	/
		Block	2	/	/	/
		Primary Shoot Length	1	27.81	<0.001	+***
		Diversity : Block	12	31.25	0.0018	**
	2017 Hucking	Provenance	2	/	/	/
		Diversity	6	/	/	/
		Block	2	21.33	<0.001	***
		Primary Shoot Length	1	37.28	<0.001	+***
		Tree Height	1	27.74	<0.001	+***
		Provenance : Diversity	7	35.15	<0.001	***
	2017 Hartshorne	Provenance	2	/	/	/
		Diversity	6	/	/	/
		Block	2	/	/	/

		Primary Shoot Length	1	13.52	<0.001	+***
		Apparency	1	42.31	<0.001	+***
		Provenance : Diversity	7	20.23	0.0051	**
		Diversity : Block	12	51.55	<0.001	***
Leaf Miners	2016 Hucking	Diversity	6	14.98	0.020	*
		Primary Shoot Length	1	15.56	<0.001	+***
		Block	2	12.75	0.0017	**
	2017 Hucking	Diversity	6	/	/	/
		Block	2	/	/	/
		Primary Shoot Length	1	20.11	<0.001	+***
		Apparency	1	8.77	0.0031	+**
		Diversity: Block	12	42.35	<0.001	***
	2017 Hartshorne	Provenance	2	6.28	0.043	*
		Diversity	6	/	/	/
		Block	2	/	/	/
		Primary Shoot Length	1	31.18	<0.001	+***
		Apparency	1	53.33	<0.001	+***
		Diversity : Block	12	29.12	0.0022	**
Leaf Manipulators	2016 Hucking	Primary Shoot Length	1	11.64	<0.001	+***
	2017 Hucking	Provenance	2	6.62	0.037	*
		Diversity	6	/	/	/
		Block	2	/	/	/
		Primary Shoot Length	1	15.18	<0.001	+***
		Tree Height	1	19.73	<0.001	+***

	Diversity: Block	12	32.23	0.0013	**
2017 Hartshorne	Diversity	6	21.39	0.0016	**
	Block	2	103.48	<0.001	***
	Primary Shoot Length	1	6.28	0.012	+*
	Apparency	1	9.49	0.0021	+**

Table 4: Summary of results of GLMs of oak insect herbivores. Results shown are likelihood ratio tests (Chi-squared tests) for each variable retained in minimal adequate models. Stars denote level of significance in Chi-squared tests (* = $p < 0.05$; ** = $p < 0.01$, *** = $p < 0.001$) with +/- indicating the direction of effect (positive or negative) for continuous variables. Note that for models with interaction effects, likelihood ratio tests cannot be calculated on the main effect without removing the interaction term from the model, so are not reported here.

Impacts of plant apparency

At Hucking, tree height (and its quadratic term) was a better predictor of powdery mildew infection than apparency (Table 3). At Hartshorne, apparency was a better single predictor of powdery mildew than tree height (Fig. 5, E and F) though both remained significant when included in a single model (Table 3). For gallers, tree height was a better predictor of gall abundance than apparency at Hucking, while at Hartshorne, apparency was a better predictor (Table 4). For leaf miners, apparency was a better predictor than tree height at both sites (Table 4). For leaf manipulators, at Hucking tree height was a better predictor than apparency (Table 4). At Hartshorne, the main effect of tree height was nonsignificant, however,

apparency was a significant predictor.

Plant apparency was not systematically linked to diversity treatment at either site, apart from mixed species plots at Hartshorne in 2017, where oak apparency was higher than other treatments, due to poor growth of other planted tree species (Fig. S4).

Insect herbivore abundance as a predictor of oak powdery mildew infection

At Hucking in 2016 there was a significant positive correlation between leaf miner abundance on primary growth, and mildew infection on lammas growth (Fig. 6). However, this result was not consistent between years, as in 2017, none of the insect herbivore groups were significant predictors of mildew infection (Table 3).

Discussion

We found inconsistent impacts of admixing species and provenances on oak insect herbivory and powdery mildew between years and sites. We did not find consistent evidence to support local adaptation by foliar organisms to local tree genotypes, compared to regionally climate-matched tree provenances, though provenance identity impacted both gallers and powdery mildew. We found strong support for the Plant Vigour Hypothesis across insect herbivore groups, and plant vigour also significantly predicted powdery mildew infection. It is

likely that both individual plant vigour and growing context (plant apparency) jointly influence foliar damage organisms on oak.

Inconsistent effects of tree species diversity

Previous studies have found that admixing oak with other tree species can have significant associational effects on insect herbivore and mildew damage (Castagneyrol et al., 2013; Hantsch et al., 2014; Moore et al., 1991). Based on these previous findings, in our study we expected that both insect herbivore abundance and powdery mildew infection would decrease in mixed species plots compared to all others across sites and years, due to increased dilution of susceptible oaks by non-host species. Although associational resistance was observed in some comparisons between mixed species plots and oak monocultures, no group of foliar organisms was consistently reduced across sites and years in mixed species plots. Inconsistent tree diversity effects on insect herbivores between years within the same diversity experiment have also been observed in other studies (Castagneyrol et al. 2013; Castagneyrol et al. 2017). Like Climate Match, most tree diversity experiments used in such studies are still at the establishment stage (Verheyen et al., 2016). Variation between survey years, and between trial sites, could have included ontological changes to tree physiology over time, and contrasting edaphic conditions differentially impacting tree growth and apparency relative to understorey vegetation and surrounding trees. These temporal and spatial sources of variation between years and geographical locations could have outweighed potential dilution effects on pests and pathogens

caused by tree diversity. Moreover, differing local climatic conditions between sites and years could have impacted plot microclimate and tree traits, altering diversity effects on insect herbivores and powdery mildew (Castagneyrol, Jactel, et al., 2018; Walter et al., 2012). Our results therefore emphasise the potential importance of environmental context modulating the outcome of associational effects on pests and pathogens (Castagneyrol, Jactel, et al., 2018).

Provenance matters

French and Italian provenances planted at the Climate Match experimental sites were selected to be adapted to UK climates 50 and 80 years in the future respectively (Broadmeadow et al, 2005). We found that provenance identity impacted insect herbivory and powdery mildew, but with contrasting results between groups. Contrary to the prediction that insect herbivores and mildew will be adapted to exploit local host tree phenotypes, non-local provenances were not always less susceptible to either insect herbivory or powdery mildew infection. Although Italian provenance trees consistently escaped herbivory by gallers across sites and years compared to Local and French provenances, they did not escape herbivory by other insect guilds or powdery mildew infection. In fact in both years powdery mildew infection at one site (Hucking) was higher on Italian provenance trees compared to Local provenance. This contrasts with a reciprocal transplant study which found evidence for local adaptation by mildew populations to individual oaks (Roslin et al., 2007). However, this study took place at a different spatial scale, with alternative host trees sourced from the same

island population, rather than different geographic regions as in our study. Cynipid gallwasp larvae develop inside oak tissue which they physiologically manipulate to form a gall which provides both physical protection and a food source (Harper et al., 2004; Stone & Schönrogge, 2003; Stone et al., 2001). By comparison, leaf miners and manipulators do not exercise the same level of control over the plant tissue on which they feed, and these guilds were therefore expected to demonstrate a stronger response to variation in plant tissue quality across provenances (Huguet, Stone, & Body, 2016; Sopow, Shorthouse, Strong, & Quiring, 2003). Contrary to this, we found that gallwasps showed a stronger response to provenance than both leaf miners and leaf manipulators.

Phenological differences between provenances may explain the contrasting responses of gallwasps and powdery mildew infection to tree provenance. Italian provenance trees had more lammas shoots compared to Local and French trees by the same date in July (Fig. S3). Kleinschmit (1993) suggested that oak provenances from more southerly latitudes had earlier emergence of lammas shoots and thus higher cross-continental susceptibility to powdery mildew. Although our findings partially support this hypothesis, at Hartshorne, Italian and French provenances were not more susceptible to powdery mildew, despite showing earlier emergence of lammas shoots compared to Local trees (Fig. S3). Gallwasps require host tissues to be at the correct physiological age for successful gall induction (Weis, 1988), and local adaptation to bud burst has been observed in gallwasp species (Egan and Ott 2007). Sinclair et al (2015) found variation in gallwasp abundance across sessile oak (*Q. petraea*) provenances differing in the timing of

spring budburst. It is possible that phenological asynchrony of Italian provenances with local galler populations may explain why Italian trees in our study had lowest gall abundance across sites and years. Some galler groups are also known to respond positively to plant module size (Flaherty & Quiring, 2008; Kopelke et al., 2003). We found differential resource allocation to plant modules across provenances; local trees invested in longer primary shoots, non-local provenances produced more/longer lammas growth (Fig. S1 and S3). These differences may partly explain variation in provenance susceptibility to insect herbivores and mildew. However, as is usual for correlational studies, other non-measured differences between provenances could also contribute to variation in herbivory and mildew infection, such as leaf chemistry (foliar nutrition) and defence (Pearse, 2011). As local and non-local provenances did not consistently differ in herbivory or mildew, this could explain the lack of consistent associational effects seen in mixed provenance plots compared to provenance monocultures.

Positive impacts of tree vigour and apparency

Tree vigour and apparency were consistent predictors of insect herbivory and powdery mildew infection. More vigorous trees (taller, with longer lammas shoots, Fig. 5), and more apparent trees (Fig. 5) had higher levels of powdery mildew infection at both sites. As biotrophic pathogens, powdery mildews may respond positively to vigorous hosts with a higher photosynthetic rate (Schnathorst, 1965). To our knowledge, our study is the first to show a positive

association between oak powdery mildew and tree vigour, as well as tree apparency. Our results are also consistent with the Plant Vigour Hypothesis for insect herbivores, which predicts higher insect abundance on more vigorous hosts, potentially due to trade-offs between growth and defence (Price, 1991). But, tree apparency was a better predictor than tree height in most models of insect herbivory, and in one case (leaf manipulator abundance at Hartshorne in 2017) apparency was a significant predictor while tree height was not.

Unlike previous work, tree apparency was not systematically linked to diversity treatment in these trials (Castagneyrol et al. 2013). Here, we found that taller trees experienced higher herbivory and mildew, especially when growing in the context of less vigorous surrounding trees. For insect herbivores, apparency may determine host searching efficiency, as has been suggested previously for the pine processionary moth (Régolini et al., 2014). For powdery mildew, a passive, wind-dispersed pathogen (Marçais & Desprez-Loustau, 2014), spore interception may be more efficient when hosts are taller and therefore more prominent in the landscape.

No clear association between insects and powdery mildew

We did not find a consistent association between insect herbivores and powdery mildew, although at one site in one year (Hucking in 2016) leaf miner abundance on primary shoots was positively correlated with powdery mildew infection on lammas growth. This suggests either that leaf miners and powdery mildew

preferred the same trees, or that there is a direct interaction between the two groups. Previous work has suggested that early season herbivore pressure can promote subsequent bud flushes, increasing the amount of leaf material susceptible to mildew infection (Marçais and Desprez-Loustau 2014). Alternatively, an interaction between damage agents could occur through cross-talk between plant defence signalling pathways (Schultz, Appel, Ferrieri, & Arnold, 2013). Meta-analysis has shown that herbivores inducing the jasmonic acid (JA) pathway (including leaf miners) have an antagonistic effect on subsequent herbivores inducing the salicylic acid (SA) pathway, which is also induced by biotrophic pathogens (Moreira et al., 2018a). Understanding interactions between insects and pathogens is important as many plant health problems, such as oak decline, can have multiple causative agents (Denman et al., 2018; Thomas, 2008). More work is required to establish the strength and consistency of the interaction between insects and powdery mildew, and to establish potential underlying mechanisms in terms of plant phenology and defence.

Conclusion

We found that the effects of both tree species and genetic diversity were variable, suggesting that forest diversity effects on pests and pathogens can be unpredictable across space and time. In addition, we show that individual tree vigour and growth relative to surrounding trees can drive colonisation of tree

hosts by herbivores and pathogens, providing compelling support for both the Plant Vigour and Plant Apparency hypotheses.

Acknowledgements

We thank those who assisted with the collection of field data: Tara Love, Rebecca Garnett, Ellie Scopes, and the Forest Research Technical Support Unit: Mark Oram, Rob Cullen and Nick Moody. The Climate Match experimental trials have been sponsored by the National Forest Company, the Forestry Commission and the European Union (European Regional Development Fund ERDF) within the framework of the European INTERREG IV A 2 Mers Seas Zeeën Cross-border Cooperation Programme 2007-2013 (Project 090316 016-FR MULTIFOR: Management of Multi-Functional Forests). The donation of land for the trials and site management is gratefully acknowledged from the Woodland Trust and the Forestry Commission. EF's DPhil is funded through the Oxford-NERC Doctoral Training Partnership in Environmental Research and a CASE studentship with Forest Research. NB, MG & KS were supported by a grant jointly funded by the UK's Biotechnology and Biological Sciences Research Council, Department for Environment, Food and Rural affairs, Economic and Social Research Council, Forestry Commission, Natural Environment Research Council and the Scottish Government, under the Tree Health and Plant Biosecurity Initiative (THAPBI; grant number: BB/N022645/1).

CHAPTER 3: ASSOCIATIONAL RESISTANCE TO BOTH INSECT AND PATHOGEN DAMAGE IN MIXED FORESTS IS MODULATED BY TREE NEIGHBOUR IDENTITY AND DROUGHT

Field, E.[1*], Castagneyrol, B.[2], Gibbs, M.[3], Jactel, H.[2], Barsoum, N[4]; Schönrogge, K.[3], & Hector, A.[1]

[1] Department of Plant Sciences, South Parks Road, University of Oxford, Oxford, OX1 3RB, United Kingdom

[2] INRA (French National Institute for Agricultural Research), UMR 1202 BIOGECO, University of Bordeaux, 33610 Cestas, France.

[3] Centre for Ecology & Hydrology, MacLean Building, Crowmarsh Gifford, Wallingford, OX10 8BB, United Kingdom.

[4]Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10, 4LH, United Kingdom.

Article status

This chapter was published in *Journal of Ecology* in March 2020.

Citation information:

Field, E., Castagneyrol, B., Gibbs, M., Jactel, H., Barsoum, N., Schönrogge, K., & Hector, A. (2020). Associational resistance to both insect and pathogen damage in mixed forests is modulated by tree neighbour identity and drought. *Journal of Ecology* 108: 1511–1522. https://doi.org/10.1111/1365-2745.13397

Data accessibility

Data from this study is available for download from the Dryad repository (https://doi.org/10.5061/dryad.zw3r2285j) or alternatively from the NERC Environmental Information Data Centre (https://doi.org/10.5285/35bb7278-a5c4-4819-9b1a-efa84a479d68).

Author contributions

EF, AH, BC, & HJ conceived the study. All authors contributed to study design. EF, BC and HJ carried out fieldwork. EF analysed the data with input from BC & AH. EF wrote the manuscript. All authors commented on previous and final versions of the manuscript.

Abstract

Tree health declines can be caused by interactions between pests and pathogens and many studies have shown a reduction in their damage in mixed species forests compared to monocultures. Yet few authors have considered tree diversity effects on both groups simultaneously. Moreover, it is unclear whether diversity effects on tree pests and pathogens are robust to changes in abiotic conditions, such as drought. We addressed tree diversity effects on foliar insect herbivory, oak powdery mildew and their interaction under contrasting water regimes in a large-scale tree diversity experiment in SW France. Using an irrigation treatment that alleviated drought conditions, we were able to experimentally assess the

effects of tree diversity under contrasting abiotic environments. We surveyed plots along a richness gradient from 1 – 4 tree species, in which a focal study species of oak (*Q. robur*) was mixed with other oak species (*Q. pyrenaica* and *Q. ilex)* and a taller, broadleaved species (*Betula pendula*). Increasing tree species richness lowered leaf miner abundance, leaf chewer damage and oak powdery mildew infection, consistent with a protective effect of resource dilution. However, richness effects on leaf miners were stronger in irrigated compared to non-irrigated blocks, indicating that environmental conditions can modulate diversity effects. Separate from the effect of tree species richness, the presence of birch in a plot increased damage by leaf chewers and powdery mildew, but lowered leaf miner damage, suggesting additional tree neighbour identity effects potentially linked to modulation of microclimate. We found a negative association between leaf miner abundance and oak powdery mildew, consistent with antagonism between oak damage agents. Overall, our study illustrates the importance of considering both tree diversity and composition (neighbour identity) in designing forests more resistant to pest and pathogen damage.

Introduction

The importance of biodiversity for ecosystem functioning and services is being increasingly recognised globally (Cardinale et al. 2012; van der Plas et al. 2016; Isbell et al. 2017). In spite of this, most new forests are still planted as monocultures rather than mixed species forests (Nichols et al., 2006; Paquette et al., 2018). Over recent decades, much evidence has accumulated to suggest that

mixed species forests can have higher levels of productivity (Chamagne et al., 2016; Jactel et al., 2018), stability to environmental changes (Morin et al., 2014; Plas et al., 2016) and resistance to abiotic and biotic disturbances (Jactel et al., 2017). Planting mixed species forests is often reported as reducing forest insect damage compared to monocultures, which has been supported by meta-analyses of evidence from planted and natural forests, and biodiversity-ecosystem functioning experiments (Castagneyrol et al., 2014; Jactel & Brockerhoff, 2007). The trend towards lower damage to hosts in more diverse ecosystems is termed associational resistance, as opposed to associational susceptibility (Barbosa et al., 2009). The impact of mixed forests on pathogens has been less extensively studied, but several papers report cases of associational resistance (Hantsch et al., 2014; Pautasso et al., 2005). However, previous studies showed mixed forests do not always show associational resistance to pests and pathogens (Kambach et al., 2016; Schuldt et al., 2010), suggesting that associational effects may be context dependent, for instance, on abiotic conditions (Jactel, Poeydebat, van Halder, & Castagneyrol, 2019).

There are at least three proposed mechanisms hypothesised to underpin reduced damage in mixed forests that can apply to both insect pests and pathogens. Firstly, when total tree density is approximately constant, increasing tree diversity will reduce susceptible host density. This thereby reduces the availability of breeding substrate for a pest or pathogen, meaning it is less likely to build up to population epidemic levels - the dilution effect (Civitello et al., 2015). Secondly, associational resistance in mixed stands may be driven by a reduction in herbivore ability to

locate and reach its resource in the presence of non-host neighbour trees. Reduced physical apparency of a focal host when surrounded by taller heterospecific neighbours has been shown to reduce insect herbivory (Castagneyrol et al., 2013; Damien et al., 2016). Plant apparency may also be reduced through interference with chemical cues used by insects to locate hosts. For instance, Jactel et al (2011) found that the release of non-host volatile organic compounds impacted the ability of the pine processionary moth to find its host in mixed species stands. Thirdly, and leading from the second hypothesis, tree diversity, and the particular species composition of mixed forests, can lead to alterations to microclimatic conditions (Ehbrecht, Schall, Ammer, & Seidel, 2017) with knock-on effects on pests and pathogens. In particular, differences in tree height between species can lead to alterations in canopy closure between plots differing in tree diversity (Muiruri & Koricheva, 2017). Alterations to the abiotic environment (such as changes to light availability, temperature and humidity) can all have direct impacts on pests and pathogens' survival or performance (Dillon & Meentemeyer, 2019; Jactel, Koricheva, & Castagneyrol, 2019; Stoepler & Lill, 2013), as well as indirect effects via changes to tree leaf traits, such as secondary metabolites (Castagneyrol, Jactel, et al., 2018; Forey et al., 2016b; Walter et al., 2012).

Previous studies of the impacts of tree diversity on pest organisms have mostly considered either insect herbivores or fungal pathogens in isolation rather than both groups simultaneously on the same trees (for instance, on oak: Moore, Warrington and Whittaker, 1991; Castagneyrol *et al.*, 2013; Hantsch *et al.*, 2014). Yet, fungi and insects can have reciprocal interactions on each other's

performance through multiple biological mechanisms (Fernandez-Conradi et al., 2018; Tack, Gripenberg, & Roslin, 2012). This reduces our ability to understand if the same mechanisms of associational effects apply to both pests and pathogens, and if multi-trophic interactions alter the outcome of associational effects. Initial damage by primary pests or pathogens may lead to opportunistic colonisation of a stressed tree by secondary pests or pathogens, for instance by altering leaf chemical defences or phenology (Adams et al., 2013; Marçais & Bréda, 2006). For example, it has been reported that high leaf insect defoliation on pedunculate oak (*Quercus robur)* early in the season can induce changes to oak phenology by promoting the growth of second flush shoots ("lammas shoots") that are infected by oak powdery mildew, a specialist biotrophic (developing on living host tissue) pathogen (Delatour, 1983; Glazebrook, 2005; Marçais & Desprez-Loustau, 2014). Sequential attack by insect herbivores and pathogens on the same host plant may trigger different plant defence signalling pathways, leading to cross-talk between pathways and antagonistic or facilitative interactions between attackers (Erb, Meldau, & Howe, 2012; Pieterse et al., 2012). For instance, a meta-analysis showed that herbivores inducing the jasmonic acid defence signalling pathway (such as leaf miners and chewers) reduced subsequent damage by herbivores inducing the salicylic acid defence pathway, the pathway also used by biotrophic pathogens (Moreira, Abdala-Roberts, & Castagneyrol, 2018b). Dependent on the study system, any effect of tree diversity on insects may therefore have indirect effects on pathogens, and *vice versa*. Unravelling the complex interactions between insect pests and fungal pathogens will shed light on the still poorly understood

mechanisms underpinning the effects of tree diversity on resistance to biotic hazards (Castagneyrol et al., 2017; Schuldt et al., 2017).

The environmental context in which biotic interactions are embedded has recently been shown to influence the effects of tree diversity on pests and pathogens. For example, Castagneyrol, Jactel, et al. (2018) found that leaf insect herbivory on birch increased with tree species richness, but only under drought conditions. Drought stress can have strong plant-mediated effects on both insects and pathogens (Desprez-Loustau et al., 2006; Jactel et al., 2012).The Plant Stress hypothesis predicts an increase in insect damage under conditions of plant water stress, for instance, due to changes to leaf defensive chemistry or C:N ratio (Walter et al., 2012; White, 1974). Similarly, for pathogens, the "predisposition concept" (Schoenweiss, 1986) argues that water stress can predispose plants to infection by opportunistic plant pathogens. There is also emerging evidence that drought impacts on forests are altered by tree diversity (Grossiord, 2019). Drought could modulate diversity effects on insects and pathogens, for instance, if water stress impacts tree growth, in turn impacting associational effects. So-called "neighbour modulated" or "bottom-up" impacts of drought on diversity effects, where neighbours modify plant responses to drought, have been shown in several studies, although the mechanisms at play are still elusive (Castagneyrol, Moreira, et al., 2018; Forrester, Theiveyanathan, Collopy, & Marcar, 2010; Grossiord et al., 2014).

In this study we used a tree diversity experiment in SW France to investigate how different components of tree diversity (species richness, host dilution, tree apparency) altered insect herbivore and fungal pathogen damage. We further asked whether tree diversity effects on a specialist fungal pathogen (oak powdery mildew) were influenced by tree diversity effects on insect herbivory. Finally, we asked whether independent and interactive effects of tree diversity on insect herbivore and pathogen damage were altered under drought (ambient drought conditions versus irrigation treatment). We tested the following hypotheses on focal oak hosts (*Quercus robur* L.):

(1) increasing species richness and/or increasing resource dilution reduces damage by both oak insect herbivores and oak powdery mildew;

(2) reducing plant apparency as a result of mixing oak with a much taller broadleaved species (birch) lowers mildew and herbivore damage more than predicted just from increasing tree species richness (impacts of neighbour identity as well as diversity);

(3) more vigorously growing oaks (taller, with longer shoots) receive more damage by insect herbivores and higher mildew infection;

(4) oak powdery mildew infection is positively associated with insect herbivore damage (i.e., a positive interaction between plant antagonists).

(5) drought stress increases damage by insect herbivores (leaf chewers and leaf miners) and oak powdery mildew compared to irrigation conditions;

(6) drought stress could alter the strength and direction of tree diversity effects on both insect herbivory and powdery mildew infection.

By considering both the abiotic context of tree diversity effects, and the potential for multi-trophic interactions, our work extends our current understanding of the mechanisms of tree diversity effects on insects and pathogens.

Materials and Methods

Study System

Quercus robur is a widespread broadleaved tree species throughout the Western Palearctic harbouring a diverse insect herbivore community (Brandle & Brandl, 2001; Eaton, Caudullo, Oliveira, & de Rigo, 2016). In SW France, the main defoliating insects are leaf miners, chewers and skeletonisers, along with oak powdery mildew (*Erysiphe* spp.), a specialist foliar pathogen that is also the most common foliar pathogen on oaks in Europe (Marçais & Desprez-Loustau, 2014). It is caused by multiple cryptic species of ascomycete fungi of the genus *Erysiphe* with different phenological niches: *E. quercicola* infects oak buds and leaves, while *E. alphitoides* infects only leaves (Desprez-Loustau et al., 2018; Mougou et al., 2008). However, in the absence of molecular identification of mildew species, we

use the term "oak powdery mildew" to describe the pathogen.

In SW France, we observed that insect herbivore and oak powdery mildew damage were temporally and spatially separated on trees, due to oaks exhibiting indeterminate growth with multiple bud flushes occurring over the growing season. Most insect herbivore damage occurred on primary shoots from the first bud burst in late spring (April-May), with oak leaf quality then declining sharply over time for insects (Feeny, 1970; Tikkanen & Julkunen-Tiitto, 2003). Oak powdery mildew infection occurred on "lammas" shoots resulting from subsequent bud bursts in mid-summer (July-August), as mildew ascospores are released from overwintering chasmothecia from June onwards (Marçais, Kavkova, & Desprez-Loustau, 2009). Oak powdery mildew infection success also declines rapidly with leaf age (Ayres & Edwards, 1982). Mildew infection success is therefore highest on young leaves produced from lammas shoots, with July generally being the peak period for mildew infection (Marçais & Desprez-Loustau 2014, M-L Desprez-Loustau, pers. com).

Experimental design

Data collection took place at a large tree diversity experiment in SW France planted in 2008, ORPHEE (44°440 N, 00°460 W). The ORPHEE experiment follows a randomised blocked design (see Fig. S10). Eight blocks covering 12 ha were

established using five native tree species: three oak species (*Quercus robur, Quercus pyrenaica, Quercus ilex)* one other broadleaved tree species, silver birch (*Betula pendula)* and one conifer species, maritime pine (*Pinus pinaster*). The experiment consists of 8 blocks of 32 treatments each, in a fully factorial design for tree species richness spanning a gradient from monocultures to five species mixtures with all possible combinations. Each treatment combination is replicated once per block, plus an additional replication of the five species mixture giving a total of 32 treatment plots randomly distributed within each block. Each plot consists of 10 rows of 10 trees planted at 2 m spacing (100 trees per plot). Tree species were planted according to a systematic alternate pattern, so that each tree of a given species is surrounded by at least one tree of all other tree species present in a given composition. In addition, from 2015 an annual irrigation treatment has been applied at the block level from May – October to half of the blocks (irrigation + rainfall versus rainfall only). Irrigation consists of sprinkling the equivalent of 3 mm precipitation per night and per experimental plot from a 2.30 m pole at the centre of each plot. This treatment is designed to alleviate the severe summer drought that is typical of the climate in this region. The irrigation treatment was previously shown to significantly increase the water content of plants and soil, and to increase summer predawn water potential (Castagneyrol et al., 2017; Castagneyrol, Jactel, et al., 2018) so will not be further described here.

We selected plots to survey along a tree diversity gradient, including all mixtures with the focal host *Q. robur* except those with pine, in order to focus on broadleaved species only. This spanned from *Q. robur* monoculture, three 2-species mixes with *Q. robur*, three 3-species mixes with *Q. robur*, and the 4-species

mix of the three oak species and birch, for a total of 8 treatments. We chose these treatments in order to span a gradient of oak apparency, with apparency defined as the height of focal oaks relative to the mean height of surrounding trees (Castagneyrol et al., 2013). Birches are faster growing than oaks, thus at ORPHEE at the time of observation, birches were significantly taller than all three oak species (Fig. S11; Appendix 3). The apparency of focal oaks was therefore primarily driven by the proportion of birch in a plot, which we also corroborated by comparing canopy closure between treatments with and without birch (Fig. S11; Appendix 3). This allowed us to compare plots identical in tree species richness but differing in oak apparency. We surveyed blocks 1-6 (3 irrigated, 3 non-irrigated blocks). Within each plot we randomly selected four *Q. robur* trees for damage assessment. We focused on *Q. robur* as it is an important species economically in Europe (Eaton et al., 2016), and is the most frequently studied of the three oak species planted, for example in relation to tree diversity, insect herbivory and oak powdery mildew, enabling better comparison with previous studies.

Data Collection

We recorded insect herbivory on primary shoots, and powdery mildew infection on lammas shoots, with the shoot types being easily differentiated by a ring scar separating bud flushes. At the time of the survey, *Q. robur* was the only susceptible species to oak powdery mildew. No mildew was observed on *Q. ilex*, since evergreen oaks are known to be resistant to mildew (Marçais & Desprez-

Loustau, 2014). No mildew was observed on *Q. pyrenaica* during the survey period, likely due to the later date of bud flush of this species (Marçais & Desprez-Loustau, 2014). Lammas shoots had not yet been produced on *Q. pyrenaica* when spores of oak mildew were released. For insect herbivores, we assessed damage by leaf chewers, skeletonisers and miners. Leaf gallers were found to be very rare in the experiment at the time of surveying. Most leaf chewing and skeletonising species present at ORPHEE have been found to be generalist species feeding on both oak and birch, while the leaf mining species present are specialists feeding on a narrow range of hosts within Fagacae (Castagneyrol et al., 2013; Giffard, Jactel, Corcket, & Barbaro, 2012). Therefore, while *Q. robur* and birch may overlap in terms of leaf chewing and skeletonising species, leaf miners are likely to feed only on the oak species present at ORPHEE.

As insect herbivory begins soon after primary bud flush in early spring, while powdery mildew infection begins later in the season, on lammas growth, we assumed that insect herbivory influenced powdery mildew infestation rather than the other way around. On each tree, we haphazardly chose eight shoots at a range of heights and angles around the tree, with both primary and lammas growth. Per primary shoot we haphazardly selected five leaves and recorded the number of mines per leaf, which was then summed at tree level (over 40 leaves). We classified damage by leaf chewers and leaf skeletonisers taken together into seven percentage classes per leaf: 0 (0%); 1 (1-5%); 2 (6-15%); 3 (16-25%); 4 (26-50%); 5 (51-75%); 6 (more than 76%), as chewing damage was far more abundant than skeletonisation, and some species act as both chewers and skeletonisers during

different larval stages (BC, pers. obs.) We refer to this group hereafter as "chewers". Five leaves were haphazardly chosen on each lammas shoot, and percentage cover by mildew mycelium classified into five classes: 0 (0%); 1 (1-25%); 2 (26-50%); 3 (51 – 75%); 4 (76-100%), after previous authors (Bert et al., 2016; Field et al., 2019). Percentage damage by chewers and mildew was then averaged at the tree level (40 leaves) using the median point of each damage class. We also measured the length of each primary and lammas shoot (mm), as well as tree height (cm), to use as additional covariates.

Statistical Analysis

All analyses were completed in R version 3.5.1 (R Core Team, 2020). We analysed miner abundance, chewer damage and mildew infection at tree level using Generalised Linear Mixed Effect Models (GLMMs, using lme4 package in R, glmer() function), as our design was blocked with trees nested within plots within blocks. Since irrigation was applied at the whole block level, the ORPHEE experiment is akin to a split-plot design, with the block being the experimental unit for irrigation (Fig. S10). In addition to the tree level analysis, we performed the same analysis at shoot level (over 5 leaves), to compare the results from identical models at different ecological levels. Results were almost identical between the sets of models, but residual checking plots showed that tree level models performed better in terms of model assumptions. Thus for the sake of clarity, we report tree level models only here, and results of shoot level models can be found in supplementary material (Table S4). The following starting model

equation was used (R syntax, variables in bold are fixed effects, * indicates interaction terms, (1 | X) denotes random effects):

Y ~ **log(Shoot Length) + Tree Height + Irrigation * (Resource Dilution + Richness + Birch) +** (1 | Plot) + (1 | Block)

To analyse data at tree level, we averaged shoot length over the eight primary (herbivores) or lammas (mildew) shoots sampled, and log transformed to improve normality. Models of mildew included both primary and lammas shoot length, herbivory only primary shoot length. No continuous variables were sufficiently strongly correlated to prevent inclusion in the same model (Fig. S12). To assess the potential for a direct effect of herbivory on mildew, we included miner abundance and chewer damage at the tree level as explanatory terms in models of mildew. For miners, we fitted a negative binomial GLMM with log link, as initial GLMMs fitted using the Poisson distribution were overdispersed (using the glmer.nb() function in R, from the lme4 package). All other models were gaussian GLMMs with identity link.

We achieved minimal adequate models using backward selection of nonsignificant terms, starting with interactions. Tree species richness and resource dilution were highly correlated (Fig. S13) and minimal adequate models included whichever of the two composition variables was most significant following model selection (Table 5). We chose to test the effects of both variables, as they carried different information despite being correlated. Resource dilution refers only to the

focal host, *Q. robur,* while tree species richness (tree diversity) included the effects of adding other tree species. In other words, resource dilution was an estimate of focal host density and thus the hypothesised resources available to build up pest and pathogen populations, at least for leaf miners and mildew. Tree species richness, on the other hand, described the barriers to host colonization by dispersing propagules as a result of adding more non-host trees. We estimated model fit by calculating marginal (fixed effects only, R^2m) and conditional (fixed and random effects, R^2c) R^2 for each model, as in Nakagawa & Schielzeth (2013). We used residual checking plots to check whether model assumptions were met.

Results

Insect Herbivory

Leaf Miners

The mean number of mines per tree was 8.60 (SE = 0.41). The effects of tree species richness and birch presence on miners were contingent on irrigation, with significant interactions between these variables and irrigation (Table 5). Increasing tree species richness was associated with lower mine abundance, but only in irrigated blocks (Fig. 7A). Overall, birch presence lowered mine abundance (Fig. 7B). There were also differences in the effect of irrigation when birch was present or absent, with lower mine abundance in drought compared to irrigated conditions, but only in the presence of birch (Fig. 7B). None of the other composition variables or their interactions with irrigation were significant

predictors (Table 5). Primary shoot length had a highly significant positive effect on the number of mines (Table 5).

Leaf Chewers

Mean chewer damage per tree was 13.54 % (SE = 0.58). Chewer damage significantly decreased with increased tree species richness (Fig. 8A). However, the presence of birch in a plot increased chewer damage (Fig. 8A). Chewer damage also decreased with decreasing oak density, i.e. increasing resource dilution (Table 5), but not when included in the same model as richness, likely as the two variables were correlated (Fig. S13). Chewer damage was significantly lower in irrigated compared to non-irrigated blocks (Fig. 8B, Table 5). None of the interactions between composition variables and irrigation were significant, nor were the other covariates tree height and shoot length (Table 5).

Oak Powdery Mildew

Mean mildew infection per tree was 17.85 % (SE = 1.29). Increasing tree diversity had a negative effect on oak powdery mildew infection (Table 5, Fig. 9A). Including resource dilution of *Q. robur* as a predictor *post hoc* instead of tree species richness also showed a consistent and significant negative effect on mildew (Table 5). Mildew infection significantly increased in the presence of birch (Fig. 9B). Irrigation had no significant effect on mildew infection, either as main or interactive effect.

Longer primary shoots were associated with lower mildew infection, measured on lammas shoots on the same trees (Table 5). Longer lammas shoots were associated with higher mildew infection, but the effect was only marginally significant (Table 5). Tree height was not a significant predictor of mildew infection (Table 5).

Powdery mildew significantly decreased with increasing abundance of leaf mines, measured on primary shoots on the same trees (Fig. 10). The amount of leaf chewer damage did not impact mildew infection (Table 5). One route for the negative association between primary shoot length and mildew could be an indirect effect, via the direct positive effect of primary shoot length on leaf miner abundance (Table 5). However, the fact that both primary shoot length and miner abundance had significant effects on powdery mildew rather suggests the two had independent direct effects (Table 5).

	Estimate	SE	$\chi 2$	DF	P-value	Direction	R^2m (R^2c)
LEAF MINER ABUNDANCE (*N = 190)*							0.12 (0.15)
Intercept	1.01	0.34					
Richness	0.084	0.096		1			
Presence of Birch	-0.26	0.16		1			
Irrigation	0.58	0.30		1			
Log(Primary Shoot Length)	0.29	0.073	15.92	1	<0.001	+	
Richness:Irrigation	-0.35	0.14	7.28	1	0.0070	-	
Presence of Birch:Irrigation	0.46	0.23	4.25	1	0.039	+	
LEAF CHEWER DAMAGE (*N = 190)*							0.12 (0.15)
Intercept	2.87	0.15					
Resource Dilution	-0.51	0.21	5.64	1	0.018	-	
Presence of Birch	0.20	0.093	4.57	1	0.032	+	
Irrigation	-0.34	0.16	4.46	1	0.035	-	
OAK POWDERY MILDEW (*N = 187)*							0.12 (0.28)
Intercept	47.26	9.31					
Richness	-4.62	1.79	6.60	1	0.010	-	
Presence of Birch	6.40	3.04	4.51	1	0.034	+	

Leaf Miner Abundance	-0.62	0.22	8.00	1	0.0047	-
Log(Lammas Shoot Length)	4.31	2.26	3.79	1	0.051	+
Log(Primary Shoot Length)	-9.52	2.41	15.45	1	<0.001	-

Table 5: Results of GLMMs testing the effects of plot composition and tree traits on oak powdery mildew and insect herbivory at shoot and tree level. Given are estimates, standard errors, likelihood ratio test results (χ2 value, degrees of freedom and p value), and effect direction (+ indicating a positive effect of a variable, - indicating a negative effect of a variable), for minimal adequate models. Estimates for leaf miner and leaf chewer models are on the log scale. Note that for models with interaction effects, likelihood ratio tests cannot be calculated on the main effect without removing all interaction terms from the model, so are not reported here. R^2 values for each model are given in the final column, (R^2m) = marginal R^2 including fixed effects only; (R^2c) = conditional R^2 including both fixed and random effects.

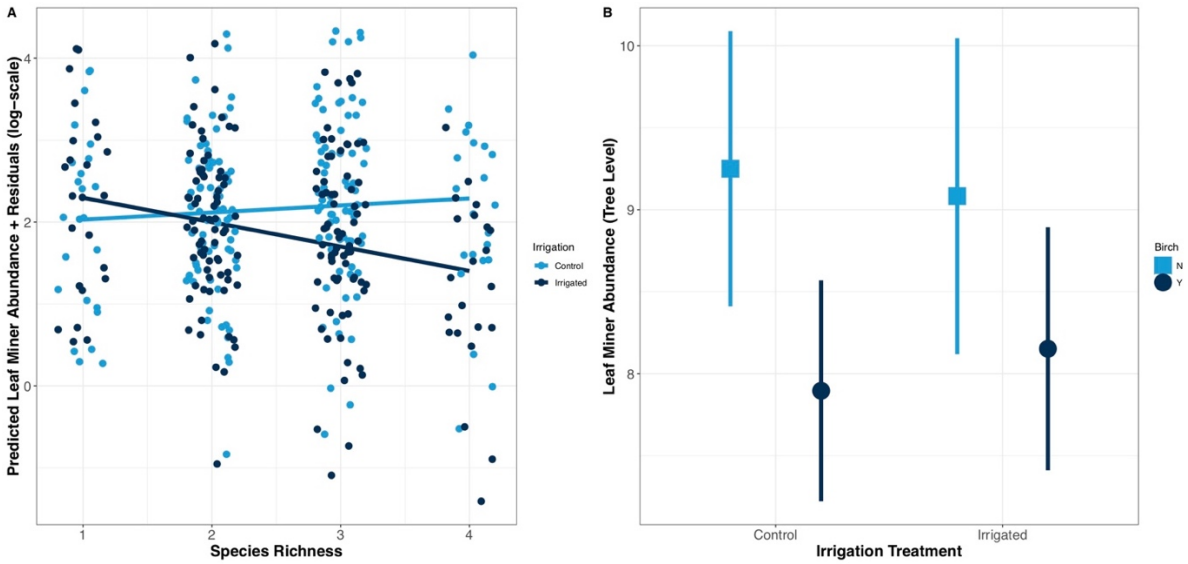


Figure 7: The effect of tree species richness and irrigation (A) and irrigation and birch presence (B) on leaf miner abundance. (A) Partial residual plot with the regression lines showing the strength of the relationship between tree species richness and leaf miner abundance when all other variables are held constant in the model. Model predictions are generated using observed values of miner abundance while holding all other continuous variables in the model constant at a mean value, and random effects set to zero. Points plotted are model predictions to which residuals from the minimal adequate model are added. (B) Points show mean of raw data (tree level), error bars are +/- 1 standard error of the mean.

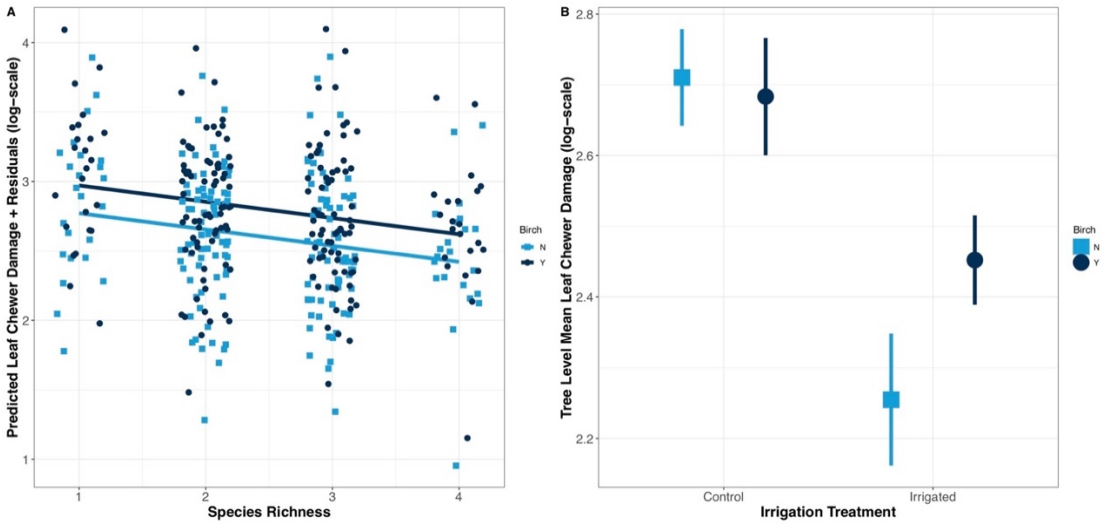


Figure 8: The effect of tree species richness and birch presence (A) and the effect of irrigation and birch presence (B) on leaf chewer damage. (A) Partial residual plot with the regression lines showing the strength of the relationship between species richness and leaf chewer damage when all other variables are held constant in the model. Model predictions are generated using observed values of chewer damage while holding all other continuous variables in the model constant at a mean value, and random effects set to zero. Points plotted are model predictions to which residuals from the minimal adequate model are added. (B) Points show mean of raw data (tree level), error bars are +/- 1 standard error of the mean.

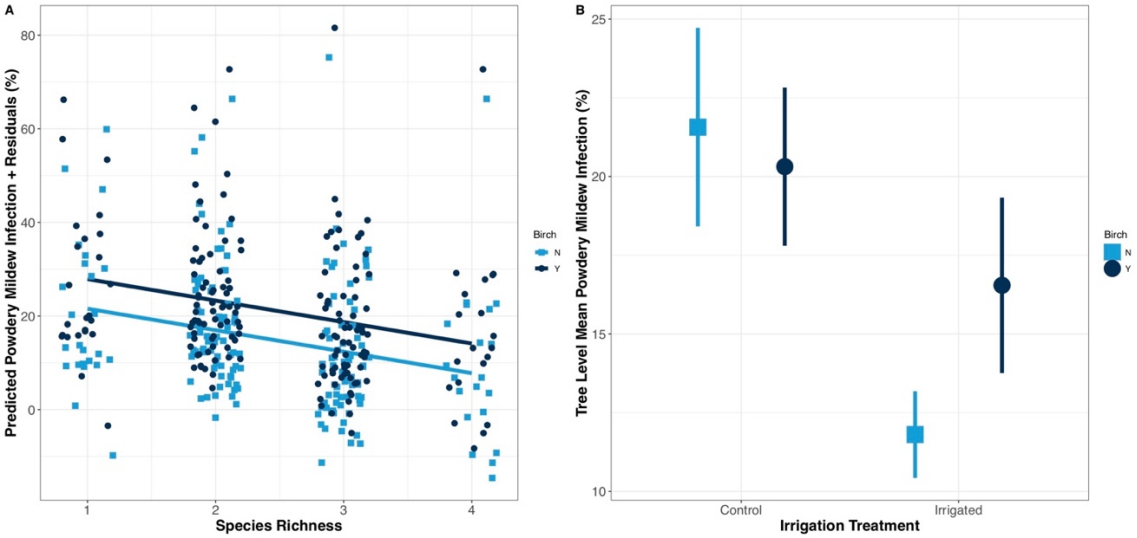


Figure 9: Partial residual plot with the regression lines showing the strength of the relationship between tree species richness and mildew infection when all other variables are held constant in the model. Model predictions are generated using observed values of species richness while holding all other continuous variables in the model constant at a mean value, and random effects set to zero. Points plotted are model predictions to which residuals from the minimal adequate model are added. (B) Points show mean of raw data (tree level), error bars are +/- 1 standard error of the mean.

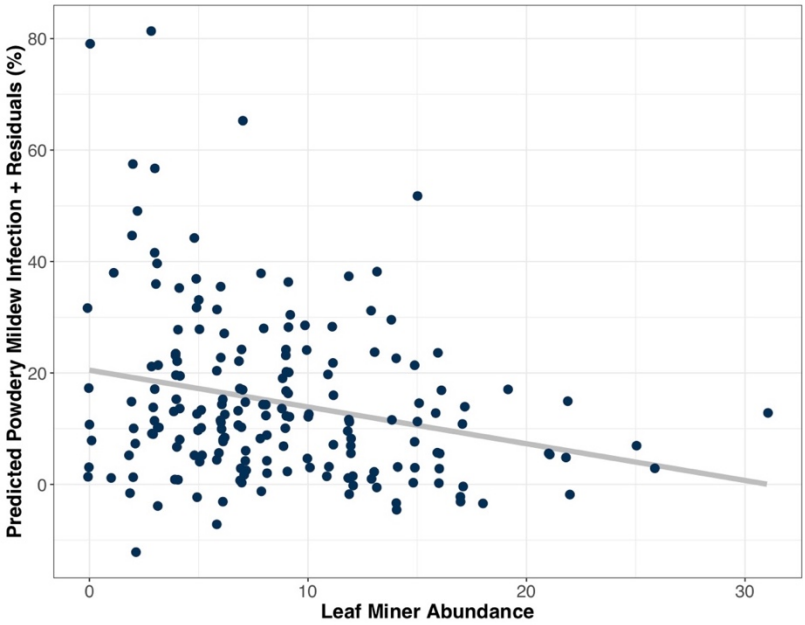


Figure 10: The relationship between leaf miner abundance and oak powdery mildew infection. Partial residual plot with the regression line showing the strength of the relationship between miner abundance and mildew infection when all other variables are held constant in the model. Model predictions are generated using observed values of miner abundance while holding all other continuous variables in the model constant at a mean value, and categorical variables constant at a single level. Points plotted are model predictions to which residuals from the minimal adequate model are added.

Discussion

In this study of a tree diversity experiment in SW France, increasing tree diversity reduced both oak insect herbivory and powdery mildew infection, while the presence of birch independently increased damage by all groups. In the case of oak leaf miners, tree diversity and neighbour identity effects were context

dependent on drought stress. Our results show that tree diversity effects are dependent upon both abiotic context and the identity of species neighbours.

Tree diversity promotes associational resistance to both herbivory and pathogen damage

In line with our hypothesis, increasing tree species richness lowered damage by oak insect herbivores and oak powdery mildew, which confirms the general pattern of associational resistance in temperate, mixed species forests and highlights its relevance to both pests and pathogens (Jactel et al., 2017). Earlier studies on oak in the same experiment (Castagneyrol et al., 2013) and elsewhere also found support for associational resistance with increasing tree species richness, for insect herbivores (Alalouni, Brandl, Auge, & Schädler, 2014; Moore et al., 1991; Setiawan et al., 2014) and oak powdery mildew (Hantsch et al. 2014). It is likely that associational resistance to both herbivores and mildew was partially driven by increasing host dilution of *Q. robur* as richness increased, the two being conflated in this experiment (Castagneyrol et al., 2013, Fig. S4). Indeed, resource dilution was a more significant predictor of leaf chewer damage than tree species richness, although both composition variables were significant predictors of leaf chewers and oak powdery mildew.

Tree diversity effects can be dependent on environmental context

We hypothesised that the presence of birch, a taller tree species that is a non-host for oak powdery mildew and has the most separate insect herbivore community, would reduce focal oak apparency and thus lower mildew infection and herbivore damage on *Q. robur*. We found support for this hypothesis for miners, consistent with a previous study (Castagneyrol et al, 2013). However, both chewer damage and mildew infection were greater in the presence of birch (Figs 8-9), which could have occurred through both direct and indirect effects. Previous observations suggest that leaf chewers at ORPHEE are more likely to be generalist herbivore species than leaf miners, with several species feeding on both *Quercus* and *Betula* (Castagneyrol et al, 2013). Thus, the presence of birch could have led to some spill-over onto neighbouring oak hosts, leading to associational susceptibility in the presence of birch (Barbosa et al., 2009). However, White and Whitham (2000) observed that associational susceptibility was only likely to occur when the preferred host was heavily defoliated, which is generally not the case for birches at ORPHEE (Castagneyrol, Jactel, et al., 2018).

