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Influence of recent global change on the pollen season in Europe

Chiara Ziello

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SUPERVISORS:
Univ.-Prof. Dr. A. Menzel
Univ.-Prof. D. P. Ankerst, Ph.D.

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Ohana means family.
Family means nobody gets left behind, or forgotten.

— Lilo & Stitch

To my father, who taught me how quality is important
to make a difference.

ABSTRACT

Background and objectives

The reality of marked changes in current climate conditions is no longer under discussion. Climate change is now widely recognized as the major problem facing the planet, impacting animal and plant life, ecosystems, and the whole environment. Increasing risk of pollinosis (hay fever) is one of the most anticipated consequences of climate change on human health. In fact, a progressive global increase in the burden of allergic diseases has affected the industrialized world over the last half century. The clinical evidence reveals a general increase in both incidence and prevalence of respiratory diseases, such as allergic rhinitis (common hay fever) and asthma. Such phenomena, still not completely explained, may be related not only to air pollution and changes in lifestyle, but also to an actual increase in airborne quantities of allergenic pollen. Phenology, the science of recurrent seasonal natural events, can help understand changes in flowering time and duration (flowering is a developmental phase directly connected to pollen production, and one of the most affected by climate change) due to the influence of global warming.

There are important aspects to investigate for achieving a more complete overview of the problem. On the one hand, from an ecological perspective, wind-pollinated plants have a deep allergological relevance, since they include species with the highest capability of triggering allergic symptoms in humans. Therefore, changes in wind-pollinated species may reflect impacts of climate change on allergenic plants. On the other hand, there is a need to assess the actual impact of climate change on mountain areas, regarded in the past as a safer place for hay fever sufferers, due to the low presence of airborne pollen relative to lowland regions. But in recent years, mountains seem to have been more strongly affected by climate change than other regions, especially the European Alps. Global warming could cause biodiversity changes on mountain tops, and rearrangements of the alpine species' composition, due to elevational shift of species range, are expected.

Therefore, in order to investigate changes in airborne pollen amounts, the research questions to address are:

- Does altitude a.s.l. influence phenology and phenological trends, and hence aerobiological data in turn?
- Do plant traits influence the phenological responses of vegetation to climate change? If so, which ones? And how?
- Are there changes in the intensity and duration of the pollen season? If present, are those changes connected to recent climate change?

MATERIALS AND METHODS

This research is based on the use of two main data sets: i) a phenological one, collected within the COST action 725, comprising more than 125,000 digitally available observational series of various phases in 542 plant and 19 animal species from 21 European countries, for the years 1971-2000, used in the first two publications [Ziello et al., 2009, 2012a, chapter two and chapter three of this thesis respectively], and ii) a palynological one, consisting of 1,221 pollen time series at 97 locations in 13 European countries from 23 pollen taxa, with series time spans ranging from 10 to 28 years in the period 1977 to 2009, used in its entirety in the third publication [Ziello et al., 2012b, chapter four of this thesis]. The E-Obs data set from the EU-FP6 project ENSEMBLES for data of monthly mean temperatures in Europe was also used, in order to study the correlation between temperature data and phenological or palynological data.

For the analysis of the first publication [Ziello et al., 2009, chapter two of this thesis], widely distributed plants in Europe including some important allergenic trees and grasses have been selected from the COST phenological data set. A selection was made of stations located over an area delimited by 46° N to 49° N latitude and 5° E to 15° E longitude, which includes major parts of the Alpine region, and phenological phases were restricted to flowering. Among the phenological series provided by the stations available in the area under exam (located as follows: 948 in Germany, 277 in Austria, 152 in Switzerland and 22 International Phenological Gardens, for a total of 1,399 stations with altitude ranging between 100 m and 1,900 m above sea level), only those ones longer than 15 years have been selected.

For the analysis of the second publication [Ziello et al., 2012a, chapter three of this thesis], 29 species that were represented in more than six stations in Europe, with records longer than 28 years during 1971-2000, were selected for analysis from the COST phenological data set. The size of the analyzed data set comprises 5,971 records based on over 170,000 single phenological observations of flowering phases, from 983 phenological stations. The related geographic coverage spans a considerable part of Europe (13 countries).

The main statistical procedures adopted within the research are linear regressions for calculation of phenological and palynological trends (obtained as linear coefficients), linear mixed models assessing the statistical significance of trends while adjusting for spatial correlation, and statistical tests for multiple comparisons.

