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Impacts of environmental factors on pollen production of birch across different climatic conditions in Europe

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To Sagun

For coming into my life and for all the inspiration, motivation, and support

during years full of ups and downs

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Abstract

Pollen production in birch trees (*Betula* spp.) is a crucial factor influencing plant reproduction and public health, as birch pollen is a major aeroallergen. However, the complex interactions between environmental factors and birch pollen production are not fully understood. This doctoral thesis aims to investigate the impacts of genetic, climatic, and other environmental drivers on pollen production across different birch species and climatic conditions in Europe. The research therefore gains major importance since climate change conditions are believed to alter the risk and severity of allergic diseases and may change conditions for reproduction in forest tree species.

The major questions addressed in this PhD thesis are:

1. What are the pollen production estimates for *Betula* species? In other words, how many inflorescences and pollen are produced by distinct types of the tree species?
2. Is there a year-to-year variation in birch pollen production?
3. How do genetic and environmental factors influence birch pollen production at local and regional scales?

The research presented in this thesis is based on three peer-reviewed publications that employed a multi-faceted approach to address the overarching research questions. The first study (Chapter 4) assessed pollen production in cloned individuals of weeping birch (*Betula pendula*) over three consecutive years (2019-2021) in a seed plantation in Germany, with catkin samples collected from 28 birch trees. The second study (Chapter 5) investigated pollen production of downy birch (*Betula pubescens*) along an altitudinal gradient of 522 meters in the European Alps during 2020 and 2021, with catkin samples collected from 17 birch individuals at nine different altitudinal locations. The third study (Chapter 6) evaluated the continental-scale impacts of meteorology (temperature, precipitation) and atmospheric gases (ozone, carbon dioxide) on downy birch pollen production across 37 International Phenological Gardens (IPGs) in Europe, with catkin samples collected over three consecutive years (2019-2021) and tree age was approximated by stem circumference.

We adopted a standardized method to extract pollen from catkins and maintained methodological consistency across all three studies. For Chapter 4 and Chapter 5, environmental factors such as air temperature, air quality, and solar radiation were monitored at the study sites. For Chapter 6, high-resolution (0.1°) gridded meteorological data were obtained from the E-OBS dataset, and concentrations of O_3, NO_2 and CO_2 were derived from Copernicus Atmosphere Monitoring Service (CAMS) reanalysis.

The major statistical analyses included non-parametric tests such as the Kruskal-Wallis test, Mann-Whitney U test, Spearman's correlation, and mean regression. Additionally, the research incorporated a distribution-free quantile regression method, where the quantiles of the response distribution (pollen production) were modeled directly. This allowed for assessing the impacts of factors such as temperature and O_3 levels across different quantiles of the response variable. All statistical analyses were carried out using R versions 4.1.2, 4.2.2, and 4.3.2.

We estimated mean pollen production for two birch species: 1.66 million pollen grains per catkin for *B. pendula* (Chapter 4) and between 0.4 and 8.3 million pollen grains per catkin for *B. pubescens* (Chapter 5 and Chapter 6). Moreover, significant annual fluctuations were noted in both pollen and catkin production. Our findings also include indication of masting behaviour in the selected birch trees (Chapter 4 and Chapter 5), suggesting that annual variations in pollen production could be influenced by masting, which may mask the effects of environmental factors such as temperature.

At the seed plantation site, we observed that genetic variability in birch trees leads to increased variability in pollen production (Chapter 4). Consequently, to effectively investigate the effects of environmental factors, we employed a research design that minimized genetic variability among trees. This approach was applied in our study across Europe using IPG birches, which exhibit reduced genetic variability (Chapter 6).

The results from studies in the European Alps (Chapter 5) and across Europe (Chapter 6) showed significant positive correlations between air temperature and pollen production in birch. However, no correlations were observed with altitude (Chapter 5)

or CO_2 concentration levels (Chapter 5). Increasing O_3 concentration levels tended to decrease pollen production (Chapter 6). The findings suggest that further climate warming could worsen the adverse effects on individuals with pollen allergies.

This doctoral research provides novel insights into the complex environmental controls on pollen production in birch species, contributing to a more detailed understanding of the potential impacts of climate change on plant reproduction and public health.

Kurzfassung

Die Pollenproduktion der Birke (*Betula* spp.) ist ein entscheidender Faktor für die Fortpflanzung der Pflanzen und die menschliche Gesundheit, da Birkenpollen ein wichtiges Aeroallergen darstellen. Die komplexen Wechselwirkungen zwischen Umweltfaktoren und der Pollenproduktion der Birke sind jedoch noch nicht vollständig geklärt. Ziel dieser Doktorarbeit ist es, die Auswirkungen genetischer, klimatischer und anderer Umweltfaktoren auf die Pollenproduktion verschiedener Birkenarten und Klimabedingungen in Europa zu untersuchen. Diese Forschungsarbeiten gewinnen große Bedeutung, da angenommen wird, dass der Klimawandel das Risiko und die Schwere allergischer Erkrankungen verändert und die Fortpflanzungsbedingungen von Waldbäumen beeinflussen könnte.

Die wichtigsten Fragen, die in dieser Dissertation behandelt werden, sind:

1. Wie hoch ist die Pollenproduktion von *Betula*-Arten? Mit anderen Worten: Wie viele Blütenstände und Pollen werden von unterschiedlichen Birkenarten produziert?
2. Gibt es jährliche Schwankungen in der Pollenproduktion der Birke?
3. Wie beeinflussen genetische Faktoren sowie Umweltfaktoren die Pollenproduktion der Birke auf lokaler und regionaler Ebene?

Die in dieser Arbeit vorgestellten Forschungsergebnisse basieren auf drei von Experten begutachteten Veröffentlichungen, die einen vielschichtigen Ansatz zur Beantwortung der übergeordneten Forschungsfragen verfolgten. In der ersten Studie (Kapitel 4) wurde die Pollenproduktion von geklonten Individuen der Hängebirke (*Betula pendula*) in drei aufeinanderfolgenden Jahren (2019-2021) in einer Saatgutplantage in Deutschland

untersucht, wobei Kätzchenproben von 28 Birken gesammelt wurden. Die zweite Studie (Kapitel 5) untersuchte die Pollenproduktion der Moorbirke (*Betula pubescens*) entlang eines Höhengradienten von 522 Metern in den europäischen Alpen in den Jahren 2020 und 2021, wobei Kätzchenproben von 17 Birken an neun verschiedenen Höhenstandorten gesammelt wurden. Die dritte Studie (Kapitel 6) untersuchte die kontinentalen Auswirkungen von Meteorologie (Temperatur, Niederschlag) und atmosphärischen Gasen (Ozon, Kohlendioxid) auf die Pollenproduktion von Moorbirken in 37 Internationalen Phänologischen Gärten (IPGs) in Europa, wobei Kätzchenproben in drei aufeinanderfolgenden Jahren (2019-2021) gesammelt wurden und das Baumalter anhand des Stammumfangs approximiert wurde.

Wir haben ein standardisiertes Verfahren zur Gewinnung von Pollen aus Kätzchen angepasst und diese Methodik konsistent in allen drei Studien angewandt. In den Kapiteln 4 und 5 wurden Umweltfaktoren wie Lufttemperatur, Luftqualität und Sonneneinstrahlung an den Untersuchungsstandorten überwacht. Für Kapitel 6 wurden meteorologische Daten mit hoher räumlicher Auflösung (0,1°) aus dem E-OBS-Datensatz gewonnen, und die Konzentrationen von O_3, NO_2 und CO_2 wurden aus der Reanalyse des Copernicus Atmosphere Monitoring Service (CAMS) abgeleitet.

Zu den wichtigsten statistischen Analysen gehörten nicht-parametrische Tests wie der Kruskal-Wallis-Test, der Mann-Whitney-U-Test, die Spearman-Korrelation und die Mittelwertregression. Außerdem wurde eine verteilungsfreie Quantilregression angewandt, bei der die Quantile der Antwortverteilung (Pollenproduktion) direkt modelliert wurden. Auf diese Weise konnten die Auswirkungen von Faktoren wie Temperatur und O_3-Gehalt über verschiedene Quantile der Antwortvariablen hinweg bewertet werden. Alle statistischen Analysen wurden mit den R-Versionen 4.1.2, 4.2.2 und 4.3.2 durchgeführt.

Wir haben die durchschnittliche Pollenproduktion für zwei Birkenarten geschätzt: 1,66 Millionen Pollenkörner pro Kätzchen für *B. pendula* (Kapitel 4) und zwischen 0,4 und 8,3 Millionen Pollenkörner pro Kätzchen für *B. pubescens* (Kapiteln 5 und 6). Außerdem wurden sowohl bei der Pollen- als auch bei der Kätzchenproduktion erhebliche jährliche Schwankungen festgestellt. Unsere Ergebnisse enthalten auch Hinweise auf ein

Mastverhalten der ausgewählten Birken (Kapiteln 4 und 5), was darauf hindeutet, dass die jährlichen Schwankungen in der Pollenproduktion durch das Mastverhalten beeinflusst werden könnten, was die Auswirkungen von Umweltfaktoren wie der Temperatur möglicherweise überdeckt.

Am Standort der Saatgutplantage haben wir festgestellt, dass die genetische Variabilität der Birken zu einer erhöhten Variabilität der Pollenproduktion führt (Kapitel 4). Um die Auswirkungen von Umweltfaktoren effektiv zu untersuchen, wendeten wir daher ein Forschungsdesign an, das die genetische Variabilität zwischen den Bäumen minimiert. Dieser Ansatz wurde in unserer europaweiten Studie mit IPG-Birken angewandt, welche eine geringere genetische Variabilität aufweisen (Kapitel 6).

Die Ergebnisse der Untersuchungen in den europäischen Alpen (Kapitel 5) und in ganz Europa (Kapitel 6) ergaben signifikante positive Korrelationen zwischen der Lufttemperatur und der Pollenproduktion der Birke. Es wurden jedoch keine Korrelationen mit dem Höhengradienten (Kapitel 5) oder der CO_2-Konzentration (Kapitel 6) festgestellt. Mit zunehmender O_3-Konzentration ging die Pollenproduktion tendenziell zurück (Kapitel 6). Die Ergebnisse deuten darauf hin, dass eine weitere Klimaerwärmung die negativen Auswirkungen auf Pollenallergiker noch verstärken könnte.

Diese Doktorarbeit bietet neue Einblicke in das komplexe Zusammenspiel der Umwelteinflüsse auf die Pollenproduktion der Birke und trägt zu einem genaueren Verständnis der möglichen Auswirkungen des Klimawandels auf die Pflanzenreproduktion und die menschliche Gesundheit bei.

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Abbreviations

AIC	Akaike information criterion
ANOVA	Analysis of variance
CAMS	Copernicus Atmosphere Monitoring Service
CO_2	Carbon dioxide
C_{vuc}	Catkins per volume unit of crown
C_{su}	Catkins per crown sampling unit
CV	Coefficient of Variance
DFG	DeutscheForschungsgemeinschaf
DWD	German Meteorological Service
ECA&D	European Climate Assessment & Dataset
EU-FP6	European Union's 6th Framework Programme
GDD	Growing degree days
Fl_{ca}	Flowers per catkin
Fl_{vuc}	Flowers per volume unit of crwon
IPCC	Intergovernmental Panel on Climate Change
IPGs	International Phenological Gardens
IQR	Interquartile range
KOH	Potassium hydroxide
m a.s.l	meters above sea level
NO_2	Nitrogen dioxide

NO$_x$	Nitrogen oxides
O$_3$	Ozone
P_{ca}	Pollen grains per catkin
P_{cr}	Pollen per volume unit of crown
P_{fl}	Pollen per flower
P_{in}	Pollen production per tree
P_{vuc}	Pollen per volume unit of crown
R^2	Coefficient of determination
r	Pearson coefficient
r$_s$	Spearman rank correlation coefficient
SD	Standard deviation
SFT	Space-for-time
T$_{max}$	Maximum temperature
T$_{mean}$	Mean temperature
T$_{min}$	Minimum temperature
UERRA	Uncertainties in Ensembles of Regional ReAnalyses
WAO	World Allergy Organization
WHO	World Health Organization

1   General introduction

1.1   Pollen, the prevalence of allergic diseases and climate change

1.1.1   Pollen

Pollen (singular and plural) is (are) male gametophyte(s) of seed plants (spermatophytes), either angiosperms or gymnosperms. Plants generate pollen as a part of their reproductive process (Frenguelli 2004). The reproductive success and overall fitness of plant populations are largely dependent on pollen (Friedman 1993). They are developed in anthers (angiosperms) or in microsporangia (gymnosperms) and are transported to the stigma of the same or another flower to fertilize the female gamete (Mascarenhas 1989). Pollen plays a critical role in the sexual reproduction and survival of many plant species (Cai & Del Duca 2019), as well as the pollinators that rely on them (Lawson *et al.* 2021).

Pollen also contain allergens and constitute an important human health issue (Huynen *et al.* 2003) as they can trigger an allergic response in some individuals (Gadermaier *et al.* 2004; D'Amato *et al.* 2007). This phenomenon occurs when the immune system mistakenly identifies pollen as a harmful substance, which initiates an inflammatory reaction. Upon inhalation, the pollen particles come into contact with the mucous membranes of the respiratory system, leading to the release of histamine and other inflammatory mediators. This cascade of events results in the characteristic symptoms associated to hay fever, such as sneezing, nasal congestion, itchy and watery eyes, and a runny nose (Bousquet *et al.* 2008).

1.1.2   Prevalence of allergic diseases

In Europe, grass pollen are by far the most common cause of pollinosis, as they are widely distributed and the different species are highly cross-reactive (D'Amato *et al.* 1998). Some of the most allergenic grass (Poaceae family) species include *Poa*, *Lolium*, *Dactylis*, and *Festuca* (D'Amato *et al.* 2007; Davies *et al.* 2016). In addition, tree species such as *Betula*, *Olea*, *Fraxinus*, *Alnus* and *Quercus* are represented as the main source of allergenic tree pollen (D'Amato *et al.* 2007).

The World Allergy Organisation (WAO) estimates that up to 40% of the world's population is sensitized to at least one allergen, while between 10-30% suffer from an allergy (Pawankar *et al.* 2013). Particularly in Europe, the prevalence of hay fever has increased substantially (D'Amato *et al.* 2020). For example, the prevalence of respiratory allergies in Germany has increased to 31% (Simoleit *et al.* 2016) and 18% of the German population is sensitized to the major allergen (Bet v 1) of birch (*Betula* spec.) (Beutner *et al.* 2021; Li *et al.* 2022).

Numerous studies indicate a rising prevalence of pollen sensitization across Europe. Lake *et al.* (2017; 2018) projected a substantial increase in common ragweed pollen sensitization, with the greatest proportional increases anticipated in countries where sensitization rates are currently low. Their estimates suggest that by 2041-2060, ragweed sensitization will more than double from 33 million to 77 million people in Europe. This aligns with the findings of Warm *et al.* (2013), who observed a statistically significant upward trend in birch pollen sensitization in Sweden over recent decades.

1.1.3   Climate change

Recent climate change, the long-term alteration of Earth's average weather patterns, including increases in global temperatures and shifts in precipitation, is largely attributed to human activities such as the burning of fossil fuels and deforestation (Kerr 2011; Trenberth 2018). The main driver causing climate change is the rise in atmospheric carbon dioxide (CO_2) levels that has mainly occurred since the start of the industrial era about 1750. In 2023, the Mauna Loa Observatory in Hawaii recorded an average annual atmospheric CO_2 concentration of 421.08 ppm, a significant increase from the pre-industrial value of 280 ppm (National Oceanic and Atmospheric Administration (NOAA) 2023). Other greenhouse gases such as methane and nitrous oxide, have also risen sharply (Thoning *et al.* 2022). As a result of the increase in these greenhouse gases, the global surface temperature in 2011-2020 exceeded the 1850-1900 level by 1.09°C, while the increased rate of temperature since 1970 has surpassed any other 50-year period in the last 2000 years (Lee *et al.* 2024). Projections of future climate change (2021-2100) suggest that the global surface temperature could rise by up to 4 °C relative to 1850-1900 (Masson-Delmotte *et al.* 2021).

Climate change has already showed impacts on various aspects of pollen biology and aerobiology. Observed effects include shifts in the onset and length of pollen seasons, elevated airborne pollen loads, enhanced pollen production, increased pollen allergenicity, and alterations in the geographical distribution of allergenic plant species (Beggs 2004; D'Amato *et al.* 2020). However, the associations between climate warming and pollen characteristics (for e.g. pollen production) are not satisfactorily understood. The sign and magnitude of the respective relationships can differ in some years or areas (e.g., Damialis *et al.* 2011; Guedes *et al.* 2009; Jochner *et al.* 2011; Jochner *et al.* 2013b; Jetschni *et al.* 2023) documenting a high variability probably due to a combination of numerous influencing factors. To obtain a comprehensive understanding of the relationship between pollen production, pollen allergenicity, and influential factors such as temperature, it is essential to study this phenomenon at multiple scales in real-life ecosystems ranging from small areas (with trees exposed to identical or similar climatic condition) to large geographic regions (with a variety of climatic conditions) while considering/minimizing possible confounding effects of age and genetic differences among trees. By examining pollen production in a small seed plantation, along an altitude gradient, and at the continental scale, we can better elucidate the underlying mechanisms and environmental drivers. Thus, potential changes in pollen characteristics with further climate warming can be better predicted.

1.2 Pollen production

1.2.1 Definition and categorization

The amount of pollen grains produced by a plant is an important trait. Faegri & Iversen (1989) have defined pollen production as the amount of pollen produced by individual plants as well as the plant community as a whole. Pollen production in angiosperms is commonly referred to and measured as the quantity of pollen produced per anther, flower, or inflorescence, while in gymnosperms it is measured per male cone (Galán *et al.* 2017). Alternatively, pollen production can be measured per plant or per unit area. Pollen production is an important factor in forest communities' reproductive success and fitness (Opedal *et al.* 2023).

Different types of pollination, such as zoophilous (animal-pollinated), entomophilous (insect-pollinated), and anemophilous (wind-pollinated), exhibit distinct characteristics in terms of pollen production and dispersal mechanisms. For example, zoophilous plants often produce relatively large-sized pollen grains and entomophilous plants produce pollen that are moderately sized (Vieira *et al.* 2012; Lu *et al.* 2022). Such pollen are sticky in nature (Faegri 1966), so that they could adhere to the bodies of the pollinating vectors such as birds, bees, butterflies, bats, and insects (Frankie & Thorp 2009). The pollen produced by such plants is usually in smaller to moderate quantities compared to anemophilous plants. Anemophilous plants, on the other hand, have the most distinctive feature of producing an enormous quantity of pollen followed by wide dispersal to increase the probability of effective fertilisation (Subba Reddi & Reddi 1986; Molina *et al.* 1996; Piotrowska 2008).

1.2.2   Scope of pollen production studies

Numerous studies have accessed pollen production across various plant taxa. **Table 1.1** summarizes selected previous research on pollen production estimates for tree, grass, and herbaceous species. However, many studies are constrained by limited sample sizes, particularly for non-woody species.

The general findings indicate that pollen production per anther in grass species is typically lower than in tree species. For instance, in the studied set of anemophilous grasses (e.g. *Avena sterilis, Cynodon dactylon, Dactylis glomerata*), the mean pollen production per anther was estimated to range from 2,000 (Subba Reddi & Reddi 1986) to 2,500 (Prieto-Baena *et al.* 2003) pollen grains. For anemophilous tree species (eg. *Pinus, Ulmus, Platanus, Quercus*), the mean pollen production per anther was approximately 25,000 pollen grains (Molina *et al.* 1996).

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Table 1.1 Selected previous studies on pollen production

Tree species	Grass species	Herb species
• *Acer* (Molina *et al.* 1996; Katz *et al.* 2020) • *Alnus* (Moe 1998) • *Betula* (Jochner *et al.* 2011; Katz *et al.* 2020; Piotrowska 2008; Kolek 2021; Jato *et al.* 2007; Jetschni *et al.* 2023) • *Cedrus* (Khanduri & Sharma 2009) • *Corylus* (Damialis *et al.* 2011) • *Cupressus* (Hidalgo *et al.* 1999; Damialis *et al.* 2011) • *Fraxinus* (Molina *et al.* 1996; Castiñeiras *et al.* 2019; Eisen *et al.* 2024) • *Juniperus* (Pers-Kamczyc *et al.* 2020) • *Olea* (Molina *et al.* 1996; Ferrara *et al.* 2007; Damialis *et al.* 2011; Aguilera & Valenzuela 2012) • *Platanus* (Damialis *et al.* 2011; Katz *et al.* 2020) • *Pinus* ((Ladeau & Clark 2006; Charalampopoulos *et al.* 2013; Khanduri & Sharma 2002) • *Quercus* (Molina *et al.* 1996; Gómez-Casero *et al.* 2004; Charalampopoulos *et al.* 2013; Kim *et al.* 2018; Fernández-González *et al.* 2020; Katz *et al.* 2020) • *Tectona* (Khanduri 2012)	Subba Reddi & Reddi 1986; Prieto-Baena *et al.* 2003; Aboulaich *et al.* 2009; Tormo-Molina *et al.* 2015; Jung *et al.* 2018; Romero-Morte *et al.* 2018; Ali *et al.* 2022; Severova *et al.* 2022; Piotrowska 2008; Jetschni *et al.* 2023	• *Artemisia* (Bogawski *et al.* 2016; Piotrowska 2008) • *Ambrosia* (Ziska & Caulfield 2000; Wayne *et al.* 2002; Rogers *et al.* 2006) • *Parietaria* (Fotiou *et al.* 2011) • *Plantago* (González-Parrado *et al.* 2015; Piotrowska 2008; Jetschni *et al.* 2023) • *Rumex* (Piotrowska 2008) • Others (Mondal & Mandal 1998)

The quantification of pollen production of various plant species holds significant implications, whether related to reproduction or not. For example, in the forestry and agricultural sectors, they can aid in predicting future crop yields, as well as the extent of fruit and seed production (Faegri & Iversen 1989; Allison 1990; González-Fernández *et al.* 2020). Numerous studies have demonstrated that pollen production is highly responsive to fluctuations in environmental conditions (Jablonski *et al.* 2002; Rogers *et*

al. 2006; Damialis *et al.* 2011; Jochner *et al.* 2013b). Due to this sensitivity, monitoring pollen from different plant species that exhibit a strong response to environmental changes could serve as an indicator for assessing local and global environmental shifts. However, there has been no long-term monitoring of pollen production. Additionally, analyzing the composition and quantities of airborne and deposited pollen can provide valuable insights into the current and historical vegetation patterns in a given region (Subba Reddi & Reddi 1986; Moore *et al.* 1991; Kouli *et al.* 2015). Detailed knowledge on the pollen production of a species is crucial for improving pollen forecasting (Jato *et al.* 2007). This metric plays a pivotal role in regulating the concentration of airborne pollen, alongside factors such as plant abundance, meteorological conditions, and atmospheric patterns (Skjøth *et al.* 2013). Finally, in public health, the estimation of pollen production levels within allergenic plant taxa gains heightened importance due to the potential to trigger immune reactions and allergic symptoms in sensitized individuals when exposed to airborne pollen from these specific plant species (D'Amato *et al.* 2017; Damialis *et al.* 2019a). Over the last two decades, the importance of pollen production studies in herbs, grasses and trees has witnessed a substantial threefold increase (**Figure 1.1**).
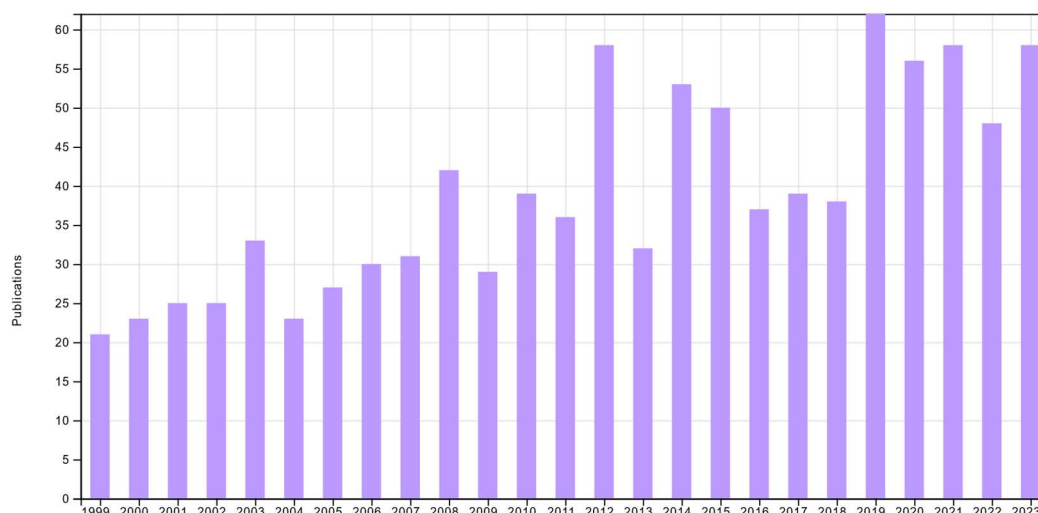
Figure 1.1 Number of publications per year (1999 to 2023 September) based on search for the topic "pollen production" (Web of Science; accessed on 27 May 2024).

1.2.3 Factors affecting pollen production

Pollen production is a complex process influenced by both genetic and environmental factors (Subba Reddi & Reddi 1986). Numerous studies have evidenced that underlying genetic factors exhibit strong associations with plant traits encompassing phenological patterns (Rousi & Pusenius 2005), morphological characteristics (Possen *et al.* 2011), physiological processes (Bradshaw & Stettler 1995; Wullschleger *et al.* 2005), reproduction efficiency (anther residuals and seed production) (Rousi *et al.* 2019), and distributional ranges (Bothwell *et al.* 2021). It was suggested that the amount of pollen grains produced per anther and the number of anthers per flower are genetically fixed and does not vary substantially (Subba Reddi & Reddi 1986; Hidalgo *et al.* 1999; Fernández-González *et al.* 2020). However, there remains a research gap in exploring how genetic variation within a species may impact pollen production.

Further variation in pollen production may be related to changes in several environmental conditions (Subba Reddi & Reddi 1986). Numerous studies on this topic have shown that various environmental factors influence the production of pollen, flowers and catkins (Wayne *et al.* 2002; Ziska & Caulfield 2000; Rogers *et al.* 2006; Damialis *et al.* 2011; Jochner *et al.* 2011; Zhao *et al.* 2017; Ladeau & Clark 2006; Jato *et al.* 2007). The most prominent factors are air temperature (Hicks *et al.* 1994; Moe 1998; Ziska *et al.* 2003; Rogers *et al.* 2006; Jochner *et al.* 2011; Jochner *et al.* 2013b; Barnes 2018; Kolek 2021; Zhang & Steiner 2022) and air pollution (Jablonski *et al.* 2002; Wayne *et al.* 2002; Darbah *et al.* 2008; Jochner *et al.* 2013b; Zhao *et al.* 2017; Kim *et al.* 2018). These factors, particularly air temperature and air pollution, can cause stressful conditions and can significantly alter pollen production.

Some studies examining the relationship between temperature and pollen production have suggested that warmer conditions result in higher pollen quantities. For example, experimental studies indicated that an increase in temperature (Ziska *et al.* 2003), elevated CO_2 (Ziska & Caulfield 2000; Wayne *et al.* 2002; Ziska *et al.* 2003) and elevated NO_2 (Zhao *et al.* 2017) increased the pollen production of herbaceous common ragweed (*Ambrosia artemisiifolia*). In contrast, other studies have found a negative correlation between temperature and pollen production. A study in the greater Munich area

examined the pollen production of urban and rural birch trees and found a significantly higher pollen production in rural compared to urban areas in 2010. Pollen production was negatively correlated with temperature, as well as with foliar potassium and iron concentration, and atmospheric NO_2 concentration (Jochner *et al.* 2013b). These findings suggest that urban environmental conditions with extreme temperatures and air pollution might not promote pollen production. The fundamental differences in temperature effect on pollen production found in these studies might be related to a combination of influential factors, species-specific differences in temperature response, or even differences in methodologies used.

Additionally, site conditions (Faegri & Iversen 1989; González-Parrado *et al.* 2015; Pers-Kamczyc *et al.* 2020; Eisen *et al.* 2024), including edaphic factors (Moe 1998; Lau & Stephenson 1993), could alter flower and/or pollen production. Silvicultural activities can cause stress on plant physiology, potentially leading to an increased reproductive output (Viherä-Aarnio & Ryynänen 1994; Stoehr *et al.* 1995; Li *et al.* 2021). Further influential factors are related to masting (Ranta *et al.* 2008; Crone & Rapp 2014). Other induced physiological stresses on plants due to pathogen attack, drought, and nutrient deficiency (Pandey *et al.* 2015; Li *et al.* 2021; Seleiman *et al.* 2021), could potentially modulate pollen production, either enhancing or decreasing the amount of pollen. However, the role of these and other variables influencing pollen production are poorly known.

Apart from genetic and environmental factors, differences in the reported estimates of pollen production could also result from methodological inadequacies (Damialis *et al.* 2011), as field sampling techniques, laboratory pollen extraction protocols, and further quantification lack uniformity, and certain methods appear to lack complete reliability (Moore *et al.* 1991).

1.3   Birch ecology and distribution

Birch (cf. German *Birke*) refers to a small to medium-sized deciduous tree of the genus *Betula* belonging to the Betulaceae family, and is widely distributed across various temperate and boreal regions in the Northern Hemisphere (Atkinson 1992). This tree is known for its distinct papery bark and delicate leaves. Birches often feature slender

trunks and branches, and their leaves are typically small and serrated (Beck *et al.* 2016; Hynynen *et al.* 2010; Vakkari 2009).

The most common birch species are *Betula pendula*, *Betula pubescens* and *Betula papyrifera*. Before, the species of Eurasian birches were known as *Betula alba* L. They were later classified as diploid silver birch (*Betula pendula*, with a somatic count of 28) and tetraploid hairy birch (*Betula pubescens*, with a somatic count of 56) (Meier-Dinkel 1992). The popular names for *B. pendula* Roth (= *B. verrucosa* Ehrh.) are **silver birch** or white birch. *B. pubescens* Ehrh. is also known as **downy birch**, hairy birch or pubescent birch.

Betula pendula and *Betula pubescens* have a broad natural range across Europe extending to central Siberia (Beck *et al.* 2016). *B. pubescens* is characterized by one of the most northerly and easterly distributions of European tree species while *B. pendula* grows in southern part of Europe such as the Iberian Peninsula, southern Italy, and Greece (Beck *et al.* 2016) (see **Figure 1.2** and **Figure 1.3**).

Figure 1.2 Distribution map of *B. pubescens*. Native range and isolated populations (Caudullo *et al.* 2017) are shown in green.
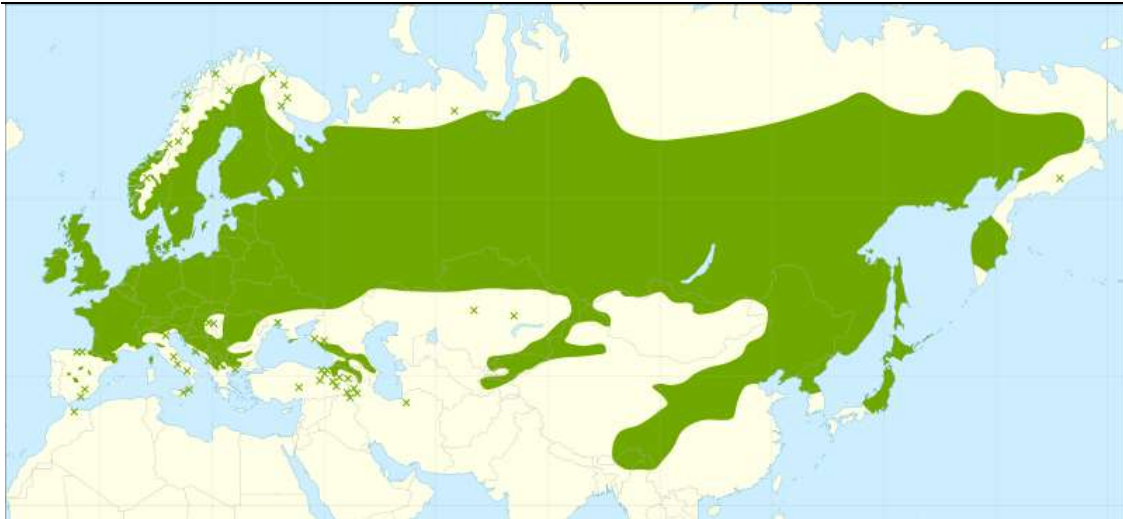
Figure 1.3 Distribution map of *B. pendula*. Native range and isolated populations (Caudullo *et al.* 2017) are shown in green.

Birch trees can survive on compact soils and wet peatlands (Hynynen *et al.* 2010). They often grow in lowlands as well as occur at higher altitudes (Emberlin *et al.* 2002). In Germany, birch is found up to an altitude of approx. 1,800 m a.s.l. (DWD 1991). The latest citizen-science generated data demonstrated that *B. pubescens* can occur at altitudes as high as 1,840 m a.s.l. and *B. pendula* was found at a maximum altitude of 1,610 m a.s.l. in the Bavarian Alps (BAYSICS Webportal 2022). Due to climate change, the birch distribution shown in **Figure 1.2** and **Figure 1.3** might change, and in the future, some localities may have diminished or increased prevalence of birch trees. It has been anticipated that, owing to the multifaceted impacts of climate change, birch trees may become less common at lower elevations but could shift their treeline and become more dominant at higher elevations in the Bavarian Alps over the next half-century, as postulated by Rojo *et al.* (2021).

Birch pollen are highly allergenic (D'Amato *et al.* 2007; Smith *et al.* 2014) presenting a major source of allergic rhinitis in Europe (Biedermann *et al.* 2019). Due to its aesthetic value, birch is a frequently used tree species in urban green space planning in Europe (Krišāns *et al.* 2022; Battisti *et al.* 2019). The abundance of birches, however, is problematic for many people who are allergic to pollen (Piotrowska 2008). Being an anemophilous tree species, birch produces large amount of pollen and previous studies have reported the estimates from 4.8 million to 10 million pollen grains per inflorescence (Erdtman 1954; Jato *et al.* 2007; Piotrowska 2008).

1.4 International Phenological Gardens

The widespread ecological importance of birch species, combined with their role as allergenic pollen producers and their adaptability to diverse climatic conditions, underscores the necessity of understanding their phenological development across varying environments. To investigate such phenological patterns in birches and but also in other forest tree species, the International Phenological Gardens (IPG) network was established. This network monitors genetically identical plant individuals across Europe, providing unique insights into the effects of climate and geographic variability on plant phenology.

The IIPG network monitors vegetatively propagated plant individuals exhibiting the same genotypes from 23 plant species (including downy birch) at 63 active IPGs across 19 European countries (Renner & Chmielewski 2021). The idea of the phenological gardens originated as early as 1953 in the Commission for Agricultural Meteorology of the World Meteorological Organization - the mother garden was also established at that time. The basic principle of the IPGs is that genetically identical trees and shrubs are planted throughout Europe and their phenological development is then observed using a standardized method.

The initial and major goal of the IPGs was to conduct phenological research. The vast geographic extent of the network and genetically identical plants provide data that illustrate the large-scale temperature effects on plant phenology (Caffarra & Donnelly 2011; Olsson *et al.* 2017; Wenden *et al.* 2020; Linkosalo *et al.* 2019). Olsson *et al.* (2017) have used IPG data (23 IPGs and 1506 records from 1968 until 2013) to develop a phenological model of budburst in Norway spruce (*Picea abies*). Wenden *et al.* (2020) conducted a study that utilized clones of *Fagus sylvatica* and *Quercus robur* to investigate whether there are consistent patterns in how clones perform when planted along a gradient of increasing mean temperatures. Another study examined the impact of light and temperature on bud burst among *Betula pubescens*, *Fagus sylvatica*, *Salix smithiana*, and *Tilia cordata* (Caffarra & Donnelly 2011). Linkosalo *et al.* (2019) investigated the efficacy of transplanting valuable nursery trees to areas with varying spring temperatures. The goal was to establish a thermal time model for *Tilia cordata*

by utilizing accurate phenological observations from a single clone of this species that was used in all IPGs. In addition, IPG plants were also used to study biogenic volatile organic compound (BVOCs) emission patterns along a latitudinal gradient (van Meeningen *et al.* 2016).

The spatial gradients covered by these IPGs are, in some cases, even larger than the natural distribution of some plant species. For example, birches in IPG Mostar, IPG Sarajevo (Bosnia and Herzegovina), and IPG Skopje (North Macedonia) are located beyond the natural distribution range of *B. pubescens*, as shown in **Figure 1.2** and **Figure 2.1**. These latitudinal and longitudinal gradients present a valuable opportunity for investigating the impact of climate on plants through natural experiments (Frenne *et al.* 2013). Research on pollen production of allergenic plants, however, has not been applied so far in this unique network.

The latest IPG was established at Catholic University of Eichstätt-Ingolstadt, Germany on 25 July 2023. At the university campus, 29 trees and shrubs have been recently planted. Presently, the IPG network is being coordinated by Professor Susanne Jochner-Oette (see https://ipg.ku.de/). Previously, Prof. Dr. Frank Chmielewski from the Institute of Agricultural and Horticultural Sciences at Humboldt University Berlin had headed the network for more than 25 years.

1.5 Research gaps and research questions

The existing research on birch pollen production suffers from several limitations and present substantial research gaps. The reported birch pollen production estimates rely on small sample sizes, often examining only a few trees over a limited period of one or two years, rendering their estimates unreliable. Furthermore, the factors influencing variability in birch pollen production are not well comprehended (see Chapter 1.2.3). The fluctuations in birch pollen production pose an important challenge from both ecological and public health standpoints, underscoring an urgent necessity for comprehensive investigations to quantify reliable birch pollen production estimates, explore potential stressors, including temperature effects, and elucidate their temporal and spatial dynamics.

There is a hypothesis that stressful environments could increase pollen production as a defensive response or due to altered plant physiology. The major birch pollen allergen, Bet v 1, belongs to the pathogen-related protein (PR10) family involved in plant defense and pollen-stigma recognition (Plaza *et al.* 2016). PR proteins exhibit upregulated expression under abiotic stress, pathogen attack, or wounding (Liu *et al.* 2003; Srivastava *et al.* 2004). Consequently, stressful conditions might enhance Bet v 1 expression, potentially affecting pollen and inflorescence production, as reported by some studies (Damialis *et al.* 2011; Plaza *et al.* 2016; Jochner *et al.* 2013b). However, the magnitude of such changes and their consistency across varying European environmental conditions are unknown.

For this reason, the following key research questions are to be addressed in this PhD thesis:

1. What are the pollen production estimates for *Betula* species? In other words, how many inflorescences and pollen are produced by distinct types of the tree species?
2. Is there a year-to-year variation in birch pollen production?
3. How do genetic and environmental factors influence birch pollen production at local and regional scales?

The studies done in this PhD thesis are part of the interdisciplinary project "pollenPALS: Biotic and abiotic effects on pollen production and allergenicity of birch and related health impacts (655850)" funded by the German Research Foundation (Deutsche Forschungsgemeinschaft) (DFG).

1.6 Outline of thesis

The thesis is structured as follows:

The introductory Chapter 1 provided a comprehensive overview of pollen, prevalence of allergic diseases, climate change, factors affecting pollen production, as well as a general overview of birch species and the International Phenological Gardens. The following chapters describe the study sites (Chapter 2) and the data collection

methods (Chapter 3). This thesis contains three scientific publications in Chapters 4, 5 and 6. All publications have been peer-reviewed and published.

The first publication (Chapter 4) "**Is Pollen Production of Birch Controlled by Genetics and Local Conditions?**" (Ranpal *et al.* 2022, published in International Journal of Environmental Research and Public Health) assessed the pollen production of eleven groups of cloned weeping birch (*Betula pendula* Roth) individuals (n = 28) in three consecutive years (2019–2021). This paper checked for differences between years as well as between and within clones and considered their synchronicity of pollen production levels. Based on the results, we discussed the implications of selecting clones producing a high/low level of pollen for seed plantations/urban planting. We present a robust mean for the pollen production of *Betula pendula* (1.66 million pollen grains per catkin). Our findings showed temporal and clonal variations in pollen production. We conclude that synchronized high or low pollen production is not utterly site-specific and, in addition, not strictly dependent on genotypes. We suggest that appropriate clone selection based on application (seed plantation, urban planting) might be advantageous and encourage long-term monitoring.

The second publication (Chapter 5) "**Pollen production of downy birch (*Betula pubescens* Ehrh.) along an altitudinal gradient in the European Alps**" (Ranpal *et al.* 2023, published in International Journal of Biometeorology) assessed pollen production of 17 birch (*Betula pubescens* Ehrh.) individuals along an altitudinal gradient in the European Alps. We sampled catkins at nine locations in the years 2020-2021 and monitored air temperatures. This paper investigated how birch pollen, flowers, and inflorescences are produced in relation to thermal factors at various elevations. We found that mean pollen production of *Betula pubescens* Ehrh. varied between 0.4 million to 8.3 million pollen grains per catkin. We did not observe any significant relationships between the studied reproductive metrics and altitude. However, minimum temperature of the previous summer was found to be significantly correlated to pollen, flower and catkin production per volume unit of crown. Therefore, we suggest that temperature variability even at such small scales are very important for studying the response related to pollen production.

The third publication (Chapter 6) "**Continental-scale Evaluation of Downy Birch Pollen Production: Estimating the Impacts of Global Change**" (Ranpal et al. 2024, published in Environmental Research) assessed the impact of meteorology (e.g., temperature and precipitation) and atmospheric gasses (e.g., ozone (O_3) and carbon-dioxide (CO_2)) on pollen and catkin production of *Betula pubescens* Ehrh trees planted in IPGs across Europe, while controlling for tree age approximated by stem circumference on pollen production. The results revealed a substantial geographic variability in mean pollen production, ranging from 1.9 to 2.5 million pollen grains per catkin. Regression analyses indicated that elevated average temperatures of the previous summer corresponded to increased pollen production, while higher O_3 levels led to a reduction. Additionally, catkins' number was positively influenced by preceding summer's temperature and precipitation but negatively by O_3 levels. The investigation of quantile effects revealed that the impacts of mean temperature and O_3 levels from the previous summer varied throughout the conditional response distribution. This comprehensive study along large spatial gradients sheds light on potential impacts of climate change on downy birch pollen production, which is crucial for reproduction and public health.

In the Discussion (Chapter 7), the main findings from the studies of this PhD are interpreted and discussed in relation to the existing body of published literature on the topic. It evaluates the results in relation to the research questions and places them within the broader scientific context. This analysis highlights the importance and implications of the research and outlines future research directions. Chapter 8 summarizes and concludes the key findings from the three scientific publications presented in this thesis.

Chapter 9 presents a consolidated list of references cited in the Chapters 1, 2, 3 and 7, allowing the reader to easily access the full details of the sources used. Chapter 10 outlines the publications and candidate's individual contributions to the published works.

2   Birch study sites

This doctoral research encompasses three study sites: a small plantation in Germany, a cross-border altitudinal gradient in Germany and Austria, and International Phenological Gardens (IPGs) spanning across Europe (**Figure 2.1**). For the investigation on the influence of genetics and local conditions on pollen production (Chapter 4), a *Betula pendula* seed plantation near Wildberg was selected. To examine pollen production of *Betula pubescens* along an elevational gradient (Chapter 5), the study area encompassed the Bavarian Alps region. To comprehensively assess *Betula pubescens* pollen production across broader spatial scales (Chapter 6), IPGs distributed throughout Europe were incorporated into the study design.
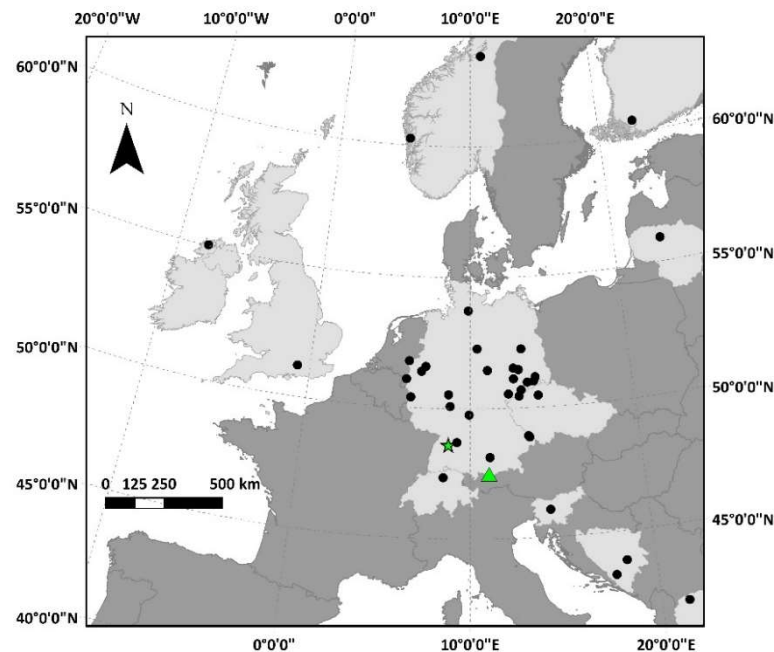
Figure 2.1 Location of birch study sites included in this PhD thesis. Green star shows the location of the seed plantation, green triangle locates the region selected in the Bavarian Alps and black points represent the International Phenological Gardens across European countries.

2.1   Seed plantation

The birch seed plantation is situated near Wildberg (48°36'44" N, 8°42'37" E, 500 m a.s.l.) in Baden-Württemberg, Germany (**Figure 2.1**; green star). The plantation was established in 2005 on an 1-hectare area with 7 m x 7 m planting grid and is managed by Forst Baden-Württemberg. This area experienced an average annual temperature of 8.6°C and receives 892 mm of precipitation annually (German Meteorological Service

station "Neubulach-Oberhaugstett", 1991-2020) (DWD 2022). The plantation occupies a west-facing slope with a 2°-6° inclination, and the soil type is classified as Cambisol (LGRB 2021). Initially comprising 215 trees from 44 distinct clones planted in 13 rows and 17 columns, the plantation underwent thinning, leaving 113 birch trees from 44 clones (with one to six representatives each) currently present on-site. The clones and individual trees were spatially randomized throughout the plantation.

2.2 Bavarian Alps

The selected gradient is located in southern Bavaria (Germany) and Tyrol (Austria), within the topographically complex Zugspitze region (**Figure 2.1**; green triangle). At 2,962 m.a.s.l., the Zugspitze is the highest mountain in Germany and is part of the Northern Limestone Alps in the Wetterstein Mountains (Jochner *et al.* 2012). The selected birch trees were situated along an altitudinal gradient spanning Garmisch-Partenkirchen (700 m a.s.l.) to the Eibsee lake (1,000 m a.s.l.), Ehrwald in Austria (1,100 m a.s.l.), and the Ehrwald Cable Car Station (1,220 m a.s.l.), covering an elevational range of 522 m. Lower elevations were dominated by meadows, while forests with spruce as the primary tree species prevailed up to approximately 1,800 m. Garmisch-Partenkirchen experienced an average annual temperature of 7.7°C and precipitation of 1,373 mm during 1991–2020. From 2019 to 2021, the average temperature and total precipitation were 7.9°C and 1,315 mm (2019), 6.1°C and 1,419 mm (2020), and 7.4°C and 1,434 mm (2021), respectively.

2.3 IPGs across Europe

This study was carried out at 37 IPGs located in eleven European countries (see **Figure 2.1**; black points). The IPGs spanned a latitudinal range of over 2,500 km, from Skopje, North Macedonia, to Trondheim, Norway, and a longitudinal extent of approximately 2,000 km, from Glenveagh, Ireland, to Šiauliai, Lithuania. The maximum distance between two IPGs was 2,600 km (Skopje, North Macedonia to Glenveagh, Ireland). The minimum distance was 7.2 km (Freyung-Schönbrunn and Freyung-Waldhäuser, Germany). Nearly all sampled birches fell within the natural range of *Betula pubescens* (Caudullo *et al.* 2017), except those from Bosnia and Herzegovina (Mostar and Sarajevo) and North Macedonia (Skopje). The sites were mostly located in rural areas (we

calculated the urban index (Jochner *et al*. 2012) for each IPG, but these indices are not included in this thesis). Additionally, soil conditions varied across the sites but were not assessed in this study.