A knock-on effect of birch on plot microclimate may therefore provide a stronger explanation for the positive effect of birch on herbivory. Increasing shading by adding birch could have directly modified the microclimate of plots; plots without birch had open canopies and thus high light intensity, as all three oak species were similar in height and had not yet formed closed canopies (Fig. S11; Appendix 3). High light has frequently been observed to negatively impact insect herbivores, by promoting a hot, dry microclimate unfavourable for insect development (Stiegel, Entling, & Mantilla-Contreras, 2017; Stoepler & Lill, 2013).

However, some studies in temperate environments have also found evidence for increased insect activity in sunny versus shade conditions, in contrast to our finding for leaf chewers (Dudt & Shure, 1994; Mooney, Tiedeken, Muth, & Niesenbaum, 2009). For oak powdery mildew, previous studies reported conflicting results on the impact of light (Giertych & Suszka, 2010; Kelly, 2002). Microclimatic measurements taken from 2016-2018 suggested that the presence of birch buffered some climatic variability, which could have led to a more beneficial environment for leaf chewers and powdery mildew (Fig. S15). Our finding that irrigation increased miner abundance, but only in plots containing birch, also suggests that the effect of birch could have been mediated by an effect on microclimate, interacting with irrigation.

Differences in insect and mildew damage in the presence of birch could also be explained by indirect effects of birch via changes to host plant traits (Dudt & Shure, 1994; Muiruri et al., 2019; Roberts & Paul, 2006). For instance, Roberts and Paul (2006) found that shading by other trees often increased foliar nutrition and reduced anti-herbivore foliar defences in focal trees, with correlated increases in insect abundance in shaded environments. Future work should prioritise linking changes in tree species composition with differences in microclimatic conditions, through to subsequent modification of plant traits, with implications for pest and pathogen damage.

We found that the effect of tree diversity on leaf miners was contingent on irrigation treatment. Mine abundance decreased with tree species richness, but

only under irrigation conditions (Fig. 7A). Neighbour-modulated effects on water use by trees have been noted by previous authors, for instance, differences in soil water uptake in mixed stands (Grossiord, 2019) as well as water use efficiency (Forrester, 2015). The interaction between stand composition and drought has in turn been shown to influence structural and chemical plant traits (Forey et al., 2016b) and some studies have suggested further cascading effects on insect herbivores (Castagneyrol, Moreira, et al., 2018; Kambach et al., 2016; Walter et al., 2012). Measurement of plant traits was not a main aim of our study, but future work should focus on measurement of multiple plant traits in differing plot compositions and abiotic contexts, to better disentangle the underlying mechanisms leading to associational effects on plant antagonists (Castagneyrol, Jactel, et al., 2018; Forrester et al., 2010; Grossiord, Gessler, et al., 2014).

We observed lower damage by leaf chewers in irrigated compared to non-irrigated blocks (Fig. 8). This is consistent with the Plant Stress Hypothesis, which predicts greater defoliation under conditions of drought stress. This can be due to direct effects of the physical environment on the survival or performance of herbivores, and/or effects on leaf traits that impact plant palatability and nutritional quality for insect herbivores (Castagneyrol, Moreira, et al., 2018; Jactel et al., 2012; White, 1974). Microclimatic data indicated that irrigated blocks had lower variability in both summer temperature and humidity, which could have had a positive direct effect on leaf chewers (Fig. S15). We did not measure plant traits associated with water availability on study trees, which was a limitation of our study. Despite a trend towards lower oak powdery mildew infection in

irrigated blocks, this effect was nonsignificant in our models (Table 5). Again, along with light and humidity, the effect of host water stress on powdery mildews is subject to debate (Marçais & Desprez-Loustau, 2014), and it may be that the effective sample size of 6 (irrigation being applied at the block level) was not large enough in our experiment to disentangle water stress effects on oak powdery mildew.

Impacts of shoot length and tree height

We predicted that both tree height and shoot length would be positive predictors of insect herbivory and oak powdery mildew, as has been seen in previous studies (Castagneyrol et al., 2013; Field et al., 2019; Gripenberg et al., 2007). We found partial support for this hypothesis, as at both the individual shoot and tree level, leaf mines were more abundant on longer shoots, and individual shoot length positively predicted mildew infection. This supports the Plant Vigour hypothesis, which predicts higher preference and/or performance of insects on more vigorous plant modules (Cornelissen et al., 2008; Price, 1991). However, larger (taller) oaks were not significantly more attacked by leaf miners nor powdery mildew, in contrast to a previous study of both groups on *Q. robur* (Field et al., 2019). Longer shoots may be older shoots, with a longer time period to accumulate damage, but this would not explain why the same relationship was not observed for chewers, despite both miners and chewers being active from early spring. Alternatively, longer shoots could have differed in their defensive chemistry and/or C:N ratio

with knock-on benefits on insects and mildew (Hajji, Dreyer, & Marçais, 2009; Hewitt & Ayres, 1976; Lerdau, Litvak, & Monson, 1994).

Interaction between insect herbivory and pathogen damage

Contrary to our hypothesis and previous studies, we did not find evidence for a positive association between insect defoliation on primary growth and oak powdery mildew infection on lammas shoot growth (Field et al., 2019; Marçais & Desprez-Loustau, 2014). Leaf chewer damage was the main contributor to leaf defoliation (Fig. S16) but was not a significant predictor of oak powdery mildew infection (Table 5). It may be that the level of defoliation we observed on primary growth was not high enough to significantly modify oak phenology, for instance, by promoting lammas shoot growth and therefore resource availability for mildew, one proposed mechanism for the interaction between herbivory and mildew (Delatour, 1983). We instead observed a negative effect of leaf miner abundance on oak powdery mildew (Table 5). The mechanism behind the negative effect of miner abundance on oak powdery mildew has not yet been investigated. However, previous work suggests that mining herbivores and mildew (biotrophic pathogens) induce separate plant defence cascades (jasmonic acid and salicylic acid signalling pathways respectively) that can be antagonistic, which could be tested experimentally in future (Moreira et al., 2018b). As miner abundance and mildew infection both decreased with increasing tree species richness (Figs 7 and 9), at higher richness levels, the negative effect of miners on mildew could have been alleviated. However, as mildew infection continued to

decline with increasing tree species richness regardless of mine abundance, this provides support for the importance of the dilution effect for this pathogen, as observed elsewhere (Hantsch et al., 2014).

Conclusion

In addition to providing support for associational resistance in mixed forests to both insect herbivores and a specialist pathogen, we show that the identity of neighbouring species can impact upon associational effects. Adding birch, a fast-growing species that lowered oak apparency, reduced leaf mine abundance. In contrast, adding birch increased leaf chewer damage and powdery mildew infection. This could have resulted from spill-over from neighbouring birch hosts in the case of leaf chewers, and/or modified microclimatic conditions under birch canopy, leading to direct and indirect effects on oak chewers and mildew. Moreover, we found that the effect of tree diversity on leaf miners was contingent on water availability, suggesting bottom-up effects of water relations on tree growth that impacted associational effects. In addition, we found evidence for an antagonistic relationship between miners and mildew. Future work to disentangle the mechanisms underlying associational effects in tree diversity experiments could aim (i) to follow a more holistic approach and consider the response of the whole community of insects and microbes interacting with trees and (ii) to characterise microclimatic differences and concurrent modifications to plant traits that are dependent on plot composition. This would provide support for the

importance of considering both tree species diversity and composition elements when designing mixed species plantations.

Acknowledgements

We thank everyone from INRA research station at Pierroton who assisted with fieldwork: in particular, Yasmine Kadiri, and also Elena Valdes-Correcher and Tomas Damestoy. We thank Marie-Laure Desprez-Loustau for her advice and input on studying oak powdery mildew in the field. Authors acknowledge UEFP 0570, INRA, 69 route d'Arcachon, 33612, CESTASEUFP-0570 for the management of the ORPHEE experiment. Irrigation was supported by EquipEx Xyloforest ANR-10-EQPX-16-01. BC and HJ received support from the French National Research Agency (project DiPTiCC, ANR-16-CE32-0003-01). EF gratefully acknowledges funding from the Oxford-NERC Doctoral Training Partnership in Environmental Research and a CASE studentship with Forest Research, UK.

CHAPTER 4: EFFECTS OF WATER STRESS ON GROWTH CHARACTERISTICS, PHENOLOGY AND OAK POWDERY MILDEW INFECTION OF *QUERCUS ROBUR*

Field, E.[1*], Schönrogge, K.[2] & Gibbs, M.[2]

[1] Department of Plant Sciences, South Parks Road, University of Oxford, Oxford, OX1 3RB, United Kingdom

[2] Centre for Ecology & Hydrology, MacLean Building, Crowmarsh Gifford, Wallingford, OX10 8BB, United Kingdom.

Article status

This chapter is presented in as yet unpublished manuscript form.

Author contributions

EF, KS & MG conceived the study and its methodology. EF, KS & MG obtained the materials. EF carried out the greenhouse experiments with assistance from MG. EF analysed the data and wrote the manuscript, with input from MG regarding statistical analyses. All authors commented on the manuscript.

Abstract

Climate change may lead to increased frequency and severity of forest pathogen outbreaks, as plant water stress can increase both susceptibility and suitability of plant tissue for pathogen growth. Oak declines in Europe have occurred repeatedly over the past century, and two potentially contributing factors are drought and oak powdery mildew. In this experiment, we used potted *Quercus robur* saplings that were exposed to severe or moderate water deprivation, or waterlogging, and inoculated them with oak powdery mildew (*Erysiphe* spp.). Corroboration of our treatments showed that they had the desired impact on soil moisture content of pots. Plants exposed to severe water deprivation had lower shoot biomass and gravimetric water content of shoots compared to the moderate and control groups, consistent with effects of drought stress. However, due to factors such as earlier than expected bud burst of potted saplings, and a severe greenhouse aphid outbreak, the saplings were almost completely resistant to oak powdery mildew infection at the time of inoculation. This highlights the importance of foliar age and quality on infection success by mildew, in line with observations of other authors. Despite saplings all being of the same provenance, they demonstrated a high degree of phenological plasticity, producing between one and four bud bursts over the growing season, which could be one consequence of high aphid damage early in the season. We also found that saplings with earlier spring bud burst produced significantly fewer primary shoots compared to saplings

with later initial bud burst, possibly representing growth conservatism of these saplings. Although water stress did not have a significant impact on the number of bud bursts, none of the waterlogged plants produced any fourth flush shoots, suggesting that this treatment may have impacted oak developmental biology. Although the lack of oak powdery mildew infection meant we were unable to test the effect water stress on the host-pathogen interaction, the study demonstrated the diversity of oak responses to different biotic and abiotic stressors. We also developed a new method for inducing and maintaining drought and water logging on potted oak saplings.

Introduction

Under conditions of climate change in the Western hemisphere, including increased drought frequency and intensity, the impacts of many forest pests and pathogens are predicted to increase in severity (Jactel et al., 2012; Sturrock et al., 2011). Drought impacts on forest pathogens likely vary with factors such as pathogen life history, and while canker and root pathogens have been studied extensively both in the field and experimentally in the context of drought, there have been few studies of drought impacts on foliar pathogens such as rusts and powdery mildews (Desprez-Loustau et al, 2006). A review of the literature in 2006 found just 5 studies on foliar pathogens, of which 2 (40%) reported an increase in disease severity under drought stress (Desprez-Loustau et al., 2006).

Oak powdery mildew (*Erysiphe* spp.) is an emerging forest pathogen in Western Europe (Lonsdale 2015). It has been present in Europe since at least the late nineteenth century but the impact of the pathogen has been increasing in recent decades (Lonsdale, 2015). Oak powdery mildew is not believed to cause oak mortality on its own, but in combination with other biotic and abiotic factors: oak powdery mildew severity has frequently been linked to earlier defoliation by insects (Marçais & Desprez-Loustau, 2014). Records of oak powdery mildew that are associated with episodes of oak decline date from as far back as the 1900s, in combination with other fungal pathogens including *Collybia fuspies, Armillaria* sp. and *Phytophthora* sp. (Delatour, 1983; Klimesch, 1924; Marçais & Bréda, 2006). Manion (1991) introduced the "spiral" model of tree health decline in which damage agents can be categorised into three groups acting sequentially to cause tree health decline: *predisposing, inciting* and *contributing* factors. Using this model, drought or other deleterious abiotic factors are *predisposing* factors; insect defoliation and oak powdery mildew are *inciting* factors; and root and canker pathogens such as *C. fusipes* and *Armillaria* sp. are *contributing* factors.

Based on observations from previous studies and plant pathology theory, we can hypothesise that drought stress may lead to an increase in oak powdery mildew infection severity. Desprez-Loustau et al (2006) suggested three main mechanisms for drought stress impacts on pathogens: direct effects on pathogen growth, indirect effects via changes to other community

members, and interactions between multiple stress agents. Considering each of these in turn: in terms of direct effects, high humidity promotes oak powdery mildew germ tube elongation (Pap, Ranković, & Maširević, 2013), suggesting drought will negatively impact mildew infection. However, powdery mildews are xerophytic pathogens, that do not require free water to germinate (Schnathorst, 1965) meaning that compared to other biotrophic pathogens, survival and reproduction of powdery mildews is not so tightly coupled to water availability. Secondly, considering indirect effects via other community members, there is compelling evidence that the activity of primary foliar feeding insects (including those on *Q. robur*) is promoted by drought stress (Field et al., 2020; Jactel et al., 2020, 2012). If oak powdery mildew is also promoted by high primary insect defoliation, drought will have an indirect positive effect on the pathogen. Thirdly, considering the interactions of multiple stress agents on plant susceptibility, drought stress can lead to changes to plant tissue chemistry and defence which could impact upon mildew. These could include reduced concentrations of structural carbohydrates, as is often observed under drought conditions, which lower oak powdery mildew germination success (Edwards & Ayres, 1981; Jactel et al., 2012) and increased mobilisation of glucose, a key substrate for oak powdery mildew (Hewitt & Ayres, 1976; Wargo, 1996). Overall, consideration of different aspects of drought effects on plant, pathogen and environment suggest that drought may promote more severe oak powdery mildew infection.

Despite predictable positive impacts of drought stress on infection severity by oak powdery mildew, there have been no studies directly linking water stress to pathogen infection under experimental conditions. Dillen et al (2017) attempted to induce drought stress on oak sapling phytometers using a rainfall reduction gradient, and measured mildew severity on saplings, but the rainfall reduction gradient did not significantly alter soil moisture. A previous study by the authors in the field found no significant impacts of irrigation on oak powdery mildew infection, potentially due to multiple interacting abiotic and biotic factors (Field et al. 2020). In this study, we aimed to test the hypothesis that drought stress would increase oak powdery mildew infection severity, using potted *Q. robur* saplings.

The impacts of oak powdery mildew are thought to be highest on young oak trees, making it an acute nursery pest (Lonsdale, 2015). Using three-year-old saplings of *Q. robur* ensured our study was of relevance to pathogen management. Both British native deciduous oak species, *Q. robur* and *Q. petraea,* are susceptible to oak powdery mildew (although there is speculative evidence that *Q. petraea* may be less susceptible (Delatour, 1983). However, *Q. robur* is generally believed to be less drought tolerant than *Q. petraea* (Ponton, Dupouey, Breda, & Dreyer, 2002), and may therefore be more vulnerable to predicted impacts of climate change.

An additional aim of our study was to develop a reliable, straightforward method for inducing different water stress conditions in potted oak

saplings. Much of the literature on drought impacts on pathogens considers only two levels: stressed and non-stressed, with stress levels induced normally representing extremes beyond those likely to occur under seasonal extremes or future conditions of climate change (Desprez-Loustau et al., 2006). We chose to induce drought stress via manipulation of the field capacity (here defined as the maximum water carrying capacity of soil, after Banfield-Zanin and Leather, 2014). This allowed us to induce moderate (50% field capacity) as well as severe (entirely un-watered) drought stress compared to controls that were maintained close to 100% field capacity. Moreover, water logging, which induces anoxia in plant roots (Parelle et al., 2006; Parent et al., 2008), has not been studied in the context of oak powdery mildew infection. Waterlogging has been associated with oak decline canker symptoms in preliminary data (Liz Shaw, unpublished data). The interaction between oak powdery mildew and waterlogging may therefore be an important additional factor to consider in the onset of oak decline under different hydric conditions. Thus, we included waterlogging as an additional treatment.

We tested the following hypotheses:

(1) Water stress treatment would impact oak powdery mildew infection severity, with contrasting effects for different water stress treatments. Mild water stress (moderate drought treatment) would lead to increased powdery mildew infection. Severe water stress (severe drought

treatment) would lead to reduced powdery mildew infection, if oak growth was severely affected (for example, if the availability of susceptible leaf tissue for powdery mildew was reduced as a result of water stress).

(2) Water stress treatments would lead to alterations in oak growth. Drought and waterlogged trees would have reduced height and stem growth and lower absolute height and stem diameter, compared to controls. Drought stressed trees would have increased root biomass and reduced shoot:root ratio compared to control saplings, while waterlogged trees would have increased shoot biomass and increased shoot:root ratio compared to control saplings.

(3) Water stress treatments would lead to alterations to oak phenology, with fewer bud bursts occurring in drought and waterlogged saplings compared to controls.

Materials and Methods

Plant Material

We obtained three-year-old saplings of oak (*Quercus robur)* saplings from the University of Oxford Zoology department, where they had been grown in greenhouse conditions from seed since summer 2015. Acorns had been obtained from Forestart Ltd (UK) from a plus tree stand near Moczkowo, Poland (region of provenance no. 153). In December 2017 we transferred

saplings to the GroDome facility at UK CEH Wallingford. The GroDome is a compartmentalised, controlled growing environment, where saplings were kept at indoor ambient temperature and natural light conditions. In March 2018 we re-potted saplings into identical 3 L pots using general all-purpose compost (peat-based). We monitored the growth and development of the saplings until summer 2018, when we imposed the drought treatments.

Initial assessments of oak growth and development

Bud burst began in March 2018, earlier than expected for this species, which normally undergoes budburst in late April-May in the UK (Cole & Sheldon, 2017). The earlier timing was likely due to the oak saplings being kept at indoor temperature conditions above freezing from late December the previous year, as warmer springs have been observed to lead to an advancement of *Q. robur* bud burst date (Visser & Holleman, 2001). On a single date in March (15/3/18) we assessed the bud burst stage of all oak saplings. We followed an adapted version of the protocol used by Crawley & Akhteruzzaman (1988) using 5 stages ranging from stage 1 (dormant bud) to stage 5 (leaves fully unfurled and mature dark green). See Figure S17 for details and photos of the bud burst stages used.

Prior to the imposition of water stress treatments, we also measured the height and stem diameter of all saplings.

Calibration and imposition of water stress treatments

We adapted the protocol used by Banfield-Zanin & Leather (2014) in which pots were kept at a constant percentage of the maximum field capacity of the soil. We spread a large quantity of soil (the same as had been used for repotting) for use in the experiment out on plastic sheeting in the greenhouse and left it to completely dry for several weeks. Following this, we sieved the soil using a 7 mm sieve to remove large objects such as plant roots and macroinvertebrates. We then repotted the saplings using the fully dried soil, to obtain pots that were of the same weight (580 g +/- 0.1 g). This weight was then set to be 0% field capacity.

Treatment	**Number of saplings**	**Conditions**
Ambient Conditions	**10**	Exposed to ambient rainfall for the duration of the experiment
Severe Drought	**10**	Water exclusion for the duration of the experiment, un-watered
Moderate Drought	**10**	Maintained at 50% field capacity
Control	**10**	Maintained at 100% field capacity
Waterlogging	**10**	Maintained above field capacity

Table 6: Treatments used in the water stress experiment.

Soil volumetric water content measures were not possible at this stage, as the soil was too dry. We then added enough water to reach saturation point

for each pot (3.5 L for each sapling), and left pots to drain for 2 hours. We measured the weight of each pot after draining and took 3 surface measurements of soil volumetric moisture content using a SM150T probe with HH2 water meter, both from Delta-T devices (Cambridge, UK). These were then averaged to produce pot level soil moisture measurements. This measurement was then set as 100% field capacity. From these two reference weights, we could calculate the weight of pots at different field capacities. Pots were then left to dry out, and weights monitored over time, to estimate the length of time required for pots to reach 50% field capacity. We calculated that to obtain weights of +/- 1 standard deviation of the target 50% and 100% field capacity weights, we needed to add 0.5 L water per week to the 50% treatment group, and 1 L water per week to the 100% treatment group.



Figure 11: Experimental set up of oak saplings on pallets, showing the plastic skirt coverings (July 2018). **Photograph by the author.**

In July 2018 we randomly assigned plants to 5 treatment groups of 10 plants each (Table 6). We ensured that saplings of different bud burst stages were distributed evenly between treatment groups, so that each treatment group contained 2 plants of stage 1, 2 plants of stage 2 etc. This was to ensure that there was no bias of developmental stages between groups of plants. Aside from the ambient conditions group, we fitted each plant with a secure transparent plastic skirt made from plastic sheeting, which covered the top of the pot and sides, preventing the input of ambient rainfall. This was secured with a plastic band around the pot (similar to the protocol used by Banfield-Zanin & Leather, 2014). Prior to fitting the skirts we tested that they waterproofed saplings, by pouring a large quantity of water over a protected sapling and ensuring no weight change occurred. Skirts were checked regularly (2-3 times/week) for the duration of the experiment to check that they were secure and that no breakages had occurred.

Water treatments imposed were as follows (Table 6): ambient conditions (exposed to ambient rainfall, with no plastic skirt covering), severe drought (water excluded for the duration of the experiment), moderate drought (plants kept at 50% field capacity, receiving 0.5 L water per week), control (plants kept at 100% field capacity, receiving 1 L water per week) and waterlogged (plants maintained above field capacity, receiving 1 L water per week and kept in a plastic tray filled with water to ensure roots remained waterlogged at all times).

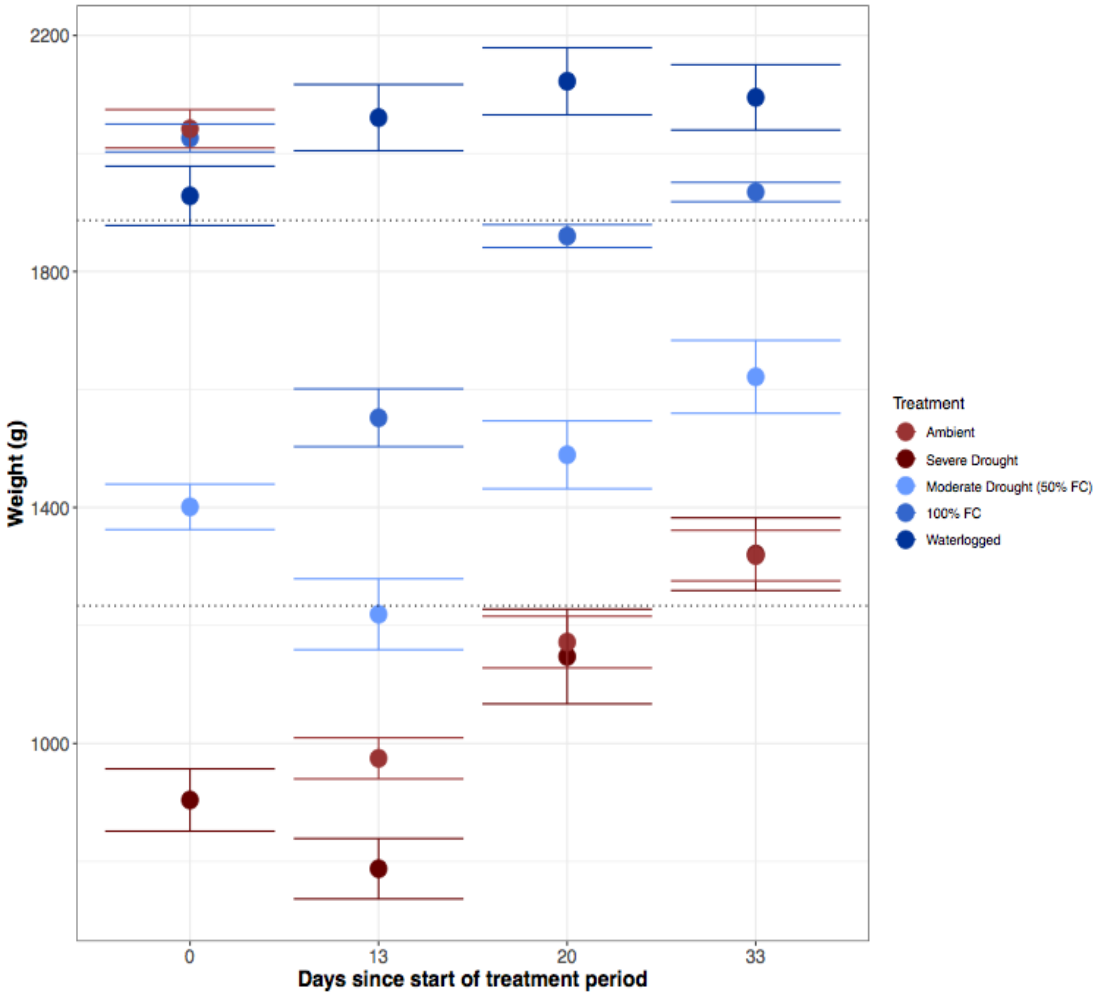


Figure 12: Weight monitoring of pots in different experimental treatments during the experimental period. Plotted are means (points) +/- standard errors for each treatment group. The dashed horizontal lines represent the target weights for the 50 % FC treatment (dashed line at 1233.4 g) and 100% FC treatment (dashed line at 1886.5 g).

Plants were transferred outside on raised pallets on gravel, to ensure that they did not absorb any water from the soil. Each treatment group was kept on a separate pallet (Fig. 11). Plants were monitored regularly to ensure that they were kept close to target weights (Fig. 12), and the amount of water

given weekly was adjusted accordingly. The duration of the experimental period was 5 weeks (July – August 2018).

Inoculation with oak powdery mildew

We carried out the experiment during July-August, as this is the peak infection window for oak powdery mildew in the UK (EF, KS and MG, pers. obs). At the start of the experimental period, we collected branches from two *Q. robur* trees on site at CEH Wallingford that were heavily infected with oak powdery mildew. Plants were then inoculated by surrounding each pot with a cardboard cone and 'tapping' branches on top of the cone to release spores, with the same infection intensity for each plant. This inoculation was repeated twice in the first week of the experimental period.

Measurements of oak growth characteristics

We monitored the development of infection by oak powdery mildew on saplings weekly. At the end of the experiment (end August 2018), we measured the final pot weight and soil moisture content of pots. We measured the height and stem diameter of all plants. For each sapling, we counted the number of separate bud bursts that had occurred over the growing season and the number of shoots in each bud burst class (ranging from 1-4). We counted the number of leaves with oak powdery mildew and

on which type of shoots they occurred (primary or secondary – lammas – shoots).

We harvested the saplings and separated the above and belowground biomass. Fresh aboveground biomass was weighed. Plant material was then dried fully in a 60 °C drying oven for 24 hours. The dry weight of above and belowground biomass was measured. From this we calculated the gravimetric water content of each shoot (Weight wet biomass – Weight dry biomass / Weight dry biomass).

Data Analysis

All statistical analyses took place in R version 5.3.1 (R Core Team, 2020). At the end of the experimental period, the sample size had reduced to n= 46 plants, as 4 plants had died during the treatment period. Using one-way least squares ANOVA, we corroborated differences between treatments by analysing the final soil moisture and pot weight of different treatments. We used least squares ANOVA to analyse the following response variables to determine if they differed between treatments:

- Final sapling height (mm)
- Final stem diameter (mm)
- Growth change (change in height, mm)
- Growth change (change in stem diameter, mm)

- Number of bud bursts completed
- Number of second, third and fourth burst shoots produced
- Total number of lammas shoots produced (sum of second-fourth burst shoots produced)
- Shoot biomass (g)
- Root biomass (g)
- Shoot:Root biomass ratio
- Gravimetric water content of shoots (g H_20 / g biomass shoots)

We also tested whether the initial bud burst date of plants (the stage of bud burst when assessed in March) predicted the number of shoots from different bud bursts that subsequently occurred, by including it as an additional variable along with treatment in the linear models of second, third and fourth flush shoots and total number of secondary shoots. Due to the number of shoots of a given bud burst that a sapling produced being intrinsically constrained by the number of shoots of the previous bud burst, we included these as additional predictors in the analysis of number of second, third and fourth flush shoots, before fitting the impact of initial bud burst (for instance, in R syntax for three-way factorial ANOVA: *lm*(No Second Flush Shoots ~ No. Primary Shoots + Budburst + Treatment).

We used residual plots to check the assumptions of the linear modelling framework were met.

Results

Corroborating treatments.

The treatments were all significantly different from each other in terms of both final pot weight and final soil moisture content, in the direction expected (Fig. 13 and Table 7; severe drought < moderate drought < control < waterlogged). This corroborated that we had produced the different hydric conditions expected during the experimental period. Summer 2018 was one of the hottest and driest years on record (Met Office, 2018). At the experimental site, recorded rainfall in July was below the 1980-2018 average and maximum temperature was above the 1980-2018 average (Fig. 14). The severity of ambient conditions meant that the severe drought treatment and ambient treatment did not significantly differ in either weight or soil moisture content (Fig. 13).

Oak Powdery Mildew infection.

We were virtually unable to induce powdery mildew infection on our saplings, despite inoculating them multiple times using an established method. We observed only very light powdery mildew infection on 4 out of the 50 plants in the experiment, meaning that we were unable to analyse powdery mildew infection variability across treatments.

Impacts of treatment on oak sapling growth characteristics.

Height and stem diameter. There were no significant effects of water stress treatments on oak sapling height, stem diameter, height change, or diameter change (Fig. 15, Table 7).

Biomass measurements. There was a significant effect of treatment on shoot biomass. Plants in the severe drought treatment group had lower shoot biomass compared to plants in the moderate drought and control groups, but were not significantly different to either the ambient or waterlogged groups, which did not differ from each other (Fig. 16, Table 7). There was no significant effect of treatment on root biomass, nor shoot:root biomass ratio (Fig. 16, Table 7).

Gravimetric water content of shoots. There was a significant effect of treatment on the gravimetric water content of shoots. Plants in the severe drought treatment group had significantly lower shoot water content compared to all other treatments (Fig. 16, Table 7). Otherwise, there were no significant differences between treatments.

Oak phenology. There were no significant effects of water stress treatments on the number of bud bursts that occurred per sapling (Fig. 17A). There were no significant effects of treatment on the number of second, third or fourth flush shoots produced, nor an effect on the total number of lammas shoots

produced, although none of the waterlogged plants produced any fourth flush shoots (Fig. 17B-F, Table 7).

Impacts of spring bud burst stage on subsequent bud bursts of oak saplings.

The number of shoots in the previous bud burst stage was positively correlated with the number of second, third and fourth shoots produced (Table 7). The timing of oak primary bud burst in March was a significant predictor of the number of primary and third flush shoots produced (Table 7, Figs. 18A and B). The earlier the primary bud burst date of oak saplings (higher bud burst score when recorded in March) the fewer overall primary shoots were produced by end August, and the more third flush shoots were produced. There was no significant effect of spring bud burst timing on the number of second or fourth flush shoots produced by oak saplings.

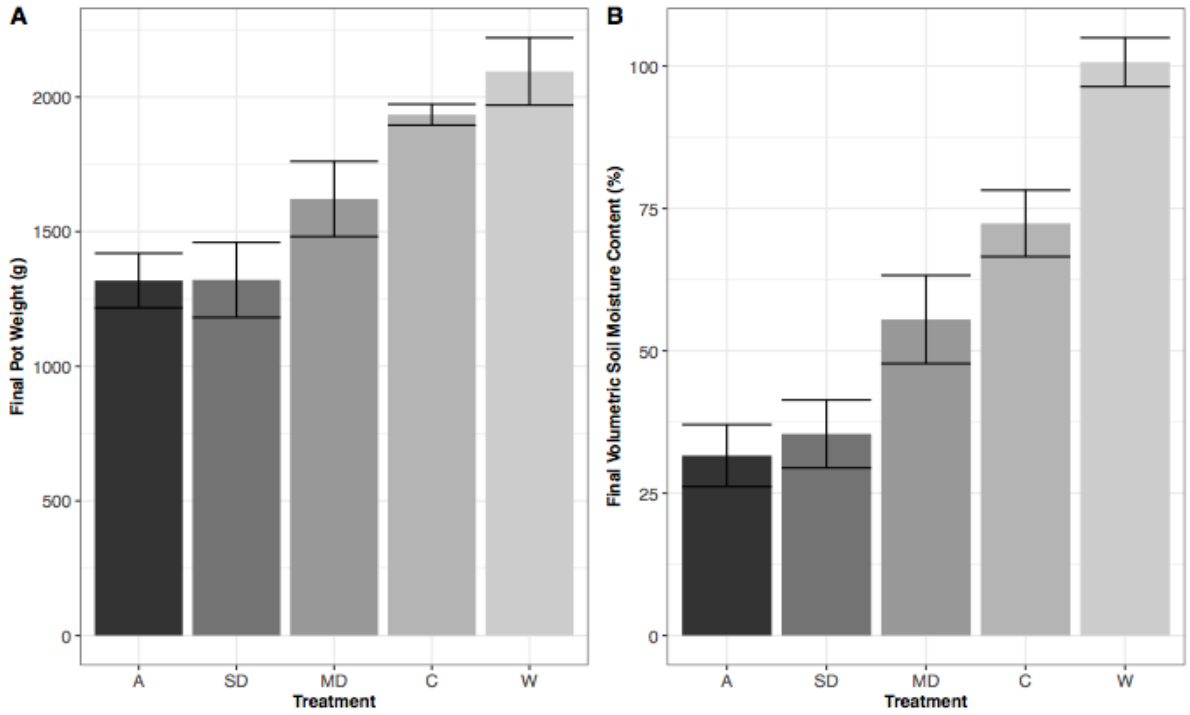


Figure 13: Differences in pot weight (g) and volumetric soil moisture content (%) between treatments at the end of the experimental period. Treatment codes on the x axis are as follows: A = Ambient, SD = Severe drought, MD = Moderate drought, C = Saturated control (100% FC), W = Waterlogged. Bar plots show means, error bars are +/- 95% confidence intervals (calculated as 1.98*SEM).

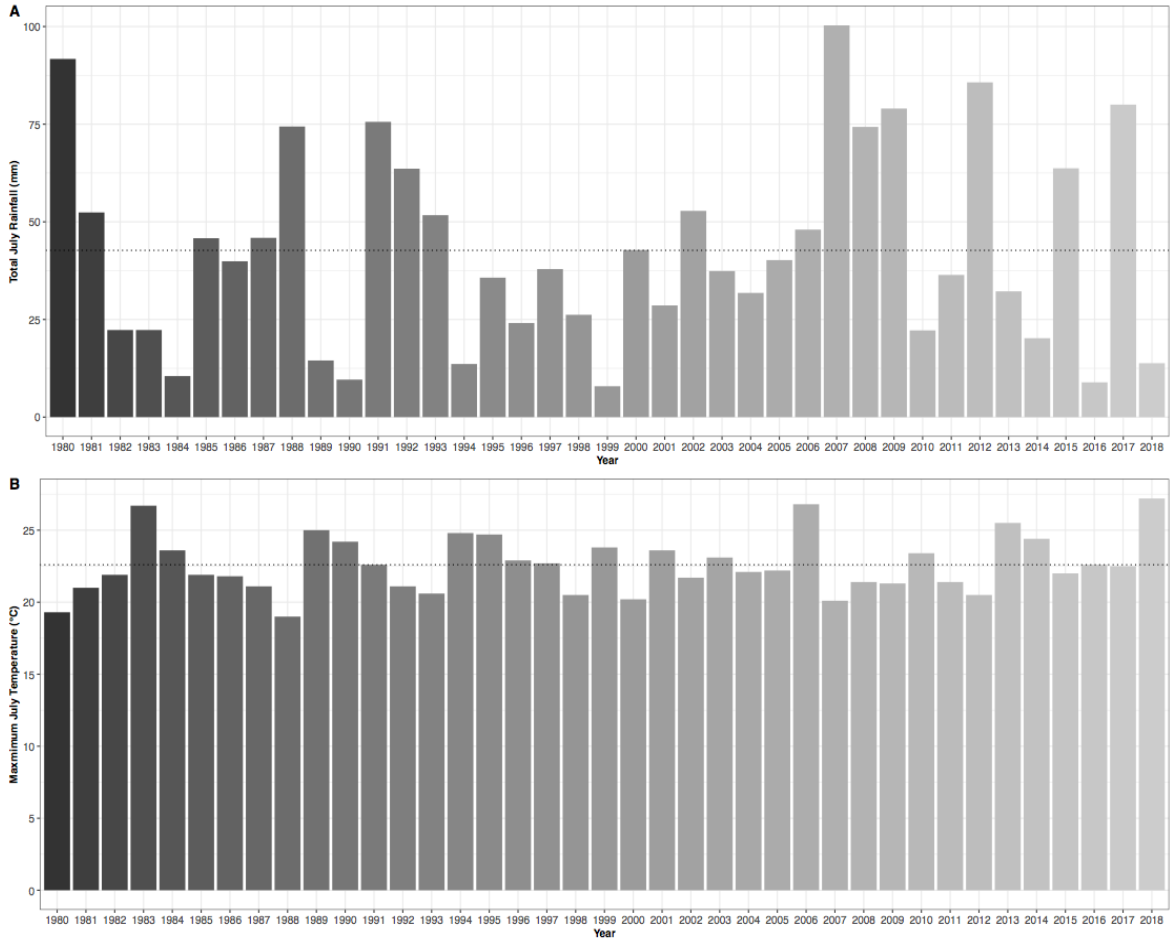


Figure 14: Total July rainfall (A) and maximum July temperature (B) recorded at the CEH Met Office Weather Station, 1980 - 2018. The horizontal dotted lines represent averages for the period (average maximum temperature = 22.6 °C, rainfall = 42.7 mm).

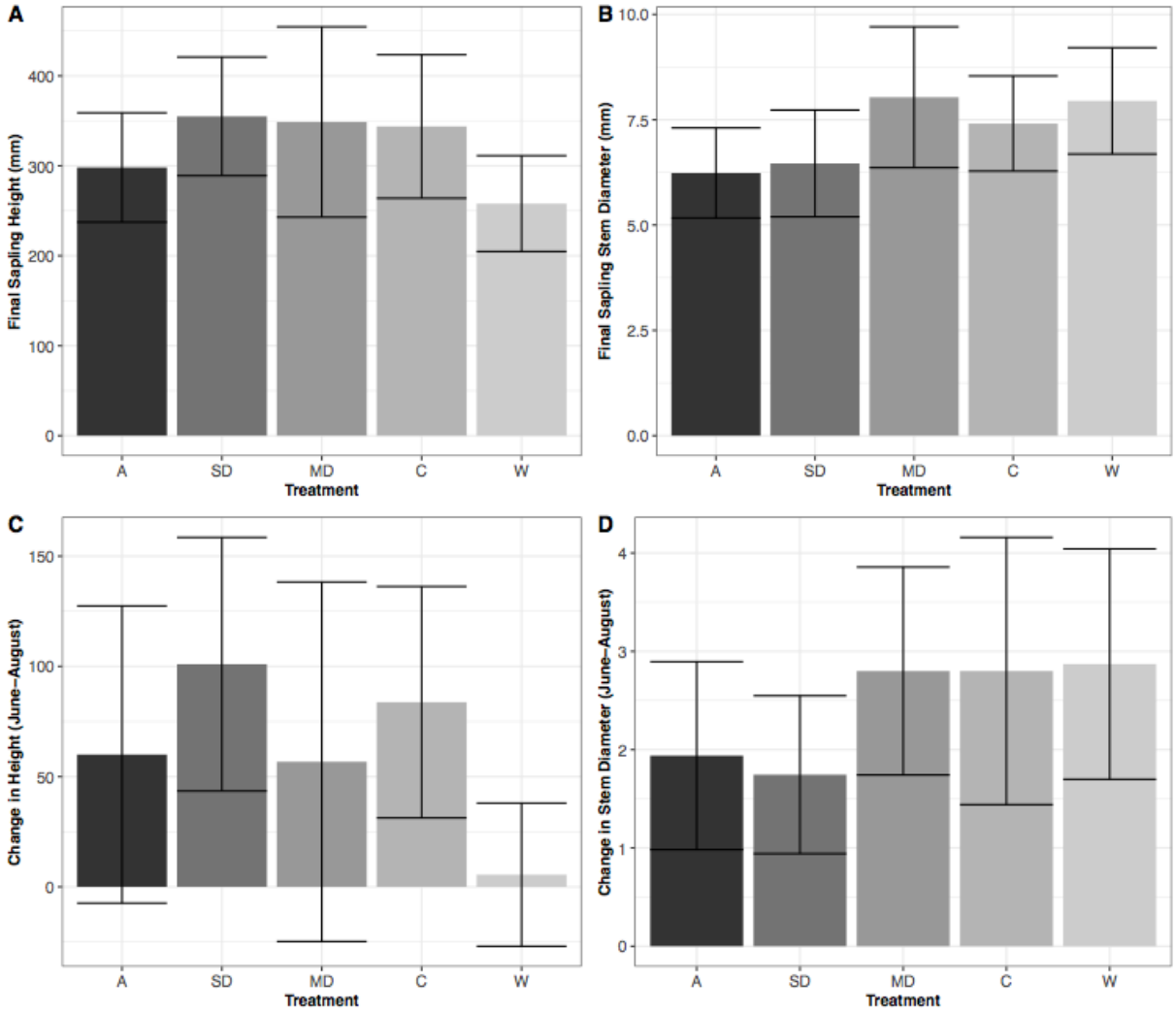


Figure 15: Differences between oak height (A), stem diameter (B) and the change in height (C) and change in stem diameter (D) over the period assessed. Treatment codes on the x axis are as follows: A = Ambient, SD = Severe drought, MD = Moderate drought, C = Saturated control (100% FC), W = Waterlogged. Bar plots show means, error bars are +/- 95% confidence intervals (calculated as 1.98*SEM).

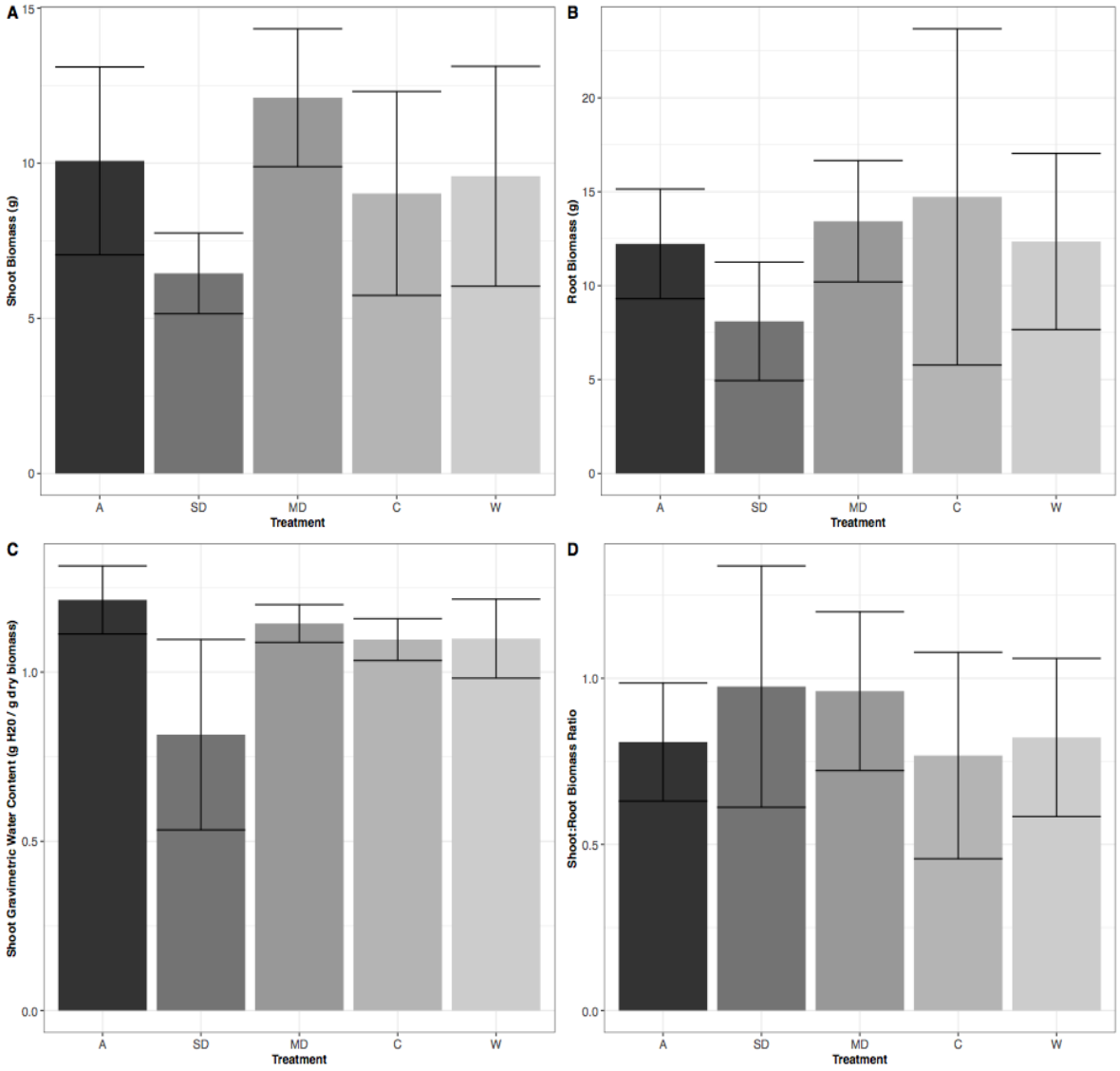


Figure 16: Differences in oak sapling shoot biomass, root biomass, gravimetric water content of shoots, and shoot:root biomass ratio between treatments. Treatment codes on the x axis are as follows: A = Ambient, SD = Severe drought, MD = Moderate drought, C = Saturated control (100% FC), W = Waterlogged. Bar plots show means, error bars are +/- 95% confidence intervals (calculated as 1.98*SEM).

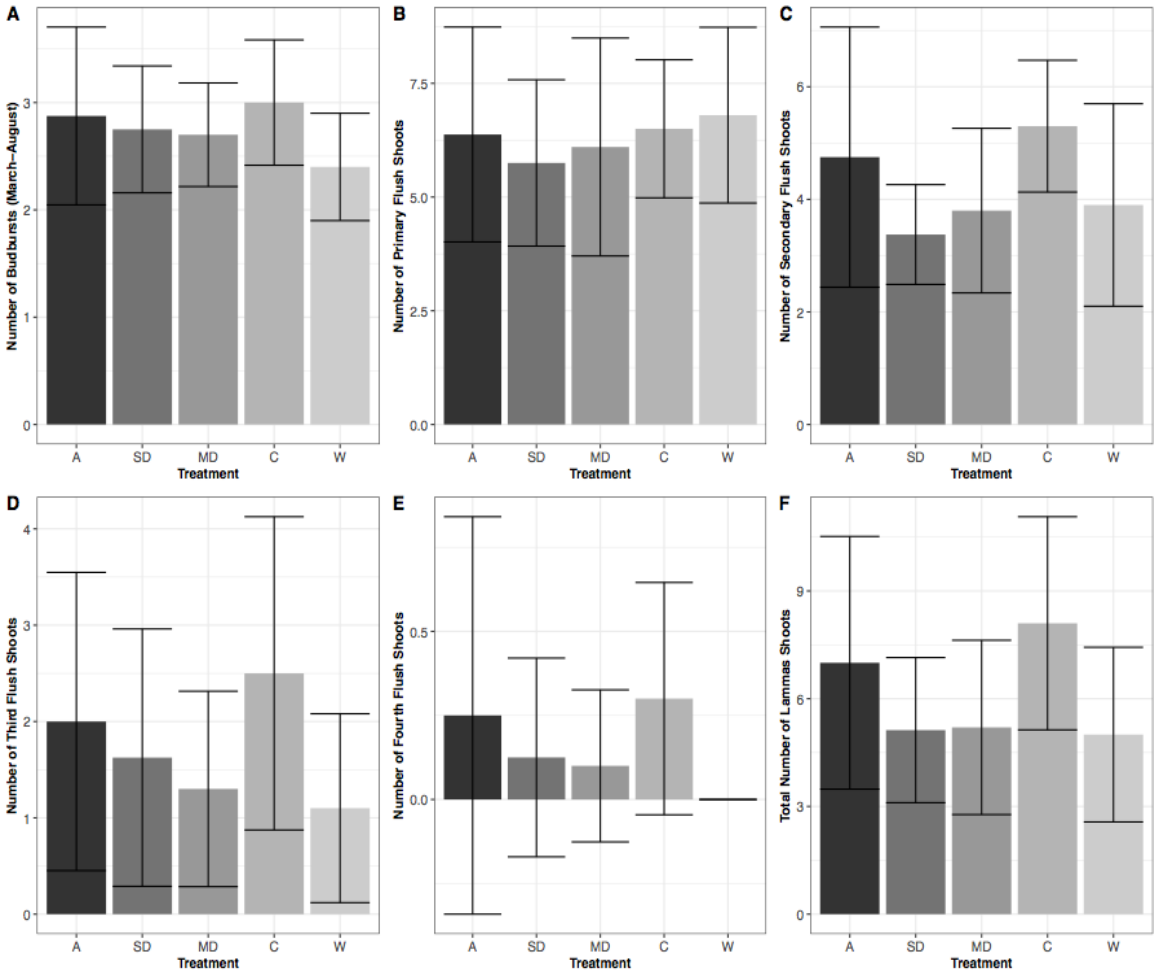


Figure 17: Differences in the number of bud bursts per sapling (A) number of primary flush shoots (B), secondary flush shoots (C), third flush shoots (D), fourth flush shoots (E) and the total number of lammas (second-fourth flush shoots, F) between treatments. Treatment codes on the x axis are as follows: A = Ambient, SD = Severe drought, MD = Moderate drought, C = Saturated control (100% FC), W = Waterlogged. Bar plots show means, error bars are +/- 95% confidence intervals (calculated as 1.98*SEM).