RESULTS AND CONCLUSIONS

In the first publication [Ziello et al., 2009, chapter two of this thesis], the analysis of the influence of altitude on plant phenological dates reveals a statistically significant positive linear dependence of flowering dates on elevation for each phenological phase analyzed. Phenological temporal trends and thermal temporal trends show a less strong dependence on altitude, predominantly not significant. However, the prevalence of negative mean phenological trends suggests a stronger advance of flowering phases at higher altitudes.

In the second publication [Ziello et al., 2012b, chapter three of this thesis], the main results indicate that the onset of flowering of wind-pollinated plants advanced more than for insect-pollinated plants, while full flowering phases tended to advance less. The detected findings suggest a lengthening of the flowering period in general, which might lead to an increasing time of exposure to airborne pollen of allergic subjects, with consequent likely increment in severity and incidence of allergic symptoms.

In the third publication [Ziello et al., 2012b, chapter four of this thesis], the analysis of the continental-scale pollen data set reveals an increasing trend in the yearly amount of airborne pollen for many taxa in Europe, which is more pronounced in urban than semi-rural/rural areas. Climate change may contribute to these changes, however increased temperatures do not appear to be a major influencing factor. Instead, the anthropogenic rise of atmospheric CO_2 levels may be influential. Since a further increase in atmospheric CO_2 is expected during the coming years, future changes may result in further increases in pollen amounts leading, in turn, to a greater exposure of humans to pollen allergens, with potentially serious consequences for public health.

PUBLICATIONS

The work presented in the current thesis has appeared previously in the following publications:

[1] - C. Ziello, N. Estrella, M. Kostova, E. Koch, and A. Menzel. Influence of altitude on phenology of selected plant species in the Alpine region (1971- 2000). *Clim. Res.*, 39(3):227-234, 2009.

> *Author contributions* - N.E. and M.K. provided a quality checked version of the phenological COST data set. C.Z. performed data analysis by means of SAS®scripting, interpreted the results, and wrote the paper. N.E., E.K. and A.M. contributed with suggestions for statistical analyses, proof editing and reading. C.Z. did about 90% of the work.

[2] - C. Ziello, A. Böck, N. Estrella, D. Ankerst, and A. Menzel. First flowering of wind-pollinated species with the greatest phenological advances in Europe. *Ecography*, 35(11):1017-1023, 2012.

> *Author contributions* - C.Z. and A.M. conceived the analysis. A.B. developed the idea of applying linear mixed models to the COST data set, and contributed with R scripting. C.Z. performed the analysis, interpreted the results, an wrote the paper. N.E., D.A. and A.M. contributed with suggestions for statistical analyses, proof editing and reading. C.Z. did about 85% of the work.

[3] - C. Ziello, T.H. Sparks, N. Estrella, J. Belmonte, K.C. Bergmann, E. Bucher, M.A. Brighetti, A. Damialis, M. Detandt, C. Galán, R. Gehrig, Ł. Grewling, A.M. Gutiérrez Bustillo, M. Hallsdóttir, M.-C. Kockhans-Bieda, C. De Linares, D. Myszkowska, A. Pàldy, A. Sánchez, M. Smith, M. Thibaudon, A. Travaglini, A. Uruska, R.M. Valencia-Barrera, D. Vokou, R. Wachter, L.A. de Weger, and A. Menzel. Changes to airborne pollen counts across Europe. *PLoS ONE*, 7(4):e34076, 2012.

Author contributions - A.M. conceived the general idea of the paper. A.S. help collect the data from several international providers. J.B., K.C.B., E.B., M.A.B., A.D., M.D., C.G., R.G., L.G., A.M.G.B., M.H., M.-C.K.-B., C.D.L., D.M., A.P., M.S., M.T., A.T., A.U., R.M.V.-B., D.V., R.W., and L.A. d.W. contributed pollen data (pollen trends). C.Z. analyzed the data by means of R scripting and wrote the paper. T.H.S., N.E., and A.M. contributed with suggestions for statistical analyses, proof editing and reading. C.Z. did about 85% of the work.