3 Short summary of the applied methods

3.1 Sampling and analyses for the estimation of pollen production

To assess pollen production in *Betula* species, male catkins with mature, closed anthers were harvested during spring, after catkin elongation initiation but before anthesis. Sampling was conducted at reachable heights (1.5 to 2 m above ground) across all cardinal directions. Tree parameters such as height, stem circumference, and crown dimensions were measured using a Suunto PM-5/1520PC Height Meter and a measuring tape. A 50 cm × 50 cm × 50 cm sampling cuboid, representing typical catkin distribution as suggested by Damialis *et al.* (2011), was positioned within the crown. Catkins within this cuboid were counted for extrapolating pollen production to crown volumes. Upon arrival at the laboratory facilities, the collected catkin samples were preserved at -20°C.

Employing a standardized methodology for studying pollen production and maintaining its methodological consistency are crucial to obtain precise pollen production estimates and facilitate meaningful comparisons across studies. In the studies conducted for this PhD thesis, we adapted the standardized pollen production study methodology proposed by Damialis *et al.* (2011). Several recent investigations (Damialis *et al.* 2011; Fotiou *et al.* 2011; Charalampopoulos *et al.* 2013; Kolek 2021; Jetschni *et al.* 2023; Eisen *et al.* 2024) have employed the same approach, facilitating direct comparisons.

The pollen extraction method involved immersing birch catkins in a 10% KOH solution overnight, followed by boiling and mechanically disrupting the softened catkins to release pollen grains (Faegri & Iversen 1989; Moore *et al.* 1991). A 70% glycerol solution was added to prevent pollen clumping (Shivanna & Rangaswamy 1992). Aliquots were taken from the homogenized suspension, stained with safranin, and pollen grains were counted under a microscope (Damialis *et al.* 2011). Pollen production was quantified at different scales, including per catkin, flower, crown volume unit, and individual tree, by extrapolating from the counted aliquots using established formulae (Damialis *et al.* 2011).

3.2 Environmental data

3.2.1 Meteorological data

To assess meteorological influences on pollen production, different approaches were employed across the studies. For Chapters 4 and 5, temperature loggers (HOBO Pro v2 U23-001, Onset, Bourne, MA, USA) with radiation shields were installed at 2 m height at the northern side of trees, recording data at 10-minute intervals throughout the study period. To estimate potential solar radiation received by the trees located at the seed plantation in Chapter 4, an unmanned aerial vehicle Phantom 4 Pro was used.

Chapter 6 relied on gridded meteorological data from the E-OBS dataset (version 23.1e), sourced from the UERRA project, Copernicus Climate Change Service, and ECA&D providers (Cornes *et al.* 2018). This high-resolution (0.1°) gridded dataset encompassed variables such as mean, minimum, and maximum temperatures, relative humidity, precipitation, and solar radiation. Considering the importance of the preceding summer for catkin development and elongation (Dahl & Strandhede 1996), meteorological data from June to August were aggregated through averaging or summing, as appropriate for each variable.

3.2.2 Air pollutants data

In Chapter 4 and Chapter 5, were we focused at relatively small geographical regions, the air quality (NO_2, NO_x and O_3) was monitored using passive samplers for a week in summer 2020. For Chapter 6, in which a European wide distribution of trees was involved, O_3, NO_2 and CO_2 concentrations data were derived from Copernicus Atmosphere Monitoring Service (CAMS) reanalysis.

3.3 Statistical analyses

This PhD thesis employed a robust statistical approach combining nonparametric tests and quantile regression to investigate relationships between various factors and pollen production. Nonparametric tests, not relying on normality assumptions, were utilized: Kruskal-Wallis test assessed differences across multiple groups, Mann-Whitney U test for pairwise comparisons, and Spearman's correlation quantified associations between variables. Importantly, quantile regression, a distribution-free method, modeled

quantiles of the pollen production distribution directly. This allowed examining how factors such as temperature and ozone levels influenced different quantiles, providing insights into potential heterogeneous effects across the response distribution. The analyses leveraged the latest R versions (4.1.2, 4.2.2, 4.3.2), ensuring up-to-date methods and software. This comprehensive approach, combining nonparametric tests for group comparisons and correlation analyses with quantile regression for distribution-wide assessments, enabled a rigorous exploration of the complex relationships between environmental factors and pollen production dynamics.

In summary, the methods employed in this study, encompassing standardized sampling and pollen quantification techniques, meteorological monitoring, and a set of statistical analyses, provide a comprehensive framework for investigating the multifaceted relationships between pollen production and environmental factors.

4   Is Pollen Production of Birch Controlled by Genetics and Local Conditions?

Authors   **Surendra Ranpal, Miriam Sieverts, Verena Wörl, Georgia Kahlenberg, Stefanie Gilles, Maria Landgraf, Kira Köpke, Franziska Kolek, Daria Luschkova, Tobias Heckmann, Claudia Traidl-Hoffmann, Carmen Büttner, Athanasios Damialis and Susanne Jochner-Oette**

Article

Is Pollen Production of Birch Controlled by Genetics and Local Conditions?

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Abstract: Intraspecific genetic variation might limit the relevance of environmental factors on plant traits. For example, the interaction between genetics and (a-)biotic factors regulating pollen production are still poorly understood. In this study, we investigated pollen production of 28 birch (*Betula pendula* Roth) individuals in the years 2019–2021. We sampled catkins of eleven groups of genetically identical trees, which were partially topped, but of the same age and located in a seed plantation in southern Germany characterized by similar microclimatic conditions. Furthermore, we monitored environmental factors such as air temperature, characterized air quality (NO_2, NO_x and O_3), and assessed potential solar radiation. We especially checked for differences between years as well as between and within clones and assessed the synchronicity of years with high/low pollen production. We present a robust mean for the pollen production of *Betula pendula* (1.66 million pollen grains per catkin). Our findings show temporal (H(2) = 46.29, $p < 0.001$) and clonal variations (H(4) = 21.44, $p < 0.001$) in pollen production. We conclude that synchronized high or low pollen production is not utterly site-specific and, in addition, not strictly dependent on genotypes. We suggest that appropriate clone selection based on application (seed plantation, urban planting) might be advantageous and encourage a long-term monitoring.

Keywords: *Betula pendula*; genotypes; reproduction; seed orchard

1. Introduction

Genetic variation among plant species is believed to limit the explanatory power of abiotic or biotic influential factors on certain plant traits. Several studies have revealed that plant traits, e.g., related to phenology, morphology, physiology, reproduction, and distribution are associated with genetic controls. Neophytou et al. [1] found a significant variation in the timing of bud burst among different Douglas fir progenies. Likewise, previous studies on poplar hybrids have reported that the patterns of tree biomass distribution above- and below-ground were genetically controlled [2,3]. Furthermore, naturally regenerated birch and aspen populations showed a variation between genotypes in the acclimatization to soil moisture conditions by altering biomass, root and leaf morphology,

water potential, and gas exchange [4]. Rousi et al. [5] documented significant variations in intraspecific reproduction efficiency (anther residuals and seed production) among individuals of *B. pubescens* in two neighboring stands in Northern Finland. In addition, information on intraspecific genetic variations plays a crucial role to improve species distribution models [6]. Under varying environmental conditions, an exposed genotype has the ability to express phenotypic plasticity [7]. Studies on birch revealed phenotypic plasticity in leaf morphology of transplanted trees related to edaphic conditions [8] and larger phenotypic plasticity of juvenile above-ground growth traits in response to soil nutrient conditions [9]. Such findings indicate that traits of plant individuals of the same species growing under similar or different environmental conditions must be understood with the consideration of intraspecific variations.

Pollen are developed in anthers (angiosperms) or in microsporangia (gymnosperms) and their quantity per inflorescence is regarded as pollen production [10]. Pollen production may be controlled by the genes of taxa, species or varieties. It was suggested that the amount of pollen grains produced per anther and the number of anthers per flowers are genetically fixed and does not vary substantially [11–13]. In addition, any further variations could be related to changes in environmental conditions [12] such as meteorology, primarily air temperature [14–16], and edaphic factors [15,17], which alter the number of flowers and/or pollen production per flower. However, the role of these and other variables influencing pollen production are poorly known.

Most studies on pollen production of woody plants are limited to genera or species. Yet, a small number of studies have focused on the intraspecific level, for example, related to *Cupressus sempervirens* varieties [18] or *Theobroma cacao* clones [19]. Although Adams and Kunze [20] studied clonal variations in seed production in spruce, there has been little discussion on pollen production of genetically identical trees.

In general, genetically identical trees are preferentially used for various applications in science because it is assumed that they show the same behavior, e.g., related to phenology [21,22]. Long-term phenological observation networks such as the International Phenological Gardens in Europe (IPG) standardized phenological studies by establishing gardens with cloned plant individuals to exclude genetic effects [21,23]. Such phenological investigations based on cloned tree species assure that observed variances are due to environmental causes rather than genotypic differences between plants [24]. There have been attempts to explore the influences or exclusion of genetics on other pollen properties such as allergenicity. Ahlholm et al. [25] investigated the allergenicity of mountain birch pollen collected from trees of ten half-sib families growing in northern Finland and found that the concentration of the major birch pollen allergen (Bet v 1) is genetically controlled. In addition, concentrations of the allergen Cry j 1 produced by pollen of Japanese cedar were reported to be significantly different between trees of eight clones [26]. Similarly, Fernández-Caldas et al. [27] demonstrated considerable variations in pollen allergenicity (Ole e 1) of different varieties of *Olea europaea*.

However, studies related to pollen production compared for different clones in birch are lacking and are in general very sparse related to other species of the plant kingdom. Veilleux and Lauer [28] studied potato (*Solarium phurejas*) clones and suggested that plants of the same genotype respond similarly to the environment and produce the same amount of unreduced pollen grains. Panda et al. [29] observed a wide variation in pollen production per anther, pollen size and pollen viability among selected banana (*Musa* spp.) genotypes. Information on the variability of pollen production of genetically identical wind-pollinated plants is, however, largely lacking.

Detailed knowledge on the pollen production of a species is crucial for improving pollen forecasting [30]. Such forecasts have agronomical importance as seed production and, therefore, harvest outcomes often rely on pollen production [31]. Pollen production also plays a vital role in allergology. In the past few years, phenological, biometeorological, and aerobiological studies on allergenic plants have become more important due to the high prevalence of allergies around the world. According to the World Allergy Organization

(WAO) up to 40% of the global population suffers from allergic sensitization [32], which could further increase by a parallel increase in pollen production [33–36].

Birch has a wide range of distribution in the Northern Hemisphere [37] and its pollen are highly allergenic [38,39] presenting a major source of allergic rhinitis in Europe [40]. Due to its aesthetic value, silver birch is a frequently used tree species in urban green space planning in Europe [41,42]. The abundance of birches, however, is problematic for many people who are allergic to pollen [43]. Studies on genotypic variations of pollen production of such allergenic tree species could identify clones, which are characterized by a lower pollen production. The breeding of such clones, e.g., for planting in urban green spaces, might also imply a reduction of atmospheric pollen concentration. On the other hand, seed plantations, in which a high pollen production of trees is desirable for a high quantity of seeds, may profit from those clones that are associated with a higher production of pollen. Most important, knowledge on the genetic variability of pollen production will allow for better evaluating the influence of environmental factors/climate change.

In this study, we assessed the pollen production of eleven groups of cloned weeping birch (*Betula pendula* Roth) individuals ($n = 28$) in three consecutive years (2019–2021). Since natural birch populations show a high grade of hybridization [44], we sampled inflorescences of genetically identical trees of the same age from a seed plantation (Baden-Württemberg, Germany), assessed the ambient microclimatic conditions and monitored any silvicultural treatments. We especially checked for differences between years as well as between and within clones and considered their synchronicity of pollen production levels. Based on the results, we discussed the implications of selecting clones producing a high/low level of pollen for seed plantations/urban planting.

2. Materials and Methods

For this study, we selected a birch seed plantation located near Wildberg (48°36′44″ N, 8°42′37″ E, 500 m a.s.l.) in Baden Württemberg, Germany (Figure 1). The average annual temperature is 8.6 °C and the precipitation sum is 892 mm (German Meteorological Service (DWD) station "Neubulach-Oberhaugstett", 1991–2020 [45]). The plantation is located on a west-exposed slope with an inclination of approximately 2°−6° and the soil type is Cambisol [46]. This 1-hectare sized plantation was established in 2005 and additional birch trees were planted in 2012, resulting in a 7 m × 7 m seedling cluster, which is managed by Forst Baden-Württemberg (Forst BW; territory number 3, Nagoldtal). Initially, 215 trees belonging to 44 different clones were planted in a total of 13 rows and 17 columns. The clones and trees were randomized spatially throughout the site. Until now, almost half of the birch trees were removed as a thinning measure: 113 birch trees from 44 clones (with one to six individuals) are still present in the plantation.

The study was conducted in three successive years (2019–2021). We focussed on 28 trees, all planted in 2005, representing eleven clones from six different geographic origins (Table 1). These clone origins are, however, located nearby, within approximately 45 km to 130 km from the study site. The trees were selected based on the reachability of twigs and, therefore, inflorescences. The number of studied trees per clone, therefore, varied between one to four.

Male catkins were harvested in March after the beginning of catkin elongation and prior to anthesis. Samples were collected from different branches at 1.5 to 2 m above ground from all cardinal directions. In addition, we measured growth traits: (a) the perimeter at breast height, (b) the height of the tree and crown by use of Suunto PM-5/1520PC Height Meter, and (c) the crown diameter, which was calculated by averaging two perpendicular diameters of the crown at its widest portion.
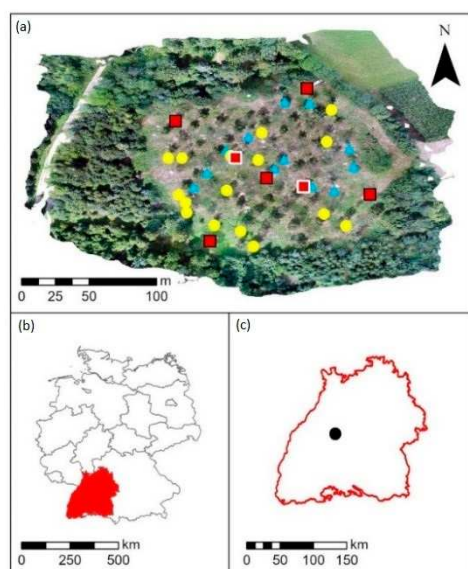
Figure 1. Study areas: (**a**) seed plantation near Wildberg (48°36′44″ N, 8°42′37″ E, 500 m a.s.l.) including measurement sites: blue triangles—topped sampled trees; yellow circles—non-topped sampled trees; red squares with black border—air temperature loggers; red squares with white border—passive samplers, (**b**) Baden-Württemberg in Germany (red fill) and (**c**) location in Baden-Württemberg (black circle).

Table 1. Studied clones and their geographic origins.

Clone Number	Number of Individuals per Clone	Origin	Latitude (N)	Longitude (E)
55–04	1			
55–07	4			
55–10	4	Lahr	48°21′	7°52′
55–46	2			
55–21	4	Nürtingen	48°37′	9°20′
55–24	2	Karlsruhe	49°00′	8°24′
55–30	2	Kehl	48°35′	7°51′
55–33	1			
55–38	3	Kandern	47°43′	7°39′
55–42	3			
55–47	2	Villingen-Schwenningen	48°04′	8°24′

We counted the number of catkins within a sampling cuboid (50 cm × 50 cm × 50 cm) in the crown, which was considered to characterize the average distribution of catkins in the tree [18]. We selected an ovoid shape of the crown to estimate pollen production per tree.

In July 2018, tree topping (cutting of the apical parts of the main trunk), which is an intended measure to increase seed production [47], was carried out in the seed plantation. Therefore, the sampled trees were categorized as topped (*n* = 12) and non-topped (*n* = 16). Six more sampled trees were topped in July 2020; however, male catkins were already formed in those trees and, therefore, no large effect on pollen production was assumed.

Air temperature and precipitation data were obtained from a 5 km distant DWD climate station "Neubulach-Oberhaugstett" [45]. In addition, we installed five temperature loggers (HOBO Pro v2 U23-001, Onset, Bourne, MA, USA) from spring 2019 (8 April) until summer 2021 (20 June). One logger was installed in the center and four at the northern, eastern, southern, and western borders of the plantation (red squares with black border in

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Figure 1) to determine temperature differences within the site. Each logger was placed in a radiation shield and mounted at a height of 2 m at the northern side of a birch tree. The loggers' data were retrieved and processed using HOBOware (Version 3.7.23) from Onset, Bourne, MA, USA.

The air quality of the study site was characterized by the measured values of nitrogen dioxide (NO_2), nitrogen oxides (NO_x) and ozone (O_3) concentrations monitored directly at the stem of the birch trees ($n = 2$, red squares with white border in Figure 1). Passive sampling of these pollutants lasted one week in summer 2020 (25 June to 2 July). The passive samplers were supplied and evaluated by Passam AG (Männedorf, Switzerland).

For estimating the potential solar radiation around each tree, the unmanned aerial vehicle Phantom 4 Pro, DJI, Nanshan, Shenzhen, China was used, which features an onboard RGB camera with a sensor resolution of 12 megapixels and a focal length of 24 mm. The flight altitude was 35 m above ground level. During the flight, which took place on 10 August 2019 and lasted approximately 17 min, 712 photos were taken with an overlap of 80%. A digital elevation model was generated using Metashape Professional (Version 1.8.1) from Agisoft LLC, St. Petersburg, Russia. In ArcGIS Pro (version 2.7.0) software from ESRI, Redlands, CA, USA, the spatial analyst tool "Solar radiation (area)" was used to calculate the potential solar radiation (W/m^2) on the surface depending on the time of day and position of the sun as well as the latitude for each pixel of the digital elevation model. We calculated solar radiation for each pixel as a sum for the period 1 May until 31 August as this period is critical for the start and development of the following year's catkin [48]. We selected a buffer of two meters around each tree and calculated the mean solar radiation. We assume only minor differences in the canopy of the surrounding forest and, therefore, use the data gained in 2019 for a general site characteristic for the whole study period.

To extract pollen grains, we adapted the method proposed by Damialis et al. [18]. For each year, one average-sized inflorescence from each cardinal direction and per tree was selected, its length and width were measured (at the widest point), and the number of flowers was counted. Then, each catkin was soaked in a 10% KOH solution [31,49] and boiled at 120 °C the following day. Afterwards, the plant material was crushed with a glass rod to break up plant tissues and to allow pollen release. To prevent pollen clumping [50], we added glycerol (70%), a bipolar solvent, to a volume of 20 mL; safranin was added as a stain. Two aliquot samples (10 μL each) per suspension were obtained using a VITLAB® micropipette while stirring it vigorously to ensure homogeneity. Subsequently, the extraction was put on microscope slides and covered with slips. Pollen grains on these slides were subsequently counted at 100× magnification (Zeiss AXIO Lab.A1, Germany). In case of a large difference between the pollen counts obtained from these two slides (>30%), the procedure was repeated in order to increase the homogeneity of the suspension.

We estimated pollen production at various scales [18]: The number of pollen grains per catkin (P_{ca}) was calculated using Equation (1):

$$P_{ca} = \frac{V_{su}}{V_{sa}} p \tag{1}$$

where V_{su} and V_{sa} are the volumes of the suspension (in mL) and the sample taken (in μL), respectively, and p is the number of pollen grains counted per 10 μL solution.

The number of pollen grains per flower (P_{fl}) was estimated as follows (Equation (2)):

$$P_{fl} = \frac{P_{ca}}{fl} \tag{2}$$

where fl is the number of flowers per catkin.

The number of pollen grains per volume unit (m^3) of crown (P_{cr}) was estimated using Equation (3):

$$P_{cr} = P_{ca} \frac{C_{su}}{M} \tag{3}$$

where C_{su} is the number of catkins per crown sampling unit (cuboid) and M is the volume of the sampling unit.

The number of pollen grains per individual (P_{in}) was estimated using Equation (4):

$$P_{in} = P_{cr}V \qquad (4)$$

where P_{cr} is the number of pollen grains per crown volume unit (see Equation (3)) and V is the total volume (in m^3) of the crown. The volume of an ovoid tree Crown can be calculated as follows (Equation (5)):

$$V = \frac{\pi d_1 d_2 h_c}{6} \qquad (5)$$

where $\pi \approx 3.14$, d_1 and d_2 are two perpendicular diameters of the crown, at its widest part, and h_c is the crown height.

Pollen production per flower, catkin, and volume unit of crown, as well as flowers per catkin and catkins per crown sampling unit, were descriptively analyzed. These reproductive metrics were non-normally distributed according to Shapiro–Wilk test. We checked for differences among sampling years and clones using the Kruskal–Wallis test and post-hoc (Dunn) test. Correlation analyses between the reproduction metrics and between solar radiation and pollen production were conducted using Spearman's correlation test. The differences between topped and non-topped trees were analyzed using Mann–Whitney U test. The variation within non-topped clones was assessed by comparing the coefficient of variances (CVs). For indicating if one specific clone can be proposed as "good" or "poor" regarding pollen production, we averaged the crown metrics (crown height and crown width) of all non-topped trees and calculated a mean crown volume. We considered that this computed crown dimension would represent an average non-topped birch tree in the seed plantation. Similarly, we calculated the mean P_{ca} and mean C_{su} obtained from the non-topped trees during the study years. These values allowed us to quantify the total P_{in} for an average tree (using Equation (5)). Further, we used average P_{ca} and C_{su} of each clone along with the crown volume of an average tree to calculate the pollen produced by each clone under mean growth parameters to compare the pollen produced by each clone to an average birch tree. All statistical analyses and visualizations were performed in RStudio (version 4.1.2) from RStudio, PBC, Boston, MA, USA, ArcGIS Pro (version 2.7.0) or Microsoft Excel 2016 from Microsoft, Washington, DC, USA.

3. Results

3.1. Descriptive Statistics and Correlation Analyses among Reproductive Metrics

The average pollen production per catkin (P_{ca}) for all selected 28 trees and all study years (2019–2021) was 1.66 ± 1.28 million pollen grains (see Table 2). P_{ca} varied within a wide range from 48,000 to 8.27 million pollen grains, especially in the year 2019. P_{ca} in 2020 was 11% higher and 28% lower compared to 2019 and 2021 when regarding mean values. P_{ca} in 2021 was 54% higher compared to 2019.

The number of catkins in a crown sampling unit (C_{su}; 0.125 m^3) ranged between 3 and 120 with an average of 29 catkins. C_{su} in 2020 were 191% and 232% higher compared to 2019 and 2021 and 17% lower in 2021 compared to 2019. Statistics for all analyzed levels (P_{ca}, P_{fl}, P_{cr}, fl and C_{su}) are presented in the Appendix A (Table A1).

We detected a statistically significant difference between P_{ca} among the three study years (H (2) = 46.29, $p < 0.001$). A post-hoc test revealed that there were significant differences between all pairs of years (Figure 2). The same applied for C_{su} (H (2) = 200.78, $p < 0.001$; boxplots not shown).

Table 2. Descriptive statistics of pollen production per catkin (P_{ca}) and catkins per crown sampling unit (C_{su}; 0.125 m^3) (minimum, maximum, mean, median and standard deviation) estimated from 28 selected birch trees of the seed plantation Wildberg during 2019–2021.

Year	Minimum	Maximum	Mean	Median	Standard Deviation
		Pollen production per catkin (P_{ca})			
2019	48,000	8,270,000	1,359,049	983,500	1,245,134
2020	108,000	4,172,000	1,511,170	1,360,000	892,862
2021	184,000	8,018,000	2,090,888	1,796,000	1,495,281
2019–2021	48,000	8,270,000	1,658,846	1,356,000	1,277,605
		Catkins per crown sampling unit (C_{su})			
2019	10	45	23	22	8
2020	10	120	44	35	26
2021	3	60	19	20	12
2019–2021	3	120	29	23	20
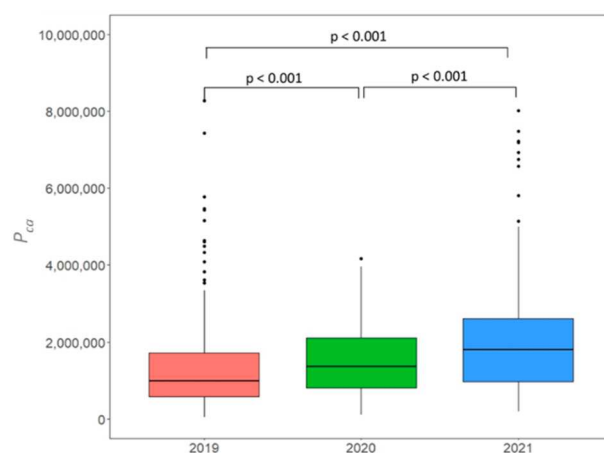
Figure 2. Boxplots based on pollen production per catkin (P_{ca}) (eight replications per tree) estimated for 28 trees in the seed plantation in Wildberg for 2019, 2020 and 2021. The interquartile range is represented by the height of the boxes, maximum and minimum values by the upper and lower whiskers, the median by bold horizontal lines in the boxes, points indicate outliers, lines above boxplots indicate pair of years, which were significantly different (Kruskal–Wallis test and Dunn's multiple comparison's tests).

Correlations between different reproductive metrics from all study years are shown in Table 3. The highest Spearman's correlation coefficient was found for P_{fl} and P_{ca} (r$_s$ = 0.980, $p < 0.001$). fl was associated with a negative correlation with P_{fl} (r$_s$ = -0.230, $p < 0.001$) and a positive correlation with C_{su} (r$_s$ = 0.200, $p < 0.001$). P_{fl} or P_{ca} did not show any significant correlations with C_{su}.

Table 3. Spearman correlations between averaged reproduction metrics for all studied years and 28 birch individuals of the Wildberg seed plantation. r_s: Spearman's correlation coefficient, p: significance.

Reproductive Metrics	P_{fl}		P_{ca}		fl	
	r_s	p	r_s	p	r_s	p
P_{ca}	0.980	0.000				
fl	-0.230	0.000	-0.040	ns		
C_{su}	-0.060	ns	-0.020	ns	0.200	0.000

The temporal variations of pollen production (Table 2, Figure 2) probably include some abiotic and biotic influential factors, which are described below.

3.2. Meteorological Differences in the Study Years

Figure 3 shows the meteorological conditions at Neubulach-Oberhaugstett, near the plantation site, for the period 2018–2020. In addition, we calculated averages for months that are especially important for the initiation and formation of catkin of the following year (i.e., May until August of the preceding summer; [48]). We estimated the lowest average P_{ca} in 2019 (Table 2, Figure 2), which was following a relatively high temperature (17.6 °C) and moderate precipitation (63.5 mm) during those specified four months in 2018 (compared to 2019 and 2020). Mean P_{ca} was higher in 2020 and linked to a preceding period with a moderate temperature (16.3 °C), but a high precipitation sum (77.1 mm) was recorded during May–August 2019. The average numbers of pollen grains per catkin estimated in 2021 was the highest among all study years; the preceding period in 2020 was associated with the lowest temperature mean (16.1 °C) and precipitation sum (54.7 mm) compared to 2018 and 2019. The selected period of the year was on average warmer but received less precipitation in all study years compared to 1991–2020 (15.7 °C; 82.3 mm).
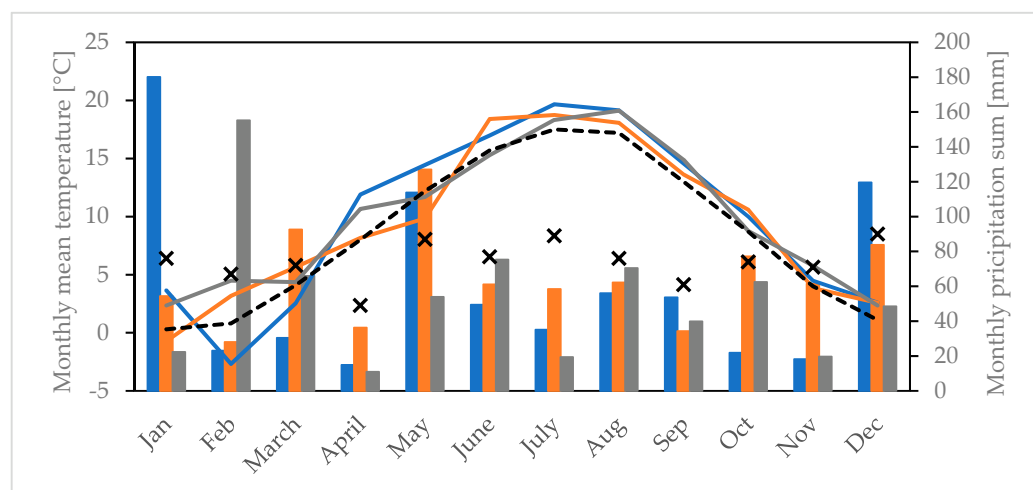
Figure 3. Monthly average temperature (lines) and monthly precipitation sum (bars) for the years 2018 (blue), 2019 (orange) and 2020 (grey) recorded at a nearby weather station (DWD station Neubulach-Oberhaugstett). *x*-axis: months, left *y*-axis: monthly mean temperature in °C, right *y*-axis: monthly precipitation sum in mm. Mean values (1991–2020) are displayed as black dashed lines (temperature) and crosses (precipitation).

Site-specific temperature data (8 April 2019 to 20 June 2021) at five different locations within the plantation (see Figure 3) were found to be not significantly different according to ANOVA tests (daily mean temperature: $F_{(4, 4020)} = 0.73$, $p = 0.570$, monthly mean temperature: $F_{(4, 125)} = 0.03$, $p = 0.990$). In addition, air pollutants sampled at two sites (see Figure 1) were almost identical: site 1—$NO_2 < 6.5$ µg/m^3, $NO_x = 2.4$ µg/m^3 and $O_3 = 33.2$ µg/m^3; site 2—$NO_2 < 6.5$ µg/m^3, $NO_x = 2.6$ µg/m^3 and $O_3 = 36.2$ µg/m^3.

Incoming shortwave radiation, expressed as the sum of radiation in the months May to August in W/m^2, varies within the seed plantation due to the surrounding forested area and is generally lower in the southern part (Figure 4). However, we found no statistically significant correlation between mean P_{ca} (2019–2021) and solar radiation ($r_s = -0.111$, $p = 0.574$) when regarding all 28 selected birch trees. For single years, we detected an alternating (but still not significant) signal: in 2019 and 2020, the correlations were positive ($r_s = 0.201$, $p = 0.304$ and $r_s = 0.076$, $p = 0.702$, respectively) and in 2021 the correlation was negative ($r_s = -0.149$, $p = 0.4489$).

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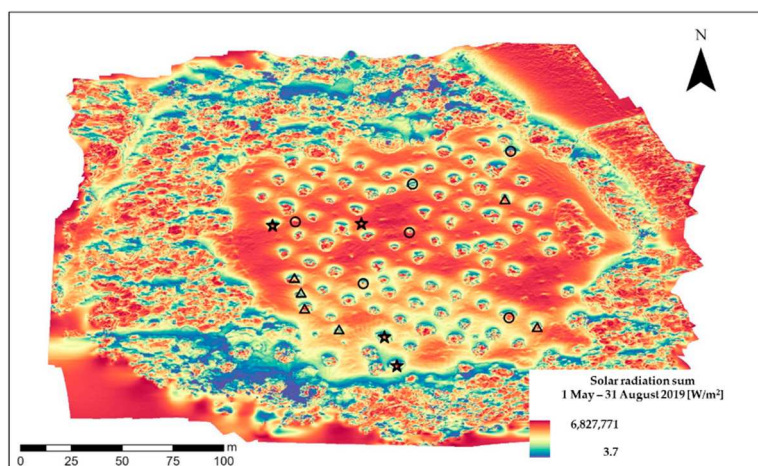
Figure 4. Solar radiation map and synchrony of pollen production levels of non-topped trees (*n* = 16). Circles—group 1 (*n* = 6, trees with maximum P_{ca} in 2020); triangles—Group 2 (*n* = 4, trees with P_{ca} extraordinarily high in 2021) and stars—group 3 (*n* = 6, almost constant P_{ca} values and/or minimum values in 2020).

3.3. Tree Condition

The differences in pollen production between trees that were topped in 2018 and non-topped trees were compared for 2020 and 2021 (Table 4). Pollen production in 2019 was considered unaffected by tree topping since this intervention was carried out after the formation of catkins.

Table 4. Reproductive metrics (mean and median) of topped (*n* = 12) and non-topped trees (*n* = 16) in 2020 and 2021 and comparisons (Mann–Whitney U test) between them.

Reproductive Metrics	Group	2020			2021		
		Mean	**Median**	***p***	**Mean**	**Median**	***p***
P_{ca}	Topped	1,252,938	1,116,000	0.000	2,143,096	2,098,000	0.016
	Non-topped	1,704,844	1,654,000		2,048,469	1,486,000	
P_{fl}	Topped	10,564	9,617	0.000	19,271	17,362	0.039
	Non-topped	15,935	14,742		19,266	13,309	
P_{cr}	Topped	485,992,500	399,840,000	ns	405,397,846	306,200,000	0.000
	Non-topped	541,242,500	384,200,000		238,281,125	169,840,000	
fl	Topped	122	117	0.000	112	112	0.030
	Non-topped	112	112		107	107	
C_{su}	Topped	53	45	0.009	23	20	0.002
	Non-topped	38	35		16	17	

Mann–Whitney U tests revealed that there were significant differences between topped compared to non-topped trees. The first year with potential effects of tree topping (2020) was associated with a significantly lower pollen production and a significantly higher flower and catkin formation compared to non-topped trees. For example, P_{ca} was 27% lower, P_{fl} was 34% lower, *fl* were 9% higher and C_{su} were 40% higher for these damaged trees. The effect of tree topping was most obvious in 2021 since all metrics were associated with significantly higher mean values. For example, P_{ca} was 5% higher, P_{cr} was 70% higher, *fl* were 5% higher and C_{su} were 44% higher for topped compared to non-topped trees. In 2021, the effect on P_{cr} was most pronounced, especially when bearing in mind that this last study year presented a year with poor catkin formation (see Table 2).

3.4. Synchrony of Pollen Production Levels

Due to the effects of topping, the assessment of synchrony in pollen production levels was carried out for all non-topped trees (n = 16) for which the temporal development was evaluated and classified into three different groups (Figure 5). The classification was performed visually according to the maximum in pollen production and the variation among years.
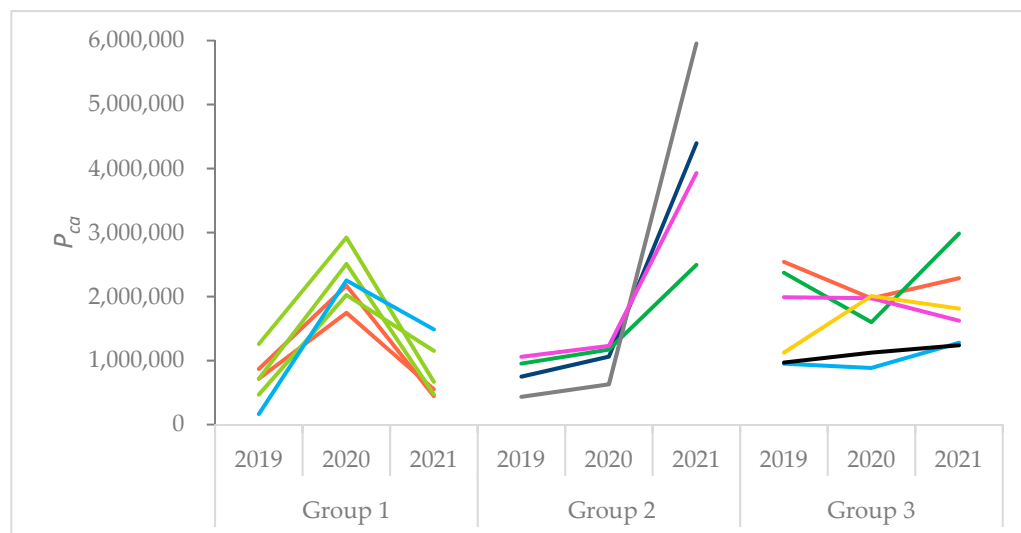
Figure 5. Pollen production per catkin (P_{ca}) (*y*-axis) in 2019–2021 assessed for the selected non-topped trees at the seed planation Wildberg and categorized in three groups with similar temporal behavior. The color of the lines symbolizes trees of the same clone.

Group 1 includes the trees with maximum P_{ca} in 2020 (n = 6). Group 2 consists of trees whose P_{ca} was extraordinarily high in 2021 (n = 4). Group 3 has almost constant P_{ca} values and/or minimum values in 2020 (n = 6). Only one clone (clone number 21) with three replications was always categorized to the same group (group 1). The trees of all other clones were distributed in more than one group.

These three different groups are highlighted in Figure 4 by different symbols. A one-way ANOVA did not reveal significant differences in cumulative solar radiation between the groups (F (2, 13) = 0.637, p = 0.545).

3.5. Differences within and among Clones

Based on the results that showed significant differences between topped and non-topped trees (Table 4), we selected five clones (12 trees) having at least two or more non-topped trees to further investigate the differences among clones, i.e., clone number 7 (n = 3), 21 (n = 3), 24 (n = 2), 30 (n = 2) and 42 (n = 2). The estimated pollen production of each year from those trees was analyzed to derive mean values and coefficient of variance (CV) (Table 5).

According to the calculated CV across all study years, clone 42 is the most consistent clone regarding P_{ca} (CV = 0.57). Clone 42 was also found to produce the highest mean value for P_{ca} and the lowest C_{su} linked to the smallest CV (CV = 0.30). Similarly, clone 30 produced the lowest average pollen but was associated with a higher coefficient of variance (CV = 0.81) and a higher C_{su} with a moderate coefficient of variation (CV = 0.60) compared to other clones.

Flowers per catkin (*fl*) were linked to lower CV values and was therefore most consistent compared to other reproductive metrics. Clone 7 had the lowest mean (96 *fl*) and CV (CV = 0.10) and clone 42 had the highest mean (127 *fl*). Clone 21 and clone 42 were linked to the highest CV (CV = 0.13) at the level of *fl*.

Table 5. Mean values for reproductive metrics and associated coefficients of variance (CV) of clones with non-topped trees in the seed plantation Wildberg for 2019–2021.

Clone	*fl*	P_{fl}	P_{ca}	C_{su}	P_{cr}
7	96 (0.10)	15,366 (0.66)	1,478,347 (0.71)	27 (0.48)	333,661,556 (0.95)
21	106 (0.13)	13,164 (0.71)	1,354,889 (0.69)	29 (0.49)	385,906,000 (1.06)
24	98 (0.11)	20,362 (0.70)	1,929,146 (0.65)	25 (0.88)	331,433,333 (0.87)
30	120 (0.11)	9732 (0.81)	1,169,417 (0.81)	39 (0.60)	444,390,000 (1.26)
42	127 (0.13)	15,787 (0.54)	1,967,167 (0.57)	18 (0.30)	312,689,333 (0.79)
16 non-topped trees	112 (0.17)	14,994 (0.84)	1,612,250 (0.81)	26 (0.56)	332,198,375 (1.04)

Interestingly, the CV is higher (for *fl*) and equal or higher (for P_{ca}) when not splitted for each clone but when calculated for all the 16 non-topped trees (Table 5). Nevertheless, it is moderate in the case of C_{su}.

There was a statistically significant difference between P_{ca} estimated for clones (H (4) = 21.44, *p* < 0.001) (see Figure 6). The post-hoc tests revealed that clone 30 was significantly different from clone 24 (*p* = 0.008) and clone 42 (*p* = 0.001). Clone 21 and clone 42 were also significantly different (*p* = 0.023).
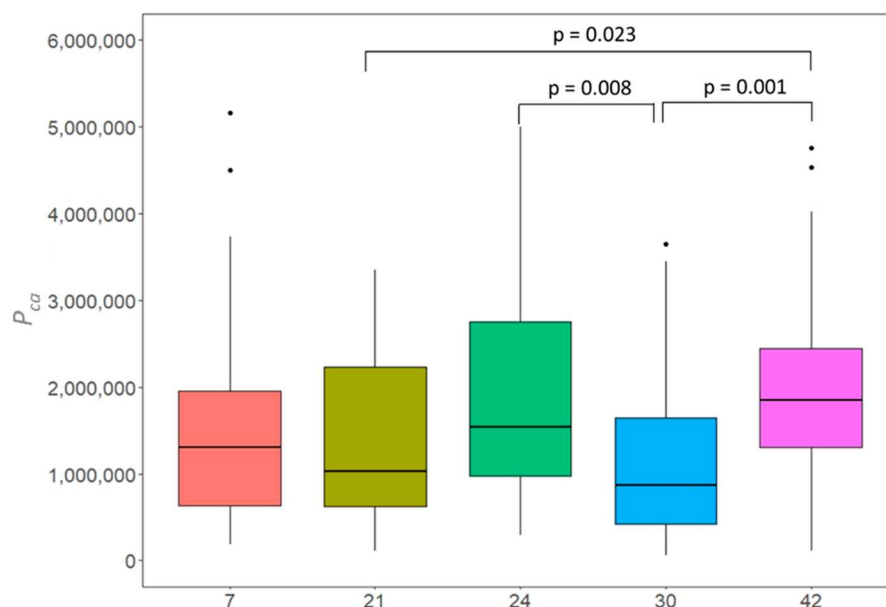
Figure 6. Boxplots based on pollen production per catkin (P_{ca}) (eight replications per tree) estimated for five selected clones with at least two non-topped trees in the seed plantation in Wildberg for 2019, 2020 and 2021. Clones 7 and 21 consist of three trees each. Clone 24, 30 and 42 consist of two trees each. The interquartile range is represented by the height of the boxes, maximum and minimum values by the upper and lower whiskers, the median by bold horizontal lines in the boxes, points indicate outliers, lines above boxplots indicate pair of clones, which were significantly different (Kruskal–Wallis test and Dunn's multiple comparison's tests).

For an average non-topped birch tree in the seed plantation, a mean crown volume was calculated as 81.55 m^3 (average crown height = 6.28 m and average crown width = 4.98 m). This mean crown volume along with mean values of C_{su} and P_{ca} (in Table 5) were used to calculate mean number of catkins and mean P_{in} for different clones and for an average birch tree (Table 6).

Table 6. Mean number of catkins and mean pollen production for different clones and for an average tree. The last column shows the equivalence of the selected clones' trees to an average tree.

Clone	Mean Catkins per Tree	Mean Pollen Production Per Tree (P_{in})	Equivalent to 100 Average Trees
Average tree	16,962	27,347,187,742	100
7	17,615	26,040,366,183	105
21	18,919	25,633,543,942	107
24	16,310	31,463,863,118	87
30	25,443	29,753,697,858	92
42	11,743	23,100,462,441	118

Table 6 demonstrates that clone 42 reproduces fewer catkins per tree (11,743) compared to other clones and 118 trees would be needed to produce the same amount of pollen produced by 100 average trees (based on mean values of all 16 trees). Clone 24 was found to produce almost the same number of catkins per tree as an average tree; however, it produces more pollen per tree. Therefore, 87 trees of clone 24 could produce the same amount of pollen as 100 average trees. Clone 30 produces a higher number of catkins per tree (25,443) and 92 trees would be needed to produce the same amount of pollen as 100 average trees.

4. Discussion

Our study investigating pollen production of 28 birch trees in three consecutive years is unique since we examined a large number of male birch inflorescences and assessed the internal variability of pollen production regarding genetic differences and similarities. In addition, this study excludes (major) environmental differences as well as age effects.

We estimated pollen production values at the level of catkins ranging from 48,000 pollen grains to 8.3 million pollen grains (mean 1.66 million). Some studies have already estimated pollen production values for *Betula pendula* (syn. *Betula alba*, *Betula verrucosa*). Erdtman [51] reported an estimate of 5.5 million pollen grains per inflorescence for *B. verrucose*. Jato et al. [30] estimated values ranging between 8.2 million and 4.8 million pollen grains per inflorescence, sampled from six trees of *B. alba* in northwestern Spain in 2002 and 2003, respectively. Piotrowska [43] estimated a mean value of 10 million pollen grains per inflorescence on the basis of 30 catkins deriving from three individuals. Although these studies have reported higher values compared to the mean P_{ca} estimated in this study, they were based on either a few trees or estimated only for a single or two study years. Consequently, it is not known if sampling took place in a masting or non-masting years. For this reason, our study can be regarded as important since we have sampled 28 trees for three years and present a robust estimate for the mean pollen production of *Betula pendula*.

We found that birch catkins with fewer flowers produce more pollen and *vice versa*. This could be considered as an internal compensation since the plant aims at upregulating pollen production when the flower amount is low. Molina et al. [52] studied ten anemophilous species of aerobiological importance (*Betula* ssp. not included) and found a significant decrease in pollen per flower with a higher number of flowers per inflorescence. They suggested that there is a more or less constant amount (within a defined margin) for pollen production in anemophilous tree species. These species tend to compensate for reproductive characteristics (e.g., pollen per anther, flowers per tree, and inflorescences per tree) by increasing some and decreasing others. Our analysis showed that the number of flowers is the most homogenous value since a low coefficient of variance was associated to this measure, e.g., in clonal comparisons.

Our study shows an annual variation in pollen production with the lowest mean values in 2019 and the highest in 2021. Such alterations could be caused by yearly changes in the meteorological conditions of the locality. Some studies examining the relationship between temperature and pollen production suggested that warmer conditions result in higher pollen quantities. For example, experimental studies indicated that an increase in temperature [16] but also an increase in atmospheric CO_2 concentration [16,53,54] was

associated with a higher pollen production of common ragweed (*Ambrosia artemisiifolia*). However, it was also found that pollen production of birch (*Betula pendula* Roth) along an urban-rural gradient was negatively correlated with temperature [55]. The authors argue that the physiological performance of birch, which mainly grows at lower temperatures in mid to high latitudes, might be affected by (very) high temperatures and in turn react with a decrease in pollen production, as also suggested by Ziello et al. [56]. However, any differences in pollen production found in natural environments might also be affected by other factors, which attenuate or diminish the influence of temperature. In addition, the response to temperature might also be species-specific and strongly dependent on the methodologies used.

Although many studies have examined the temporal change in birch pollen concentrations based on pollen trap monitoring, there is no study presenting long-term changes in pollen production assessed using the same birch trees. Detecting the influence of temperature on pollen production based on the data presented in this study is not feasible, since (a) we only cover a period of three years and (b) a small spatial extent (1 ha) with similar temperature conditions, as documented using five installed temperature loggers. Many other environmental factors such as soil type and edaphic conditions as well as air pollutants are regarded to be similar as well. Especially the latter is also supposed to affect pollen production, as documented by Jochner et al. [55]. In their study, atmospheric NO_2 levels were negatively associated to pollen production.

However, we found differences in solar radiation, which arise mainly from the forested surrounding of the seed plantation. During the study years, the correlations between pollen production and solar radiation did not vary much in magnitude, but they did shift in sign. Therefore, we calculated the correlation coefficient for mean (2019–2021) pollen production, but the association to solar radiation was no longer discernible. Thus, solar radiation, which is known to lead to higher stem and tissue temperatures [57] might also be inadequate to explain variations of pollen production at a small spatial scale. This was also evident when comparing solar radiation values with the association of birch trees to groups with similar pollen production patterns across the study years.

We did not detect a high synchrony of pollen production levels of birch trees within the birch plantation since we found that six trees exhibited the highest pollen production in 2020, four trees a very high pollen production in 2021 and six trees an almost constant pollen production across the study years. The birch trees allocated to one of these three groups did not necessarily belong to one clone. Thus, a coherence on the level of clones was not evident, except for one clone group.