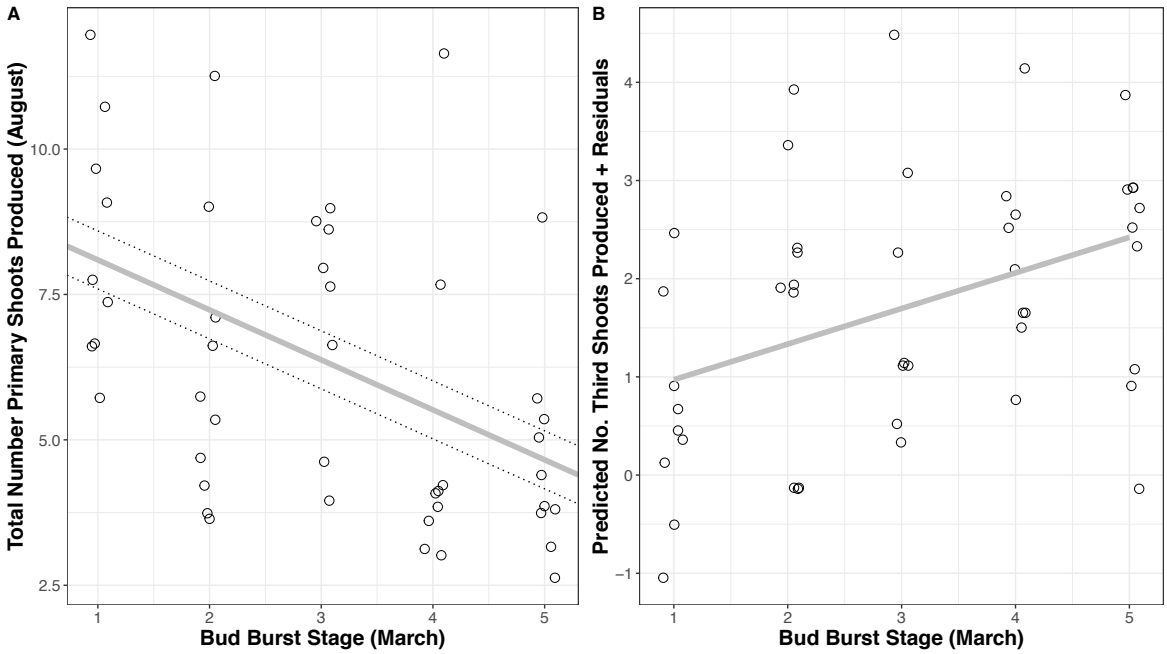


Figure 18: Correlations between initial bud burst stage of saplings and secondary bud flushes. (A) The initial bud burst stage in March was a significant negative predictor of the number of primary shoots produced by oak. Plotted is the raw data plus regression line and 95% confidence intervals of the slope (intercept = 8.95, slope = -0.86, standard error of the slope = 0.25). (B) Partial residual plot showing the conditional impact of initial bud burst stage on the predicted number of third flush shoots, after controlling for treatment and the number of second shoots produced. Due to the number of third flush shoots being highly correlated with the number of second flush shoots, a plot of the raw data (marginal relationship) would not have revealed the correlation, which is significant ($F_{1,39} = 6.18$, $p = 0.017$).

Model Response Variable	Explanatory Variables	F value	DF	P value
Final Pot Weight	Treatment	43.67	4,41	***<0.001***
Final Soil Moisture Content	Treatment	118.27	4,41	***< 0.001***
Final sapling height	Treatment	1.63	4,41	0.19
Final stem diameter	Treatment	2.03	4,41	0.11
Height change	Treatment	1.96	4,41	0.12
Stem diameter change	Treatment	1.36	4,41	0.26
No. Budbursts	Treatment	0.80	4,40	0.53
	Budburst	0.03	1,40	0.86
No. Primary Shoots	Treatment	0.25	1,40	0.91
	Budburst	11.64	4,40	***0.0015***
No. Second Shoots	No. Primary Shoots	42.55	1,39	***<0.001***
	Treatment	2.01	4,39	0.11
	Budburst	0.00060	1,39	0.98
No. Third Shoots	No. Secondary Shoots	32.17	1,39	***<0.001***
	Treatment	0.57	4,39	0.69
	Budburst	6.18	1,39	***0.017***
No. Fourth Shoots	No. Third Shoots	13.44	1,39	***<0.001***
	Treatment	0.24	1,39	0.91
	Budburst	2.25	4,39	0.14
Total No. Lammas Shoots	No. Primary Shoots	3.02	1,39	0.09
	Treatment	1.79	1,39	0.15
	Budburst	0.81	4,39	0.37
Shoot biomass	Treatment	3.16	4,41	***0.024***

Root biomass	Treatment	1.47	4,41	0.23
Gravimetric water content of shoots	Treatment	4.95	4,41	***0.0024***
Shoot:Root Ratio	Treatment	0.61	4,41	0.66

Table 7: Results of factorial ANOVAs (F tests) conducted to test the impacts of treatment and bud burst on response variables. Significant results are in bold and italics

Discussion

We were unable to induce oak powdery mildew infection on saplings, regardless of water stress treatment. This could have been for several reasons:

(1) Ambient conditions during the experimental period were unfavourable for oak powdery mildew germination and development. For instance, ambient temperatures may have been too high for successful spore germination on saplings. The dry, exceptionally hot weather also potentially meant that even though plants were maintained at different field capacities, oaks may have experienced heat stress symptoms, with many of the plants showing symptoms of light stress on their leaves by the end of the summer (KS, pers. obs.). Although direct light has been found to be favourable for powdery mildew germination (Pap et al., 2013), it could change the

physiological properties of oak leaves over the long-term in ways that could negatively impact powdery mildew infection success. Moreover, temperature and humidity also contribute to conidial development of oak powdery mildew; one study found that germ tube elongation was highest at greater humidity, and was reduced at temperatures higher than 25 °C (Pap et al. 2013). The maximum temperature in July 2018 at the site was 27.2 °C, while in August it was 23.1 °C, both well above the 1962-2018 averages for these months (22.2 °C for July and 21.7 °C for August).

(2) The availability of free powdery mildew spores was lower given how hot and dry ambient conditions were, meaning that despite direct inoculation with infected branches these spores could no longer have been viable.

(3) Saplings underwent bud burst earlier than expected, including secondary bud bursts, which occurred as early as April/May for some saplings (typical timing in the UK is June/July (EF, pers. obs.). As a result, leaf material may not have been at the correct physiological stage for successful germination of powdery mildew spores by the time plants were inoculated. Observations and experiments by previous authors suggest that oak leaf susceptibility declines rapidly within the first three weeks following bud burst (Ayres & Edwards, 1982; Kerling, 1965). By the time of the start of the experimental period, which was 4 months following bud burst for most saplings, most leaves (even those of later flushes) would have accumulated higher levels of tannins and increased lignification, potentially inhibiting

powdery mildew infection (Edwards & Ayres, 1981; Feeny & Bostock, 1968; Tikkanen & Julkunen-Tiitto, 2003).

(4) In the GroDome at CEH during the spring/summer period there was an intense aphid outbreak, meaning that for several months (from approx. April – June) oak leaf material was heavily infested with aphids. The aphid infection was consistent across all oak saplings in the GroDome. This could also have lowered the susceptibility of plant material to oak powdery mildew, as the surface of leaves became coated in sugary 'honeydew', previously found to inhibit both oak powdery mildew germination and hyphae formation (Ayres & Edwards, 1982). Aphid infection has also been shown to alter the production of plant defence compounds and may have had unexpected indirect impacts on oak powdery mildew infection success (Khan, Ulrichs, & Mewis, 2011). Many studies have shown that plant pathogens and insects co-occurring on host plants can have both indirect and direct impacts on each other's growth and performance (Fernandez-Conradi et al., 2018; Stout, Thaler, & Thomma, 2006; Tack et al., 2012; van Dijk et al. , 2020). A recent experimental study found that when *Q. robur* saplings were sequentially infected with first aphids, then oak powdery mildew, mildew coverage was significantly lower than without aphids, as we also observed in our study (van Dijk et al., 2020). It is therefore likely that multiple factors contributed to the lack of powdery mildew infection observed, including that saplings had already been exposed to at least one biotic stressor (high aphid infection) prior to being transferred to the

experimental set up and inoculated with mildew. Our experiment therefore highlights the importance of leaf quality for powdery mildew infection.

We therefore discuss the results in the context of all saplings being equally exposed to severe aphid infection prior to the imposition of water stress. Polycyclism (the production of multiple bud flushes) commonly occurs in *Q. robur*, and may in fact be a response to defoliation by insects (Marçais & Desprez-Loustau, 2014; Wargo, 1996). Although aphids do not entirely defoliate trees, they coat the surface of oak leaves in sticky "honeydew". This could in itself induce polycyclism if photosynthesis was negatively impacted by aphid infection, potentially explaining the large number of bud flushes that occurred in our saplings (with most oak saplings producing at least three bud flushes). However, a previous study in sugar beet found no significant effects of aphid infection or honeydew on stomatal functioning (Hurej & Werf, 1993).

We did not find that the water stress treatments imposed significantly altered oak phenology, although this is not particularly surprising as the experimental period occurred in midsummer, and most saplings had already produced second and even third flush shoots before the start of the experimental period. The number of bud flushes and the number of shoots of different flushes was not significantly impacted by water stress treatment, although waterlogged plants did not produce any fourth flush shoots. This

could potentially be due to water logging leading to a cessation of oak growth, as observed in other studies (Parelle et al., 2006).

Observations on oak phenology

We expected that saplings with earlier spring bud burst would produce greater numbers of secondary shoots due to an increased length of growing period for indeterminate leaf development. The total number of bud bursts of saplings was not predicted by initial bud burst timing (Table 7), but there was a positive correlation between timing of spring bud burst and the number of third flush shoots produced (Table 7, Fig. 18B), providing partial support for this hypothesis. In addition, we found that saplings undergoing earlier spring bud burst produced fewer overall primary shoots (Table 7, Fig. 18A). *Quercus robur* exhibits determinate growth of primary buds, with resource allocation to next year's primary growth determined at the end of the growing season and primary buds already developed by the following spring (Kerling, 1965; Thomas, 2014). It may be that saplings which underwent earlier bud burst in spring 2018 had a more conservative growing strategy, potentially due to growing stress in the previous growing season and therefore lower resource allocation to next year's primary growth. Prior to repotting, saplings were obtained in different sized pots, and many had pot bound roots, which could also have led to differences in the initial number of buds on different saplings, despite them all being of the same provenance. Our study also suggests that the number of both

primary and lammas shoots can be predetermined by previous growing season conditions; i.e. the earlier the primary bud burst date in Spring, the more third flush shoots were produced, although this was not the case for second flush shoots (Fig. 18B). This has implications for understanding oak powdery mildew susceptibility of trees between years, as it may be partly predetermined by prior conditions and resultant numbers of lammas shoots produced the next summer, in addition to variables such as insect defoliation occurring during spring (Marçais & Desprez-Loustau, 2014). However, the same climatic variables that impact oak resource allocation, such as drought and temperature variables, may also impact oak powdery mildew chlamydospore overwintering between years (Desprez-Loustau, Hamelin, & Marçais, 2019; Kerling, 1965; Day, 1927). This could make it difficult to disentangle drivers of susceptibility to oak powdery mildew between years, on the one hand due to direct impacts of climate on the fungus, and on the other due to indirect impacts of climate via oak growth. One study found that oak powdery mildew responded less strongly than oak host bud burst to changes in climate along an elevation gradient, leading to increasing phenological asynchrony between hosts and parasite (Dantec et al., 2015). This suggests that oak host phenology may be more responsive to environmental conditions than parasite phenology, and thus indirect effects may be stronger drivers of powdery mildew susceptibility than direct effects, although this hypothesis remains to be tested.

Challenges to future research on mildew - drought interactions

As leaf age and phenology are so crucial for understanding this pathosystem, to test the interaction between drought stress and oak powdery mildew requires oak leaves to be in the correct phenological condition for infection. Higher spring temperatures have led to an advancement of *Q. robur* spring bud burst (Roberts, Tansey, Smithers & Phillimore, 2015; Visser & Holleman, 2001), and in our study older, aphid damaged leaves were no longer suitable substrate for mildew infection. The trade-off for researchers in such an instance is whether to keep saplings outside during the winter, risking exposure to frost damage, or keep them in a greenhouse environment, where warmer temperatures may result in abnormal phenology. Our study highlights the challenge of creating experimental systems that adequately mimic the natural environment in order to provide useful outcomes for practitioners.

Improvements to a future study

A future study would require infectious cultures of oak powdery mildew to be guaranteed, which could be brought about by maintaining cultures on living oak tissue over the winter period to the following growing season, meaning that infection of oak saplings could occur soon after primary bud burst the following year regardless of its timing. This would result in a more controlled study but carried out under more artificial conditions, as the entire experiment would be carried out under controlled environmental

conditions in a greenhouse. This would enable more ease in controlling the hydric stress conditions, as there would be no need to control ambient rainfall inputs. However, it is likely that oak bud burst would occur earlier than if saplings were kept outside, which would then test the infection intensity of oak powdery mildew on primary growth, rather than on lammas growth as more commonly occurs under natural conditions. Previous work has suggested that differences exist in the structure and physiology of primary compared to secondary oak growth, which are still poorly understood (Beikircher & Mayr, 2013). Infecting primary rather than lammas tissue would not reflect the reality of the phenology of the system in natural ecosystems. This would limit the potential application of the results to real-world management issues.

Conclusions

We developed a new method for inducing and maintaining water stress treatments on potted oak saplings, which we showed to be effective in altering soil moisture and lowering shoot biomass and water content, despite the relatively short period of the experimental treatment. Saplings pre-exposed to high aphid infection early in the growing season were almost entirely resistant to oak powdery mildew infection later in the summer, preventing a direct test of drought effects on oak powdery mildew. We found compelling evidence for an association between primary bud burst date and the number of third flush shoots produced, suggesting

predetermination of oak phenology can occur beyond initial primary bud burst. Moreover, waterlogged plants may have been inhibited from producing further growth during the experimental period. This suggests a role for water stress in altering oak phenology.

Acknowledgments

We thank Sofia Gripenberg and Kristina Visakorpi for providing us with the oak sapling material from the University of Oxford Zoology department. Thank you to all the staff at UK CEH Wallingford for their maintenance of the GroDome facilities for the duration of our study.

Chapter 5: What are the impacts of mixed forest stands on forest pathogens? A systematic review and meta-analysis

Elsa Field[1], Andrew Hector[1] & Julia Koricheva[2]

[1] Department of Plant Sciences, South Parks Road, University of Oxford, Oxford, OX1 3RB, United Kingdom

[2] Department of Biological Sciences, Royal Holloway University of London, Egham, Surrey

Article status

This chapter is presented in as yet unpublished manuscript form.

Author contributions

EF & JK conceived the study and the methodology. EF carried out the data searches, data extraction and evidence synthesis. EF carried out the meta-analysis with input from JK and AH. EF wrote the manuscript. All authors commented on this version of the manuscript.

Abstract

Diversifying planted forests to reduce the risks associated with large scale disturbances is a major aim of sustainable forest management. Previous meta-analysis has shown that insect pest damage may be lower in mixed forests stands compared to monocultures, but the same has not been shown for pathogens. Here, we provide the first systematic review and meta-analysis specifically testing the effects of tree diversity on pathogen damage. Relevant studies were retrieved using bibliographic databases and internet searches, as well as previously unpublished data sets contributed by stakeholders. We found that more diverse forest stands overall had significantly lower pathogen damage, and that this result was most pronounced in temperate forests for which the most studies were available. Although in some cases tree diversity had a strong effect, this was not universal and was influenced neither by pathogen specialism, nor by the presence of alternative hosts in stands. Instead, we found that tree neighbour identity rather than species richness emerged as a crucial variable impacting pathogen damage in mixed stands. Neighbour identity effects reported in studies were far-ranging, including impacts on the microclimate of stands and on the microbiome surrounding host trees. Future work should focus on mechanistic explanations that could underpin neighbour identity effects in mixed forests. We suggest the use of the disease triangle as a tool for considering the multiple factors that can influence pathogen damage in mixed forest stands.

Introduction

Plant health threats range from the chronic to the severe, with several devastating plant pathogens worldwide (Fisher et al., 2012). Even small, chronic loss of leaf tissue due to herbivory has been shown on a global scale to lower forest productivity by 2-15% (Zvereva, Zverev, & Kozlov, 2012). There is a clear need for durable strategies to reduce infection transmission and improve overall forest productivity and resilience to disease (Ennos, 2015). Planting stands of mixtures of tree species is often promoted as a way to reduce pest and pathogen damage to forest stands and thus promote forest productivity (Forrester & Bauhus, 2016; Jactel et al., 2017).

The negative relationship between plant diversity and infectious disease has been widely shown in agricultural systems and experimental grassland communities (Mitchell, Tilman, & Groth, 2002; Mundt, 2005). Disease theory suggests that as the density of a given host reduces in mixture, there is lower potential for pathogen populations to build up, disperse and cause disease, leading to lower infection and damage rates – the dilution effect (Keesing et al., 2010; Mundt, 2005). A recent meta-analysis provided evidence of the dilution effect occurring across diverse study systems ranging from animals to plants (Civitello et al., 2015), however, this study included just one paper on forests. Likewise, Liu et al. (2020), in a meta-analysis of plant diversity effects on disease, found a reduction in pathogen damage overall in mixtures, which was nonsignificant in forests. However, this study included only six papers considering pathogens on trees, five

from Europe and one from Mexico. Whether tree diversity positively or negatively impacts pathogen damage has yet to be the focus of a comprehensive, global meta-analysis or systematic review.

It has been suggested that effects of plant diversity on pathogen infection could be smaller in forests compared to other plant communities because the large size of trees may lead to increased frequency of autoinfection (multiple infections on the same host plant), reducing the effectiveness of mixtures (Mundt, 2002). Furthermore, given the comparatively long generation time of most forest plantations, the ability of a mixed stand to avoid and resist disease progression over the entire length of the rotation has to be questioned.

There is strong evidence that tree diversity has a negative impact on specialist insect herbivores – associational resistance (Castagneyrol et al., 2014; Jactel & Brockerhoff, 2007; Vehviläinen et al., 2007). Moreover, when the composition of trees in a mixture are closer in phylogenetic relationship to one another, tree diversity has less of an effect on insects (Castagneyrol et al., 2014). This suggests that both pest specialism and tree neighbour identity have important roles in determining tree diversity effects on insects.

We currently lack an understanding of whether associational resistance in mixed forests is as dominant a trend for pathogens as it is for insects. This is even more important in light of the fact that trees are often under threat from multiple abiotic and biotic factors simultaneously (Jactel et al., 2017; Manion & Lachance, 1992). A

literature review of biodiversity effects on pathogens in naturally assembled communities found that neutral/positive relationships between host diversity and pathogen damage were just as common for non-specialist pathogens as were negative relationships, while for specialist pathogens, negative relationships outweighed positive/neutral ones (van der Plas, 2019). This suggests that specialism may play an important role for pathogens as it does for insects, although the study did not quantify this using meta-analysis. Previous work in mixed forests also found important tree identity as well as diversity effects on pathogens (Hantsch et al., 2014; Hantsch et al., 2013). The evidence on the different factors which impact upon host diversity-pathogen damage relationships in forests has yet to assimilated.

In this study, we focused on forests and undertook a global systematic review and meta-analysis of the English language forestry literature relating to mixed forest stands and pathogens. We included both experimental and observational studies from across the globe and searched both bibliographic databases and the grey literature. We tested the following specific hypotheses in the meta-analysis:

1) Overall, mixed species forest stands would have lower pathogen damage than monocultures, consistent with evidence for a dilution effect.

2) Pathogen specialists would be more strongly affected (larger negative effect size) than pathogen generalists by mixed forest stands.

3) The presence of alternative hosts in mixed stands would lead to either reduced damage on focal hosts or an increase in damage, depending on whether alternative hosts were more or less susceptible than the focal host. Alternative hosts being less susceptible would increase host dilution, leading to associational resistance. More competent hosts could lead to an associational susceptibility effect, where spill over occurs onto the focal host from more susceptible hosts, reversing the protective effect of mixed stands on pathogen damage to the focal host (Barbosa et al., 2009). Therefore, the overall effect of presence of alternative hosts may be positive or negative.

In addition to these more general hypotheses, we also summarised results from studies containing qualitative information and observations on the topic and focused on extracting information on mechanisms that could underpin diversity-disease effects.

Materials and Methods

We followed the established protocol for systematic reviews from the Collaboration for Environmental Evidence (Pullin & Stewart, 2006). Our systematic review protocol was independently peer-reviewed blind by a panel of experts recruited through the CEE network (by handling editor Neal Haddaway). We summarise the methodology used here, and further detail is available in the

protocol (Field, Petrokofsky and Koricheva, 2018) and the supplementary material to this paper.

Literature searches and inclusion criteria

We used the PECO framework (components of the primary question, comprising population, exposure, comparator and outcome) as the basis for searching for studies and study inclusion criteria. To be judged relevant to the review, a study needed to include all four components. We identified the *population* as tree species, including both long and short rotation crops and fruit trees. The *exposure* (treatment) was mixed tree species stands, the *comparator* (control) was pure stands, and the *outcomes* were metrics of pathogen damage on the focal tree species. We included both observational / longitudinal studies from natural (unplanted) forests, and experimental studies from tree diversity experiments and forest plantations. We included studies only where the English full text of the paper was available. No restrictions were given on study publishing date.

We searched three major bibliographic databases: Scopus, Web of Science (relevant databases only, listed in Appendix I) and CAB Abstracts. We also searched Google Scholar, as Haddaway et al. (2015) showed only poor overlap between results from Web of Science and Google Scholar when similar search strings were used. We first conducted a scoping study using Scopus, refining search terms based upon whether they could return articles in the test library of known to be relevant studies (Appendices I-II). The final search terms used

included a range of keywords relevant to each component of the primary question (Table S5). Details of searches conducted and numbers of hits in each database are given in Table S6.

A range of stakeholders known to be working in the field were also contacted and asked to contribute relevant citations, as well as the personal libraries of contributors (Appendix III). We also searched the citations of articles found to be relevant at the level of full text, to find further studies.

We also received unpublished data from TreeDivNet, the experimental long term forest diversity network (Verheyen et al., 2016). Data was contributed along with standardised metadata sheets, submitted by each contributor (Appendix III), and these underwent the same process of screening as for all other studies.

The total number of studies brought forward to the screening stage was 11,470 (Fig. 19).

Article screening

Studies were assessed for their relevance for inclusion first based on title, then title and abstract, then full text (Fig. 20). Screening at both title level and abstract level was completed using the online tool Abstrackr: (http://abstrackr.cebm.brown.edu/). The majority of article screening was undertaken by the same reviewer (EF) but we performed kappa tests (CEE, 2013)

at both title and abstract screening stages to check for consistency. All reviewers received the same list of inclusion criteria for assessing studies. A kappa value > 0.6 indicated that reviewers were consistent in their assessments of study relevance. If k < 0.6, then reviewers discussed the possible sources of inconsistency and repeated until k > 0.6. A randomised subset of 200 articles were used for kappa tests at both title and abstract level.

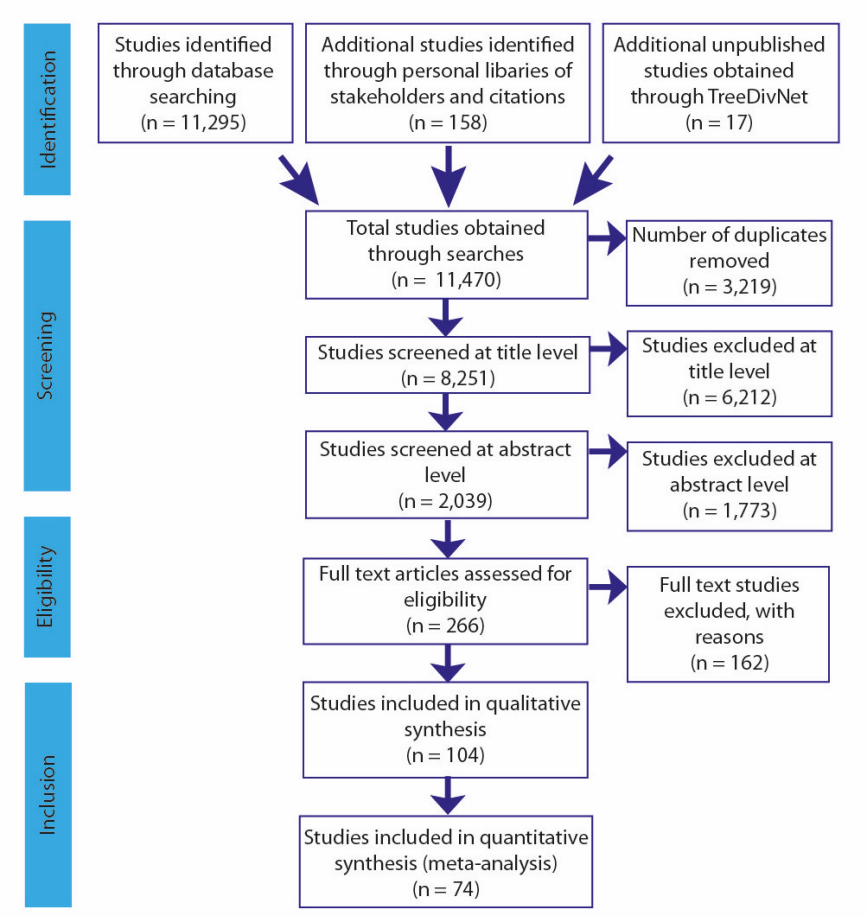


Figure 19: PRISMA record workflow of studies included in the systematic review. Divided into sections: Identification of studies, Screening of studies, Eligibility of studies, Inclusion of studies. The number of studies at each stage is indicated in brackets by *n*.

Data Extraction

We used standardised spreadsheets for extracting all data from publications (Field, Petrokosky & Koricheva, 2018). First, we assessed whether a publication contained sufficient quantitative data for inclusion in the meta-analysis. If so, then publication metadata and quantitative data were extracted, along with any additional qualitative data. Where data were available only in graphs, we used the online tool Web Plot Digitizer (https://automeris.io/WebPlotDigitizer/) to extract data. The same reviewer (EF) carried out data extraction for all studies. If insufficient quantitative data was available for the meta-analysis, we extracted metadata and qualitative data only.

We used Standardised Mean Difference (Hedges' d) as the effect size to test for differences in measures of pathogen damage between monospecific and mixed species stands (Hedges, 1981). We chose to use Hedges' d as the measure of effect size, as:

(1) Many studies included comparisons between monocultures and just one level of multispecies mixtures (for example, two-species mixtures), rather than continuous relationships between tree species richness and pathogen damage.

(2) It is possible to convert to *d* to/from other statistics that may be available from studies (such as *t*-statistics and correlation coefficients) using standard equations (Koricheva et al., 2013)

(3) Hedges' *d* has commonly been used in similar meta-analyses assessing plant diversity effects on plant attackers, which enhances the comparability of our study with others in the field (e.g. Jactel & Brockerhoff, 2007; Koricheva & Hayes, 2018).

To calculate Hedges' *d* we extracted means, sample size and standard deviation of treatment and control groups from each publication. A positive effect size indicated that pathogen damage was higher in mixed species stands compared to monocultures, while a negative effect size indicated that mixed species stands had a protective effect.

In some cases, multiple effect sizes could be calculated from the same article, i.e. the same article was split into multiple studies. This was the case for instance if damage by multiple pathogen species was measured in the same article, or if a paper contained data from multiple study sites that had not been pooled.

Wherever possible, we calculated effect sizes directly from data available in the study. For studies where a continuous relationship between more than one level of tree diversity had been compared to monoculture, we used standard equations to convert between correlation coefficients and Hedges' *d* (Koricheva et al., 2013). If insufficient data was available to calculate effect size directly, we firstly

contacted the authors for additional data. If additional data were unavailable, we obtained Hedges' *d* and its variance by interconversion between effect sizes from the data available in the study using standard equations (Koricheva et al., 2013). In the case of unpublished studies from TreeDivNet, we calculated effect sizes directly from the raw data provided by authors rather than from summary statistics.

We extracted the following metadata from studies when available in text, tables or graphs.

- Study type (primary research or review)
- Study year
- Study geographical location: continent, country, region
- Latitude and longitude of study site
- Biome (boreal, temperate or tropical)
- Size of plots / stands (m^2)
- Average age of trees at time of sampling
- Focal tree species on which pathogen recorded
- Other species of tree present in mixed stands
- Type of monoculture (broadleaved or conifer)
- Type of mixture (broadleaved, conifer or mixed i.e. containing both broadleaved and conifer species)
- Pathogen species recorded on focal trees
- Pathogen type (fungal, bacterial, viral or oomycete)

- Pathogen specialism (generalist or specialist, where specialist pathogens were defined as being able to colonise only hosts in the same genera as the focal host)
- Pathogen life history (biotrophic, necrotrophic or both/unknown if multiple pathogens recorded)
- Type of pathogen damage recorded. This fell into three categories: incidence,
- Incidence, abundance or severity of pathogen damage depending on how the study was undertaken. Incidence studies described observations of disease presence or absence on trees or in plots, or proportion presence in plots. Abundance studies measured the abundance of infections per plant or plot e.g. the number of lesions per leaf or tree. Severity studies were measures of severity of disease on individual trees e.g. percentage dieback of the crown or percentage coverage by fungal mycelium on leaves.
- Experimental versus observational studies
- Whether summary estimates for pathogen damage were recorded at the level of single trees, or at plot level

Critical Appraisal of Studies

Studies eligible for inclusion at full text were subjected to a critical appraisal of study validity. Studies were assigned into two groups: low or high risk of bias. As there were no geographical or temporal limitations on the review, the critical appraisal focused on internal validity (risk of bias) of studies, rather than external

validity (relevance to the review). The same reviewer (EF) carried out critical appraisal of all studies.

Studies were assigned a score based upon the following 2 categories:

1. Presence of treatment replication: use of blocking to account for spatial variability OR presence of multiple study sites: Both (2) / Either (1) / Neither (0)
2. Randomisation: randomisation of tree selection for assessing pathogen damage: Y (1) / N (0)

Studies were then split into two categories – high risk of bias (score of 0-1) or low risk of bias (score of 2-3).

Statistical Analyses

Meta-analysis

The total number of studies available for the meta-analysis was k = 74 studies, from 44 articles. We used a random effects meta-analysis model as significant heterogeneity between studies was expected. A restricted maximum likelihood approach was used to estimate the parameters of the meta-analysis model. All meta-analyses were conducted using the *metafor* package (Viechtbauer, 2010) in R version 3.5.3 (R Core Team, 2020). We calculated the total heterogeneity in effect sizes (Q_t) as well as the I^2 statistic, which estimates the proportion of heterogeneity

that is present due to between-study variance. Due to the potential non-independence of studies coming from the same paper, we included study ID as a random effect in the meta-analysis (random effect with 44 levels, Nakagawa et al., 2017). We used the rma.mv() function in metafor, which enables fitting of multi-level meta-analytic models.

We first tested for the overall significance of the effect of mixed species stands on pathogen damage. Next, we used meta-regression to investigate the impact of categorical moderators on between-study variance. There were only a few categorical variables extracted from studies that we chose to analyse as additional covariates in models, due to *a priori* hypotheses of interest as well as data on those variables being available for most studies. These were: biome, pathogen specialism, presence of alternative hosts and pathogen life history. We also ran exploratory analyses to test for effects of several other variables extracted from papers: type of diversity metric, single tree versus plot level studies, and experimental versus observational studies.

Location information varied per study and we were only able to extract the exact latitude for $k = 43$ studies. Latitude has shown to be a driver of species diversity effects on both pests (Kambach et al., 2016) and pathogens (Liu et al., 2020) previously. Biome was the only location variable available for all studies, and likely included an overall effect of latitude in combination with other environmental factors. For instance, most studies of conifers occurred in the boreal

biome (25/30 studies of conifers), while broadleaves were mainly studied in the temperate biome (36/43 studies of broadleaves).

Rather than include moderators together in the same meta-regression model, we chose to fit separate models testing the individual impact of each moderator on effect size. This was for two reasons:

(1) Not all studies had data available on all moderators, so fitting a model with all moderators would have excluded several studies (reducing the sample size from 74 to 49 studies for a model with all 4 moderators) potentially leading to a biased subsample of studies and reduced statistical power.

(2) Using contingency tables, we found that moderators tended to be confounded. For instance, biotrophic pathogens tended to be specialist pathogens in our data set (Fig. S18). Generalist pathogens tended to be associated with studies where alternative hosts were present in mixed stands. This makes sense, as the likelihood of an alternative host being present in mixed stands will be higher for generalist pathogens (Fig. S18). Specialism was also confounded with biome; more specialist pathogens were present in temperate versus boreal and tropical biomes (Fig. S18).

With several confounded categorical variables, we focused instead on which variable could explain the most variation in effect size on its own, potentially giving us a clue towards underlying mechanisms. For the model including Pathogen Specialism as a moderator, we excluded studies that tested the effect of

both necrotrophic and biotrophic pathogens in the same study (k = 6 studies), to provide a clear contrast between necrotrophs and biotrophs only. For the model including climate as a covariate, we removed studies that took place across multiple biomes (across both Boreal and Temperate biomes, k = 3 studies).

Sensitivity analysis

Following the main analysis, we conducted sensitivity analyses to assess the sensitivity of the overall effect size to aspects of study design that we had previously assumed did not influence the outcome. To test the effect of study bias on effect size, we ran a meta-regression of overall effect size, including risk of bias score (low or high) as a moderator. We also tested whether conversion between effect sizes had an influence on outcome using meta-regression (i.e., whether or not the metric of effect size (Hedges' d) had been calculated directly or by interconversion from other effect sizes).

Publication bias

We tested for potential publication bias using funnel plots, which display effect size plotted against standard error, and inspected them for visual asymmetry. Potential publication bias is indicated by asymmetry in the plot, with studies reporting small or nonsignificant effects missing from the opposite side of the plot to the true effect size. However, funnel plots have limitations, as asymmetry can be caused by several other factors (Lau et al., 2006). We tested for the significance

of asymmetry of the funnel plot using Egger's regression (function *regtest()* in *metafor*). We also calculated fail safe numbers using Rosenberg's weighted method to assess the robustness of results to potential publication bias (Rosenberg, 2005). The failsafe number indicates the number of additional studies having an effect size of 0 that would be required to add in order to make the overall effect size statistically non-significant (Rosenberg, 2005).

Results

General patterns

Our meta-analysis of the effect of mixed species stands on pathogen damage was based on 44 papers yielding 74 comparisons of the effect of tree species mixtures compared to monocultures. We also extracted qualitative data from a further 30 papers.

The majority of quantitative studies (40 out of 74 studies) took place in the temperate biome, 26 were from boreal regions and 5 from tropical regions. This pattern was similar for studies containing only qualitative data, of which 16 out of 30 studies were from temperate regions, 8 from boreal and 4 from tropical regions, with 2 studies spread across both temperate and boreal countries. Fig. 20 shows the patterns of study distribution by geographical country (several studies contained data from more than one country). Quantitative data was concentrated

in Europe (with 77 observations in different countries), with studies mostly concentrated in Germany and Finland (19 and 17 studies, respectively). Across other continents, North America had 11 studies in total, South America two studies, Asia had one study (from China), and Africa one study. In studies containing only qualitative data (with 38 observations in different countries), there were 20 studies from Europe, 10 in the USA and the remaining 8 studies between Asia, Africa and South America.

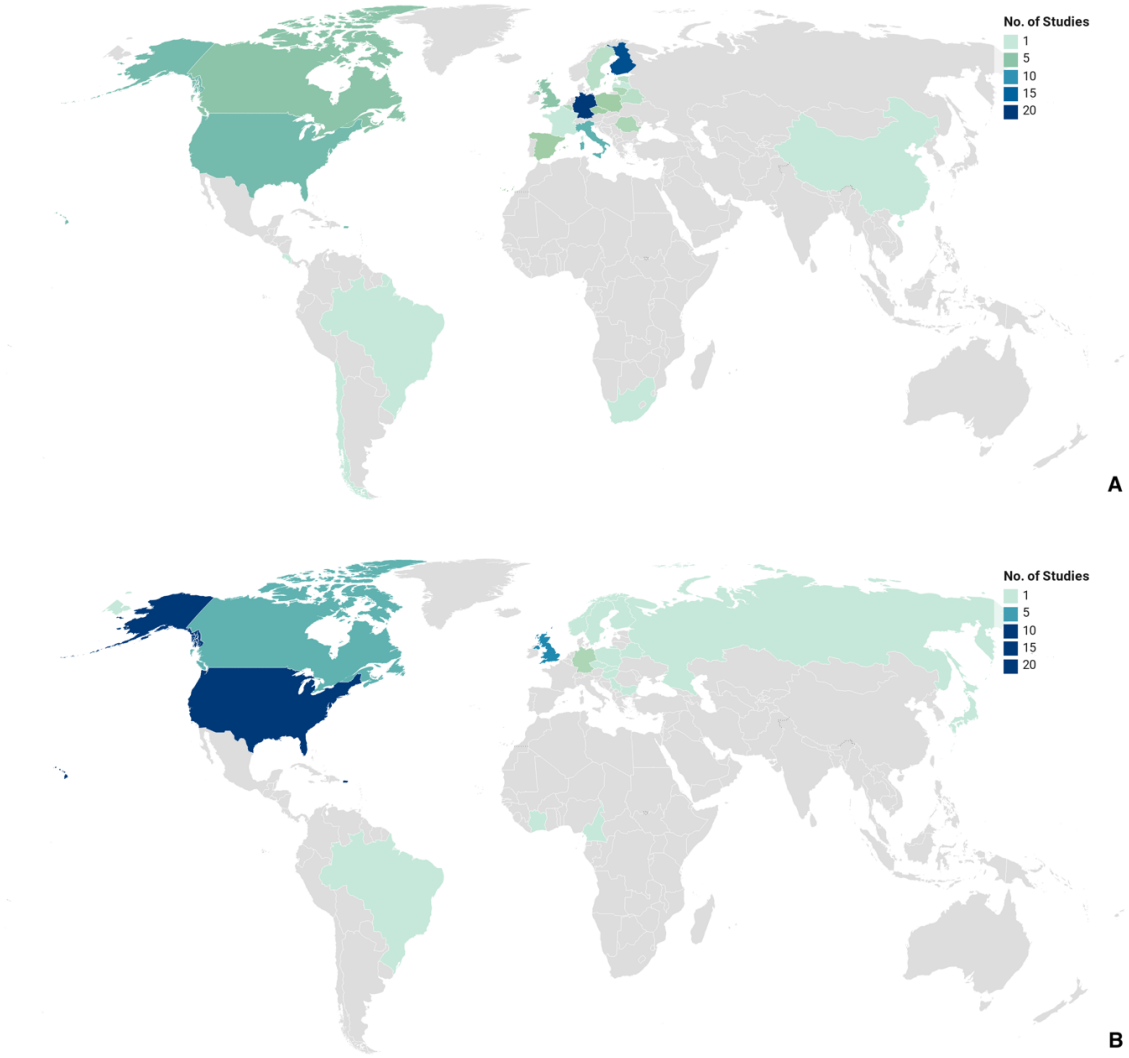


Figure 20: Geographical distribution of countries for which quantitative or qualitative data was extracted. Grey regions are countries with zero studies. Some studies contained data from more than one country. **A)** Quantitative studies only, *n = 93* observations in different countries, total no. of studies *(k)* = 74. **B)** Qualitative studies only, *n* = 38 observations in different countries, total no. of studies (*k*) = 30.

Fungal pathogens were by far the most commonly studied group, making up 68 out of 74 comparisons for the meta-analysis (92%) and 26 out of 30 qualitative papers (87%). The remaining 4 qualitative papers were concerning oomycetes (all *Phytophthora*). One study in the meta-analysis concerned bacteria, three concerned oomycetes (all *Phytophthora ramorum*) and two assessed general pathogen damage symptoms (Fig. 21).

Hosts were divided between broadleaved and coniferous trees. Among quantitative studies, 43 considered broadleaved hosts, 30 considered coniferous hosts, and one study was of both broadleaved and coniferous hosts combined (Ampoorter et al., 2019). Among qualitative studies, 17 considered broadleaved hosts and 13 considered coniferous hosts (Fig. 21).

Among quantitative studies, pathogens were most commonly studied on leaves, whereas for qualitative studies, work was more evenly distributed between the different damage organs of the host trees (Fig. 21).

For quantitative studies, the most commonly studied pathogens were rusts and powdery mildews (29 out of 74 studies), in particular the powdery mildew pathogens *Erysiphe* on *Quercus,* which were the focal pathogens of 9 studies. Root rot pathogens were another major group studied; 19 out of 74 studies were of root rot pathogens, all of which were reported on coniferous species.

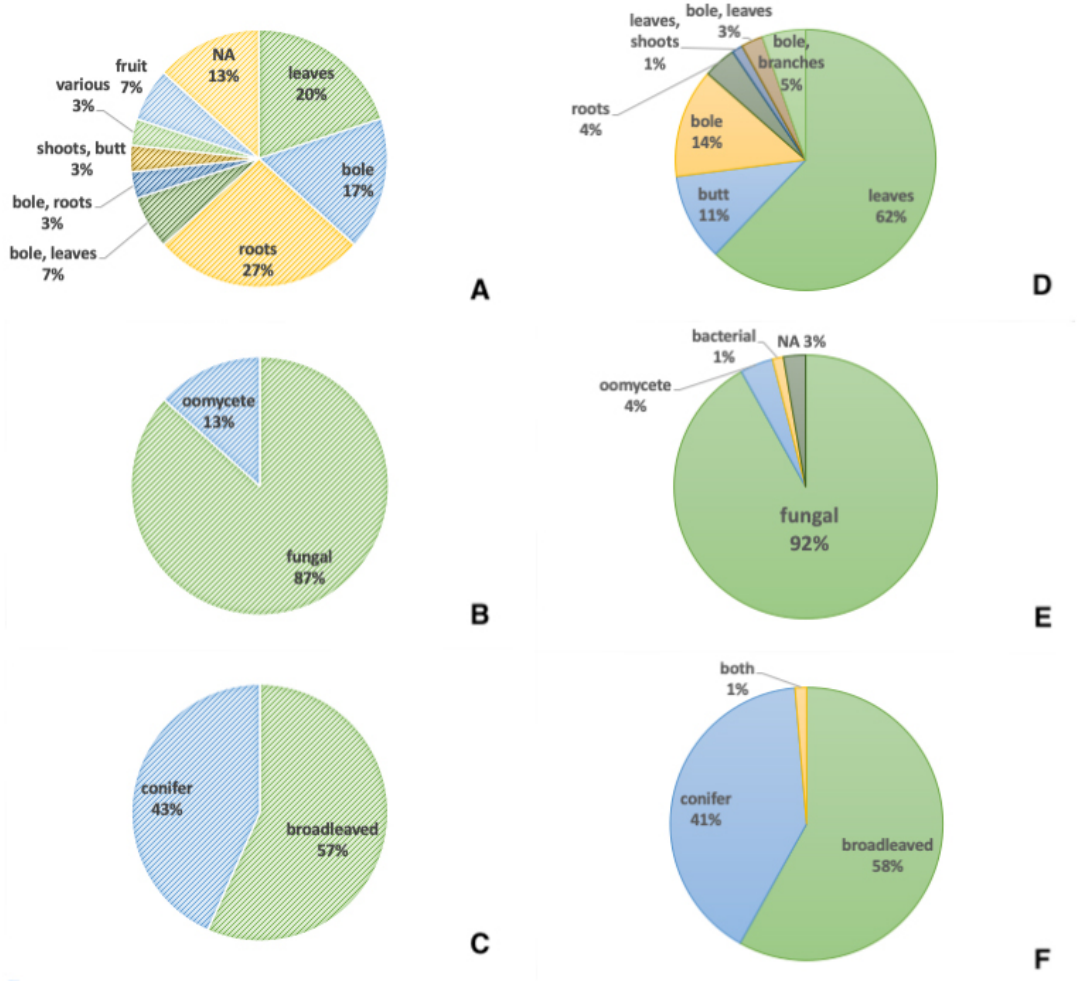


Figure 21: Pie charts showing the distribution of studies by damage organ, pathogen type and host type. **A-C)** Qualitative studies. **D-F)** Quantitative Studies. Explanation of terms used: Butt = the base of the tree stem. Bole = the main portion of the tree stem.

Meta-analysis

Overall, we found that mixed species stands had lower pathogen damage compared to monocultures (d = -0.27, 95% CI -0.49 to -0.046, k = 74 studies, Fig. 22). According to Cohen's original benchmarks for the interpretation of d (Cohen, 1970) this corresponds to a small effect size. Between study heterogeneity was high (Q_t = 835.67, $p < 0.001$, df = 73), and 98% of heterogeneity was due to between study variance. This therefore justified the inclusion of additional explanatory moderators using meta-regression.

The effect of mixed species stands differed depending on whether the study took place in a boreal, temperate or tropical region (Q_m for Biome = 13.17, p = 0.0014, df = 2, k = 71 studies, Fig. 23). Significant reduction in pathogen damage in mixed stands compared to monocultures was observed only in temperate forests, whereas no significant effects of forest diversity on pathogen damage were detected in boreal forests. Results of studies in tropical forests varied but tended to indicate reduction of pathogen damage in mixed stands similarly to temperate forests (Fig. 23).

None of the other moderators (Specialism, Life History or Alternative Hosts) had a significant influence on effect size (Figs 24-26). Damage by generalist pathogens was significantly lower in mixed forests compared to monocultures, but the effect was not significantly different from specialist pathogens (Fig. 24). Similarly,

damage by biotrophic pathogens was significantly lower in mixed forests, but the effect was not significantly different from necrotrophic pathogens, which were not significantly affected by tree diversity (Fig. 26).

Different levels of moderators were not significantly different from each other for any of the other variables that were extracted as metadata in our exploratory analysis (see Figs S19-S22).

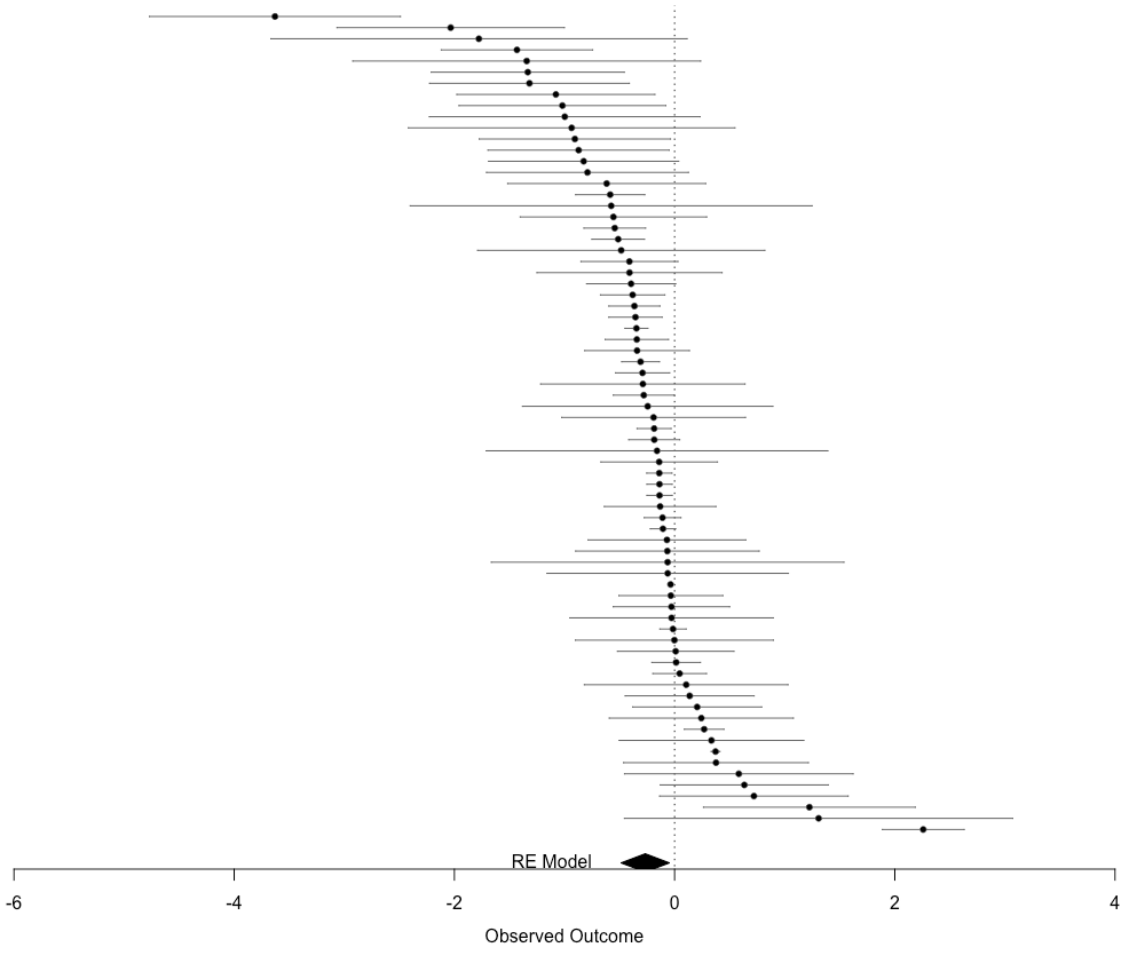


Figure 22. Caterpillar plot showing effect sizes and associated 95% confidence intervals, in order of effect size for all 74 studies included in the meta-analysis.

The x axis displays effect size with the vertical dashed line indicating an effect size of 0. Effect sizes are significantly different from 0 if their confidence intervals do not include zero. The bottom polygon shows the overall result from the random effects model (*d* = -0.27, 95% CI -0.49 to -0.046, *k* = 74 studies).