> *Many of the events of the annual cycle recur year after year in a regular order. A year-to-year record of this order is a record of the rates at which solar energy flows to and through living things. They are the arteries of the land.*
> *By tracing their responses to the sun, phenology may eventually shed some light on that ultimate enigma, the land's inner workings.*
>
> — Aldo Leopold, *A Phenological Record for Sauk and Dane Counties*, Wisconsin, 1935-1945 (1947)

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1

GENERAL INTRODUCTION

Motivation

There is no longer a debate on the real occurrence of recent climatic changes; Their exact drivers, modes and timing probably represent the research challenge of the current century from several points of view [Parry et al., 2007]. In this context, airborne pollen is a key topic, mainly due to its importance to allergic subjects. There is a need for studying and investigating the effective occurrence of changes in the pollen season under changing climate conditions. In fact, the burden of allergic diseases is getting higher in all the "westernized" countries, not only in terms of the symptoms of diseases as for example allergic rhinitis, but also in terms of the resulting absenteeism from work and the (sometimes also heavily) reduced quality of life of allergy sufferers [Beggs, 2004; Beggs and Bambrick, 2005]. Moreover, in an increasing number of cases, allergic patients develop a consequent asthma [Beggs, 2004; Beggs and Bambrick, 2005].

Worldwide organizations like WAO (World Allergy Organization) and WHO (World Health Organization) are now clearly talking about an "allergy epidemic" affecting both developed and developing countries, and the last statistical reports bring to our attention a dramatic picture: Hundreds of millions of people in the world suffer from diseases somehow connected with atopy (i.e., allergy). This affects in Europe up to 40% of the total population, according to the European Academy of Allergy and Clinical Immunology (EAACI). These impressively large numbers alone tell us about the real importance of the problem.

If, on the one hand, there is compelling evidence in the literature of the occurrence of higher incidence and prevalence of allergic diseases in general, and pollen-related allergies in particular, in the last 30-40 years, on the other hand causes and mechanisms are not clear. There is contradictory evidence about the impact of recent climate change on the pollen season, which is expected to become longer and more intense for many allergenic plants, and a worldwide pattern is currently difficult to detect. It is not even unambiguously shown, but of course strongly suggested by the experts, whether an increasing trend in the amount of the pollen grains transported by tropospheric air masses would lead to a major number of clinical cases, as a consequence of an increment in both duration and intensity of exposure to pollen allergens. It is hence evident that there is a need for the detection of actual changes in any of the factors that, contributing to the chain of events finally leading to the onset (or the exacerbation) of pollen allergy in humans, can become crucially important for public health.

1.1 CLIMATE CHANGE AND HUMAN HEALTH

Climate Change in a few words

There is evidence of changes in climatic conditions at planetary level [Parry et al., 2007]. Increased surface and sea temperatures during the last decades are unequivocally reported, and are consistent with a steep rise, of anthropogenic origin, in the atmospheric concentration of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), halocarbons (among which are CFCs connected to the ozone layer depletion), and nitrous oxide (N_2O). These changes occurred in the latter half of the 20th century after a period of about 10,000 years in which the amount of such gases in the atmosphere has been relatively stable [Solomon et al., 2007]. The observed effects of a changing climate range from snow and ice cover melt, with consequent sea level rise, to an increment in the frequency of extreme weather events such as floods, heat waves, or heavy precipitation episodes, including the increase in the amount of land areas affected by drought. The projections associated with these changes suggest more pronounced effects in this direction in future years, with levels that could be also dramatic, depending on the scenario used to calculate predictions [Bernstein et al., 2007]. Many ecosystems have started showing the first changes, very likely linked to climate change. Some of the most observed consequences are the earlier timing of spring events in terrestrial ecosystems, or the range shifts in the distribution of a number of animal and plant species in both terrestrial and aquatic (marine as well as freshwater) ecosystems [Rosenzweig et al., 2007].

Implications for human health

But changing climate conditions also have, and will likely have in future, a heavy impact on human health. The augmented frequency of heat wave events, together with increased night temperatures, causes a marked mortality; Floods, often associated with windstorms, cause excessive deaths, injuries, and also increased probability to contract infectious diseases following contamination of water supplies; Droughts lead to deaths and malnutrition, as well as infectious and respiratory diseases; High temperatures have a role in the communication of some bacterial diseases, also via food poisoning (e.g., salmonellosis); Alteration in the water cycles, such as in rainfall, surface water and water quality, could affect the burden of water-related diseases (e.g., cholera) [Confalonieri et al., 2007; Hegerl et al., 2007; Kundzewicz et al., 2007]. Moreover, the impact of extreme events (temperature and precipitation) have already been shown to establish unstable environmental and socio-economic conditions, finally leading to increased occurrence of mental health problems [Berry et al., 2010].