Masting behavior, the inherent year-to-year variation in pollen production by plant populations [58,59], can be observed in several tree species [60,61] including birch [14,30]. Flowering and annual pollen sums in birch were reported to fluctuate from year to year [62]. Using aerobiological data gathered from pollen traps that assess the pollen concentration of the ambient outdoor air, a biennial [63] as well as triennial rhythm [64] of masting can be observed. Related to *Betula* species, Ranta et al. [59] found that male flowering shows synchronized annual fluctuations among stands at a regional scale; however, stand-specific catkin number during the masting year varies considerably, which in turn might also influence the pollen produced. This is also in accordance with our findings since the numbers of catkins varied (mean C_s (SD) = 23 (8), 44 (26) and 19 (12) in 2019, 2020, and 2021, respectively, Table 2) within the plantation.

Asynchronous pollen production levels, which were found in our study might be caused by the resource balance of an individual tree. If the initial resource stock and the resource gained afterwards differ from one individual to the next in the stand, masting synchronization might not occur [65,66], even under the same environmental conditions [65]. In addition, plant-pathogen and plant-mycorrhizosphere interactions may reduce or enhance the impacts of abiotic stress on resource allocation [67] which could be specific to each tree.

Effects on pollen production and catkin formation were especially obvious two years after topping. Topping and pruning have been considered as adequate tree crown management techniques to enhance seed production, specifically in conifer seed orchards, or to promote the branching of the trees [47,68,69]. Viherä-Aarnio and Ryynänen [47] studied seed production of silver birch individuals that were topped in the second year in a greenhouse experiment. In the fourth year, a ten times higher amount of seeds per plant (compared to the previous year) was obtained. This was followed by a year with poor flowering and seed production. In our study, we cannot conclude on any effects in upcoming years; therefore, we recommend a longer monitoring of pollen production after topping in further studies.

Birch clones characterized by on average lower pollen production could be an opportunity to reduce the prevalence of allergies. In an experiment, transgenic birch grown in a greenhouse showed the ability to prevent flowering in silver birch trees [70]. However, such preventions might be associated with adverse side effects such as aberrant branching and growth disturbance. Therefore, we suggest selecting birch clones associated with low pollen production. We estimated P_{ca} ranging between 1.17 million (clone 30) and 1.97 million (clone 42) pollen. Clones producing less pollen might contribute to lower pollen concentrations in the atmosphere. Therefore, clone 42 could be recommended for urban plantations. Similarly, clone 24 needs 87 trees to produce the same pollen amount as 100 average trees. This clone could be suitable in seed plantations to increase seed production. Since variations within clones were especially obvious when comparing pollen production levels across years (Chapter 3.4), we highly recommend monitoring pollen production for a longer term in order to create robust averages for different clones.

5. Conclusions

Our study revealed considerable differences in pollen, flower and catkin productions by birch trees among the study years. Moreover, we found topped birches were associated to higher reproductive outputs, especially two years after the intervention. We conclude that synchronicity of pollen production levels is not utterly site-specific and, in addition, not strictly dependent on genotypes. The detected variations in solar radiation within the plantation were found to be not responsible for asynchrony. Since we revealed significant differences in pollen production between clones, we propose that a wise selection of plants depending on their application (seed plantation, urban planting) might be advantageous. As these conclusions are based on three years of investigation, we recommend a longer monitoring period to further extend our knowledge related to pollen production of anemophilous tree species. In addition, further experimental studies with intended treatment such as pruning and topping under different climatic conditions are highly desirable.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Appendix A

Table A1. Descriptive statistics of pollen production per catkin (P_{ca}), pollen production per flower (P_{fl}), pollen production per volume unit of crown (P_{cr}), flowers per catkin (*fl*) and catkins per crown sampling unit (C_{su}; 0.125 m^3) (minimum, maximum, mean, median and standard deviation) estimated from 28 selected birch trees of the seed plantation Wildberg during 2019–2021.

Reproductive Metrics	Minimum	Maximum	Mean	Median	Standard Deviation
All years					
P_{ca}	48,000	8,270,000	1,658,846	1,356,000	1,277,605
P_{fl}	407	80,291	15,018	12,093	12,008
P_{cr}	4,992,000	2,333,440,000	359,736,647	244,592,000	348,107,774
Fl	77	173	113	112	18
C_{su}	3	120	29	23	20
2019					
P_{ca}	48,000	8,270,000	1,359,049	983,500	1,245,134
P_{fl}	407	80,291	12,001	8691	11,360
P_{cr}	4,992,000	1,654,000,000	250,112,679	164,292,000	261,586,850
Fl	77	173	116	113	19
C_{su}	10	45	23	22	8
2020					
P_{ca}	108,000	4,172,000	1,511,170	1,360,000	892,862
P_{fl}	788	37,250	13,633	12,093	8729
P_{cr}	12,960,000	2,333,440,000	517,563,929	388,160,000	409,661,684
Fl	82	173	117	117	19
C_{su}	10	120	44	35	26
2021					
P_{ca}	184,000	8,018,000	2,090,888	1,796,000	1,495,281
P_{fl}	2000	71,589	19,268	16,102	14,030
P_{cr}	8,880,000	1,731,360,000	313,195,517	207,240,000	300,234,937
Fl	78	142	109	107	16
C_{su}	3	60	19	20	12

References

1. Neophytou, C.; Hasenauer, H.; Kroon, J. Molecular Genetic Identification Explains Differences in Bud Burst Timing among Progenies of Selected Trees of the Swedish Douglas Fir Breeding Programme. *Forests* **2022**, *13*, 895. [CrossRef]
2. Bastiaanse, H.; Zinkgraf, M.; Canning, C.; Tsai, H.; Lieberman, M.; Comai, L.; Henry, I.; Groover, A. A comprehensive genomic scan reveals gene dosage balance impacts on quantitative traits in *Populus* trees. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 13690–13699. [CrossRef]
3. Sun, P.; Jia, H.; Zhang, Y.; Li, J.; Lu, M.; Hu, J. Deciphering Genetic Architecture of Adventitious Root and Related Shoot Traits in *Populus* Using QTL Mapping and RNA-Seq Data. *Int. J. Mol. Sci.* **2019**, *20*, 6114. [CrossRef] [PubMed]
4. Possen, B.J.H.M.; Oksanen, E.; Rousi, M.; Ruhanen, H.; Ahonen, V.; Tervahauta, A.; Heinonen, J.; Heiskanen, J.; Kärenlampi, S.; Vapaavuori, E. Adaptability of birch (*Betula pendula* Roth) and aspen (*Populus tremula* L.) genotypes to different soil moisture conditions. *For. Ecol. Manag.* **2011**, *262*, 1387–1399. [CrossRef]
5. Rousi, M.; Possen, B.J.M.H.; Pulkkinen, P.; Mikola, J. Using long-term data to reveal the geographical variation in timing and quantity of pollen and seed production in silver and pubescent birch in Finland: Implications for gene flow, hybridization and responses to climate warming. *For. Ecol. Manag.* **2019**, *438*, 25–33. [CrossRef]

6.　Bothwell, H.M.; Evans, L.M.; Hersch-Green, E.I.; Woolbright, S.A.; Allan, G.J.; Whitham, T.G. Genetic data improves niche model discrimination and alters the direction and magnitude of climate change forecasts. *Ecol. Appl.* **2021**, *31*, e02254. [CrossRef]

7.　Pigliucci, M.; Murren, C.J.; Schlichting, C.D. Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* **2006**, *209*, 2362–2367. [CrossRef]

8.　Davy, A.J.; Gill, J.A. Variation due to environmental and heredity in birch transplanted between heath and bog. *New Phytol.* **1984**, *97*, 489–505. [CrossRef]

9.　Eriksson, G.; Black-samuelsson, S.; Jensen, M.; Myking, T.; Rusanen, M.; Skrøppa, T.; Vakkari, P.; Westergaard, L. Genetic Variability in Two Tree Species, *Acer platanoides* L. and *Betula pendula* Roth, With Contrasting Life-history Traits. *Scand. J. For. Res.* **2003**, *18*, 320–331. [CrossRef]

10.　Galán, C.; Ariatti, A.; Bonini, M.; Clot, B.; Crouzy, B.; Dahl, A.; Fernandez-González, D.; Frenguelli, G.; Gehrig, R.; Isard, S.; et al. Recommended terminology for aerobiological studies. *Aerobiologia* **2017**, *33*, 293–295. [CrossRef]

11.　Fernández-González, M.; González-Fernández, E.; Ribeiro, H.; Abreu, I.; Rodríguez-Rajo, F.J. Pollen Production of Quercus in the North-Western Iberian Peninsula and Airborne Pollen Concentration Trends during the Last 27 Years. *Forests* **2020**, *11*, 702. [CrossRef]

12.　Subba Reddi, C.; Reddi, N.S. Pollen Production in Some Anemophilous Angiosperms. *Grana* **1986**, *25*, 55–61. [CrossRef]

13.　Hidalgo, P.J.; Galán, C.; Domínguez, E. Pollen production of the genus *Cupressus*. *Grana* **1999**, *38*, 296–300. [CrossRef]

14.　Hicks, S.; Helander, M.; Heino, S. Birch pollen production, transport and deposition for the period 1984–1993 at Kevo, northern-most Finland. *Aerobiologia* **1994**, *10*, 183–191. [CrossRef]

15.　Moe, D. Pollen production of *Alnus incana* at its south Norwegian altitudinal ecotone. *Grana* **1998**, *37*, 35–39. [CrossRef]

16.　Ziska, L.H.; Gebhard, D.E.; Frenz, D.A.; Faulkner, S.; Singer, B.D.; Straka, J.G. Cities as harbingers of climate change: Common ragweed, urbanization, and public health. *J. Allergy Clin. Immunol.* **2003**, *111*, 290–295. [CrossRef] [PubMed]

17.　Lau, T.-K.; Stephenson, A.G. Effects of Soil Nitrogen on Pollen Production, Pollen Grain Size, and Pollen Performance in *Cucurbita pepo* (Cucurbitaceae). *Am. J. Bot.* **1993**, *80*, 763–768. [CrossRef]

18.　Damialis, A.; Fotiou, C.; Healley, J.M.; Vokou, D. Effects of environmental factors on pollen production in anemophilous woody species. *Trees* **2011**, *25*, 253–264. [CrossRef]

19.　García-Cruzatty, L.C.; Vera-Pinargote, L.; Zambrano-Gavilanes, F.; Zamora-Macías, A.; Cedeño-Ortega, J. Pollen Production in *Theobroma cacao* L. Genotypes National Type and CCN-51 and Its Relationship with Climatic Factors on the Ecuadorian Coast. *Acta Agrobot.* **2020**, *73*. [CrossRef]

20.　Adams, G.W.; Kunze, H.A. Clonal variation in cone and seed production in black and white spruce seed orchards and management implications. *For. Chron.* **1996**, *72*, 475–480. [CrossRef]

21.　Schnelle, F.; Volkert, E. Vorschläge zur Einrichtung Internationaler Phänologischer Gärten als Stationen eines Grundnetzes für internationale phänologische Beobachtungen. *Meteorol. Rundsch.* **1957**, *10*, 130–133.

22.　Rötzer, T.; Chmielewski, F.M. Phenological maps of Europe. *Clim. Res.* **2001**, *18*, 249–257. [CrossRef]

23.　Renner, S.S.; Chmielewski, F.-M. The International Phenological Garden network (1959 to 2021): Its 131 gardens, cloned study species, data archiving, and future. *Int. J. Biometeorol.* **2021**, *66*, 35–43. [CrossRef] [PubMed]

24.　Caffarra, A.; Donnelly, A. The ecological significance of phenology in four different tree species: Effects of light and temperature on bud burst. *Int. J. Biometeorol.* **2011**, *55*, 711–721. [CrossRef] [PubMed]

25.　Ahlholm, J.U.; Helander, M.L.; Savolainen, J. Genetic and environmental factors affecting the allergenicity of birch (*Betula pubescens* ssp. *czerepanovii* [Orl.] Hämet-ahti) pollen. *Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol.* **1998**, *28*, 1384–1388. [CrossRef] [PubMed]

26.　Goto, Y.; Kondo, T.; Hayashi, E.; Kuramoto, N.; Takahashi, M.; Yasueda, H. Influences of genetic and environmental factors on the concentration of the allergen Cry j 1 in sugi (*Cryptomeria japonica*) pollen. *Tree Physiol.* **2004**, *24*, 409–414. [CrossRef]

27.　Fernández-Caldas, E.; Carnés, J.; Iraola, V.; Casanovas, M. Comparison of the allergenicity and Ole e 1 content of 6 varieties of *Olea europaea* pollen collected during 5 consecutive years. *Ann. Allergy Asthma Immunol.* **2007**, *98*, 464–470. [CrossRef]

28.　Veilleux, R.E.; Lauer, F.I. Variation for 2n pollen production in clones of *Solanum phureja* Juz. and Buk. *Theoret. Appl. Genet.* **1981**, *59*, 95–100. [CrossRef]

29.　Panda, A.K.; Soorianathasundaram, K.; Vijayakumar, R.M. Screening for male fertility status in selected banana genotypes. *Electron. J. Plant Breed.* **2019**, *10*, 1309–1316. [CrossRef]

30.　Jato, F.V.; Rodriguez-Rajo, F.J.; Aira, M.J. Use of phenological and pollen-production data for interpreting atmospheric birch pollen curves. *Ann. Agric. Environ. Med.* **2007**, *14*, 271–280.

31.　Faegri, K.; Kaland, P.E.; Krzywinski, K. *Textbook of Pollen Analysis*; Wiley: Hoboken, NJ, USA, 1989; ISBN 0471921785.

32.　Pawankar, R.; Canonica, G.W.; Holgate, S.T.; Lockey, R.F.; Blaiss, M. World Allergy Organization (WAO) White Book on Allergy: Update 2013. Available online: https://www.worldallergy.org/wao-white-book-on-allergy (accessed on 25 November 2021).

33.　Ozasa, K.; Hama, T.; Dejima, K.; Watanabe, Y.; Hyo, S.; Terada, T.; Araki, N.; Takenaka, H. A 13-year Study of Japanese Cedar Pollinosis in Japanese Schoolchildren. *Allergol. Int.* **2008**, *57*, 175–180. [CrossRef]

34.　Honda, K.; Saito, H.; Fukui, N.; Ito, E.; Ishikawa, K. The Relationship between Pollen Count Levels and Prevalence of Japanese Cedar Pollinosis in Northeast Japan. *Allergol. Int.* **2013**, *62*, 375–380. [CrossRef]

35.　Yoshida, K.; Adachi, Y.; Akashi, M.; Itazawa, T.; Murakami, Y.; Odajima, H.; Ohya, Y.; Akasawa, A. Cedar and cypress pollen counts are associated with the prevalence of allergic diseases in Japanese schoolchildren. *Allergy* **2013**, *68*, 757–763. [CrossRef]

36. Damialis, A.; Traidl-Hoffmann, C.; Treudler, R. Climate Change and Pollen Allergies. In *Biodiversity and Health in the Face of Climate Change*; Marselle, M.R., Stadler, J., Korn, H., Irvine, K.N., Bonn, A., Eds.; Springer: Cham, Switzerland, 2019; pp. 47–66; ISBN 978-3-030-02317-1.

37. Atkinson, M.D. *Betula pendula* Roth (*B. Verrucosa* Ehrh.) and *B. pubescens* Ehrh. *J. Ecol.* **1992**, *80*, 837. [CrossRef]

38. D'Amato, G.; Cecchi, L.; Bonini, S.; Nunes, C.; Annesi-Maesano, I.; Behrendt, H.; Liccardi, G.; Popov, T.; van Cauwenberge, P. Allergenic pollen and pollen allergy in Europe. *Allergy* **2007**, *62*, 976–990. [CrossRef]

39. Smith, M.; Jäger, S.; Berger, U.; Šikoparija, B.; Hallsdottir, M.; Sauliene, I.; Bergmann, K.C.; Pashley, C.H.; de Weger, L.; Majkowska-Wojciechowska, B.; et al. Geographic and temporal variations in pollen exposure across Europe. *Allergy* **2014**, *69*, 913–923. [CrossRef]

40. Biedermann, T.; Winther, L.; Till, S.J.; Panzner, P.; Knulst, A.; Valovirta, E. Birch pollen allergy in Europe. *Allergy* **2019**, *74*, 1237–1248. [CrossRef]

41. Krišāns, O.; Čakša, L.; Matisons, R.; Rust, S.; Elferts, D.; Seipulis, A.; Jansons, Ā. A Static Pulling Test Is a Suitable Method for Comparison of the Loading Resistance of Silver Birch (*Betula pendula* Roth.) between Urban and Peri-Urban Forests. *Forests* **2022**, *13*, 127. [CrossRef]

42. Battisti, L.; Pille, L.; Wachtel, T.; Larcher, F.; Säumel, I. Residential Greenery: State of the Art and Health-Related Ecosystem Services and Disservices in the City of Berlin. *Sustainability* **2019**, *11*, 1815. [CrossRef]

43. Piotrowska, K. Pollen production in selected species of anemophilous plants. *Acta Agrobot.* **2008**, *61*, 41–52. [CrossRef]

44. Wilsey, B.J.; Haukioja, E.; Koricheva, J.; Sulkinoja, M. Leaf Fluctuating Asymmetry Increases with Hybridization and Elevation in Tree-Line Birches. *Ecology* **1998**, *79*, 2092–2099. [CrossRef]

45. DWD. Deutscher Wetterdienst/German Meteorological Service. Available online: https://www.dwd.de/DE/Home/home_node.html (accessed on 12 February 2022).

46. LGRB. LGRB-Kartenviewer: —Layer Bodenkunde: Reliefparameter. Available online: https://maps.lgrb-bw.de/ (accessed on 2 December 2021).

47. Viherä-Aarnio, A.; Ryynänen, L. Seed production of micropropagated plants, grafts and seedlings of birch in a seed orchard. *Silva Fenn.* **1994**, *28*, 5538. [CrossRef]

48. Dahl, Å.; Strandhede, S.-O. Predicting the intensity of the birch pollen season. *Aerobiologia* **1996**, *12*, 97–106. [CrossRef]

49. Moore, P.D.; Webb, J.A.; Collinson, M.E. *Pollen Analysis*, 2nd ed.; Blackwell Science: Oxford, UK; Malden, MA, USA, 1991; ISBN 0865428956.

50. Shivanna, K.R.; Rangaswamy, N.S. *Pollen Biology: A Laboratory Manual*, 1st ed.; Springer: Berlin/Heidelberg, Germany, 1992; ISBN 9783642773068.

51. Erdtman, G. *An Introduction to Pollen Analysis*, 2nd ed.; Chronica Botanica: Waltham, MA, USA, 1954.

52. Molina, R.T.; Rodríguez, A.M.; Palaciso, I.S.; López, F.G. Pollen production in anemophilous trees. *Grana* **1996**, *35*, 38–46. [CrossRef]

53. Ziska, L.H.; Caulfield, F.A. Rising CO_2 and pollen production of common ragweed (*Ambrosia artemisiifolia* L.), a known allergy-inducing species: Implications for public health. *Funct. Plant Biol.* **2000**, *27*, 893–898. [CrossRef]

54. Wayne, P.; Foster, S.; Connolly, J.; Bazzaz, F.; Epstein, P. Production of allergenic pollen by ragweed (*Ambrosia artemisiifolia* L.) is increased in CO_2-enriched atmospheres. *Ann. Allergy Asthma Immunol.* **2002**, *88*, 279–282. [CrossRef]

55. Jochner, S.; Höfler, J.; Beck, I.; Göttlein, A.; Ankerst, D.P.; Traidl-Hoffmann, C.; Menzel, A. Nutrient status: A missing factor in phenological and pollen research? *J. Exp. Bot.* **2013**, *64*, 2081–2092. [CrossRef]

56. Ziello, C.; Sparks, T.H.; Estrella, N.; Belmonte, J.; Bergmann, K.C.; Bucher, E.; Brighetti, M.A.; Damialis, A.; Detandt, M.; Galán, C.; et al. Changes to Airborne Pollen Counts across Europe. *PLoS ONE* **2012**, *7*, e34076. [CrossRef]

57. Derby, R.W.; Gates, D.M. The temperature of tree trunks—calculated and observed. *Am. J. Bot.* **1966**, *53*, 580–587. [CrossRef]

58. Kelly, D. The evolutionary ecology of mast seeding. *Trends Ecol. Evol.* **1994**, *9*, 465–470. [CrossRef]

59. Ranta, H.; Hokkanen, T.; Linkosalo, T.; Laukkanen, L.; Bondestam, K.; Oksanen, A. Male flowering of birch: Spatial synchronization, year-to-year variation and relation of catkin numbers and airborne pollen counts. *For. Ecol. Manag.* **2008**, *255*, 643–650. [CrossRef]

60. Cuevas, J.; Polito, V.S. The role of staminate flowers in the breeding system of *Olea europaea* (Oleaceae): An andromonoecious, wind-pollinated taxon. *Ann. Bot.* **2004**, *93*, 547–553. [CrossRef] [PubMed]

61. Nussbaumer, A.; Waldner, P.; Etzold, S.; Gessler, A.; Benham, S.; Thomsen, I.M.; Jørgensen, B.B.; Timmermann, V.; Verstraeten, A.; Sioen, G.; et al. Patterns of mast fruiting of common beech, sessile and common oak, Norway spruce and Scots pine in Central and Northern Europe. *For. Ecol. Manag.* **2016**, *363*, 237–251. [CrossRef]

62. Ranta, H.; Oksanen, A.; Hokkanen, T.; Bondestam, K.; Heino, S. Masting by *Betula*-species; applying the resource budget model to north European data sets. *Int. J. Biometeorol.* **2005**, *49*, 146–151. [CrossRef] [PubMed]

63. Latałowa, M.; Miętus, M.; Uruska, A. Seasonal variations in the atmospheric *Betula* pollen count in Gdańsk (southern Baltic coast) in relation to meteorological parameters. *Aerobiologia* **2002**, *18*, 33–43. [CrossRef]

64. Detandt, M.; Nolard, N. The fluctuations of the allergenic pollen content of the air in Brussels (1982 to 1997). *Aerobiologia* **2000**, *16*, 55–61. [CrossRef]

65. Isagi, Y.; Sugimura, K.; Sumida, A.; Ito, H. How Does Masting Happen and Synchronize? *J. Theor. Biol.* **1997**, *187*, 231–239. [CrossRef]

Int. J. Environ. Res. Public Health **2022**, *19*, 8160

18 of 18

66. Crone, E.E.; Rapp, J.M. Resource depletion, pollen coupling, and the ecology of mast seeding. *Ann. N. Y. Acad. Sci.* **2014**, *1322*, 21–34. [CrossRef]
67. Matyssek, R.; Agerer, R.; Ernst, D.; Munch, J.-C.; Oßwald, W.; Pretzsch, H.; Priesack, E.; Schnyder, H.; Treutter, D. The Plant's Capacity in Regulating Resource Demand. *Plant Biol.* **2005**, *7*, 560–580. [CrossRef]
68. Stoehr, M.; Hollefreund, C.; Webber, J.; Hewson, C.; Ross, S. Effects of crown-pruning on seed and pollen cone production in two lodgepole pine seed orchards in British Columbia. *New For.* **1995**, *10*, 133–143. [CrossRef]
69. Li, Y.; Li, X.; Zhao, M.-H.; Pang, Z.-Y.; Wei, J.-T.; Tigabu, M.; Chiang, V.L.; Sederoff, H.; Sederoff, R.; Zhao, X.-Y. An Overview of the Practices and Management Methods for Enhancing Seed Production in Conifer Plantations for Commercial Use. *Horticulturae* **2021**, *7*, 252. [CrossRef]
70. Lemmetyinen, J.; Keinonen, K.; Sopanen, T. Prevention of the flowering of a tree, silver birch. *Mol. Breed.* **2004**, *13*, 243–249. [CrossRef]

5 Pollen production of downy birch (*Betula pubescens* Ehrh.) along an altitudinal gradient in the European Alps

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ORIGINAL PAPER

Pollen production of downy birch (*Betula pubescens* Ehrh.) along an altitudinal gradient in the European Alps

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Abstract

High-altitude environments are highly susceptible to the effects of climate change. Thus, it is crucial to examine and understand the behaviour of specific plant traits along altitudinal gradients, which offer a real-life laboratory for analysing future impacts of climate change. The available information on how pollen production varies at different altitudes in mountainous areas is limited. In this study, we investigated pollen production of 17 birch (*Betula pubescens* Ehrh.) individuals along an altitudinal gradient in the European Alps. We sampled catkins at nine locations in the years 2020–2021 and monitored air temperatures. We investigated how birch pollen, flowers and inflorescences are produced in relation to thermal factors at various elevations. We found that mean pollen production of *Betula pubescens* Ehrh. varied between 0.4 and 8.3 million pollen grains per catkin. We did not observe any significant relationships between the studied reproductive metrics and altitude. However, minimum temperature of the previous summer was found to be significantly correlated to pollen ($r_s = 0.504$, $p = 0.039$), flower ($r_s = 0.613$, $p = 0.009$) and catkin ($r_s = 0.642$, $p = 0.005$) production per volume unit of crown. Therefore, we suggest that temperature variability even at such small scales is very important for studying the response related to pollen production.

Keywords Plant ecology · Elevation · Mountain-valley gradient · Thermal factors · Reproduction · Air temperature

Introduction

Plant traits such as phenology and tree growth have been repeatedly reported to be very sensitive to ongoing climate change (Dobbertin 2005; Menzel et al. 2006, 2020). For plant species, this sensitivity is amplified at

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the limits of species distribution (Mellert et al. 2016), where the ecological conditions do not meet the optimal requirements for plant survival and growth. Cold-adapted plant species growing at higher elevations in the European Alps are especially susceptible to the effects of climate change (Albrich et al. 2020; Engler et al. 2011), and tree species at the tree line were reported to be more sensitive to environmental changes (Wielgolaski et al. 2017). In general, mountain ecosystems allow studying climate change impacts as they cover a variety of changes related to abiotic and biotic factors along the elevational gradient (Tito et al. 2020). For instance, air temperature decreases by on average 0.5 °C for every 100 m of elevation, as reported for the Bavarian Alps, Germany (Kirchner et al. 2013). Studying plant responses using such lapse rates can be easily translated into thermal responses as often applied in phenological research (Cerlini et al. 2022; Damialis et al. 2020; Jochner et al. 2012). The plants' behavior along an altitudinal gradient provides indications on potential impacts of climate change at small horizontal distances (Damialis et al. 2011; Jochner et al. 2012).

It is already widely documented that the flowering time of many spring flowering species has shifted earlier in the year due to increases in temperature (Khanduri et al. 2008; Menzel et al. 2020; Ziello et al. 2009). In addition to this well-known response in phenology, pollen production of different species was found to be affected by factors related to global change (Ladeau and Clark 2006; Ziska et al. 2003). However, most of these studies refer to warming experiments, and only very few studies assessed climate change impacts in real-life ecosystems, either in urban or rural and mountainous environments (e.g. Charalampopoulos et al. 2013; Damialis et al. 2011, 2020; Jochner et al. 2012).

Knowledge of changes in pollen production is important for *inter alia* predicting crop yield in agriculture (González-Fernández et al. 2020) and seed production in forestry (Allison 1990). Furthermore, exposure to airborne pollen of certain plant taxa provoke immune responses and allergic symptoms in sensitized individuals (Buters et al. 2012; D'Amato et al. 2007; Damialis et al. 2019). Even though there are sophisticated European models of airborne pollen abundance and timing (e.g. SILAM, Sofiev et al. 2015) including inter-seasonal variations of the potential amount of pollen emission (Verstraeten et al. 2019), most of them have not incorporated information on pollen production, which undoubtedly plays a vital role in forecasting the intensity of the airborne pollen season and associated allergic symptoms. Information on individual-specific values of pollen production can help understand the involved processes that contribute to modifications of pollen concentrations and hence might be important for implementation in pollen forecasting systems.

The assessment of pollen production and the extraction methods are not standardized, and most previous studies were descriptive in nature reporting quantitative estimates of single species and/or single locations (Fernández-González et al. 2020; Hidalgo et al. 1999; Khanduri and Sharma 2009; Molina et al. 1996; Subba Reddi and Reddi 1986). The spatial extent is larger (horizontally or vertically) when examining the influence of urbanization or altitude as these studies are based on environmental gradients (Damialis et al. 2011; Fotiou et al. 2011; Jochner et al. 2011; Ziska et al. 2003). However, there is still limited research on flower and/or pollen production along elevation gradients. This understanding is important since it would give information on the plant's plasticity and how different environmental conditions impact reproductive traits (Charalampopoulos et al. 2013). Few studies assessed and attempted to explain pollen production of several woody species along elevation gradients, namely *Corylus avellana*, *Cupressus sempervirens*, *Olea europaea*, *Pinus halepensis*, *Platanus orientalis* and *Quercus coccifera*, mostly in Mediterranean regions (Aguilera and Valenzuela 2012; Charalampopoulos et al. 2013; Damialis et al. 2011; Rojo et al. 2015), and *Alnus incana* in the Nordic region (Moe 1998). Reproduction studies conducted along altitudinal gradients mainly focus on characteristics of seeds, e.g. seed quality, germination rate or weight (Allen et al. 2012; 2014). For birch species, Holm (1994) studied the reproductive patterns along an altitudinal gradient in Northern Sweden. So far and to the best of our knowledge, no previous study has investigated the variation of pollen production of birch at different altitudes. There has been a general lack of studies examining the pollen production of anemophilous species within alpine ecosystems as well as in the European Alps. In contrast, there are some studies on differences in birch pollen concentration in ambient air along altitudinal gradients in the Alps (Gehrig and Peeters 2000; Jochner et al. 2012; Wörl et al. 2022) and on pollen abundance and its correlation with allergic symptoms and immune reactions in sensitised patients (Damialis et al. 2019).

Birch (*Betula* spp.) trees are widely distributed across the Northern Hemisphere (Atkinson 1992), and their pollen are highly allergenic (D'Amato et al. 2017) and present a major cause of allergic rhinitis in central and northern Europe (Biedermann et al. 2019). They often grow in lowlands, although they are also present at higher altitudes (Emberlin et al. 2002). In Germany, birch is found up to an altitude of approx. 1800 m a.s.l. (DWD 1991). The latest citizen-science generated data demonstrated that *Betula pubescens* (downy birch) can occur at altitudes as high as 1840 m a.s.l., and *Betula pendula* (silver birch) was found at a maximum altitude of 1610 m a.s.l. in the Bavarian Alps (BAYSICS Webportal). Based on future projections using IPCC scenarios, birch trees in Bavaria are anticipated to become less common at lower elevations but shift their

treeline and become more dominant at higher elevations in the Bavarian Alps over the next half century (Rojo et al. 2021). Spatiotemporal studies on birch pollen concentrations in the Bavarian Alps have also shown the effect of differing meteorological conditions such as wind patterns on birch pollen concentration (Jochner et al. 2012). Therefore, the estimation of actual and prospective pollen production and knowledge on spatial and temporal variations are important for forecasting future effects on respiratory allergies.

In the current work, we studied pollen production of *Betula pubescens* along a valley-mountain gradient in the Bavarian Alps for 2 consecutive years (2020 and 2021). The main aim of this work was to quantify the production of birch pollen, flowers and inflorescences (i.e. catkins) at sites ranging from 700 to 1220 m a.s.l. In addition, the relationship between reproductive metrics and thermal parameters was studied.

Materials and methods

Study area

The study area was located in southern Bavaria (Germany) and Tyrol (Austria) in the topographically complex region of the Zugspitze area (Fig. 1). With 2962 m a.s.l., the Zugspitze, which belongs to the Northern Limestone Alps in the Wetterstein Mountains, presents the highest mountain in Germany

(Jochner et al. 2012). The birch trees were located in the city of Garmisch-Partenkirchen (700 m a.s.l.) and followed an altitudinal gradient up to the lake Eibsee (1000 m a.s.l.) and Ehrwald in Austria (1100 m a.s.l.). The highest location was at 1220 m a.s.l. (Ehrwald Cable Car Station); thus, the study covers an elevational gradient of 522 m. At lower sites, meadows are dominating; at higher elevations until approx. 1800 m, forests with spruce as the dominating tree species.

The average annual temperature recorded at Garmisch-Partenkirchen is 7.7 °C and the average precipitation sum amounts to 1373 mm (1991–2020). For the years 2019–2021, the average temperature and total precipitation at Garmisch-Partenkirchen are 7.9 °C and 1315 mm (in 2019), 6.1 °C and 1419 mm (in 2020), and 7.4 °C and 1434 mm (in 2021), respectively (Fig. 2) (DWD 2022).

Birch tree selection and inflorescence sampling

For assessing pollen production, we studied the species *Betula pubescens* Ehrh. The selection of individual trees was based on their presence, and the criteria of accessibility of the site and the reachability of catkins. Consideration was given to have at least one site for every 100 m of difference in elevation and to have representatives especially at the lowest (700–900 m; nine individuals) and highest sites (> 1100 m; six individuals) (Table 1). We collected inflorescence samples from 17 trees at nine locations (Table 1) in spring 2020 and 2021.
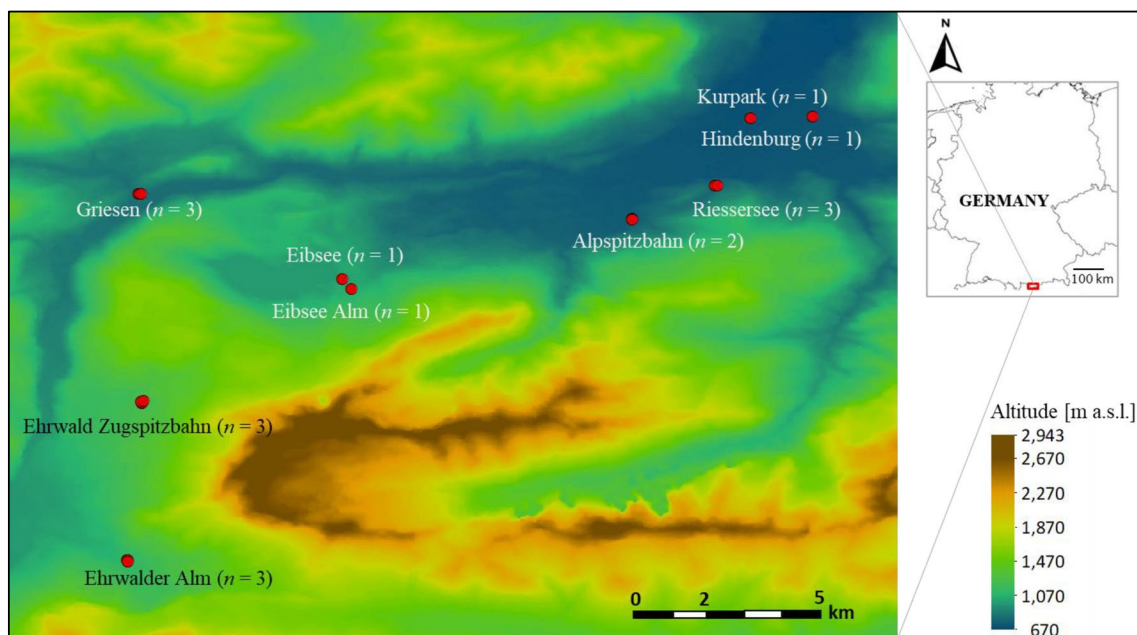

Fig. 1 Location of the study sites in Germany/Austria (Eurostat GISCO) and in the Zugspitze region (NASA JPL 2020). Red dots: nine birch tree locations (with in total 17 birch individuals). White font locations are in Germany and black font locations are in Austria
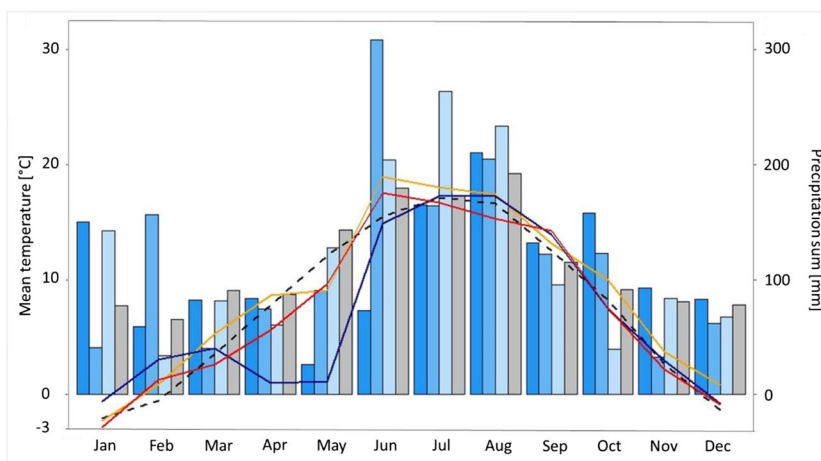
Fig. 2 Monthly meteorological data recorded at DWD station Garmisch-Partenkirchen for the years 2019–2021. Solid lines show monthly average temperature: 2019 (orange), 2020 (dark blue) and 2021 (red) and blue bars the monthly precipitation sum: 2019 (dark blue), 2020 (blue) and 2021 (light blue). *x*-axis: months, left *y*-axis: monthly mean temperature in °C, right *y*-axis: monthly precipitation sum in millimetres. Mean values (1991–2020) are displayed as black dashed line (temperature) and grey bars (precipitation sum). Data: DWD 2022

After catkin elongation was initiated and before anthesis, male catkins with mature and closed anthers were collected in March and April 2020 and 2021. Catkins were harvested in all four cardinal directions from various branches at reachable heights (1.5 to 2 m a.g.l.). We also assessed tree parameters that were used for the extrapolation of pollen production from catkins to the crown volume: the height of the crown using Suunto PM-5/1520PC Height Meter and the crown diameter, which was computed by averaging the crown's two widest perpendicular diameters. We counted the number of catkins inside a sampling cuboid in the crown with a volume of 50 cm × 50 cm × 50 cm and selected those areas of the tree crown that represent the typical distribution of catkins (Damialis et al. 2011).

Table 1 Description of the location of the trees selected for studying their pollen production, including their coordinates, mean altitude (m a.s.l.) and number of the trees at each site in the Garmisch-Partenkirchen area

Location	Coordinates	Altitude (m a.s.l.)	*n* trees
Kurpark	N 47°29′46″E 11°05′26″	696	1
Hindenburg	N 47°29′47″E 11°06′19″	706	1
Alpspitzbahn	N 47°28′18″E 11°03′40″	749	2
Riessersee	N 47°28′47″ E 11°04′53″	781	2
Griesen	N 47°28′40″ E 10°56′27″	824	3
Eibsee	N 47°27′38″ E 10°59′14″	982	1
Eibsee Alm	N 47°27′16″ E 10° 59′34″	1,011	1
Ehrwalder Alm	N 47°23′17″ E 10°56′17″	1,102	3
Ehrwald Zugspitzbahn	N 47°25′36″ E 10°56′30″	1,218	3

Pollen extraction method

We adapted the method of Damialis et al. (2011) for the extraction of birch pollen grains from closed inflorescences. The length and width (at the broadest point) of one medium-sized inflorescence from each cardinal direction and per tree were measured, and the number of flowers was counted. Then, each catkin was immersed in a 10% KOH solution overnight (Faegri et al. 1989; Moore et al. 1991; Ranpal et al. 2022). After boiling the solution the next day at 120 °C for 10 min, the soft catkin was mashed with a glass rod to discharge pollen. We added a bipolar solvent, glycerol (70%), to a volume of 20 mL (Ranpal et al. 2022) to prevent pollen from clustering (Shivanna and Rangaswamy 1992); safranin was applied as a stain. Using a VITLAB® micropipette, two aliquot samples (10 µL each) of each suspension were taken while the mixture was continuously stirred to achieve homogeneity. The extraction was then placed on microscope slides and covered with slips. We then counted the pollen grains on these slides using a $100 \times$ magnification (Zeiss AXIO Lab.A1, Germany). In case of a substantial difference in the pollen counts between these two slides ($> 30\%$), the progress was repeated to increase homogeneity of the suspension.

We estimated pollen production for different scales following the formulae mentioned by Damialis et al. (2011). The number of pollen grains per catkin (P_{ca}) was calculated by multiplying the number of pollen grains on a microscope slide with the ratio of the volumes of the suspension (20 mL) and the sample taken (10 µL). Following, the number of pollen grains per flower (P_{fl}) was derived as a quotient of

P_{ca} divided by the numbers of flowers per catkin (Fl_{ca}). The number of pollen grains per volume unit (1 m^3) of crown (P_{vuc}) was determined by multiplying P_{ca} with the ratio of the number of catkins per crown sampling unit (C_{su}) and the volume of the sampling unit (0.125 m^3). In addition, the number of flowers (Fl_{vuc}) and catkins (C_{vuc}) per volume unit of crown was extrapolated.

Environmental data

To assess the influence of temperature on pollen production, we positioned nine loggers with radiation shields (HOBO Pro v2 U23-001, Onset, Bourne, MA, USA). Each logger was set up at a height of 2 m a.g.l. on the northern side of one birch tree from one location, which recorded temperature at 10-min intervals from February 2020 until June 2021. A new HOBO logger was installed at the location Griesen in January 2021 as the previous one was lost, and no data were available for this site until December 2020. The location's missing daily temperature data were interpolated applying linear regression with daily temperatures and altitudes of the other eight loggers. The root-mean-square errors (RMSE) between predicted and observed daily mean temperature during January until June 2021 were 1.2. The software package HOBOware (Version 3.7.23; Onset, Bourne, Massachusetts, USA) was used to download the data from the loggers and to export the raw data as text files.

We focussed on temperatures measured in the summer previous of flowering, since this period is assumed to be important for pollen production (Ranpal et al. 2022), as catkins already start to develop and elongate (Dahl and Strandhede 1996). Thus, we were able to compare temperature data of 2020 with pollen production of 2021. For the first study year, we cannot resort to 2019 data; thus, we link pollen production of 2020 to March temperatures of 2020. For comparison, we also link 2021 pollen data to 2021 March temperatures.

Furthermore, we calculated growing degree-days (GDD; in °C) of summer 2020 (June–August) by cumulating positive differences between the daily mean temperature (derived as an average of T_{max} and T_{min}) and a threshold temperature. In our study, we used a base temperature of 5 °C (Bucher et al. 2018; Estrella and Menzel 2006).

Statistical analyses

All levels of flower, catkin and pollen production were checked for normality using Shapiro–Wilk test, which revealed that these reproductive measures were not normally distributed. Non-normality was dealt with by using non-parametric tests.

We examined differences between sampling years using the non-parametric Mann–Whitney U test and applied Spearman's correlations to analyse association between altitude and reproductive metrics. In addition, the influence of the altitude on the tree-specific differences in reproductive metrics between 2021 and 2020 was checked. To investigate the effect of environmental factors on pollen production of birch along the elevational gradient, we compared reproductive metrics with temperature variables (T_{mean}, T_{min}, T_{max}, GDD).

All statistical analyses were carried out with R version 4.2.2 (R Core Team 2020).

Results

Pollen, flowers and catkins production

Pollen production per catkin (P_{ca}) for all selected 17 birch trees in the area of Garmisch-Partenkirchen was 5.23 ± 1.52 million pollen grains in 2020 and 2.51 ± 1.23 million pollen grains in 2021 (see Table 2). P_{ca} varied within a wide range from approx. 400,000 (minimum of 2021) to 8.3 million pollen grains (maximum of 2020). P_{ca} in 2021 was 52% lower compared to 2020 when regarding mean values. The number of catkins in a crown sampling unit (C_{su}; 0.125 m^3) ranged between 1 (minimum of 2021) and 50 (maximum of 2020) with an average of 28 catkins in 2020 and 5 catkins in 2021 (-82%). In addition, all other estimated parameters, i.e. pollen production per flower (P_{fl}), per volume unit of crown (P_{vuc}) and the number of flowers per catkin (Fl_{ca}), were consistently higher in 2020, which does not only apply to mean, but also to minimum and maximum values (Table 2).

Year-to-year variation in reproductive metrics

The Mann–Whitney U test revealed that the means of all reproductive metrics except for flowers per catkin (Fl_{ca}) ($p = 0.214$) were significantly different between 2020 and 2021. In each case, the percentage change was positive, i.e. the highest values were measured in 2020. Figure 3 shows exemplary the differences of P_{fl}, P_{ca}, P_{vuc}, Fl_{ca}, Fl_{vuc} and C_{vuc} between 2020 and 2021.

Effects of altitude and temperature on reproductive metrics

Altitude

In 2020, there were no significant correlations between the reproductive metrics and altitude (Table 3). In 2021, some correlation coefficients increased in magnitude, but were not statistically significant (marginally significant for Fl_{vuc}, $r_s = -0.446$, $p = 0.073$ and C_{vuc}, $r_s = -0.443$, $p = 0.075$).

Table 2 Descriptive statistics of pollen production per flower (P_{fl}), per catkin (P_{ca}) and per volume unit of crown (P_{vuc}); flower production per catkin (F_{ca}) and per volume unit of crown (Fl_{vuc}) and catkin production per crown sampling unit (C_{su}; 0.125 m³) and per volume unit of crown (C_{vuc}) estimated for 17 selected birch trees along an altitudinal gradient in the Garmisch-Partenkirchen area during 2020–2021. The second last column indicates the results of the Mann–Whitney U test for comparison of means

Reproductive metric	Year	Minimum	Maximum	Mean	Median	Standard deviation	W statistic (p value)	Difference 2020 to 2021 (in %)
Pollen production								
P_{fl}	2020	20,442	79,007	45,738	44,886	13,041	256 ($\leq$0.001)	48%
	2021	4,445	56,139	23,639	23,154	12,798		
P_{ca}	2020	2,197,500	8,257,667	5,228,025	5,255,750	1,521,924	262 ($\leq$0.001)	52%
	2021	398,667	5,409,250	2,507,427	2,412,500	1,230,822		
P_{vuc}	2020	187,620,000	2,039,200,000	1,095,620,550	929,480,000	562,490,982	287 ($\leq$0.001)	91%
	2021	6,378,672	482,500,000	96,997,804	69,294,000	109,138,708		
Flower production								
Fl_{ca}	2020	84	146	115	114	16	181 (0.214)	6%
	2021	77	125	108	112	14		
Fl_{vuc}	2020	4560	42,560	25,163	24,240	12,085	278 ($\leq$0.001)	82%
	2021	896	20,800	4314	2712	4800		
Catkin production								
C_{su}	2020	5	50	28	25	14	276 ($\leq$0.001)	82%
	2021	1	25	5	3	6		
C_{vuc}	2020	40	400	223	200	108	276 ($\leq$0.001)	82%
	2021	8	200	41	24	46		
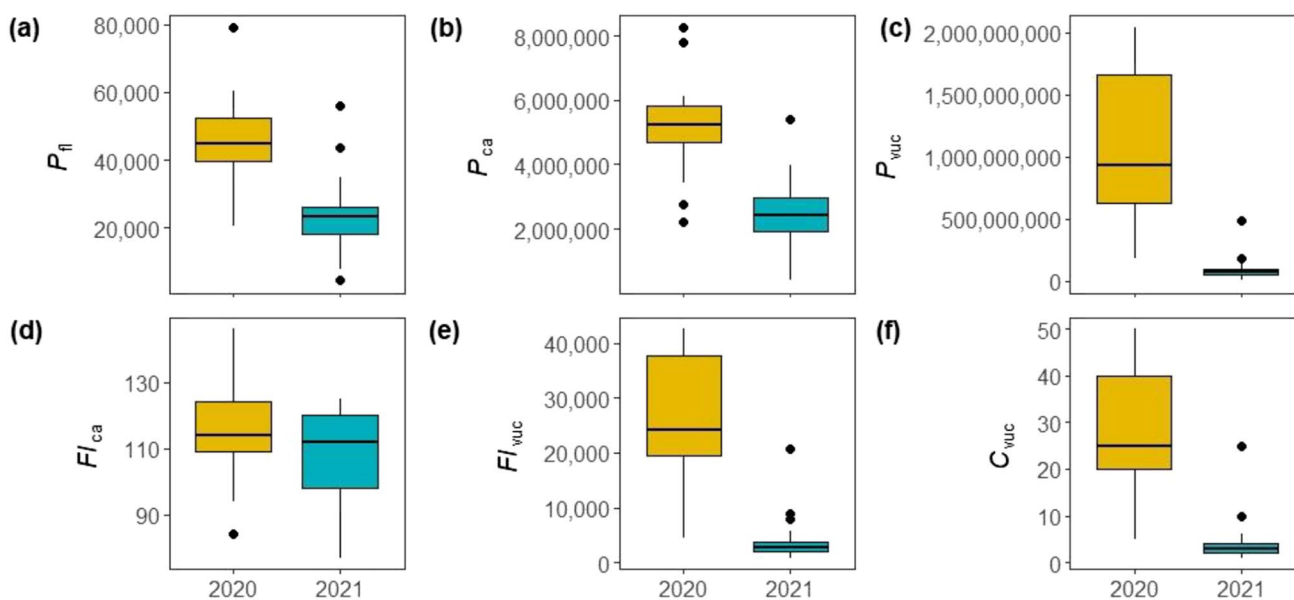
Fig. 3 Boxplots based on (**a**) P_{fl}, (**b**) P_{ca}, (**c**) P_{vuc}, (**d**) Fl_{ca}, (**e**) Fl_{vuc} and (**f**) C_{vuc} estimated for 17 trees along an altitudinal gradient in the Garmisch-Partenkirchen area for 2020 and 2021. The interquartile range (IQR) is represented by the height of the boxes, maximum and minimum values by the upper and lower whiskers, the median by bold horizontal lines in the boxes, dots represent observations exceeding or falling below 1.5 times the IQR

Figure 4 illustrates the relationship between altitude and reproductive metrics estimated in 2020 and 2021. Especially in 2020, but also in 2021, there is a large scattering, which was also reflected by the non-significant relationship (Table 3). Regression lines were added in case of marginally significant relationships.