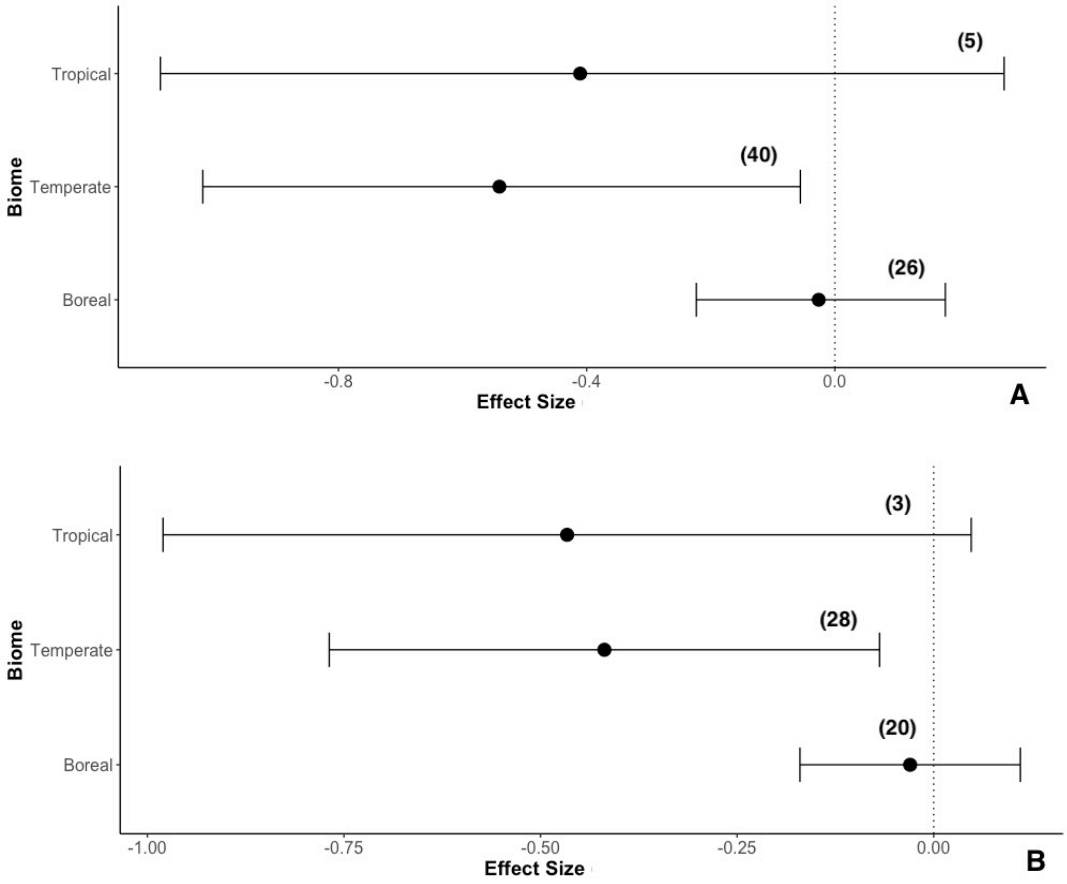


Figure 23: Effect of biome on effect size (Hedges' *d* – mean effect size and 95% confidence intervals for each biome; tropical, temperate or boreal). In brackets is the number of studies in each group. A) Results for all studies reporting data on biome (total *k* = 71 studies). Biome was a significant predictor of effect size (Q_m for Biome = 13.17, *p* = 0.0014, *df* = 2). B) Results for low bias studies only (total *k* = 51 studies). Biome was a significant predictor of effect size (Q_m for Biome = 15.22, *p* = <0.001, *df* = 2).

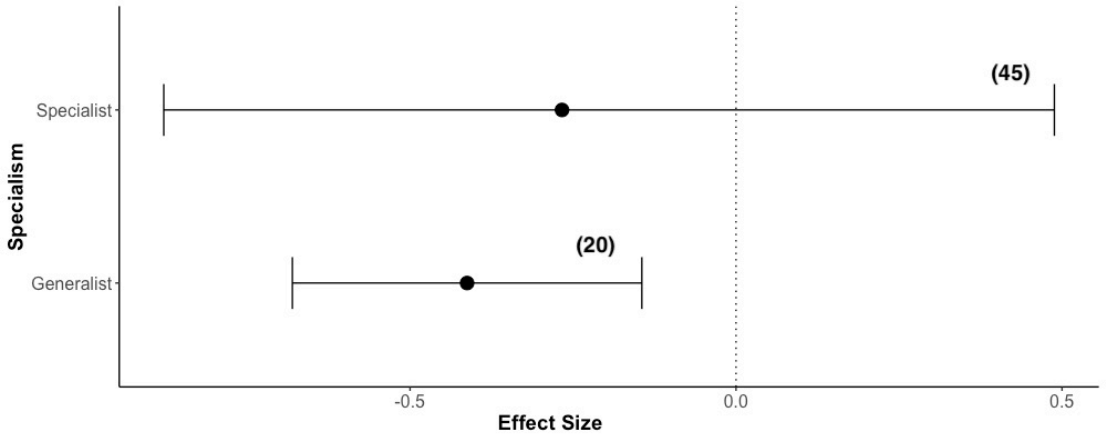


Figure 24. Effect of pathogen specialism on effect size (Hedges' *d*). Shown is the mean effect size and 95% confidence intervals for pathogen specialists and generalists. In brackets is the number of studies in each group (total $k = 65$ studies). Pathogen specialism was a non-significant predictor of effect size ($Q_M = 0.69$, $p = 0.41$, $df = 1$).

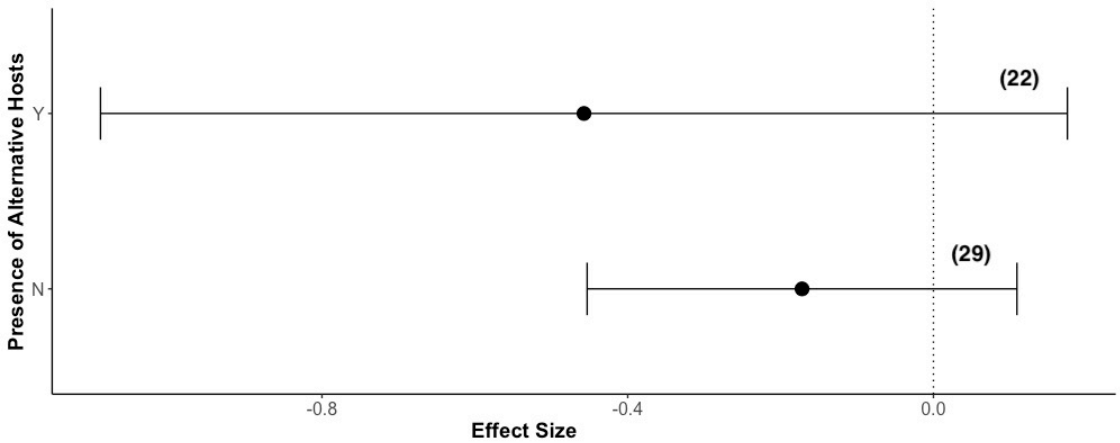


Figure 25. Effect of presence of alternative hosts (Y/N) on effect size (Hedges' *d*). Shown is the mean effect size and 95% confidence intervals for each group. In brackets is the number of studies in each group (total $k = 51$ studies). Presence of alternative hosts was a non-significant predictor of effect size ($Q_M = 2.53$, $p = 0.11$, $df = 1$).

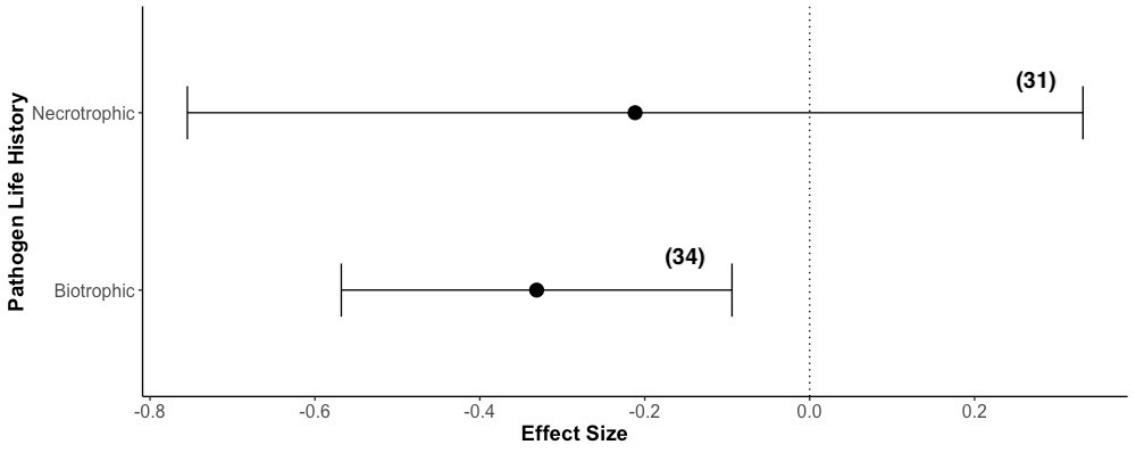


Figure 26. Effect of pathogen life history on effect size (Hedges' *d*). Shown is the mean effect size and 95% confidence intervals for necrotrophs and biotrophs. In brackets is the number of studies in each group (total k = 65 studies). Pathogen life history was a non-significant predictor of effect size $Q_M = 0.58$, $p = 0.45$, $df = 1$).

Sensitivity analysis

Studies with a high versus low risk of bias did not differ significantly in their effect sizes (Fig. 27). However, studies with high risk of bias had significantly negative effect sizes whereas results from studies with low bias varied greatly. To test whether the main conclusions still held when high bias studies were removed, we re-ran the meta-analysis on low bias studies only. Removal of high bias studies removed the significant protective effect of mixed stands compared to monocultures (d = -0.17, 95% CI -0.39 to 0.048, k = 54 studies). But when low bias studies were again split into groups by biome (again removing studies occurring across biomes) there was a significant effect of mixed stands in temperate regions only, as the results of the main analysis had shown (Fig. 23B).

Studies with converted/non-converted effects did not significantly differ in their effect sizes (Fig. S22).

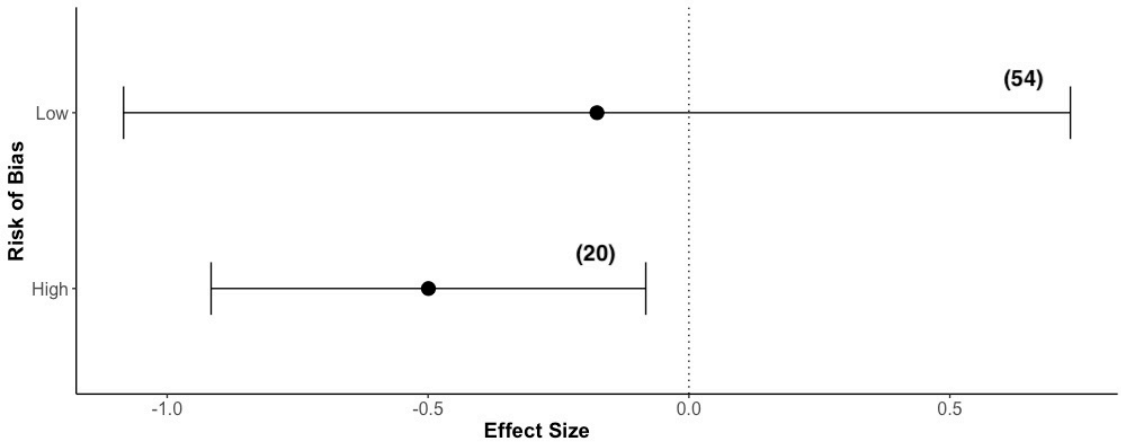


Figure 27. The effect of risk of bias on effect size. Shown is the mean effect size and 95% confidence intervals for low and high risk studies. Risk of bias as assessed *a priori* was a non-significant predictor of effect size (Q_M = 1.66, p = 0.20, df = 1).

Publication bias

Funnel plots suggested that there could be a negative correlation between effect size and standard error, as shown by the empty space in the bottom right side of the funnel plot of effect size against study standard error (Fig. S23). This space should have been populated by small, nonsignificant studies where effect size was positive but standard error large, i.e., studies that may have been subject to the "file-drawer" problem. Egger's regression test confirmed the significance of funnel plot asymmetry (t = -2.10, p = 0.039). This indicates that there may be some potential publication bias in this field. However, the overall mean effect (Hedges'

d = -0.27) was robust to publication bias, as shown by the high number of additional studies that would need to be added to render the overall effect size non-significant (Rosenthal's failsafe N = 1310).

Qualitative evidence synthesis

Qualitative studies did not contain sufficient quantitative data comparing mixed stands with monocultures to be included in the meta-analysis, but many reported both increases and decreases in pathogen damage in mixed stands. Several qualitative studies were early field studies containing field reports (e.g. Day, 1927; Osmaston, 1927). Other qualitative studies were reviews of previous literature, such as reviews of long-term experiments or groups of experiments with the same theme (McCracken & Dawson, 1998; McCracken, Dawson, & Carlisle, 2005; Mundt, 2005). Some reviews cited other work, much of which was not available at full text in English when citations were searched (Korhonen et al., 1998; Pautasso et al., 2005). Most qualitative studies fell into groups of the same purported mechanisms to explain diversity effects on pathogens (Table 8).

Mechanism	Relevant Citations
AR - Host dilution (reduction in host density in mixed stands)	*(Busby, Newcombe, Dirzo, & Whitham, 2013; Cobb, Slaughter, Rowney, & DeMars, 1982; Day, 1927; Evans, 1999; Gerlach, Reich, Puettmann, & Baker, 1997; Mccracken & Dawson, 1997; McCracken & Dawson, 1998; McCracken et al., 2005; Mundt, 2005)*
AR – Effects of Microclimate	*(D. Lonsdale & Wainhouse, 1987; Louw, 2015; Northrop, 2006; Osmaston, 1927; Shaw, Oester, & Filip, 2009; Tarrant & Trappe, 1971)*
AR – Encounter Reduction (physical barriers to host colonisation)	*(Gerlach et al., 1997; Korhonen et al., 1998; Lindén & Eko, 2003; Pautasso et al., 2005)*
AR – Tree health better in mixed stands	*(Gosselin, 1944)*
AR – Effects on microbiome	*(DeLong, Lewis, Simard, & Gibson, 2002)*
AS – Disease vector population higher in mixed stands	*(Gidoin et al., 2014)*
Either AR or AS - Disease requires more than one host	*(Chard, 1962; Davis, Moss, Montana, & Director, 1940)*
Either AR or AS – Depends on host competence	*(Cobb et al., 2010; Rank, Cushman, Meentemeyer, & Rizzo, 2010)*

Table 8. A breakdown of the mechanisms behind diversity-disease effects reported in qualitative papers, with citations provided for which that mechanism was reported. See discussion for description of the mechanisms cited. AR = Associational resistance; AS = Associational susceptibility.

Discussion

Mixed stands lower pathogen damage in temperate forests

Overall, we found a significant reduction in pathogen damage in mixed stands compared to monocultures, although the effect size varied from strongly negative to strongly positive (Fig 22). A significant negative effect size was only found in the temperate biome (Fig. 23), where 40 out of 74 studies were conducted. The reduction in pathogen damage in mixed stands was consistent in temperate forests, regardless of study bias (Fig. 23B). This suggests that the result for temperate biomes is the most robust pattern.

To our knowledge, this is the first meta-analysis providing evidence for a significant negative effect of tree diversity on pathogen damage in any biome. Liu et al. (2020) in a meta-analysis of plant diversity effects on infectious diseases had previously reported a nonsignificant result in forests. They suggested that for forest trees, the identity of immediate neighbours is more important than overall plant community diversity in predicting disease damage, as several papers had noted the importance of tree neighbour identity effects on pathogens (Dillen et al., 2016; Hantsch et al., 2013). However, their meta-analysis included estimates from only six papers on forests, all from higher latitudes (northern hemisphere), likely as they had searched just two databases for sources of studies (Web of Science and China National Knowledge Infrastructure).

We also found an overall trend towards studies from higher latitudes (Fig. 20A-B) as was also found by Liu et al (2020). Unfortunately, we could not test for effects of abiotic variables such as temperature and precipitation, as this would have required accurate latitudinal data to be available for all studies. Moreover, as the latitudinal gradient of studies was skewed towards the northern hemisphere, the gradient of precipitation and temperature that could have been tested would have been fairly narrow. Interestingly, although fewer studies were conducted in boreal forests, they showed less variance than both tropical and temperate forests (Fig. 23). This suggests that the nonsignificant result for boreal versus temperate forests is likely to be reliable (Fig. 23).

The importance of pest specialisation: a false paradigm?

Previous meta-analyses found that the effect of tree diversity on insects was stronger for specialist (monophagous) insect herbivores compared to generalist (polyphagous) insect herbivores (Castagneyrol et al., 2014; Jactel & Brockerhoff, 2007). This supports the hypothesis that dilution effects are stronger when there is a lower likelihood of alternative hosts for a pest being present in a stand. However, we did not find evidence that the same trend held true for forest pathogens. Neither pathogen specialism nor the presence of alternative hosts had significant effects on pathogen damage in mixed stands compared to monocultures. However, studies of generalist pathogens (utilising hosts from more than one genus) did have significant negative effect sizes, whilst no effect was found in studies of specialist pathogens.

This initially surprising result for generalist pathogens could have several potential explanations. The majority of studies of generalist pathogens (16 out of 20 studies) were studies of two important groups of generalist forest pathogens. These were the generalist forest pathogen *Phytophthora ramorum* (3 studies), and the root rot pathogens *Armillaria* sp. and *Heterobasidion annosum* (5 and 8 studies respectively) that affect multiple conifer and broadleaved species. The negative effect of tree diversity on *Phytophthora ramorum* damage to host species has been well documented in landscape-scale studies from California, USA (Dillon & Meentemeyer, 2019; Haas et al., 2016; Haas, Hooten, Rizzo, & Meentemeyer, 2011). Previous work by the same group had also noted that the local abundance of the most infectious and transmissible host, bay laurel, predicted time to infection in all three host species, and that infection was lowest in the presence of a high proportion of non-infective conifer species such as Douglas fir and redwood (Cobb et al., 2010; Rank et al., 2010). However, later studies confirmed the overall trend of a reduction in pathogen damage with increasing tree diversity and higher host dilution (Dillon & Meentemeyer, 2019; Haas et al., 2016; Haas et al., 2011). Although *P. ramorum* is a generalist pathogen with multiple hosts from different genera, tree diversity negatively impacts this pathogen at the landscape scale. Many tree diversity experiments are carried out in small plots, meaning that edge effects could erode the impacts of tree diversity (McCracken et al., 2005). It may be that studies at a plot-to-landscape scale (such as those conducted for *P. ramorum)* are able to provide a more accurate view of whether tree diversity positively or

negatively impacts upon a particular pathogen, rather than smaller-scale studies from a single experiment or site with just a few replicates.

The negative impact of tree diversity on root rot pathogens, which impact numerous coniferous species worldwide causing substantial economic losses, has also been well documented in previous reviews, although occasional increases in damage have also been observed (Korhonen et al., 1998; Pautasso et al., 2005). One hypothesis is that there is a reduction in disease transmissibility due to encounter reduction in the presence of alternative species, as root rot pathogens are spread via root-to-root contacts between susceptible hosts (Korhonen et al., 1998; Pautasso et al., 2005, Table 1). Several qualitative studies of root rot pathogens suggested the importance of considering tree neighbour identity. These studies suggest that in particular, increasing proportions of broadleaved species in stands can have a a stronger negative effect as these are likely to be non-hosts for the disease (Lindén & Eko, 2003; Pautasso et al., 2005). However, 2 out of 3 studies of root rot pathogens with positive effect sizes in the meta-analysis had coniferous species planted in mixtures, rather than broadleaves, suggesting this is not a universal mechanism to explain associational resistance (Arhipova et al., 2011; Korhonen et al., 1992; Whitney, 1973). Moreover, in the meta-analysis, the presence of alternative hosts in stands did not significantly influence effect size. Although the presence of alternative hosts may reduce the impact of tree diversity in individual cases, it is not a general trend that can be relied upon for predictive forestry.

Rather than the presence of alternative hosts, other direct or indirect effects of tree neighbour identity, such as their effect on soil microbiome, or microclimatic conditions, may underlie their associational effect on forest pathogens. For instance, Tarrant & Trappe (1971) suggested that the presence of alder in mixed stands could reduce damage by the root rot pathogen *Phellinus weirii* on *Pseudotsuga menziesii* and other conifers, by producing phenolics and other ammonia-containing compounds to suppress fungal growth. In another example, the presence of paper birch in mixed stands with Douglas Fir has long been documented to reduce *Armillaria* damage to Douglas Fir (Baleshta, Simard, Guy, & Chanway, 2005; Louw, 2015). DeLong et al., (2002) found that *Armillaria*-inhibiting populations of pseudomonad bacteria increased around birch roots, suggesting that paper birch was altering the microbiome to create a "suppressive soil" for *Armillaria*. In a case of associational susceptibility in mixed stands, the authors suggested that the presence of hemlock in mixture with beech increased the humidity of stands and protected beech boles from winter freezing, both of which would benefit the scale insect vector of beech bark disease (Twery & Patterson, 1983). A previous study by the authors (Field et al., 2020) also found that the composition of mixed stands altered the microclimate (temperature and humidity) and was correlated with oak powdery mildew infection (a more stable microclimate increased mildew damage). The environmental conditions created in mixed stands, dependent on the identity of neighbouring tree species, may therefore be as important as their potential as alternative hosts for disease. That being the case, multiple hosts are required to complete the pathogen life cycle, the impact of mixed stands will depend upon whether alternate hosts are planted or

not. This is the case, for instance, for some rust species such as white pine blister rust (*Cronartium ribicola)* that requires both *Pinus strobus* (the aecial host) and *Ribes* sp. (the telial host) to complete its life cycle. In the first half of the twentieth century in the United States and United Kingdom, large-scale removal of naturally regenerating *Ribes* sp was one of the few known treatments for white pine blister rust, but was eventually abandoned as an approach for economic reasons (Chard, 1962; Davis et al., 1940). In this case, it does not matter whether white pine is planted as a monoculture, as the disease cannot complete its life cycle without the presence of the alternative host. The same is true for pine twist rust (*Melampsoa pinitorqua*) for which the other main host is aspen. A study from Finland therefore found when *Pinus strobus* was planted in mixture with aspen, associational susceptibility was the result compared to monoculture (Mattila, 2005).

Impacts on rusts and powdery mildews

We found that biotrophic pathogens overall had significantly lower damage in mixed stands, whilst necrotrophic pathogens were not significantly affected. While necrotrophic pathogens varied, studies of biotrophic pathogens (that rely on a living host to complete their life cycle) were exclusively of powdery mildew and rust species (34 out of 34 studies). This group of pathogens has been extensively studied in forest, crop and grassland systems (Mundt, 2005), perhaps as they are easy to identify and score in the field with strong visual symptoms. As rust and powdery mildew species are most commonly passively wind-dispersed

species, epidemiological theory would suggest that the success of dispersal will depend upon the distance between susceptible hosts, although this theory has yet to be tested in the forest environment (Mundt, 2005).

Host, pathogen, and environment

The disease triangle, a key concept in plant pathology, describes the outcome of a disease to be dependent upon triangular interactions between host, pathogen and environment (Francl, 2001; Stevens, 1960). Assimilation of the evidence on mixed stands and forest pathogens reveals the sheer variety of outcomes that are possible when increasing tree diversity, and the many potential mechanisms underpinning such effects on pathogens. We suggest the use of the disease triangle again, as an aid for ecologists and foresters who wish to conduct hypothesis-focused research. Figure 28 shows how such research could begin by considering the impacts of mixed stands on these three areas for any given pathosystem. Host, pathogen and environment may all be impacted in different ways by the planting of species mixtures, with the overall outcome requiring assessment of all three areas.

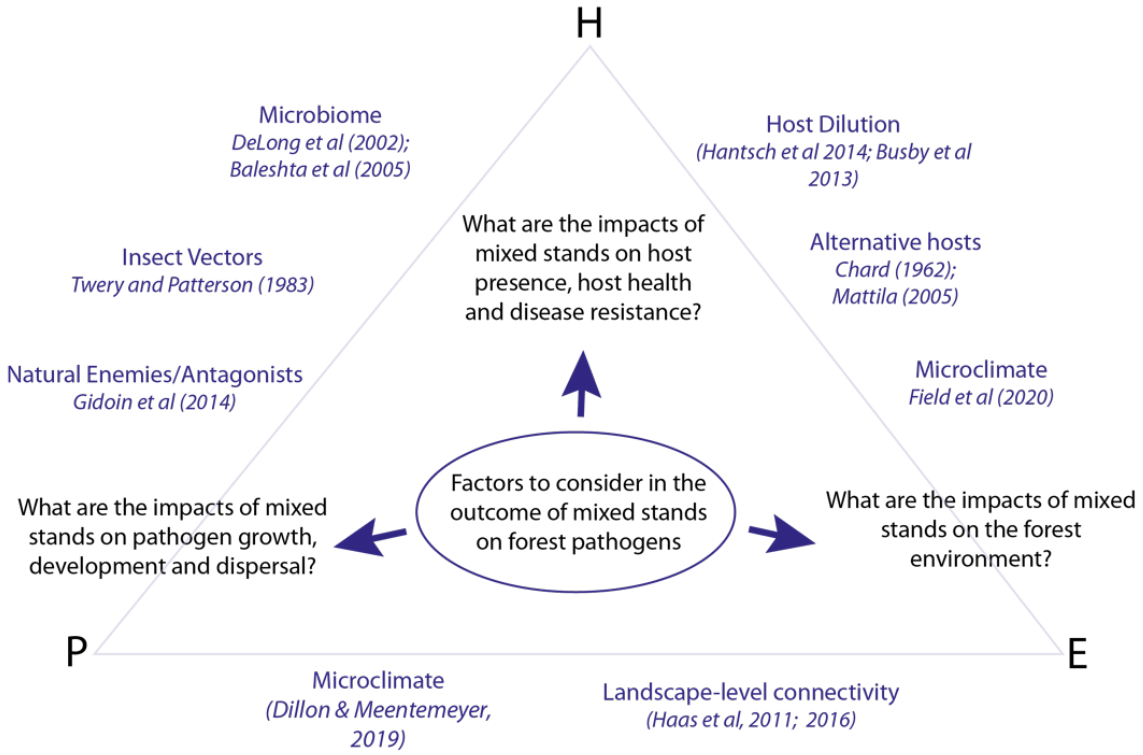


Figure 28: Summary of factors to consider in the outcome of mixed stands on forest pathogens, arranged around the classical disease triangle of pathogen, host and environment. Words in blue indicate mechanisms by which host, pathogen and environment influence one another along these three axes, and citations are example papers which illustrate a mechanism.

Knowledge gaps

There was an obvious latitudinal bias present in our studies, with just 5 out of 74 studies from the tropical biome included in the meta-analysis and a further 4 out of 30 qualitative studies from the tropics. There was no significant effect of mixed stands on pathogens in tropical biomes, but the sample size was perhaps too small to ascertain if this was a real trend with only 5 studies from the tropics (Fig. 24). In

contrast to our results, Liu et al. (2020) had suggested weaker plant diversity effects on pathogens with increasing latitude, although their study did not contain any tropical studies. They had postulated that greater competition due to higher overall plant diversity at lower latitudes could have led to higher host specialisation and therefore strong effects of resource dilution (Liu et al., 2020). Our results find no evidence to support this conclusion. More research on the effect of mixtures on disease in tropical plantations could have a beneficial impact for small-scale farmers. For instance, Dumont et al., (2014) found in a social study of cocoa farmers that 2% of farmers reported an increase in black pod disease with increasing tree diversity, while 6.8% reported decreases, showing that a large proportion of farmers remained neutral or lacked sufficient knowledge to make such planting decisions.

Unsurprisingly, studies were also biased almost entirely towards fungi, which are the most widespread and economically important group of plant pathogens on the planet (Fisher et al., 2012). There were only a small number of studies of *Phytophthora* (oomycete) species (7 studies total), despite this genus including some of the most lethal group of plant pathogens in the world (Brasier, 2008). The one study of bacteria that was included in the meta-analysis was also one of only two quantitative studies from South America (Chile), reporting a negative but nonsignificant effect of tree diversity on *Pseudomonas siringae pv. Mors-prunorum* damage to *Prunus avium* (Loewe, González, & Balzarini, 2013). There is therefore clear potential for future research on the effects of mixed stands on bacterial and oomycete species.

In our results, there was a notable lack of studies with small, nonsignificant positive effect sizes (Fig. S23). This is consistent with the predictions of a "file drawer problem" – that small, nonsignificant studies are rarely published due to institutional and journal bias against such studies (Rosenthal, 1979). The current trend in mixed stand literature is to overwhelmingly discuss the benefits of mixed stands on different types of pest damage (e.g. Jactel et al., 2017). While this may be a real trend, we also found a bias against studies with large standard errors, suggesting that some publication bias could also be at play. A possible consequence of this publication bias could be an overestimation of the true effect in the literature.

Study limitations and suggestions for future work

In our study, we could not directly test whether one of the major mechanisms suggested by authors of previous studies (Table 8) held true: that associational resistance was driven by a reduction of host density (resource dilution) in mixed stands. This was due to host density often not being reported in studies, although we may be able to assume that host dilution often increased with increasing tree diversity. However, the fact that both pathogen specialism and presence of alternative hosts had nonsignificant impacts on the effect size, and that a significant effect of mixed stands was still seen for generalist pathogens, suggests that the effect of mixed stands on forest pathogens is not always as simple as a host dilution effect even if host density is lower at greater diversity levels. Instead,

we found several studies suggesting the importance of tree neighbour identity for influencing pathogen damage on focal hosts. In fact, one study actually reported an increase in disease with a reduction in host abundance. The main vector of black pod disease (*Phytophthora megakarya*) of cacao trees (*Theobroma cacao*) are ants, which were found in lower numbers with decreasing plant diversity, therefore potentially lowering disease in monocultures (Gidoin et al., 2014). This also highlights the importance of considering how a disease is vectored, a step which is not so common for insects but that for pathogens, may also include other trophic levels.

Another major limitation of our study was the inability to mine data from papers that were not available in English. Several studies relevant at abstract level were not available at full text in English, in particular, many forestry studies from Eastern Europe, cited in reviews such as Korhonen *et al* (1998). These studies would almost certainly have been a source of additional data for meta-analysis. Forestry researchers should note the large quantity of studies from Eastern Europe that are not available for English systematic reviews and meta-analyses, representing a "black hole" of literature as yet unincorporated into the English-speaking areas of the scientific world. A future collaborative study between several multi-lingual scientists, with the aim of including this literature, could be highly valuable. For instance, a previous study of the invasive pest literature highlighted the importance of data mining literature from Chinese to English, when modelling the spread of insect pests in China (Bebber et al., 2019).

Conclusions. It is commonly assumed that, as is the case for insect pests, increasing tree diversity in forest plantations has a negative effect on forest pathogens. Using meta-analysis, we found a small but significant negative effect of mixed stands on tree disease, that was particularly clear in temperate forests for which the most data was available. For some pathogens (for instance, *Armillaria, H. annosum, P. ramorum*), there was clear and plentiful evidence that increasing tree diversity reduced pathogen damage. The importance of tree neighbour identity rather than resource dilution on its own became clear in our synthesis of both the quantitative and qualitative evidence. In the planting of mixed stands, it is therefore important to understand specific aspects of the study system at hand, rather than a general recommendation to plant mixed stands. Future experimental work in mixed forests could therefore focus on unravelling associational effects on a focal host, that are dependent on the effects of neighbouring tree species on aspects such as the forest microclimate and microbiome.

Acknowledgments

We thank Hervé Jactel and colleagues who provided a list of relevant studies that helped develop the scoping searches. Thank you to all the authors who provided additional data from previously published studies for the meta-analysis: Pilar Fernandez-Conradi, Mike Cruickshank, Whalen Dillon, Diem Nguyen, Sarah Haas and Ross Meentemeyer. We also thank additional researchers from TreeDivNet for providing additional unpublished data: Matthias Dillen and Martin Schädler. This systematic review would not have been possible without the support of EF's lab group, the Hector-Turnbull Plant Ecology lab in Oxford. Emma Jardine, Emily Warner, and April Burt all assisted in the full text and abstract screening stages as additional reviewers for kappa tests. We are also grateful too to Gill Petrokofsky in the Long-term Ecology Lab, Oxford, for her advice and support in the development of systematic review methodology and to Neal Haddaway and independent peer-reviewers from the Centre for Environmental Evidence who reviewed the systematic review protocol. EF would like to thank her other PhD supervisors, Melanie Gibbs, Karsten Schönrogge and Nadia Barsoum for commenting on earlier versions of the manuscript.

CHAPTER 6: GENERAL DISCUSSION

In this thesis, I have shown that tree diversity can lower pathogen damage, and that interactive abiotic and biotic factors are also important. Individual tree traits that increase or decrease susceptibility to pests and pathogens may be influenced by factors such as tree provenance, or drought stress, and thus, these factors can have indirect effects on pathogen damage. Moreover, interactions between sequential damage agents (insect herbivores and pathogens) can also influence the overall damage on host trees, although responses may be challenging to disentangle and predict. Below, I outline how my results relate to the initial research objectives of the thesis, as stated in the General Introduction.

Research Objective 1 – How does tree genetic and species diversity influence oak powdery mildew infection on* Quercus robur*? (Chapters 2-3)

Both the planting of improved provenances and of mixed forests are currently discussed as strategies to improve resilience of forests in the face of climate change and increased pressure from pest and disease. I used tree diversity experiments with differing designs in order to investigate both aspects. Chapter 2 focussed on the impact of **host tree identity** (tree genetic provenance, tree vigour) on oak pest and pathogen damage, while Chapter 3 focussed on the impact of **tree neighbour identity** on oak pest and pathogen damage.

In Chapter 2, tree diversity treatments (species and provenance mixtures) had variable effects that proved challenging to disentangle statistically (see Appendix I). Provenance identity rather than diversity emerged as a strong predictor of both leaf galler abundance and oak powdery mildew infection intensity. While local UK provenance trees were more likely to be selected by leaf gallers, this was not true for powdery mildew, where the highest infection levels could be found on Italian provenance trees.

By contrast, in Chapter 3, I found evidence for associational resistance to oak insect pests and pathogens with increasing tree diversity. The density of the focal host *Quercus robur* decreased with greater tree diversity, so this result provides evidence for a dilution effect. However, I also found evidence for tree species identity effects driving both damage by oak insect herbivores and oak powdery mildew. Although tree diversity overall lowered damage, the presence of birch in mixture increased damage by powdery mildew and leaf chewers. Birch buffered some environmental variability in plots, indicating that effects of tree neighbour identity may, in part, be modulated via effects on the microclimate.

Research Objective 2 – How are diversity effects on oak powdery mildew and oak insect herbivores moderated by environmental variation? (Chapters 2-3)

In Chapter 2, I found that inter-annual differences and both intra- and inter-site variability had strong effects on the foliar oak insect herbivore and pathogen community. This highlighted the importance of environmental context as well as

the influence of scale on tree pests and pathogens. This study complements earlier findings of Roy (2019) working in a UK-based provenance trial, who also did not find consistent evidence for local adaptation of the oak insect herbivore community to local provenance trees. Sources of environmental variation were also largely unmeasured in Roy's study. Roy also compared the insect herbivore communities of adjacent *Q. robur* and *Q. petraea* trees, finding that the strongest interspecific differences were found for the leaf galler community. In my study, this was the group of herbivores most consistently impacted by provenance, again suggesting that the more intimate the relationship between host plant and insect, the greater the importance of tree identity versus environmental context.

In Chapter 3, I quantified some of the environmental variation using a drought treatment and showed that drought modified diversity effects on leaf miners. Diversity effects were stronger under control versus drought conditions. This study therefore contributes to the growing body of literature showing evidence for diversity effects moderated by climate and environmental context (Grossiord, Granier, et al., 2014; Jactel et al., 2018; Ratcliffe et al., 2017). However, contrary to our hypothesis that drought may have significant positive impacts on oak powdery mildew, I did not find an effect of drought stress on pathogen damage.

Overall, the results of the field-based component of the thesis illustrate the multitude of interactive abiotic and biotic variables that can influence insect and pathogen damage on host trees, even in a single pathosystem. Although tree diversity can lower pathogen damage (as seen in Chapter 3), results can vary

spatially and temporally (Chapter 2). Moreover, in Chapter 3, due to the greater replication of treatments and consistent block design, I was able to use a Generalised Linear Mixed Modelling Framework, partitioning variance into random and fixed effects. I was therefore able to partition variance due to intra-site spatial differences into the random effect "Block", which explained more of the variance for powdery mildew infection than for leaf miners or leaf chewers (consider the marginal versus conditional R^2 values presented in Table 5). In Chapter 2, however, I was unable to use mixed models due to the lack of replication of treatments, which potentially restricted the power of our statistical approach as I had to perform separate GLMs for each site and each year (Gelman & Hill, 2007). In a world where statistical trends are changing rapidly, designing a long-term experiment that can stand the test of time statistically is a challenging job. Designs such as the ORPHEE experiment, with at least 5 levels of each random effect, are preferred, as these permit the use of mixed models (Gelman & Hill, 2007).

Insect-pathogen interactions

In Chapters 2-3, I considered the simultaneous impacts of oak powdery mildew and insect herbivory so that I could look for interactions between multiple damage agents on the same trees. As phenological differences mean that the majority of insect herbivory occurs earlier than powdery mildew damage, I achieved this by including insect damage as a predictor in models of oak powdery mildew. Interestingly, in Chapters 2 and 3, I obtained contrasting results. The

result of Chapter 2, that powdery mildew was promoted by increasing herbivory by leaf miners, is consistent with the **phenological** theory that high insect defoliation on primary growth promotes mildew infection of lammas growth (Marçais & Desprez-Loustau, 2014). However, the result of Chapter 3, that powdery mildew declined with increasing herbivory by leaf miners, is consistent with a **plant defence signalling** theory that reciprocal antagonism can occur between sequential attackers on the same plant (Moreira, Abdala-Roberts, & Castagneyrol, 2018, see Fig. 29). Interestingly, in both studies it was the same guild of oak insect herbivores (leaf miners) that were found to interact with oak powdery mildew, and not the other guilds measured (leaf gallers or leaf chewers). Roy (2019) also found evidence for both facilitative or antagonistic interactions between fungal endophytes and insect herbivores on host *Q. robur* trees, suggesting that tripartite interactions may be complex and challenging to disentangle mechanistically.

Research Objective 3 – How does water stress impact oak powdery mildew infection on Quercus robur? (Chapters 3 & 4)

Although previous work would suggest that drought stress would have a positive impact on the pathogen (see Chapter 1), I did not find evidence that soil moisture deficiency influenced oak powdery mildew infection, nor evidence for diversity-drought interactions (Chapter 3). In Chapter 4, I was unable to induce oak powdery mildew infection, likely due to phenological asynchrony between greenhouse grown plants and natural mildew populations, along with a previous

aphid outbreak. Thus, although the results of Chapter 3 suggest that this pathogen is not significantly impacted by water stress, I was unable to test the robustness of this result experimentally.

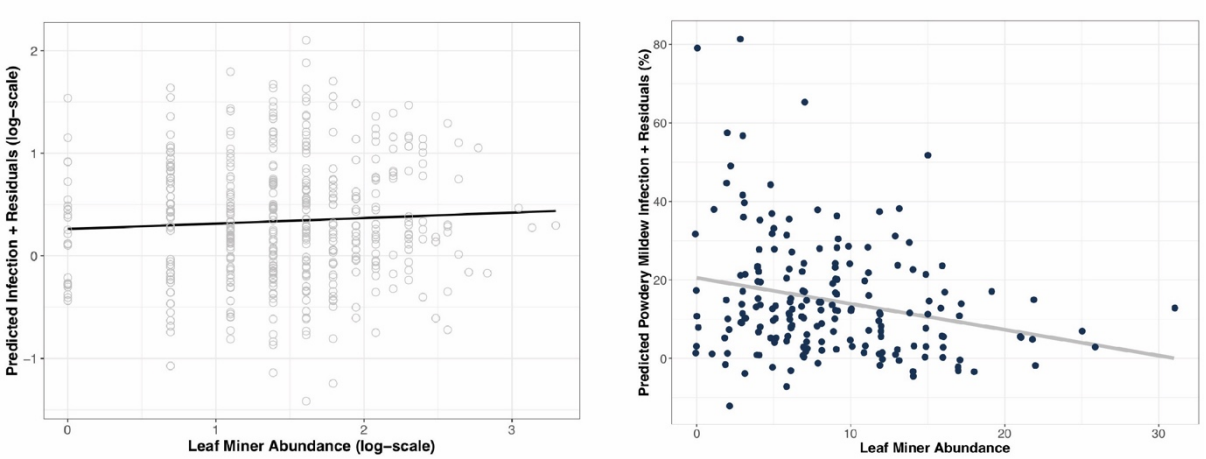


Figure 29: Partial residual plots from Chapter 2 (left-hand plot) and Chapter 3 (right-hand plot), presented side by side. In Chapters 2 and 3 I measured leaf miner abundance and oak powdery mildew infection using the same protocols at the tree level in two different tree diversity experiments (Climate Match, left-hand plot, and ORPHEE, right-hand plot). In Chapter 2, I log-transformed both powdery mildew infection and leaf miner abundance in order to better fit the assumptions of the GLM framework, while in Chapter 3 these same transformations were not necessary, so the two graphs are not directly comparable numerically. However, presenting them side by side illustrates how in Chapter 2, I found a significant positive correlation between herbivory and mildew infection, while in Chapter 3, I found the opposite (see main text for contrasting explanatory hypotheses).

The importance of individual tree traits

As well as partitioning variance due to diversity treatment, in Chapters 2-3 I also showed that individual tree traits impacted upon pest and pathogen damage. In Chapter 2, regardless of tree provenance, taller trees with longer shoots had higher pest and pathogen loads. Tree apparency, rather than tree height, was a stronger predictor of pest and pathogen loads but was not systematically influenced by tree diversity treatment. Plant growth traits could also have provided an explanation for the provenance effects observed, as local provenances had longer primary shoots (preferred by leaf gallers) while non-local provenances invested more in lammas shoots (preferred by oak powdery mildew). Overall, I showed that plant traits at the level of individual trees, potentially influenced by (unmeasured) abiotic variables as well as the genetic identity of trees, can be more important than stand structure at the scale of individual forest stands. In Chapter 3, by contrast, while tree diversity consistently lowered herbivory and pathogen damage, individual tree traits had a more variable effect. I did not find a significant effect of lammas shoot length nor tree height on oak powdery mildew infection. However, as in Chapter 2, I did also find a positive effect of shoot length on leaf miners. Tree height was not a significant predictor of oak pest and pathogen loads in Chapter 3.

These results are consistent with the work of Roy (2019) on oak trees of a similar age and size class, who showed that trees with higher DBH and longer primary shoots to support more herbivore activity. However, in Chapter 3 I also found

evidence for the Plant Stress Hypothesis for leaf miners – suggesting that the two hypotheses are not mutually exclusive, as proposed by White (2009).

In Chapter 4, although I was unable to test the impacts of drought and waterlogging on powdery mildew, I observed patterns of oak growth that could have implications for disease development. I found a positive correlation between the number of primary shoots produced by oak saplings in the spring (which is predetermined by the previous year's bud-set) and the number of third flush shoots later produced – suggesting that predetermination of both primary and lammas growth shoots can occur. In order to predict damage by oak powdery mildew (and thereby, other foliar diseases) between years, it may therefore be important to consider the previous year's climatic variability.

Research Objective 4 – What are the overall effects of planting mixed species stands on forest resilience to pathogens?

In Chapter 5, I aimed to provide a much greater sense of scale to the issue, by assimilating evidence at a global scale as has never before been achieved for this topic. I showed that tree diversity lowers pathogen damage in temperate ecosystems. Contrary to my predictions, neither pathogen specialism nor the presence of alternative hosts were consistent predictors of pathogen damage in mixed stands, however, the influence of tree neighbour identity on factors such as stand microclimate and soil microbiome was important to consider. This complemented the results of Chapter 2-3 which showed that there was no

consistent effect of tree diversity, but that managers need to consider individual site conditions and what else is known about the impact of environmental conditions on host and pathogen.

The systematic review also revealed that the most robust result came from temperate ecosystems and fungal pathogens, where the most studies were available, and that knowledge gaps remain around the influence of stand diversity in boreal and tropical systems, and on bacterial and viral pathogens.

Limitations to the thesis

In ecology, there is often a trade-off between measuring a limited number of variables in detail and exploring underlying mechanisms that can explain the relationships between measured variables. In Chapters 2 and 3, I restricted the number of abiotic and biotic variables that were measured and correlated with pest and pathogen damage. Although I observed some interesting correlations (for instance, that longer shoots suffered higher powdery mildew infection (Chapter 2) or that plots containing birch had higher mildew infection (Chapter 3) I was unable to provide definitive mechanistic explanations for these correlations, but merely suggest likely causes. I was able to show, for instance, in Chapter 3, that leaf miner damage mediated powdery mildew via a direct, rather than indirect effect, but not the underlying mechanism behind any such direct effect. This is the advantage of an experimental approach where many more variables can be controlled – the purpose of Chapter 4. However, even in this chapter,

although I successfully managed to adequately control the abiotic stressor (water availability), the biotic stressor (oak powdery mildew) proved more challenging to induce.

The need for greater mechanistic explanations behind observations was also a theme in Chapter 5, the systematic review and meta-analysis. Although many qualitative papers reported a decrease in tree diversity and suggested mechanisms behind such effects (see Table 8), very few papers actually attempted to measure these mechanisms.

The challenge of disentangling what can be a complex network of indirect and direct effects in biodiversity-ecosystem functioning experiments can be met in several ways. Statistical approaches such as structural equation modelling (Lefcheck, 2016) can be used to determine whether variables are acting directly or indirectly, as has been done for tree diversity experiments (Muiruri & Koricheva, 2017; Schuldt, Bruelheide, et al., 2017). Another method is to perform direct experiments in the highly controlled environment of a lab or greenhouse to complement field observations, although as I illustrated in Chapter 4, such experiments also carry their logistical challenges. However, without complementary observations from field experiments, the conclusions that can be drawn from experimental work may be limited. For instance, DeLong et al (2002) found that the soil surrounding birch roots contained *Armillaria*-suppressive pseudomonads than that taken from surrounding Douglas Fir roots, but they did not test whether this lowered *Armillaria* damage in mixed stands of Douglas Fir

and birch. Finally, measurement of multiple plant traits, such as plant defence and growth traits, in tandem with measurement of insect herbivory or pathogen damage along diversity gradients, provides an integrative approach to disentangling indirect and direct effects (e.g. Castagneyrol et al., 2017; Castagneyrol, Moreira, et al., 2018). This style of study, where diversity effects are linked to cascading effects on other feeding guilds and plant growth traits, provides a means to uncover the mechanistic explanations behind associational effects.

Suggestions for future work

Scaling up our observations and exploring underlying mechanisms could be achieved in various ways. The classical "disease triangle", a paradigm in plant pathology, describes the onset of a disease as being necessitated by a susceptible host, virulent pathogen and favourable environment for disease development (Stevens, 1960). The work presented in Chapter 2 suggested that genetic identity of trees as well phenotypic traits (vigour, phenology) can predict fungal disease development on oaks. However, we currently lack an understanding of how abiotic aspects alter the development of disease, and the potential for gene-by-environment interactions (Boshier & Buggs, 2015). To greater understand the role of gene-by-environment interactions influencing tree health, studies such as that conducted in Chapter 2 could be replicated at multiple provenance trials. The REINFORCE network of replicated trials planted with the same pool of provenances throughout Europe, provides one such opportunity.

Studies at the regional scale have previously shown how diversification can reduce tree disease at the local scale (Haas et al., 2016; Haas, Hooten, Rizzo, & Meentemeyer, 2011). A study such as this on *Q. robur* would then complement our results from tree diversity experiments, allowing us to explore the generality of the patterns we observed from site to landscape level. For instance, a network of observational plots could be established to measure multiple damage agents on *Q. robur* over multiple years. Stand-level variables such as tree diversity could also be measured, coupled with microclimatic measurements and measurement of plant growth and defence traits. Multiple abiotic and biotic variables could then be linked together in a structural equation modelling framework or alternative approach. In the UK, there is an increasing concern about the most recent onset of oak decline (Denman et al., 2014). An advantage of measurements over multiple years would be that cumulative effects on tree health could also be measured, allowing us to gain insight into the role of different damage agents and abiotic factors in the onset of oak decline (Denman et al., 2018). Although multiple agents are often found to have a cumulative impact on tree health (De Grandpré et al., 2019), as observed in this thesis, insect and pathogens may also have antagonistic effects on each other (result from Chapter 2). Likewise, in this thesis I found that drought did not impact pathogen damage on oak, but that it did increase herbivory (Chapter 3). All of these observations could be explored in more detail, using the same model system, in a more extensive study such as that suggested above.

Moreover, in this thesis, I did not evaluate any reciprocal impacts of insect or pathogen damage on water relations. In the ORPHEE experiment at the time of our study, the irrigation treatment had not yet had a significant impact on oak growth (tree height), however, the impacts of irrigation on leaf miners and chewers suggests irrigation impacted other plant traits, indirectly affecting herbivory (Chapter 3). By contrast, in Chapter 4, I found that even a short period of water deficit could significantly lower oak shoot biomass. A recent study found that insect damage actually offset the impacts of water stress on tree growth (Itter et al., 2019). Following on from the water stress experiment in Chapter 4, a future experimental study on oak could evaluate the modification of plant traits under sequential impacts of water stress, followed by insect/pathogen damage, and vice versa. Such a study would be invaluable in understanding how climate change is likely to alter the potential impacts of pest outbreaks on European forests.

Another route to exploring the generality of tree diversity effects on pathogens, and the potential effects of climatic change, would be to scale up our approach geographically. For my chosen study system of the foliar oak insect herbivore and pathogen community, there are many more studies in TreeDivNet that could be included in a cross-European study. Although the climatic variation gradient would not be huge at just the continental scale, such a study could allow us to explore in more detail the interaction between abiotic variables and oak powdery mildew infection by including temperature and humidity in a mixed model analysis of multiple tree diversity experiments. Yet another approach to understanding the generality of diversity-disease effects in forests would be to

scale up the meta-analytic approach introduced in Chapter 5. I observed a large amount of unmined literature from Eastern Europe and Russia which could have provided additional information, particularly in the boreal region, on tree diversity effects on pathogens. An obvious future study would be to attempt to mine more of this literature through collaboration with multi-lingual scientists, an approach which was out of reach of the scope of this DPhil where I was limited to studies whose full texts were available in English.