Air quality

In addition to the other already mentioned factors, air quality has also being crucially affected by climate change and is known to affect human morbidity and mortality. The atmospherical concentration of pollutants is driven by climatic conditions: For example, temperature and humidity can influence their production, especially that of fine particulate matter (PM), or high-pressure systems can prevent their dispersion and diffusion, favoring the formation of persistent air masses that are heavily polluted [Confalonieri et al., 2007]. Phenomena of long-range transport can contribute to the general picture, carrying from remote areas desert windblown dust, including fungal spores or bacteria harmful to humans, as well as aerosols, carbon monoxide (CO), mould spores, pesticides [Confalonieri et al., 2007]. Air pollutants from forest fires, such as toxic gaseous and particulate air pollutants, can also be released into the atmosphere [Confalonieri et al., 2007; Hegerl et al., 2007; Kundzewicz et al., 2007].

Ground-level ozone (O_3)

Ozone (O_3) deserves special attention that, naturally occurring in the lowest layers of the atmosphere (tropospheric O_3), can have toxic effects on humans when reaching higher concentrations. In urban environments, an enhanced presence of O_3 results from chemical reactions, favored by solar ultraviolet radiation (specifically UVB) and high temperature, between nitrogen oxides (NO_x) and volatile organic compounds (VOCs) emitted by motor vehicles, which are a significant component of the dome of warm air over the cities also known as the “urban heat island” [Nilsson et al., 2001a,b; Krzyzanowski et al., 2005].

The urban planning of green areas can be of help in mitigating the negative effects of urban pollution, but unfortunately in such areas, as well as in natural forest environments, biogenic VOCs (BVOCs) are released by trees in a quantity that differs according to species. Such BVOCs can also react with NO_x and contribute to the total amount of O_3 available in the troposphere [Peñuelas and Staudt, 2010]. The harmfulness of O_3 is well known: if inhaled, the respiratory track irritation can lead to a wide range of diseases such as pneumonia or chronic obstructive pulmonary disease, with a documented increase in heart and lung disease mortality following exposure [Bell et al., 2005, 2006; WHO, 2006; Bell et al., 2007]. Moreover, heat increases the respiratory frequency and the volume of air inhaled per breath in humans, as a consequence of unbalanced physiological functions [Parsons, 2007].

Communicable diseases

Last but not least, one of the most dreaded effects of climate change on air quality are the geographical shifts of the distributions for a number of vector-borne (carried by insects, e.g. ticks and mosquitoes, like malaria or dengue) and rodent-borne (like leptospirosis) infectious diseases. Many of such diseases have already shown some changes in their distributional patterns, but the role of climatic conditions has still not been assessed in full (see Confalonieri et al. [2007, pp. 403–405] for an extensive review).

The role of pollen and its influence on the climate-driven effects on human health will be discussed in the following sections.

1.2 POLLEN AS AEROALLERGEN: IMPACTS ON AIR QUALITY

What is pollen?

A good technical description of pollen can be found in the American Heritage® Science Dictionary, reported in the following:

> *Powdery grains that contain the male reproductive cells of most plants. In gymnosperms, pollen is produced by male cones or conelike structures. In angiosperms, pollen is produced by the anthers at the end of stamens in flowers. Each pollen grain contains a generative cell, which divides into two nuclei (one of which fertilizes the egg), and a tube cell, which grows into a pollen tube to conduct the generative cell or the nuclei into the ovule. The pollen grain is the male gametophyte generation of seed-bearing plants. In gymnosperms, each pollen grain*

also contains two sterile cells (called prothallial cells), thought to be remnants of the vegetative tissue of the male gametophyte.