Table 3 Spearman's correlations between altitudes, temperature metrics, and reproductive metrics of 17 birch trees located in the Garmisch-Partenkirchen area in 2020 and 2021. r_s Spearman's correlation coefficient, p significance value

2020		Altitude	T_{min} March 2020	T_{mean} March 2020	T_{max} March 2020	GDD March 2020
P_{fl}	r_s	−0.071	0.093	−0.006	−0.014	−0.009
	p	0.786	0.723	0.981	0.959	0.974
P_{ca}	r_s	−0.148	0.190	0.087	0.059	0.020
	p	0.570	0.464	0.741	0.821	0.940
P_{vuc}	r_s	−0.221	0.085	0.117	0.068	0.209
	p	0.395	0.745	0.654	0.795	0.421
Fl_{ca}	r_s	0.122	−0.170	−0.138	−0.157	−0.185
	p	0.642	0.515	0.599	0.546	0.478
Fl_{vuc}	r_s	−0.104	−0.049	0.030	−0.020	0.022
	p	0.691	0.851	0.910	0.940	0.932
C_{vuc}	r_s	−0.165	0.006	0.078	0.031	0.068
	p	0.527	0.981	0.765	0.906	0.794

2021		Altitude	T_{min} March 2021	T_{mean} March 2021	T_{max} March 2021	GDD March 2021	T_{min} Summer 2020	T_{mean} Summer 2020	T_{max} Summer 2020	GDD Summer 2020
P_{fl}	r_s	0.405	−0.147	−0.234	−0.189	0.115	−0.288	−0.399	−0.328	−0.330
	p	0.107	0.573	0.367	0.467	0.660	0.262	0.112	0.199	0.196
P_{ca}	r_s	0.363	−0.119	−0.220	−0.171	0.121	−0.279	−0.354	−0.289	−0.289
	p	0.152	0.650	0.396	0.513	0.643	0.278	0.164	0.260	0.260
P_{vuc}	r_s	−0.303	0.361	**0.512**	*0.455*	**0.774**	**0.504**	0.232	−0.010	0.272
	p	0.237	0.155	0.036	0.067	0.000	0.039	0.369	0.970	0.291
Fl_{ca}	r_s	−0.032	0.170	0.133	0.100	0.159	−0.004	0.101	0.224	0.153
	p	0.903	0.513	0.610	0.703	0.541	0.987	0.700	0.388	0.557
Fl_{vuc}	r_s	*−0.446*	*0.470*	**0.635**	**0.509**	**0.650**	**0.613**	0.391	0.173	*0.425*
	p	0.072	0.057	0.006	0.037	0.005	0.009	0.121	0.507	0.089
C_{vuc}	r_s	*−0.443*	Not shown since catkin development is prior to March 2021				**0.642**	0.400	0.145	*0.425*
	p	0.075					0.005	0.112	0.578	0.089

Significant at the 0.05 level (bold values) and marginally significant (at the 0.1 level; italics values)
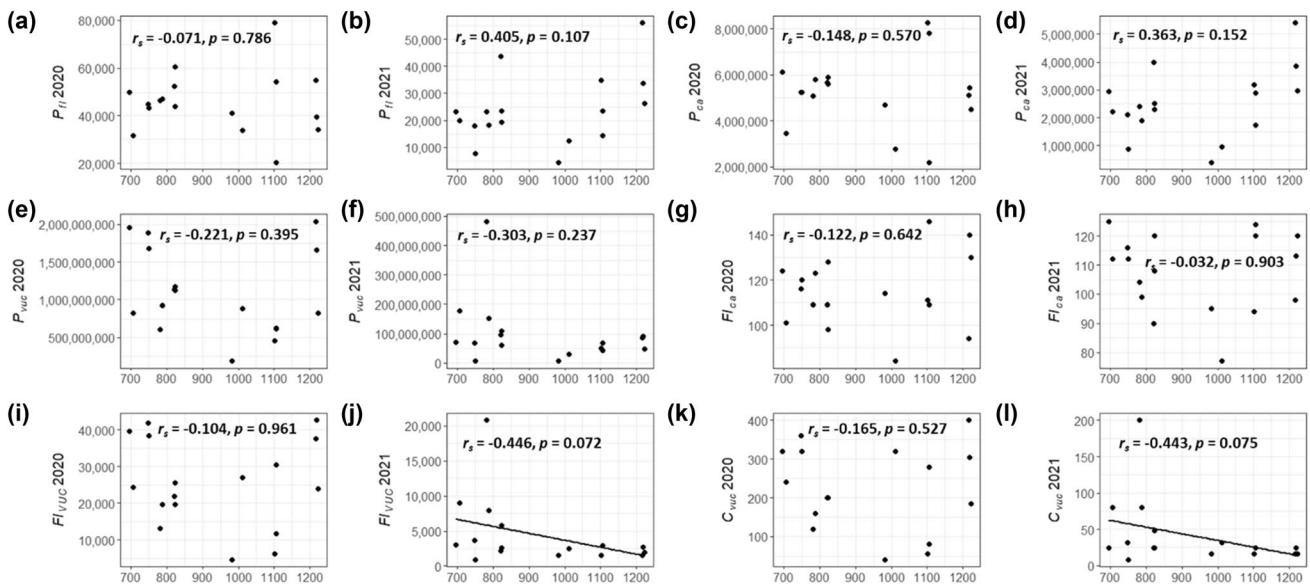
Fig. 4 Scatterplots of altitude and P_{fl} in 2020 (**a**), 2021 (**b**); P_{ca} in 2020 (**c**), 2021 (**d**); P_{vuc} in 2020 (**e**), 2021 (**f**); Fl_{ca} in 2020 (**g**), 2021 (**h**); Fl_{vuc} in 2020 (**i**), 2021 (**j**); and C_{vuc} in 2020 (**k**), 2021 (**l**) esti- mated for 17 trees in the Garmisch-Partenkirchen area. Regression lines were added in case of marginally significant relationships

Figure 5 demonstrates the differences in the selected reproductive metrics (P_{ca}, Fl_{ca} and C_{vuc}) between 2020 and 2021. Though the correlations are all non-significant, some interesting patterns can be revealed: Only two trees, located above 1100 m a.s.l., were linked to a negative P_{ca} value, i.e. higher pollen production, in 2021 (Fig. 5a). Three trees at the highest location (Ehrwald Zugspitzbahn; EZ) showed relatively small differences, but two trees at Ehrwalder Alm (EA) the largest differences. No or a very small differences in Fl_{ca} between 2020 and 2021 were obtained for trees at Alpspitzbahn (AB; 749 m a.s.l.) and Kurpark (KP; 696 m

a.s.l.). Four trees were linked to less flowers in 2021, the rest to more flowers. A clearer pattern was seen for the differences in C_{vuc} between 2020 and 2021. Here, only one tree (located at Riessersee; RS) was associated to a lower number of catkins in 2020. However, correlation analyses revealed no significant relation to altitude.

Temperature

In general, mean summer temperatures (June–August 2020) recorded at each site were negatively and strongly correlated

Fig. 5 Scatterplots of the difference in the selected reproductive metrics (**a**) P_{ca}, (**b**) Fl_{ca} and (**c**) C_{vuc} between 2020 and 2021 estimated for 17 trees in the Garmisch-Partenkirchen area (locations: AB, Alp- spitzbahn; EA, Ehrwalder Alm; EZ, Ehrwald Zugspitzbahn; KP, Kur- park; and RS, Riessersee) and respective altitudes

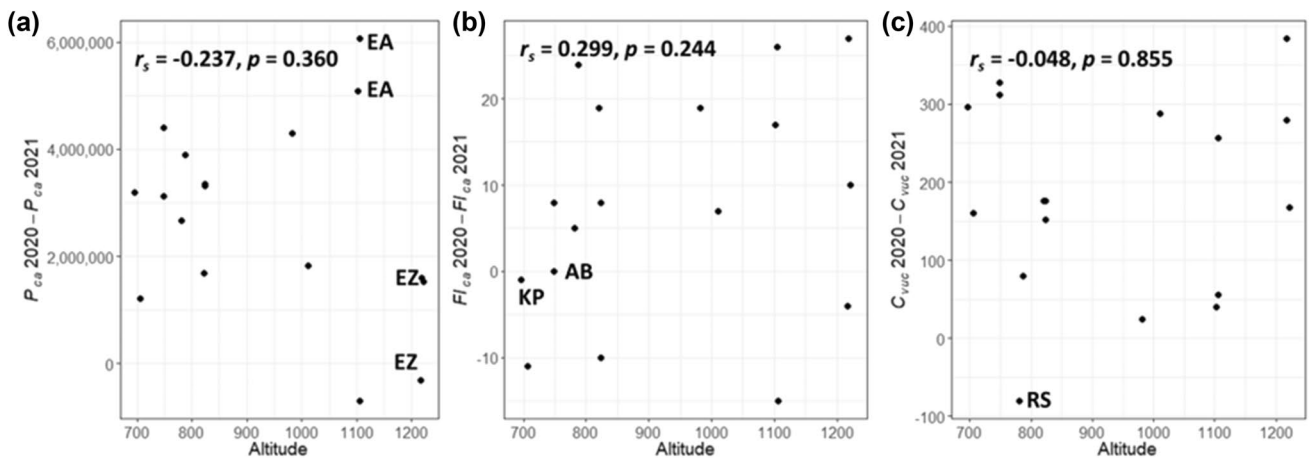with altitude ($r_s = -0.940$, $p < 0.001$). GDD of the same period was also negatively and strongly correlated with altitude ($r_s = -0.880$, $p < 0.001$). The highest temperature mean (17.8 °C), considered for the period of June–August 2020, was measured at Hindenburg (706 m a.s.l.), which is one of the lowest locations of the study. The lowest mean annual temperature (14.8 °C) was recorded at the highest site, at Ehrwald Zugspitzbahn (1218 m a.s.l.) (Table 1). The sites with the highest (Hindenburg; 5.0 °C) and the lowest (Ehrwald Zugspitzbahn; 1.7 °C) temperature mean recorded in March 2020 were the same as mentioned above.

The relationships with reproduction metrics and temperature variables (minimum, mean, maximum temperature and GDD) were not statistically significant in 2020, the year in which the highest pollen and catkin production was observed. For 2021, however, we found some significant correlations. C_{vuc} was significantly ($p = 0.005$) correlated to summer T_{min} ($r_s = 0.642$). Since the amount of catkins in a sampling volume also influences the reproduction measures Fl_{vuc} and P_{vuc}, positive and significant relationships were also derived in these cases: Fl_{vuc} and summer T_{min} ($r_s = 0.613$, $p = 0.009$), P_{vuc} and summer T_{min} ($r_s = 0.504$, $p = 0.039$). Interestingly, other temperature variables calculated for the period June to August (mean and maximum temperatures, GDD) were not significantly associated to any of the reproductive metrics. Instead, March temperatures were sometimes superior in describing the relationship. The highest correlation was achieved with P_{vuc} and GDD ($r_s = 0.774$, $p = 0.000$). In summary, warmer conditions were related to higher pollen and flower production, which was only obvious for higher levels, i.e. for the volume unit of the crown, as a result of the temperature dependency of catkin numbers in 2021.

For visualization (Fig. 6), we focused on the relationships with reproductive metrics estimated in 2021 and minimum summer temperature in 2020. The subplots (a), (b) and (d) in Fig. 6 demonstrate that pollen and flower production of male inflorescences in 2021 was random with respect to the T_{min} of the previous summer. However, an increase in the number of pollen, flowers and catkins per volume unit of crown was observed with higher values of T_{min} in summer 2020 (Fig. 6c, e, f).

Discussion

The value of gradient studies in pollen research

Long-term studies can profit from almost constant site conditions (soil type, edaphic regime) and varying meteorological conditions across years that may allow for calculating trends or response rates based on the same individual and therefore excluding genetic variability (Jochner et al. 2013a). Studies along altitudinal gradients can be affected by several factors such as complex environmental heterogeneity and extreme geography (Körner 2007). As the space-for-time approach also includes a multitude of different individuals at various sites, differences in local environmental factors, e.g. soil conditions, nutrient availability and water supply as well as differences in pollution or other factors related to local climate and genetics might exert additional influences. Thus, gradient studies do not only account for differences in
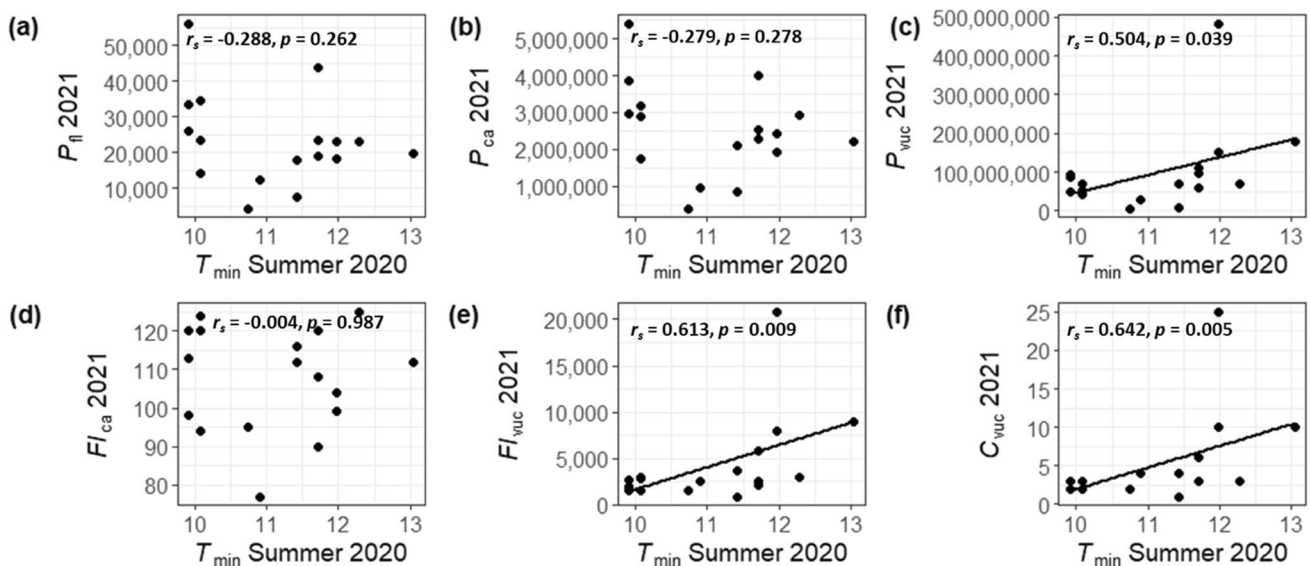
Fig. 6 Relationship between (**a**) P_{fl} 2021, (**b**) P_{ca} 2021, (**c**) P_{vuc} 2021, (**d**) Fl_{ca} 2021, (**e**) Fl_{vuc} 2021 and (**f**) C_{vuc} 2021, and summer T_{min} of 2020 for 17 birches along the altitudinal gradient in the Garmisch-Partenkirchen area. Regression lines were added in case of significant relationships

temperature, which is, however, the most important variable when the effect of climate change is aimed to be assessed.

Pollen, flowers and catkins production

In the present study, we investigated how reproductive metrics of *Betula pubescens* trees differ in 2 consecutive years along an altitudinal gradient in the Bavarian Alps. We estimated mean pollen production values at the level of catkins ranging between 5 and 2.5 million. This estimate is comparable to that published by Erdtman (1954), who reported a value of 6 million pollen grains per inflorescence for *B. pubescens*. Our mean value, however, is an average of the estimates from 17 downy birch trees growing in various elevations for 2 years ($n = 34$). According to Ranpal et al. (2022), a catkin from *Betula pendula* (silver birch) produces on average 1.7 million pollen grains. Such estimations of pollen production might be important to assist pollen emission parameterization since commonly, their proxies were only related to plant characteristics such as leaf area index and canopy height (Helbig et al. 2004).

Estimation of pollen production by counting all pollen on microscope slides is a labour- and time-intensive method. Improved pollen counting methods, such as a cell counter (Kakui et al. 2020) or automatic identification of the number of pollen grains on microscopic slides (Kadaikar et al. 2019), would make the method more efficient.

Year-to-year variation in reproductive metrics

The year 2020 was found to be a pollen- and catkin-rich year: We estimated on average 109% more pollen grains per catkin than in the following year. In addition, the number of catkins per volume unit of crown was 460% higher in 2020, but the number of flowers per catkin was only changed slightly (+6%) and associated to a non-significant difference.

Flower numbers were not substantially different between years. The number of anthers per flower is genetically fixed and does not vary substantially (Fernández-González et al. 2020; Hidalgo et al. 1999; Subba Reddi and Reddi 1986). In the case of birch, flowers per catkin seem to have the most homogenous value among clones and years (Ranpal et al. 2022). However, the number of flowers per volume unit of crown was also calculated by multiplying the number of flowers of single catkins by the abundance of catkins within the volume. Thus, the number of catkins, which can largely differ between trees and years, is the most decisive factor for the value obtained for flowers per volume unit of a crown.

In general, reproductive metrics in birch trees can vary greatly from year to year, as found by Jato et al. (2007), Damialis et al. (2011) and Ranpal et al. (2022). Alternating patterns of flower (and seed) production are related to masting behaviour, an inherent common feature in temperate tree species that occurs, in the case of birch, every second or third year (Detandt and Nolard 2000; Latałowa et al. 2002). Given that the catkin (more than 10 times) and pollen production (3 times) were extraordinarily high in 2020, one may assume that this year was a masting year. At a seed plantation in Baden-Württemberg, Germany (distance to Garmisch-Partenkirchen approx. 210 km), Ranpal et al. (2022) also found that mean C_{su} of a total of 28 trees in 2020 was two times higher than in the preceding year and the subsequent year. Data obtained from our pollen monitoring site in Eichstätt, Bavaria (distance to Garmisch-Partenkirchen approx. 160 km), also indicated that 2020 was linked to a high pollen load in the air: Here, an APIn (annual pollen integral) of 8720 pollen grains*day/m^3 was measured, compared to only 1923 pollen grains*day/m^3 in the following year (unpublished data). In general, for defining mast years, a longer time-series would be needed for a detailed identification and evaluation (LaMontagne and Boutin 2009). The delineation of mast years is mostly based on concepts that include the coefficient of variation that accounts for the mean and standard deviation, but consistent and generally applicable methods are not available (LaMontagne and Boutin 2009). In the case of pollen, one reason might be the underrepresentation of studies addressing flower masting (Pearse et al. 2016; Satake and Iwasa 2002).

Thus, the lack of studies related to flower masting along altitudinal gradients is not surprising. This is in contrast to seed masting, where changes of temporal patterns of masting were *inter alia* already linked to the variation in climatic conditions along elevational gradients (Masaki et al. 2020). The authors found that mean fruiting density and fruiting frequency of *Quercus crispula* decreased with elevation, while the annual variation in fruiting density increased. Therefore, harsh environmental conditions (e.g. low temperatures) at high elevations might be linked to a reduced photosynthetic production and increased masting (Masaki et al. 2020). In our study, which was only based on two consecutive years, we found no significant dependency between the deviation from 2020 and 2021 in pollen, flower or catkin production and altitude (Fig. 5). In 2020, the vast majority of selected trees synchronously produced a higher amount of pollen, flowers and catkins. However, it was obvious that two trees located at high elevations were the only exceptions showing a negative deviation (i.e. higher flower and pollen production per catkins in 2021). The number of catkins produced in 2021 for those trees, however, was very low as well; thus, the pollen or flower production based on larger units (i.e. volume unit of the crown) was still higher in 2020. These findings also point to the need for defining and categorizing (flower) masting since reproductive metrics can be altered differently. For this reason, a larger dataset including more observation years and more birch trees, e.g. located at even harsher sites, would be desirable.

It should be noted (but must remain unevaluated) that the masting year has occurred (in 2020) after the year (2019) with the highest temperature (7.9 °C) and lowest precipitation sum (1315 mm), registered in Garmisch-Partenkirchen in the period of 2019–2021. This also calls for the installation of a long-term monitoring in order to be able to understand the influence of meteorology on masting years in more detail.

Effects of altitude and temperature on reproductive metrics

Altitude

We found that there were no prominent changes in the analysed reproductive traits with increasing altitude (Table 3; Fig. 4). The results of very few prior studies studying pollen production along altitudinal gradients showed that there is no conclusive evidence on the associated relationships with increased elevation since a decrease in pollen production (Markgraf 1980; Moe 1998), an increase (Aguilera and Valenzuela 2012) or no significant change (Charalampopoulos et al. 2013; Hasegawa et al. 2022) was observed. Aguilera and Valenzuela (2012) argued that higher olive pollen production observed at elevated regions might be related to intrinsic mechanisms of these trees to compensate for a limited pollination efficiency and a shorter growing period. However, these results may also be affected by human interventions (cutting) that may have a masking effect on pollen production. However, in some of the studied species at Mount Olympos, pollen and inflorescence traits at the higher reproduction level (e.g. per individual tree) were decreased with increasing altitude (Charalampopoulos et al. 2013). In this study, we decided not to integrate the number of pollen, flowers or inflorescences per individual, since this measure is strongly dependent on the age and height of a tree that considerably varies along the gradient under investigation. In addition, extrapolating production estimates to the whole tree is based on the assumption of a simplified geometric shape of the tree (Molina et al. 1996). However, this potential geometric shape differs from its original form to a certain extent, implying uncertainties in the estimation for the level of an individual tree. All variables based on a specific volume are believed to be superior indicators of pollen production, since they account for the pollen produced per catkins and the number of catkins in a standard volume (1 m^3). Some studies, such as those by Bogawski et al. (2019) and Katz et al. (2020), have used LiDAR data to determine crown parameters, which were used for estimating pollen production per tree or tree stand.

The result of this study indicated that the number of male inflorescences per crown sampling unit (C_{su}) in 2021 decreased along the gradient ($r_s = -0.443$, $p = 0.075$,

Table 3). Thus, compensation for pollen limitation might more strongly affect the pollen produced by single inflorescences. Fernández-González et al. (2020) found that smaller sized tree species of the genus *Quercus* attempt to produce a higher amount of pollen per anther to ensure fertilization.

Temperature

Although we found a strong and significant relation with temperature and altitude, those variables associated to temperature showed stronger and more significant correlations than altitude alone. This also points to the fact that temperature measurement should be implemented in any altitudinal gradient studies.

In 2021, we detected an increased catkin formation at warmer (lower) locations, which was also reflected in the reproductive metrics whose computations were based on the number of catkins. Our results indicated that minimum temperature was superior in any statistical analyses than mean and maximum temperatures or even GDD.

Non-significant relationships with temperature were found in 2020, the assumed mast year. The reason might be that the amount of pollen and inflorescences produced by the selected birch trees in our study was most probably regulated by the resource balance of the trees, and masting-associated parameters masked other influences and variability present in normal reproductive years. According to the resource budget model, masting can occur due to plants' resource balance even in the absence of interannual environmental variations (Isagi et al. 1997). In general, pollen concentration and therefore pollen availability is reduced at higher elevations due to a decreasing prevalence of birch trees (Charalampopoulos et al. 2013; Jochner et al. 2012). A lower availability of birch pollen might also cause low seed production. Following the resource storage hypothesis, this may affect resource accumulation resulting in more flowering/fruiting (Bogdziewicz et al. 2020) as observed in our study in 2020. Therefore, the fact that birch is only seldom represented at higher altitudes in our study area, might also affect its resource budget, which could mask the influence of environmental factors such as temperature.

Existing studies indicate varying relationships between temperature and pollen production. Jochner et al. (2013b) found a significant reduction in pollen production per catkin in silver birch (*Betula pendula* Roth) at urban locations (under higher temperatures) in Munich (Germany). The authors argued that conditions in urban areas might have a negative effect on the physiology of birch and thus on pollen production. On the other hand, an urban gradient study indicated that an increase in temperature increased the pollen production of other species such as common ragweed (*Ambrosia artemisiifolia*, Ziska et al. 2003).

In general, microclimate is believed to have a strong impact on pollen production (Aguilera and Valenzuela 2012); therefore, a variability is quite expected and would be even more pronounced when studying a larger altitudinal gradient. With the calculated temperature lapse rates in this study (season-depended varying between 0.4 and 0.6 °C; not shown in the results section) and the given gradient of 522 m, a temperature difference of 2.1 and 3.1 °C might be too low to observe strong effects on pollen, flower and inflorescence production.

Effects of other environmental parameters

In addition, other information than air temperature might be important: A study in alpine environments by Scherrer et al. (2011) found significant fluctuations in soil temperature of up to 4 °C depending on slope aspect and topography. Because of this, even trees at the same location and similar altitude are exposed to different microclimatic conditions that might affect reproductive traits.

Air pollutants such as nitrogen dioxide (NO_2) might reduce (Jochner et al. 2013b) or increase pollen production of birch (Zhao et al. 2017). In addition, ozone (O_3) was also found to affect birch reproduction (Darbah et al. 2008). Birch trees growing in areas with higher NO_2 levels were found to be more often affected with birch idaeovirus (Gilles et al. 2023), and such biotic stress could further influence the reproduction of infected trees. These pollutants are likely to change with increasing elevation and should also be incorporated in further studies. In the present study, we only measured NO_2 and O_3 during a 1-week period in late spring 2020 and found significant correlations between NO_2 and P_{vuc} 2020 ($r_s = 0.520$, $p = 0.032$) as well as between O_3 and P_{ca} 2020 ($r_s = -0.519$, $p = 0.033$) and P_{fl} 2020 ($r_s = -0.489$, $p = 0.047$) (not shown). Since these results are only based on a short measurement duration, we decided not to incorporate these findings in the "Results" section but encourage further research to specifically focus on pollution as potential influential factor. Since the effects of pollution might also be species-specific, there is also a strong need to compare different plant species.

Moreover, other factors can have an influence on pollen production, such as artificial pruning/topping, since the induction of stress results in a higher reproductive output (Ranpal et al. 2022). In addition, site characteristics such as stand density and exposure (Faegri et al. 1989) and genetics (Ranpal et al. 2022) were found to be relevant in the discussion on pollen production.

Knowledge derived from seed masting studies suggest that nutrient availability which usually declines with elevation as a result of decreased organic matter decomposition and nutrient mineralization (Sundqvist et al. 2013) might also affect seed availability (Allen et al. 2014). Related to birch pollen production per catkin, it was found that iron concentration (assessed in birch leaves) was linked to a decrease (Jochner et al. 2013b), but other information on the influence on nutrients, specially assessed in the soil, is largely lacking.

Low temperature and high moisture availability 2 years before seed fall was linked to a higher amount of seed production (Richardson et al. 2005). Relationships with reproduction variables related to pollen based on lag effects, however, are not commonly evaluated in existing research and highlights the need for long-term studies.

In addition, more experimental studies may be best suited to disentangle the influence of temperature and other factors influencing reproduction traits of plants and their magnitude free from masked effects. Birch trees become sexually mature (and bear male catkins) from the age of approx. 10–15 years (Perala and Alm 1990). Therefore, in the case of birch, experimental setups remain challenging since their relocation to laboratory conditions cannot easily be materialized.

In summary, future research could benefit from the inclusion of more birch trees spanning an even larger altitudinal gradient and observation years. Ideally, a long-term monitoring, which is still not established, is desirable. Spatial information on air pollution along with meteorological measurements is helpful to conclude on their influences on pollen production. Less time-consuming methods of pollen quantification should be tested and more experimental research avoiding masked effects on pollen production is suggested.

Conclusions

In conclusion, this study provides valuable insights into the production of birch pollen, flowers and inflorescences in relation to thermal parameters across an elevational gradient. The findings of this study indicate that no significant changes in the reproductive traits were detectable with increasing altitude alone. Moreover, likely due to the temperature dependency of catkin numbers in 2021, warmer sites were associated with higher pollen and flower production, which was only apparent for higher levels, i.e. for the volume unit of the crown. Temperatures further from the optimum of birch growth might be linked to more pronounced changes; thus, studying pollen production along even larger altitudinal gradients is highly relevant in future research.

Author contribution Conceptualization: Susanne Jochner-Oette. Methodology: Susanne Jochner-Oette, Surendra Ranpal and Athanasios Damialis. Software: Surendra Ranpal. Validation: Susanne

Declarations

Consent for publication All the authors have consented on their own behalf for the publication of this study.

Competing interests The authors declare no competing interests.

References

Aguilera F, Valenzuela LR (2012) Microclimatic-induced fluctuations in the flower and pollen production rate of olive trees (Olea europaea L.). Grana 51:228–239. https://doi.org/10.1080/00173134.2012.659203

Albrich K, Rammer W, Seidl R (2020) Climate change causes critical transitions and irreversible alterations of mountain forests. Glob Change Biol 26:4013–4027. https://doi.org/10.1111/gcb.15118

Allen RB, Mason NWH, Richardson SJ, Platt KH (2012) Synchronicity, periodicity and bimodality in inter-annual tree seed production along an elevation gradient. Oikos 121:367–376. https://doi.org/10.1111/j.1600-0706.2011.19306.x

Allen RB, Hurst JM, Portier J, Richardson SJ (2014) Elevation-dependent responses of tree mast seeding to climate change over 45 years. Ecol Evol 4:3525–3537. https://doi.org/10.1002/ece3.1210

Allison TD (1990) Pollen production and plant density affect pollination and seed production in Taxus Canadensis. Ecology 71:516–522. https://doi.org/10.2307/1940305

Atkinson MD (1992) Betula pendula Roth (B. *verrucosa* Ehrh.) and B. pubescens Ehrh. The Journal of Ecology 80:837. https://doi.org/10.2307/2260870

BAYSICS Webportal BAYSICS Webportal: Tree Line Analyses. https://www.portal.baysics.de/map/. Accessed 17 November 2022

Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E (2019) Birch pollen allergy in Europe. Allergy 74:1237–1248. https://doi.org/10.1111/all.13758

Bogawski P, Grewling Ł, Dziób K, Sobieraj K, Dalc M, Dylawerska B, Pupkowski D, Nalej A, Nowak M, Szymańska A, Kostecki Ł, Nowak MM, Jackowiak B (2019) Lidar-derived tree crown parameters: are they new variables explaining local birch (Betula sp.) pollen concentrations? Forests 10:1154. https://doi.org/10.3390/f10121154

Bogdziewicz M, Ascoli D, Hacket-Pain A, Koenig WD, Pearse I, Pesendorfer M, Satake A, Thomas P, Vacchiano G, Wohlgemuth T, Tanentzap A (2020) From theory to experiments for testing the proximate mechanisms of mast seeding: an agenda for an experimental ecology. Ecol Lett 23:210–220. https://doi.org/10.1111/ele.13442

Bucher SF, König P, Menzel A, Migliavacca M, Ewald J, Römermann C (2018) Traits and climate are associated with first flowering day in herbaceous species along elevational gradients. Ecol Evol 8:1147–1158. https://doi.org/10.1002/ece3.3720

Buters JT, Thibaudon M, Smith M, Kennedy R, Rantio-Lehtimäki A, Albertini R, Reese G, Weber B, Galan C, Brandao R, Antunes CM, Jäger S, Berger U, Celenk S, Grewling Ł, Jackowiak B, Sauliene I, Weichenmeier I, Pusch G, Sarioglu H, Ueffing M, Behrendt H, Prank M, Sofiev M, Cecchi L (2012) Release of Bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study. Atmos Environ 55:496–505. https://doi.org/10.1016/j.atmosenv.2012.01.054

Cerlini PB, Saraceni M, Orlandi F, Silvestri L, Fornaciari M (2022) Phenological response to temperature variability and orography in Central Italy. Int J Biometeorol 66:71–86. https://doi.org/10.1007/s00484-021-02190-1

Charalampopoulos A, Damialis A, Tsiripidis I, Mavrommatis T, Halley JM, Vokou D (2013) Pollen production and circulation patterns along an elevation gradient in Mt Olympos (Greece) National Park. Aerobiologia 29:455–472. https://doi.org/10.1007/s10453-013-9296-0

D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, van Cauwenberge P (2007) Allergenic pollen and pollen allergy in Europe. Allergy 62:976–990. https://doi.org/10.1111/j.1398-9995.2007.01393.x

D'Amato G, Vitale C, Sanduzzi A, Molino A, Vatrella A, D'Amato M (2017) Allergenic pollen and pollen allergy in Europe. In: Singh AB (ed) Allergy and allergen immunotherapy: New mechanisms and strategies / edited by A.B. Singh. Apple Academic Press, Toronto, 287–306

Dahl Å, Strandhede S-O (1996) Predicting the intensity of the birch pollen season. Aerobiologia 12:97–106. https://doi.org/10.1007/bf02446601

Damialis A, Fotiou C, Healley JM, Vokou D (2011) Effects of environmental factors on pollen production in anemophilous woody species. Trees 25:253–264. https://doi.org/10.1007/s00468-010-0502-1

Damialis A, Häring F, Gökkaya M, Rauer D, Reiger M, Bezold S, Bounas-Pyrros N, Eyerich K, Todorova A, Hammel G, Gilles S, Traidl-Hoffmann C (2019) Human exposure to airborne pollen and relationships with symptoms and immune responses: indoors versus outdoors, circadian patterns and meteorological effects in alpine and urban environments. Sci Total Environ 653:190–199. https://doi.org/10.1016/j.scitotenv.2018.10.366

Damialis A, Charalampopoulos A, Lazarina M, Diamanti E, Almpanidou V, Maraidoni AM, Symeonidou A, Staikidou E, Syropoulou E, Leontidou K, Tsiripidis I, Halley JM, Vokou D (2020) Plant flowering mirrored in airborne pollen seasons? Evidence from phenological observations in 14 woody taxa. Atmos Environ 240:117708. https://doi.org/10.1016/j.atmosenv.2020.117708

Darbah JNT, Kubiske ME, Nelson N, Oksanen E, Vapaavuori E, Karnosky DF (2008) Effects of decadal exposure to interacting elevated CO2 and/or O3 on paper birch (Betula papyrifera) reproduction. Environ Pollut 155:446–452. https://doi.org/10.1016/j.envpol.2008.01.033

Detandt M, Nolard N (2000) The fluctuations of the allergenic pollen content of the air in Brussels (1982 to 1997). undefined

Dobbertin M (2005) Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. Eur J Forest Res 124:319–333. https://doi.org/10.1007/s10342-005-0085-3

DWD (1991) Anleitung für Die Phänologischen Beobachter des Deutschen Wetterdienstes: DeutscherWetterdienst: Offenbach am Main, Germany

DWD (2022) Deutscher Wetterdienst/German Meteorological Service. https://www.dwd.de/DE/Home/home_node.html. Accessed 12 February 2022

Emberlin J, Detandt M, Gehrig R, Jaeger S, Nolard N, Rantio-Lehtimäki A (2002) Responses in the start of Betula (birch) pollen seasons to recent changes in spring temperatures across Europe. Int J Biometeorol 46:159–170. https://doi.org/10.1007/s00484-002-0139-x

Engler R, Randin CF, Thuiller W, Dullinger S, Zimmermann NE, Araújo MB, Pearman PB, Le LAYG, Piedallu C, Albert CH, Choler P, Coldea G, de Lamo X, Dirnböck T, Gégout J-C, Gómez-García D, Grytnes J-A, Heegaard E, Høistad F, Nogués-Bravo D, Normand S, Puşcaş M, Sebastià M-T, Stanisci A, Theurillat J-P, Trivedi MR, Vittoz P, Guisan A (2011) 21st century climate change threatens mountain flora unequally across Europe. Glob Change Biol 17:2330–2341. https://doi.org/10.1111/j.1365-2486.2010.02393.x

Erdtman G (1954) An introduction to pollen analysis., 2nd edn. Chronica Botanica, Waltham Mass

Estrella N, Menzel A (2006) Responses of leaf colouring in four deciduous tree species to climate and weather in Germany. Climate Res 32:253–267. https://doi.org/10.3354/cr032253

Faegri K, Kaland PE, Krzywinski K (1989) Textbook of pollen analysis. Wiley

Fernández-González M, González-Fernández E, Ribeiro H, Abreu I, Rodríguez-Rajo FJ (2020) Pollen production of quercus in the North-Western Iberian Peninsula and airborne pollen concentration trends during the Last 27 Years. Forests 11:702. https://doi.org/10.3390/f11060702

Fotiou C, Damialis A, Krigas N, Halley JM, Vokou D (2011) Parietaria judaica flowering phenology, pollen production, viability and atmospheric circulation, and expansive ability in the urban environment: impacts of environmental factors. Int J Biometeorol 55:35–50. https://doi.org/10.1007/s00484-010-0307-3

Gehrig R, Peeters AG (2000) Pollen distribution at elevations above 1000 m inSwitzerland. Aerobiologia 16:69–74. https://doi.org/10.1023/A:1007607121614

Gilles S, Meinzer M, Landgraf M, Kolek F, von Bargen S, Pack K, Charalampopoulos A, Ranpal S, Luschkova D, Traidl-Hoffmann C, Jochner-Oette S, Damialis A, Büttner C (2023) Betula pendula trees infected by birch idaeovirus and cherry leaf roll virus: impacts of urbanisation and NO2 levels. Environ Pollut 327:121526. https://doi.org/10.1016/j.envpol.2023.121526

González-Fernández E, Piña-Rey A, Fernández-González M, Aira MJ, Rodríguez-Rajo FJ (2020) Prediction of grapevine yield based on reproductive variables and the influence of meteorological conditions. Agronomy 10:714. https://doi.org/10.3390/agronomy10050714

Hasegawa TM, Itagaki T, Sakai S (2022) Intraspecific variation in morphology of spiny pollen grains along an altitudinal gradient in an insect-pollinated shrub. Plant Biol. https://doi.org/10.1111/plb.13493

Helbig N, Vogel B, Vogel H, Fiedler F (2004) Numerical modelling of pollen dispersion on the regional scale. Aerobiologia 20:3–19. https://doi.org/10.1023/B:AERO.0000022984.51588.30

Hidalgo PJ, Galán C, Domínguez E (1999) Pollen production of the genus Cupressus. Grana 38:296–300. https://doi.org/10.1080/001731300750044519

Holm SO (1994) Reproductive variability and pollen limitation in three Betula taxa in northern Sweden. Ecography 17:73–81. https://doi.org/10.1111/j.1600-0587.1994.tb00078.x

Isagi Y, Sugimura K, Sumida A, Ito H (1997) How does masting happen and synchronize? J Theor Biol 187:231–239. https://doi.org/10.1006/jtbi.1997.0442

Jato FV, Rodriguez-Rajo F.J., Aira M.J. (2007) Use of phenological and pollen-production data for interpreting atmospheric birch pollen curves. Annals of Agricultural and Environmental Medicine 14

Jochner SC, Beck I, Behrendt H, Traidl-Hoffmann C, Menzel A (2011) Effects of extreme spring temperatures on urban phenology and pollen production: a case study in Munich and Ingolstadt. Clim Res 49:101–112. https://doi.org/10.3354/cr01022

Jochner S, Ziello C, Böck A, Estrella N, Buters J, Weichenmeier I, Behrendt H, Menzel A (2012) Spatio-temporal investigation of flowering dates and pollen counts in the topographically complex Zugspitze area on the German-Austrian border. Aerobiologia 28:541–556. https://doi.org/10.1007/s10453-012-9255-1

Jochner S, Caffarra A, Menzel A (2013a) Can spatial data substitute temporal data in phenological modelling? A survey using birch flowering. Tree Physiol 33:1256–1268. https://doi.org/10.1093/treephys/tpt079

Jochner S, Höfler J, Beck I, Göttlein A, Ankerst DP, Traidl-Hoffmann C, Menzel A (2013b) Nutrient status: a missing factor in phenological and pollen research? J Exp Bot 64:2081–2092. https://doi.org/10.1093/jxb/ert061

Kadaikar A, Guinot B, Trocan M, Amiel F, Conde-Cespedes P, Oliver G, Thibaudon M, Sarda-Estève R, Baisnée D (2019) Automatic pollen grains counter. In: 2019 3rd International Conference on Bio-engineering for Smart Technologies (BioSMART), 1–4

Kakui H, Tsurisaki E, Sassa H, Moriguchi Y (2020) An improved pollen number counting method using a cell counter and mesh columns. Plant Methods 16:124. https://doi.org/10.1186/s13007-020-00668-4

Katz DSW, Morris JR, Batterman SA (2020) Pollen production for 13 urban North American tree species: allometric equations for tree trunk diameter and crown area. Aerobiologia. https://doi.org/10.1007/s10453-020-09638-8

Khanduri VP, Sharma CM (2009) Cyclic pollen production in Cedrus deodara. Sex Plant Reprod 22:53–61. https://doi.org/10.1007/s00497-008-0091-y

Khanduri VP, Sharma CM, Singh SP (2008) The effects of climate change on plant phenology. Environmentalist 28:143–147. https://doi.org/10.1007/s10669-007-9153-1

Kirchner M, Faus-Kessler T, Jakobi G, Leuchner M, Ries L, Scheel H-E, Suppan P (2013) Altitudinal temperature lapse rates in an Alpine valley: trends and the influence of season and weather patterns. Int J Climatol 33:539–555. https://doi.org/10.1002/joc.3444

Körner C (2007) The use of 'altitude' in ecological research. Trends Ecol Evol 22:569–574. https://doi.org/10.1016/j.tree.2007.09.006

Ladeau SL, Clark JS (2006) Pollen production by Pinus taeda growing in elevated atmospheric CO2. Funct Ecol 20:541–547

LaMontagne JM, Boutin S (2009) Quantitative methods for defining mast-seeding years across species and studies. J Veg Sci 20:745–753. https://doi.org/10.1111/j.1654-1103.2009.01068.x

Latałowa M, Miętus M, Uruska A (2002) Seasonal variations in the atmospheric Betula pollen count in Gdańsk (southern Baltic coast) in relation to meteorological parameters. Aerobiologia 18:33–43. https://doi.org/10.1023/A:1014905611834

Markgraf V (1980) Pollen dispersal in a mountain area. Grana 19:127–146. https://doi.org/10.1080/00173138009424995

Masaki T, Abe S, Naoe S, Koike S, Nakajima A, Nemoto Y, Yamazaki K (2020) Horizontal and elevational patterns of masting across multiple species in a steep montane landscape from the perspective of forest mammal management. J for Res 25:92–100. https://doi.org/10.1080/13416979.2020.1744231

Mellert KH, Ewald J, Hornstein D, Dorado-Liñán I, Jantsch M, Taeger S, Zang C, Menzel A, Kölling C (2016) Climatic marginality: a new metric for the susceptibility of tree species to warming exemplified by Fagus sylvatica (L.) and Ellenberg's quotient. Eur J Forest Res 135:137–152. https://doi.org/10.1007/s10342-015-0924-9

Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kübler K, Bissolli P, Braslavska O, Briede A, Chmielewski FM, Crepinsek Z, Curnel Y, Dahl Å, Defila C, Donnelly A, Filella Y, Jatczak K, Måge F, Mestre A, Nordli Ø, Peñuelas J, Pirinen P, Remišová V, Scheifinger H, Striz M, Susnik A, van Vliet AJ, Wielgolaski F-E, Zach S, Zust A (2006) European phenological response to climate change matches the warming pattern. Glob Change Biol 12:1969–1976. https://doi.org/10.1111/j.1365-2486.2006.01193.x

Menzel A, Yuan Y, Matiu M, Sparks T, Scheifinger H, Gehrig R, Estrella N (2020) Climate change fingerprints in recent European plant phenology. Glob Change Biol 26:2599–2612. https://doi.org/10.1111/gcb.15000

Moe D (1998) Pollen production of Alnus incana at its south Norwegian altitudinal ecotone. Grana 37:35–39. https://doi.org/10.1080/00173139809362637

Molina RT, Rodríguez AM, Palaciso IS, FsG L (1996) Pollen production in anemophilous trees. Grana 35:38–46. https://doi.org/10.1080/00173139609430499

Moore PD, Webb JA, Collinson ME (1991) Pollen analysis, 2nd edn. Blackwell Science, Oxford, Malden MA

Pearse IS, Koenig WD, Kelly D (2016) Mechanisms of mast seeding: resources, weather, cues, and selection. New Phytol 212:546–562. https://doi.org/10.1111/nph.14114

Perala DA, Alm AA (1990) Reproductive ecology of birch: a review. For Ecol Manage 32:1–38. https://doi.org/10.1016/0378-1127(90)90104-J

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

Ranpal S, Sieverts M, Wörl V, Kahlenberg G, Gilles S, Landgraf M, Köpke K, Kolek F, Luschkova D, Heckmann T, Traidl-Hoffmann C, Büttner C, Damialis A, Jochner-Oette S (2022) Is pollen production of birch controlled by genetics and local conditions? Int J Environ Res Public Health 19:8160. https://doi.org/10.3390/ijerph19138160

Richardson SJ, Allen RB, Whitehead D, Carswell FE, Ruscoe WA, Platt KH (2005) Climate and net carbon availability determine temporal patterns of seed production by Nothofagus. Ecology 86:972–981. https://doi.org/10.1890/04-0863

Rojo J, Salido P, Pérez-Badia R (2015) Flower and pollen production in the 'Cornicabra' olive (Olea europaea L.) cultivar and the influence of environmental factors. Trees 29:1235–1245. https://doi.org/10.1007/s00468-015-1203-6

Rojo J, Oteros J, Picornell A, Maya-Manzano JM, Damialis A, Zink K, Werchan M, Werchan B, Smith M, Menzel A, Timpf S, Traidl-Hoffmann C, Bergmann K-C, Schmidt-Weber CB, Buters J (2021) Effects of future climate change on birch abundance and their pollen load. Glob Change Biol 27:5934–5949. https://doi.org/10.1111/gcb.15824

Satake A, Iwasa Y (2002) Spatially limited pollen exchange and a long-range synchronization of trees. Ecology 83:993–1005. https://doi.org/10.1890/0012-9658(2002)083[0993:SLPEAA]2.0.CO;2

Scherrer D, Schmid S, Körner C (2011) Elevational species shifts in a warmer climate are overestimated when based on weather station data. Int J Biometeorol 55:645–654. https://doi.org/10.1007/s00484-010-0364-7

Shivanna KR, Rangaswamy NS (1992) Pollen biology: a laboratory manual, 1st edn. Springer, Berlin / Heidelberg, Berlin, Heidelberg

Sofiev M, Vira J, Kouznetsov R, Prank M, Soares J, Genikhovich E (2015) Construction of the SILAM Eulerian atmospheric dispersion model based on the advection algorithm of Michael Galperin. Geoscientific Model Development 8:3497–3522. https://doi.org/10.5194/gmd-8-3497-2015

Subba Reddi C, Reddi NS (1986) Pollen production in some anemophilous angiosperms. Grana 25:55–61. https://doi.org/10.1080/00173138609429933

Sundqvist MK, Sanders NJ, Wardle DA (2013) Community and ecosystem responses to elevational gradients: processes, mechanisms, and insights for global change. Annu Rev Ecol Evol Syst 44:261–280. https://doi.org/10.1146/annurev-ecolsys-110512-135750

Tito R, Vasconcelos HL, Feeley KJ (2020) Mountain ecosystems as natural laboratories for climate change experiments. Front For Glob Change 0:38. https://doi.org/10.3389/ffgc.2020.00038

Verstraeten WW, Dujardin S, Hoebeke L, Bruffaerts N, Kouznetsov R, Dendoncker N, Hamdi R, Linard C, Hendrickx M, Sofiev M, Delcloo AW (2019) Spatio-temporal monitoring and modelling of birch pollen levels in Belgium. Aerobiologia 35:703–717. https://doi.org/10.1007/s10453-019-09607-w

Wielgolaski FE, Hofgaard A, Holtmeier FK (2017) Sensitivity to environmental change of the treeline ecotone and its associated biodiversity in European mountains. Climate Res 73:151–166

Wörl V, Jetschni J, Jochner-Oette S (2022) Birch pollen deposition and transport along an altitudinal gradient in the Bavarian Alps—a case study using gravimetric pollen traps in the pollen season 2020. Atmosphere 13:2007. https://doi.org/10.3390/atmos13122007

Zhao F, Heller W, Stich S, Durner J, Winkler JB, Traidl-Hoffmann C, Ernst D, Frank U (2017) Effects of NO2 on inflorescence length, pollen/seed amount and phenolic metabolites of common ragweed (ambrosia artemisiifolia L.)