Conclusions: Integrating tree diversity into forestry strategy to control pathogens

In this thesis, I used meta-analysis to show that there is evidence that in temperate eco-systems, increasing tree diversity lowers overall pathogen damage. However, I would hesitate before recommending the general planting of mixed stands, without consideration of other factors and further study. In this thesis I have not considered the economic impacts, nor any other biological impacts of planting mixed stands, such as natural disturbances other than drought or the multifunctionality of stands. These, too, will be important factors to consider in the application of tree species mixtures by foresters, depending on the management objectives of the stand.

As explored in the Discussion of Chapter 5, practitioners may need to consider all aspects of the **disease triangle** in their decision-making process regarding planting mixed stands. Based on the results of this thesis, I would suggest asking the following questions, if the purpose is to lower damage by a specific pathogen:

(1) How much is known about the microclimate that is preferred for **pathogen** growth, reproduction and dispersal? Stand microenvironment (potentially influenced by pre-existing site conditions) should be altered to minimise pathogen growth.

(2) How much is known about the microclimate that is preferred for **host tree** health? Separate from the consideration of pathogen growth, stand microenvironment should be altered in order to maximise host tree health (for instance, by reducing drought stress).

(3) How is climate change likely to alter the site conditions, and is there any evidence for how climatic variables such as temperature and humidity impact upon host tree health and pathogen growth, survival and reproduction?

(4) Are there other pests and pathogens (such as insect herbivores) that are currently affecting stand health, or are likely to do so in the future? Is there evidence for how admixing species impacts these other biotic threats?

The ultimate question posed by this thesis is: how far can human influence on stand structure, versus the entropy of nature, impact upon plant health? In this thesis, I have shown that human intervention can alter pathogen development significantly, and that mixed stands will have an important role to play as part of a sustainable forestry strategy.

References

Aakala, T., Kuuluvainen, T., De Grandpré, L., Gauthier, S., Aas, E., Gossner, G., … Leuschner, C. (2016). *Forest Ecology and Management*, *10*(1), doi: 10.1111/j.1365-2745.2010.01659.x

Adams, A. S., Aylward, F. O., Adams, S. M., Erbilgin, N., Aukema, B. H., Currie, C. R., … Raffa, K. F. (2013). Mountain pine beetles colonizing historical and naive host trees are associated with a bacterial community highly enriched in genes contributing to terpene metabolism. *Applied and Environmental Microbiology*, *79*(11), 3468–3475. doi: 10.1128/AEM.00068-13

Alalouni, U., Brandl, R., Auge, H., & Schädler, M. (2014). Does insect herbivory on oak depend on the diversity of tree stands? *Basic and Applied Ecology*, *15*(8), 685–692. doi: 10.1016/j.baae.2014.08.013

Ampoorter, E., Barbaro, L., Jactel, H., Baeten, L., Boberg, J., Carnol, M., … Allan, E. (2019). Tree diversity is key for promoting the diversity and abundance of forest-associated taxa in Europe. *Oikos*, 133–146. doi: 10.1111/oik.06290

Arhipova, N., Gaitnieks, T., Donis, J., Stenlid, J., & Vasaitis, R. (2011). Butt rot incidence, causal fungi, and related yield loss in picea abies stands of latvia. *Canadian Journal of Forest Research*, *41*(12), 2337–2345. doi: 10.1139/X11-141

Aust, H., & Hoyningen-Huene, J. V. (1986). Microclimate in relation to epidemics of powdery mildew. *Annual Review of Phytopathology*, *24*(1), 491–510.

Ayres, P. G., & Edwards, M. C. (1982). Seasonal changes in resistance of Quercus petraea (sessile oak) leaves to Microsphaera alphitoides. *Transactions of the*

British Mycological Society, *78*(3), 569–571. doi: 10.1016/S0007-1536(82)80174-5

Baeten, L., Verheyen, K., Wirth, C., Bruelheide, H., Bussotti, F., Finér, L., … Scherer-Lorenzen, M. (2013). A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. *Perspectives in Plant Ecology, Evolution and Systematics*, *15*(5), 281–291. doi: 10.1016/j.ppees.2013.07.002

Baleshta, K. E., Simard, S. W., Guy, R. D., & Chanway, C. P. (2005). Reducing paper birch density increases Douglas-fir growth rate and Armillaria root disease incidence in southern interior British Columbia. *Forest Ecology and Management*, *208*(1–3), 1–13. doi: 10.1016/j.foreco.2004.07.076

Banfield-Zanin, J. A., & Leather, S. R. (2014). Frequency and intensity of drought stress alters the population size and dynamics of *Elatobium abietinum* on Sitka spruce. *Annals of Applied Biology*, *165*(2), 260–269. doi: 10.1111/aab.12133

Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A., & Szendrei, Z. (2009). Associational Resistance and Associational Susceptibility: Having Right or Wrong Neighbors. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 1–20. doi: 10.1146/annurev.ecolsys.110308.120242

Barsoum, N. (2015). Mixed Provenance and Mixed Species trials: Informing the debate on how to prepare native tree species for climate change in England. *Quarterly Journal of Forestry*, *109*(3), 201–207.

Basova, S. (1987). Seasonal dynamics of powdery mildew on English oaks at a seed grafting plantation. *Mikologiya Fitopatologiya 21*(3), 269–273.

Bebber, D. P., Field, E., Gui, H., Mortimer, P., Holmes, T., & Gurr, S. J. (2019). Many unreported crop pests and pathogens are probably already present.

Global Change Biology, *25*(8), 2703–2713. doi: 10.1111/gcb.14698

Beikircher, B., & Mayr, S. (2013). Winter peridermal conductance of apple trees: Lammas shoots and spring shoots compared. *Trees - Structure and Function*, *27*(3), 707–715. doi: 10.1007/s00468-012-0826-0

Benneter, A., Forrester, D. I., Bouriaud, O., Dormann, C. F., & Bauhus, J. (2018). Tree species diversity does not compromise stem quality in major European forest types. *Forest Ecology and Management*, *422*, 323–337. doi: 10.1016/j.foreco.2018.04.030

Bert, D., Lasnier, J.-B., Capdevielle, X., Dugravot, A., & Desprez-Loustau, M.-L. (2016). Powdery mildew decreases the radial growth of oak trees with cumulative and delayed effects over years. *PLOS ONE*, *11*(5), e0155344. doi: 10.1371/journal.pone.0155344

Boshier, D., & Buggs, R. J. A. (2015). The potential for field studies and genomic technologies to enhance resistance and resilience of British tree populations to pests and pathogens. *Forestry*, *88*(1), 27–40. doi: 10.1093/forestry/cpu046

Boyd, I. L., Freer-Smith, P. H., Gilligan, C. A., & Godfray, H. C. J. (2013). The consequence of tree pests and diseases for ecosystem services. *Science (New York, N.Y.)*, *342*(6160), 1235773. doi: 10.1126/science.1235773

Brandle, M., & Brandl, R. (2001). Species richness of insects and mites on trees: expanding Southwood. *Journal of Animal Ecology*, *70*(3), 491–504. doi: 10.1046/j.1365-2656.2001.00506.x

Brasier, C. M. (2008). The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology*, *57*(5), 792–808. doi: 10.1111/j.1365-3059.2008.01886.x

Bravo-Oviedo, A., Barreiro, S., Strelcova, K., & Pretzsch, H. (2014). Eumixfor introduction: Integrating scientific knowledge in sustainable management of mixed forests. *Forest Systems*, *23*(3), 515–517. doi: 10.5424/fs/2014233-07050

Broadmeadow, M. S. J. (2005). Climate change and the future for broadleaved tree species in Britain. *Forestry*, *78*(2), 145–161. doi: 10.1093/forestry/cpi014

Brown, B. J., & Ewel, J. J. (1987). Herbivory in complex and simple tropical successional ecosystems. *Ecology*, *68*(1), 108–116. doi: 10.2307/1938810

Bucharova, A., Bossdorf, O., Hölzel, N., Kollmann, J., & Prasse, R. (2018). Mix and match : regional admixture provenancing strikes a balance among di ff erent seed-sourcing strategies for ecological restoration. *Conservation Genetics*, *0*(0), 0. doi: 10.1007/s10592-018-1067-6

Bucharova et al. (2017). Are local plants the best for ecosystem restoration? It depends on how you analyze the data. *Ecology and Evolution*, *7*, 10683. doi: 10.1002/ece3.3585

Busby, P. E., Newcombe, G., Dirzo, R., & Whitham, T. G. (2013). Genetic basis of pathogen community structure for foundation tree species in a common garden and in the wild. *Journal of Ecology*, *101*(4), 867–877. doi: 10.1111/1365-2745.12112

Camy, C., Villebonne, D., Delatour, C., & Marcais, B. (2003). Soil factors associated with infection by Collybia fusipes and decline of oaks. *Forest Pathology*, *33*(4), 253–266. doi: 10.1046/j.1439-0329.2003.00333.x

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., … Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, *486*(7401), 59–67. doi: 10.1038/nature11148

Cardinale, B. J., Srivastava, D. S., Emmett Duffy, J., Wright, J. P., Downing, A. L., Sankaran, M., & Jouseau, C. (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, *443*(7114), 989–992. doi: 10.1038/nature05202

Cardoza, Y. J., Alborn, H. T., & Tumlinson, J. H. (2002). In vivo volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage. *Journal of Chemical Ecology*, *28*(1), 161–174. doi: 10.1023/A:1013523104853

Cardoza, Y. J., Lait, C. G., Schmelz, E. A., Huang, J., & Tumlinson, J. H. (2003). Fungus-induced biochemical changes in peanut plants and their effect on development of beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae. *Environmental Entomology*, *32*(1), 220–228. doi: 10.1603/0046-225X-32.1.220

Castagneyrol, B., Bonal, D., Damien, M., Jactel, H., Meredieu, C., Muiruri, E. W., & Barbaro, L. (2017). Bottom-up and top-down effects of tree species diversity on leaf insect herbivory. *Ecology and Evolution*, *7*(10), 3520–3531. doi: 10.1002/ece3.2950

Castagneyrol, B., Giffard, B., Péré, C., & Jactel, H. (2013). Plant apparency, an overlooked driver of associational resistance to insect herbivory. *Journal of Ecology*, *101*(2), 418–429. doi: 10.1111/1365-2745.12055

Castagneyrol, B., Jactel, H., & Moreira, X. (2018). Anti-herbivore defences and insect herbivory: Interactive effects of drought and tree neighbours. *Journal of Ecology*, *106*(5), 2043–2057. doi: 10.1111/1365-2745.12956

Castagneyrol, B., Jactel, H., Vacher, C., Brockerhoff, E. G., & Koricheva, J. (2014).

Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. *Journal of Applied Ecology*, *51*(1), 134–141. doi: 10.1111/1365-2664.12175

Castagneyrol, B., Lagache, L., Giffard, B., Kremer, A., & Jactel, H. (2012). Genetic Diversity Increases Insect Herbivory on Oak Saplings. *PLOS ONE*, *7*(8), e44247. doi: 10.1371/journal.pone.0044247

Castagneyrol, B., Moreira, X., & Jactel, H. (2018). Drought and plant neighbourhood interactively determine herbivore consumption and performance. *Scientific Reports*, *8*(1), 5930. doi: 10.1038/s41598-018-24299-x

Chamagne, J., Tanadini, M., Frank, D., Matula, R., Paine, C. E. T. T., Philipson, C. D., Hector, A. (2016). Forest diversity promotes individual tree growth in central European forest stands. *Journal of Applied Ecology*, *54*(1), 71–79. doi: 10.1111/1365-2664.12783

Chard, R. (1962). Silviculture of Weymouth Pine. *Forestry*, *34*(1), 1–10. doi: 10.1093/forestry/34.1.1

Chen, J., Saunders, S. C., Crow, T. R., Naiman, R. J., Brosofske, K. D., Mroz, G. D., Franklin, J. F. (1999). Microclimate in Forest Ecosystem and Landscape Ecology. *BioScience*, *49*(4), 288–297. doi: 10.2307/1313612

Civitello, D. J., Cohen, J., Fatima, H., Halstead, N. T., Liriano, J., McMahon, T. A., … Rohr, J. R. (2015). Biodiversity inhibits parasites: Broad evidence for the dilution effect. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(28), 8667–8671. doi: 10.1073/pnas.1506279112

Cobb, F. W., Slaughter, G. W., Rowney, D. L., & DeMars, C. J. (1982). Rate of spread of Ceratocystis wageneri in ponderosa pine stands in the central Sierra

Nevada. *Phytopathology*, *72*(10), 1359–1362.

Cobb, R. C., Filipe, J. A., Meentemeyer, R. K., Gilligan, C. A., Lynch, S. ., & Rizzo, D. M. (2010). Community and individual effects on SOD intensification in California redwood forests: implications for tanoak persistence. In S. J. Frankel, J. T. Kliejunas, & K. M. Palmieri (Eds.), *Proceedings of the Sudden Oak Death Fourth Science Symposium.* (pp. 217–222).

Cohen, J. (1970). Statistical power analysis for the behavioural sciences. *Biometrics*, *26*(3), 588. doi: 10.2307/2529115

Cole, E. F., & Sheldon, B. C. (2017). The shifting phenological landscape: Within- and between-species variation in leaf emergence in a mixed-deciduous woodland. *Ecology and Evolution*, n/a-n/a. doi: 10.1002/ece3.2718

Coll, L., Ameztegui, A., Collet, C., Löf, M., Mason, B., Pach, M., … Ponette, Q. (2018). Knowledge gaps about mixed forests: What do European forest managers want to know and what answers can science provide? *Forest Ecology and Management*, *407*, 106–115. doi: 10.1016/j.foreco.2017.10.055

Cornelissen, T., Wilson Fernandes, G., & Vasconcellos-Neto, J. (2008). Size does matter: Variation in herbivory between and within plants and the plant vigor hypothesis. *Oikos*, *117*(8), 1121–1130. doi: 10.1111/j.0030-1299.2008.16588.x

Crawley, M. (2007). Generalized Linear Models. In *The R Book* (pp. 511–526). doi: 10.1002/9780470515075.ch13

Crawley, M. J., & Akhteruzzaman, M. (1988). Individual variation in the phenology of oak trees and its consequences for herbivorous insects. *Functional Ecology*, *2*(3), 409. doi: 10.2307/2389414

Damien, M., Jactel, H., Meredieu, C., Régolini, M., van Halder, I., & Castagneyrol,

B. (2016). Pest damage in mixed forests: Disentangling the effects of neighbor identity, host density and host apparency at different spatial scales. *Forest Ecology and Management*, *378*, 103–110. doi: 10.1016/j.foreco.2016.07.025

Dantec, C. F., Ducasse, H., Capdevielle, X., Fabreguettes, O., Delzon, S., & Desprez-Loustau, M.-L. (2015). Escape of spring frost and disease through phenological variations in oak populations along elevation gradients. *Journal of Ecology*, *103*(4), 1044–1056. doi: 10.1111/1365-2745.12403

Davis, K. P., Moss, V. D., Montana, M., & Director, M. (1940). Blister rust control in the management of western white pine. In *Station Paper No. 3. Missoula, MT: U.S. Department of Agriculture, Forest Service, Northern Rocky Mountain Forest and Range Experiment Station. 34 p.*

Day, W. R. (1927). The oak mildew microsphaera quercina (schw.) burrill and armillaria mellea (vahl) quel. in relation to the dying back of oak. *Forestry*, *1*, 108–112.

De Grandpré, L., Kneeshaw, D. D., Perigon, S., Boucher, D., Marchand, M., Pureswaran, D., & Girardin, M. P. (2019). Adverse climatic periods precede and amplify defoliator-induced tree mortality in eastern boreal North America. *Journal of Ecology*, *107*(1), 452–467. doi: 10.1111/1365-2745.13012

Delatour, C. (1983). Les dépérissements de chênes en Europe. *Revue Forestière Française*, *35*(4), 265–282.

DeLong, R. L., Lewis, K. J., Simard, S. W., & Gibson, S. (2002). Fluorescent pseudomonad population sizes baited from soils under pure birch, pure Douglas-fir, and mixed forest stands and their antagonism toward *Armillaria ostoyae* in vitro. *Canadian Journal of Forest Research*, *32*(12), 2146–2159. doi:

10.1139/x02-141

Denman, S., Brown, N., Kirk, S., Jeger, M., & Webber, J. (2014). A description of the symptoms of Acute Oak Decline in Britain and a comparative review on causes of similar disorders on oak in Europe. *Forestry*, *87*(4), 535–551. doi: 10.1093/forestry/cpu010

Denman, S., Doonan, J., Ransom-Jones, E., Broberg, M., Plummer, S., Kirk, S., … McDonald, J. E. (2018). Microbiome and infectivity studies reveal complex polyspecies tree disease in Acute Oak Decline. *ISME Journal*, *12*(2), 386–399. doi: 10.1038/ismej.2017.170

Desprez-Loustau, M., Hamelin, F., & Marçais, B. (2019). The ecological and evolutionary trajectory of oak powdery mildew in Europe. In K. Wilson, A. Fenton, & D. Tompkins (Eds.), *Wildlife Disease Ecology: Linking Theory to Data and Application*. doi: 10.1017/9781316479964.015

Desprez-Loustau, M.-L., Vitasse, Y., Delzon, S., Capdevielle, X., Marçais, B., & Kremer, A. (2010). Are plant pathogen populations adapted for encounter with their host? A case study of phenological synchrony between oak and an obligate fungal parasite along an altitudinal gradient. *Journal of Evolutionary Biology*, *23*(1), 87–97. doi: 10.1111/j.1420-9101.2009.01881.x

Desprez-Loustau, M. L. (2014). Oak powdery mildew changes growth patterns in its host tree: host tolerance response and potential manipulation of host physiology by the parasite. *Annals of Forest Science*.

Desprez-Loustau, M.-L., Marçais, B., Nageleisen, L.-M., Piou, D., & Vannini, A. (2006). Interactive effects of drought and pathogens in forest trees. *Annals of Forest Science*, *63*(6), 597–612. doi: 10.1051/forest:2006040

Desprez-Loustau, M.-L., Massot, M., Toïgo, M., Fort, T., Aday Kaya, A. G., Boberg, J., … Tack, A. J. (2018). From leaf to continent: The multi-scale distribution of an invasive cryptic pathogen complex on oak. *Fungal Ecology*, *36*, 39–50. doi: 10.1016/J.FUNECO.2018.08.001

Dickson, L. L., & Whitham, T. G. (1996). Genetically-based plant resistance traits affect arthropods, fungi, and birds. *Oecologia*, *106*(3), 400–406. doi: 10.1007/BF00334568

Dillen, M., Smit, C., Buyse, M., Höfte, M., Clercq, P. De, & Verheyen, K. (2017). Stronger diversity effects with increased environmental stress: A study of multitrophic interactions between oak, powdery mildew and ladybirds. *PLOS ONE*, *12*(4), e0176104. doi: 10.1371/journal.pone.0176104

Dillen, M., Smit, C., & Verheyen, K. (2017). How does neighbourhood tree species composition affect growth characteristics of oak saplings? *Forest Ecology and Management*, *401*, 177–186. doi: 10.1016/j.foreco.2017.07.016

Dillen, M., Verheyen, K., & Smit, C. (2016). Identity rather than richness drives local neighbourhood species composition effects on oak sapling growth in a young forest. *Forest Ecology and Management*, *380*, 274–284. doi: 10.1016/j.foreco.2016.09.004

Dillon, W. W., & Meentemeyer, R. K. (2019). Direct and indirect effects of forest microclimate on pathogen spillover. *Ecology*, *100*(5), e02686. doi: 10.1002/ecy.2686

Dudt, J. F., & Shure, D. J. (1994). The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, *75*(1), 86–98. doi: 10.2307/1939385

Eaton, E., Caudullo, G., Oliveira, S., & de Rigo, D. (2016). Quercus robur and

Quercus petraea in Europe: distribution, habitat, usage and threats. In *European Atlas of Forest Tree Species* (pp. 160–163).

Edwards, M. C., & Ayres, P. G. (1981). Cell Death and Cell Wall Papillae in the Resistance of Oak Species to Powdery Mildew Disease. *New Phytologist*, *89*(3), 411–418. doi: 10.1111/j.1469-8137.1981.tb02322.x

Edwards, M. C., & Ayres, P. G. (1982). Seasonal changes in resistance of Quercus petraea (sessile oak) leaves to Microsphaera alphitoides. *Transactions of the British Mycological Society*, *78*(3), 569–571. doi: 10.1016/S0007-1536(82)80174-5

Egan, S. P., & Ott, J. R. (2007). Host plant quality and local adaptation determine the distribution of a gall-forming herbivore. *Ecology*, *88*(11), 2868–2879. doi: 10.1890/06-1303.1

Ehbrecht, M., Schall, P., Ammer, C., & Seidel, D. (2017). Quantifying stand structural complexity and its relationship with forest management, tree species diversity and microclimate. *Agricultural and Forest Meteorology*, *242*, 1–9. doi: 10.1016/j.agrformet.2017.04.012

Ekholm, A., Roslin, T., Pulkkinen, P., & Tack, A. J. M. (2017). Dispersal, host genotype and environment shape the spatial dynamics of a parasite in the wild. *Ecology*, *98*(10), 2574–2584. doi: 10.1002/ecy.1949

Elton, C. (1958). *The ecology of invasions by animals and plants*. English Language Book Society.

Ennos, R. A. (2015). Resilience of forests to pathogens: an evolutionary ecology perspective. *Forestry*, *88*(1), 41–52. doi: 10.1093/forestry/cpu048

Ennos, R., Cottrell, J., Hall, J., & O'Brien, D. (2019). Is the introduction of novel exotic forest tree species a rational response to rapid environmental change? –

A British perspective. *Forest Ecology and Management*, *432*, 718–728. doi: 10.1016/J.FORECO.2018.10.018

Erb, M., Meldau, S., & Howe, G. A. (2012, May). Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science*, Vol. 17, pp. 250–259. doi: 10.1016/j.tplants.2012.01.003

Evans, J. (1999). Sustainability of forest plantations - the evidence. *The International Forestry Review*, 865–872. doi: 10.1016/B0-12-145160-7/00219-2

Faraway, J. J., & J., J. (2005). *Extending the linear model with R : generalized linear, mixed effects and nonparametric regression models*. Chapman & Hall/CRC.

Feau, N., Lauron-Moreau, A., Piou, D., Marçais, B., Dutech, C., & Desprez-Loustau, M.-L. (2012). Niche partitioning of the genetic lineages of the oak powdery mildew complex. *Fungal Ecology*, *5*(2), 154–162. doi: 10.1016/j.funeco.2011.12.003

Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a case of spring feeding by winter moth caterpillars. *Ecology*, *51*(4), 565–581. doi: 10.2307/1934037

Feeny, P. (1976). Plant apparency and chemical defense. In J. W. Wallace & R. L. Mansell (Eds.), *Biochemical Interaction Between Plants and Insects* (pp. 1–40). Springer US.

Feeny, P. P., & Bostock, H. (1968). Seasonal changes in the tannin content of oak leaves. *Phytochemistry*, *7*(5), 871–880. doi: 10.1016/S0031-9422(00)84845-1

Fernandez-Conradi, P., Jactel, H., Robin, C., Tack, A. J. M., & Castagneyrol, B. (2018). Fungi reduce preference and performance of insect herbivores on challenged plants. *Ecology*, *99*(2), 300–311. doi: 10.1002/ecy.2044

Fichtner, A., Härdtle, W., Bruelheide, H., Kunz, M., Li, Y., & Von Oheimb, G. (2018). Neighbourhood interactions drive overyielding in mixed-species tree communities. *Nature Communications*, *9*(1), 1–8. doi: 10.1038/s41467-018-03529-w

Field, E., Castagneyrol, B., Gibbs, M., Jactel, H., Barsoum, N., Schönrogge, K., & Hector, A. (2020). Associational resistance to both insect and pathogen damage in mixed forests is modulated by tree neighbour identity and drought. *Journal of Ecology*, *108*(4), 1511–1522. doi: 10.1111/1365-2745.13397

Field, E., Petrokofsky, G. & Koricheva, J. (2018). What are the impacts of mixed forest stands on forest pathogens? A systematic review protocol. Available at: https://www.researchgate.net/publication/322528517_What_are_the_impacts_of_mixed_forest_stands_on_forest_pathogens_A_systematic_review_protocol [Accessed 3/11/20].

Field, E., Schönrogge, K., Barsoum, N., Hector, A., & Gibbs, M. (2019). Individual tree traits shape insect and disease damage on oak in a climate-matching tree diversity experiment. *Ecology and Evolution*, ece3.5357. doi: 10.1002/ece3.5357

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012, April 12). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, Vol. 484, pp. 186–194. doi: 10.1038/nature10947

Flaherty, L., & Quiring, D. (2008). Plant module size and dose of gall induction stimulus influence gall induction and galler performance. *Oikos*, *117*(11), 1601–1608. doi: 10.1111/j.1600-0706.2008.16555.x

Forey, E., Langlois, E., Lapa, G., Korboulewsky, N., Robson, T. M., & Aubert, M. (2016a). Tree species richness induces strong intraspecific variability of beech

(Fagus sylvatica) leaf traits and alleviates edaphic stress. *European Journal of Forest Research*, *135*(4), 707–717. doi: 10.1007/s10342-016-0966-7

Forey, E., Langlois, E., Lapa, G., Korboulewsky, N., Robson, T. M., & Aubert, M. (2016b). Tree species richness induces strong intraspecific variability of beech (Fagus sylvatica) leaf traits and alleviates edaphic stress. *European Journal of Forest Research*, *135*(4), 707–717. doi: 10.1007/s10342-016-0966-7

Forrester, D. I. (2015). Transpiration and water-use efficiency in mixed-species forests versus monocultures: effects of tree size, stand density and season. *Tree Physiology*, *35*(3), 289–304. doi: 10.1093/TREEPHYS/TPV011

Forrester, D. I., & Bauhus, J. (2016). *A Review of Processes Behind Diversity-Productivity Relationships in Forests*. doi: 10.1007/s40725-016-0031-2

Forrester, D. I., Theiveyanathan, S., Collopy, J. J., & Marcar, N. E. (2010). Enhanced water use efficiency in a mixed Eucalyptus globulus and Acacia mearnsii plantation. *Forest Ecology and Management*, *259*(9), 1761–1770. doi: 10.1016/J.FORECO.2009.07.036

Francl, L. J. (2001). The disease triangle: a plant pathological paradigm revisited. *The Plant Health Instructor*. doi: 10.1094/phi-t-2001-0517-01

Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., Kjellander, P., … Bengtsson, J. (2013). Higher levels of multiple ecosystem services are found in forests with more tree species. *Nature Communications*, *4*, 1340. doi: 10.1038/ncomms2328

Gelman, A., & Hill, J. (2007). *Data analysis using regression and multilevel/hierarchical models*. Cambridge University Press.

Gelman, A., Hill, J., & Yajima, M. (2012). Why we (usually) don't have to worry

about multiple comparisons. *Journal of Research on Educational Effectiveness*, *5*, 189–211. doi: 10.1080/19345747.2011.618213

Gerlach, J. P., Reich, P. B., Puettmann, K., & Baker, T. (1997). Species, diversity, and density affect tree seedling mortality from *Armillaria* root rot. *Canadian Journal of Forest Research*, *27*(9), 1509–1512. doi: 10.1139/x97-098

Gidoin, C., Babin, R., Bagny Beilhe, L., Cilas, C., ten Hoopen, G. M., & Bieng, M. A. N. (2014). Tree spatial structure, host composition and resource availability influence mirid density or black pod prevalence in cacao agroforests in Cameroon. *PLoS ONE*, *9*(10), e109405. doi: 10.1371/journal.pone.0109405

Giertych, M. J., & Suszka, J. (2010). Influence of cutting off distal ends of quercus robur acorns on seedling growth and their infection by the fungus erysiphe alphitoides in different light conditions. *Dendrobiology*, *64*, 73–77.

Giffard, B., Jactel, H., Corcket, E., & Barbaro, L. (2012). Influence of surrounding vegetation on insect herbivory: A matter of spatial scale and herbivore specialisation. *Basic and Applied Ecology*, *13*(5), 458–465. doi: 10.1016/J.BAAE.2012.08.004

Glassmire, A. E., Jeffrey, C. S., Forister, M. L., Parchman, T. L., Nice, C. C., Jahner, J. P., … Dyer, L. A. (2016). Intraspecific phytochemical variation shapes community and population structure for specialist caterpillars. *New Phytologist*, *212*(1), 208–219. doi: 10.1111/nph.14038

Glavaš, M. (2011). Protection of oak stands from powdery mildew (Microsphaera alphitoides Griff. et Maubl.). *Croatian Journal of Forest Engineering*, *32*(1), 205-210,209,209.

Glazebrook, J. (2005). Contrasting Mechanisms of Defense Against Biotrophic and

Necrotrophic Pathogens. *Annual Review of Phytopathology*, *43*(1), 205–227. doi: 10.1146/annurev.phyto.43.040204.135923

Goodenough, A. E., & Goodenough, A. S. (2012). Development of a Rapid and Precise Method of Digital Image Analysis to Quantify Canopy Density and Structural Complexity. *ISRN Ecology*, 1–11. doi: 10.5402/2012/619842

Gosselin, R. (1944). Studies in Polystictus circinatus and its relation to butt rot of Spruce. *Bull. Serv. For. Queb.*, (10 n.s.).

Gripenberg, S., Morriën, E., Cudmore, A., Salminen, J.-P., & Roslin, T. (2007). Resource selection by female moths in a heterogeneous environment: what is a poor girl to do? *Journal of Animal Ecology*, *76*(5), 854–865. doi: 10.1111/j.1365-2656.2007.01261.x

Gripenberg, S., Ovaskainen, O., Morriën, E., & Roslin, T. (2008). Spatial population structure of a specialist leaf-mining moth. *Journal of Animal Ecology*, *77*(4), 757–767. doi: 10.1111/j.1365-2656.2008.01396.x

Grossiord, C. (2019). Having the right neighbors: how tree species diversity modulates drought impacts on forests. *New Phytologist*. doi: 10.1111/nph.15667

Grossiord, C., Gessler, A., Granier, A., Berger, S., Bréchet, C., Hentschel, R., Bonal, D. (2014). Impact of interspecific interactions on the soil water uptake depth in a young temperate mixed species plantation. *Journal of Hydrology*, *519*, *Part*, 3511–3519. doi: 10.1016/j.jhydrol.2014.11.011

Grossiord, C., Granier, A., Gessler, A., Jucker, T., & Bonal, D. (2014). Does drought influence the relationship between biodiversity and ecosystem functioning in boreal forests? *Ecosystems*, *17*(3), 394–404. doi: 10.1007/s10021-013-9729-1

Grossman, J. J., Vanhellemont, M., Barsoum, N., Bauhus, J., Bruelheide, H., Castagneyrol, B., … Verheyen, K. (2018). Synthesis and future research directions linking tree diversity to growth, survival, and damage in a global network of tree diversity experiments. *Environmental and Experimental Botany*. doi: 10.1016/j.envexpbot.2017.12.015

Guyot, V., Castagneyrol, B., Vialatte, A., Deconchat, M., & Jactel, H. (2016). Tree diversity reduces pest damage in mature forests across Europe. *Biology Letters*, *12*(4), 20151037. doi: 10.1098/rsbl.2015.1037

Haas, S E, Cushman, J. H., Dillon, W. W., Rank, N. E., Rizzo, D. M., & Meentemeyer, R. K. (2016). Effects of individual, community, and landscape drivers on the dynamics of a wildland forest epidemic. *Ecology*, *97*(3), 649–660.

Haas, Sarah E, Hooten, M. B., Rizzo, D. M., & Meentemeyer, R. K. (2011). Forest species diversity reduces disease risk in a generalist plant pathogen invasion: Species diversity reduces disease risk. *Ecology Letters*, *14*(11), 1108–1116. doi: 10.1111/j.1461-0248.2011.01679.x

Haddaway, N. R., Collins, A. M., Coughlin, D., Kirk, S., Moen, J., & Pullin, A. (2015). The role of Google Scholar in evidence reviews and its applicability to grey literature searching. *PLOS ONE*, *10*(9), e0138237. doi: 10.1371/journal.pone.0138237

Hajji, M., Dreyer, E., & Marçais, B. (2009). Impact of Erysiphe alphitoides on transpiration and photosynthesis in Quercus robur leaves. *European Journal of Plant Pathology*, *125*(1), 63–72. doi: 10.1007/s10658-009-9458-7

Hambäck, P. A., Ågren, J., & Ericson, L. (2000). Associational resistance: Insect

damage to purple loosestrife reduced in thickets of sweet gale. *Ecology*, *81*(7), 1784–1794. doi: 10.1890/0012-9658(2000)081[1784:ARIDTP]2.0.CO;2

Hantsch, L., Bien, S., Radatz, S., Braun, U., Auge, H., & Bruelheide, H. (2014). Tree diversity and the role of non-host neighbour tree species in reducing fungal pathogen infestation. *Journal of Ecology*, *102*(6), 1673–1687. doi: 10.1111/1365-2745.12317

Hantsch, L., Braun, U., Scherer-Lorenzen, M., & Bruelheide, H. (2013). Species richness and species identity effects on occurrence of foliar fungal pathogens in a tree diversity experiment. *Ecosphere*, *4*(7), 1–12. doi: 10.1890/ES13-00103.1

Harper, L. J., Schönrogge, K., Lim, K. Y., Francis, P., & Lichtenstein, C. P. (2004). Cynipid galls: Insect-induced modifications of plant development create novel plant organs. *Plant, Cell and Environment*, *27*(3), 327–335. doi: 10.1046/j.1365-3040.2004.01145.x

Hayward, A., & Stone, G. N. (2005). Oak gall wasp communities: Evolution and ecology. *Basic and Applied Ecology*, *6*(5), 435–443. doi: 10.1016/J.BAAE.2005.07.003

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M. C., Diemer, M., Dimitrakopoulos, P. G., Lawton, J. H. (1999). Plant diversity and productivity experiments in european grasslands. *Science (New York, N.Y.)*, *286*(5442), 1123–1127. doi: 10.1126/SCIENCE.286.5442.1123

Hector, Andy. (1998). The effect of diversity on productivity: detecting the role of species complementarity. *Oikos*, *82*(3), 597. doi: 10.2307/3546380

Hedges, L. V. (1981). Distribution Theory for Glass's Estimator of Effect size and Related Estimators. *Journal of Educational Statistics*, *6*(2), 107–128. doi:

10.3102/10769986006002107

Hemery, G., Hurst, J., Petrokofsky, G., (2019). Bringing children closer to nature: report of a survey on Forest School and outdoor learning in England. 23pp. Available at: www.sylva.org.uk/forestschools [Accessed 3/11/20].

Herbert, R., Samuel, S., & Patterson, G. (1999). Using local stock for planting native trees and shrubs. *Forestry Commission Practice Note 8*. Forestry Commission, Edinburgh, UK.

Hewitt, H. G., & Ayres, P. G. (1976). Effect of infection by Microsphaera alphitoides (powdery mildew) on cabohydrate levels and translocation in seelings of Quercus robur. *New Phytologist*, *77*(2), 379–390. doi: 10.1111/j.1469-8137.1976.tb01527.x

Hinks, A. E., Cole, E. F., Daniels, K. J., Wilkin, T. A., Nakagawa, S., & Sheldon, B. C. (2015). Scale-dependent phenological synchrony between songbirds and their caterpillar food source. *The American Naturalist*, *186*(1), 84–97. doi: 10.1086/681572

Hubert, J., & Cottrell, J. (2007). The role of forest genetic resources in helping British forests. *Forestry Commission Information Note*. Forestry Commission, Edinburgh, UK.

Huberty, A. F., & Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, *85*(5), 1383–1398. doi: 10.1890/03-0352

Huguet, E., Stone, G. N., & Body, M. (2016). Insect-induced effects on plants and possible effectors used by galling and leaf-mining insects to manipulate their

host-plant. *Journal of Insect Physiology*, *84*, 70–89. doi: 10.1016/J.JINSPHYS.2015.12.009

Hurej, M., & Werf, W. van der. (1993). The influence of black bean aphid, Aphis fabae Scop., and its honeydew on the photosynthesis of sugar beet. *Annals of Applied Biology*, *122*(2), 189–200. doi: 10.1111/j.1744-7348.1993.tb04026.x

Isbell, F., Adler, P. R., Eisenhauer, N., Fornara, D., Kimmel, K., Kremen, C., … Scherer-Lorenzen, M. (2017). Benefits of increasing plant diversity in sustainable agroecosystems. *Journal of Ecology*, *105*(4), 871–879. doi: 10.1111/1365-2745.12789

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., … Eisenhauer, N. (2015). Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature*, *526*(7574), 574–577. doi: 10.1038/nature15374

Itter, M. S., D'Orangeville, L., Dawson, A., Kneeshaw, D., Duchesne, L., & Finley, A. O. (2019). Boreal tree growth exhibits decadal-scale ecological memory to drought and insect defoliation, but no negative response to their interaction. *Journal of Ecology*, *107*(3), 1288–1301. doi: 10.1111/1365-2745.13087

Jactel, H., Birgersson, G., Andersson, S., & Schlyter, F. (2011). Non-host volatiles mediate associational resistance to the pine processionary moth. *Oecologia*, *166*(3), 703–711. doi: 10.1007/s00442-011-1918-z

Jactel, H., Gritti, E. S., Drössler, L., Forrester, D. I., Mason, W. L., Morin, X., … Castagneyrol, B. (2018). Positive biodiversity–productivity relationships in forests: climate matters. *Biology Letters*, *14*(4), 20170747. doi: 10.1098/rsbl.2017.0747

Jactel, H., Bauhus, J., Boberg, J., Bonal, D., Castagneyrol, B., Gardiner, B., … Brockerhoff, E. G. (2017). Tree diversity drives forest stand resistance to natural disturbances. *Current Forestry Reports*, *3*(3), 223–243. doi: 10.1007/s40725-017-0064-1

Jactel, H., & Brockerhoff, E. G. (2007). Tree diversity reduces herbivory by forest insects. *Ecology Letters*, *10*(9), 835–848. doi: 10.1111/j.1461-0248.2007.01073.x

Jactel, H., Desprez-Loustau, M. L., Battisti, A., Brockerhoff, E., Santini, A., Stenlid, J., … Zalucki, M. P. (2020). Pathologists and entomologists must join forces against forest pest and pathogen invasions. *NeoBiota*, *58*, 107–127. doi: 10.3897/neobiota.58.54389

Jactel, H., Koricheva, J., & Castagneyrol, B. (2019). Responses of forest insect pests to climate change: not so simple. *Current Opinion in Insect Science*, *35*, 103–108. doi: 10.1016/j.cois.2019.07.010

Jactel, H., Nicoll, B. C., Branco, M., Gonzalez-Olabarria, J. R., Grodzki, W., L\a angström, B. (2009). The influences of forest stand management on biotic and abiotic risks of damage. *Annals of Forest Science*, *66*(7), 1–18.

Jactel, H., Petit, J., Desprez-Loustau, M. L., Delzon, S., Piou, D., Battisti, A., & Koricheva, J. (2012). Drought effects on damage by forest insects and pathogens: A meta-analysis. *Global Change Biology*, *18*(1), 267–276. doi: 10.1111/j.1365-2486.2011.02512.x

Jactel, H., Poeydebat, C., van Halder, I., & Castagneyrol, B. (2019). Interactice effects of tree mixing and drought on a primary forest pest. *Frontiers in Forests and Global Change*, *2*, 77. doi: 10.3389/ffgc.2019.00077

Johnson, M. T. J., Lajeunesse, M. J., & Agrawal, A. A. (2006). Additive and

interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters*, *9*(1), 24–34. doi: 10.1111/j.1461-0248.2005.00833.x

Kambach, S., Kühn, I., Castagneyrol, B., & Bruelheide, H. (2016). The impact of tree diversity on different aspects of insect herbivory along a global temperature gradient - a meta-analysis. *PLOS ONE*, *11*(11), e0165815. doi: 10.1371/journal.pone.0165815

Keenan, R. J., Reams, G. A., Achard, F., De Freitas, J. V, Grainger, A., & Lindquist, E. (2015). Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. *Forest Ecology and Management*, *352*, 9–20. doi: 10.1016/j.foreco.2015.06.014

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., … Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, *468*(7324), 647–652. doi: 10.1038/nature09575

Kelly, D. L. (2002). The regeneration of Quercus petraea (sessile oak) in southwest Ireland: a 25-year experimental study. *Forest Ecology and Management*, *166*, 207–226. doi: 10.1007/BF02099177

Kerling, L. C. P. (1965). The hibernation of the oak mildew. *Acta Botanica Neerlandica*, *15*(1), 76–83. doi: 10.1111/j.1438-8677.1966.tb00214.x

Khan, M. A. M., Ulrichs, C., & Mewis, I. (2011). Water stress alters aphid-induced glucosinolate response in Brassica oleracea var. italica differently. *Chemoecology*, *21*(4), 235–242. doi: 10.1007/s00049-011-0084-4

Kleinschmit, J. (1993). Intraspecific variation of growth and adaptive traits in European oak species. *Ann. For. Sci.*, *50*, 166s-185s. doi:

10.1051/forest:19930716

Klimesch, J. (1924). Eichensterben in Jugoslavien. *Wie Allg. Forst-u. Jagd-Zeitung*, *46*(271–3).

Kopelke, J. P., Amendt, J., & Schönrogge, K. (2003). Patterns of interspecific associations of stem gallers on willows. *Diversity and Distributions*, *9*(6), 443–453. doi: 10.1046/j.1472-4642.2003.00037.x

Korhonen, K., Bobko, I., Hanso, S., Piri, T., & Vasiliauskas, A. (1992). Intersterility groups of Heterobasidion annosum in some spruce and pine stands in Byelorussia, Lithuania and Estonia. *European Journal of Forest Pathology*, *22*(6–7), 384–391. doi: 10.1111/j.1439-0329.1992.tb00311.x

Korhonen, K., Delatour, C., Greig, B., & Schonhar, S. (1998). Silvicultural Control. In *Heterobasidion annosum: biology, ecology, impact, and control*. CAB International, Wallingford, UK.

Koricheva, J., Gurevitch, J., & Mengersen, K. (2013). *Handbook of Meta-analysis in Ecology and Evolution*. Princeton University Press, Princeton, U.S.A..

Koricheva, J., & Hayes, D. (2018). The relative importance of plant intraspecific diversity in structuring arthropod communities: A meta-analysis. *Functional Ecology*, *32*(7), 1704–1717. doi: 10.1111/1365-2435.13062

Koricheva, J., & Larsson, S. (1998). Insect performance on experimentally stressed woody plants: a meta-analysis. *Annual review of entomology*, *43*(1), 195-216.

Koricheva, J., Vehviläinen, H., Riihimäki, J., Ruohomäki, K., Kaitaniemi, P., & Ranta, H. (2006). Diversification of tree stands as a means to manage pests and diseases in boreal forests: myth or reality? *Canadian Journal of Forest Research*, *36*(2), 324–336. doi: 10.1139/x05-172

Larsson, S. (1989). Stressful Times for the Plant Stress: Insect Performance Hypothesis. *Oikos*, *56*(2), 277. doi: 10.2307/3565348

Lau, J., Ioannidis, J. P. A., Terrin, N., Schmid, C. H., & Olkin, I. (2006, September 16). The case of the misleading funnel plot. *British Medical Journal*, Vol. 333, pp. 597–600. doi: 10.1136/bmj.333.7568.597

Lefcheck, J. S. (2016). piecewiseSEM : Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573–579. doi: 10.1111/2041-210X.12512

Lerdau, M., Litvak, M., & Monson, R. (1994). Plant chemical defense: monoterpenes and the growth-differentiation balance hypothesis. *Trends in Ecology & Evolution*, *9*(2), 58–61. doi: 10.1016/0169-5347(94)90269-0

Lindén, M., & Eko, P. M. (2003). Increment and yield in mixed stands with Norway spruce in southern Sweden. In *Acta Universitatis Agriculturae Sueciae. Silvestria* (Vol. 260).

Liu, X., Chen, L., Liu, M., García-Guzmán, G., Gilbert, G. S., & Zhou, S. (2020). Dilution effect of plant diversity on infectious diseases: latitudinal trend and biological context dependence. *Oikos*, oik.07027. doi: 10.1111/oik.07027

Loewe M., V., González O., M., & Balzarini, M. (2013). Wild cherry tree (Prunus avium L.) growth in pure and mixed plantations in South America. *Forest Ecology and Management*, *306*, 31–41. doi: 10.1016/j.foreco.2013.06.015

Lonsdale, D., & Wainhouse, D. (1987). Beech Bark Disease. *Forestry Commission Bulletin*. doi: 10.1038/218315c0

Lonsdale, David. (2015). Review of oak mildew, with particular reference to mature and veteran trees in Britain. *Arboricultural Journal*, *37*(2), 61–84. doi:

10.1080/03071375.2015.1039839

Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, *412*(6842), 72–76. doi: 10.1038/35083573

Louw, D. (2015). Interspecific interactions in mixed stands of paper birch (Betula papyrifera) and interior Douglas-fir (Pseudotsuga mensiezii , var . glauca). *Doctoral Dissertation, University of British Columbia,* pp. 169.

Manion, P.D.; Lachance, D. L. (1992). Forest decline concepts: an overview. In D. L. Manion, P.D.; Lachance (Ed.), *Forest decline concepts* (pp. 181–190). Minnesota, USA: APS Press.

Manion, P. D. (1991). *Tree disease concepts*. New Jersey, USA: Prentice Hall.

Marçais, B, & Bréda, N. (2006). Role of an opportunistic pathogen in the decline of stressed oak trees. *Journal of Ecology*, *94*(6), 1214–1223.

Marçais, B., & Desprez-Loustau, M.-L. (2014). European oak powdery mildew: impact on trees, effects of environmental factors, and potential effects of climate change. *Annals of Forest Science*, *71*(6), 633–642. doi: 10.1007/s13595-012-0252-x

Marçais, B., Kavkova, M., & Desprez-Loustau, M.-L. (2009). Phenotypic variation in the phenology of ascospore production between European populations of oak powdery mildew. *Annals of Forest Science*, *66*(8), 814. doi: 10.1051/forest/2009077

Marris, E. (2009). Planting the forest of the future. *Nature*, *459*(June).

Mattila, U. (2005). *Probability models for pine twisting rust (Melampsora pinitorqua) damage in Scots pine (Pinus sylvestris) stands in Finland*. *35*(1), 9–21. doi: 10.1111/j.1439-0329.2004.00382.x

Mccracken, A. R., & Dawson, W. M. (1997). Growing clonal mixtures of willow to reduce effect of Melampsora epitea var. epitea. *European Journal of Forest Pathology*, *27*(5), 319–329.

McCracken, A. R., & Dawson, W. M. (1998). Short rotation coppice willow in Northern Ireland since 1973: development of the use of mixtures in the control of foliar rust (Melampsora spp.). *European Journal of Forest Pathology*, *28*(4), 241–250. doi: 10.1111/j.1439-0329.1998.tb01179.x

McCracken, A. R., Malcolm Dawson, W., & Carlisle, D. (2005). Short-rotation coppice willow mixtures and rust disease development. In *Rust Diseases of Willow and Poplar* (pp. 184–194).

Mitchell, C. E., Tilman, D., & Groth, J. V. (2002). Effects of Grassland Plant Species Diversity, Abundance, and Composition on Foliar Fungal Disease. *Ecology*, *83*(6), 1713–1726. doi: 10.1890/0012-9658(2002)083[1713:EOGPSD]2.0.CO;2

Mitchell, R. J., Bellamy, P. E., Ellis, C. J., Hewison, R. L., Hodgetts, N. G., Iason, G. R., … Taylor, A. F. S. (2019). Collapsing foundations: The ecology of the British oak, implications of its decline and mitigation options. *Biological Conservation*, (December 2018), 0–1. doi: 10.1016/j.biocon.2019.03.040

Mooney, E. H., Tiedeken, E. J., Muth, N. Z., & Niesenbaum, R. A. (2009). Differential induced response to generalist and specialist herbivores by Lindera benzoin (Lauraceae) in sun and shade. *Oikos*, *118*(8), 1181–1189. doi: 10.1111/j.1600-0706.2009.17228.x

Moore, R., Warrington, S., & Whittaker, J. B. (1991). Herbivory by Insects on Oak Trees in Pure Stands Compared with Paired Mixtures. *The Journal of Applied Ecology*, *28*(1), 290. doi: 10.2307/2404130

Moreira, X., Abdala-Roberts, L., & Castagneyrol, B. (2018a). Interactions between plant defence signalling pathways: Evidence from bioassays with insect herbivores and plant pathogens. *Journal of Ecology*. doi: 10.1111/1365-2745.12987

Moreira, X., Abdala-Roberts, L., & Castagneyrol, B. (2018b). Interactions between plant defence signalling pathways: Evidence from bioassays with insect herbivores and plant pathogens. *Journal of Ecology*, *106*(6), 2353–2364. doi: 10.1111/1365-2745.12987

Moreira, X., Abdala-Roberts, L., Parra-Tabla, V., & Mooney, K. A. (2014). Positive Effects of Plant Genotypic and Species Diversity on Anti-Herbivore Defenses in a Tropical Tree Species. *PLOS ONE*, *9*(8), e105438. doi: 10.1371/journal.pone.0105438

Moreira, X., Abdala-Roberts, L., Rasmann, S., Castagneyrol, B., & Mooney, K. A. (2016). Plant diversity effects on insect herbivores and their natural enemies: current thinking, recent findings, and future directions. *Current Opinion in Insect Science*, *14*, 1–7. doi: 10.1016/j.cois.2015.10.003

Morin, X., Fahse, L., de Mazancourt, C., Scherer-Lorenzen, M., & Bugmann, H. (2014). Temporal stability in forest productivity increases with tree diversity due to asynchrony in species dynamics. *Ecology Letters*, *17*(12), 1526–1535. doi: 10.1111/ele.12357

Mougou, A., Dutech, C., & Desprez-Loustau, M.-L. (2008). New insights into the identity and origin of the causal agent of oak powdery mildew in Europe. *Forest Pathology*, *38*(4), 275–287. doi: 10.1111/j.1439-0329.2008.00544.x

Muiruri, E. W., Barantal, S., Iason, G. R., Salminen, J.-P., Perez-Fernandez, E., &

Koricheva, J. (2019). Forest diversity effects on insect herbivores: do leaf traits matter? *New Phytologist*, *221*, 2250–2260. doi: 10.1111/nph.15558

Muiruri, E. W., & Koricheva, J. (2017). Going undercover: increasing canopy cover around a host tree drives associational resistance to an insect pest. *Oikos*, *126*(3), 339–349. doi: 10.1111/oik.03307

Mundt, C C. (2002). Use of Multiline Cultivars and Cultivar Mixtures for Disease Management. *Annual Review of Phytopathology*, *40*(1), 381–410. doi: 10.1146/annurev.phyto.40.011402.113723

Mundt, Chris C. (2005). Host Diversity, Epidemic Progression and Pathogen Evolution. In *Rust diseases of willow and poplar* (p. 175).