Ziello C, Estrella N, Kostova M, Koch E, Menzel A (2009) Influence of altitude on phenology of selected plant species in the Alpine region (1971–2000). Climate Res 39:227–234. https://doi.org/10.3354/cr00822

Ziska LH, Gebhard DE, Frenz DA, Faulkner S, Singer BD, Straka JG (2003) Cities as harbingers of climate change: common ragweed, urbanization, and public health. J Allergy Clin Immunol 111:290–295. https://doi.org/10.1067/mai.2003.53

6   Continental-scale Evaluation of Downy Birch Pollen Production: Estimating the Impacts of Global Change

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Continental-scale evaluation of downy birch pollen production: Estimating the impacts of global change

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ABSTRACT

The high prevalence of hay fever in Europe has raised concerns about the implications of climate change-induced higher temperatures on pollen production. Our study focuses on downy birch pollen production across Europe by analyzing 456 catkins during 2019–2021 in 37 International Phenological Gardens (IPG) spanning a large geographic gradient. As IPGs rely on genetically identical plants, we were able to reduce the effects of genetic variability. We studied the potential association with masting behavior and three model specifications based on mean and quantile regression to assess the impact of meteorology (e.g., temperature and precipitation) and atmospheric gases (e.g., ozone (O_3) and carbon-dioxide (CO_2)) on pollen and catkin production, while controlling for tree age approximated by stem circumference. The results revealed a substantial geographic variability in mean pollen production, ranging from 1.9 to 2.5 million pollen grains per catkin. Regression analyses indicated that elevated average temperatures of the previous summer corresponded to increased pollen production, while higher O_3 levels led to a reduction. Additionally, catkins number was positively influenced by preceding summer's temperature and precipitation but negatively by O_3 levels. The investigation of quantile effects revealed that the impacts of mean temperature and O_3 levels from the previous summer varied throughout the conditional response distribution. We found that temperature predominantly affected trees characterized by a high pollen production. We therefore suggest that birches modulate their physiological processes to optimize pollen production under varying temperature regimes. In turn, O_3 levels negatively affected trees with pollen production levels exceeding the conditional median. We conclude that future temperature increase might exacerbate pollen production while other factors may modify (decrease in the case of O_3 and amplify for precipitation) this effect. Our comprehensive study sheds light on potential impacts of climate change on downy birch pollen production, which is crucial for birch reproduction and human health.

1. Introduction

The World Allergy Organisation (WAO) estimates that up to 40% of the world's population is sensitized to at least one allergen, while between 10 and 30% suffers from an allergy (Pawankar et al., 2013). Particularly in Europe, the prevalence of hay fever has increased substantially in the past (D'Amato et al., 2020) and may continue to rise with increasing levels of pollen production (Beggs, 2004; Damialis et al., 2019). In addition, future pollen loads, such as those from birch, are expected to change regionally in response to altered plant distribution driven by changing growing conditions under climate change (Rojo et al., 2021). Nowadays, the prevalence of allergic sensitization in Germany to the major allergen (Bet v 1) of birch (*Betula* spec.) is 18% (Beutner et al., 2021; Li et al., 2022). Examining potential environmental factors influencing pollen production is essential to estimate forthcoming changes. Therefore, plant individuals sharing identical genetic backgrounds can reveal environmental impacts, which are otherwise confounded by high inter-individual genetic variability (Ranpal et al., 2022).

The network of the International Phenological Gardens (IPG) includes a set of 23 plant species at 63 active IPGs across 19 European countries (Renner and Chmielewski, 2021). The peculiarity of this network is that every individual of a plant species was propagated vegetatively from a single mother tree, thereby eliminating genetic variability. The vast geographic extent of the network has been used to document large-scale temperature effects on plant phenology (Caffarra and Donnelly, 2011; Olsson et al., 2017; Linkosalo et al., 2019; Wenden et al., 2020) or to study the effects of biogenic volatile organic compound (BVOCs) emission patterns along the latitudinal gradient of these gardens (van Meeningen et al., 2016). The latitudinal and longitudinal gradients, which are covered by the IPG sites, present a valuable basis for investigating the impact of climate on plants through natural experiments (Frenne et al., 2013). Research on pollen production of allergenic plants, however, has not been applied so far in this unique network.

Birch is a wind-pollinated species that produces large amounts of pollen (Piotrowska, 2008; Geburek et al., 2012; Ranpal et al., 2022). Its pollen are, together with those of grass (Poaceae) species, the most common cause for allergic symptoms such as rhinitis and asthma (D'Amato et al., 1998; Biedermann et al., 2019). *Betula pubescens* Ehrh. (downy birch), frequently planted in the IPG network, is a tree species with a broad natural distribution across Europe, characterized by one of the most northerly and easterly distributions among European tree species (Beck et al., 2016). It can survive on compact soils and wet peatlands (Hynynen et al., 2010) and can be found at higher elevations and in European boreal forests (Beck et al., 2016). In addition, several varieties of birch are frequently cultivated as ornamental trees in temperate cities (Rojo et al., 2021).

Studies on birch flowering usually show a large year-to-year variation in the amount of male and female catkins and the produced pollen and seeds, respectively (Ranta et al., 2008), which is known as masting behaviour (Kelly, 1994). Aerobiological studies examining the amount of atmospheric birch pollen concentrations suggest that such masting events can occur every second (Latałowa et al., 2002) or third year (Detandt and Nolard, 2000). There is still only a limited understanding of the individual-specific pollen production of birch, its spatiotemporal patterns, and its response to environmental stressors. Previous studies have primarily focused on estimating birch pollen production within relatively small geographic areas (Jato et al., 2007; Ranpal et al., 2022) or along environmental gradients in urban (Jochner et al., 2013; Kolek, 2021; Jetschni et al., 2023) or mountainous regions (Ranpal et al., 2023). In general, especially higher temperatures and CO_2 concentrations were linked to higher levels of pollen production in various plant species (Ziska and Caulfield, 2000; Wayne et al., 2002; Albertine et al., 2014). Positive correlations were documented for previous summer's temperatures along an altitudinal gradient in non-masting years (Ranpal

et al., 2023). However, in two urban-rural gradient studies, birch pollen production was found to be negatively correlated to temperature (Jochner et al., 2013; Jetschni et al., 2023) as well as to foliar potassium and iron concentration and atmospheric NO_2 concentration (Jochner et al., 2013). In addition, it has to be considered that plant viral infections have an impact on the quantity of the birch pollen production, namely a significantly lower amount of pollen was found in catkins with virus infected pollen (Gilles et al., 2023). These findings point to the fact that the relationship between pollen production and environmental factors is likely to be multifaceted, whereas not only a single stressor exerts an influence on pollen production. Different levels of urbanisation and air pollution may change the plant responses non-linearly, i.e., between central Europe (Germany) and the Mediterranean (Greece) (Damialis et al., 2011). We had previously reported considerable differences in pollen production among same-aged birch trees growing under similar microclimatic conditions in a small geographic area (Ranpal et al., 2022). While it is key to understand the influence of environmental factors for ascertaining the possible influence of climate change, only large-scale studies can fully capture the potential variability in birch pollen production in diverse environments. Detailed information on the factors affecting pollen production can help in developing models for pollen production prediction and in devising strategies to mitigate the adverse effects of allergenic pollen on human health.

In this study, we considered the effects of various environmental variables on pollen production of birch trees (2019–2021) planted in IPGs across Europe. Our aim was to obtain a more comprehensive understanding of the determinants of birch pollen production. Therefore, we carried out a detailed analysis of pollen production as a function of local environmental variables and accounted for the potential heterogeneity of the effects of the environmental variables across the conditional pollen production distribution.

2. Materials and methods

2.1. Study sites

This study was carried out during three consecutive years (2019–2021) at 37 IPGs located in eleven European countries (see Appendix Table 1). Catkin samples were obtained from up to 44 birch trees per year as some of the gardens had between one and three birch individuals.

The IPGs were located along a latitudinal gradient spanning over 2500 km, from Skopje, North Macedonia, in the Balkan region, to Trondheim, in northern Norway, and along a longitudinal gradient covering approximately 2000 km, from Glenveagh, Ireland, to Šiauliai, Lithuania (Fig. 1, Appendix Table 1). The largest distance between two

Table 1

Considered models (M) employing environmental variables of the previous summer: mean temperature (Tavg$_{t-1}$), precipitation sum (PPsum$_{t-1}$), ozone (O$_{3,t-1}$) concentration, carbon dioxide (CO$_{2,t-1}$) concentration and stem circumference (S$_{cir}$).

Models	Description	Independent variables
M1	Meteorology of previous summer	Tavg$_{t-1}$ and PPsum$_{t-1}$
M2	Meteorology and atmospheric gases of previous summer	Tavg$_{t-1}$, PPsum$_{t-1}$, O$_{3,t-1}$ and CO$_{2,t-1}$
M3	Meteorology, atmospheric gases of previous summer and stem circumference	Tavg$_{t-1}$, PPsum$_{t-1}$, O$_{3,t-1}$, CO$_{2,t-1}$ and S$_{cir}$

The explanatory variables considered in the models included mean air temperatures ranging from 13.8 °C (Trondheim, Norway) to 22.8 °C (Skopje, North Macedonia), total precipitation ranging from 69 mm (London, UK) to 462 mm (Bergen, Norway), ozone concentration levels ranging from 51.0 μg m^{-3} (Trondheim, Norway) to 90.8 μg m^{-3} (Freyung Waldhäuser, Germany), and CO_2 concentration levels ranging from 405.05 ppm (Šiauliai, Lithuania) to 413.04 ppm (Waldfeucht, Germany).
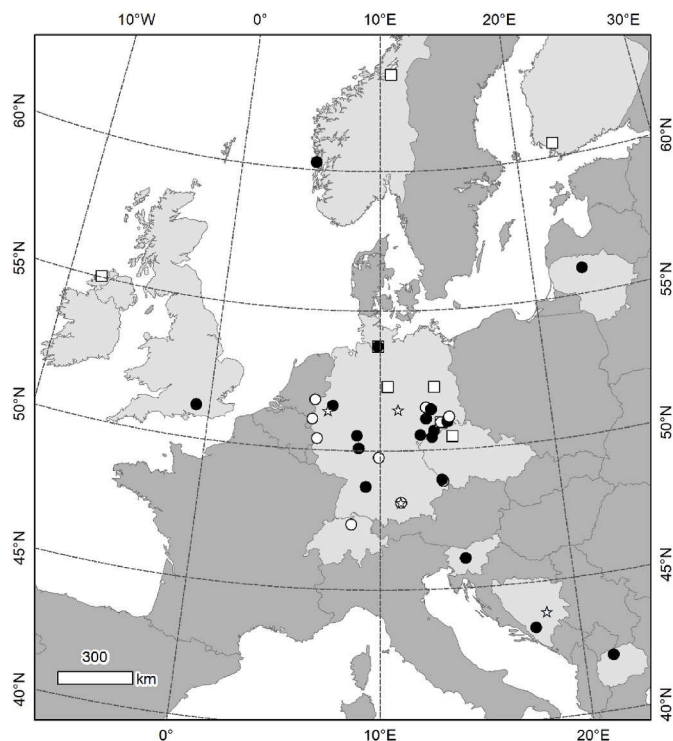
Fig. 1. Location of International Phenological Gardens across European countries. Circles are the IPG trees identified as clone 1, squares are identified as clone 2, stars are identified as clone 3 and black points represent other individuals with unique genotypes. Some circles/squares are overlapped. Light grey countries are included in this study.

IPGs was 2600 km (Skopje, North Macedonia to Glenveagh, Ireland). The shortest distance was 7.2 km (Freyung-Schönbrunn and Freyung-Waldhäuser, Germany).

Almost all selected IPG birches are within the natural distribution range of *B. pubescens*, as mapped by the European Commission Joint Research Centre (Caudullo et al., 2017), except trees from Bosnia and Herzegovina (Mostar and Sarajevo) and North Macedonia (Skopje). The trees in Bosnia and Herzegovina are the youngest, as they were planted in 2013 and 2014, respectively. The oldest trees are from Stuttgart (Germany), Tharandt-Hartha (Germany), and Ljubljana (Slovenia), which were planted in 1960.

2.2. Assessment of genetic identity

The IPG network was initially designed to only include individuals sharing the same genetic background (Schnelle and Volkert, 1957). However, this could not be maintained for all individuals of one plant since propagation methods and sites (mother gardens) changed in the past (Renner and Chmielewski, 2021). It is evident that propagation by cuttings is more favourable compared to grafting since the latter method is prone for a failed propagation, for example when the stock, which derives from another plant, gets dominant over the scion of the plant that should be propagated (Renner and Chmielewski, 2021). We used cambium samples from the twigs of which the inflorescences were obtained to determine the genetic identity of the trees. Genotyping was performed by the Bavarian Office for Forest Genetics (AWG; Teisendorf, Germany) at eight microsatellite loci (four alleles per locus). The analysis revealed two major clones (clone 1 as circles and clone 2 as squares in Fig. 1) with ten individuals in each clone group. Other clones with unique genotypes had up to four individuals, whereas 14 birch trees were not associated to a replicate genotype. This can be probably attributed to a failure of the grafting method or to the fact that the sampled birches were mistakenly assigned to the IPG network.

2.3. Pollen extraction method and pollen production metrics

To obtain catkin samples from the birches located at the IPGs, we instructed the local phenological observers of the respective gardens. We maintained regular communication with each IPG, provided them with the sampling material that included a detailed manual (https://www.ku.de/fileadmin/150303/Forschung/PollenPALS/Instruction_manual_pollenPALS_5th_Edition.pdf) to ensure a standardization in the catkin collection process. Male catkins with mature and closed anthers were collected each year in spring, after catkin elongation was commenced and before anthesis. Catkins were collected from different branches at attainable heights (1.5–2 m a.g.l.) in all four cardinal directions. The number of catkins inside a 50 cm × 50 cm × 50 cm sample cuboid in the crown were counted and utilized for extrapolating pollen production from catkins to the crown volume. Once the samples were received via express shipping, they were immediately stored at −20 °C until they were processed in the laboratory.

Extraction of downy birch pollen grains from closed inflorescences was performed following the method of Damialis et al. (2011), with the modification described in Ranpal et al. (2022): one medium-sized inflorescence from each cardinal direction and per tree was selected, and the length and width (at the broadest point) were measured. The number of flowers of each catkin was counted, and the catkin was then immersed in a 10% KOH solution overnight. On the following day, the solutions were boiled at 120 °C for 10 min, and the soft catkin was mashed with a glass rod to extract the pollen. To prevent pollen from clustering, a bipolar solvent, glycerol (70%), was added to a volume of 20 mL, and safranin was applied as a stain. The mixture was homogenized by continuous stirring, and two aliquots (10 μL each) of each suspension were taken using a VITLAB® micropipette. The samples were placed on microscope slides, covered with slips, and counted using a 100× magnification (Zeiss AXIO Lab.A1, Germany). The extraction method was repeated on another catkin of the same tree when there was a substantial difference between the pollen counts obtained from these two slides (>30%).

2.4. Meteorological data

The meteorological data used in this study were obtained from the E-OBS dataset (version 23.1e) from the EU-FP6 project UERRA (http://www.uerra.eu), the Copernicus Climate Change Service, and the data providers in the ECA&D project (https://www.ecad.eu) as documented by Cornes et al. (2018). These data are based on daily gridded values of meteorological variables, including mean, minimum, and maximum temperatures, relative humidity, precipitation, and global solar radiation. The dataset is based on a high-resolution 0.1° regular grid, which ensures accurate and detailed representation of meteorological variables. As catkins of the subsequent year develop during the preceding summer (Dahl and Strandhede, 1996), we considered the meteorological data of the previous summer. We aggregated (averaged or summed up in case of precipitation) the daily data for the period June to August. Relative humidity and sum of global radiation were excluded for further analyses due to their high correlations (r > 0.6) with temperature.

2.5. O_3, NO_2 and CO_2 data

Average data for ozone (O_3 at surface in μg m^{-3}), nitrogen dioxide (NO_2 at surface in μg m^{-3}) and atmospheric carbon dioxide (CO_2 column mean molar fraction in ppm) concentrations were derived from Copernicus Atmosphere Monitoring Service (CAMS) reanalysis. For O_3 and NO_2, monthly files containing 1-hourly analyses with a horizontal resolution of 0.1° × 0.1° were obtained. In case of CO_2, the available monthly data of 0.75° regular grid was used. We calculated mean values of the previous summer from the obtained data. NO_2 was excluded as it showed a high correlation (r > 0.6) with temperature.

2.6. Stem circumference

We included stem circumference as a proxy for the age of the tree. We inserted this variable since it was most accurately measured compared to alternative information such as tree height or planting year. For the former, a photograph with a measuring rod (as reference) placed in front of the trunk was used for deriving estimates; for the latter, planting year (which is known) does not equate to tree age (which is not known).

2.7. Data processing and statistical analyses

2.7.1. Data processing

Although we found that the selected trees also derive from different genotypes, we decided not to discard any of the trees, but instead to modify our statistical analyses to account for heterogenous effects, which may also be driven by factors such as genetics. We investigated 93 samples, as we had to remove 21 samples from the dataset due to diseased trees and a very low pollen production. To estimate pollen production, we used the mathematical method described by Damialis et al. (2011) and calculated it for different scales. In brief, we calculated the number of pollen grains per catkin (P_{ca}) by multiplying the number of pollen grains observed on a microscope slide by the ratio of the suspension volume (in mL) to the sample volume taken (10 μL). Next, we determined the number of pollen grains per flower (P_{fl}) by dividing P_{ca} by the number of flowers per catkin (Fl). Finally, to estimate the number of pollen grains per volume unit (m^3) of crown (P_{vuc}), we multiplied P_{ca} by the ratio of the number of catkins per crown sampling unit (C_{su}) and the volume of the sampling unit (0.125 m^3).

We also analyzed the temporal pattern of pollen and catkin production based on a subset of the 93 samples. To account for potential masting effects, we only considered the trees for which pollen production metrics were available for all study years. This was the case for 20 trees (resulting in 60 samples), and we assigned those trees to three different groups: i.e., group 1 with maximum values of pollen or catkin production in 2019, group 2 with maximum values in 2020, and group 3 with maximum values in 2021.

2.7.2. Mean regression

We investigated the effect of the environmental variables on pollen production based on three different model specifications of the form

$$Y = X_1 \beta_1 + \ldots + X_P \beta_P + U. \tag{1}$$

In (1), Y denotes the pollen production metric, $\mathbf{X} = (X_1, \ldots, X_P)$ are the environmental variables and the stem circumference with corresponding coefficients $\beta_1, \ldots, \beta_P$, and U is an error term. We modeled the conditional expectation:

$$E(Y|\mathbf{X}) = X_1 \beta_1 + \ldots + X_P \beta_P, \tag{2}$$

and assumed $E(U|\mathbf{X}) = 0$. This requires that the systematic effect of the environmental variables on pollen production is captured by the right-hand-side of (2). Parameter estimates were obtained by least squares, where the residual sum of squares is minimized.

The employed model specifications are summarized in Table 1 and were contrasted in terms of interpretations and model selection criteria. Model M1 includes the meteorological variables mean air temperature ($Tavg_{t-1}$) and sum of total precipitation ($PPsum_{t-1}$) of June, July, and August of the previous year (or previous summer). Model M2 additionally includes mean concentrations of ozone ($O_{3,t-1}$) and carbon dioxide ($CO_{2,t-1}$) of the previous summer. Model M3 is model M2 augmented by the stem circumference (S_{cir}).

2.8. Quantile regression

Due to the reduced but still existent genetic variability and the wide geographic distribution of the analyzed downy birch trees, we also employed quantile regression (for introductions and reviews see Koenker and Hallock, 2001; Cade and Noon, 2003; Koenker, 2017; for an extensive treatment, see Koenker, 2005). Quantile regression is a distribution-free method, where the quantiles of the response distribution are modeled directly (Koenker, 2005). The approach allows the coefficient estimates of the different explanatory variables to vary across the conditional response distribution, which alleviates the problem of model misspecification (Cade and Noon, 2003). Similar to mean regression, a model structure of the form (1) is assumed, but instead of the conditional expectation (2), the conditional quantile

$$Q_{(Y|\mathbf{X})}(\tau) = X_1 \beta_{1\tau} + \ldots + X_P \beta_{P\tau} \tag{3}$$

is modeled. In (3), $Q_{(Y|\mathbf{X})}(\tau)$ denotes the conditional τ-quantile of the response variable Y given the explanatory variables $\mathbf{X}$ and the coefficients of (3) may vary across the different conditional quantiles. Identification of the conditional τ-quantile requires that $Q_{(U|\mathbf{X})}(\tau) = 0$ (Koenker, 2005). In quantile regression, coefficient estimates result from minimizing the absolute sum of errors through linear programming (Portnoy and Koenker, 1997; Koenker and Hallock, 2001). For all plots of the quantile effects, heteroscedasticity robust standard errors proposed by Powell (1991), were used.

All statistical analyses were carried out in R version 4.3.2 (R Core Team, 2022) using the package quantreg (Koenker et al., 2023). The visualization in the paper were created using the packages cowplot (Wilke, 2024), gridExtra (Auguie and Antonov, 2017), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2023), ggspatial (Dunnington et al., 2023) and RColorBrewer (Neuwirth, 2022).

3. Results

3.1. Pollen, flower and catkin production

Pollen production per catkin (P_{ca}) for all selected birch trees in the IPGs across Europe was 1.92 ± 1.29 million pollen grains in 2019, 2.07 ± 1.42 million pollen grains in 2020 and 2.53 ± 1.53 million pollen grains in 2021 (see Table 2). P_{ca} of individual trees varied within a wide range from 227,625 (minimum in 2021) to 7 million pollen grains (maximum in 2019). In 2021, mean P_{ca} was 22% higher compared to 2020 and 32% higher compared to 2019. In addition, the mean pollen production per flower (P_{fl}) and per volume unit of crown (P_{vuc}) in 2021 were higher compared to both 2019 and 2020. Specifically, P_{fl} in 2021 showed a 35% increase compared to 2019 and a 37% increase compared to 2020. Similarly, P_{vuc} in 2021 exhibited a 64% increase against 2019 and an increase by 8% compared to 2020 (Table 2).

For other flower and catkin metrics, their maxima were mostly registered in 2020. The number of flowers per catkin (Fl_{ca}) ranged between 65 and 145 with an average of 113 in 2019, 117 in 2020 and 107 in 2021. The number of catkins in a crown sampling unit (C_{su}; 0.125 m^3) ranged between 5 and 100 with an average of 30 in 2019, 40 in 2020 and 32 in 2021.

Table 2 shows that the null hypothesis of the Kruskal Wallis test was never rejected at a significance level of $\alpha = 0.05$. According to the test results, there were no indications that the samples of reproductive metrics were generated by different distributions across years. For flowers per catkin (Fl_{ca}), it was marginally significant (p = 0.069). Fig. 2 suggests that the underlying distribution was non-normal and that there were potential outliers in the pollen production metrics. These were indicated by the observations exceeding or falling below the whiskers of the boxplots, where the maximum length or the whiskers is 1.5 times the interquartile range (dots in Fig. 2).

3.2. Geographic distribution of pollen production

Fig. 3 displays the geographic distribution of P_{ca} at the selected IPGs

Table 2
Descriptive statistics of pollen, flower and catkin production: Pollen production per flower (P_{fl}), catkin (P_{ca}) and volume unit of crown (P_{vuc}); flower production per catkin (Fl_{ca}) and volume unit of crown (Fl_{vuc}), and catkin production per crown sampling unit (C_{su}; 0.125 m^3) and volume unit of crown (C_{vuc}) for the IPG birch trees across Europe during 2019–2021. Kruskal Wallis test (p-value) indicating that the central tendency of the distribution of the variables is identical across the years 2019–2021.

Reproductive metric	Year	Minimum	Maximum	Mean	Median	Standard deviation	Kruskal Wallis (p-value)
Pollen production							
P_{fl}	2019	3926	58,458	17,602	13,908	11,700	0.123
	2020	3172	43,085	17,333	14,489	10,861	
	2021	1711	47,692	23,715	24,160	13,936	
P_{ca}	2019	413,000	7,029,625	1,918,125	1,597,407	1,286,134	0.229
	2020	290,250	6,118,000	2,074,821	1,828,500	1,420,317	
	2021	227,625	5,580,000	2,526,685	2,271,000	1,530,021	
P_{vuc}	2019	21,648,000	1,730,560,000	495,982,353	431,818,000	441,787,251	0.568
	2020	21,670,000	4,032,800,000	754,305,379	446,964,000	913,352,274	
	2021	13,476,000	4,464,000,000	813,038,640	399,452,000	1,000,533,451	
Flower production							
Fl_{ca}	2019	79	145	113	113	14	0.069
	2020	91	142	117	118	14	
	2021	65	133	107	109	15	
Fl_{vuc}	2019	4512	76,560	28,525	24,240	19,211	0.185
	2020	5080	106,400	38,072	34,080	24,979	
	2021	4464	93,600	28,974	26,600	22,195	
Catkin production							
C_{su}	2019	5	66	30	30	19	0.286
	2020	5	100	40	38	24	
	2021	6	100	32	30	23	
C_{vuc}	2019	40	528	244	240	150	0.286
	2020	40	800	318	304	193	
	2021	48	800	258	240	187	
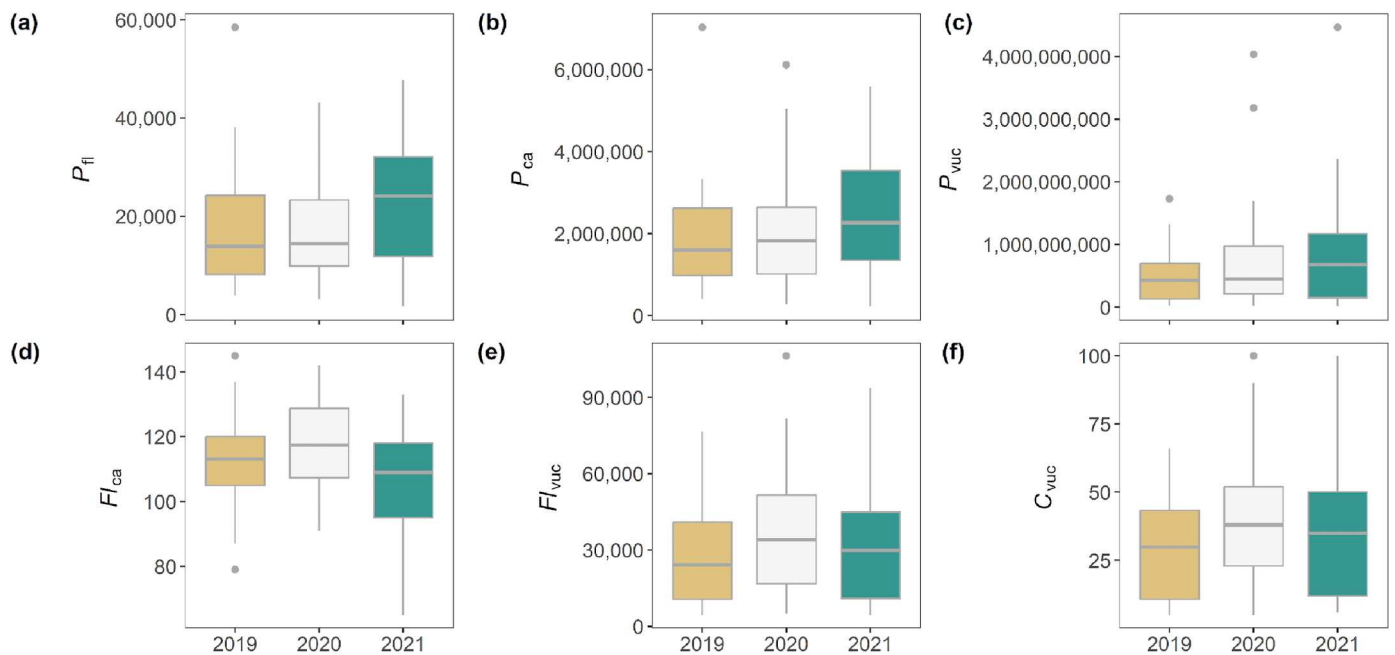
Fig. 2. Boxplots of pollen production per (a) flower (P_{fl}), (b) catkin (P_{ca}) and (c) volume unit of crown (P_{vuc}); flower production per (d) catkin (Fl_{ca}) and (e) volume unit of crown (Fl_{vuc}), and (f) catkin production per volume unit of crown (C_{vuc}) (ordinate) estimated for selected IPG birches across Europe during 2019–2021 (abscissa). Interquartile range (IQR) represented by height of boxes, median by bold horizontal lines, upper (lower) whiskers indicate minimum of maximum (minimum) of metric and 1.5 times IQR, dots represent observations exceeding or falling below 1.5 times IQR.

for the three years. In 2019, the highest P_{ca} was estimated for Waldfeucht (approx. 7 million pollen grains per catkin; marked by the triangle across the three panels). For this IPG tree, the P_{ca} estimates showed a notable decline, with a decrease of 69% in 2020 and 47% in 2021 compared to 2019. As an example, the neighbouring station Kleve (marked by the diamond), located 78 km away from Waldfeucht, was found to have the highest P_{ca} in 2021 (ca. 5 million pollen grains per

catkin). This represents a substantial increase of 160% compared to the P_{ca} value in 2020, and an 83% increase compared to the P_{ca} value observed in 2019 for the same tree. Similarly, a distinct pattern was observed for the two nearest stations (within 7.2 km), Freyung-Schönbrunn and Freyung-Waldhäuser (marked by the overlapped cross signs). Freyung-Schönbrunn revealed its highest P_{ca} (ca. 2 million) in 2021 (0.45 million in 2019 and 0.5 million in 2020), while Freyung-
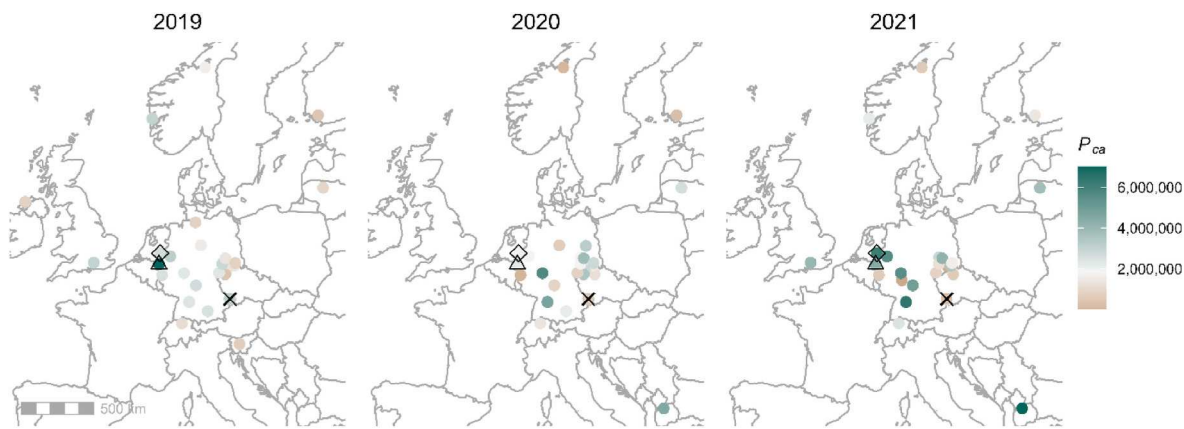
Fig. 3. Geographic distribution of P_{ca} across IPG birches from 2019 to 2022. Color scheme reflects pollen production (brown for low levels, grey for average levels and dark green for high levels). Triangle: Waldfeucht, diamond: Kleve, and crosses (overlapped): Freyung-Schönbrunn and Freyung-Waldhäuser.

Waldhäuser had the highest P_{ca} (ca. 3 million) in 2019 (0.73 million in 2020 and 0.68 million in 2021). This illustrates the high variability in pollen production across both space and time.

3.3. Tree-wise temporal patterns

Fig. 4 shows the temporal pattern of P_{ca} and of C_{su} from 2019 to 2021 for those 20 trees, which were sampled in every study year. Trees with highest P_{ca} values in 2019 (i.e., group 1, N = 6) had on average 68% less pollen in the following year (min = 37%; max = 89%) and 52% less pollen in the second following year (min = 18%; max = 79%). For those trees that had the maximum P_{ca} in 2020 (i.e., group 2, N = 4), we found that P_{ca} was reduced by on average 51% in the preceding year (min = 34%; max = 70%) and 44% in the following year (min = 11%; max = 83%). The birches that were linked to the highest pollen production in

Fig. 4. Pollen production per catkin (P_{ca}) (ordinate of top display) and catkin production per sampling unit (C_{su}) (ordinate of bottom display) in 2019–2021 (abscissa) assessed for the trees sampled in all years at IPG stations and categorized in three groups with similar temporal behavior.

2021 (i.e., group 3, N = 10) had on average 41% less pollen in 2020 and 55% less pollen in 2019.

Regarding C_{su}, trees with the highest C_{su} values in 2019 (group 1, N = 7) were found with an average reduction of 35% in catkins in 2020 (min = 0%; max = 80%) and 2021 (min = 8%; max = 80%). Trees with C_{su} maximum in 2020 (Group 2, N = 10) were estimated with an average decline of 55% in catkins in the preceding year (min = 10%; max = 84%) and a 56% reduction in the following year (ranging from 25% to 90%). Birch trees associated with the highest catkin production in 2021 (group 3, N = 3) displayed an average reduction of 16% in 2020 and 64% in 2019.

3.4. Mean regression

In the following subsection, the results of regressions of the pollen production metrics P_{ca}, C_{su} and P_{vuc} are summarized. Note that all statements made about the effects of the explanatory variables on the response hold on average and ceteris paribus. For P_{ca}, specification M1, which includes only meteorological variables of the previous summer, singled out mean temperature as the most important explanatory variable (p = 0.005). The effect of temperature was found to be positive, suggesting that, P_{ca} increases with increasing temperature. Adding atmospheric gas variables in specification M2 showed that $O_{3,t-1}$ (in addition to $Tavg_{t-1}$) was different from zero (p = 0.013). The effect was found to be negative, which indicates that P_{ca} decreases with increasing levels of ozone. The highest adjusted R^2 (18.2%) resulted for specification M3 when also taking the stem circumference into account.

For C_{su}, the model based solely on meteorological variables (M1) had the lowest adjusted R^2 (6.8%), while the two explanatory variables included in the model, $Tavg_{t-1}$ and $PPsum_{t-1}$, were shown to exert a positive effect on the response (p = 0.029, p = 0.009). Adding atmospheric gas concentration variables (M2) resulted in an improvement in the model fit (adj. R^2 = 11.3%), maintaining qualitatively identical conclusions for the meteorological variables temperature (p = 0.009) and precipitation (p = 0.040). Additionally, a negative effect of $O_{3,t-1}$ (p = 0.02) on C_{su} was observed in the model. This also holds true for specification M3, which adds the further explanatory variable stem circumference. For this model, the adjusted R^2 (10.6%) decreased compared to specification M2.

When considering P_{vuc} as the response, the lowest adjusted R^2 was found when only accounting for meteorological variables (6.7%). R^2 was highest for specification M2, in which meteorological and pollutant concentration variables were included (17.1%). Similar to the results found for C_{su}, specification M3 was associated with a lower adjusted R^2 (16.2%) compared to M2.

In summary, the mean regression results indicate that the preceding summers' mean temperature and O_3 concentration levels are most
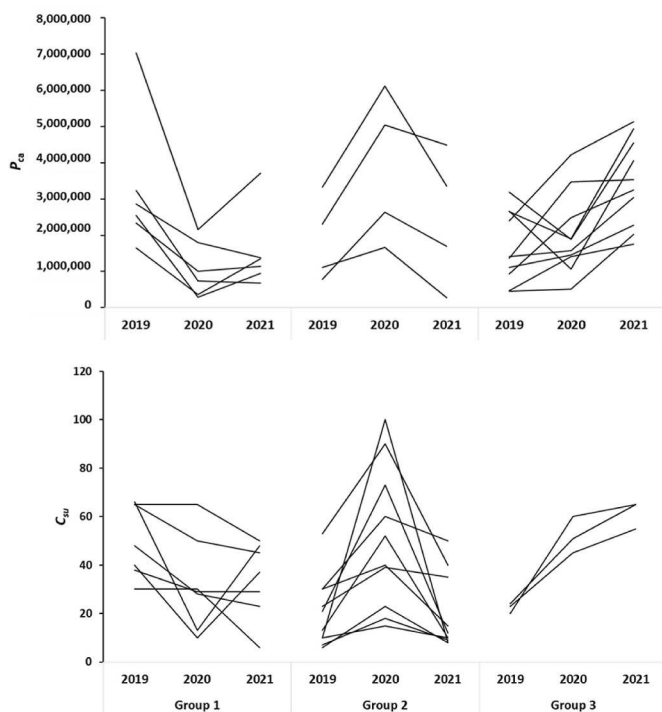
important in the production of pollen per catkin (P_{ca}). Our results suggest that elevated $Tavg_{t-1}$ corresponds to an increase in P_{ca}, while an increase in $O_{3,t-1}$ has the opposite effect. Catkin quantities, on the other hand, are influenced by temperature, precipitation, and O_3 concentration levels of the preceding summer. Higher values in these meteorological variables lead to increased catkin production (C_{su}), while an elevated $O_{3,t-1}$ is associated with decreased C_{su}. Pollen production at the larger scale (at the level of the volume of the crown) is therefore also mostly influenced by temperature and ozone.

Among the considered specifications, M2 has the lowest AIC values (see Table 3) across all three dependent variables. We focus on this model variant in the following and describe the results for P_{ca}.

3.5. Quantile regression

The documented non-normality in the pollen production metrics (see Table 2 and Fig. 2; Shapiro-Wilk tests rejected the null of normality for all three metrics with p-values <0.001), and the presence of genetic variability and potential outliers may render the linear regression results and their interpretations unreliable due to specification bias and/or inconsistent standard error estimates. We therefore chose quantile regression as modeling alternative.

In the following, we describe the quantile regression results for specification M2. Fig. 5 summarizes the results via quantile plots, which show the coefficient estimates across the conditional τ-quantiles of the response distribution separately for each explanatory variable. The plots indicate the coefficient estimates together with the 90%-confidence region, which is illustrated by the shaded area. The considered quantiles range from $\tau = 0.2$ to $\tau = 0.8$. Via the horizontal lines, the plots also include the mean regression results (dotted line) and the corresponding 90% confidence region (dashed lines) for reference.

Fig. 5 illustrates the presence of mild quantile effects across the conditional response distribution. For $Tavg_{t-1}$, for example, the effect of the variable on the response – when conditioning for all other explanatory variables – increases across τ for all considered quantiles. The effect is indistinguishable from zero for $\tau = 0.2$ at a significance level of $\alpha = 0.1$ and positive for $\tau \in (0.3, 0.8)$. This implies that the mean temperature of the previous summer exerts a positive effect on pollen production for all trees, except the ones with the lowest pollen production levels. For $O_{3,t-1}$, similar statements hold, but here, the effects of the variable on the response are zero for $\tau < 0.5$ and negative for all other values of τ. This suggests that the effects of $O_{3,t-1}$ on pollen production is negative for the trees with a pollen production equal to or higher than the median level. For $PPsum_{t-1}$ and $CO_{2,t-1}$, the effects are indistinguishable from zero for all quantiles. The centercept can be considered as a prediction of the τ-quantile of pollen production for the IPG trees with the explanatory variables set to their respective medians (see Appendix Table 2 which summarizes the descriptive statistics for the meteorological variables). Note that when considering the mean regression effects only, similar conclusions result for variables $PPsum_{t-1}$

and $CO_{2,t-1}$. For $Tavg_{t-1}$ and $O_{3,t-1}$, however, the (global) positive and negative effects indicated by the mean regression results may be an oversimplification.

For C_{su}, the effects of $Tavg_{t-1}$ are positive for $\tau > 0.7$. The effect of $O_{3,t-1}$ is negative for $\tau > 0.5$, while for $PPsum_{t-1}$, the effects are indistinguishable from zero for all quantiles (see Appendix Fig. 1). For P_{vuc}, the effects of $Tavg_{t-1}$ are positive for $\tau > 0.7$ and negative for O_3 concentrations when $\tau > 0.5$ (see Appendix Fig. 2).

4. Discussion

4.1. Pollen, flower and catkin production

This study investigated the reproductive metrics of downy birch trees from IPGs across Europe along spatial gradients over a period of three years. We measured the mean pollen production of catkins (P_{ca}) in the range of 1.9 million to 2.5 million. These measures are an important estimation range when representing pollen production of downy birch across its distribution range. However, this estimate was even higher (between 2.5 million to 5 million) when studied along an altitudinal gradient in the European Alps (Ranpal et al., 2023). In the contemporary study years, pollen production estimations of *Betula pendula* (silver birch) with comparable sample sizes and methods were estimated with <1 million (Kolek, 2021), <1.6 million (Jetschni et al., 2023) and up to 1.7 million (Ranpal et al., 2022). Based on these estimates, we suggest that *B. pendula* produce less P_{ca} as compared to *B. pubescens*.

4.2. Year-to-year variation in pollen production

Based on mean values of all selected IPGs across Europe, our analysis revealed the lowest means for P_{ca} in 2019 and the highest in 2021. This year-to-year variation, however, was not significant in all the studied reproductive metrics of pollen, flowers, and catkins. However, substantial differences between single sites across the years get obvious, as discussed for Fig. 3. At a small geographical scale, Ranpal et al. (2022, 2023) also found annual variations in pollen production during the studied years.

Masting can play a substantial role in influencing reproductive metrics as it can mask the influences of environmental stressors, for example, temperature (Ranpal et al., 2023). Masting can either be limited to the population level (Ranta et al., 2005) or can be observed simultaneously in larger regions (Ranta et al., 2008) as well as continents (Bogdziewicz et al., 2021). In our study, maximum values of P_{ca} or C_{su} were not simultaneously observed for all trees in one specific year. For most of the trees (N = 10), maximum P_{ca} values were obtained in 2021; regarding C_{su}, most trees (N = 10) produced the highest catkin numbers in 2020. These findings suggest that the observed year-to-year variations in pollen and catkin production are not solely driven by genetic factors, as each group of trees categorized in Fig. 4 comprises individuals of different genotypes. As the difference in P_{ca} and C_{su} between

Table 3

Mean regression models according to the specifications given in Table 1. Columns detail explanatory variables, residual standard error, degrees of freedom, adjusted R^2, p-value of overall F-test that all coefficients are jointly zero, Akaike information criterion (AIC).

| Model	Explanatory variables (° p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001)	Residual standard error	Degrees of freedom	Adjusted R^2	p-value	AIC
P_{ca}						
M1	$Tavg_{t-1}$**, $PPsum_{t-1}$	1,367,000	90	0.089	0.015	2896.702
M2	$Tavg_{t-1}$***, $PPsum_{t-1}$, $O_{3,t-1}$*, $CO_{2,t-1}$°	1,288,000	88	0.174	0.0003	2887.490
M3	$Tavg_{t-1}$**, $PPsum_{t-1}$, $O_{3,t-1}$*, $CO_{2,t-1}$°, $S_{cir,t}$	1,281,000	87	0.182	0.0004	2887.529
C_{su}						
M1	$Tavg_{t-1}$*, $PPsum_{t-1}$**	22.40	89	0.068	0.016	838.129
M2	$Tavg_{t-1}$**, $PPsum_{t-1}$*, $O_{3,t-1}$*, $CO_{2,t-1}$	21.86	87	0.113	0.006	835.537
M3	$Tavg_{t-1}$**, $PPsum_{t-1}$*, $O_{3,t-1}$*, $CO_{2,t-1}$, $S_{cir,t}$	21.95	86	0.106	0.012	837.201
P_{vuc}						
M1	$Tavg_{t-1}$**, $PPsum_{t-1}$°	766,900,000	89	0.067	0.017	4030.291
M2	$Tavg_{t-1}$***, $PPsum_{t-1}$, $O_{3,t-1}$**, $CO_{2,t-1}$	722,900,000	87	0.171	0.0004	4021.32
M3	$Tavg_{t-1}$***, $PPsum_{t-1}$, $O_{3,t-1}$**, $CO_{2,t-1}$, $S_{cir,t}$	726,700,000	86	0.162	0.001	4023.225
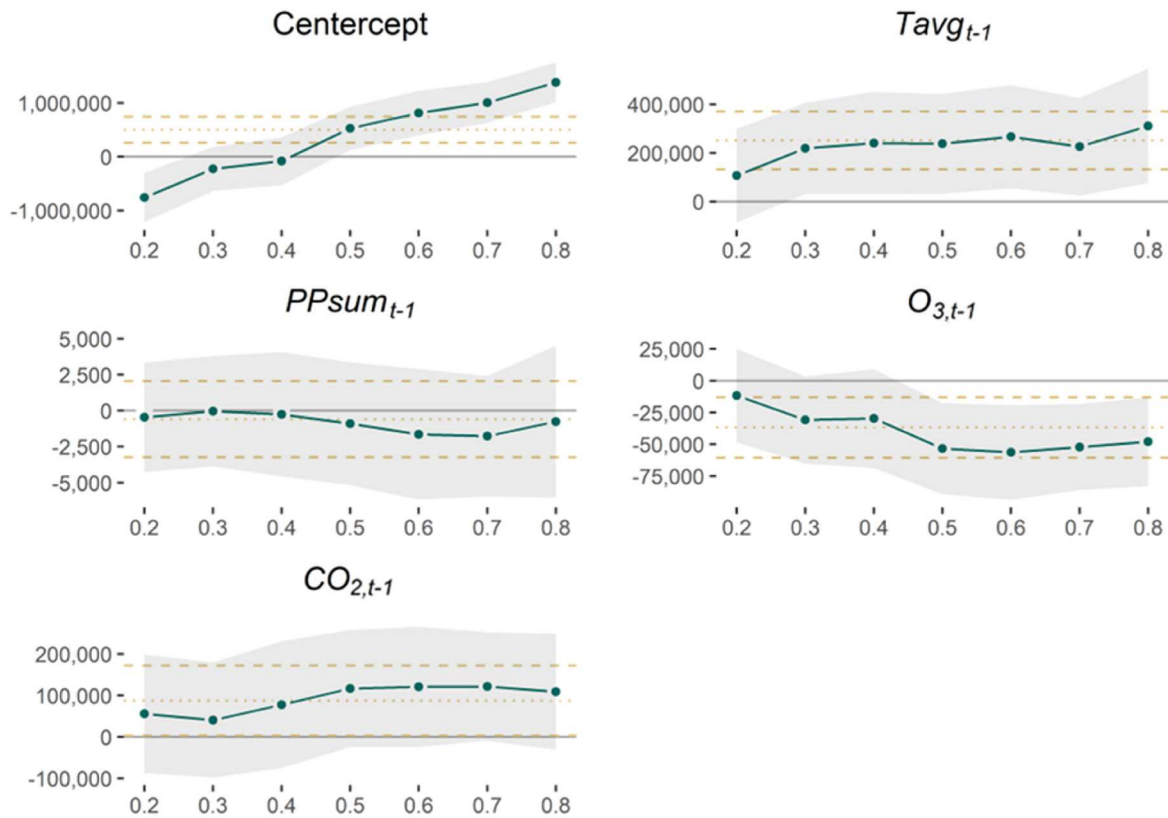
Fig. 5. Quantile plots for specification M2; effects of environmental variables (ordinate) on P_{ca} across conditional response distribution for τ-quantiles, τ ϵ (0.2, 0.8) (abscissa) shown via points. Shaded area represents 90%-confidence region. Horizontal lines indicate mean regression effect (dotted) and corresponding 90%-confidence region (dashed).

the years before and after the registered maxima were quite large, longer time-series are needed to identify and analyze masting events in more detail.

4.3. Effects of environmental variables on pollen production

The understanding of environmental determinants of pollen production is crucial for investigating trends in the face of future climate change. In this study, we assessed the influences of different environmental variables on birch pollen production, while also controlling for stem circumference (in one model specification).

Our models that combine atmospheric gases and meteorological data appeared most appropriate for modeling the pollen production metrics. We found that temperature of the previous summer had positive effects on both, pollen production and catkin formation, while precipitation increased the number of catkins. In contrast, O_3 concentrations had a negative effect on pollen and catkin production. The relationship between temperature and pollen production found in this study are in line with the findings of previous studies (Damialis et al., 2011; Ranpal et al., 2023). We had previously reported that a higher pollen production of downy birch during a non-masting year was associated with higher temperature of the preceding summer in birch trees along an altitudinal gradient (Ranpal et al., 2023). Investigations on other species, such as the herbaceous common ragweed (*Ambrosia artemisiifolia*), have demonstrated that increases in temperature were associated with a higher pollen production (Ziska et al., 2003). However, our findings are in contrast with the results of Jochner et al. (2013) and Jetschni et al. (2023), who observed a notable decrease in pollen production per catkin in silver birch within urban areas (associated with elevated temperatures and higher pollutant concentrations). Our study indicates that birch trees could modulate their physiological processes to optimize

pollen production under varying temperature regimes. Especially those trees which were characterized by high pollen production levels were associated with increases of P_{ca} under high mean temperature of the previous summer. It may be possible that birch trees show an initial boost in pollen production as temperatures rise, but there could be a threshold beyond which further temperature increases might hinder their physiological performance. Our findings could be interpreted such that the examined temperature range is still within the realm of positive impact on reproduction.

Plant biomass of Scots pine individuals have been reported to be adversely affected by elevated O_3 in fumigation experiments (Manninen et al., 2009). However, knowledge on the effect of O_3 on pollen production of birch is largely lacking. Our study has revealed that O_3 has adverse effects on both pollen and catkin production, and these effects vary across different parts of the conditional distribution of the reproductive metrics. Quantile regressions revealed that the effect of O_3 on P_{ca} was negative for quantiles τ ≥ 0.5 (Fig. 5), while being indistinguishable from zero for τ < 0.5. Similarly, in the case of C_{su} and P_{vuc}, O_3 exerted a negative effect for τ ≥ 0.6 (see Appendix Figs. 1 and 2). Regarding reproductive outputs, Darbah et al. (2008) found reduced seed mass and germination rates of *Betula papyrifera* under the elevated O_3 condition. In another study, Ziska (2002) did not find any significant difference in floral biomass of ragweed under elevated O_3 concentrations. O_3 is a gaseous pollutant that enters plants via the stomata. High levels of O_3 can lead to oxidative stress in plants and can disrupt photosynthesis (Gandin et al., 2021) which could potentially also limit pollen production. Further studies, e.g., based on experimental approaches, are needed to investigate the role of O_3 on reproduction of downy birch in more detail.

Rising levels of CO_2 in the atmosphere can fertilize vegetation (Kudeyarov et al., 2006), increase the ability of plants to

photosynthesize (Drake et al., 1997) and was found to induce higher pollen production in some plants such as ragweed (Rauer et al., 2021; Rogers et al., 2006; Ziska and Caulfield, 2000) and timothy grass (Albertine et al., 2014) as well as in trees, such as pine (Ladeau and Clark, 2006) and oak (Kim et al., 2018; Ladeau and Clark, 2006). As we do not have a longer time-series and therefore cannot cover a period with a large increase in CO_2, we can only relate the regional differences (range: 8 ppm) of CO_2 based on a coarse resolution ($0.75° \times 0.75°$) to pollen production. In this case, our result only revealed an association between elevated CO_2 and P_{ca} at marginal significance.

The observation of quantile effects in this study offers novel insights into the relationship between environment and pollen production. Our results indicate that the effects of mean temperature and O_3 in the previous summer vary across the conditional response distribution. While the effects of temperature are zero for trees with low levels of pollen production and positive for trees with high levels of pollen production, the effects of O_3 are zero for trees with low pollen production and negative for trees with pollen production levels above the conditional median. The large confidence regions in our analysis emphasizes the importance of expanding the sample sizes in future studies to enhance the reliability of the conclusions. Overall, our results highlight the benefits of expanding the analysis of pollen production metrics beyond the conditional mean.

The study's geographic scope is constrained by the uneven distribution of the IPGs across Europe, limiting the representation of birches in certain regions. Additionally, the similar environmental conditions within each IPG site restrict the variation in the explanatory variables. Future research should expand sampling beyond the IPG network to capture a more comprehensive representation of birch habitats and environmental factors influencing pollen production. Despite these limitations, the study provides valuable insights into continental-scale patterns and drivers of birch pollen production, increasing our understanding of the potential impacts of global change on this important aeroallergen.

4.4. Genetic effects

Studying IPG plants can be compared with an experimental approach since all birch individuals originate from Germany and were transferred to other sites in Europe (Renner and Chmielewski, 2021). Translocated individuals do not alter their DNA sequence to adapt to new environmental conditions (Amaral et al., 2020; Richards et al., 2017). However, small RNAs might participate in mechanisms that trigger epigenetic gene expression changes in response to environmental cues (Duempelmann et al., 2020) and such changes in small RNA components could be passed over to bud cells and therefore to the next generation (Vanden Broeck et al., 2018). Therefore, apart from environmental influences, the variations in reproductive metrics of IPG birches could be due to clonal differences and potential epigenetic effects. Although, we found differences in genetics of the IPG trees, many birches present a distinct genotype. Thus, we conclude that the variability of genetic effects is extremely reduced in this study. This assertion holds true for all studies incorporating data of the International Phenological Gardens network. Therefore, our approach can be considered a sophisticated space-for-time (SFT) approach, as the genetic variability, which often poses a disadvantage in SFT studies, is largely reduced in our study.

5. Conclusion

The present research is novel as the study site covers IPGs in a large geographical region across Europe. We did not observe any clear spatial pattern in pollen production which reflects the complex interactions between birch trees and their environment. The findings of this paper indicate that birch pollen production increases with higher mean air temperature but decreases with elevated ozone concentrations. Moreover, quantile regression enabled us to quantify the effects of the environmental variables across different pollen production levels. Future research should focus on how individuals and communities can adapt to increased pollen and allergen levels, e.g., using improved pollen forecasts or urban planning that considers allergen-free vegetation. Collaboration between climatologists, ecologists, biologists, medical researchers, public health experts, and statisticians as demonstrated by this publication is crucial to address the multifaceted nature of these challenges.

CRediT authorship contribution statement

Surendra Ranpal: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Susanne von Bargen:** Writing – review & editing. **Stefanie Gilles:** Writing – review & editing. **Daria Luschkova**[3]**:** Writing – review & editing. **Maria Landgraf:** Writing – review & editing. **Pawel Bogawski:** Writing – review & editing, Visualization, Methodology. **Claudia Traidl-Hoffmann:** Writing – review & editing, Funding acquisition, Conceptualization. **Carmen Büttner:** Writing – review & editing, Funding acquisition, Conceptualization. **Athanasios Damialis:** Writing – review & editing, Validation, Methodology. **Markus Fritsch:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis. **Susanne Jochner-Oette:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.
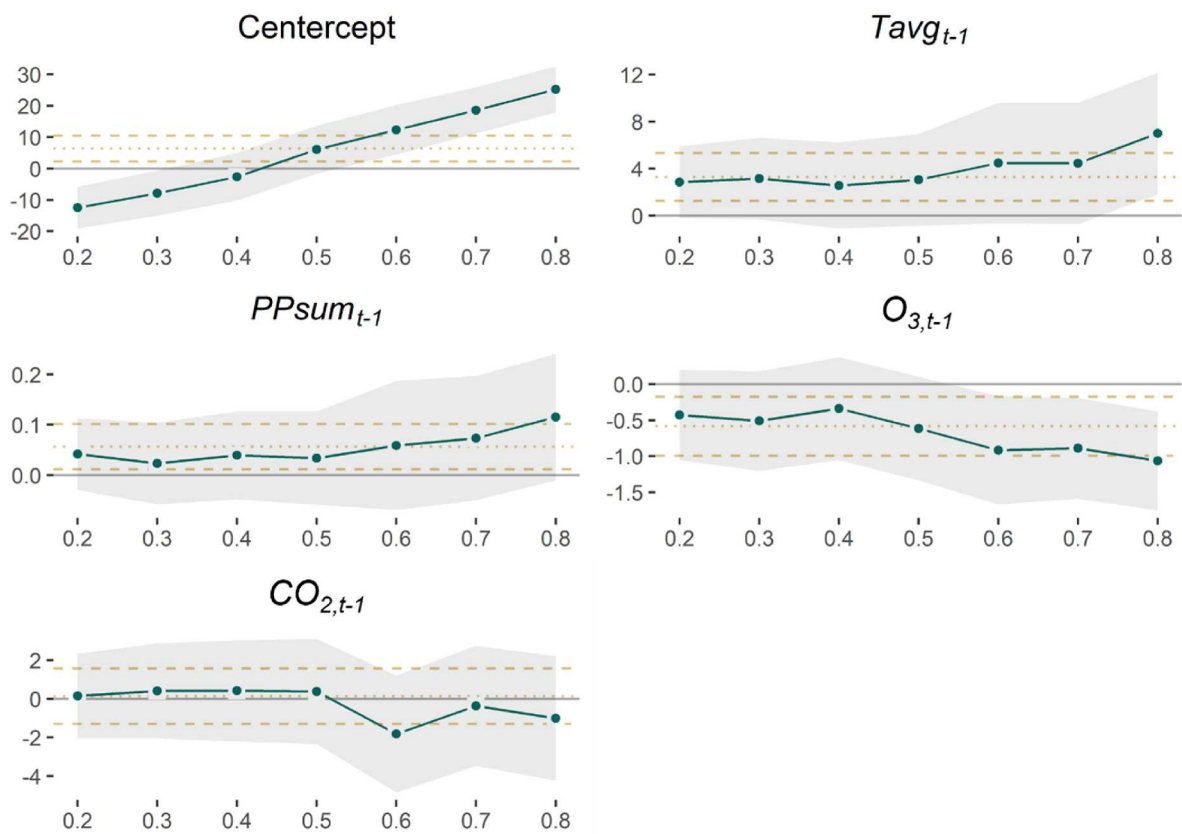
Appendix

Appendix Table 1
List of International Phenological Gardens and countries from which up to three downy birch trees were selected for this study. Years (1 = 2019, 2 = 2020 and 3 = 2021) in which samples were available; elevation (meters above sea level); triannual mean air temperature (Tavg) from 2018 to 2020 (°C) and triannual mean precipitation sum (PPsum) from 2018 to 2020 (mm).

IPG	Site	Country	Years	Coordinates	Elevation	no. of trees	Plantation year	Tavg	PPsum
1	Trondheim	Norway	1, 2, 3	N 63° 29′ E 10° 52′	69	2	1963, 2007	5.99	978
2	Bergen-Fana	Norway	1, 3	N 60° 16′ E 5° 21′	50	2	1994, 1999	8.05	2441
8	Turku	Finland	1, 2, 3	N 60° 29′ E 22° 45′	45	2	1965	6.92	637
24	Offenbach	Germany	1, 2, 3	N 50° 05′ E 08° 46′	114	1	2012	11.76	543
27	Stuttgart-Hohenheim	Germany	1, 2, 3	N 48° 43′ E 09° 13′	380	2	1960	11.36	592
36	München-Grafrath	Germany	1, 2, 3	N 48° 08′ E 11° 09′	569	2	1996, 2009	9.75	785
38	Freyung-Schönbrunn	Germany	1, 2, 3	N 48° 52′ E 13° 31′	820	1	2012	8.35	999
40	Freyung-Waldhäuser	Germany	1, 2, 3	N 48° 55′ E 13° 27′	967	1	2005	6.70	1014
42	Tharandt-Hartha	Germany	1, 2, 3	N 50° 58′ E 13° 32′	358	2	1960, 1969	9.84	557
46	Zürich-Birmensdorf	Switzerland	1, 2, 3	N 47° 21′ E 08° 27′	544	1	1965	10.65	983
55	Ljubljana	Slovenia	1	N 46° 04′ E 14° 30′	299	3	1960,1990, 2007	12.18	1274
62	Skopje	North Macedonia	2, 3	N 42° 01′ E 21° 24′	294	1	1980	13.73	660
72	London	United Kingdom	1, 3	N 51° 12′ W 00° 46′	83	1	2005	11.47	731
77	Hamburg-Quickborn	Germany	1	N 53° 44′ E 09° 52′	13	2	1988	10.28	736
80	Thyrow	Germany	1, 2	N 52° 15′ E 09° 52′	43	1	1998	11.19	406
85	Praha-Doksany	Czech Republic	2, 3	N 50° 27′ E 14° 10′	158	1	2000	10.73	389
90	Hellenthal	Germany	1, 2, 3	N 50° 24′ E 06° 22′	686	1	2009	8.97	905
112	Glenveagh	Ireland	1	N 55° 01′ W 07° 58′	118	1	2007	9.47	1847
152	Siauliai	Lithuania	1, 2, 3	N 55° 55′ E 23° 16′	126	1	2005	8.61	510
189	Linden	Germany	1, 2, 3	N 50° 31′ E 08° 41′	171	1	2003	10.83	567
190	Graupa	Germany	1, 2, 3	N 51° 00′ E 13° 55′	181	1	2004	10.67	540
191	Heinzebank	Germany	1, 2	N 50° 41′ E 13° 08′	605	1	2005	8.67	715
192	Kretscham-Rothensehma	Germany	1, 2	N 50° 27′ E 12° 59′	852	1	2005	6.93	827
193	Doberschütz	Germany	1, 2, 3	N 51° 31′ E 12° 41′	99	1	2005	11.25	443
194	Eich	Germany	1, 2, 3	N 50° 33′ E 12° 20′	449	1	2005	9.61	634
195	Leipzig	Germany	1, 2, 3	N 51° 07′ E 12° 41′	201	1	2009	10.99	495
196	Taura	Germany	1, 2, 3	N 51° 28′ E 13° 00′	127	1	2005	10.96	428
210	Sarajevo	Bosnia and Herzegovina	1, 2, 3	N 43° 52′ E 18° 25′	633	1	2013	9.72	899
212	Mostar	Bosnia and Herzegovina	1	N 43° 20′ E 17° 47′	98	1	2014	14.09	1158
220	Würzburg	Germany	1, 2, 3	N 49° 45′ E 09° 56′	193	1	2010	11.37	476
221	Roßla	Germany	1, 2, 3	N 51° 27′ E 11° 04′	148	1	2011	10.35	452
224	Kleve	Germany	1, 2, 3	N 51° 47′ E 06° 10′	13	1	2009	11.46	682
226	Essen	Germany	1, 2, 3	N 51° 24′ E 06° 57′	155	1	2008	11.93	723
227	Recklinghausen	Germany	1, 2, 3	N 51° 35′ E 07° 13′	54	1	2009	11.53	695
228	Ohorn	Germany	1, 2, 3	N 51° 10′ E 14° 02′	306	1	2011	10.34	556
230	Waldfeucht	Germany	1, 2, 3	N 51° 05′ E 06° 03′	30	1	2011	11.74	625
231	Braunschweig	Germany	1, 2	N 52° 17′ E 10° 26′	82	1	2011	11.19	493

Appendix Table 2
Descriptive statistics for the meteorological variables for IPG sites included in this study analysis and Kruskal Wallis test (p-value) indicating that the central tendency of the distribution of the variables is identical across years 2019–2021.

Meteorological variables	Description	Year	Minimum	Maximum	Mean	Median	Standard deviation	Kruskal Wallis (p-value)
$Tmin_{t-1}$	Minimum temperature (°C) of the preceding summer	2018	1.5	8.1	5.4	5.5	1.6	0.597 (0.742)
		2019	1.8	7.7	5.2	5.6	1.5	
		2020	2.6	7.8	5.5	5.8	1.4	
$Tavg_{t-1}$	Average temperature (°C) of the preceding summer	2018	5.7	12.3	10	10.5	1.8	0.386 (0.825)
		2019	5.5	13.9	9.9	10.5	1.9	
		2020	6.6	13.4	10.1	10.6	1.8	
$Tmax_{t-1}$	Maximum temperature (°C) of the preceding summer	2018	10.1	17.3	14.,6	15.1	2.3	0.401 (0.818)
		2019	9.6	19.8	14.5	15.1	2.3	
		2020	10.7	19.1	14.5	15.5	2.4	
$PPsum_{t-1}$	Total precipitation (mm) of the preceding summer	2018	329	2102	757	590	461	2.58 (0.276)
		2019	427	1054	692	683	182	
		2020	411	2945	856	684	608	
RH_{t-1}	Relative humidity (%) of the preceding summer	2018	70.3	84.3	76.3	75.7	4	0.333 (0.847)
		2019	70.7	82.,9	76.2	75.3	3.2	
		2020	70.5	87.1	76.4	75	4.6	
GR_{t-1}	Global solar radiation (W/m^2) of the preceding summer	2018	35468	54538	48023	49296	5131	31.722 (0.000)
		2019	29649	60432	38329	37069	6110	
		2020	34382	55748	46399	47530	5937	

Appendix Fig. 1. Quantile plots for specification M2; effects of environmental variables (ordinate) on C_{su} across conditional response distribution for τ-quantiles, $\tau \in$ (0.2, 0.8) (abscissa) shown via points. Shaded area represents 90%-confidence region. Horizontal lines indicate mean regression effect (dotted) and corresponding 90%-confidence region (dashed).
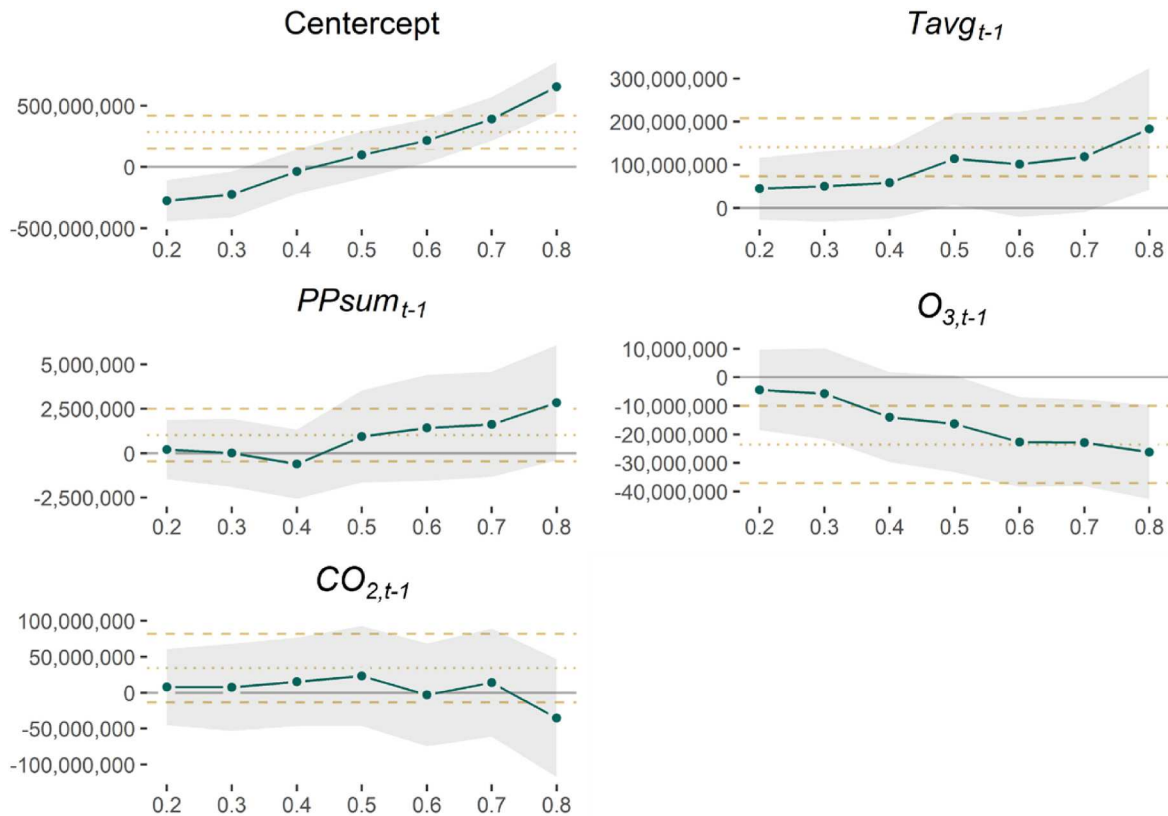
Appendix Fig. 2. Quantile plots for specification M2; effects of environmental variables (ordinate) on P_{vuc} across conditional response distribution for τ-quantiles, τ ∈ (0.2, 0.8) (abscissa) shown via points. Shaded area represents 90%-confidence region. Horizontal lines indicate mean regression effect (dotted) and corresponding 90%-confidence region (dashed).

References

Albertine, J.M., Manning, W.J., DaCosta, M., Stinson, K.A., Muilenberg, M.L., Rogers, C. A., 2014. Projected carbon dioxide to increase grass pollen and allergen exposure despite higher ozone levels. PLoS One 9, e111712. https://journals.plos.org/plosone /article?id=10.1371/journal.pone.0111712.

Amaral, J., Ribeyre, Z., Vigneaud, J., et al., 2020. Advances and promises of epigenetics for forest trees. Forests 11, 976.

Auguie, B, Antonov, A, 2017. gridExtra: Miscellaneous Functions for "Grid" Graphics [R package version 2.3]. https://cran.r-project.org/web/packages/gridExtra/index. html.

Beck, P., Caudullo, G., Rigo, D de, Tinner, W., 2016. Betula Pendula, Betula Pubescens and Other Birches in Europe: Distribution, Habitat, Usage and Threats. Publication Office of the European Union, Luxembourg, pp. 70–73.

Beggs, P.J., 2004. Impacts of climate change on aeroallergens: past and future. Clin. Exp. Allergy 34, 1507–1513. https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.136 5-2222.2004.02061.x.

Beutner, C., Werchan, B., Forkel, S., Gupta, S., Fuchs, T., Schön, M.P., Geier, J., Buhl, T., 2021. Sensitization rates to common inhaled allergens in Germany - increase and change patterns over the last 20 years. Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG 19, 37–44.

Biedermann, T., Winther, L., Till, S.J., Panzner, P., Knulst, A., Valovirta, E., 2019. Birch pollen allergy in Europe. Allergy 74, 1237–1248. https://onlinelibrary.wiley.com/ doi/10.1111/all.13758.

Bogdziewicz, M., Hacket-Pain, A., Ascoli, D., Szymkowiak, J., 2021. Environmental variation drives continental-scale synchrony of European beech reproduction. Ecology 102, e03384. https://esajournals.onlinelibrary.wiley. com/doi/full/10.1002/ecy.3384#ecy3384-bib-0045.

Broeck, an Vanden, Cox, K., Brys, R., et al., 2018. Variability in DNA methylation and generational plasticity in the lombardy poplar, a single genotype worldwide distributed since the eighteenth century. Front. Plant Sci. 9, 1635. https://www.fron tiersin.org/articles/10.3389/fpls.2018.01635/full.

Cade, B.S., Noon, B.R., 2003. A gentle introduction to quantile regression for ecologists. Front. Ecol. Environ. 1, 412–420. https://pubs.usgs.gov/publication/1015331.

Caffarra, A., Donnelly, A., 2011. The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. Int. J. Biometeorol. 55, 711–721. https://link.springer.com/article/10.1007%2Fs00484-0 10-0386-1.

Caudullo, G., Welk, E., San-Miguel-Ayanz, J., 2017. Chorological maps for the main European woody species. Data Brief 12, 662–666.

Cornes, R.C., van der Schrier, G., van den Besselaar, E.J., Jones, P.D., 2018. An ensemble version of the E-OBS temperature and precipitation data sets. J. Geophys. Res. Atmos. 123, 9391–9409.

D'Amato, G., Spieksma, F.T., Liccardi, G., et al., 1998. Pollen-related allergy in Europe. Allergy 53, 567–578.

D'Amato, G., Chong-Neto, H.J., Monge Ortega, O.P., et al., 2020. The effects of climate change on respiratory allergy and asthma induced by pollen and mold allergens. Allergy 75, 2219–2228. https://onlinelibrary.wiley.com/doi/full/10.1111/all .14476.

Dahl, Å., Strandhede, S.-O., 1996. Predicting the intensity of the birch pollen season. Aerobiologia 12, 97–106. https://doi.org/10.1007/bf02446601.

Damialis, A., Fotiou, C., Healley, J.M., Vokou, D., 2011. Effects of environmental factors on pollen production in anemophilous woody species. Trees (Berl.) 25, 253–264.

Damialis, A., Traidl-Hoffmann, C., Treudler, R., 2019. Climate change and pollen allergies. In: Marselle, M.R., Stadler, J., Korn, H., Irvine, K.N., Bonn, A. (Eds.), Biodiversity and Health in the Face of Climate Change. Springer, Cham, pp. 47–66.

Darbah, J.N., Kubiske, M.E., Nelson, N., Oksanen, E., Vapaavuori, E., Karnosky, D.F., 2008. Effects of decadal exposure to interacting elevated CO2 and/or O3 on paper birch (Betula papyrifera) reproduction. Environ. Pollut. 155, 446–452.

Detandt, M., Nolard, N., 2000. The fluctuations of the allergenic pollen content of the air in Brussels (1982 to 1997). undefined. https://www.semanticscholar.org/paper/The -fluctuations-of-the-allergenic-pollen-content-Detandt-Nolard/76f019a0802f65df7 3b5ccee04798bb608cc7376.

Drake, B.G., Gonzalez-Meler, M.A., Long, S.P., 1997. More efficient plants: a consequence of rising atmospheric CO2? Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 609–639.

Duempelmann, L., Skribbe, M., Bühler, M., 2020. Small RNAs in the transgenerational inheritance of epigenetic information. Trends Genet. 36, 203–214. https://www.cell. com/trends/genetics/fulltext/S0168-9525(19)30259-8.

Dunnington, D, Thorne, B, Hernangómez, D. ggspatial: Spatial Data Framework for ggplot2 [R package version 1.1.9]. https://cran.r-project.org/web/packages/ggspa tial/index.html.

Frenne, P., Graae, B.J., Rodríguez-Sánchez, F., et al., 2013. Latitudinal gradients as natural laboratories to infer species' responses to temperature. J. Ecol. 101, 784–795. https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2745.12 074.

Gandin, A., Dizengremel, P., Jolivet, Y., 2021. Integrative role of plant mitochondria facing oxidative stress: the case of ozone. Plant Physiol. Biochem. : PPB (Plant

Physiol. Biochem.) 159, 202–210. https://www.sciencedirect.com/science/article/pii/S0098194282030642.

Geburek, T., Hiess, K., Litschauer, R., Milasowszky, N., 2012. Temporal pollen pattern in temperate trees: expedience or fate? Oikos 121, 1603–1612.

Gilles, S., Meinzer, M., Landgraf, M., et al., 2023. Betula pendula trees infected by birch idaeovirus and cherry leaf roll virus: impacts of urbanisation and NO2 levels. Environ. Pollut. 327, 121526. https://www.sciencedirect.com/science/article/pii/S0269749123005286.

Hynynen, J., Niemistö, P., Viherä-Aarnio, A., Brunner, A., Hein, S., Velling, P., 2010. Silviculture of birch (Betula pendula roth and Betula pubescens Ehrh.) in northern Europe. Forestry: Int. J. Financ. Res. 83, 103–119. https://academic.oup.com/forestry/article/83/1/103/546795.

Jato, F.V., Rodriguez-Rajo, F.J., Aira, M.J., 2007. Use of phenological and pollen-production data for interpreting atmospheric birch pollen curves. Ann. Agric. Environ. Med. 14.

Jetschni, J., Fritsch, M., Jochner-Oette, S., 2023. How does pollen production of allergenic species differ between urban and rural environments? Int. J. Biometeorol. 67, 1839–1852.

Jochner, S., Höfler, J., Beck, I., Göttlein, A., Ankerst, D.P., Traidl-Hoffmann, C., Menzel, A., 2013. Nutrient status: a missing factor in phenological and pollen research? J. Exp. Bot. 64, 2081–2092. https://academic.oup.com/jxb/article/64/7/2081/581703.

Kassambara, A. ggpubr: 'ggplot2' Based Publication Ready Plots [R package version 0.6.0]. https://cran.r-project.org/web/packages/ggpubr/index.html.

Kelly, D., 1994. The evolutionary ecology of mast seeding. Trends Ecol. Evol. 9, 465–470.

Kim, K.R., Oh, J.-W., Woo, S.-Y., Seo, Y am, Choi, Y.-J., Kim, H.S., Lee, W.Y., Kim, B.-J., 2018. Does the increase in ambient CO2 concentration elevate allergy risks posed by oak pollen? Int. J. Biometeorol. 62, 1587–1594. https://pubmed.ncbi.nlm.nih.gov/29748910/.

Koenker, R., 2005. Quantile Regression. Cambridge University Press, Cambridge.

Koenker, R., 2017. Quantile regression: 40 Years on. Annual Review of Economics 9, 155–176.

Koenker, R., Hallock, K.F., 2001. Quantile regression. J. Econ. Perspect. 15, 143–156. https://www.aeaweb.org/articles?id=10.1257/jep.15.4.143.

Koenker, R., Portnoy, S., Ng, P.T., et al., 2023. Quantile Regression [R Package Quantreg Version 5.97]. Comprehensive R Archive Network (CRAN).

Kolek, F., 2021. Spatial and Temporal Monitoring of Betula Pollen in the Region of Augsburg. Bavaria, Germany. Dissertation.

Kudeyarov, V.N., Biel, K., Blagodatsky, S.A., Semenov, V.M., Dem'yanova, E.G., Dorodnikov, M.V., 2006. Fertilizing effect of the increasing CO2 concentration in the atmosphere. Eurasian Soil Sci. 39, S6–S14. https://link.springer.com/article/10.1134/S1064229306130035.

Ladeau, S.L., Clark, J.S., 2006. Pollen production by Pinus taeda growing in elevated atmospheric CO 2. Funct. Ecol. 20, 541–547.

Latałowa, M., Miętus, M., Uruska, A., 2002. Seasonal variations in the atmospheric Betula pollen count in Gdańsk (southern Baltic coast) in relation to meteorological parameters. Aerobiologia 18, 33–43. https://link.springer.com/article/10.1023/A:1014905611834.

Li, L., Chang, C., Guan, K., 2022. Birch pollen allergens. Curr. Protein Pept. Sci. 23, 731–743.

Linkosalo, T., Siljamo, P., Riikonen, A., Chmielewski, F.M., Raisio, J., 2019. Utilizing a thermal time model to estimate safe times to transplant Tilia trees. Arboric. Urban For. 45, 201–210.

Manninen, S., Huttunen, S., Vanhatalo, M., Pakonen, T., Hämäläinen, A., 2009. Inter- and intra-specific responses to elevated ozone and chamber climate in northern birches. Environ. Pollut. 157, 1679–1688. https://www.sciencedirect.com/science/article/pii/S0269749108006830.

Neuwirth, E. RcolorBrewer: ColorBrewer Palettes [R package version 1.1-3]. https://cran.r-project.org/web/packages/RColorBrewer/index.html.

Olsson, C., Olin, S., Lindström, J., Jönsson, A.M., 2017. Trends and uncertainties in budburst projections of Norway spruce in Northern Europe. Ecol. Evol. 7, 9954–9969. https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.3476.

Pawankar, R., Canonica, G.W., Holgate, S.T., Lockey, R.F., Blaiss, M., 2013. World allergy organization (WAO) white book on allergy: update 2013. Available via. https://www.worldallergy.org/wao-white-book-on-allergy, 25 Nov 2021.932Z.

Piotrowska, K., 2008. Pollen production in selected species of anemophilous plants. Acta Agrobot. 61. http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.agro-article-83572de8-adce-4e35-a08b-68c29bf27a8e.

Portnoy, S., Koenker, R., 1997. The Gaussian hare and the Laplacian tortoise: computability of squared-error versus absolute-error estimators. Stat. Sci. 12,

279–300. https://projecteuclid.org/journals/statistical-science/volume-12/issue-4/The-Gaussian-hare-and-the-Laplacian-tortoise–computability-of/10.1214/ss/1030037960.full.

Powell, JL, 1991. Estimation of monotonic regression models under quantile restrictions. In: Barnett, W, Powell, JL, Tauchen, G (Eds.), Nonparametric and semiparametric methods in econometrics and statistics. Cambridge University Press, Cambridge, UK, pp. 357–384. https://mathscinet.ams.org/mathscinet/article?mr=1174980.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ranpal, S., Sieverts, M., Wörl, V., et al., 2022. Is pollen production of birch controlled by genetics and local conditions? Int. J. Environ. Res. Publ. Health 19, 8160.

Ranpal, S., Bargen, S von, Gilles, S., et al., 2023. Pollen production of downy birch (Betula pubescens Ehrh.) along an altitudinal gradient in the European Alps. Int. J. Biometeorol. 67, 1125–1139. https://link.springer.com/content/pdf/10.1007/s00484-023-02483-7.pdf.

Ranta, H., Oksanen, A., Hokkanen, T., Bondestam, K., Heino, S., 2005. Masting by Betula-species; applying the resource budget model to north European data sets. Int. J. Biometeorol. 49, 146–151.

Ranta, H., Hokkanen, T., Linkosalo, T., Laukkanen, L., Bondestam, K., Oksanen, A., 2008. Male flowering of birch: spatial synchronization, year-to-year variation and relation of catkin numbers and airborne pollen counts. For. Ecol. Manag. 255, 643–650. http://www.sciencedirect.com/science/article/pii/S0378112707007062.

Rauer, D., Gilles, S., Wimmer, M., et al., 2021. Ragweed plants grown under elevated CO2 levels produce pollen which elicit stronger allergic lung inflammation. Allergy 76, 1718–1730.

Renner, S.S., Chmielewski, F.-M., 2021. The International Phenological Garden network (1959 to 2021): its 131 gardens, cloned study species, data archiving, and future. Int. J. Biometeorol. 1–9. https://link.springer.com/article/10.1007/s00484-021-02185-y.

Richards, C.L., Alonso, C., Becker, C., et al., 2017. Ecological plant epigenetics: evidence from model and non-model species, and the way forward. Ecol. Lett. 20, 1576–1590.

Rogers, C.A., Wayne, P.M., Macklin, E.A., Muilenberg, M.L., Wagner, C.J., Epstein, P.R., Bazzaz, F.A., 2006. Interaction of the onset of spring and elevated atmospheric CO2 on ragweed (Ambrosia artemisiifolia L.) pollen production. Environ. Health Perspect. 114, 865–869.

Rojo, J., Oteros, J., Picornell, A., et al., 2021. Effects of future climate change on birch abundance and their pollen load. Global Change Biol. 27, 5934–5949. https://onlinelibrary.wiley.com/doi/full/10.1111/gcb.15824.

Schnelle, F., Volkert, E., 1957. Vorschläge zur Einrichtung Internationaler Phänologischer Gärten als Stationen eines Grundnetzes für internationale phänologische Beobachtungen. Meteorol. Rundsch. 10, 130–133.

van Meeningen, Y., Schurgers, G., Rinnan, R., Holst, T., 2016. BVOC emissions from English oak (Quercus robur) and European beech (Fagus sylvatica) along a latitudinal gradient. Biogeosciences 13, 6067–6080. https://bg.copernicus.org/articles/13/6067/2016/bg-13-6067-2016-discussion.html.

Wayne, P., Foster, S., Connolly, J., Bazzaz, F., Epstein, P., 2002. Production of allergenic pollen by ragweed (Ambrosia artemisiifolia L.) is increased in CO2-enriched atmospheres. Ann. Allergy Asthma Immunol. 88, 279–282. http://www.sciencedirect.com/science/article/pii/S1081120610620091.

Wenden, B., Mariadassou, M., Chmielewski, F.-M., Vitasse, Y., 2020. Shifts in the temperature-sensitive periods for spring phenology in European beech and pedunculate oak clones across latitudes and over recent decades. Global Change Biol. 26, 1808–1819. https://onlinelibrary.wiley.com/doi/10.1111/gcb.14918.

Wickham, H., 2016. ggplot2. Elegant Graphics for Data Analysis. Springer, Switzerland, p. 260.

Wilke, CO.. cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2' [R package version 1.1.3]. https://cran.r-project.org/web/packages/cowplot/index.html.

Ziska, L.H., 2002. Sensitivity of ragweed (Ambrosia artemisiifolia) growth to urban ozone concentrations. Funct. Plant Biol. 29, 1365–1369. https://www.publish.csiro.au/fp/FP02039.

Ziska, L.H., Caulfield, F.A., 2000. Rising CO2 and pollen production of common ragweed (Ambrosia artemisiifolia L.), a known allergy-inducing species. implications for public health 27, 893–898. https://www.publish.csiro.au/fp/pdf/pp00032.

Ziska, L.H., Gebhard, D.E., Frenz, D.A., Faulkner, S., Singer, B.D., Straka, J.G., 2003. Cities as harbingers of climate change: common ragweed, urbanization, and public health. J. Allergy Clin. Immunol. 111, 290–295. http://www.sciencedirect.com/science/article/pii/S009167490300959X.

7 Discussion

The research described in the previous three chapters focused on pollen production of *Betula* species and their spatial and temporal variation. Chapter 4 assessed *B. pendula* in a small seed plantation. Chapter 5 dealt with *B. pubescens* at an altitudinal gradient, and Chapter 6 dealt with *B. pubescens* at a larger spatial extent across Europe. The results were analyzed taking into account different influencing factors such as genetics, meteorology and atmospheric gases. Finally, these three studies have provided answers to the research questions outlined in section 1.3, which are discussed in this chapter and can be used as a basis for future research.

7.1 Mean pollen production of birch trees

We investigated pollen production of *Betula pendula* Roth (n = 28 trees) in three consecutive years (2019 – 2021) in a small seed plantation (Chapter 4). This study was unique since a large number of male birch inflorescences (n = 336 catkins) were examined and the internal variability of pollen production regarding genetic differences and similarities were assessed. Furthermore, this study excluded large differences in the environmental conditions. All 28 trees included were of uniform age, planted in 2005, thereby eliminating potential age-related effects.

The pollen production values of *Betula pendula* at catkin level ranged from 48,000 pollen grains to 8.3 million pollen grains (mean 1.66 million). There are few studies that have already calculated pollen production for *Betula pendula* (sometimes referred as *Betula alba*, *Betula verrucosa*). Erdtman (1954) mentioned an estimate of 5.5 million pollen grains per catkin for *B. verrucose*. Jato *et al.* (2007) reported estimates ranging from 4.8 million to 8.2 million pollen grains per inflorescence for six *B. alba* trees in northwestern Spain in 2002 and 2003, respectively. Studying 30 catkins from three trees, Piotrowska (2008) calculated a mean value of 10 million pollen grains per inflorescence.

While previous research findings have indeed presented higher values in comparison to the mean P_{ca} estimated in Chapter 4, it is important to note that these specific investigations were often limited due to the comparatively few numbers of trees or

were exclusively focused on just one or two specific years. As a result, it is unknown whether sampling occurred during masting or non-masting years. For this reason, the estimates of *Betula pendula* reported in this PhD study can be regarded as important since we have sampled 28 trees for three years and present a robust estimate for the descriptive statistics including mean, minimum and maximum values.

Furthermore, in the contemporary study years, there were two more studies that conducted investigations on the pollen production of *B. pendula*. Kolek (2021) collected male inflorescence from 43 *B. pendula* trees (within the greater region of Augsburg, Germany) in 2016 and 2017 and estimated <1 million pollen grains per catkin. The second study by Jetschni *et al.* (2023) found 1.6 million ± 1 million (rural environment) and 9.4 million ± 9 million (urban environment) pollen grains per catkin of *B. pendula*. The study was focused on the greater region of Ingolstadt, Germany.

For *B. pubescens* Ehrh. reproductive metrics, we have investigated and reported the estimations in two different spatial scales in Chapter 5 and in Chapter 6. Chapter 5 was conducted for two consecutive years of 2020 and 2021 along an altitudinal gradient in the Bavarian Alps (maximum distance between the selected birch trees ~ 14 km). The mean pollen production per catkin was estimated in a range between 2.5 million to 5 million. This estimate is comparable to that published by Erdtman (1954), who reported a value of 6 million pollen grains per inflorescence for *B. pubescens*. Our mean value, however, is an average of the estimates from 17 downy birch trees growing in various elevations for two years (n = 136 catkins). The genetic characteristics of those 17 birch trees were not assessed, and no information was found indicating that they were clones; therefore, it can be assumed that they were genetically heterogeneous.

The second estimations (Chapter 6) for *B. pubescens* were made from downy birch trees of the IPG network across Europe along a spatial gradient over a period of three years (2019 - 2021). The mean pollen production of catkins (P_{ca}) was measured in the range of 1.9 million to 2.5 million, which is 32% to 100% lower than results observed along the altitudinal gradient in the Bavarian Alps. These differences could be due to the reduction in genetically induced variability in the IPGs birch trees as compared to the birches in the Bavarian Alps. Nevertheless, the measures in Chapter 6 provide an

important estimation range for representing pollen production of downy birch across its distribution range. Overall, the findings in this PhD thesis suggest that, when regional extents are disregarded, *B. pubescens* generally produce higher mean P_{ca} compared to *B. pubescens*.

7.2 Year to year variations in the reproductive metrics

Some studies have examined the temporal change in birch pollen concentrations based on pollen trap monitoring (Ranta *et al.* 2008; Piotrowska & Kubik-Komar 2012), however, there is no study presenting long-term changes in pollen production assessed using the same birch trees, which this PhD thesis has endeavored to address to a certain extent.

In our studies at small geographical scales (Chapter 4 and Chapter 5), we found annual variations in pollen production during the studied years. Chapter 4 showed an annual variation in P_{ca} and C_{su} with the lowest mean values in 2019 and the highest in 2021. In Chapter 5, though the reproductive metrics of birch in the altitudinal gradient along Bavarian Alps was studied only for two years, significant alterations in pollen and catkin production were observed between those years. The year 2020 was found to be a pollen- and catkin-rich year: We estimated on average 109% more pollen grains per catkin than in the following year. In addition, the number of catkins per volume unit of crown was 460% higher in 2020, but the number of flowers per catkin was only changed slightly (+ 6%) and was associated with a non-significant difference.

In both studies (Chapter 4 and Chapter 5), flower numbers per inflorescence were not significantly different among years. This can be explained by the fact that the number of anthers per flower is genetically fixed and does not vary substantially (Subba Reddi & Reddi 1986; Hidalgo *et al.* 1999; Fernández-González *et al.* 2020).

In Chapter 6, based on mean values of all selected IPGs across Europe, our analysis revealed the lowest means for P_{ca} in 2019 and the highest in 2021. This year-to-year variation, however, was not significant in all the studied reproductive metrics of pollen, flowers, and catkins. However, pollen production at single sites can vary substantially across years (see Chapter 6).

DISCUSSION

These year-to-year variations in pollen production could *inter alia* be induced by yearly variations in the meteorological conditions of the locality, but long-term data is lacking to prove this assumption. In addition, a key consideration in our study is the substantial role that masting behavior plays in influencing the reproductive metrics of birch trees. Masting, the synchronized and highly variable production of flowers, seeds, and pollen across years, is a common feature observed in many temperate tree species (Kelly 1994), including birch (Jato *et al.* 2007; Ranta *et al.* 2008) and might be responsible for the observed year-to-year variation in our studies.

The phenomenon of masting has been the subject of several hypotheses attempting to elucidate its proximate drivers. The resource dynamics hypotheses, including the resource matching hypothesis (Kelly 1994), the resource budget hypothesis (Isagi *et al.* 1997), and resource budget modeling (Crone & Rapp 2014), propose that plants reproduce when resources are abundant or when reserves exceed a threshold. Alternatively, the pollen coupling hypothesis (Koenig & Knops 2000) suggests that synchronization of flowering enhances pollen availability and fertilization success. The weather cueing hypothesis (Schauber *et al.* 2002) posits that unusual weather events trigger hormonal changes that induce synchronous flowering and fruiting. Furthermore, the genetic/hormonal regulation hypotheses propose that gene networks integrate environmental cues to control flowering synchrony (Crone *et al.* 2009), while hormones regulate flowering and seed maturation in response to environmental signals (Miyazaki *et al.* 2014). These diverse hypotheses highlight the complexity of masting and the potential interplay of resource dynamics, pollination ecology, environmental cues, and genetic/hormonal regulation in driving this phenomenon.

Previous research have documented the alternating patterns of flower and seed production in birch, with masting events occurring every second or third year (Damialis *et al.* 2011; Detandt & Nolard 2000; Latałowa *et al.* 2002; Jato *et al.* 2007). This masting behavior can be limited at the population level (Ranta *et al.* 2005) or synchronize across larger regional and continental scales (Ranta *et al.* 2008; Bogdziewicz *et al.* 2021). Importantly, these masting patterns have been observed for flower and pollen production as well (Gassner *et al.* 2019).

DISCUSSION

The findings of this PhD thesis provide evidence of the influence of masting on birch reproductive metrics to some extent. In Chapter 5, we observed an extraordinary increase (more than 10-fold for catkins, 3-fold for pollen) in catkin and pollen production in 2020 compared to 2021, with the majority of the selected trees synchronously exhibiting this heightened reproductive output. This strongly suggests that 2020 was a masting year in the Garmisch-Partenkirchen region.

Interestingly, we also noted that during this masting year, the typical associations between reproductive metrics and environmental factors such as temperature (see Chapter 5) were not observed. This indicates that the dominant influence of masting behavior can effectively mask the impacts of other environmental stressors on reproductive metrics. However, more data from a longer-time series would be necessary for a proper interpretation.

Further supporting evidence for the 2020 masting event was found in the data from the Wildberg seed plantation (Chapter 4), located approximately 210 km from the Garmisch-Partenkirchen. Here, the mean catkin production per sampling unit (C_{su}) was twice as high in 2020 compared to the preceding and following years. Additionally, pollen monitoring data from Eichstätt, Bavaria, roughly 160 km away, showed a dramatic increase in the annual pollen integral (APIn) in 2020 compared to 2021 (unpublished data).

In Chapter 6, the maximum values for pollen production per catkin (P_{ca}) and catkin production per sampling unit (C_{su}) were not observed simultaneously across all trees in a single year. Rather, the peak values for these metrics were distributed, with most trees (N = 10) exhibiting maximum P_{ca} in 2021 and maximum C_{su} in 2020.

In our seed plantation study (Chapter 4), we observed a lack of complete synchronization of high or low pollen production among birch trees. This asynchrony can be attributed to factors such as individual resource balance (Isagi *et al.* 1997; Crone & Rapp 2014), where variations in initial resource stock and subsequent acquisition among trees, even under similar conditions, can lead to deviations. Plant-pathogen and plant-mycorrhizosphere interactions also influence abiotic stress impacts on resource

allocation dynamics (Matyssek *et al.* 2005), contributing to tree-specific responses and potential asynchrony in masting.