Nakagawa, S., Noble, D. W. A., Senior, A. M., & Lagisz, M. (2017). Meta-evaluation of meta-analysis: Ten appraisal questions for biologists. *BMC Biology*, Vol. 15, p. 18. doi: 10.1186/s12915-017-0357-7

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*(2), 133–142. doi: 10.1111/j.2041-210x.2012.00261.x

Nichols, J. D., Bristow, M., & Vanclay, J. K. (2006). Mixed-species plantations: Prospects and challenges. *Forest Ecology and Management*, *233*(2–3), 383–390. doi: 10.1016/j.foreco.2006.07.018

Nieukerken, E. J., & Johansson, R. (2003). The Quercus feeding Stigmella species of the West Palaearctic: new species, key and distribution (Lepidoptera: Nepticulidae). *Tijdschrift Voor Entomologie*, *1*(January), 307–370.

Northrop, R. J. (2006). Dynamics of powdery mildew in a homogenous urban sand live oak (Quercus geminata) forest. *Proceedings of the 119th Annual*

Meeting of the Florida State Horticultural Society, *119*, 396.

Oliva, J., Stenlid, J., & Martínez-Vilalta, J. (2014). The effect of fungal pathogens on the water and carbon economy of trees: implications for drought-induced mortality. *New Phytologist*, *203*(4), 1028–1035. doi: 10.1111/nph.12857

Osmaston, L. S. (1927). Mortality Among Oak. *Quarterly Journal of Forestry*, 28–30.

Pap, P., Ranković, B., & Maširević, S. (2013). Effect of temperature, relative humidity and light on conidial germination of oak powdery mildew (Microsphaera alphitoides Griff. et Maubl.) under controlled conditions. *Archives of Biological Sciences*, *65*(3), 1069–1077.

Paquette, A., Hector, A., Castagneyrol, B., Vanhellemont, M., Koricheva, J., Scherer-Lorenzen, M., & Verheyen, K. (2018). A million and more trees for science. *Nature Ecology & Evolution*, *2*(5), 763–766. doi: 10.1038/s41559-018-0544-0

Parelle, J., Brendel, O., Bodénès, C., Berveiller, D., Dizengremel, P., & Jolivet, Y. (2006). Differences in morphological and physiological responses to water-logging between two sympatric oak species (Q. Petraea [Matt.] Lieb., Q. Robur L.). *Annals of Forest Science 8 (63), 849-859. (2006).*

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M., & Dat, J. (2008). An overview of plant responses to soil waterlogging. *Plant Stress*, *2*(1), 20–27.

Pautasso, M., Holdenrieder, O., & Stenlid, J. (2005). Susceptibility to Fungal Pathogens of Forests Differing in Tree Diversity. In *Forest Diversity and Function* (pp. 263–289). doi: 10.1007/3-540-26599-6_13

Pearse, I. S. (2011). The role of leaf defensive traits in oaks on the preference and performance of a polyphagous herbivore, Orgyia vetusta. *Ecological*

Entomology, *36*(5), 635–642. doi: 10.1111/j.1365-2311.2011.01308.x

Pearse, I. S., & Hipp, A. L. (2009). Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences*, *106*(43), 18097–18102. doi: 10.1073/pnas.0904867106

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal Modulation of Plant Immunity. *Annual Review of Cell and Developmental Biology*, *28*(1), 489–521. doi: 10.1146/annurev-cellbio-092910-154055

Plas, F. van der, Manning, P., Allan, E., Scherer-Lorenzen, M., Verheyen, K., Wirth, C., … Fischer, M. (2016). Jack-of-all-trades effects drive biodiversity–ecosystem multifunctionality relationships in European forests. *Nature Communications*, *7*, 11109. doi: 10.1038/ncomms11109

Plath, M., Dorn, S., Riedel, J., Barrios, H., & Mody, K. (2012). Associational resistance and associational susceptibility: specialist herbivores show contrasting responses to tree stand diversification. *Oecologia*, *169*(2), 477–487. doi: 10.1007/s00442-011-2215-6

Ponton, S., Dupouey, J.-L., Breda, N., & Dreyer, E. (2002). Comparison of water-use efficiency of seedlings from two sympatric oak species: genotype x environment interactions. *Tree Physiology*, *22*(6), 413–422. doi: 10.1093/treephys/22.6.413

Pretzsch, H., Bielak, K., Block, J., Bruchwald, A., Dieler, J., Ehrhart, H.-P., … Zingg, A. (2013). Productivity of mixed versus pure stands of oak (Quercus petraea (Matt.) Liebl. and Quercus robur L.) and European beech (Fagus sylvatica L.) along an ecological gradient. *European Journal of Forest Research*,

132(2), 263–280. doi: 10.1007/s10342-012-0673-y

Pretzsch, H., Forrester, D. I., & Bauhus, J. (2017). *Mixed-species forests : ecology and management*.

Price, P. W. (1991). The Plant Vigor Hypothesis and Herbivore Attack. *Oikos*, *62*(2), 244. doi: 10.2307/3545270

Pullin, A. S., & Stewart, G. B. (2006). Guidelines for systematic review in conservation and environmental management. *Conservation biology*, *20*(6), 1647-1656.

Quine, C., Atkinson, N., Denman, S., Desprez-Loustau, M.-L., Jackson, R., & Kirby, K. (2019). *Action Oak Knowledge Review: An assessment of the current evidence on oak health, identification of evidence gaps and prioritisation of research needs*. Action Oak.

R Core Team (2020) R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Rank, N. E., Cushman, H., Meentemeyer, R. K., & Rizzo, D. M. (2010). Establishment of Phytophthora ramorum Depends on Woodland Diversity and Species Composition. *Sudden Oak Death Fourth Science Symposium*, 356.

Ratcliffe, S., Wirth, C., Jucker, T., van der Plas, F., Scherer-Lorenzen, M., Verheyen, K., … Baeten, L. (2017). Biodiversity and ecosystem functioning relations in European forests depend on environmental context. *Ecology Letters*. doi: 10.1111/ele.12849

Régolini, M., Castagneyrol, B., Dulaurent-Mercadal, A.-M., Piou, D., Samalens, J.-C., & Jactel, H. (2014). Effect of host tree density and apparency on the

probability of attack by the pine processionary moth. *Forest Ecology and Management*, *334*, 185–192. doi: 10.1016/j.foreco.2014.08.038

Roberts, A.M.I., Tansey, C., Smithers, R.J. & Phillimore, A. B. (2015). Predicting a change in the order of spring phenology in temperate forests. *Global Change Biology*, *21*, 2603–2611.

Roberts, M. R., & Paul, N. D. (2006). Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens. *New Phytologist*, *170*(4), 677–699. doi: 10.1111/j.1469-8137.2006.01707.x

Rosenberg, M. S. (2005). The file-drawer problem revisited: A general weighted method for calculating fail-safe numbers in meta-analysis. *Evolution*, *59*(2), 464–468. doi: 10.1111/j.0014-3820.2005.tb01004.x

Rosenthal, R. (1979). The file drawer problem and tolerance for null results. *Psychological Bulletin*, *86*(3), 638–641. doi: 10.1037/0033-2909.86.3.638

Roslin, T., Laine, A.-L., & Gripenberg, S. (2007). Spatial population structure in an obligate plant pathogen colonizing oak Quercus robur. *Functional Ecology*, *21*(6), 1168–1177. doi: 10.1111/j.1365-2435.2007.01315.x

Roy, S. (2019). Evaluating the impact of tree provenance, tree phenotype and emergent disease on microbial and insect populations in tree ecosystems. Doctoral Thesis, University of Reading, Surrey, UK.

Schnathorst, W. C. (1965). Environmental relationships in the powdery mildews. *Annual Review of Phytopathology*, *3*(1), 343–366.

Schoenweiss, D. (1986). Water stress predisposition to disease - an overview. In L. Ayres, P., and Boddy (Ed.), *Water, Fungi and Plants: Symposium of the British*

Mycological Society Held at the University of Lancaster, April 1985 (pp. 157–174). Cambridge University Press.

Schuldt, A., Baruffol, M., Böhnke, M., Bruelheide, H., Härdtle, W., Lang, A. C., … Assmann, T. (2010). Tree diversity promotes insect herbivory in subtropical forests of south-east China. *Journal of Ecology*, *98*(4), 917–926. doi: 10.1111/j.1365-2745.2010.01659.x

Schuldt, A., Bruelheide, H., Buscot, F., Assmann, T., Erfmeier, A., Klein, A.-M., … Wubet, T. (2017). Belowground top-down and aboveground bottom-up effects structure multitrophic community relationships in a biodiverse forest. *Scientific Reports*, *7*(1), 4222. doi: 10.1038/s41598-017-04619-3

Schuldt, A., Hönig, L., Li, Y., Fichtner, A., Härdtle, W., von Oheimb, G., … Bruelheide, H. (2017). Herbivore and pathogen effects on tree growth are additive, but mediated by tree diversity and plant traits. *Ecology and Evolution*, *7*(18), 7462–7474. doi: 10.1002/ece3.3292

Schultz, J. C., Appel, H. M., Ferrieri, A. P., & Arnold, T. M. (2013). Flexible resource allocation during plant defense responses. *Frontiers in Plant Science*, *4*, 324. doi: 10.3389/fpls.2013.00324

Setiawan, N., Vanhellemont, M., Baeten, L., Van de Peer, T., Ampoorter, E., Ponette, Q., & Verheyen, K. (2017). Local neighbourhood effects on sapling growth in a young experimental forest. *Forest Ecology and Management*, *384*, 424–443. doi: 10.1016/j.foreco.2016.10.012

Setiawan, N., Vanhellemont, M., Baeten, L., Dillen, M., & Verheyen, K. (2014). The effects of local neighbourhood diversity on pest and disease damage of trees in a young experimental forest. *Forest Ecology and Management*, *334*, 1–9. doi:

10.1016/j.foreco.2014.08.032

Shaw, D. C., Oester, P. T., & Filip, G. M. (2009). *Managing Insects and Diseases of Oregon Conifers*. (June).

Sinclair, F. H., Stone, G. N., Nicholls, J. A., Cavers, S., Gibbs, M., Butterill, P., Schönrogge, K. (2015). Impacts of local adaptation of forest trees on associations with herbivorous insects: implications for adaptive forest management. *Evolutionary Applications*, *8*(10), 972–987. doi: 10.1111/eva.12329

Smilanich, A. M., Fincher, R. M., & Dyer, L. A. (2016). Does plant apparency matter? Thirty years of data provide limited support but reveal clear patterns of the effects of plant chemistry on herbivores. *New Phytologist*, *210*(3), 1044–1057. doi: 10.1111/nph.13875

Smith Dumont, E., Gnahoua, G. M., Ohouo, L., Sinclair, F. L., & Vaast, P. (2014). Farmers in Côte d'Ivoire value integrating tree diversity in cocoa for the provision of ecosystem services. *Agroforestry Systems*, *88*(6), 1047–1066. doi: 10.1007/s10457-014-9679-4

Sobek, S., Scherber, C., Steffan-Dewenter, I., & Tscharntke, T. (2009). Sapling herbivory, invertebrate herbivores and predators across a natural tree diversity gradient in Germany's largest connected deciduous forest. *Oecologia*, *160*(2), 279–288. doi: 10.1007/s00442-009-1304-2

Sopow, S. L., Shorthouse, J. D., Strong, W., & Quiring, D. T. (2003). Evidence for long-distance, chemical gall induction by an insect. *Ecology Letters*, *6*(2), 102–105. doi: 10.1046/j.1461-0248.2003.00410.x

Southwood, T. (1961). The numbers of species of insect associated with various trees. *The Journal of Animal Ecology*, *30*(1), 1. doi: 10.2307/2109

Stevens, R. B. (1960). *Plant Pathology: An Advanced Treatise.*

Stiegel, S., Entling, M. H., & Mantilla-Contreras, J. (2017). Reading the leaves' palm: leaf traits and herbivory along the microclimatic gradient of forest layers. *PLOS ONE*, *12*(1), e0169741. doi: 10.1371/journal.pone.0169741

Stoepler, T. M., & Lill, J. T. (2013). Direct and indirect effects of light environment generate ecological trade-offs in herbivore performance and parasitism. *Ecology*, *94*(10), 2299–2310. doi: 10.1890/12-2068.1

Stone, G. N., & Schönrogge, K. (2003). The adaptive significance of insect gall morphology. *Trends in Ecology & Evolution*, *18*(10), 512–522. doi: 10.1016/S0169-5347(03)00247-7

Stone, G. N., Schönrogge, K., Atkinson, R. J., Bellido, D., & Pujade-Villar, J. (2001). The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annual Review of Entomology*, *47*, 633–668.

Stout, M. J., Thaler, J. S., & Thomma, B. P. H. J. (2006). Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annual Review of Entomology*, Vol. 51, pp. 663–689. doi: 10.1146/annurev.ento.51.110104.151117

Sturrock, R. N., Frankel, S. J., Brown, A. V, Hennon, P. E., Kliejunas, J. T., Lewis, K. J., … Woods, A. J. (2011). Climate change and forest diseases. *Plant Pathology*, *60*(1), 133–149. doi: 10.1111/j.1365-3059.2010.02406.x

Tack, A., Gripenberg, S., & Roslin, T. (2012). Cross-kingdom interactions matter: fungal-mediated interactions structure an insect community on oak. *Ecology Letters*, *15*(3), 177–185. doi: 10.1111/j.1461-0248.2011.01724.x

Tack, A., Ovaskainen, O., Pulkkinen, P., & Roslin, T. (2010). Spatial location

dominates over host plant genotype in structuring an herbivore community. *Ecology*, *91*(9), 2660–2672. doi: 10.1890/09-1027.1

Tahvanainen, J., & Root, R. B. (1972). The influence of vegetational diversity on the population ecology of a specialized herbivore, Phyllotreta cruciferae (Coleoptera: Chrysomelidae). *Oecologia*, *10*(4), 321–346. doi: 10.1007/BF00345736

Tarrant, R. F., & Trappe, J. M. (1971). The role of Alnus in improving the forest environment. *Plant and Soil*, *35*(1), 335–348. doi: 10.1007/BF02661862

Thomas, F M, Blank, R., & Hartmann, G. (2002). Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *Forest Pathology*, *32*(4–5), 277–307.

Thomas, F. (2008). Recent advances in cause-effect research on oak decline in Europe. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *3*(37), 1–12.

Thomas, P. (2014). Chapter 6: The growing tree. In *Trees: their natural history.* Cambridge, UK: Cambridge University Press.

Tikkanen, O.-P., & Julkunen-Tiitto, R. (2003). Phenological variation as protection against defoliating insects: the case of Quercus robur and Operophtera brumata. *Oecologia*, *136*(2), 244–251. doi: 10.1007/s00442-003-1267-7

Tilman, D., Wedin, D., & Knops, J. (1996). Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, *379*(6567), 718.

Twery, M. J., & Patterson, W. A. (1983). Effects of species composition and site factors on the severity of beech bark disease in western Massachusetts and the White Mountains of New Hampshire: A preliminary report. *Proceedings,*

IUFRO Beech Bark Disease Working Party Conference; 1982, 127–133. US Department of Agriculture, Forest Service.

Utsumi, S., Ando, Y., Craig, T. P., & Ohgushi, T. (2011). Plant genotypic diversity increases population size of a herbivorous insect. *Proceedings of the Royal Society of London B: Biological Sciences*, *278*(1721), 3108–3115. doi: 10.1098/rspb.2011.0239

van Asch, M., & Visser, M. E. (2007). Phenology of Forest Caterpillars and Their Host Trees: The Importance of Synchrony. *Annual Review of Entomology*, *52*(1), 37–55. doi: 10.1146/annurev.ento.52.110405.091418

van der Plas, F. (2019). Biodiversity and ecosystem functioning in naturally assembled communities. *Biological Reviews*, *94*(4), 1220–1245. doi: 10.1111/brv.12499

van Dijk, L. J. A., Ehrlén, J., & Tack, A. J. M. (2020). The timing and asymmetry of plant–pathogen–insect interactions. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1935), 20201303. doi: 10.1098/rspb.2020.1303

Vehviläinen, H., Koricheva, J., & Ruohomäki, K. (2007). Tree species diversity influences herbivore abundance and damage: meta-analysis of long-term forest experiments. *Oecologia*, *152*(2), 287–298. doi: 10.1007/s00442-007-0673-7

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., … Scherer-Lorenzen, M. (2016). Contributions of a global network of tree diversity experiments to sustainable forest plantations. *Ambio*, *45*(1), 29–41. doi: 10.1007/s13280-015-0685-1

Visser, M. E., & Holleman, L. J. M. (2001). Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society of London B:*

Biological Sciences, *268*(1464), 289–294. doi: 10.1098/rspb.2000.1363

Walter, J., Hein, R., Auge, H., Beierkuhnlein, C., Löffler, S., Reifenrath, K., … Jentsch, A. (2012). How do extreme drought and plant community composition affect host plant metabolites and herbivore performance? *Arthropod-Plant Interactions*, *6*(1), 15–25. doi: 10.1007/s11829-011-9157-0

Wargo, P. (1996). Consequences of environmental stress on oak: predisposition to pathogens. *Annales Des Sciences Forestières*, *53*(2–3), 359–368. doi: 10.1051/forest:19960218

Weis, A. (1988). Reactive Plant Tissue Sites And The Population Biology Of Gall Makers. *Annual Review of Entomology*, *33*(1), 467–486. doi: 10.1146/annurev.ento.33.1.467

White, J. A., & Whitham, T. G. (2000). Associational susceptibility of cottonwood to a box elder herbivore. *Ecology*, *81*(7), 1795–1803. doi: 10.1890/0012-9658(2000)081[1795:ASOCTA]2.0.CO;2

White, T. C. R. (1974). A hypothesis to explain outbreaks of looper caterpillars, with special reference to populations of Selidosema suavis in a plantation of Pinus radiata in New Zealand. *Oecologia*, *16*(4), 279–301. doi: 10.1007/BF00344738

White, T. C. R. (2009). Plant vigour versus plant stress: a false dichotomy. *Oikos*, *118*(6), 807–808. doi: 10.1111/j.1600-0706.2009.17495.x

Whitney, R. D. (1973). Root rot losses in upland spruce at Candle Lake, Saskatchewan. *The Forestry Chronicle*, 176–179.

Wimp, G. M., Martinsen, G. D., Floate, K. D., Bangert, R. K., & Whitham, T. G. (2005). Plant genetic determinants of arthropod community structure and

diversity. *Evolution*, *59*(1), 61–69. doi: 10.1111/j.0014-3820.2005.tb00894.x

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. doi: 10.1007/978-0-387-87458-6

Zvereva, E. L., Zverev, V., & Kozlov, M. V. (2012). Little strokes fell great oaks: minor but chronic herbivory substantially reduces birch growth. *Oikos*, *121*(12), 2036–2043. doi: 10.1111/J.1600-0706.2012.20688.X

APPENDIX 1: SUPPLEMENTARY INFORMATION FOR CHAPTER 2

Model Response Variable	**Retained Variables in Minimal Adequate Model**	**Model Type**	**Post Hoc Analyses Conducted**
Oak Powdery Mildew (Hucking 2016)	Provenance [F]; Diversity [F]; Block [F]; Lammas Shoot Length [C]; Leaf Miner Abundance [C]; Diversity:Block [I]	Gaussian GLM, log link	1) Pairwise comparison of means between provenances (z tests)
Oak Powdery Mildew (Hucking 2017)	Provenance [F]; Diversity [F]; Block [F]; Lammas Shoot Length [C]; Tree Height [C]; Tree Height^2 [C]; Provenance:Lammas Shoot Length [I]	Gaussian GLM, log link	1) Apparency included post hoc 1) in addition to 2) instead of tree height variables, likelihood ratio test performed to compare significance 2) Pairwise comparison of means between provenances (z tests) 3) Pairwise comparison of means between diversity treatments (z tests) for: • Mixed Species versus All Provenance Monocultures • 50:50 Provenance Mixtures versus All Provenance Monocultures • 75:25 Provenance Mixtures versus All Provenance Monocultures

Oak Powdery Mildew (Hartshorne 2017)	Provenance [F]; Diversity [F]; Block [F]; Lammas Shoot Length [C]; Tree Height [C]; Apparency [C]; Provenance:Block [I]; Provenance:Lammas Shoot Length [I]	Gaussian GLM, log link	1) Apparency included post hoc 1) in addition to 2) instead of tree height, likelihood ratio test performed to compare significance 2) Pairwise comparison of means between diversity treatments (z tests) for: • 33:33:33 Provenance Mixtures versus all other plots
Galler Abundance (Hucking 2016)	Provenance [F]; Diversity [F]; Block [F]; Primary Shoot Length [C]; Diversity:Block [I]	Negative binomial GLM, log link	1) Pairwise comparison of means between provenances (z tests)
Galler Abundance (Hucking 2017)	Provenance [F]; Diversity [F]; Block [F]; Primary Shoot Length [C]; Tree Height [C]; Provenance:Diversity [I]	Negative binomial GLM, log link	1) Apparency included post hoc 1) in addition to 2) instead of tree height, likelihood ratio test performed to compare significance 2) Pairwise comparison of means between provenances (z tests) 3) To test the outcome of the provenance:diversity interaction, reran the model for Italian provenance only. Pairwise comparisons of means performed to compare: • 50:50 Provenance Mixes with Italian Monocultures

			• 33:33:33 Provenance Mixes with Italian Monocultures
Galler Abundance (Hartshorne 2017)	Provenance [F]; Diversity [F]; Block [F]; Primary Shoot Length [C]; Apparency [C]; Provenance:Diversity [I]; Diversity:Block [I]	Negative binomial GLM, log link	1) Apparency included post hoc 1) in addition to 2) instead of tree height, likelihood ratio test performed to compare significance 2) Pairwise comparison of means between provenances (z tests)
Leaf Miner Abundance (Hucking 2016)	Diversity [F]; Block [F]; Primary Shoot Length [C]	Negative binomial GLM, log link	1) Pairwise comparison of means (z tests) between Mixed Species, Mixed Provenance plots compared to all others
Leaf Miner Abundance (Hucking 2017)	Diversity [F]; Block [F]; Primary Shoot Length [C]; Apparency [C]; Diversity:Block [I]	Negative binomial GLM, log link	1) Apparency included post hoc 1) in addition to 2) instead of tree height, likelihood ratio test performed to compare significance
Leaf Miner Abundance (Hartshorne 2017)	Provenance [F]; Diversity [F]; Block [F]; Primary Shoot Length [C]; Apparency [C]; Diversity:Block [I]	Poisson GLM, log link	1) Apparency included post hoc 1) in addition to 2) instead of tree height, likelihood ratio test performed to compare significance 2) Pairwise comparison of means between provenances (z tests)
Leaf Manipulator Abundance (Hucking 2016)	Primary Shoot Length [C]	Negative binomial GLM, log link	None
Leaf Manipulator	Provenance [F]; Diversity [F]; Block [F]; Primary Shoot	Negative binomial	1) Apparency included post hoc 1) in addition to 2) instead of

Abundance (Hucking 2017)	Length [C]; Tree Height [C]; Diversity:Block [I]	GLM, log link	tree height, likelihood ratio test performed to compare significance 2) Pairwise comparison of means between provenances (z tests)
Leaf Manipulator Abundance (Hartshorne 2017)	Diversity [F]; Block [F]; Primary Shoot Length [C]; Apparency [C]	Negative binomial GLM, log link	1) Apparency included post hoc (tree height nonsignificant) 2) Pairwise comparison of means between diversity treatments (z tests) for: • 75:25 Provenance Mixtures versus All Provenance Monocultures • 33:33:33 Provenance Mixtures versus All Provenance Monocultures

Table S1: Explanation of the post hoc tests and/or analyses completed for each model.

Model Response Variable	Year + Site	F value	DF	P value
Primary Shoot Length	2016 Hucking	6.47	2,417	***0.0017***
	2017 Hucking	39.67	2, 413	***< 0.001***
	2017 Hartshorne	47.80	2, 408	***< 0.001***
Lammas Shoot Length	2016 Hucking	2.20	2,417	0.11
	2017 Hucking	2.32	2, 413	0.099
	2017 Hartshorne	12.01	2, 408	***< 0.001***
Tree Height	2017 Hucking	1.33	2, 413	0.27
	2017 Hartshorne	2.24	2, 408	0.11
Lammas Shoot Availability	2017 Hucking	23.81	2,135	***< 0.001***
	2017 Hartshorne	17.76	2,131	***< 0.001***

Table S2: F tests for one-way ANOVAs testing the effect of provenance on tree phenotypic traits. Significant results are in bold and italics. DF = degrees of freedom for F tests.

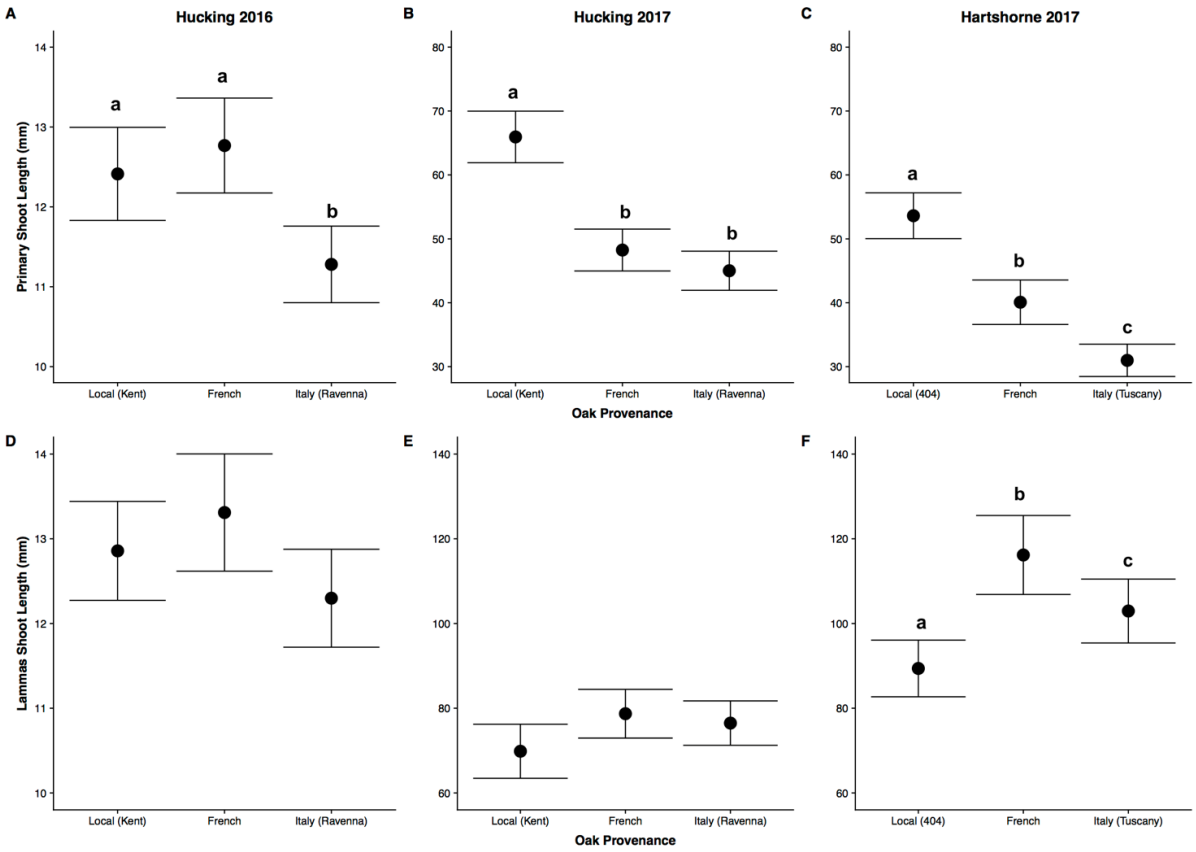


Figure S1: Differences in primary shoot length (A-C) and lammas shoot length (D-F) between provenances. A + D – Hucking 2016; B + E – Hucking 2017; C + F – Hartshorne 2017. Plotted are means (black circles) +/- 95% confidence intervals (error bars). Different letters above error bars indicate where significant differences between provenances occurred ($p < 0.05$) as calculated using one-way ANOVA.

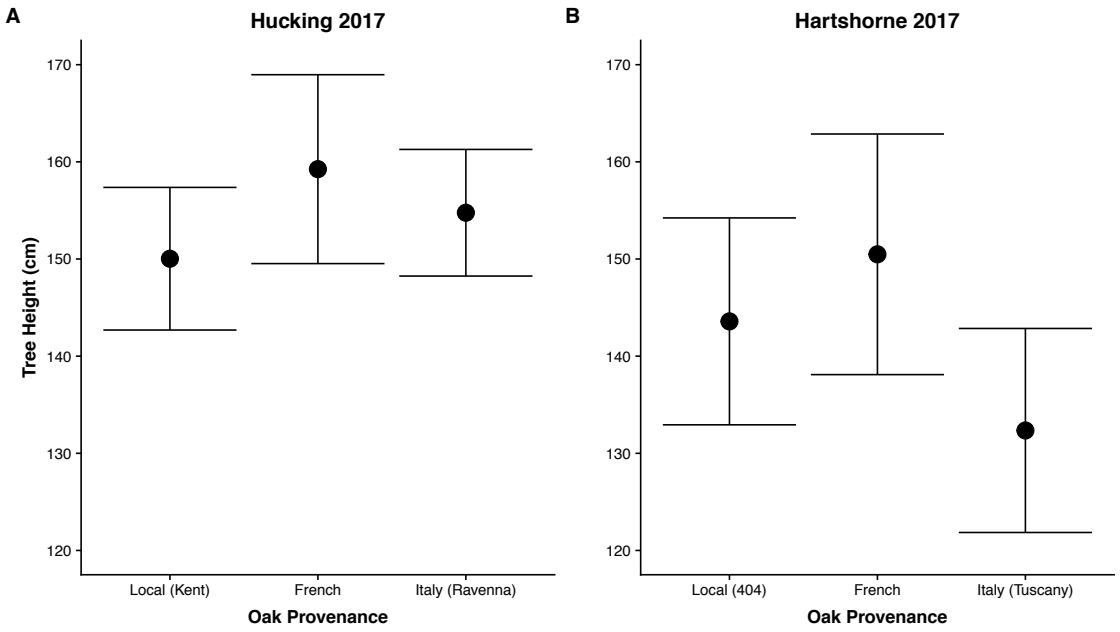


Figure S2: Differences in tree height between provenances. A = Hucking 2017, B = Hartshorne 2017. Plotted are means (black circles) +/- 95% confidence intervals (error bars).

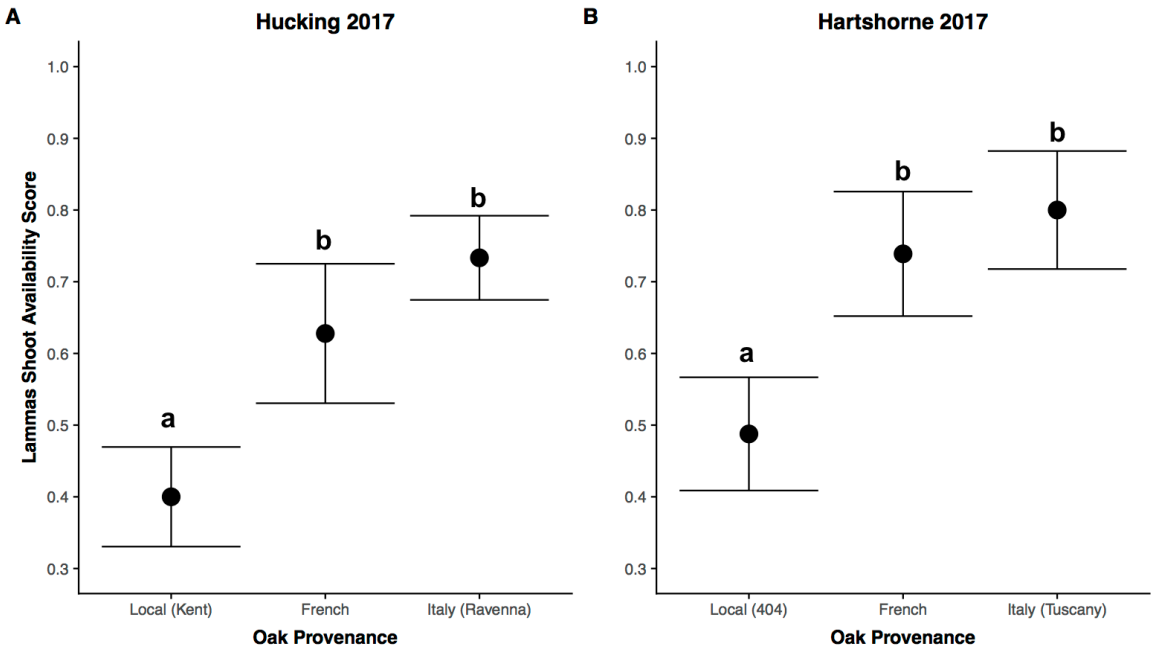


Figure S3: Differences in lammas shoot availability between provenances. A = Hucking 2017, B = Hartshorne 2017. Plotted are means (black circles) +/- 95%

confidence intervals (error bars). Different letters above error bars indicate where significant differences between provenances occurred ($p < 0.05$) as calculated using one-way ANOVA.

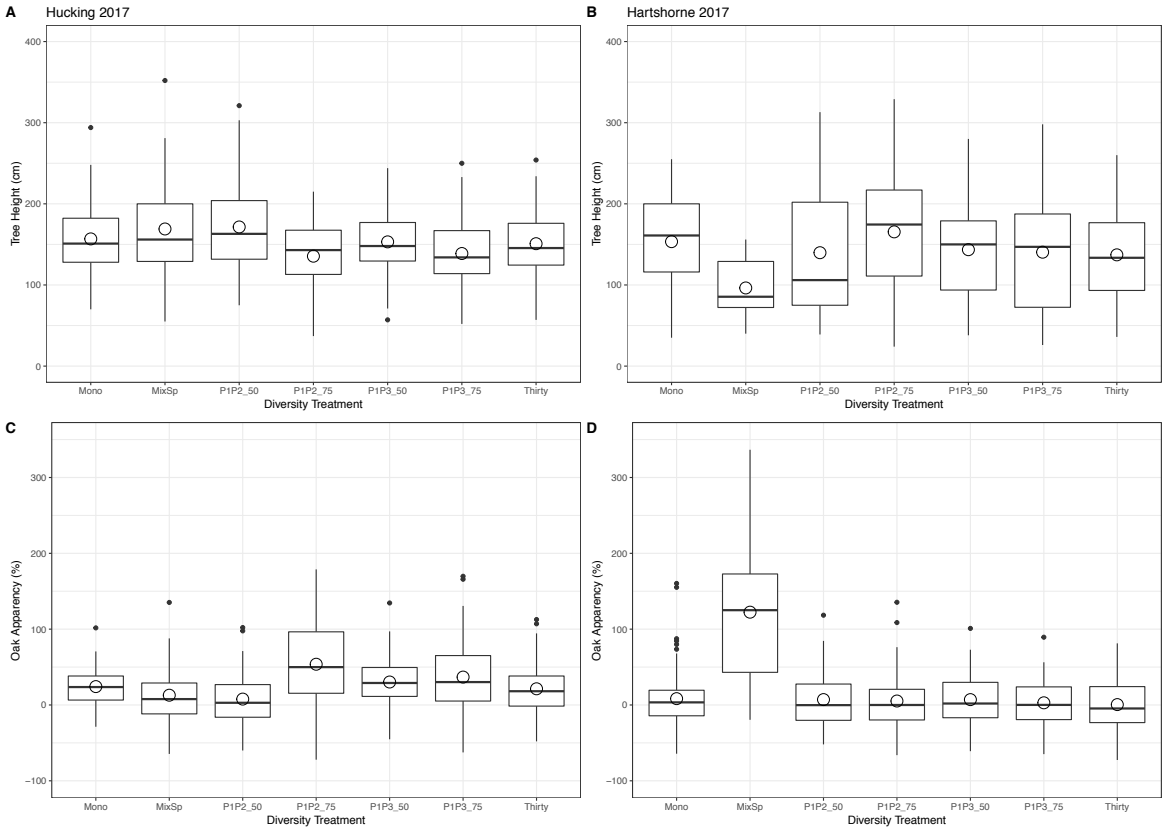


Figure S4: Boxplots showing differences in tree apparency and tree height between diversity treatments in the two sites. A & C, Hucking 2017, B & D, Hartshorne 2017. Abbreviations for diversity treatments are as follows: Mono (provenance monoculture); MixSp (mixed species, mixed provenance); P1P2_50 (Local:French 50:50 mixture); P1P2_75 (Local:French 75:25 mixture), P1P3_50 (Local:Italian 50:50 mixture); P1P3_75 (Local:Italian 75:25), Thirty (33:33:33 mixture of Local, French and Italian provenances).

HUCKING 2016

	MILDEW		GALLERS		MINERS		MANIPULATORS	
TREATMENT	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
Provenance Monoculture	1.80	0.28	31.10	8.02	4.43	0.51	11.87	0.33
Mixed species	1.77	0.13	19.83	5.11	3.11	0.31	13.61	1.65
Local:French 50:50	1.99	0.18	37.39	5.64	4.74	0.53	16.22	3.31
Local:French 75:25	1.31	0.17	37.53	5.28	3.53	0.35	9.31	1.05
Local:Italian 50:50	1.54	0.33	19.62	5.05	4.98	0.90	10.93	1.61
Local:Italian 75:25	1.65	0.38	11.72	2.17	4.34	0.23	12.36	2.98
Local:French:Italian 33:33:33	1.72	0.29	33.01	3.50	3.85	0.29	12.08	4.92

HUCKING 2017

	MILDEW		GALLERS		MINERS		MANIPULATORS	
TREATMENT	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
Provenance Monoculture	1.13	0.08	152.88	41.77	4.24	0.85	19.40	4.63
Mixed species	0.84	0.07	122.07	54.43	3.98	0.57	22.54	7.48
Local:French 50:50	0.84	0.06	282.98	27.78	4.56	0.78	16.24	2.73
Local:French 75:25	0.56	0.06	220.77	22.64	2.84	0.23	17.37	1.24
Local:Italian 50:50	1.13	0.11	150.63	20.56	3.30	0.68	20.98	4.24
Local:Italian 75:25	0.98	0.13	125.65	33.82	3.21	0.42	17.33	0.90

Local:French:Italian 33:33:33	1.06	0.08	135.29	10.79	3.92	0.46	19.17	0.44

HARTSHORNE 2017

	MILDEW		GALLERS		MINERS		MANIPULATORS	
TREATMENT	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
Provenance Monoculture	0.92	0.24	64.00	7.59	3.69	0.98	8.54	3.65
Mixed species	0.69	0.10	12.81	3.08	1.52	0.47	5.33	4.44
Local:French 50:50	0.93	0.41	70.87	6.72	3.40	0.90	8.64	4.02
Local:French 75:25	0.92	0.27	90.82	40.26	3.41	0.68	8.76	4.93
Local:Italian 50:50	1.11	0.29	33.76	12.87	3.17	1.02	9.26	2.89
Local:Italian 75:25	0.88	0.32	32.11	6.63	3.77	1.61	11.60	3.78
Local:French:Italian 33:33:33	0.73	0.30	51.38	17.20	3.11	0.32	5.60	2.10

Table S3: The effects of diversity treatment on powdery mildew infection and insect herbivore abundance at each site and year. Numbers are plot level averages, so N = 3 for each treatment at each site and year (3 blocks per site with each treatment replicated once per block), except for Hartshorne, where N = 2 for mixed species plots as they are replicated in 2/3 blocks.

Figures S5-S9: Partial Residual Plots

Partial residual plots are a type of model prediction plot that can be used for hypothesis testing, for instance, to clearly visualise the significance of multiple predictors when minimal adequate models contain interaction terms. In all plots one or two variables are varied while others are held constant at their mean value (continuous predictors) or a representative factor level (categorical predictors). Model residuals from the minimal adequate model (Table 2 and S1) are then added. For further discussion of partial residual plots, see Faraway (2005).

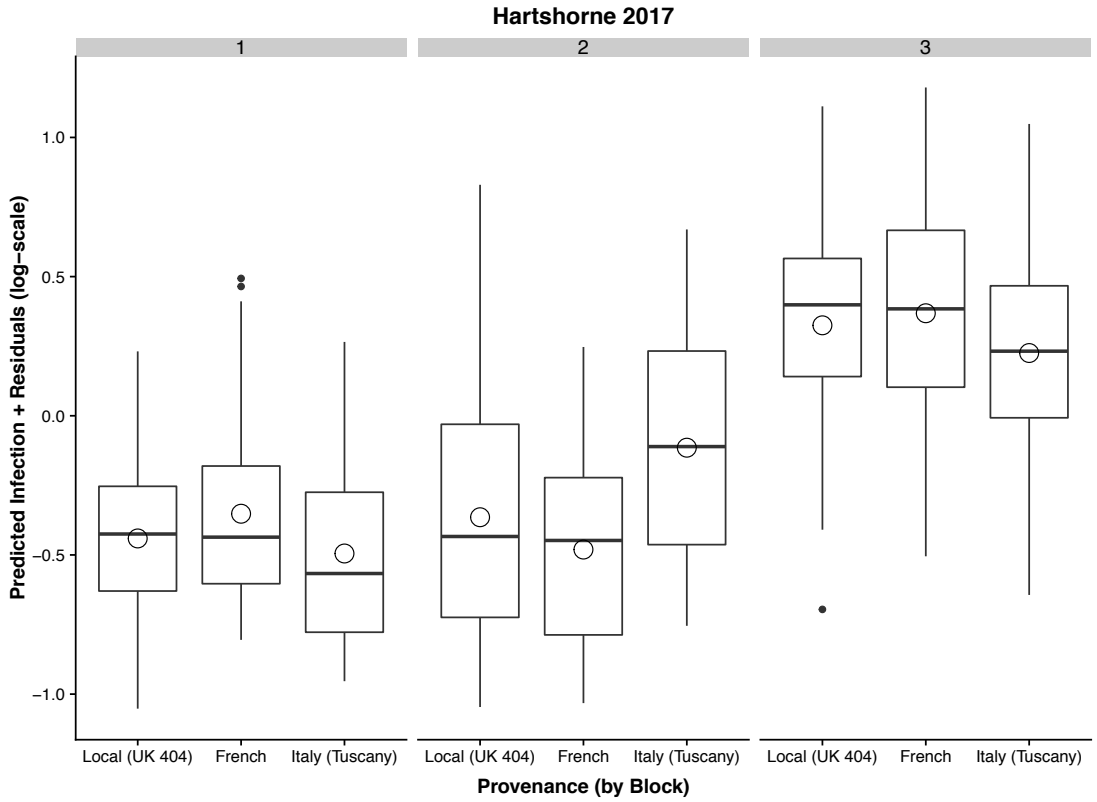


Figure S5: Partial residual plot showing the conditional effects of provenance and block on powdery mildew at Hartshorne in 2017. All other variables are held constant in the model (treatment = provenance monoculture). Different blocks (1-

3) are shown in different panels. The plot shows the lack of consistency in the effect of provenance on powdery mildew infection when the effect of block is also considered. Plotted are means (open black circles), medians (horizontal line), interquartile range (box), upper and lower quartiles (whiskers) and outliers (black dots).

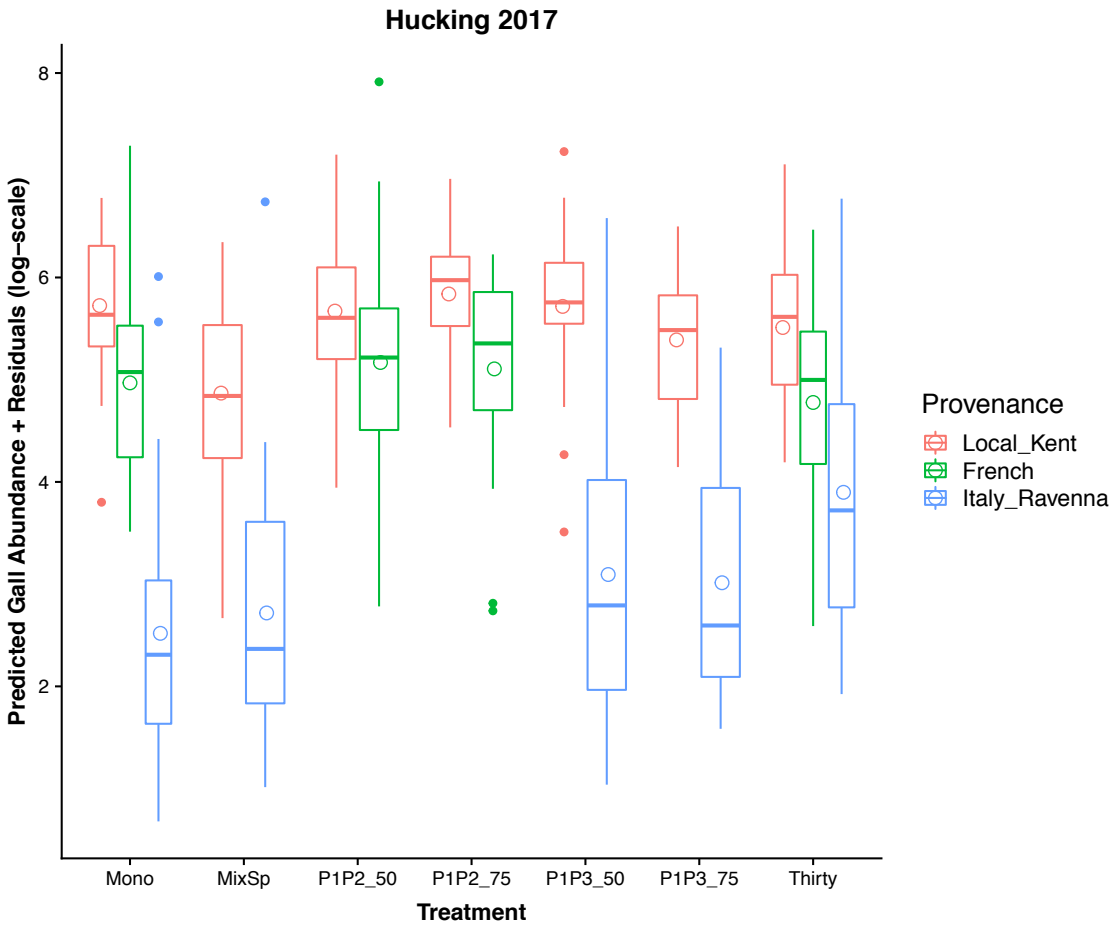
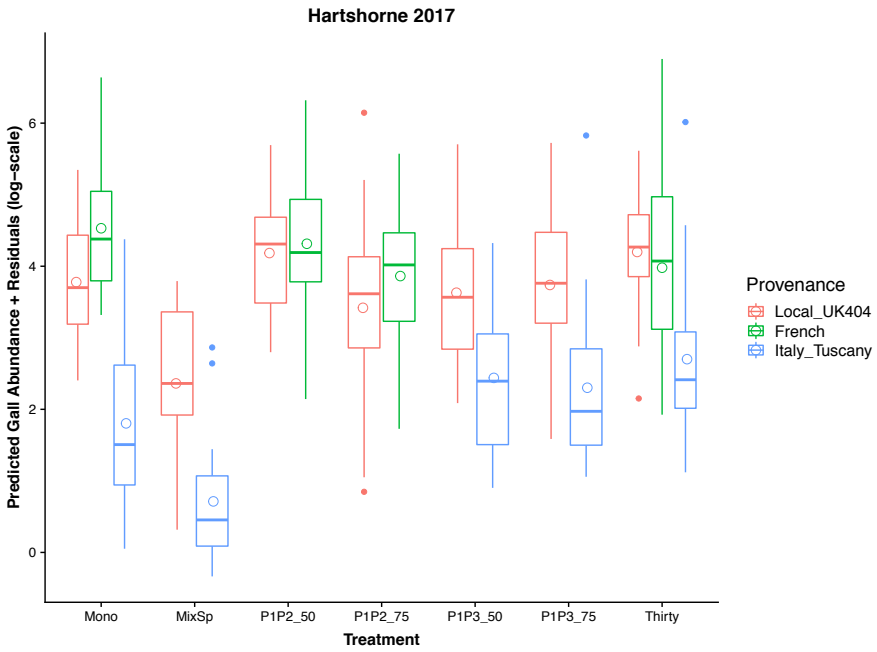


Figure S6: Partial residual plot showing the conditional effects of provenance and treatment on gall abundance at Hucking in 2017. All other variables are held constant in the model (block = 1). Plotted are medians (horizontal line), means (open circles), interquartile range (box), upper and lower quartiles (whiskers) and outliers (dots). Abbreviations for diversity treatments are as follows: Mono (provenance monoculture); MixSp (mixed species, mixed provenance); P1P2_50

(Local:French 50:50 mixture); P1P2_75 (Local:French 75:25 mixture), P1P3_50 (Local:Italian 50:50 mixture); P1P3_75 (Local:Italian 75:25), Thirty (33:33:33 mixture of Local, French and Italian provenances).

BLOCK 1



BLOCK 2

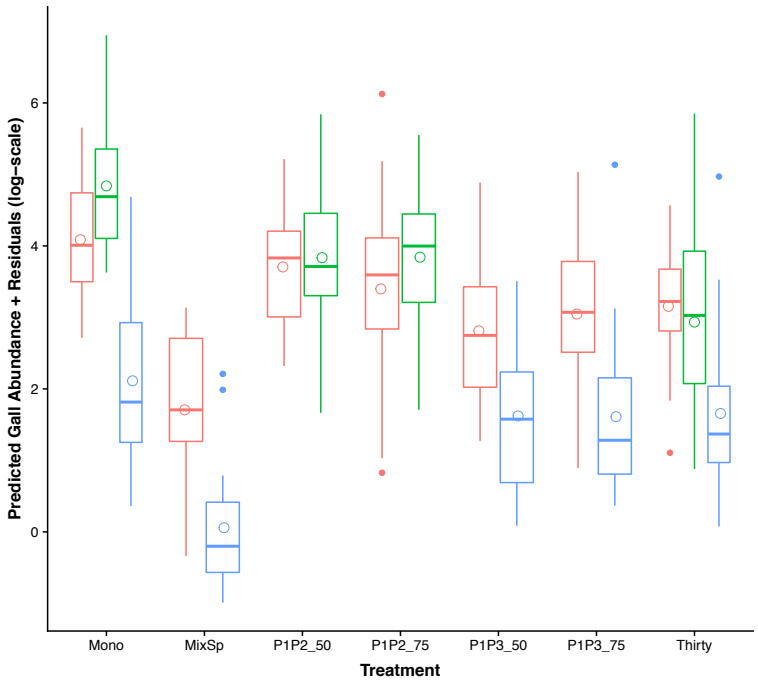


Figure S7: Partial residual plot showing the conditional effects of provenance and treatment on gall abundance at Hartshorne in 2017, in block 1 (top panel) and block 2 (bottom panel), as these two blocks contain the mixed species plots. All other variables are held constant in the model. Plotted are medians (horizontal line), means (open circles), interquartile range (box), upper and lower quartiles (whiskers) and outliers (dots). Differences between mixed species plots and provenance monocultures tested using z tests: for Local trees in Block 1: $z = -2.64$ $p = 0.0084$; for Italian trees in Block 1: $z = -2.24$ $p = 0.025$. Abbreviations for diversity treatments are as follows: Mono (provenance monoculture); MixSp (mixed species, mixed provenance); P1P2_50 (Local:French 50:50 mixture); P1P2_75 (Local:French 75:25 mixture), P1P3_50 (Local:Italian 50:50 mixture); P1P3_75 (Local:Italian 75:25), Thirty (33:33:33 mixture of Local, French and Italian provenances).

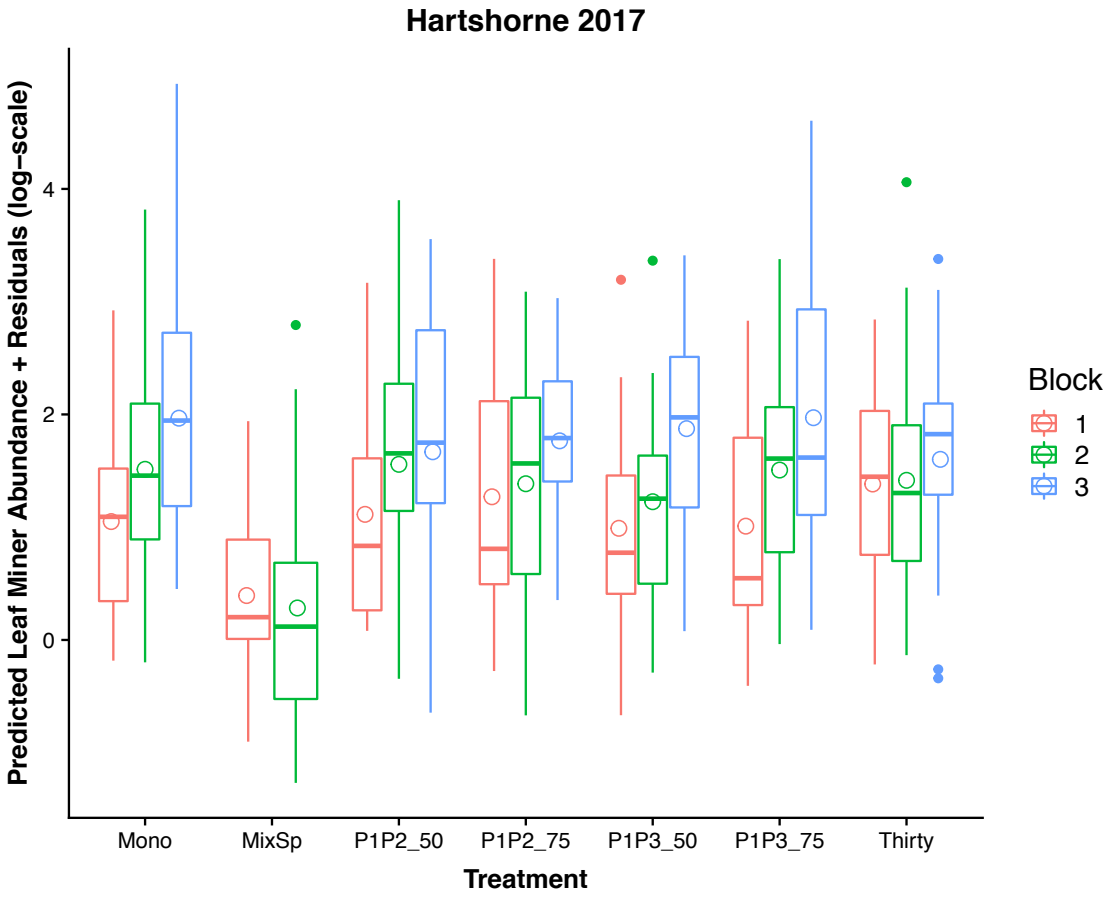
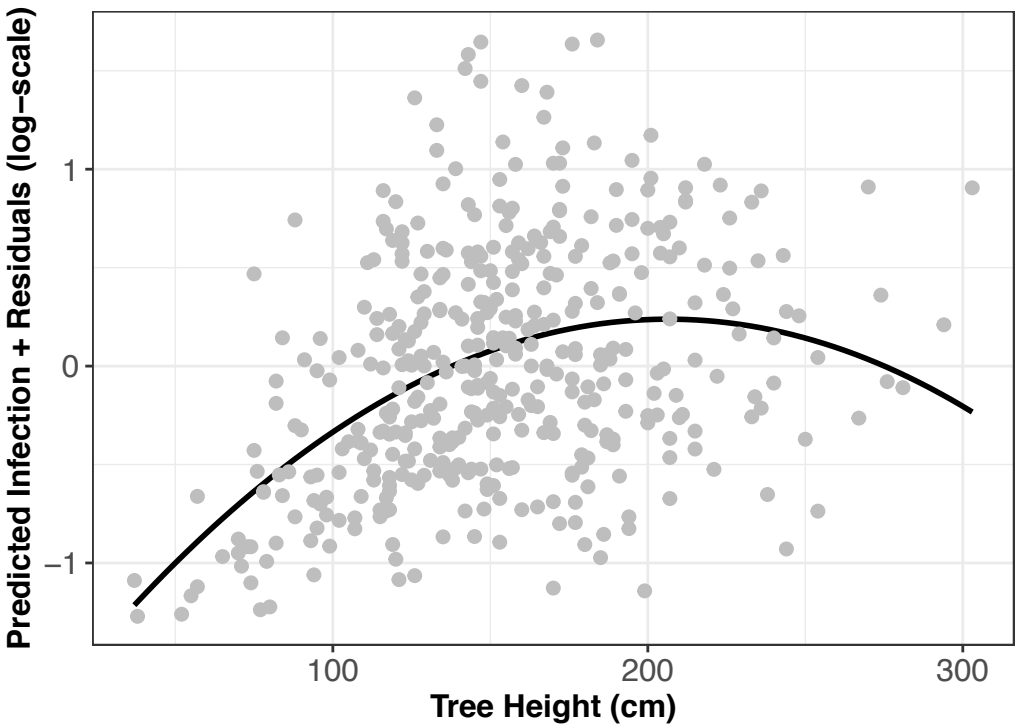


Figure S8: Partial residual plot showing the conditional effects of treatment and block on leaf miner abundance at Hartshorne in 2017. All other variables are held constant in the model (provenance = Local). Plotted are medians (horizontal line), means (open circle), interquartile range (box), upper and lower quartiles (whiskers) and outliers (dots). Abbreviations for diversity treatments are as follows: Mono (provenance monoculture); MixSp (mixed species, mixed provenance); P1P2_50 (Local:French 50:50 mixture); P1P2_75 (Local:French 75:25 mixture), P1P3_50 (Local:Italian 50:50 mixture); P1P3_75 (Local:Italian 75:25), Thirty (33:33:33 mixture of Local, French and Italian provenances).



Figurre S9: Partial residual plot showing the impact of tree height on powdery mildew infection for an updated model where outliers are removed, Hucking 2017. Two trees with heights > 310 cm (see Fig. 4D) have been removed. Grey dots show the predicted infection intensity of powdery mildew to which model residuals are added. The black regression line shows the modelled quadratic relationship between tree height and mildew infection. The quadratic term for tree height remains a significant predictor of mildew infection ($F_{(1,398)}$ = 15.310, p = 0.000107).

APPENDIX 2: SUPPLEMENTARY INFORMATION FOR CHAPTER 3

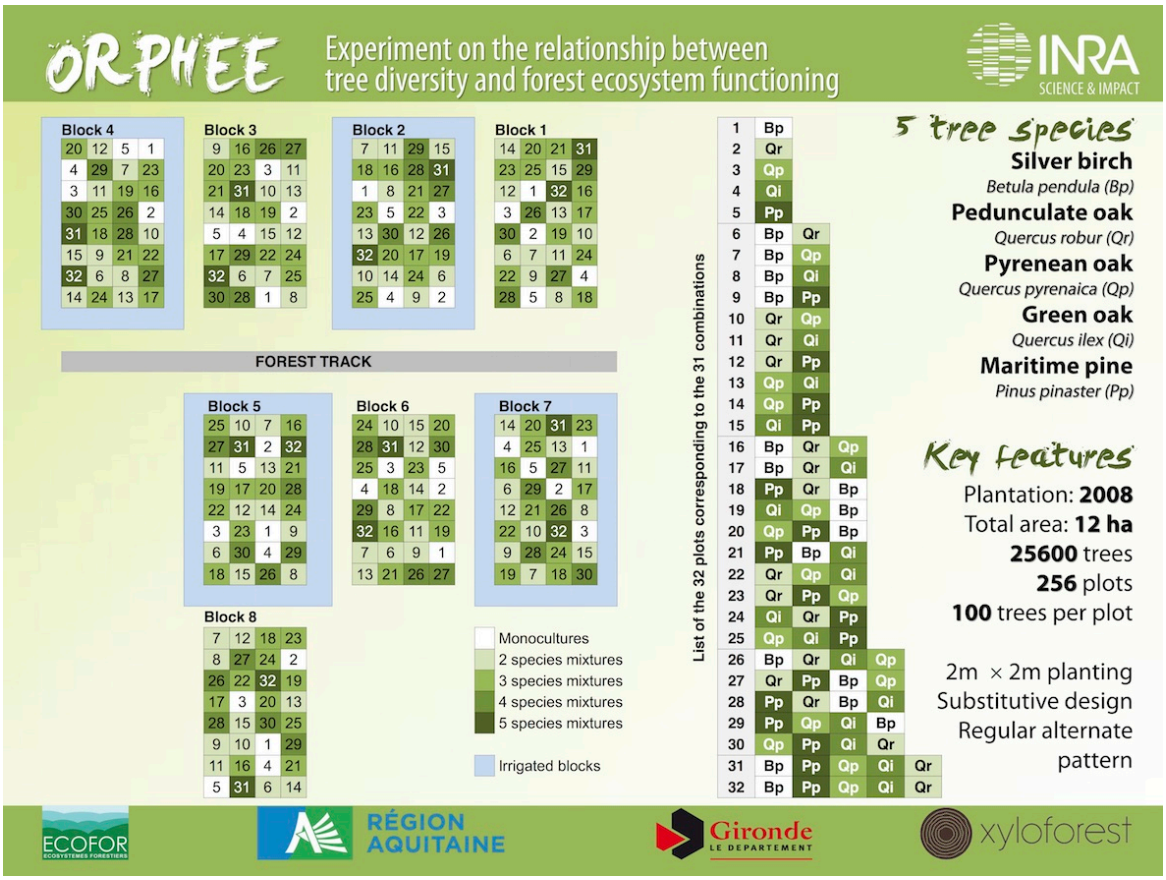


Figure S10: Experimental plan of the ORPHEE tree diversity experiment in SW France (44°440 N, 00°460 W). The experiment was planted on sandy podzolic soil, with former land use as a maritime pine plantation. The experiment consists of 8 blocks of 32 treatments each, in a fully factorial design for tree diversity spanning a gradient from monocultures to five species mixtures with all possible combinations. Irrigated blocks are highlighted in blue. In this study, we surveyed blocks 1-6 only.

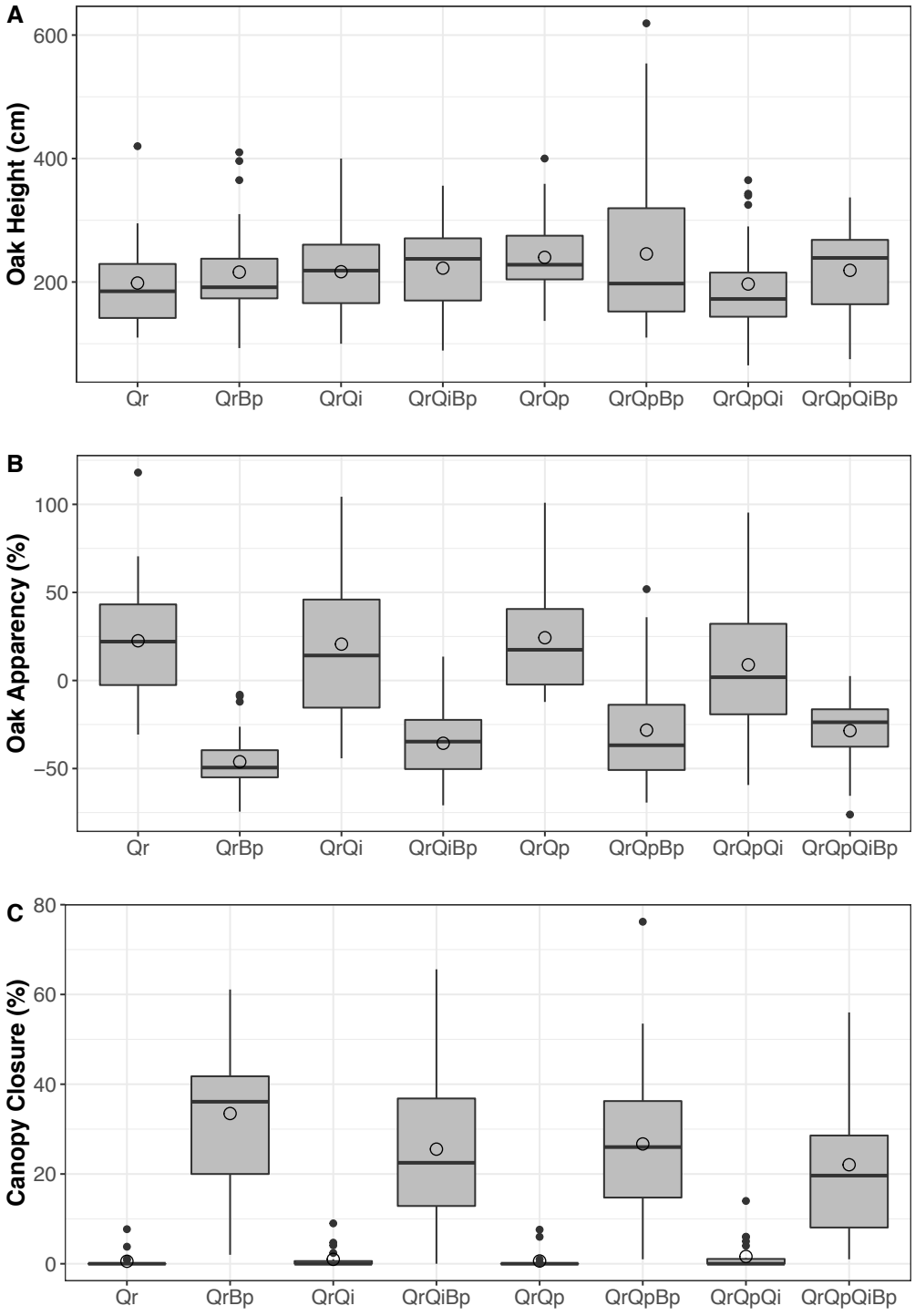
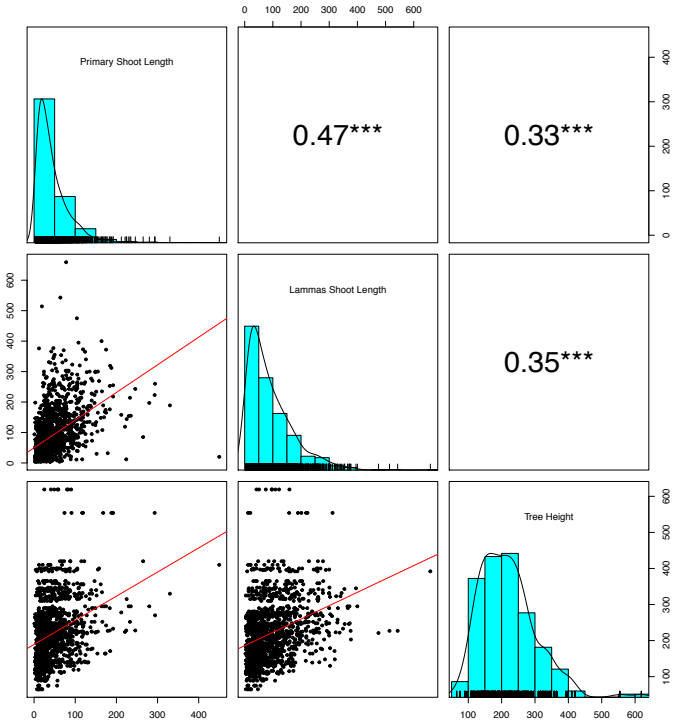
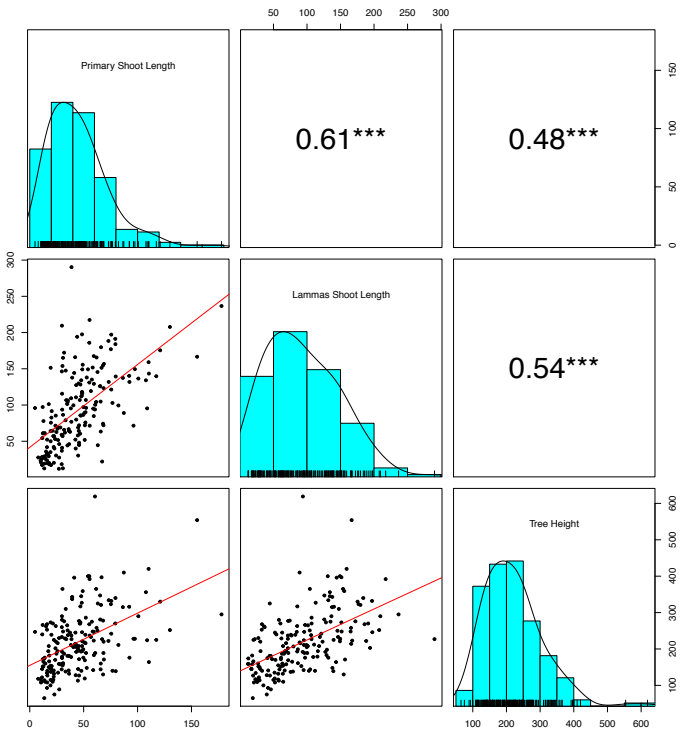


Figure S11: Boxplots showing A) oak (*Quercus robur*) height (cm), B) oak apparency (%) and C) canopy closure (%) between different plot compositions, measured July 2018. Boxes show interquartile range, solid line indicating the

median, black dots indicating the range and open circles giving the mean. Oak height did not significantly differ between plots differing in species composition, nor between irrigated and non-irrigated blocks (Appendix 3). Oak apparency was calculated as: ((Height Focal Oak – Average height of trees in plot) / Average trees in plot)*100, after Castagneyrol, Moreira & Jactel (2018). The canopy closure of the different treatments was also a proxy for apparency (with higher canopy closure indicating lower oak apparency). We assessed canopy closure using two methods (C). The first was directly in the field using the free application for android smartphone, Canopy Cover Free, which uses smartphone images and user defined thresholds. Photos were taken at the same four locations per plot in July, at a standardised height 1.5 m from the ground, and directly assessed in situ using the application's user defined settings. We compared this result with that obtained when the same photos were assessed post hoc using the open source software for Windows, CanopyDigi (Goodenough & Goodenough, 2012). This analysis showed there was no significant difference between the canopy closure assessment from the two methods, suggesting a robust result overall (File S1).



A



B

Figure S12: Correlations between continuous variables used as covariates in models of oak insect herbivory and powdery mildew. (A) Correlations at shoot

level; (B) Correlations at tree level. Upper panels report Pearson's correlation coefficients for pairwise comparisons, with significance stars representing significant level: *p*-values: 0.1 < . < 0.05 < * < 0.01 < ** < 0.001 < ***. Lower panels display pairwise linear regressions between variables, with histogram of each variable and density smoother along the diagonal.

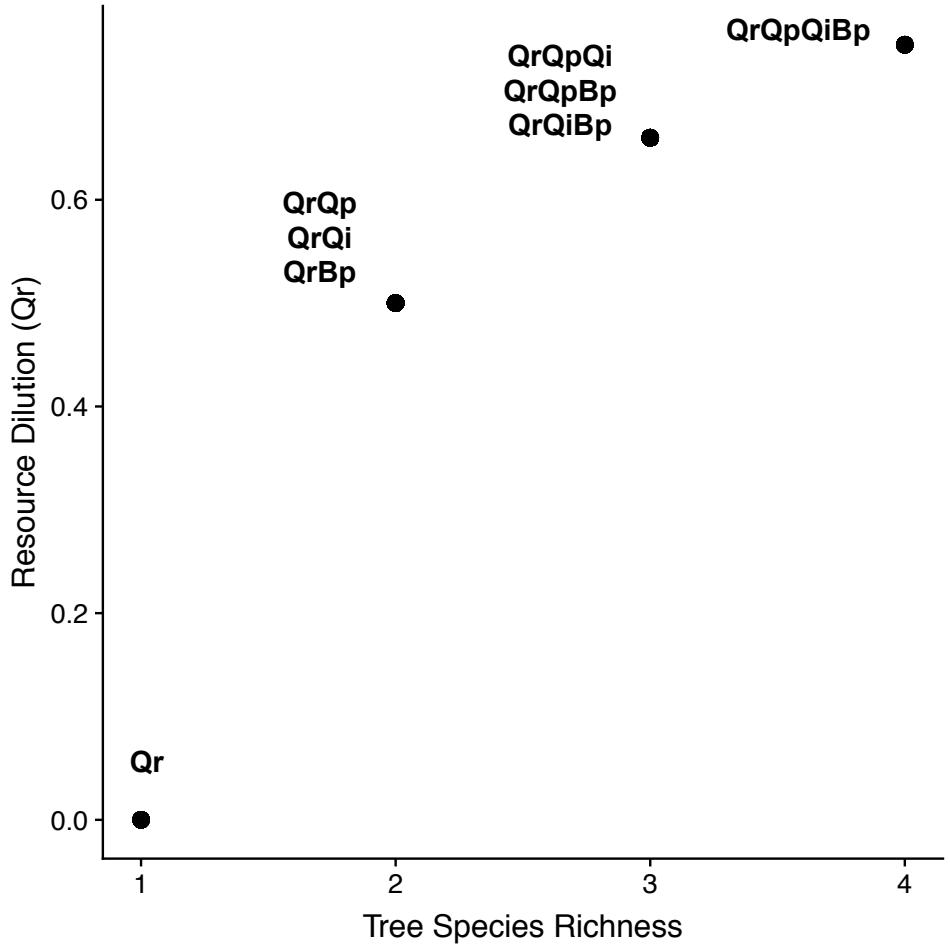


Figure S13: The relationship between tree species richness and resource dilution of *Q. robur* (Qr) in plots surveyed at the ORPHEE experiment. Text next to points represents plot compositions, other species codes are Qp = *Q. pyrenaica;* Qi = *Q.*

ilex; Bp = *Betula pendula.* Tree species richness and resource dilution were highly correlated (Pearson's correlation = 0.90).

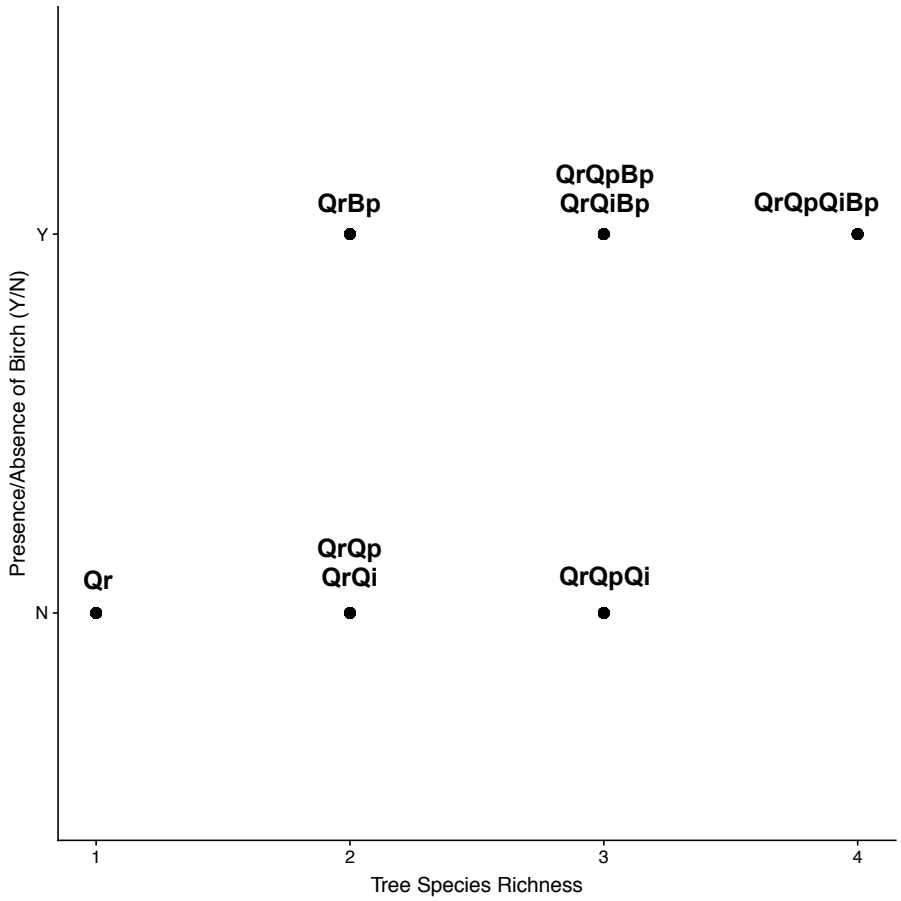


Figure S14: The relationship between tree species richness and presence/absence of birch in plots surveyed at the ORPHEE experiment. Text next to points represents plot compositions, other species codes are Qp = *Q. pyrenaica;* Qi = *Q. ilex;* Bp = *Betula pendula.*

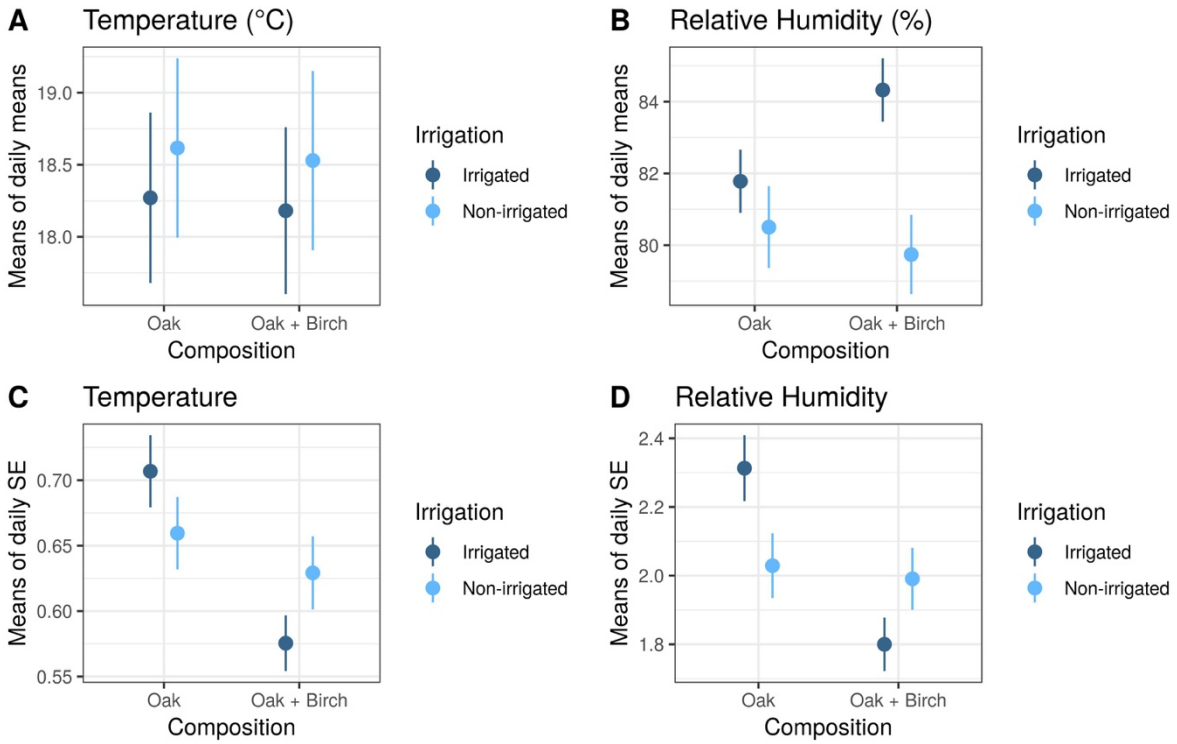


Figure S15: Comparison of microclimate in oak monocultures ('Oak') and oak-birch mixtures ('Oak + Birch'). Temperature (°C) and Relative Humidity (RH, %) were measured every hour from May-October, in 2016, 2017 and 2018. Measurements were taken at the centre of oak monocultures and oak-birch mixtures with HOBO data loggers installed on poles at 1.50m from ground level in blocks 1 to 6. We first calculated daily means per plot and then calculated means across days and blocks. Points and error bars represent means +/- 1*standard error of the mean.

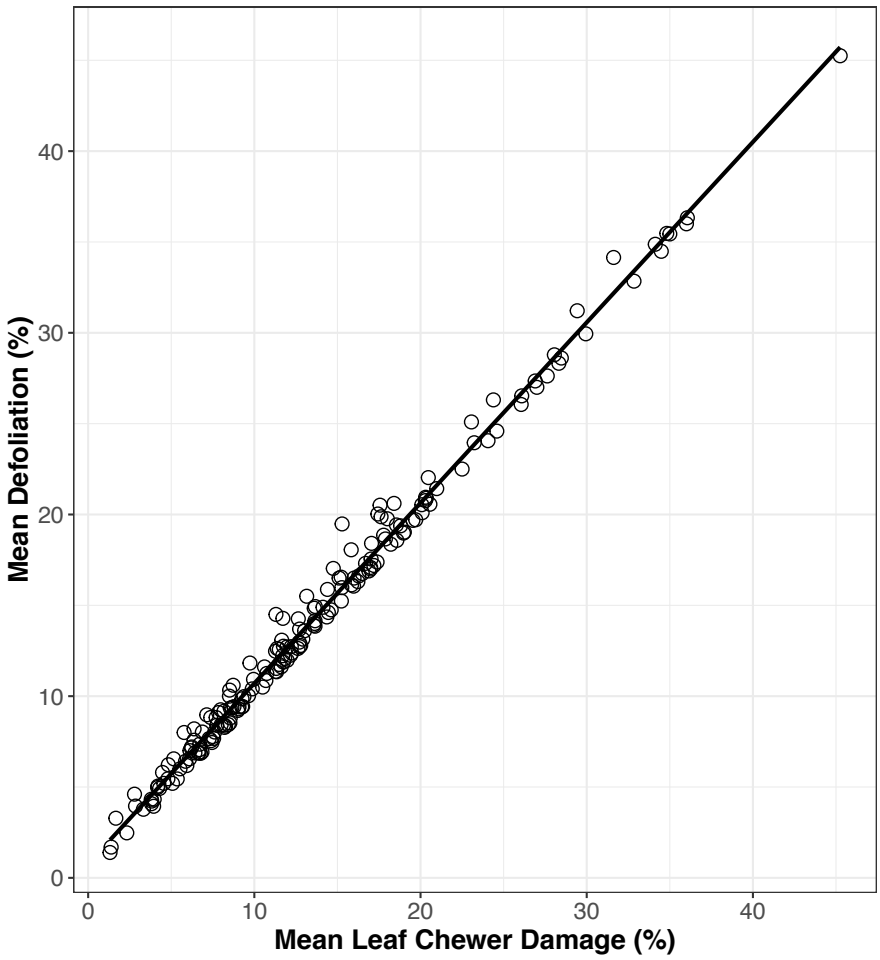


Figure S16: The relationship between mean leaf chewer damage (at tree level per leaf, %) and mean defoliation (at tree level per leaf, %). Open circles are raw data points, the black line is a least squares regression line. Due to the two being so highly correlated (Pearson's correlation coefficient = 0.99), only leaf chewer damage was analysed as a response variable in GLMMs.

	Estimate	SE	$\chi 2$	DF	P-value	Direction	R^2m (R^2c)
LEAF MINER ABUNDANCE (*N = 1520)*							0.030 (0.087)
Intercept	-1.22	0.26	/	/	/	/	
Richness	0.11	0.093	/	1	/	/	
Presence of Birch	-0.27	0.16	/	1	/	/	
Irrigation	0.64	0.30	/	1	/	/	
Log(Primary Shoot Length)	0.30	0.044	47.93	1	<0.001	+	
Richness:Irrigation	-0.38	0.13	7.47	1	0.006	-	
Presence of Birch:Irrigation	0.48	0.23	4.07	1	0.044	+	
LEAF CHEWER DAMAGE (*N = 1520)*							0.062 (0.36)
Intercept	2.72	0.17	/	/	/	/	
Resource Dilution	-0.60	0.23	6.59	1	0.010	-	
Presence of Birch	0.22	0.10	4.87	1	0.027	+	
Irrigation	-0.38	0.19	4.27	1	0.039	-	
OAK POWDERY MILDEW (*N = 1414)*							0.043 (0.52)
Intercept	25.60	6.03	/	/	/	/	
Richness	-3.70	1.88	3.91	1	0.048	-	
Presence of Birch	6.20	3.21	3.77	1	0.052	+	
Log(Lammas Shoot Length)	3.19	0.64	24.62	1	<0.001	+	

Log(Primary Shoot Length)	-1.79	0.79	5.98	1	0.014	-
Tree Height	-0.040	0.015	7.35	1	0.0067	-

Table S4: Results of GLMMs testing the effects of plot composition and tree traits on oak powdery mildew and insect herbivory at shoot level. Given are estimates, standard errors, likelihood ratio test results ($\chi 2$ value, degrees of freedom and p value), and effect direction (+ indicating a positive effect of a variable, - indicating a negative effect of a variable), for minimal adequate models. Estimates for leaf miner and leaf chewer models are on the log scale. Note that for models with interaction effects, likelihood ratio tests cannot be calculated on the main effect without removing all interaction terms from the model, so are not reported here. R^2 values for each model are given in the final column, (R^2m) = marginal R^2 including fixed effects only; (R^2c) = conditional R^2 including both fixed and random effects. The starting model equation, prior to model simplification, is given below (variables in bold are fixed effects, * indicates interaction terms, (1|X) denotes random effects):

Y ~ **log(Shoot Length) + Tree Height + Irrigation * (Resource Dilution + Richness + Birch) +** (1|Tree) + (1|Plot) + (1|Block:Irrigation)

To assess the potential for a direct effect of herbivory on mildew, we also included miner abundance and chewer damage (shoot level) as explanatory terms in models of mildew.

Notes on the shoot level analysis: Results were found to be almost identical between shoot level and tree level models (compare Table 1 and Table S1), so for the sake of clarity, only tree level models are reported in the main text as these models perform better in terms of model assumptions. The one explanatory variable for which the two sets of models differed was the model of oak powdery mildew. Tree height was nonsignificant at tree level but was a significant negative predictor at shoot level ($\chi2 = 7.35$, df = 1, $p = 0.0067$). This difference we treat as minor, as the effect of tree height was not a main question in our study, and since tree height can incorporate the effects of several environmental variables that can be a challenge to disentangle. Moreover, this variable did not emerge as a significant predictor in any other model. In addition, leaf miner abundance was a significant negative predictor of mildew at tree level ($\chi2 = 8.00$, df = 1, $p = 0.0047$), but was nonsignificant at shoot level. This difference is understandable, as the effect of leaf herbivory at shoot level on the growth of individual shoots was likely to be minor / not statistically significant, seeing as this data was zero-inflated. Moreover, we do not expect any mechanistic effect of leaf herbivory on mildew to be occurring at shoot level, but rather affecting overall tree level physiology and growth responses (see main Discussion of Chapter 3).

APPENDIX 3: SUPPLEMENTARY ANALYSIS DOCUMENT FOR CHAPTER 3

This supplementary document was submitted to the journal as an R Markdown, and is inserted as is here.

Supplementary Analysis Document - Tree Apparency, Canopy Closure, Tree Height

Elsa Field

03/07/2019

Contents

Differences in Tree Apparency Between Plots **2**
Apparency versus Plot Composition 2

Differences in Canopy Closure Between Plots **4**
Canopy Closure - Method Comparison 4
Canopy Closure versus Plot Composition 5

Differences in Tree Height between Plots **10**

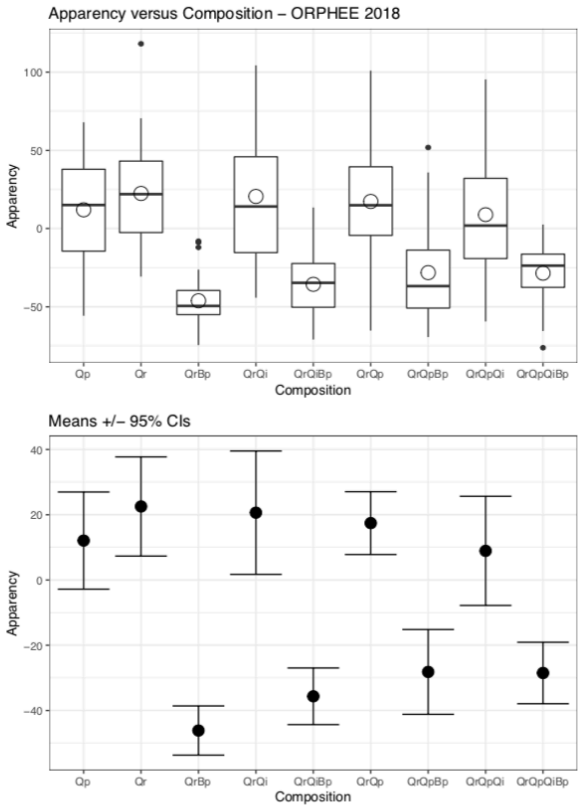
Differences in Tree Apparency Between Plots

Where apparency is defined as:

((Height Focal Tree - Mean Height Trees in Plot)/ Mean Height Trees in Plot) *100

Apparency versus Plot Composition

Differences in apparency between plots differing in composition:



In plots with birch mixed with oak, focal oaks have lower apparency, as expected.

Formalise this with a linear model of the form:

Apparency ~ Composition

```
##
## Call:
## lm(formula = Apparency ~ Composition, data = data.apparency)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -82.597 -20.985  -1.724  18.092  95.525
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)           22.543      6.618   3.407 0.000777 ***
## CompositionQp        -10.458      9.359  -1.117 0.264987
## CompositionQrBp      -68.685      9.359  -7.339 3.71e-12 ***
## CompositionQrQi       -1.899      9.359  -0.203 0.839350
## CompositionQrQiBp    -58.191      9.359  -6.218 2.36e-09 ***
## CompositionQrQp       -5.100      8.105  -0.629 0.529849
## CompositionQrQpBp    -50.717      9.359  -5.419 1.51e-07 ***
## CompositionQrQpQi    -13.612      9.359  -1.454 0.147199
## CompositionQrQpQiBp  -51.045      9.569  -5.334 2.30e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 32.42 on 229 degrees of freedom
## Multiple R-squared:  0.3958, Adjusted R-squared:  0.3747
## F-statistic: 18.75 on 8 and 229 DF,  p-value: < 2.2e-16
```

```
## Analysis of Variance Table
##
## Response: Apparency
##              Df Sum Sq Mean Sq F value    Pr(>F)
## Composition   8 157655   19707   18.75 < 2.2e-16 ***
## Residuals   229 240687    1051
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

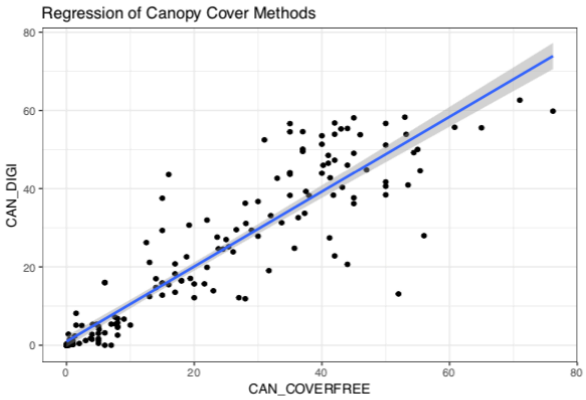
Quercus robur monocultures are only significantly different from mixtures with *Betula pendula*, as expected. Plant apparency is significantly lowered when focal *Quercus robur* trees are mixed with *B. pendula*, but not with other oak species.

Differences in Canopy Closure Between Plots

Canopy Closure - Method Comparison

We used two methods to determine canopy closure. The first was directly in the field, using the free open-source app "Canopy Cover Free" for Android, the second was post-hoc on the same smartphone images collected in the field, using the free open-source software "CanopyDigi" (link here) for Windows.

A regression plot illustrates the close relationship between the canopy closure data generated using the two methods:



We used a paired t test to compare the two methods, to show that the canopy closure estimated by the "quick and dirty" method (smartphone in the field) and the more laborious post-hoc method were not significantly different.

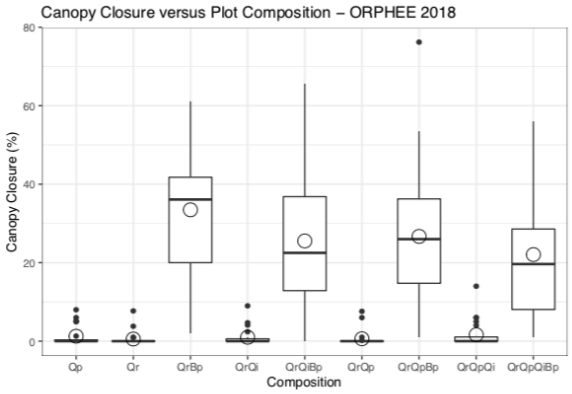
```
##
##  Paired t-test
##
## data:  can_wide_nonas$CAN_COVERFREE and can_wide_nonas$CAN_DIGI
## t = -0.40964, df = 207, p-value = 0.6825
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -1.2577509  0.8249913
## sample estimates:
## mean of the differences
##              -0.2163798
```

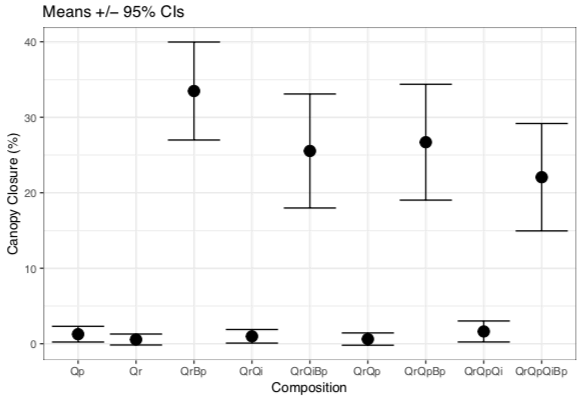
This was the case even when we conducted a t test where the photos with canopy cover = 0% were removed, as it was likely that both methods could distinguish a canopy cover of 0, regardless of the different light thresholds used between the two methods.

```
##
##  Paired t-test
##
## data:  no_z$CAN_COVERFREE and no_z$CAN_DIGI
## t = -0.40496, df = 142, p-value = 0.6861
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -1.831637  1.208784
## sample estimates:
## mean of the differences
##              -0.3114266
```

Canopy Closure versus Plot Composition

We carry out a factorial ANOVA of canopy closure against plot composition, to test the hypothesis that plots with birch have higher canopy closure (and thus, canopy closure is a proxy of oak apparency).





For the statistical analysis, we use the data collected in situ with the app Canopy Cover Free, as the t tests above showed there was no significant difference between plots.

```
##
## Call:
## lm(formula = CAN_COVER ~ Composition, data = can_comps)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -31.475  -1.625  -0.617   2.985  49.504
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)          0.55833    2.35497   0.237    0.813
## CompositionQp        0.70833    3.33044   0.213    0.832
## CompositionQrBp     32.91667    3.33044   9.884  < 2e-16 ***
## CompositionQrQi      0.42500    3.33044   0.128    0.899
## CompositionQrQiBp   24.97500    3.33044   7.499 1.86e-12 ***
## CompositionQrQp      0.05833    3.33044   0.018    0.986
## CompositionQrQpBp   26.13750    3.33044   7.848 2.23e-13 ***
## CompositionQrQpQi    1.06667    3.33044   0.320    0.749
## CompositionQrQpQiBp 21.50000    3.33044   6.456 7.53e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 11.54 on 207 degrees of freedom
## Multiple R-squared:  0.5766, Adjusted R-squared:  0.5603
## F-statistic: 35.24 on 8 and 207 DF,  p-value: < 2.2e-16
```

```
## Analysis of Variance Table
##
## Response: CAN_COVER
##              Df Sum Sq Mean Sq F value    Pr(>F)
## Composition   8  37526  4690.8  35.242 < 2.2e-16 ***
```

```
## Residuals   207  27552   133.1
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

This confirms our hypothesis of significant differences only between plots containing birch, and plots with oak.

Alternatively, we could analyse this data as a mixed effect model analysis, in order to account for the residual variation accounted for by differences between plots nested within blocks. This would be a model of the form:

$$\text{Canopy Closure} \sim \text{Composition} + (1|\text{Block/Plot})$$

```
lmer_can <- lmer(CAN_COVER ~ Composition + (1|BLOCK/PLOT), data = can_comps)
summary(lmer_can)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: CAN_COVER ~ Composition + (1 | BLOCK/PLOT)
##    Data: can_comps
##
## REML criterion at convergence: 1577.8
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.5792 -0.2817 -0.0566  0.1201  3.6064
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  PLOT:BLOCK (Intercept) 54.69    7.395
##  BLOCK      (Intercept) 10.96    3.310
##  Residual               76.02    8.719
## Number of obs: 216, groups:  PLOT:BLOCK, 54; BLOCK, 6
##
## Fixed effects:
##                     Estimate Std. Error t value
## (Intercept)          0.55833    3.75607   0.149
## CompositionQp        0.70833    4.95623   0.143
## CompositionQrBp     32.91667    4.95623   6.641
## CompositionQrQi      0.42500    4.95623   0.086
## CompositionQrQiBp   24.97500    4.95623   5.039
## CompositionQrQp      0.05833    4.95623   0.012
## CompositionQrQpBp   26.13750    4.95623   5.274
## CompositionQrQpQi    1.06667    4.95623   0.215
## CompositionQrQpQiBp 21.50000    4.95623   4.338
##
## Correlation of Fixed Effects:
##             (Intr) CmpstQ CmpsQB CompstnQrQ CmpstnQrQBp CmpstnQrQp
## CompositnQp -0.660
## CompstnQrBp -0.660  0.500
## CompostnQrQ -0.660  0.500  0.500
```

```
## CmpstnQrQBp -0.660  0.500  0.500  0.500
## CompstnQrQp -0.660  0.500  0.500  0.500       0.500
## CmpstnQrQpB -0.660  0.500  0.500  0.500       0.500       0.500
## CmpstnQrQpQ -0.660  0.500  0.500  0.500       0.500       0.500
## CmpstnQrQQB -0.660  0.500  0.500  0.500       0.500       0.500
##             CmpstnQrQpB CmpQQQ
## CompositnQp
## CompstnQrBp
## CompostnQrQ
## CmpstnQrQBp
## CompstnQrQp
## CmpstnQrQpB
## CmpstnQrQpQ  0.500
## CmpstnQrQQB  0.500       0.500
```

```
drop1(lmer_can, test = "Chi")
```

```
## boundary (singular) fit: see ?isSingular
```

```
## Single term deletions
##
## Model:
## CAN_COVER ~ Composition + (1 | BLOCK/PLOT)
##             Df    AIC   LRT   Pr(Chi)
## <none>         1640.9
## Composition  8 1694.4 69.46 6.293e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

In the mixed effect model, composition remains a highly significant predictor of canopy closure.

Note the similarity of t values between the linear model summary output and the linear mixed effect model summary output.

We can use multiple comparison of means (multcomp::glht()) to test for differences between different compositions (z tests, using default package settings).