The findings in this PhD thesis underscore the need for longer-term datasets to accurately identify and analyze masting events in birch populations. The delineation of masting years is a complex task, often relying on concepts such as the coefficient of variation, but consistent and widely applicable methods are still lacking (LaMontagne & Boutin 2009). Additionally, the potential influence of meteorological factors on masting patterns requires further investigation through long-term monitoring. Importantly, there are indications that climate change is likely to alter the frequency and dynamics of masting events. For instance, Nussbaumer *et al.* (2018) found that the relationship between weather cues and seed initiation in European beech weakened over time with increasing temperatures, resulting in progressive asynchrony of seed maturation.

Overall, the current research highlights the substantial and potentially confounding influence of masting behavior on the reproductive metrics of birch trees. Accounting for these inherent, cyclical patterns in flower, pollen and catkin production will be crucial for accurately assessing the impacts of environmental drivers and developing robust predictive models for birch pollen production.

7.3 Factors affecting birch pollen production

7.3.1 Genetic effects

The findings from Chapter 4 demonstrated that pollen production in birch trees is significantly influenced by their underlying genetics. By studying five groups of genetically identical birch clones of the same age, grown under similar environmental conditions, we observed substantial differences in pollen production per clone group, with estimates ranging from 1.17 million (clone 30) to 1.97 million (clone 42) pollen grains per catkin. We also found that pollen production differs between individuals within the same clone. For example, clone 42 was the most consistent clone regarding P_{ca} (CV = 0.57), whereas clone 30 was associated with a higher coefficient of variance (CV = 0.81). However, these results highlight the importance of genetic factors in controlling pollen production. Clones producing lower amounts of pollen could

contribute to reduced pollen concentrations in the atmosphere, potentially offering an opportunity to mitigate the prevalence of pollen-related allergies. For example, clone 42 would require 118 trees to produce the same pollen amount as 100 average trees, making it a suitable candidate for urban plantations. Conversely, clone 24, which would need only 87 trees to match the pollen production of 100 average trees, could be more suitable for seed production plantations.

The findings in Chapter 4 justify our approach presented in Chapter 6, where IPG network was considered to have birch individuals of same genetic background. However, to confirm the genetic identity, we assessed the sampled trees, which revealed different clones and a larger number of trees with no genetic replicate. Nevertheless, the variability of genetic effects is extremely reduced in our study. It has to be noted that potential epigenetic effects may still contribute to the observed differences in reproductive metrics. Small RNA-mediated mechanisms, for instance, could trigger gene expression changes in response to environmental cues, which could then be passed on to subsequent generations (Duempelmann *et al.* 2020; Vanden Broeck *et al.* 2018). Therefore, future studies should consider the potential role of epigenetic factors in modulating pollen production, in addition to the primary genetic effects. Overall, the findings from these studies emphasize the need to carefully consider the genetic composition of birch populations when investigating the environmental drivers of pollen production. The ability to select and propagate low-pollen producing birch clones could be a valuable strategy for mitigating the burden of pollen-related allergies. Additionally, maintaining genetic variability is crucial, as the adaptability of trees to climate change is higher when genetic variability is high (Leites et al. 2023).

7.3.2 Biotic factors

Biotic factors may influence pollen production by affecting plant physiology. Pollen can carry viruses (Alexander & Cilia 2016) and plant microbes, such as fungi and bacteria (Manirajan *et al.* 2018; Shrestha *et al.* 2023), which have a high potential to alter host metabolism to their advantage. Birches can be infected by various virus species, including badnaviruses, carlaviruses, ilarviruses, idaeoviruses, and nepoviruses. It is

common for birch leaves to be co-infected with multiple viruses, such as cherry leaf roll virus (CLRV) and birch idaeovirus (BIV) (Massart *et al.* 2017; Rumbou *et al.* 2020; Rumbou *et al.* 2021; Büttner *et al.* 2023). CLRV is transported by pollen and is widespread throughout Europe (Bargen *et al.* 2009; Büttner *et al.* 2011; Büttner *et al.* 2013; Rumbou *et al.* 2016). It is believed to be involved in the recent decline of birch trees in Finland (Jalkanen *et al.* 2007).

These pollen-associated viruses might exist in the host plant independent of any abiotic factors (Gilles *et al.* 2023) and might also correlate with environmental factors such as rising temperatures, drought (Roossinck 2015), and air pollutants (Obersteiner *et al.* 2016; Gilles *et al.* 2023). Such biotic stress may alter pollen allergenicity (Pallett *et al.* 2009; Obersteiner *et al.* 2016; Gilles *et al.* 2023). However, the impact of viral infections on the pollen production of affected trees remains largely unknown and a deeper understanding is crucial.

To understand the effect of biotic factors on birch pollen production, we collected leaf samples from all trees under investigation each study year. Based on molecular biological methods, we did not find any symptoms of viral infection in the birch individuals studied in Chapter 4 (results not included in the publication). These analyses were conducted at the Humboldt University of Berlin, leveraging its advanced molecular biology facilities. Analyses for the other two study sites are still ongoing.

7.3.3 Abiotic factors

The understanding of abiotic environmental determinants of pollen production is crucial for investigating trends in the face of future climate change. In this PhD, the influences of different environmental variables on birch pollen production were assessed. The studies conducted in a small seed plantation, along an altitudinal gradient in the European Alps and across Europe revealed varying impacts of temperature and other abiotic factors on birch tree pollen production. Based on these studies, environmental factors appear to affect birch pollen production in different ways, depending on the spatial scale and topographical features.

Temperature

Increasing temperatures have been observed to correlate with earlier flowering and pollination periods for multiple plant taxa (Ziska *et al.* 2003; Ziello *et al.* 2012; D'Amato *et al.* 2015). This advancement in phenology, coupled with elevated atmospheric pollen concentrations across various aeroallergenic plant species has been found to exacerbate respiratory allergies in the northern hemisphere (D'Amato *et al.* 2015; Ziska *et al.* 2019; Damialis *et al.* 2019a). Therefore, understanding the role of temperature in modulating pollen production becomes crucial for mitigating the potential public health impacts of climate change on aeroallergen exposure and allergic respiratory disease burden.

In Chapter 4, the monitored air temperature during preceding summers and the measured air quality within the study site did not vary significantly, likely due to the small study site and the consistent microclimate. Therefore, we were not able to associate air temperature with pollen production. The potential solar radiation received by each selected birch varied within the study site; however, it did not show any significant association with P_{ca}. Therefore, expanding the geographic scope and employing a sophisticated space-for-time (SFT) approach (Pickett 1989), as done in Chapter 6 and also by reducing the genetic variability, is necessary to cover different climatic conditions across regions and better elucidate the temperature-pollen relationships.

Chapter 5 includes the findings on the effects of temperature on birch pollen production. In this study, we observed an increased catkin formation in downy birches at warmer locations (at lower altitudes) during a non-masting year. This result indicates that, in years without any confounding effects of masting behavior, the temperature conditions—particularly those of the preceding summer—can enhance pollen production, as indicated by positive correlation with catkin formation, in birch trees along an altitudinal gradient. This finding is also in accordance with Frei (1998), who suggested that masting can take precedence over the effects of temperature.

The statistical analyses in Chapter 5 revealed minimum temperature as a superior temperature factor than mean and maximum temperatures or even GDD. We also observed a strong and significant association between temperature and altitude, but

the temperature variables showed stronger and more significant correlations with the studied reproductive metrics than altitude alone. This is further evidence that temperature measurements should be part of any gradient study.

The findings in Chapter 5 are specific to a valley-mountain gradient in Bavaria Alps. It has been already anticipated that, due to the multifaceted impact of climate change, birch trees become less common at lower elevations but shift their treeline and become more dominant at higher elevations in the Bavarian Alps over the next half century (Rojo *et al.* 2021). Therefore, continued monitoring and research will be crucial to further elucidate these patterns and their implications for ecosystem dynamics as well as allergy management.

Chapter 6 evaluated downy birch pollen production at a continental-scale and this study could be considered to explain the impacts of environmental factors on birch pollen production across its distribution range during the study period. The model specified by integrating meteorological data with atmospheric gasses appeared the most appropriate for modeling the pollen production metrics. We found that the mean temperature of the previous summer had positive effects on both pollen production and catkin formation, while precipitation increased the number of catkins. The results underscore the ability of birch trees to modulate their physiological processes in order to optimize pollen production under varying temperature regimes. Notably, those individuals exhibiting elevated pollen production levels were associated with increased P_{ca} values in response to higher mean temperatures during the preceding summer. This observation suggests that birch trees may initially exhibit a boost in pollen production as temperatures rise, potentially as an adaptive mechanism. However, it is plausible that there exists a temperature threshold beyond which further increases could impair their physiological performance, leading to a decline in pollen production. These findings could be interpreted such that the examined temperature range is still within the realm of positive impact on pollen production.

The relationship between temperature and pollen production found in Chapter 6 are in line with the findings of Chapter 5 and Damialis *et al.* (2011). Investigations on other species, such as the herbaceous common ragweed (*Ambrosia artemisiifolia*), have demonstrated that increases in temperature were associated with a higher pollen

production (Ziska *et al.* 2003). However, our findings are in contrast with the results of Jochner *et al.* (2013b) and Jetschni *et al.* (2023), who observed a notable decrease in pollen production per catkin in silver birch within urban areas (associated with elevated temperatures and higher pollutant concentrations). The authors argued that conditions in urban areas might have a negative effect on the physiology of birch and thus on pollen production. Birch mainly grows at lower temperatures in mid to high latitudes and might be affected by (very) high temperatures and in turn react with a decrease in pollen production, as also suggested by Ziello *et al.* (2012).

In summary, our major finding in this doctoral research provides evidence that higher temperatures lead to an increase in pollen production in birch trees, as revealed in the investigations conducted in Chapter 5 and Chapter 6. Further studies should focus on birches growing in areas with the highest temperatures to better understand how extreme temperature conditions might influence pollen production.

Carbon dioxide

In addition to the effects of temperature, numerous studies have demonstrated that increased atmospheric CO_2 concentrations can also influence pollen production in various plant species: research has shown that rising CO_2 levels can act as a fertilizer, enhancing the ability of plants to photosynthesize and allocate more resources towards reproductive structures such as pollen (Kudeyarov *et al.* 2006; Drake *et al.* 1997). The increase in pollen production under elevated CO_2 has been observed in studies of ragweed (Ziska & Caulfield 2000; Wayne *et al.* 2002; Rogers *et al.* 2006; Rauer *et al.* 2021), timothy grass (Albertine *et al.* 2014), as well as tree species such as pine (Ladeau & Clark 2006) and oak (Kim *et al.* 2018; Ladeau & Clark 2006).

In tree species, a study on paper birch (*Betula papyrifera*) found that elevated CO_2 concentrations led to a significant increase in the number of trees producing male flowers, suggesting greater pollen production potential (Darbah *et al.* 2008). Similarly, research on sawtooth oak (*Quercus acutissima*) exposed to projected future CO_2 concentration levels of 560 and 720 ppm reported a larger increase in pollen production per tree (Kim *et al.* 2018). It should be considered that those studies are CO_2 enrichment

experiments facilitated by FACE (Free Air Carbon Dioxide Enrichment) or OTCs (open-top chambers).

Our investigation was conducted in a more naturalistic setting without any experimental controls. However, our study was limited in its ability to directly assess the impacts of rising CO_2 on pollen production. The study could only examine the association between regional CO_2 concentration differences (a range of 8 ppm) and pollen production, as a longer time series of pollen production data was not available to capture the effects of larger increases in atmospheric CO_2 over time. It is noteworthy that atmospheric CO_2 levels have risen substantially, increased by over 140 ppm from the pre-industrial era to 2023, primarily due to human activities (National Oceanic and Atmospheric Administration (NOAA) 2023). In this case, our result only revealed an association between elevated CO_2 and P_{ca} at marginal significance.

Nonetheless, the broader body of evidence suggests that, in addition to temperature, elevated CO_2 concentrations can be an important driver of increased pollen production in various plant species, including allergenic taxa. Integrating the effects of both temperature and CO_2 will be crucial for developing a comprehensive understanding of how environmental changes may influence pollen production.

Ozone

Ozone (O_3) concentrations recorded at the two locations in Chapter 4 showed negligible variation, making it unnecessary to evaluate its effect on pollen production. However, the analysis conducted for Chapter 5 unveiled a statistically significant inverse correlation between O_3 and pollen productions, namely P_{ca} and P_{fl}. Acknowledging the limited temporal duration of the measurements, we decided to exclude these findings from the final publication.

The findings presented in Chapter 6, provide valuable insights into the effects of O_3 on the pollen production of birch trees. This is an important consideration, as previous research has demonstrated that plant biomass can be adversely affected by elevated O_3 levels, as found in Scots pine (Manninen *et al.* 2009).

Our study revealed that O_3 has negative impacts on both pollen and catkin production in birch, with the effects varying across different parts of the conditional distribution of the reproductive metrics. Quantile regression analyses showed that the effect of O_3 on P_{ca} was negative for quantiles $\tau \geq 0.5$, while being indistinguishable from zero for lower quantiles ($\tau < 0.5$). Similarly, for C_{su} and P_{vuc}, O_3 exerted a negative effect for quantiles $\tau \geq 0.6$. O_3 is a gaseous pollutant that enters plants via the stomata. High levels of O_3 can lead to oxidative stress in plants and can disrupt photosynthesis (Gandin *et al.* 2021) which could limit pollen production.

These findings align with previous research on the impacts of O_3 on the reproductive outputs of birch species. For example, Darbah *et al.* (2008) found reduced seed mass and germination rates in *Betula papyrifera* under elevated O_3 conditions. In contrast, a study by Ziska (2002) did not detect significant differences in floral biomass of ragweed under high O_3 levels, suggesting species-specific responses.

The observed quantile-dependent effects of O_3 on birch pollen production offer novel insights into the complex relationships between environmental stressors and plant reproductive processes. While the effects of temperature were found to be positive for trees with high pollen production levels but indistinguishable from zero for those with low pollen output, the impacts of O_3 were negative for trees with pollen production above the conditional median but negligible for those with lower pollen levels.

These findings highlight the importance of considering the entire conditional distribution of pollen production metrics, rather than focusing solely on the mean effects. The large confidence regions in the current analysis also emphasize the need for expanded sample sizes in future studies to enhance the reliability of the conclusions.

Overall, the results from Chapter 6 underscore the adverse effects of O_3 on the pollen production of birch trees, particularly for individuals with higher pollen and catkin production. This has important implications for understanding the impacts of air quality on the availability and allergenicity of birch pollen. Further research, potentially incorporating experimental approaches, will be crucial to elucidate the mechanisms

underlying these relationships and inform strategies for mitigating the effects of O_3 on birch pollen dynamics.

For the studies mentioned in Chapter 4 and Chapter 5, air pollutants (O_3 and NO_2) were monitored only during a one-week period in late spring 2020. Air pollutants (O_3, NO_2 and NO_x) sampled in two locations inside Wildberg seed plantation were almost identical and were not considered to investigate their influence on pollen production. In the study mentioned in Chapter 5, significant correlations between O_3 and P_{ca} 2020 (r_s = -0.519, p = 0.033) and P_{fl} 2020 (r_s = -0.489, p = 0.047) as well as between NO_2 and P_{vuc} 2020 (r_s = 0.520, p = 0.032) were observed. Since these results are only based on a short measurement duration, we decided not to incorporate these findings in those two chapters but encourage further research to specifically focus on pollution as a potential influential factor.

Nitrogen dioxide

Air pollutants such as nitrogen dioxide (NO_2) have shown contrasting effects on pollen production across different plant species. While Zhao *et al.* (2017) found that NO_2 induced increased pollen production in *Ambrosia artemisiifolia* L. (common ragweed), Jochner *et al.* (2013b) revealed reduced pollen production in birch trees exposed to higher NO_2 levels in the urban environment of Munich.

In our study (Chapter 6), we initially considered including NO_2 levels in the regression analyses. However, NO_2 exhibited a high correlation (r > 0.6) with temperature. Our reasoning for focusing on temperature was that pollen production is believed to be altered under different temperatures (Sofiev *et al.* 2013; Bruffaerts *et al.* 2018), and earlier studies have also included temperature as an explanatory variable for pollen production (Ranta *et al.* 2008; Tseng *et al.* 2018). Moreover, NO_2 in the troposphere is a precursor of O_3, which we have included in our models in Chapter 6. As nearly all our study sites (IPGs) were located in rural areas with trees relatively far from traffic sources (see coordinates in Chapter 6, Annex), we generally expect higher O_3 concentrations due to reduced degradation by short-lived nitrogen oxides (NO) emitted from urban traffic and industrial sources (Sillman 1999; Fritsch & Behm 2021; Carmona-Cabezas *et*

al. 2023). Consequently, the occurrence of O_3 and NO_2 in the air is related, and we assume that including O_3 along with temperature accounts for the potential effect of NO_2 in our models.

Additional abiotic factors and methodological constraints

The effects of the above-mentioned factors on pollen production are important; nonetheless, they may not be the sole determinants of pollen production. The influence of these factors can be obscured or reduced by the presence of a variety of additional abiotic factors, the impacts of which are not yet well understood.

Furthermore, even trees at similar altitudes can be exposed to different microclimatic conditions, which might affect their reproductive traits. A study in alpine environments found significant fluctuations in soil temperature of up to 4°C depending on slope aspect and topography (Scherrer *et al.* 2011). In addition, site characteristics such as stand density and exposure can also influence pollen production (Faegri & Iversen 1989).

In summary, there might be more environmental variables that stress plants' physiology, for example, drought, soil nutrient surplus or deficiency, and solar radiation, may further influence pollen production (Pandey *et al.* 2015; Li *et al.* 2021; Seleiman *et al.* 2021). This underscores the importance of further studies considering a broader range of environmental and site-specific variables to enhance our understanding of the determinants of pollen production.

To deepen this understanding, especially in relating atmospheric gases to pollen production, site-specific measurements should be made on each individual plant sampled, rather than deriving these data from a land use regression model (Fritsch & Behm 2021). Nonetheless, land use regression models are highly useful when such data are not available.

Additionally, experimental research designed to isolate and quantify the effects of individual factors on pollen production, while avoiding potential masking effects, could provide valuable insights into the underlying mechanisms. Although conducting experimental studies on mature birch trees poses a challenge due to the difficulty in

relocating them to laboratory conditions, as they attain sexual maturity and begin producing male catkins from approximately 10 to 15 years of age (Perala & Alm 1990), some innovative approaches have been employed. For instance, FACE experiments have been utilized to investigate the effects of elevated atmospheric CO_2 concentrations on bud physiology (Riikonen *et al.* 2008) and reproduction (Darbah *et al.* 2008) in birch trees growing in natural field conditions. Such in situ experimental setups, while logistically challenging, can provide valuable insights into the responses of mature birch trees to specific environmental factors, complementing observational studies and controlled experiments on seedlings or saplings.

7.4   Temperature gradients

Temperature gradients such as altitudinal and longitudinal and latitudinal gradients are accompanied by a simultaneous shift in various abiotic and biotic parameters, and this multifaceted change in environmental conditions, rather than the gradient alone, might exert a profound influence on pollen production.

Altitudinal gradients

In our studies, we did not observe "altitude" as an influential driver of pollen production. Chapter 5 was based on an altitudinal gradient in the European Alps. In this study, we found no significant changes in the analyzed reproductive traits of *B. pubescens* with increasing altitude. Another study, which we have conducted at a seed plantation (Chapter 4) was performed at a small site (1 ha) without distinct variations in the topography. Chapter 6 included samples from trees located at 13 m a.s.l to 967 m a.s.l across Europe. However, such elevational variations lack a continuous gradient and could not be reliably used for an altitudinal gradient study. Moreover, sites at the same altitude but from different geographic regions can vary significantly due to factors such as latitude, climate, and local topography.

Previous studies investigating pollen production along altitudinal gradients have shown mixed results, with some that found a decrease in pollen production with increased elevation (Markgraf 1980; Moe 1998), others were linked to an increase (Aguilera & Valenzuela 2012), and some found no significant change (Charalampopoulos *et al.* 2013;

Hasegawa *et al.* 2022). Aguilera & Valenzuela (2012) argued that higher olive pollen production observed at higher areas might be related to intrinsic mechanisms of these trees to compensate for a limited pollination efficiency and a shorter growing period. However, human interventions (topping, pruning, cutting, etc.) which can have a masking effect on pollen production (as in Chapter 4), may also have influenced these results as olive is a highly cultivated species.

Pollen and inflorescence traits at the higher reproductive level (e.g., per individual tree) decreased with increasing altitude in some previous studies, such as on Mount Olympos (Charalampopoulos *et al.* 2013). The highest reproductive level studied in Chapter 5 was crown volume per unit (1 m^3) and the result indicated that the number of male inflorescences per crown volume unit (C_{vuc}) in 2021 decreased along the gradient. This suggests that pollen production by individual inflorescences may be more affected by pollen limitation compensation, as found in smaller *Quercus* species (Fernández-González *et al.* 2020).

Our results are based on a shorter altitudinal gradient of 522 m and fewer birches; however, we have provided valuable insights into the complex relationship between altitude and pollen production. Due to the complex topography and unique microclimate formed by the surrounding terrain, the relationship between elevation gradient and pollen production in our study area cannot be easily compared to other alpine locations.

Spatial gradient

In Chapter 6, we employed a space-for-time approach, where spatial gradients of IPGs across Europe were used as proxies for temporal changes. We illustrated the geographic distribution of P_{ca} across the selected IPGs. The discerned patterns in P_{ca} across the studied geographic underscored a substantial spatial variability, even at relatively small scales. Notably, the variation in P_{ca} values observed among trees in close proximity, such as Freyung-Schönbrunn and Freyung-Waldhäuser (located only 7.2 km apart), accentuated the impact of localized factors on pollen production, as discussed in earlier chapters. Despite the comprehensive coverage across Europe in Chapter 6, it is

imperative to acknowledge the limitation posed by the uneven spatial distribution of the studied IPGs.

Moreover, some studies across urban-rural gradients (and thus with a smaller spatial extend than our study focusing at European scale) have found significant spatial variations (Jochner *et al.* 2013b; Jetschni *et al.* 2023). Specially, those studies have highlighted pronounced spatial variations in birch pollen production across urban-rural gradients, with pollen production decreasing as urbanization and temperature levels increase. As additional influential factors, such as enhanced air pollution in urban areas, may be of higher importance for these kinds of studies, the necessity of experimental studies is highlighted as well.

7.5   Silviculture

Silvicultural activities such as topping and pruning can have an influence on pollen production since such operations might induce stress on plant physiology, which might result in a higher reproductive output. Topping and pruning have been considered as adequate tree crown management techniques to enhance seed production in seed orchards or to promote the branching of the trees (Viherä-Aarnio & Ryynänen 1994; Stoehr *et al.* 1995; Li *et al.* 2021). As found in Chapter 4, effects on pollen production and catkin formation were especially obvious two years after topping the tree crowns. For example, P_{cr} was 70% higher, and C_{su} was 44% higher for topped trees compared to non-topped trees. Sole pruning treatments were not conducted at the seed plantation, therefore its effect on pollen production could not be investigated. Information on whether silvicultural treatments were applied to the birch trees studied in Chapter 5 and Chapter 6 was not available.

Viherä-Aarnio & Ryynänen (1994) studied seed production of silver birch in a greenhouse experiment for five years. As a part of the experiment, the plant individuals were topped in the second year. A ten-time higher amount of seeds per plant was obtained in the fourth year as compared to the third year. Further, they observed the following year with poor flowering and seed production. Since the study in Chapter 4 was only conducted for three years, it was not possible to conclude on the long-term effects of topping on pollen production. A longer monitoring period is recommended in

further studies to better understand the impacts of such silvicultural practices on pollen production over time.

7.6 Methodological limitations and recommendations for future studies

While the studies presented in this PhD thesis contribute valuable insights into birch pollen production, several methodological limitations should be acknowledged. One inherent limitation lies in the estimation of pollen production through manual counting of pollen grains on microscopic slides, a process that is both time-consuming and labor-intensive. Although this method has been widely employed in many studies (Damialis *et al.* 2011; Charalampopoulos *et al.* 2013; Kolek 2021; Jetschni *et al.* 2023; Eisen *et al.* 2024), the development and integration of more efficient pollen counting techniques, such as automated cell counters (Kakui *et al.* 2021) or machine learning-based algorithms for automatic identification and quantification of pollen grains (Kadaikar *et al.* 2019), could substantially enhance the efficiency and scalability of pollen production studies.

Another limitation arises from the decision to exclude reproductive measure per individual tree from the analysis. While pollen production per tree could provide valuable insights, it is strongly dependent on the age and height of the tree, which varied considerably in Chapter 5 and 6. Moreover, the process of extrapolating pollen production estimates to the entire tree level introduces a degree of uncertainty due to the simplified geometric assumptions of the crown shape employed in the calculations (Molina *et al.* 1996). Instead, the focus was placed on volume-based variables, which are considered superior indicators of pollen production as they account for both the pollen per catkin and the number of catkins in a standardized volume. However, further studies could incorporate advanced methods such as photogrammetry or terrestrial laser scanning (TLS) to accurately capture the precise tree structure and catkin distribution across the entire tree crown. These techniques could potentially provide more accurate estimates of pollen production at the individual tree level, complementing the volume-based approach employed in the current study.

The geographic coverage of the studies is limited by a shorter range of altitudinal gradient (only 522 m), with an uneven distribution across altitudes in the Bavarian Alps

and the uneven spatial distribution of the IPGs across Europe, resulting in an inadequate representation of birch trees from certain locations and regions.

For the study mentioned in Chapter 5, our initial study site was a >1000 m altitudinal gradient in Davos, Switzerland. In summer 2019, we selected 30 birch trees along this gradient, measured tree morphometry, leaf chlorophyll, collected leaf samples, and deployed to monitor micrometeorological conditions. We obtained necessary permits from local Davos authorities. However, the 2020 sampling campaign was halted due to COVID-19 travel restrictions closing the Germany-Switzerland border. Consequently, we promptly changed the study site to the Bavarian Alps in Germany.

Despite these limitations, the studies presented in this PhD thesis offer valuable insights into spatiotemporal patterns and drivers of birch pollen production, contributing to our understanding of the potential impacts of global change on this important aeroallergen.

Future research endeavors would greatly benefit from incorporating larger altitudinal gradients across multiple sites in the European Alps and other regions beyond the IPGs, spanning observations over several years. Furthermore, in natural settings, there exists a broader genetic diversity, demanding the incorporation of samples from diverse provenances to capture the potential range of birch pollen production values across Europe. Although, the study conducted at the IPGs was important to get detailed information on the importance of single influential variables, it does not account for local adaption to the prevailing environmental conditions. Thus, further studies on pollen production including genetic variability would broaden our understandings. In fact, as birch is not the only allergenic species that is of importance in Europe or the world, researchers should be encouraged to focus on more species. The outlined methods and approaches of this thesis may be of great help.

8   Conclusions

This thesis provided a comprehensive investigation into the key factors influencing pollen, flower, and catkin production in birch species. The studies presented are novel, encompassing research sites ranging from a small seed plantation, through an altitudinal gradient along the Bavarian Alps, to a large geographical region across Europe. We presented robust estimates of reproductive outputs for birch species. Our findings reveal that, in general, *B. pendula* produces lower mean P_{ca} compared to *B. pubescens*. Additionally, considerable year-to-year variations were observed in pollen and catkin production.

The strategic control of genetic variabilities within clone groups in the study from Chapter 4 yielded promising insights, highlighting the meaningful influence of genetics on pollen production in birch trees. This allowed us to apply these findings and select IPG birch trees with reduced genetic variability for the study in Chapter 6. The main findings of this thesis highlighted that higher temperatures generally increase birch pollen production. However, other environmental stressors, such as O_3, and inherent biological processes such as masting behavior also were important in affecting pollen production of birch trees.

We concluded that further climate warming might exacerbate the adverse effects on allergic individuals. This thesis contributes to a comprehensive understanding of the complex interplay between genetic and environmental factors governing pollen, flower, and catkin production in birch trees, providing valuable insights for future research and potential mitigation strategies. Pollen production estimates can also be used as input for pollen transport models, which simulate the dispersal and movement of pollen through the atmosphere. Future research should prioritize how individuals and communities can adapt to increasing pollen and allergen levels, for instance, by using advanced pollen forecasts or designing urban areas with allergen-free vegetation. This thesis also highlights the critical need for collaboration among climatologists, ecologists, biologists, medical researchers, public health professionals, and statisticians to effectively address these multifaceted challenges.

9   References

Aboulaich N, Bouziane H, Kadiri M, Del Mar Trigo M, Riadi H, Kazzaz M, Merzouki A (2009) Pollen production in anemophilous species of the Poaceae family in Tetouan (NW Morocco). *Aerobiologia*, **25**, 27–38. https://link.springer.com/article/10.1007/s10453-008-9106-2.

Adams GW, Kunze HA (1996) Clonal variation in cone and seed production in black and white spruce seed orchards and management implications. *The Forestry Chronicle*, **72**, 475–480.

Aguilera F, Valenzuela LR (2012) Microclimatic-induced fluctuations in the flower and pollen production rate of olive trees (*Olea europaea* L.). *Grana*, **51**, 228–239.

Ahlholm JU, Helander ML, Savolainen J (1998) Genetic and environmental factors affecting the allergenicity of birch (*Betula pubescens* ssp. *czerepanovii* [Orl.] Hämet-ahti) pollen. *Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology*, **28.** https://pubmed.ncbi.nlm.nih.gov/9824411/.

Albertine JM, Manning WJ, DaCosta M, Stinson KA, Muilenberg ML, Rogers CA (2014) Projected carbon dioxide to increase grass pollen and allergen exposure despite higher ozone levels. *PLOS ONE*, **9**, e111712. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0111712.

Albrich K, Rammer W, Seidl R (2020) Climate change causes critical transitions and irreversible alterations of mountain forests. *Global Change Biology*, **26**, 4013–4027. https://onlinelibrary.wiley.com/doi/full/10.1111/gcb.15118.

Alexander MM, Cilia M (2016) A molecular tug-of-war: Global plant proteome changes during viral infection. *Current Plant Biology*, **5**, 13–24. https://www.sciencedirect.com/science/article/pii/S2214662815000146.

Ali AM, Rooney P, Hawkins JA (2022) Automatically counting pollen and measuring pollen production in some common grasses. *Aerobiologia*, **38**, 433–455. https://link.springer.com/article/10.1007/s10453-022-09758-3.

Allen RB, Hurst JM, Portier J, Richardson SJ (2014) Elevation-dependent responses of tree mast seeding to climate change over 45 years. *Ecology and evolution*, **4**, 3525–3537. https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.1210.

Allen RB, Mason NWH, Richardson SJ, Platt KH (2012) Synchronicity, periodicity and bimodality in inter-annual tree seed production along an elevation gradient. *Oikos*, **121**, 367–376.

Allison TD (1990) Pollen Production and Plant Density Affect Pollination and Seed Production in *Taxus canadensis*. *Ecology*, **71**, 516–522.

Amaral J, Ribeyre Z, Vigneaud J *et al.* (2020) Advances and Promises of Epigenetics for Forest Trees. *Forests*, **11**, 976.

Atkinson MD (1992) *Betula pendula* Roth (*B. Verrucosa* Ehrh.) and *B. pubescens* Ehrh. *The Journal of Ecology*, **80**, 837.

Auguie B, Antonov A gridExtra: Miscellaneous Functions for "Grid" Graphics [R package gridExtra version 2.3]. Available via https://cran.r-project.org/web/packages/gridExtra/index. html.

Bargen S, Grubits E, Jalkanen R, Büttner C (2009) Cherry leaf roll virus – an emerging virus in Finland? *Silva Fennica*, **43**.

Barnes CS (2018) Impact of Climate Change on Pollen and Respiratory Disease. *Current allergy and asthma reports*, **18**, 59.

Battisti L, Pille L, Wachtel T, Larcher F, Säumel I (2019) Residential Greenery: State of the Art and Health-Related Ecosystem Services and Disservices in the City of Berlin. *Sustainability*, **11**, 1815. https://www.mdpi.com/2071-1050/11/6/1815.

REFERENCES

BAYSICS Webportal (2022) BAYSICS Webportal. Tree Line Analyses. Available via https://www.portal.baysics.de/map/. Accessed 17 Nov 2022.

Beck P, Caudullo G, Rigo D de, Tinner W (2016) *Betula pendula*, *Betula pubescens* and other birches in Europe: distribution, habitat, usage and threats. Publication Office of the European Union, Luxembourg, 70-73.

Beggs PJ (2004) Impacts of climate change on aeroallergens: past and future. *Clinical & Experimental Allergy*, **34**, 1507–1513. https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2222.2004.02061.x.

Beutner C, Werchan B, Forkel S, Gupta S, Fuchs T, Schön MP, Geier J, Buhl T (2021) Sensitization rates to common inhaled allergens in Germany - increase and change patterns over the last 20 years. *Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG*, **19**, 37–44.

Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E (2019) Birch pollen allergy in Europe. *Allergy*, **74**, 1237–1248. https://onlinelibrary.wiley.com/doi/10.1111/all.13758.

Bogawski P, Grewling Ł, Dziób K *et al.* (2019) Lidar-Derived Tree Crown Parameters: Are They New Variables Explaining Local Birch (*Betula* sp.) Pollen Concentrations? *Forests*, **10**, 1154. https://www.mdpi.com/1999-4907/10/12/1154/htm#B78-forests-10-01154.

Bogawski P, Grewling Ł, Frątczak A (2016) Flowering phenology and potential pollen emission of three *Artemisia* species in relation to airborne pollen data in Poznań (Western Poland). *Aerobiologia*, **32**, 265–276. https://link.springer.com/article/10.1007/s10453-015-9397-z.

Bogdziewicz M, Ascoli D, Hacket-Pain A *et al.* (2020) From theory to experiments for testing the proximate mechanisms of mast seeding: an agenda for an experimental ecology. *Ecology Letters*, **23**, 210–220. https://onlinelibrary.wiley.com/doi/10.1111/ele.13442.

Bogdziewicz M, Hacket-Pain A, Ascoli D, Szymkowiak J (2021) Environmental variation drives continental-scale synchrony of European beech reproduction. *Ecology*, **102**, e03384.

Bothwell HM, Evans LM, Hersch-Green EI, Woolbright SA, Allan GJ, Whitham TG (2021) Genetic data improves niche model discrimination and alters the direction and magnitude of climate change forecasts. *Ecological Applications*, **31**, e02254. https://esajournals.onlinelibrary.wiley.com/doi/full/10.1002/eap.2254.

Bousquet J, Khaltaev N, Cruz AA *et al.* (2008) Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*, **63 Suppl 86**, 8–160.

Bradshaw HD, Stettler RF (1995) Molecular genetics of growth and development in populus. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. *Genetics*, **139**, 963–973. http://dx.doi.org/10.1093/genetics/139.2.963.

Bruffaerts N, Smedt T de, Delcloo A *et al.* (2018) Comparative long-term trend analysis of daily weather conditions with daily pollen concentrations in Brussels, Belgium. *International Journal of Biometeorology*, **62**, 483–491. https://link.springer.com/content/pdf/10.1007/s00484-017-1457-3.pdf.

Bucher SF, König P, Menzel A, Migliavacca M, Ewald J, Römermann C (2018) Traits and climate are associated with first flowering day in herbaceous species along elevational gradients. *Ecology and evolution*, **8**, 1147–1158.

Buters JT, Thibaudon M, Smith M *et al.* (2012) Release of Bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study. *Atmospheric Environment*, **55**, 496–505. https://www.sciencedirect.com/science/article/pii/S1352231012000751.

Büttner C, Bargen S von, Bandte M, Mühlbach HP (2013) Forest diseases caused by viruses. In: *Infectious forest diseases* (eds Gonthier P, Nicolotti G), pp 50–75. CABI, Wallingford, UK, Boston, MA.

REFERENCES

Büttner C, Bargen S von, Bandte M, Myrta A (2011) CHAPTER 24: Cherry leaf roll virus. In: *Virus and virus-like diseases of pome and stone fruits* (eds Hadidi A, Barba M, Candresse T, Jelkmann W), pp 119–125. American Phytopathological Society, St. Paul, Minn.

Büttner C, Landgraf M, Fernandez Colino HL, Bargen S von, Bandte M Virus diseases of forest and urban trees. Forest Microbiology, 61–97.

Cade BS, Noon BR (2003) A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment*, **1**, 412–420. https://pubs.usgs.gov/publication/1015331.

Caffarra A, Donnelly A (2011) The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. *International Journal of Biometeorology*, **55**, 711–721. https://link.springer.com/article/10.1007%2Fs00484-010-0386-1.

Cai G, Del Duca S (2019) Pollen Tube and Plant Reproduction. *International Journal of Molecular Sciences*, **20**.

Carmona-Cabezas R, Gomez-Gomez J, Ariza-Villaverde AB, Rave EG de, Jimenez-Hornero FJ (2023) Can complex networks describe the urban and rural tropospheric O_3 dynamics?

Castiñeiras P, Vázquez-Ruiz RA, Fernández-González M, Rodríguez-Rajo FJ, Aira MJ (2019) Production and viability of *Fraxinus* pollen and its relationship with aerobiological data in the northwestern Iberian Peninsula. *Aerobiologia*, **35**, 227–241. https://link.springer.com/article/10.1007/s10453-018-09553-z.

Caudullo G, Welk E, San-Miguel-Ayanz J (2017) Chorological maps for the main European woody species. *Data in Brief*, **12**, 662–666. https://www.sciencedirect.com/science/article/pii/S2352340917301981.

Cerlini PB, Saraceni M, Orlandi F, Silvestri L, Fornaciari M (2022) Phenological response to temperature variability and orography in Central Italy. *International Journal of Biometeorology*, **66**, 71–86. https://link.springer.com/article/10.1007/s00484-021-02190-1.

Charalampopoulos A, Damialis A, Tsiripidis I, Mavrommatis T, Halley JM, Vokou D (2013) Pollen production and circulation patterns along an elevation gradient in Mt Olympos (Greece) National Park. *Aerobiologia*, **29**, 455–472.

Cornes RC, van der Schrier G, van den Besselaar EJ, Jones PD (2018) An Ensemble Version of the E-OBS Temperature and Precipitation Data Sets. *Journal of Geophysical Research: Atmospheres*, **123**, 9391–9409.

Crone EE, Miller E, Sala A (2009) How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. *Ecology Letters*, **12**, 1119–1126.

Crone EE, Rapp JM (2014) Resource depletion, pollen coupling, and the ecology of mast seeding. *Annals of the New York Academy of Sciences*, **1322**, 21–34. https://nyaspubs.onlinelibrary.wiley.com/doi/full/10.1111/nyas.12465.

Cuevas J, Polito VS (2004) The role of staminate flowers in the breeding system of *Olea europaea* (Oleaceae): an andromonoecious, wind-pollinated taxon. *Annals of Botany*, **93**, 547–553.

D'Amato G, Cecchi L, Bonini S *et al.* (2007) Allergenic pollen and pollen allergy in Europe. *Allergy*, **62**, 976–990. https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1398-9995.2007.01393.x.

D'Amato G, Vitale C, Sanduzzi A, Molino A, Vatrella A, D'Amato M (2017) Allergenic Pollen and Pollen Allergy in Europe. In: *Allergy and allergen immunotherapy.* New mechanisms and strategies / edited by A.B. Singh (ed Singh AB), pp 287–306. Apple Academic Press, Toronto.

REFERENCES

Dahl Å, Strandhede S-O (1996) Predicting the intensity of the birch pollen season. *Aerobiologia*, **12**, 97–106. http://dx.doi.org/10.1007/bf02446601.

D'Amato G, Chong-Neto HJ, Monge Ortega OP *et al.* (2020) The effects of climate change on respiratory allergy and asthma induced by pollen and mold allergens. *Allergy*, **75**, 2219–2228. https://onlinelibrary.wiley.com/doi/full/10.1111/all.14476.

D'Amato G, Spieksma FT, Liccardi G *et al.* (1998) Pollen-related allergy in Europe. *Allergy*, **53**, 567–578.

D'Amato G, Vitale C, Martino A de *et al.* (2015) Effects on asthma and respiratory allergy of Climate change and air pollution. *Multidisciplinary Respiratory Medicine*, **10**, 39. https://mrmjournal.biomedcentral.com/counter/pdf/10.1186/s40248-015-0036-x.

Damialis A, Charalampopoulos A, Lazarina M *et al.* (2020) Plant flowering mirrored in airborne pollen seasons? Evidence from phenological observations in 14 woody taxa. *Atmospheric Environment*, **240**, 117708. https://www.sciencedirect.com/science/article/pii/S1352231020304404.

Damialis A, Fotiou C, Healley JM, Vokou D (2011) Effects of environmental factors on pollen production in anemophilous woody species. *Trees*, **25**, 253–264.

Damialis A, Häring F, Gökkaya M *et al.* (2019a) Human exposure to airborne pollen and relationships with symptoms and immune responses: Indoors versus outdoors, circadian patterns and meteorological effects in alpine and urban environments. *Science of The Total Environment*, **653**, 190–199. http://www.sciencedirect.com/science/article/pii/S0048969718342669.

Damialis A, Traidl-Hoffmann C, Treudler R (2019b) Climate Change and Pollen Allergies. In: *Biodiversity and Health in the Face of Climate Change* (eds Marselle MR, Stadler J, Korn H, Irvine KN, Bonn A), pp 47–66. Springer, Cham.

Darbah JN, Kubiske ME, Nelson N, Oksanen E, Vapaavuori E, Karnosky DF (2008) Effects of decadal exposure to interacting elevated CO_2 and/or O_3 on paper birch (*Betula papyrifera*) reproduction. *Environmental pollution (Barking, Essex : 1987)*, **155**, 446–452.

Davies J, Matricardi P, Schmid J (2016) Grass pollen allergy. *Molecular allergology user's guide*, 85–94.

Davy AJ, Gill JA (1984) Variation due to environmental and heredity in birch transplanted between heath and bog. *New Phytologist*, **97**, 489–505.

Derby RW, Gates DM (1966) The temperature of tree trunks—calculated and observed. *American Journal of Botany*, **53**, 580–587.

Detandt M, Nolard N (2000) The fluctuations of the allergenic pollen content of the air in Brussels (1982 to 1997). *undefined.* https://www.semanticscholar.org/paper/The-fluctuations-of-the-allergenic-pollen-content-Detandt-Nolard/76f019a0802f65df73b5ccee04798bb608cc7376.

Dobbertin M (2005) Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. *European Journal of Forest Research*, **124**, 319–333. https://link.springer.com/article/10.1007/s10342-005-0085-3.

Drake BG, Gonzalez-Meler MA, Long SP (1997) MORE EFFICIENT PLANTS: A Consequence of Rising Atmospheric CO_2? *Annual review of plant physiology and plant molecular biology*, **48**, 609–639.

Duempelmann L, Skribbe M, Bühler M (2020) Small RNAs in the Transgenerational Inheritance of Epigenetic Information. *Trends in Genetics*, **36**, 203–214. http://www.cell.com/article/S0168952519302598/pdf.

REFERENCES

Dunnington D, Thorne B, Hernangomez D Spatial Data Framework for ggplot2 [R package ggspatial version 1.1.9]. Available via https://cran.r-project.org/web/packages/ggspatial/index.html.

DWD (1991) Anleitung für Die Phänologischen Beobachter des Deutschen Wetterdienstes. DeutscherWetterdienst: Offenbach am Main, Germany.

DWD (2022-02-12) Deutscher Wetterdienst/German Meteorological Service. Available via https://www.dwd.de/DE/Home/home_node.html. Accessed 12 Feb 2022.

Eisen A-K, Buchner L, Fussi B, Jochner-Oette S (2024) Does ash dieback affect the reproductive ecology of Fraxinus excelsior L.? *Journal of Forestry Research*, **35**, 1–16. https://link.springer.com/content/pdf/10.1007/s11676-023-01670-x.pdf.

Emberlin J, Detandt M, Gehrig R, Jaeger S, Nolard N, Rantio-Lehtimäki A (2002) Responses in the start of Betula (birch) pollen seasons to recent changes in spring temperatures across Europe. *International Journal of Biometeorology*, **46**, 159–170.

Engler R, Randin CF, Thuiller W *et al.* (2011) 21st century climate change threatens mountain flora unequally across Europe. *Global Change Biology*, **17**, 2330–2341.

Erdtman G (1954) An introduction to pollen analysis. Chronica Botanica, Waltham Mass.

Eriksson G, Black-samuelsson S, Jensen M, Myking T, Rusanen M, Skrøppa T, Vakkari P, Westergaard L (2003) Genetic Variability in Two Tree Species, *Acer platanoides* L. and *Betula pendula* Roth, With Contrasting Life-history Traits. *Scandinavian Journal of Forest Research*.

Estrella N, Menzel A (2006) Responses of leaf colouring in four deciduous tree species to climate and weather in Germany. *Climate Research*, **32**, 253–267.

Faegri K (1966) Some problems of representivity in pollen analysis. http://14.139.63.228:8080/pbrep/handle/123456789/522.

Faegri K, Iversen J (1989) Textbook of pollen analysis. Wiley.

Fernández-González M, González-Fernández E, Ribeiro H, Abreu I, Rodríguez-Rajo FJ (2020) Pollen Production of Quercus in the North-Western Iberian Peninsula and Airborne Pollen Concentration Trends during the Last 27 Years. *Forests*, **11**, 702.

Ferrara G, Camposeo S, Palasciano M, Godini A (2007) Production of total and stainable pollen grains in Olea europaea L. *Grana*, **46**, 85–90.

Fotiou C, Damialis A, Krigas N, Halley JM, Vokou D (2011) *Parietaria judaica* flowering phenology, pollen production, viability and atmospheric circulation, and expansive ability in the urban environment: impacts of environmental factors. *International Journal of Biometeorology*, **55**, 35–50. https://link.springer.com/article/10.1007/s00484-010-0307-3.

Frankie GW, Thorp RW (2009) Pollination and Pollinators. In: *Encyclopedia of insects* (eds Cardé RT, Resh VH), pp 813–819. Academic Press, London.

Frei T (1998) The effects of climate change in Switzerland 1969–1996 on airborne pollen quantities from hazel, birch and grass. *Grana*, **37**, 172–179.

Frenguelli G (2004) Pollen structure and morphology. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*, **20**, 200–204. https://www.termedia.pl/Journal/-7/pdf-2107-1?filename=Pollen stru.pdf.

Frenne P de, Graae BJ, Rodríguez-Sánchez F *et al.* (2013) Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology*, **101**, 784–795.

Friedman WE (1993) The evolutionary history of the seed plant male gametophyte. *Trends in Ecology & Evolution*, **8**, 15–21.

Fritsch M, Behm S (2021) Agglomeration and infrastructure effects in land use regression models for air pollution – Specification, estimation, and interpretations. *Atmospheric Environment*, **253**, 118337.

REFERENCES

Gadermaier G, Dedic A, Obermeyer G, Frank S, Himly M, Ferreira F (2004) Biology of weed pollen allergens. *Current allergy and asthma reports*, **4**, 391–400.

Galán C, Ariatti A, Bonini M *et al.* (2017) Recommended terminology for aerobiological studies. *Aerobiologia*, **33**, 293–295. https://pennstate.pure.elsevier.com/en/publications/recommended-terminology-for-aerobiological-studies.

Gandin A, Dizengremel P, Jolivet Y (2021) Integrative role of plant mitochondria facing oxidative stress: The case of ozone. *Plant physiology and biochemistry : PPB*, **159**, 202–210.

García-Cruzatty LC, Vera-Pinargote L, Zambrano-Gavilanes F, Zamora-Macías A, Cedeño-Ortega J (2020) Pollen Production in *Theobroma cacao* L. Genotypes National Type and CCN-51 and Its Relationship with Climatic Factors on the Ecuadorian Coast. *Acta Agrobotanica*, **73**. https://pbsociety.org.pl/journals/index.php/aa/article/view/aa.7323.