```
levels(can_comps$Composition)
```

```
## [1] "Qr"       "Qp"       "QrBp"     "QrQi"     "QrQiBp"   "QrQp"
## [7] "QrQpBp"   "QrQpQi"   "QrQpQiBp"
```

```
#create vector of contrasts
vec.1 <- c(1/5,1/5,-1/4,1/5,-1/4,1/5,-1/4,1/5,-1/4)
#apply this vector to data frame
names(vec.1) <- levels(can_comps$Composition)
#run multiple comparison of means
pht.1 <- glht(model = lmer_can, linfct = mcp(Composition = vec.1))
summary(pht.1)
```

```
##
##   Simultaneous Tests for General Linear Hypotheses
```

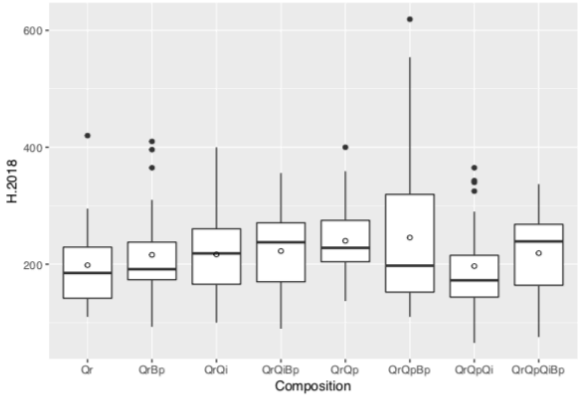
```
##
## Multiple Comparisons of Means: User-defined Contrasts
##
##
## Fit: lmer(formula = CAN_COVER ~ Composition + (1 | BLOCK/PLOT), data = can_comps)
##
## Linear Hypotheses:
##        Estimate Std. Error z value Pr(>|z|)
## 1 == 0  -25.931      2.351  -11.03   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

The multiple comparison of means compares plots with just oak (5/9 plots) to plots with oak and birch (4/9 plots). In the summary, the estimate is negative because we compared Plots with just Oak, to Oak + Birch plots. This shows us that Oak + Birch Plots have higher canopy closure than Plots with just Oak (25.931% more on average, SE = 1.496)

Our conclusion from both statistical anlalyses is that composition is a highly significant predictor of canopy cover, with the only significant differences being between mixtures with birch and mixtures with just oak species.

Differences in Tree Height between Plots

Finally, we check whether apparency and tree height are truly separated, by testing the effect of composition on tree height.



The graph above suggests that plot composition does not impact tree height.

We can use linear mixed effect models and linear models to test for significant composition effects on tree growth:

```
#TEST COMPOSITION EFFECTS ON OAK HEIGHT
m1 <- lmer(H.2018 ~ Composition + (1|Block/Plot), data = Qr)
drop1(m1, test = "Chi") #Composition not a significant predictor of tree height
```

```
## Single term deletions
##
## Model:
## H.2018 ~ Composition + (1 | Block/Plot)
##             Df    AIC    LRT Pr(Chi)
## <none>         2223.7
## Composition  7 2213.7 3.9758  0.7826
```

```
lm1 <- lm(H.2018 ~ Composition, data = Qr)
summary(lm1) #no significant differences between Qr and Qr mixes.
```

```
##
## Call:
## lm(formula = H.2018 ~ Composition, data = Qr)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -143.91  -58.22  -16.71   43.90  373.42
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
```

```
## (Intercept)          198.375     17.373  11.419   <2e-16 ***
## CompositionQrBp       17.667     24.569   0.719   0.4730
## CompositionQrQi       18.292     24.569   0.745   0.4575
## CompositionQrQiBp     24.125     24.569   0.982   0.3274
## CompositionQrQp       41.583     24.569   1.693   0.0923 .
## CompositionQrQpBp     47.208     24.569   1.921   0.0562 .
## CompositionQrQpQi     -1.625     24.569  -0.066   0.9473
## CompositionQrQpQiBp   20.534     25.121   0.817   0.4148
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 85.11 on 182 degrees of freedom
## Multiple R-squared:  0.03668,    Adjusted R-squared:  -0.0003659
## F-statistic: 0.9901 on 7 and 182 DF,  p-value: 0.4399
```

We can conclude from both modelling approaches that there are no significant differences between compositions in terms of oak tree height.

```r
sessionInfo()
```

```
## R version 3.5.3 (2019-03-11)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Mojave 10.14.5
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_GB.UTF-8/en_GB.UTF-8/en_GB.UTF-8/C/en_GB.UTF-8/en_GB.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] multcomp_1.4-10 TH.data_1.0-10  MASS_7.3-51.4   survival_2.43-3
## [5] mvtnorm_1.0-10  lme4_1.1-21     Matrix_1.2-15   dplyr_0.8.0.1
## [9] ggplot2_3.1.1
##
## loaded via a namespace (and not attached):
##  [1] zoo_1.8-5           tidyselect_0.2.5    xfun_0.6
##  [4] purrr_0.3.2         splines_3.5.3       lattice_0.20-38
##  [7] colorspace_1.4-1    htmltools_0.3.6     yaml_2.2.0
## [10] base64enc_0.1-3     rlang_0.3.4         pillar_1.3.1
## [13] nloptr_1.2.1        foreign_0.8-71      glue_1.3.1
## [16] withr_2.1.2         RColorBrewer_1.1-2  plyr_1.8.4
## [19] stringr_1.4.0       munsell_0.5.0       gtable_0.3.0
## [22] htmlwidgets_1.3     codetools_0.2-16    evaluate_0.13
## [25] labeling_0.3        latticeExtra_0.6-28 knitr_1.22
## [28] htmlTable_1.13.1    Rcpp_1.0.1          acepack_1.4.1
## [31] backports_1.1.4     checkmate_1.9.3     scales_1.0.0
## [34] Hmisc_4.2-0         gridExtra_2.3       digest_0.6.18
## [37] stringi_1.4.3       grid_3.5.3          tools_3.5.3
## [40] sandwich_2.5-1      magrittr_1.5        lazyeval_0.2.2
## [43] tibble_2.1.1        Formula_1.2-3       cluster_2.0.7-1
## [46] crayon_1.3.4        pkgconfig_2.0.2     data.table_1.12.2
## [49] rstudioapi_0.10     assertthat_0.2.1    minqa_1.2.4
## [52] rmarkdown_1.12      rpart_4.1-13        R6_2.4.0
## [55] boot_1.3-20         nnet_7.3-12         nlme_3.1-137
## [58] compiler_3.5.3
```

Appendix 4: Supplementary Information for chapter 4



Figure S17: Oak Bud Burst stages (as assessed in March 2018).

From left to right, top to bottom:

STAGE 1: dormant bud

STAGE 2: green visible below bud scales, bud has swollen, no leaves visible

STAGE 3: leaves protrude beyond

STAGE 4: leaves have begun to fully unfurl beyond edge of bud

STAGE 5: leaves fully unfurled and are taking on mature dark green colour

Appendix 5: Supplementary Information for chapter 5

Description of scoping searches and main literature search

A scoping study conducted using the database Scopus was used to identify the search terms for use in bibliographic databases and internet searches. This was done by testing search strings in Scopus using the Field Tag (TITLE-ABS-KEY) to search for relevant titles, abstracts and keywords. Search terms were sourced from previous meta-analyses and systematic reviews on themes of mixed forests and/or insect herbivory and forest pathogens, and from the keywords of studies identified *a priori* as highly relevant (see Appendix I). Search results were ordered based on relevance and limited to studies in the 'Agriculture and Biological sciences' and 'Environmental Sciences' topics to remove results from non-relevant subjects. For each search combination, the total number of hits and the number of relevant papers (assessed based on title) in the first 200 hits was recorded. In addition, each search combination was scrutinised for its ability to retrieve a list of **12** studies identified a priori as highly relevant to the primary question (test library, see below). These studies were sourced from the personal libraries of the authors and stakeholders. Although all contain information relevant to the primary question, they utilise a range of observational and experimental study systems and the phrasing related to the primary question differs in each case; not all combinations of search terms were able to retrieve all of these papers. The final search terms were able to retrieve all of these papers, and achieved a good balance between relevant and non-relevant studies in the first 200 results.

The final search terms and their relevance to each of the PECO question elements is summarised in Table S5. Table S6 shows the exact search terms used in each database, the number of hits returned when initially searched, and search date. The large discrepancy in numbers of hits returned from Scopus compared to Web of Science and CABI is likely to be due to the restricted field tag used (TITLE-ABS-KEY) combined with differences in the literature available from Scopus, as without use of this field tag the number of hits returned by this database are typically extremely high (a comparison search using the same search terms without this field tag in Scopus returned over 37,000 hits).

Due to the large number of search results from irrelevant topics, the results of the Scopus search was limited to journals in the "Agricultural and Biological Sciences" and "Environmental Science" categories. In Web of Science, results of searches was limited to the following research areas in order to narrow the search: Agriculture OR Forestry OR Environmental Sciences OR Ecology OR Plant sciences.

PECO question component	Terms	Search terms
Population	Forest	tree* OR forest* OR plantation OR wood* OR orchard OR coppice OR shrub OR abies OR acer OR betula OR eucalyptus OR fagus OR fraxinus OR picea OR pinus OR populus OR quercus
Exposure	Mixed Stands	"mixed species" OR "mixed-species" OR "mixed stand*" OR "tree divers*" OR "tree species divers*" OR "host species divers*" OR "host divers*" OR "community divers*" OR "forest divers*" OR "forest species divers*" OR "divers* stand" OR "divers* plant*" OR "divers* forest" OR "divers* wood*" OR "tree species rich*" OR "host species rich*" OR "forest species rich*" OR polyculture* OR "landscape heterogen*" OR "heterogen* landscape" OR "species mix*" OR "diversity-disease" OR "species composition"
Comparator	Forest Pathogen Damage	pathogen OR disease OR infection OR "root rot" OR "butt rot" OR fung* OR lesion OR canker OR bacteria* OR viral OR virus OR Phytophthora OR Armillaria OR Heterobasidion

Table S5: List of search strings used in bibliographic databases and their relationship to each component of the primary question. Each section was combined using 'AND' (for exact search strings with Boolean operators used in each database, see Table S2).

Database	Search string used	Number of hits	Date
Scopus	(TITLE-ABS-KEY ((tree* OR forest* OR plantation OR wood* OR orchard OR coppice OR shrub OR abies OR acer OR betula OR eucalyptus OR fagus OR fraxinus OR picea OR pinus OR populus OR quercus) AND ("mixed species" OR "mixed-species" OR "mixed stand*" OR "tree divers*" OR "forest divers*" OR "tree species divers*" OR "host species divers*" OR "host divers*" OR "community divers*" OR "forest species divers*" OR "divers* stand" OR "divers* plant*" OR "divers* forest" OR "divers* wood*" OR "tree species rich*" OR "host species rich*" OR "forest species rich*" OR polyculture* OR "landscape heterogen*" OR	1,744 (limited to Topics: Agricultural/Environmental Science and Biological Sciences)	13th December 2017

	"heterogen* landscape" OR "species mix*" OR "diversity-disease" OR "species composition") AND (pathogen OR disease OR infection OR "root rot" OR "butt rot" OR fung* OR lesion OR canker OR bacteria* OR viral OR virus OR phytophthora OR armillaria OR heterobasidion))) AND (LIMIT-TO (SUBJAREA , "AGRI ") OR LIMIT-TO (SUBJAREA , " ENVI "))		
Web of Science (All databases: WoS Core Collection 1945-present, BioSIS Citation Index, Current Contents Connect, Data Citation Index,	TS = (tree* or forest* or plantation or wood* or orchard or coppice or shrub or abies or acer or betula or eucalyptus or fagus or fraxinus or picea or pinus or populus or quercus) AND TS = ("mixed species" or "mixed-species" or "mixed stand*" or "tree divers*" or "tree species divers*" or "forest divers*" or "host species divers*" or "host divers*" or "community divers*" or "forest species divers*" or "divers* stand" or "divers* plant*" or "divers* forest" or "divers*	5,703 (limited to Research Areas within Science and Technology: (Agriculture OR Forestry OR Environmental Sciences OR Ecology OR Plant sciences)	13th December 2017

Derwent Innovations Index, Inspec, KCI-Korean Journal Database, MEDLINE, Russian Science Citation Index, SciELO Citation Index, Zoological Record)	wood*" or "tree species rich*" or "host species rich*" or "forest species rich*" or polyculture* or "landscape heterogen*" or "heterogen* landscape" or "species mix*" or "diversity-disease" or "species composition") AND TS = (pathogen or disease or infection or "root rot" or "butt rot" or fung* or lesion or canker or bacteria* or viral or virus or Phytophthora or Armillaria or Heterobasidion)		
CAB Abstracts (Database: 1910 – 2017)	(tree* or forest* or plantation or wood* or orchard or coppice or shrub or abies or acer or betula or eucalyptus or fagus or fraxinus or picea or pinus or populus or quercus) AND ("mixed species" or "mixed-species" or "mixed stand*" or (mixed adj1 stand) or "tree divers*" or "tree species divers*" or "host species divers*" or "host divers*" or "community divers*" or	2,756 Searching abstract, title, original title, broad terms, heading words, identifiers, cabicodes	13th December 2017

	"forest divers*" or "forest species divers*" or "tree species divers*" or (forest adj1 divers*) or (tree adj1 divers*) or "divers* stand" or "divers* plant*" or "divers* forest" or "divers* wood*" or "tree species rich*" or "host species rich*" or "forest species rich*" or polyculture* or "landscape heterogen*" or "heterogen* landscape" or "species mix*" or "diversity-disease" or "species composition") AND (pathogen or disease or infection or "root rot" or "butt rot" or fung* or lesion or canker or bacteria* or viral or virus or Phytophthora or Armillaria or Heterobasidion)		

Table S6: List of search strings used in each major database queried during the systematic review, with number of hits returned and date initially searched.

NB: Google Scholar does not permit the use of Boolean operators, so search terms were modified and the first 1000 results downloaded from Google Scholar. We tried several search term combinations in Google Scholar to maximise the number of test library studies found while minimising duplication with the rest of the

literature searches. Final search terms used in Google Scholar were: ' "mixed stand" forest pathogen ' (Search completed on 21st February 2018).

Test Library used in Scoping Searches

1. Dillen, Mathias, Kris Verheyen, and Christian Smit. 2016. Identity rather than Richness Drives Local Neighbourhood Species Composition Effects on Oak Sapling Growth in a Young Forest. Forest Ecology and Management 380 (November): 274–84. doi:10.1016/j.foreco.2016.09.004.

2. DeLong RL, Lewis KJ, Simard SW, Gibson S. (2002). Fluorescent pseudomonad population sizes baited from soils under pure birch, pure Douglas-fir, and mixed forest stands and their antagonism toward Armillaria ostoyae in vitro. Can. J. For. Res. 32:2146–59.

3. Filip, G. M., Bronson, J. J., Chadwick, K. L., Filip, J. B., Frankel, S. J., Goheen, D. J et al (2015). Precommercial Thinning in Mixed-Species Conifer Plantations Affected by Armillaria and Heterobasidion Root Diseases in West-Central Oregon and Washington: 30-Year Results. Forest Science.

4. Gerlach, J P, P B Reich, K Puettmann, and T Baker. 1997. Species, Diversity, and Density Affect Tree Seedling Mortality from Armillaria Root Rot. Canadian Journal of Forest Research 27 (9): 1509–12. doi:10.1139/x97-098.

5. Haas, Sarah E., Mevin B. Hooten, David M. Rizzo, and Ross K. Meentemeyer. 2011. Forest Species Diversity Reduces Disease Risk in a Generalist Plant Pathogen Invasion. Ecology Letters 14 (11): 1108–16. doi:10.1111/j.1461-0248.2011.01679.x.

6. Hantsch, Lydia, Steffen Bien, Stine Radatz, Uwe Braun, Harald Auge, and Helge Bruelheide. 2014. Tree Diversity and the Role of Non-Host Neighbour Tree Species in Reducing Fungal Pathogen Infestation. Journal of Ecology 102 (6): 1673–87. doi:10.1111/1365-2745.12317.

7. Hantsch, Lydia, Uwe Braun, Michael Scherer-Lorenzen, and Helge Bruelheide. 2013. Species Richness and Species Identity Effects on Occurrence of Foliar Fungal Pathogens in a Tree Diversity Experiment. Ecosphere 4 (7): 1–12.

8. Jules ES, Kauffman MJ, Ritts WD, Carroll AL. (2002). Spread of an Invasive Pathogen over a Variable Landscape: A Nonnative Root Rot on Port Orford Cedar. Ecology 83:3167–81.

9. Morrison, D. J., M. G. Cruickshank, and A. Lalumière. 2014. Control of Laminated and Armillaria Root Diseases by Stump Removal and Tree Species Mixtures: Amount and Cause of Mortality and Impact on Yield after 40 Years. Forest Ecology and Management 319 (May): 75–98. doi:10.1016/j.foreco.2014.02.007.

10. Nguyen D, Castagneyrol B, Bruelheide H, Bussotti F, Guyot V, Jactel H, et al. (2016). Fungal disease incidence along tree diversity gradients depends on latitude in European forests. Ecol. Evol. 2016;n/a-n/a.

11. Lindén, Magnus, and Gudmund Vollbrecht. 2002. Sensitivity of Picea Abies to Butt Rot in Pure Stands and in Mixed Stands with Pinus Sylvestris in Southern Sweden. Silva Fennica 36 (4). doi:10.14214/sf.519.

12. Piri T., Korhonen K., Sairanen A., 1990. Occurrence of Heterobasidion annosum in pure and mixed spruce stands in Southern Finland. Scand. J. For. Res. 5, 113-125

List of stakeholders consulted during the literature searching stage

Centre for International Forest Research (CIFOR)

EU COST action on Mixed Forests (EUMIXFOR)

EUFORGEN

European Forest Institute

Food and Agriculture Organisation of the United Nations

Forest Europe

Forest Research (UK)

French National Institute for Agricultural Research (INRA)

International Union of Forestry Research Organisations (IUFRO)

United States Department of Agriculture

World Agroforestry Centre

Metadata Form sent to stakeholders from TreeDivNet contributing unpublished data sets

TreeDivNet Pathogen Data for Systematic Synthesis	
Name:	Institution:
Position:	Contact email:
Contact address:	Experiment name:
Location of experiment (latitude and longitude):	Dates of data collection:
Year planted:	
Who collected the data?	Contact email of the person who collected the data (if different from above):
Tree species planted in experiment:	Tree species sampled for pathogens:
Please provide a brief description of sampling protocol, including the number of plots sampled and treatment levels, number of trees sampled per plot, number of leaves/shoots sampled per tree.	
How were pathogens identified (e.g. by eye in the field, microscopically from sampled leaves, molecular methods)?	
What is the metric of pathogen damage recorded? (e.g. % damage per leaf, number of stem lesions, etc).	
Further comments	

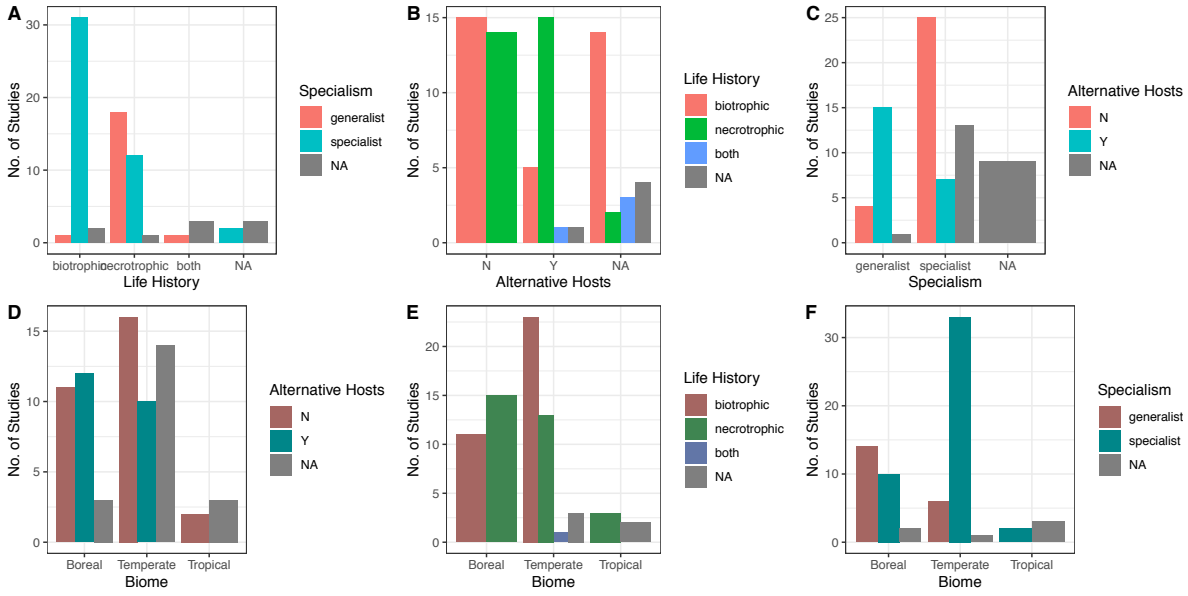


Figure S18. Bar charts showing categorical moderators used in mixed effects meta-analysis models, plotted against number of studies. Shown in each plot are the numbers of studies in each category, for two categorical moderators. Several moderators are well confounded (A, C, E, F). We therefore chose to analyse moderators separately rather than including them in the same models. Total no. of studies *(k)* = 74.

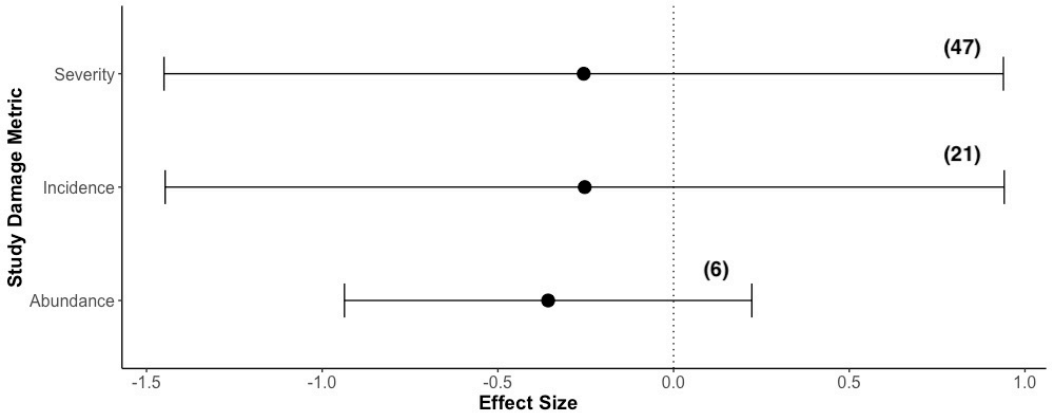


Figure S19: Effect of diversity metric used in studies on effect size (Hedges' *d*). Shown is the mean effect size +/- 95% confidence intervals for each group. Metrics of pathogen damage fell into two main categories: pathogen incidence (*k* = 21 studies) and pathogen severity (*k* = 47 studies), with a smaller number (*k* = 6 studies) that assessed pathogen abundance. Diversity metric was a non-significant predictor of effect size $Q_M = 0.11$, $p = 0.95$, $df = 2$). In brackets is the number of studies in each group (total *k* = 72 studies).

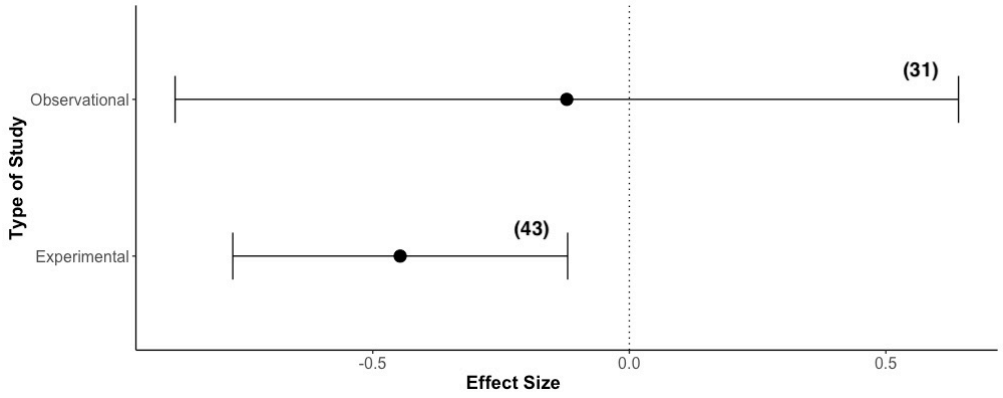


Figure S20: Effect of study type (experimental versus observational) on effect size (Hedges' *d*). Shown is the mean effect size +/- 95% confidence intervals for each group. Study type was a non-significant predictor of effect size $Q_M = 2.12$, $p = 0.14$, $df = 1$). Damage in experimental studies was significantly lower in mixed forests compared to monocultures, but the effect was not significantly different from observational studies (Fig. 6). In brackets is the number of studies in each group (total $k = 72$ studies).

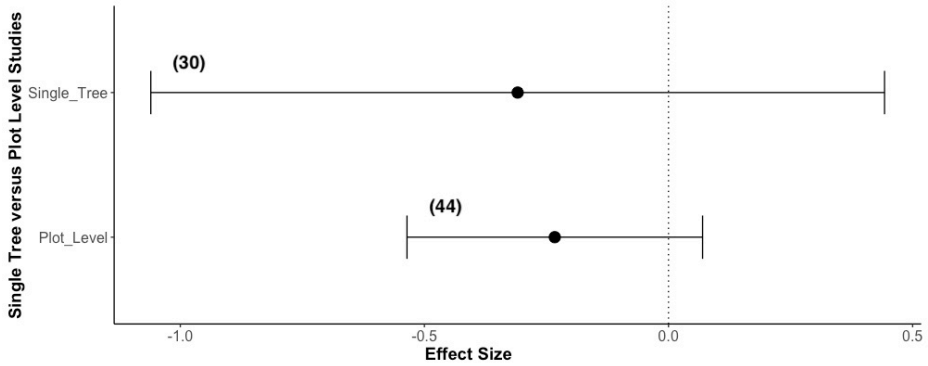


Figure S21: Effect of study level (single tree level versus plot level estimates) on effect size (Hedges' *d*). Shown is the mean effect size +/- 95% confidence intervals for each group. Study type was a non-significant predictor of effect size $Q_M = 0.11$,

p = 0.74, df = 1). In brackets is the number of studies in each group (total k = 72 studies).

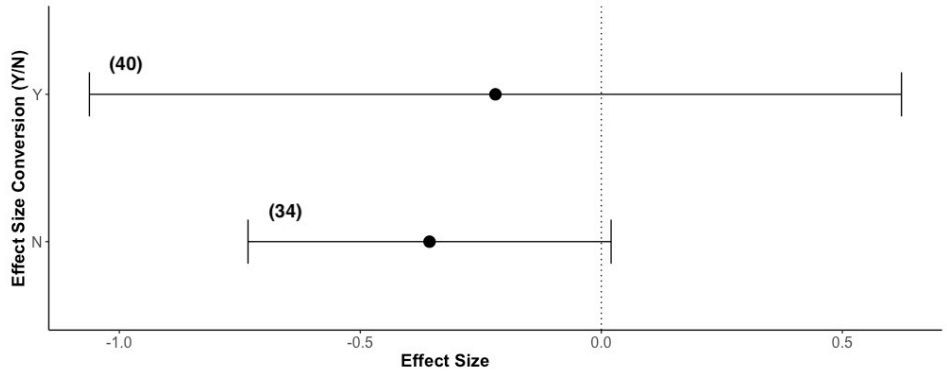


Figure S22: Comparison of directly calculated effect sizes and those converted from other metrics of effect size. Conversions were mostly from correlation coefficients, in some cases p-values and $\chi 2$ statistics. Shown is the mean effect size +/- 95% confidence intervals for each group. Effect size conversion was a non-significant predictor of effect size Q_M = 0.33, p = 0.57, df = 1). In brackets is the number of studies in each group (total k = 72 studies).

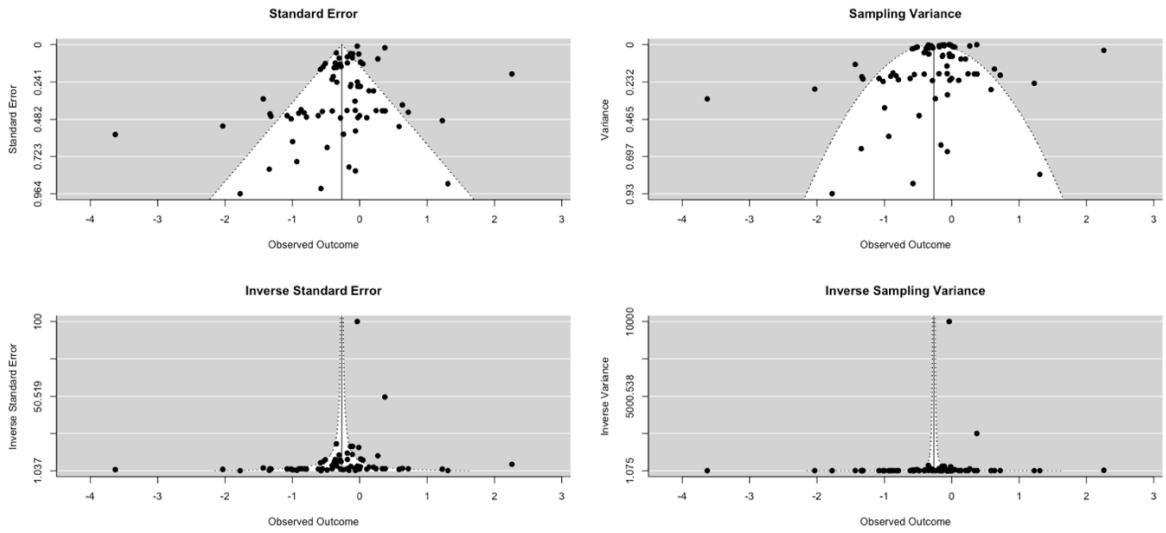


Figure S23. Funnel plots showing observed outcome (study effect sizes) against study standard error, variance, and inverse standard error/inverse variance. Plotted are the effect sizes. The black vertical line indicates the overall effect size, with the white region showing the boundaries within which studies would lie given perfect symmetry.

Appendix 6: Additional Resources on Tree Health for Educators

Tree Pest and Disease Factsheet: Ash *(Fraxinus excelsior)*

What to look out for on *Ash*

1. Ash Dieback (*Hymenoscyphus fraxineus*)



healthy leaf (left); diseased leaf (right)

Key facts

- Fungal disease introduced from Asia into Europe
- First observed in the UK in 2012
- Can kill young trees quickly, takes longer on mature trees. Stressed trees and those in ash dominated woodlands are likely to decline more quickly than trees in open settings.
- There is no cure, but some trees will be tolerant to the disease



canopy dieback

Key symptoms - Summer

- Blackened and wilting leaves
- Dead branches high up in the canopy
- Diamond-shaped lesions (wounds) on the bark
- Clumps of new growth towards the centre of the canopy



wilting, dying leaves

Key symptoms - Winter

- Diamond-shaped lesions on the bark
- Dead branches



diamond-shaped stem lesion

What to do if you suspect ash dieback on your site

- Report your sighting to TreeAlert (see below)
- If you suspect you have a resistant ash tree, report it to (?)
- Do not remove your ash trees unless they are becoming a health and safety hazard, as ash trees support many other species of plants and animals.

2. Emerald Ash Borer *(Agrilus planipennis)*



- This pest is **NOT** present in the UK yet.
- It's an exotic beetle native to East Asia that has been spreading and has been reported west of Russia. In North America, it has become a major pest of ash.
- Beetle activity on trees is common and can be caused by several species. If you observe this distinctive green beetle, report your sighting immediately to TreeAlert.

Pest and Disease Reporting

- If you spot a pest or disease, report your concerns through the Forestry Commission's online reporting tool TreeAlert at treealert.forestresearch.gov.uk
- Make sure to clean boots and equipment before and after site visits, to remove any mud and debris that can transmit pests and diseases.



SYLVA FOUNDATION

Produced by the Sylva Education team, with support from the Forestry Commission. Made possible with funding from the Ernest Cook Trust. Photo credits (clockwise from top): Barnaby Wylder / Forestry Commission; Matthew Woodcock / Forestry Commission; Mick Biddle / Forestry Commission; Leah Bauer (USDA, Bugwood.org); Joan Webber / Forest Research

ECT · Ernest Cook Trust · learning from the land

Forestry Commission

Tree Pest and Disease Factsheet: Oak *(Quercus robur)*

What to look out for on *Oak*

1. Acute Oak Decline



Canopy decline



Agrilus beetle exit hole on bark

Key facts

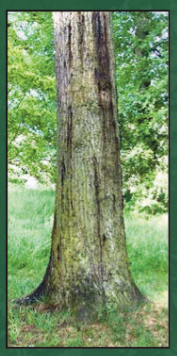
- Impacting thousands of oak trees across England and Wales
- Trees suddenly decline and can die within a few years
- Oak decline has multiple causes: drought and insect outbreaks can weaken a tree, making it more susceptible to bacterial and fungal disease

Key symptoms

- Dark weeping patches of fluid on the trunk (stem bleeds)
- Stem bleeds may be dry or oozing
- Dead or dying branches
- D-shaped exit holes of the beetle *Agrilus biguttatus* may be visible on the trunk. The beetle is thought to weaken trees and may transmit disease

What to do if you suspect oak decline on your site

- Report your sighting to TreeAlert (see below)
- If you have a tree with stem bleeds, do not remove the tree unless it is a safety hazard. Monitor the symptoms as trees can recover
- To avoid transmitting the disease to healthy trees, do not touch stem bleeds and then touch other trees





Stem bleeds (above) and closeup (below)

2. Oak Powdery Mildew *(Erysiphe sp.)*



Key facts and symptoms

- Fungal disease present all over the UK
- Coats the leaves in a white powder that develops throughout summer
- Doesn't kill the tree but can weaken it, and stunts the growth of young trees
- Report sightings to TreeAlert

3. Oak Processionary Moth *(Thaumetopoea processionea)*



Key facts and symptoms

- Caterpillars "process" along trees in Spring and feed on leaves, weakening the tree
- Contact with the caterpillar hairs causes skin and eye irritation
- Currently present in London and the South East
- Any sightings must be reported immediately so nests can be destroyed

Pest and Disease Reporting

- If you spot a pest or disease, report your concerns through the Forestry Commission's online reporting tool TreeAlert at treealert.forestresearch.gov.uk
- Make sure to clean boots and equipment before and after site visits, to remove any mud and debris that can transmit pests and diseases.

SYLVA FOUNDATION

Produced by the Sylva Education team, with support from the Forestry Commission. Made possible with funding from the Ernest Cook Trust. Photo credits: Forestry Commission (oak decline); Henry Kuppen (OPM), Forest Research (mildew)

Forestry Commission

Tree Pest and Disease Factsheet: Horse Chestnut *(Aesculus hippocastanum)*

What to look out for on *Horse Chestnut*

1. Horse Chestnut Leaf Miner *(Cameraria ohridella)*

Key facts

- Leaf mining moth native to Southern Europe, introduced to the UK
- Now widespread across England, Wales and southern Scotland
- Impacts over several years can reduce tree growth and conker size in autumn

Key symptoms

- Caterpillars feed on leaves from June onwards, forming "leaf mines"
- Leaf mines are blotchy, first white then turning brown/orange
- Leaves dry up, turn brown and can drop early in autumn
- There are several **lookalike** pests and diseases (see photos below)



leaf mine symptoms

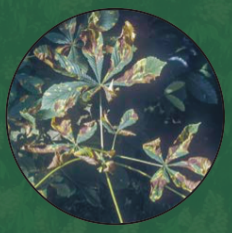
What to do if you suspect horse chestnut leaf miner on your site

- The Forestry Commission encourage TreeAlert reports of the pest north of Leicester.
- Collecting the fallen leaves and burning them in autumn can reduce pest build up on isolated trees

from left to right:
(1) leaf mine symptoms;
(2) leaf miner closeup;
lookalikes:
(3) *Guignardia* leaf blotch;
(4) squirrel damage









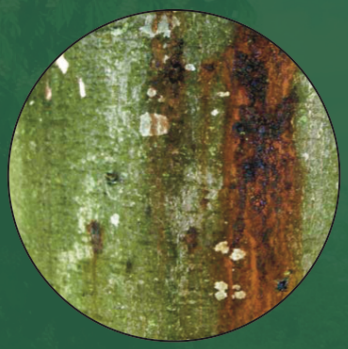
2. Horse Chestnut Bleeding Canker

Key facts

- Caused by at least two different pathogens
- Widespread across the UK on all ages of tree

Key symptoms

- Bleeding cankers: cracks in the bark that ooze black/brown/orange fluid
- Cankers can occur on the trunk or branches



What to do if you suspect bleeding canker on your site

- Report your sighting to TreeAlert (see below)
- Trees can recover so removal is unnecessary unless they pose a safety hazard
- Infected branches can be removed to slow disease spread

Pest and Disease Reporting

- If you spot a pest or disease, report your concerns through the Forestry Commission's online reporting tool TreeAlert at treealert.forestresearch.gov.uk
- Make sure to clean boots and equipment before and after site visits, to remove any mud and debris that can transmit pests and diseases.

SYLVA FOUNDATION

Produced by the Sylva Education team, with support from the Forestry Commission. Made possible with funding from the Ernest Cook Trust. Photo credits (clockwise from top): Forestry Commission; Barry Lambsdown / Forestry Commission; Joan Webber / Forest Research; Robert Strouts / Forestry Commission; Forestry Commission; Suzanne Sancisi-Frey / Forest Research

ERNEST COOK TRUST · learning from the land

Forestry Commission

Tree Pest and Disease Factsheet: Sweet Chestnut *(Castanea sativa)*



What to look out for on *Sweet Chestnut*

1. Sweet Chestnut Blight *(Cryphonectria parasitica)*

Key facts

- Fungal disease introduced from Asia into Europe
- First identified in the UK in 2011
- Impact sweet chestnut, not horse chestnut trees

Key symptoms

- Orange coloured cankers (areas of dead bark) on smooth bark
- Blister-like cracks on the tree's bark as the infection progresses
- Orange fruiting bodies of the fungus on the bark
- Leaf wilting above the cankers
- New shoots are produced below the area of dead bark

What to do if you suspect chestnut blight on your site

- To prevent disease spread, any sightings must be reported immediately so infected material can be destroyed
- Do not move infected plants, bark or wood
- Report your sighting to the Forestry Commission using TreeAlert (see below)



canker on bark



split bark and new shoots



fruiting bodies on bark



split bark

2. Oriental Chestnut Gall Wasp *(Dryocosmus kuriphilus)*

Key facts and symptoms

- Recently intoduced to the UK, where it is widespread
- Native to China
- Causes galls (swellings) on buds, leaves, and leaf stalks that can reduce tree growth over time
- Sightings must be reported immediately to TreeAlert so infected material can be destroyed



leaf galls in summer (above) and autumn (below)

3. *Phytophthora ramorum*

Key facts and symptoms

- Fungal-like organism that can affect many species of tree, in the UK mainly larch and sweet chestnut
- Symptoms include leaf lesions and canopy die-back
- Report sightings to TreeAlert



leaf lesions

Pest and Disease Reporting

- If you spot a pest or disease, report your concerns through the Forestry Commission's online reporting tool TreeAlert at treealert.forestresearch.gov.uk
- Make sure to clean boots and equipment before and after site visits, to remove any mud and debris that can transmit pests and diseases.

SYLVA FOUNDATION

Produced by the Sylva Education team, with support from the Forestry Commission. Made possible with funding from the Ernest Cook Trust. Pest photo credits (clockwise from top): Mick Biddle / Forestry Commission; Ana Perez-Sierra / Forest Research; Daniel Rigling / WSL Switzerland; Forestry Commission (x3); Barnaby Wylder / Forestry Commission

ECT Ernest Cook Trust

Forestry Commission

Leaf Insect Herbivore Identification Guide

Which critters have been feeding on the leaves in your woodland? Use our key to find out!

Leaf webbers and rollers

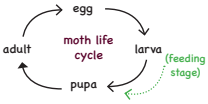


Leaf webbers glue together several leaves as they feed on them, even from separate branches!



Leaf rollers make their home by rolling up leaves as they feed.

For both rollers and webbers, larvae (caterpillars) develop in the leaf folds, before they pupate and emerge as mature adult moths.



SYLVA FOUNDATION
trees and people together

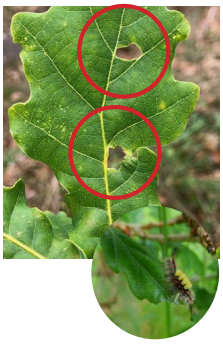
Photos by Shymali Roy, University of Reading & Elsa Field, University of Oxford

Leaf skeletonisers



Leaf skeletonisers are grazing on the leaf, leaving only the leaf veins (leaf "skeleton") behind. Hold a leaf up to the light to see evidence of leaf skeletonisers.

Leaf chewers



Leaf chewers are munching on the leaf itself, leaving holes behind!

Activity ideas

The best time of year to observe leaf herbivory is from late Spring onwards, from May to September. You'll see autumn generation leaf galls from late July onwards. For older children, you could create a collage of photos or ask them to draw their favourite galls or mines!

As a group activity, try a game where groups get points for finding different types of herbivore damage on the trees at your site.

You could also compare the numbers or types of herbivores occurring on different species of tree. For instance, gallers will be most abundant on oak trees, which support many species.

For more education resources: www.sylva.org.uk

Leaf miners

Leaf miners are species of moth, whose larvae develop inside mines in the leaf - eating the leaf from the inside out. Leaf mines come in all shapes and sizes! Later in the season, you might see "erupted" mines once the insect has left the mine.



Blotch mines on upper leaf surface



Serpentine mine on upper leaf surface



Erupted tent mine on the upper leaf surface



Lower leaf surface, showing the same mine

Leaf gallers





Oak spangle galls on the underside of leaves

Leaf gallers are tiny wasps that lay their eggs on leaves, which develop into structures called galls in which the caterpillars grow, before emerging as wasps. Most gallers are have spring and autumn generations. The autumn generation galls tend to be larger, and are shown here.



Oak cherry gall wasp

Bud and acorn gallers

Galls can also develop on branch and stem buds and acorns. Look out for a diversity of structures and colours!



Oak marble galls develop on oak buds. Look out for small exit holes where the wasp has left the gall!



Acorn knopper gall wasp develops on oak acorns. This is an invasive species

SYLVA FOUNDATION
trees and people together

Forest School Impact Assessment Guide

This guide provides examples of common positive and negative impacts of Forest School activities, for use in preparing an Ecological Impact Assessment for your site. You may have some or all of the following impacts on your Forest School site! Use this guide as a prompt.

Negative impacts on site ecology ☹

Activity and Impact	Ecological layer	Photo(s)	Management actions
Fire pits, leading to burnt vegetation and change in chemical composition of soil.	soil layer		• Enclose fire pit so fires are well contained, or use a fire bowl. • Limit the frequency of fires to allow soil to recover. • If the site is large enough, rotate fire locations. Fires should be at least 4 metres from surrounding trees.
Digging soil / mud kitchens that can damage soil structure and tree roots.	soil layer	 	• Contain area of digging and reduce frequency of activity. • Move areas of digging away from tree roots. • Replant/resow areas of digging to allow plants to regrow. • Talk to children about how to look after bug life in the soil and any plants that they find. • Identify tree roots and talk to children about the role of roots and how we can protect them.
Footfall in popular areas of the site, leading to erosion and compaction of soil.	soil layer	 	• Divert or create new paths. • Woodchip paths to reduce soil erosion - but beware importing woodchip from other sites, due to the risk of introducing pests and diseases. • Rotate popular play areas and allow areas to recover. • Use multiple paths through sites to spread impact.
Woodland play that can lead to trampling of plants / young trees.	field / understorey layer		• Identify young plants with children. • Mark them with a stake/tube or other method. • Agree a plan with the children a plan to avoid damaging plants through play. • Put brash (branches) or logs around young plants to protect them. Consider fencing for large areas of young trees (> 20 trees).

Negative impacts on site ecology ☹			
Description of Impact	Ecological layer	Photo(s)	Management actions
Den building, which removes dead wood from field layer reducing available insect fodder and habitat .	field layer	 den building	• Reduce size and number of dens built. • Create a pile of den building wood that children know is OK to use. • Create other dead wood piles on site, and agree with the children that these are for wildlife habitat, not den building.
Collecting fire wood and kindling, reducing available deadwood habitat.	field layer	 firewood pile	• Reduce amount of firewood collected. • Consider buying local firewood from sustainably harvested sources instead, or ask for donations. • Encourage the creation of new dead wood habitats on site and agree with the children that these are for wildlife, not firewood.
Breaking tree branches through play, tree swings, ropes and slack lines.	understorey layer	 rope swings	• Talk with children about how to care for and look after the tree and why. • Remove swings from any damaged branches and engage a tree surgeon/ arboriculturist to advise on tree health. • Take down ropes at the end of each session for the safety of other users of the site, and protection of the tree.
Bark damage: bark stripping through play, and/or damage through tree swings, ropes and slack lines .	understorey layer	 rope damage to bark	• Talk with children about how to care for and look after the tree. • Engage a tree surgeon / arboriculturist to advise on tree health. • Buy or make tree protectors that pad between the tree and the rope/slack line. • Take down ropes at the end of sessions for safety and tree protection.
Tree guards breaking / plastic waste from tree guards which can harm wildlife.	understorey	 broken tree guard	• Remove tree guards once the cover becomes a tight fit on the tree. • Periodically remove guards if mortality has occurred. • Engage children with activities about reducing plastic waste and recycling. • Use other natural materials to protect tree such as brash, bramble and nettle.
Heat from fire damaging tree canopy.	canopy layer	 fire pit	• Light fires at least 4 metres away from large trees and any overhanging branches. • Reduce frequency of fires / spread locations to reduce impact.

Positive impacts on site ecology ☺

Description of Impact	Ecological layer	Photo(s)	Management actions
Pruning fruit trees to promote new growth, more blossom for insects and improve fruit yield. Planting new fruit trees to increase food for pollinators, birds and other wildlife.	canopy layer	 apple harvest	• Engage with local volunteer groups who may have knowledge to share. • Attend pruning training days or employ an arboriculturist to prune trees on your site. • Harvest a share of the fruit (leaving some for the birds!) and share amonst school community or use in Forest School activities.
Minimise the spread of tree pests and diseases on your site.	canopy layer	 butt rott at the base of tree  ash dieback symptoms	• Employ an arboriculturist or tree surgeon to remove diseased trees where appropriate, to slow spread of diseases that are spread via root contact between trees, such as honey fungus and Dutch elm disease. • Practice good biosecurity such as cleaning boots and equipment after site visits. Install a boot washing station for yourself and the children. • Report sightings of pests and diseases to the relevant authority (in the UK, the online Forestry Commission TreeAlert service).
Coppicing and pollarding tree species such as willow and hazel, increasing light to forest floor, promoting ground flora diversity and creating valuable insect, bird and mammal habitat.	canopy layer	 coppicing	• Engage with local volunteer groups who may have knowledge to share, or employ an arboriculturist to coppice/ pollard trees on your site. • Retain wood harvested for creative projects / firewood / deadwood habitat. • Fence newly coppiced areas to protect trees from rabbits / deer.
Creating new habitats for birds.	canopy layer	 bird box in situ	• Plan a bird box creation and monitoring activity with the children. • Create activities to identify birds, observe their behaviour and chart biodiversity change over the seasons. • Make bird feeders with children and ensure bird feeders are available during the wwwinter for birds.

Log your positive and negative impacts on ***myForest for Education:*** a free online tool enabling educators to create maps and management plans for Forest School sites.
Visit: www.sylva.org.uk/myforest

Produced by the Education team at the Sylva Foundation with support and funding from the Ernest Cook Trust

Positive impacts on site ecology ☺			
Description of Impact	Ecological layer	Photo(s)	Management actions
Pond creation, creating new habitats for invertebrates, frogs and other animal and plant pond-life.	soil /field layer	 pond creation	• Engage children with creating new ponds on site. Explore natural options such as clay for lining ponds. • Introduce pond plants and make ponds frog friendly. • Survey the wildlife in your pond regularly with children. Ponds are one of the most biodiversity enhancing features you can add to a site! • Remove excess leaves from pond regularly to prevent build up.
Creating new deadwood habitats for insects including bees, small mammals including hedgehogs, and other animal life.	field / understorey layer	 deadwood pile  stump pile	• Engage children with creating new habitats from deadwood piles and old wood. • Research how to encourage different types of wildlife through habitat creation on your site. • Survey wildlife with the children to show the positive impact the new habitats are bringing.
Promoting plant diversity by clearing brambles and invasive plant species such as rhododendron, Japanese knotweed *etc.** *ensure that all invasive plant material is disposed of in accordance with government regulations.	field layer	 bramble patch	• Identify any invasive species on your site. • Organise a day for parents and volunteers (or even older children) to clear overgrown areas. • Ensure some overgrown areas of brambles, nettles etc are left for wildlife.
Sowing wildflower seeds, promoting biodiverse meadow habitat.	field layer	 wildflower meadow	• Sow new wildflower meadows with the children. • Talk with children about caring for meadows, not pulling up flowers *etc.* • Create a plant identification activity once the wildflowers emerge in Spring. Survey the nature present with children regularly to show the positive impact meadows can bring.
Tree planting / new woodland creation. Benefits include: • Creation of new play areas • Biodiversity increase (plant, bird, and invertebrate habitats) • Climate change mitigation through carbon absorption.	understorey / canopy layer	 new willow structure created by Forest School	• Involve children in tree planting and care of young trees. • Try planting willow, hazel and other fast growing species to create small structures and play areas. • Keep trees protected with tree guards when small. • Monitor pest / disease impact on young plants. • Survey the nature of these areas regularly with the children (birds, insects, *etc*).