Gassner M, Schmid-Grendelmeier P, Clot B (2019) Ash pollen allergy and aerobiology. *Allergo Journal International*, **28**, 289–298. https://link.springer.com/content/pdf/10.1007/s40629-019-00105-6.pdf.

Geburek T, Hiess K, Litschauer R, Milasowszky N (2012) Temporal pollen pattern in temperate trees: expedience or fate? *Oikos*, **121**, 1603–1612.

Gehrig R, Peeters AG (2000) Pollen distribution at elevations above 1000 m inSwitzerland. *Aerobiologia*, **16**, 69–74. https://link.springer.com/article/10.1023/A:1007607121614.

Gilles S, Meinzer M, Landgraf M *et al.* (2023) *Betula pendula* trees infected by birch idaeovirus and cherry leaf roll virus: Impacts of urbanisation and NO_2 levels. *Environmental pollution (Barking, Essex : 1987)*, **327**, 121526. https://www.sciencedirect.com/science/article/pii/S0269749123005286.

Gómez-Casero MT, Hidalgo PJ, García-Mozo H, Domínguez E, Galán C (2004) Pollen biology in four Mediterranean *Quercus* species. *Grana*, **43**, 22–30.

González-Fernández E, Piña-Rey A, Fernández-González M, Aira MJ, Rodríguez-Rajo FJ (2020) Prediction of Grapevine Yield Based on Reproductive Variables and the Influence of Meteorological Conditions. *Agronomy*, **10**, 714. https://www.mdpi.com/2073-4395/10/5/714/htm.

González-Parrado Z, Fernández-González D, Vega-Maray AM, Valencia-Barrera RM (2015) Relationship between flowering phenology, pollen production and atmospheric pollen concentration of Plantago lanceolata (L.). *Aerobiologia*, **31**, 481–498. https://link.springer.com/article/10.1007/s10453-015-9377-3.

Goto Y, Kondo T, Hayashi E, Kuramoto N, Takahashi M, Yasueda H (2004) Influences of genetic and environmental factors on the concentration of the allergen Cry j 1 in sugi (*Cryptomeria japonica*) pollen. *Tree Physiology*, **24**, 409–414. https://academic.oup.com/treephys/article/24/4/409/1682603.

Guedes A, Ribeiro N, Ribeiro H, Oliveira M, Noronha F, Abreu I (2009) Comparison between urban and rural pollen of *Chenopodium alba* and characterization of adhered pollutant aerosol particles. *Journal of Aerosol Science*, **40**, 81–86. https://www.sciencedirect.com/science/article/pii/S0021850208001717.

Hasegawa TM, Itagaki T, Sakai S (2022) Intraspecific variation in morphology of spiny pollen grains along an altitudinal gradient in an insect-pollinated shrub. *Plant Biology.* https://onlinelibrary.wiley.com/doi/full/10.1111/plb.13493.

Helbig N, Vogel B, Vogel H, Fiedler F (2004) Numerical modelling of pollen dispersion on the regional scale. *Aerobiologia*, **20**, 3–19. https://idp.springer.com/authorize/casa?redirect_uri=https://link.springer.com/article/10.

REFERENCES

1023/b:aero.0000022984.51588.30&casa_token=bvlotuppsfaaaaaa:tlfkkmzkcajvztdd3dxyyq chrrgxsakc3j650x0edap29bwxpoydvw3p7fqszypnb_6ql12rrf74nhlz.

Hicks S, Helander M, Heino S (1994) Birch pollen production, transport and deposition for the period 1984–1993 at Kevo, northernmost Finland. *Aerobiologia*, **10**, 183–191. http://dx.doi.org/10.1007/bf02459234.

Hidalgo PJ, Galán C, Domínguez E (1999) Pollen production of the genus *Cupressus*. *Grana*, **38**, 296–300.

Holm SO (1994) Reproductive variability and pollen limitation in three *Betula* taxa in northern Sweden. *Ecography*, **17**, 73–81.

Huynen M, Menne B, Behrendt H *et al.* (2003) Phenology and human health: allergic disorders. https://research.wur.nl/en/publications/phenology-and-human-health-allergic-disorders.

Hynynen J, Niemistö P, Viherä-Aarnio A, Brunner A, Hein S, Velling P (2010) Silviculture of birch (*Betula pendula* Roth and *Betula pubescens* Ehrh.) in northern Europe. *Forestry: An International Journal of Forest Research*, **83**, 103–119. https://academic.oup.com/forestry/article/83/1/103/546795.

Isagi Y, Sugimura K, Sumida A, Ito H (1997) How Does Masting Happen and Synchronize? *Journal of Theoretical Biology*, **187**, 231–239.

Jablonski LM, Wang X, Curtis PS (2002) Plant reproduction under elevated CO 2 conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist*, **156**, 9–26.

Jalkanen R, Büttner C, Bargen S von (2007) Cherry leaf roll virus abundant on Betula pubescens in Finland. http://jukuri.luke.fi/bitstream/10024/532640/1/cherry.pdf.

Jato V, Rodriguez-Rajo FJ, Aira MJ (2007) Use of phenological and pollen-production data for interpreting atmospheric birch pollen curves. *Annals of Agricultural and Environmental Medicine*, **14**. https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-article-18f5be1d-8c5f-4d24-8c9f-2ede616935e6/c/14271.pdf.

Jetschni J, Fritsch M, Jochner-Oette S (2023) How does pollen production of allergenic species differ between urban and rural environments? *International Journal of Biometeorology*, **67**, 1839–1852.

Jochner S, Caffarra A, Menzel A (2013a) Can spatial data substitute temporal data in phenological modelling? A survey using birch flowering. *Tree physiology*, **33**, 1256–1268.

Jochner S, Höfler J, Beck I, Göttlein A, Ankerst DP, Traidl-Hoffmann C, Menzel A (2013b) Nutrient status: a missing factor in phenological and pollen research? *Journal of Experimental Botany*, **64**, 2081–2092. https://academic.oup.com/jxb/article/64/7/2081/581703.

Jochner S, Ziello C, Böck A, Estrella N, Buters J, Weichenmeier I, Behrendt H, Menzel A (2012) Spatio-temporal investigation of flowering dates and pollen counts in the topographically complex Zugspitze area on the German–Austrian border. *Aerobiologia*, **28**, 541–556.

Jochner SC, Beck I, Behrendt H, Traidl-Hoffmann C, Menzel A (2011) Effects of extreme spring temperatures on urban phenology and pollen production: a case study in Munich and Ingolstadt. *Climate Research*, **49**, 101–112.

Jung S, Estrella N, Pfaffl MW, Hartmann S, Handelshauser E, Menzel A (2018) Grass pollen production and group V allergen content of agriculturally relevant species and cultivars. *PLOS ONE*, **13**, e0193958. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0193958.

Kadaikar A, Guinot B, Trocan M *et al.* (2019) Automatic Pollen Grains Counter. In: *BioSMART 2019 proceedings.* 3rd International Conference on Bio-engineering for Smart Technologies, Paris, 24th-26th April, 2019 (ed Nait-Ali A), pp 1–4. IEEE, Piscataway, NJ.

REFERENCES

Kakui H, Tsurisaki E, Sassa H, Moriguchi Y (2020) An improved pollen number counting method using a cell counter and mesh columns. *Plant Methods*, **16**, 124. https://plantmethods.biomedcentral.com/articles/10.1186/s13007-020-00668-4.

Kakui H, Tsurisaki E, Shibata R, Moriguchi Y (2021) Factors Affecting the Number of Pollen Grains per Male Strobilus in Japanese Cedar (*Cryptomeria japonica*). *Plants*, **10**, 856. https://www.mdpi.com/2223-7747/10/5/856.

Kassambara A 'ggplot2' Based Publication Ready Plots [R package ggpubr version 0.6.0]. Available via https://cran.r-project.org/web/packages/ggpubr/index.html.

Katz DS, Morris JR, Batterman SA (2020) Pollen production for 13 urban North American tree species: allometric equations for tree trunk diameter and crown area. *Aerobiologia*.

Kelly D (1994) The evolutionary ecology of mast seeding. *Trends in Ecology & Evolution*, **9**, 465–470.

Kerr RA (2011) Climate change. Humans are driving extreme weather; time to prepare. *Science (New York, N.Y.)*, **334**, 1040.

Khanduri VP (2012) Annual variation in floral phenology and pollen production in a 25-year-old plantation of *Tectona grandis*. *Nordic Journal of Botany*, **30**, 82–89. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1756-1051.2011.01157.x.

Khanduri VP, Sharma CM (2002) Pollen productivity variations. Pollen-ovule ratio and sexual selection in *Pinus roxburghii*. *Grana*, **41**, 29–38.

Khanduri VP, Sharma CM (2009) Cyclic pollen production in *Cedrus deodara*. *Sexual Plant Reproduction*, **22**, 53–61. https://link.springer.com/article/10.1007/s00497-008-0091-y.

Khanduri VP, Sharma CM, Singh SP (2008) The effects of climate change on plant phenology. *The Environmentalist*, **28**, 143–147. http://dx.doi.org/10.1007/s10669-007-9153-1.

Kim KR, Oh J-W, Woo S-Y, Seo Y am, Choi Y-J, Kim HS, Lee WY, Kim B-J (2018) Does the increase in ambient CO_2 concentration elevate allergy risks posed by oak pollen? *International Journal of Biometeorology*, **62**, 1587–1594. https://pubmed.ncbi.nlm.nih.gov/29748910/.

Kirchner M, Faus-Kessler T, Jakobi G, Leuchner M, Ries L, Scheel H-E, Suppan P (2013) Altitudinal temperature lapse rates in an Alpine valley: trends and the influence of season and weather patterns. *International Journal of Climatology*, **33**, 539–555.

Koenig WD, Knops JM (2000) Patterns of Annual Seed Production by Northern Hemisphere Trees: A Global Perspective. *The American naturalist*, **155**, 59–69.

Koenker R (2005) Quantile regression. Cambridge University Press, Cambridge.

Koenker R (2017) Quantile Regression: 40 Years On. *Annual Review of Economics*, **9**, 155–176.

Koenker R, Hallock KF (2001) Quantile Regression. *Journal of Economic Perspectives*, **15**, 143–156. https://www.aeaweb.org/articles?id=10.1257/jep.15.4.143.

Koenker R, Portnoy S, Ng PT *et al.* (2023) Quantile Regression [R package quantreg version 5.97]. Comprehensive R Archive Network (CRAN).

Kolek F (2021) Spatial and temporal monitoring of *Betula* pollen in the region of Augsburg, Bavaria, Germany. Dissertation.

Körner C (2007) The use of 'altitude' in ecological research. *Trends in Ecology & Evolution*, **22**, 569–574. https://www.sciencedirect.com/science/article/pii/S0169534707002819.

Kouli K, Masi A, Mercuri AM, Florenzano A, Sadori L (2015) Regional Vegetation Histories: An Overview of the Pollen Evidence from the Central Mediterranean. *Late Antique Archaeology*, **11**, 69–82.

Krišāns O, Čakša L, Matisons R, Rust S, Elferts D, Seipulis A, Jansons Ā (2022) A Static Pulling Test Is a Suitable Method for Comparison of the Loading Resistance of Silver Birch (*Betula pendula* Roth.) between Urban and Peri-Urban Forests. *Forests*, **13**, 127.

REFERENCES

Kudeyarov VN, Biel K, Blagodatsky SA, Semenov VM, Dem'yanova EG, Dorodnikov MV (2006) Fertilizing effect of the increasing CO_2 concentration in the atmosphere. *Eurasian Soil Science*, **39**, S6-S14.

Ladeau SL, Clark JS (2006) Pollen production by Pinus taeda growing in elevated atmospheric CO_2. *Functional Ecology*, **20**, 541–547.

Lake I, Colon F, Jones N (2018) Quantifying the health effects of climate change upon pollen allergy: a combined cohort and modelling study. *The Lancet Planetary Health*, **2**, S16.

Lake IR, Jones NR, Agnew M *et al.* (2017) Climate Change and Future Pollen Allergy in Europe. *Environmental Health Perspectives*, **125**, 385–391.

LaMontagne JM, Boutin S (2009) Quantitative methods for defining mast-seeding years across species and studies. *Journal of Vegetation Science*, **20**, 745–753.

Latałowa M, Miętus M, Uruska A (2002) Seasonal variations in the atmospheric *Betula* pollen count in Gdańsk (southern Baltic coast) in relation to meteorological parameters. *Aerobiologia*, **18**, 33–43. https://link.springer.com/article/10.1023/A:1014905611834.

Lau T-K, Stephenson AG (1993) Effects of Soil Nitrogen on Pollen Production, Pollen Grain Size, and Pollen Performance in *Cucurbita pepo* (Cucurbitaceae). *American Journal of Botany*, **80**, 763–768.

Lawson SP, Kennedy KB, Rehan SM (2021) Pollen composition significantly impacts the development and survival of the native small carpenter bee, Ceratina calcarata. *Ecological Entomology*, **46**, 232–239.

Lee H, Calvin K, Dasgupta D *et al.* (2024) CLIMATE CHANGE 2023 Synthesis Report Summary for Policymakers. *CLIMATE CHANGE 2023 Synthesis Report: Summary for Policymakers*.

Leites L, Benito Garzón M (2023) Forest tree species adaptation to climate across biomes: Building on the legacy of ecological genetics to anticipate responses to climate change. Global Change Biology, 29, 4711-4730.

Lemmetyinen J, Keinonen K, Sopanen T (2004) Prevention of the flowering of a tree, silver birch. *Molecular Breeding*, **13**, 243–249. https://link.springer.com/article/10.1023/B:MOLB.0000022525.96200.53.

LGRB (2021-12-02) LGRB-Kartenviewer: —Layer Bodenkunde: Reliefparameter. Available via https://maps.lgrb-bw.de/. Accessed 02 Dec 2021.

Li L, Chang C, Guan K (2022) Birch Pollen Allergens. *Current protein & peptide science*, **23**, 731–743.

Li Y, Li X, Zhao M-H *et al.* (2021) An Overview of the Practices and Management Methods for Enhancing Seed Production in Conifer Plantations for Commercial Use. *Horticulturae*, **7**, 252. https://www.mdpi.com/2311-7524/7/8/252/htm#B73-horticulturae-07-00252.

Linkosalo T, Siljamo P, Riikonen A, Chmielewski F, Raisio J (2019) Utilizing a Thermal Time Model to Estimate Safe Times to Transplant Tilia Trees. *Arboriculture & Urban Forestry*, **45**.

Liu J-J, Ekramoddoullah AK, Yu X (2003) Differential expression of multiple PR10 proteins in western white pine following wounding, fungal infection and cold-hardening. *Physiologia Plantarum*, **119**, 544–553.

Lu X, Ye X, Liu J (2022) Morphological differences between anemophilous and entomophilous pollen. *Microscopy research and technique*, **85**, 1056–1064.

Manirajan BA, Maisinger C, Ratering S, Rusch V, Schwiertz A, Cardinale M, Schnell S (2018) Diversity, specificity, co-occurrence and hub taxa of the bacterial-fungal pollen microbiome. *FEMS microbiology ecology*, **94**.

Manninen S, Huttunen S, Vanhatalo M, Pakonen T, Hämäläinen A (2009) Inter- and intra-specific responses to elevated ozone and chamber climate in northern birches. *Environmental pollution (Barking, Essex : 1987)*, **157**, 1679–1688.

REFERENCES

Markgraf V (1980) Pollen Dispersal in a Mountain Area. *Grana*, **19**, 127–146.

Masaki T, Abe S, Naoe S, Koike S, Nakajima A, Nemoto Y, Yamazaki K (2020) Horizontal and elevational patterns of masting across multiple species in a steep montane landscape from the perspective of forest mammal management. *Journal of Forest Research*, **25**, 92–100.

Mascarenhas JP (1989) The Male Gametophyte of Flowering Plants. *The Plant cell*, **1**, 657–664.

Massart S, Candresse T, Gil J *et al.* (2017) A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies. *Frontiers in microbiology*, **8**, 45.

Masson-Delmotte V, Zhai P, Pirani A *et al.* (2021) Climate change 2021: the physical science basis. *Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change*, **2**, 2391.

Matyssek R, Agerer R, Ernst D *et al.* (2005) The Plant's Capacity in Regulating Resource Demand. *Plant Biology*, **7**, 560–580.

Meier-Dinkel A (1992) Micropropagation of Birches (*Betula* spp.). In: *High-Tech and Micropropagation II* (ed Bajaj YPS), pp 40–81. Springer Berlin Heidelberg, Berlin, Heidelberg.

Mellert KH, Ewald J, Hornstein D *et al.* (2016) Climatic marginality: a new metric for the susceptibility of tree species to warming exemplified by *Fagus sylvatica* (L.) and Ellenberg's quotient. *European Journal of Forest Research*, **135**, 137–152. https://link.springer.com/article/10.1007/s10342-015-0924-9.

Menzel A, Sparks TH, Estrella N *et al.* (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology*, **12**, 1969–1976. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2486.2006.01193.x.

Menzel A, Yuan Y, Matiu M, Sparks T, Scheifinger H, Gehrig R, Estrella N (2020) Climate change fingerprints in recent European plant phenology. *Global Change Biology*, **26**, 2599–2612. https://onlinelibrary.wiley.com/doi/full/10.1111/gcb.15000.

Miyazaki Y, Maruyama Y, Chiba Y *et al.* (2014) Nitrogen as a key regulator of flowering in *Fagus crenata*: understanding the physiological mechanism of masting by gene expression analysis. *Ecology Letters*, **17**, 1299–1309.

Moe D (1998) Pollen production of *Alnus incana* at its south Norwegian altitudinal ecotone. *Grana*, **37**, 35–39.

Molina RT, Rodríguez AM, Palaciso IS, López Fs (1996) Pollen production in anemophilous trees. *Grana*, **35**, 38–46.

Mondal AK, Mandal S (1998) Pollen production in some terrestrial angiosperms. *Current Science*, **74**, 906–910. http://www.jstor.org/stable/24101098.

Moore PD, Webb JA, Collinson ME (1991) Pollen analysis. Blackwell Science, Oxford, Malden MA, viii, 216.

National Oceanic and Atmospheric Administration (NOAA) (2023) Mauna Lao CO_2 annual mean data. Available via https://gml.noaa.gov/webdata/ccgg/trends/co2/co2_annmean_mlo.txt.

Neuwirth E ColorBrewer Palettes [R package RColorBrewer version 1.1-3]. Available via https://cran.r-project.org/web/packages/RColorBrewer/index.html.

Nussbaumer A, Waldner P, Apuhtin V *et al.* (2018) Impact of weather cues and resource dynamics on mast occurrence in the main forest tree species in Europe. *Forest Ecology and Management*, **429**, 336–350. https://www.sciencedirect.com/science/article/pii/S0378112718304833.

Nussbaumer A, Waldner P, Etzold S *et al.* (2016) Patterns of mast fruiting of common beech, sessile and common oak, Norway spruce and Scots pine in Central and Northern Europe. *Forest Ecology and Management*, **363**, 237–251.

REFERENCES

Obersteiner A, Gilles S, Frank U *et al.* (2016) Pollen-Associated Microbiome Correlates with Pollution Parameters and the Allergenicity of Pollen. *PLoS ONE*, **11**.

Olsson C, Olin S, Lindström J, Jönsson AM (2017) Trends and uncertainties in budburst projections of Norway spruce in Northern Europe. *Ecology and evolution*, **7**, 9954–9969.

Opedal ØH, Pérez-Barrales R, Brito VL, Muchhala N, Capó M, Dellinger A (2023) Pollen as the link between floral phenotype and fitness. *American Journal of Botany*, **110**, e16200.

Pallett DW, Soh E, Edwards M-L *et al.* (2009) Proof of concept pilot study: prevalence of grass virus infection and the potential for effects on the allergenic potency of pollen. *Environmental Health*, **8 Suppl 1**, S10. https://ehjournal.biomedcentral.com/counter/pdf/10.1186/1476-069X-8-S1-S10.

Panda AK, Soorianathasundaram K, Vijayakumar RM (2019) Screening for male fertility status in selected banana genotypes. *Electronic Journal of Plant Breeding*, **10**, 1309–1316.

Pandey P, Sinha R, Mysore KS, Senthil-Kumar M Combined Stresses in Plants. Physiological, Molecular, and Biochemical Aspects. Springer International Publishing, Cham. Available via https://link.springer.com/content/pdf/10.1007/978-3-319-07899-1_10.pdf.

Pauleit S, Duhme F (2000) Assessing the environmental performance of land cover types for urban planning. *Landscape and Urban Planning*, **52**, 1–20. http://dx.doi.org/10.1016/s0169-2046(00)00109-2.

Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss M. (2013) World Allergy Organization (WAO) White Book on Allergy: Update 2013. Available via https://www.worldallergy.org/wao-white-book-on-allergy. Accessed 25 Nov 2021.932Z.

Pearse IS, Koenig WD, Kelly D (2016) Mechanisms of mast seeding: resources, weather, cues, and selection. *New Phytologist*, **212**, 546–562.

Perala DA, Alm AA (1990) Reproductive ecology of birch: A review. *Forest Ecology and Management*, **32**, 1–38. http://www.sciencedirect.com/science/article/pii/037811279090104J.

Pers-Kamczyc E, Tyrała-Wierucka Ż, Rabska M, Wrońska-Pilarek D, Kamczyc J (2020) The higher availability of nutrients increases the production but decreases the quality of pollen grains in *Juniperus communis* L. *Journal of Plant Physiology*, **248**, 153156. https://www.sciencedirect.com/science/article/pii/S0176161720300456.

Pickett ST Long-Term Studies in Ecology. Approaches and Alternatives. Springer New York, New York, NY. Available via https://link.springer.com/content/pdf/10.1007/978-1-4615-7358-6_5.pdf.

Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, **209**, 2362–2367. https://journals.biologists.com/jeb/article/209/12/2362/9393/Phenotypic-plasticity-and-evolution-by-genetic.

Piotrowska K (2008) Pollen production in selected species of anemophilous plants. *Acta Agrobotanica*, **61**. http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.agro-article-83572de8-adce-4e35-a08b-68c29bf27a8e.

Piotrowska K, Kubik-Komar A (2012) The effect of meteorological factors on airborne Betula pollen concentrations in Lublin (Poland). *Aerobiologia*, **28**, 467–479. https://link.springer.com/content/pdf/10.1007/s10453-012-9249-z.pdf.

Plaza MP, Alcázar P, Galán C (2016) Correlation between airborne *Olea europaea* pollen concentrations and levels of the major allergen Ole e 1 in Córdoba, Spain, 2012-2014. *International Journal of Biometeorology*, **60**, 1841–1847.

Portnoy S, Koenker R (1997) The Gaussian hare and the Laplacian tortoise: computability of squared-error versus absolute-error estimators. *Statistical Science*, **12**, 279–300.

REFERENCES

https://projecteuclid.org/journals/statistical-science/volume-12/issue-4/The-Gaussian-hare-and-the-Laplacian-tortoise--computability-of/10.1214/ss/1030037960.full.

Possen BJ, Oksanen E, Rousi M *et al.* (2011) Adaptability of birch (*Betula pendula* Roth) and aspen (*Populus tremula* L.) genotypes to different soil moisture conditions. *Forest Ecology and Management*, **262**, 1387–1399. https://www.sciencedirect.com/science/article/pii/S0378112711003975.

Powell J (1991) Estimation of monotonic regression models under quantile restrictions. In Barnett W, Powell JL, Tauchen G (Eds.), Nonparametric and semiparametric methods in econometrics and statistics (pp. 357–384). https://mathscinet.ams.org/mathscinet/article?mr=1174980.

Prieto-Baena JC, Hidalgo PJ, Domínguez E, Galán C (2003) Pollen production in the Poaceae family. *Grana*, **42**, 153–159.

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ranpal S, Bargen S von, Gilles S *et al.* (2023) Pollen production of downy birch (*Betula pubescens* Ehrh.) along an altitudinal gradient in the European Alps. *International Journal of Biometeorology*, **67**, 1125–1139. https://link.springer.com/article/10.1007/s00484-023-02483-7.

Ranpal S, Sieverts M, Wörl V *et al.* (2022) Is Pollen Production of Birch Controlled by Genetics and Local Conditions? *International Journal of Environmental Research and Public Health*, **19**, 8160.

Ranta H, Hokkanen T, Linkosalo T, Laukkanen L, Bondestam K, Oksanen A (2008) Male flowering of birch: Spatial synchronization, year-to-year variation and relation of catkin numbers and airborne pollen counts. *Forest Ecology and Management*, **255**, 643–650. http://www.sciencedirect.com/science/article/pii/S0378112707007062.

Ranta H, Oksanen A, Hokkanen T, Bondestam K, Heino S (2005) Masting by *Betula*-species; applying the resource budget model to north European data sets. *International Journal of Biometeorology*, **49**, 146–151.

Rauer D, Gilles S, Wimmer M *et al.* (2021) Ragweed plants grown under elevated CO_2 levels produce pollen which elicit stronger allergic lung inflammation. *Allergy*, **76**, 1718–1730.

Renner SS, Chmielewski F-M (2021) The International Phenological Garden network (1959 to 2021): its 131 gardens, cloned study species, data archiving, and future. *International Journal of Biometeorology*, 1–9. https://link.springer.com/article/10.1007/s00484-021-02185-y.

Richards CL, Alonso C, Becker C *et al.* (2017) Ecological plant epigenetics: Evidence from model and non-model species, and the way forward. *Ecology Letters*, **20**, 1576–1590.

Richardson SJ, Allen RB, Whitehead D, Carswell FE, Ruscoe WA, Platt KH (2005) Climate and net Carbon availability determine temporal patterns of seed production by *Nothofagus*. *Ecology*, **86**, 972–981.

Riikonen J, Kets K, Darbah J *et al.* (2008) Carbon gain and bud physiology in Populus tremuloides and Betula papyrifera grown under long-term exposure to elevated concentrations of CO2 and O3. *Tree Physiology*, **28**, 243–254.

Rogers CA, Wayne PM, Macklin EA, Muilenberg ML, Wagner CJ, Epstein PR, Bazzaz FA (2006) Interaction of the onset of spring and elevated atmospheric CO_2 on ragweed *(Ambrosia artemisiifolia* L.) pollen production. *Environmental Health Perspectives*, **114**, 865–869.

Rojo J, Oteros J, Picornell A *et al.* (2021) Effects of future climate change on birch abundance and their pollen load. *Global Change Biology*, **27**, 5934–5949. https://onlinelibrary.wiley.com/doi/full/10.1111/gcb.15824.

REFERENCES

Rojo J, Salido P, Pérez-Badia R (2015) Flower and pollen production in the 'Cornicabra' olive (*Olea europaea* L.) cultivar and the influence of environmental factors. *Trees*, **29**, 1235–1245. https://link.springer.com/article/10.1007/s00468-015-1203-6.

Romero-Morte J, Rojo J, Rivero R, Fernández-González F, Pérez-Badia R (2018) Standardised index for measuring atmospheric grass-pollen emission. *The Science of the total environment*, **612**, 180–191. https://www.sciencedirect.com/science/article/pii/S0048969717321393.

Roossinck MJ (2015) Plants, viruses and the environment: Ecology and mutualism. *Virology*, **479-480**, 271–277. https://www.sciencedirect.com/science/article/pii/S0042682215001816.

Rötzer T, Chmielewski FM (2001) Phenological maps of Europe. *Climate Research*, **18**, 249–257.

Rousi M, Possen BJ, Pulkkinen P, Mikola J (2019) Using long-term data to reveal the geographical variation in timing and quantity of pollen and seed production in silver and pubescent birch in Finland: Implications for gene flow, hybridization and responses to climate warming. *Forest Ecology and Management*, **438**, 25–33.

Rousi M, Pusenius J (2005) Variations in phenology and growth of European white birch *(Betula pendula)* clones. *Tree Physiology*, **25**, 201–210.

Rumbou A, Bargen S von, Demiral R, Langer J, Rott M, Jalkanen R, Büttner C (2016) High genetic diversity at the inter-/intra-host level of Cherry leaf roll virus population associated with the birch leaf-roll disease in Fennoscandia. *Scandinavian Journal of Forest Research*, **31**, 546–560.

Rumbou A, Candresse T, Marais A, Svanella-Dumas L, Landgraf M, Bargen S von, Büttner C (2020) Unravelling the virome in birch: RNA-Seq reveals a complex of known and novel viruses. *PLOS ONE*, **15**, e0221834.

Rumbou A, Vainio EJ, Büttner C (2021) Towards the Forest Virome: High-Throughput Sequencing Drastically Expands Our Understanding on Virosphere in Temperate Forest Ecosystems. *Microorganisms*, **9**.

Satake A, Iwasa Y (2002) Spatially limited pollen exchange and a long-range synchronization of trees. *Ecology*, **83**, 993–1005.

Schauber EM, Kelly D, Turchin P *et al.* (2002) Masting by eighteen New Zealand plant species: the role of temperature as a synchronizing cue. *Ecology*, **83**, 1214–1225.

Scherrer D, Schmid S, Körner C (2011) Elevational species shifts in a warmer climate are overestimated when based on weather station data. *International Journal of Biometeorology*, **55**, 645–654. https://link.springer.com/article/10.1007/s00484-010-0364-7.

Schnelle F, Volkert E (1957) Vorschläge zur Einrichtung Internationaler Phänologischer Gärten als Stationen eines Grundnetzes für internationale phänologische Beobachtungen. *Meteorologische Rundschau*, **10**, 130–133.

Seleiman MF, Al-Suhaibani N, Ali N *et al.* (2021) Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. *Plants (Basel, Switzerland)*, **10**.

Severova E, Kopylov-Guskov Y, Selezneva Y, Karaseva V, Yadav SR, Sokoloff D (2022) Pollen Production of Selected Grass Species in Russia and India at the Levels of Anther, Flower and Inflorescence. *Plants*, **11**, 285. https://www.mdpi.com/2223-7747/11/3/285.

Shivanna KR, Rangaswamy NS (1992) Pollen Biology. A Laboratory Manual. Springer Berlin / Heidelberg, Berlin, Heidelberg, 122 pp.

REFERENCES

Shrestha A, Limay-Rios V, Brettingham DJ, Raizada MN (2023) Maize pollen carry bacteria that suppress a fungal pathogen that enters through the male gamete fertilization route. *Frontiers in Plant Science*, **14**, 1286199.

Sillman S (1999) The relation between ozone, NO_x and hydrocarbons in urban and polluted rural environments. *Atmospheric Environment*, **33**, 1821–1845.

Simoleit A, Wachter R, Gauger U, Werchan M, Werchan B, Zuberbier T, Bergmann K-C (2016) Pollen season of European beech (*Fagus sylvatica* L.) and temperature trends at two German monitoring sites over a more than 30-year period. *Aerobiologia*, **32**, 489–497. https://link.springer.com/article/10.1007/s10453-016-9421-y.

Skjøth CA, Šikoparija B, Jäger S, EAN-Network (2013) Pollen Sources. In: *Allergenic Pollen,* pp 9–27. Springer, Dordrecht.

Smith M, Jäger S, Berger U *et al.* (2014) Geographic and temporal variations in pollen exposure across Europe. *Allergy*, **69**, 913–923.

Sofiev M, Siljamo P, Ranta H *et al.* (2013) A numerical model of birch pollen emission and dispersion in the atmosphere. Description of the emission module. *International Journal of Biometeorology*, **57**, 45–58. https://link.springer.com/content/pdf/10.1007/s00484-012-0532-z.pdf.

Sofiev M, Vira J, Kouznetsov R, Prank M, Soares J, Genikhovich E (2015) Construction of the SILAM Eulerian atmospheric dispersion model based on the advection algorithm of Michael Galperin. *Geoscientific Model Development*, **8**, 3497–3522. https://gmd.copernicus.org/articles/8/3497/2015/.

Srivastava S, Fristensky B, Kav NN (2004) Constitutive expression of a PR10 protein enhances the germination of *Brassica napus* under saline conditions. *Plant & cell physiology*, **45**, 1320–1324.

Stoehr M, Hollefreund C, Webber J, Hewson C, Ross S (1995) Effects of crown-pruning on seed and pollen cone production in two lodgepole pine seed orchards in British Columbia. *New Forests*, **10**, 133–143. https://link.springer.com/article/10.1007/BF00033403.

Subba Reddi C, Reddi NS (1986) Pollen Production in Some Anemophilous Angiosperms. *Grana*, **25**, 55–61.

Sundqvist MK, Sanders NJ, Wardle DA (2013) Community and Ecosystem Responses to Elevational Gradients: Processes, Mechanisms, and Insights for Global Change. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 261–280.

Thoning K, Dlugokencky E, Lan X, NOAA Global Monitoring Laboratory Trends in globally-averaged CH4, N2O, and SF6.

Tito R, Vasconcelos HL, Feeley KJ (2020) Mountain Ecosystems as Natural Laboratories for Climate Change Experiments. *Frontiers in Forests and Global Change*, **0**, 38. https://www.frontiersin.org/articles/10.3389/ffgc.2020.00038/full.

Tormo-Molina R, Maya-Manzano J-M, Silva-Palacios I, Fernández-Rodríguez S, Gonzalo-Garijo Á (2015) Flower production and phenology in *Dactylis glomerata*. *Aerobiologia*, **31**, 469–479. https://link.springer.com/article/10.1007/s10453-015-9381-7.

Trenberth KE (2018) Climate change caused by human activities is happening and it already has major consequences. *Journal of Energy & Natural Resources Law*, **36**, 463–481.

Tseng Y-T, Kawashima S, Kobayashi S, Takeuchi S, Nakamura K (2018) Algorithm for forecasting the total amount of airborne birch pollen from meteorological conditions of previous years. *Agricultural and Forest Meteorology*, **249**, 35–43. https://www.sciencedirect.com/science/article/pii/S0168192317303945.

Vakkari P (2009) *EUFORGEN* Technical Guidelines for genetic conservation and use for silver birch (*Betula pendula*). Biodiversity International, Rome, Italy.

REFERENCES

van Meeningen Y, Schurgers G, Rinnan R, Holst T (2016) BVOC emissions from English oak (*Quercus robur*) and European beech (*Fagus sylvatica*) along a latitudinalgradient. *Biogeosciences*, **13**, 6067–6080. https://bg.copernicus.org/articles/13/6067/2016/.

an Vanden Broeck, Cox K, Brys R *et al.* (2018) Variability in DNA Methylation and Generational Plasticity in the Lombardy Poplar, a Single Genotype Worldwide Distributed Since the Eighteenth Century. *Frontiers in Plant Science*, **9**, 1635. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2018.01635/pdf.

Veilleux RE, Lauer FI (1981) Variation for 2n pollen production in clones of *Solanum phureja* Juz. and Buk. *Theoretical and Applied Genetics*, **59**, 95–100. https://link.springer.com/article/10.1007/BF00285897.

Verstraeten WW, Dujardin S, Hoebeke L *et al.* (2019) Spatio-temporal monitoring and modelling of birch pollen levels in Belgium. *Aerobiologia*, **35**, 703–717. https://link.springer.com/article/10.1007/s10453-019-09607-w.

Vieira Ld, Soares TL, Rossi ML, Alves AA, Santos Fd, Souza FV (2012) Viability, production and morphology of pollen grains for different species in the genus Manihot (Euphorbiaceae). *Acta Botanica Brasilica*, **26**, 350–356.

Viherä-Aarnio A, Ryynänen L (1994) Seed production of micropropagated plants, grafts and seedlings of birch in a seed orchard. *0037-5330.* https://helda.helsinki.fi/handle/1975/9178.

Warm K, Lindberg A, Lundbäck B, Rönmark E (2013) Increase in sensitization to common airborne allergens among adults - two population-based studies 15 years apart. *Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology*, **9**, 20.

Wayne P, Foster S, Connolly J, Bazzaz F, Epstein P (2002) Production of allergenic pollen by ragweed (*Ambrosia artemisiifolia* L.) is increased in CO_2-enriched atmospheres. *Annals of Allergy, Asthma & Immunology*, **88**, 279–282. http://www.sciencedirect.com/science/article/pii/S1081120610620091.

Wenden B, Mariadassou M, Chmielewski F-M, Vitasse Y (2020) Shifts in the temperature-sensitive periods for spring phenology in European beech and pedunculate oak clones across latitudes and over recent decades. *Global Change Biology*, **26**, 1808–1819. https://onlinelibrary.wiley.com/doi/10.1111/gcb.14918.

Wickham H (2016) ggplot2. Elegant graphics for data analysis. Springer, Switzerland, 260 pp.

Wielgolaski FE, Hofgaard A, Holtmeier FK (2017) Sensitivity to environmental change of the treeline ecotone and its associated biodiversity in European mountains. *Climate Research*, **73**, 151–166. https://www.jstor.org/stable/26394433.

Wilke CO Streamlined Plot Theme and Plot Annotations for 'ggplot2' [R package cowplot version 1.1.3]. Available via https://cran.r-project.org/web/packages/cowplot/index.html.

Wilsey BJ, Haukioja E, Koricheva J, Sulkinoja M (1998) Leaf Fluctuating Asymmetry Increases with Hybridization and Elevation in Tree-Line Birches. *Ecology*, **79**, 2092–2099. http://www.jstor.org/stable/176712.

Wörl V, Jetschni J, Jochner-Oette S (2022) Birch Pollen Deposition and Transport along an Altitudinal Gradient in the Bavarian Alps—A Case Study Using Gravimetric Pollen Traps in the Pollen Season 2020. *Atmosphere*, **13**, 2007. https://www.mdpi.com/2073-4433/13/12/2007.

Wullschleger SD, Yin TM, DiFazio SP, Tschaplinski TJ, Gunter LE, Davis MF, Tuskan GA (2005) Phenotypic variation in growth and biomass distribution for two advanced-generation pedigrees of hybrid poplar. *Canadian Journal of Forest Research*, **35**, 1779–1789.

REFERENCES

Zhang Y, Steiner AL (2022) Projected climate-driven changes in pollen emission season length and magnitude over the continental United States. *Nature Communications*, **13**, 1234. https://www.nature.com/articles/s41467-022-28764-0.pdf.

Zhao F, Heller W, Stich S, Durner J, Winkler JB, Traidl-Hoffmann C, Ernst D, Frank U (2017) Effects of NO_2 on inflorescence length, pollen/seed amount and phenolic metabolites of common ragweed (*Ambrosia artemisiifolia* L.).

Ziello C, Estrella N, Kostova M, Koch E, Menzel A (2009) Influence of altitude on phenology of selected plant species in the Alpine region (1971–2000). *Climate Research*, **39**, 227–234.

Ziello C, Sparks TH, Estrella N *et al.* (2012) Changes to Airborne Pollen Counts across Europe. *PLoS ONE*, **7**.

Ziska LH (2002) Sensitivity of ragweed (Ambrosia artemisiifolia) growth to urban ozone concentrations. *Functional Plant Biology*, **29**, 1365–1369.

Ziska LH, Caulfield FA (2000) Rising CO_2 and pollen production of common ragweed (*Ambrosia artemisiifolia* L.), a known allergy-inducing species: implications for public health, **27**, 893–898. https://www.publish.csiro.au/fp/pdf/pp00032.

Ziska LH, Gebhard DE, Frenz DA, Faulkner S, Singer BD, Straka JG (2003) Cities as harbingers of climate change: Common ragweed, urbanization, and public health. *Journal of Allergy and Clinical Immunology*, **111**, 290–295. http://www.sciencedirect.com/science/article/pii/S009167490300959X.

Ziska LH, Makra L, Harry SK *et al.* (2019) Temperature-related changes in airborne allergenic pollen abundance and seasonality across the northern hemisphere: a retrospective data analysis. *The Lancet Planetary Health*, **3**, e124-e131.

10 Publications and Candidate's individual contributions

Reviewed publications

1. **Ranpal S**, Sieverts M, Wörl V, Kahlenberg G, Gilles S, Landgraf M, Köpke K, Kolek F, Luschkova D, Heckmann T, Traidl-Hoffmann C, Büttner C, Damialis A, Jochner-Oette S (2022) Is pollen production of birch controlled by genetics and local conditions? In *International Journal of Environmental Research and Public Health* 19:8160. https://doi.org/10.3390/ijerph19138160

2. **Ranpal S**, Bargen S von, Gilles S, Luschkova D, Landgraf M, Traidl-Hoffmann C, Büttner C, Damialis A, Jochner-Oette S (2023) Pollen production of downy birch (*Betula pubescens* Ehrh.) along an altitudinal gradient in the European Alps. In *International Journal of Biometeorology* 67:1125–1139. https://doi.org/10.1007/s00484-023-02483-7

3. **Ranpal S**, Bargen S von, Gilles S, Luschkova D, Landgraf M, Bogawski P, Traidl-Hoffmann C, Büttner C, Damialis A, Fritisch M, Jochner-Oette S (2024) Continental-scale evaluation of downy birch pollen production: estimating the impacts of global change. In *Environmental Research* 252 (Pt 4), p. 119114. https://doi.org/10.1016/j.envres.2024.119114

Publications in preparation

4. Luschkova D, Rauer L, Gerkhardt S, Sener A, Eggestein A, Kolek F, **Ranpal S**, Landgraf M, Koepke K, Buettner C, Jochner-Oette S, Damialis A, Traidl-Hoffmann C, Gilles S (2024) Understanding the factors influencing birch pollen allergenicity and its health effects. In preparation.

Other publications

5. Gilles, S.; Meinzer, M.; Landgraf, M.; Kolek, F.; Pack, K.; von Bargen, S.; Charalampopoulos, A.; **Ranpal, S.**; Luschkova, D.; Traidl-Hoffmann, C.; Jochner-Oette, S.; Damialis, A.; and Büttner, C. et al. *Betula pendula* trees infected by birch idaeovirus and cherry leaf roll virus: impacts of urbanisation and NO_2

levels. Environmental Pollution 2023.
https://doi.org/10.1016/j.envpol.2023.121526

Conference Proceedings and Abstracts

Oral presentations

6. **Ranpal S,** Sieverts M, Wörl V, Jetschni J, Gilles S, Köpke K, Landgraf M, Kolek F, Luschkova D, Traidl-Hoffmann C, Büttner C, Damialis A, Jochner-Oette S (2020) Pollen production of genetically identical birch trees in a seed plantation. 7th European Symposium on Aerobiology. 16th-20th November 2020 in Cordoba, Spain (Online).

7. **Ranpal S,** Sieverts M, Wörl V, Kolek F, Gilles S, Köpke K, Landgraf M, Luschkova D, Traidl-Hoffmann C, Büttner C, Damialis A, Jochner-Oette S (2022) Pollen production of birch under differing environmental regimes in International Phenological Gardens across Europe. Phenology 2022. 20th-24th June 2022 in Avignon, France.

8. **Ranpal S,** Sieverts M, Gilles S, Luschkova D, von Bargen S, Landgraf M, Sieverts M, Wörl V, Büttner C, Traidl-Hoffmann C, Damialis A, Fritsch M, Jochner-Oette S (2023) Biotic and abiotic effects on pollen production of birch assessed in International Phenological Gardens of Europe. 2023 International Congress of Biometeorology. 14th-17th May 2023 in Tempe, Arizona, USA.

Poster presentations

9. **Ranpal S,** Sieverts M, Wörl V, Kolek F, Gilles S, Köpke K, Landgraf M, Luschkova D, Traidl-Hoffmann C, Büttner C, Damialis A, Jochner-Oette S (2021) Pollen production of birch under differing environmental regimes in International Phenological Gardens across Europe. 2021 International Congress of Biometeorology. 21th-22th September 2021. (Online conference)

10. Luschkova, D, Gilles, S, Damialis, A, **Ranpal, S,** Landgraf, M, Kolek, F, Köpke, K, Sieverts, M, Wörl, V, Büttner, C and Jochner-Oette, S (2021). P011: Biotische und abiotische Effekte auf die Pollenproduktion und Allergenität der Birke und damit verbundene gesundheitliche Auswirkungen: eine europaweite Untersuchung. *Journal der Deutschen Dermatologischen Gesellschaft*, *19*(S2), p.29.

11. **Ranpal S,** Sieverts M, Wörl V, Kahlenberg G, Köpke K, Landgraf M, Gilles S, Damialis A, Traidl-Hoffmann C, Büttner C, Jochner-Oette S (2022) Biotische und abiotische Einflüsse auf die Pollenproduktion und allergenitätt der Birke und deren Auswirkungen auf die Gesundheit. Dies Academicus. 17th May 2022 Eichstätt, Germany.

12. Jochner-Oette. S, **Ranpal S**, von Bargen S, Benzing T, Bogawski P, Fritsch M, Landgraf M, Luschkova D, Büttner C, Traidl-Hoffmann C, Gilles S, Damialis A (2024) Pollen production assessed in International Phenological Gardens during 2019-2023 reveals potential impacts of climate change.2024 World Aerobiology 2024 1st -5th July 2024 in Vilnius Lithuania

Only participation

13. European Academy of Allergy and Clinical Immunology (EAACI) Digital Congress 2020. Attendee. 6th-8th June 2020

Candidate's individual contributions

Three publications were prepared for this publication-based PhD thesis. They are included in this thesis as individual chapters (Chapters 4 to 6). All the publications were submitted to different scientific journals, which ensured a peer-review process. All the publications have been published.

All publications were written at the professorship of Physical Geography/Landscape Ecology and Sustainable Ecosystem Development at the Catholic University of Eichstätt-Ingolstadt. In addition, all publications were written in collaboration with Prof. Dr. Claudia Tridal-Hoffmann and her team from the University of Augsburg and Prof. Dr.

Carmen Büttner and her team from Humboldt-University of Berlin, who are also partners of the pollenPALS project. Furthermore, the third publication (Chapter 6) was written in collaboration with Markus Fritsch of the University of Passau and with Pawel Bogawski of Adam Mickiewicz University, Poland. The individual contributions to each publication are detailed below:

1. **Ranpal**, S.; Sieverts, M.; Wörl, V.; Kahlenberg, G.; Gilles, S.; Landgraf, M.; Köpke, K.; Kolek, F.; Luschkova, D.; Heckmann, T.; Traidl-Hoffmann, C.; Büttner, C., Damialis, A.; and Jochner-Oette, S. et al. Is pollen production of birch controlled by genetics and local conditions? 2022, 19, 8160.

   Susanne Jochner-Oette conceptualized the study. Miriam Sieverts and Verena Wörl assisted in the field and lab work. Athanasios Damialis guided in the lab methodology. Tobias Heckman provided ideas on statistical analyses. Georgia Kahlenberg helped me with the GIS. Susanne and Athanasios contributed with suggestions for statistical analyses, corrections, and proofreading. Most of the work (80%), ranging from field visit, sample collection, data collection, laboratory work, statistical analyses, and the writing of the manuscript, was done by myself.

2. **Ranpal, S.**; von Bargen, S.; Gilles, S.; Luschkova, D.; Landgraf, M.; Traidl-Hoffmann, C.; Büttner, C., Damialis, A.; and Jochner-Oette, S. et al. Pollen production of downy birch (*Betula pubescens* Ehrh.) along an altitudinal gradient in the European Alps. International Journal of Biometeorology 2023, 1-15.

   Susanne Jochner-Oette theorised the study. She and Athanasios Damialis contributed with suggestions for statistical analyses, corrections, and proofreading. Some HiWi students assisted at the field to collect catkin samples. Most of the work (75%), ranging from data collection, laboratory work, statistical analyses, map drawing and the writing of the manuscript, was done by myself.

3. **Ranpal, S.**; von Bargen, S.; Gilles, S.; Luschkova, D.; Landgraf, M.; Bogawski, P.; Traidl-Hoffmann, C.; Büttner, C., Damialis, A.; Fritsch, M.; and Jochner-Oette, S. et al. Continental-scale evaluation of downy birch pollen production: estimating the impacts of global change. Environmental Research 2024, 252-119114.

Susanne Jochner-Oette coordinated the project and contributed to the paper with suggestions for the study design, statistical analyses and proofreading. Markus Fritsch suggested statistical analyses, provided the code for some of the analyses, and wrote the section about regression models. Pawel Bogawski assisted in extracting atmospheric gasses. I carried out most of the work (75%) including IPG coordination, data compilation, laboratory work, statistical analyses and writing.