But when thinking of pollen, an allergy sufferer will hardly have in mind a similar objective description. The abnormal reactions that pollen triggers in people prone to atopy can heavily impact on these subjects, compromising the life quality in the working environment as well as in private contexts. For those people, a more appropriate definition of pollen could be that provided by the Mosby's Medical Dictionary:

> *A fertilizing element of plants that travels in the air and produces seasonal allergic responses [...] such as hay fever or asthma in sensitive individuals.*

Pollen morphology

A pollen grain can present a different morphology according to the plant species by which it is produced. Considering only the most allergenic species, the diameter can vary from 15 to 70 µm. The pollen wall, the external layers where the proteins acting as allergens are mostly located, can also present morphological differences by species: It is generally formed by the exine, which consists in turn of an endexine and a ektexine, and the inner intine, but one or more of these layers are hardly distinguishable or even absent for some species or families [Diethart et al., 2007]. When inhaled, pollen grains get in contact with the moist and warm mucosal barriers of the upper respiratory ways, which imitates the stigma environment, and the subsequent hydration causes the release of the allergenic proteins from both pollen wall and cytoplasm [Diethart et al., 2007; Buters et al., 2010]. Pollen by insect pollinated plants commonly has an additional external protective layer that seals the grain, but such a lipid coating is often not present or scarcely efficient to protect the inner cytoplasm in wind pollinated species. This is the reason why anemophilous pollen can be subjected, under specific climatic conditions, to grain rupture, with consequent outflow of pollen cytoplasmic granules (PCGs) [Abou Chakra et al., 2011]. These outcoming particles, having a smaller diameter (< 5 µm), can then easily trespass the upper airways and penetrate into the lower respiratory tract, inducing symptoms of bronchial asthma, while the likelihood that whole grains reach the lungs is low. Pollen rupture mainly occurs upon light rain events or thunderstorms (provoking the so-called "thunderstorm asthma", see D'Amato et al. [2006]), but according to recent literature, the exposure of pollen grains to gaseous pollutants such as O_3 and NO_2 can also be a cause [Motta et al., 2006].

Pollen cytoplasmic granules

Thunderstorm asthma

Several studies have shown that a single pollen grain can release from 700 to 1000 PCGs [Diethart et al., 2007; Abou Chakra et al., 2011].

Cross-reactivity

Each specific pollen type or taxon can contain more than one group of allergens, and considering the fact that only 29 protein groups are allergenic out of the 2615 protein families found in seed plants, it can be easily understood how same groups can be involved in allergic reactions caused by different pollen types or even food [Diethart et al., 2007]. This fact leads to a phenomenon called cross-reactivity, which makes possible an immediate allergic reaction of non-sensitized subjects (to develop allergic diseases, a predisposed subject needs to get in touch with the allergen, and this first contact provokes the sensitization) to, e.g., pollen types or food they were not aware of being allergic to. And it also explains how the majority of allergy sufferers are allergic to wide sets of allergens belonging to the same family (e.g., grass pollen) instead of being allergic to single allergens (a specific pollen or food) [Jäger, 2008].

Allergic rhinitis

A type I allergy is a disorder of the immune system characterized by an overreaction to non-hazardous proteins mainly due to inhalation of allergenic proteins from pollen, house dust mites or animal dander [Colombo et al., 2003; Diethart et al., 2007]. Allergic rhinitis, or hay fever, or pollinosis, is the most frequently occurring airway disease of our time. More than 25% of the population of industrialized countries (almost 500 million people) suffers from it and the incidence has more than doubled in the past three decades [Colombo et al., 2003; Traidl-Hoffmann et al., 2003; Diethart et al., 2007]. These symptoms include vasodilatation (widening of the blood vessels) and hence resulting redness and swelling as well as increased mucus secretion (watery rhinorrhoea), obstruction of the nasal passages, (palatal) itching and sneezing [Lakhani et al., 2012]. Complications of allergic rhinitis include nasal polyps and bacterial sinusitis, and comorbid conditions include asthma, allergic conjunctivitis, atopic dermatitis, and in rare cases nasopharyngeal tumors [Lakhani et al., 2012]. The nasal congestion is associated with sleep-disordered breathing, a condition that can have a deep effect on mental health, including increased psychiatric disorders, depression, anxiety, even alcohol abuse [Nathan, 2007]. Furthermore, sleep-disordered breathing in childhood and adolescence is associated with increased disorders of learning performance, behavior, and attention: This is the reason why the occurrence of allergic rhinitis leads to a worsening of the quality of sleep [Leger et al., 2006; Mullol et al., 2008; Colás et al., 2012]. In the United States alone, allergic rhinitis results in 3.5 million lost workdays and 2 million lost schooldays annually [Nathan, 2007]. A consistent number of epidemiological studies have shown a reduction in quality of life in patients suffering from allergic rhinitis, who however often underestimate their symptoms, and are prone to perceive them as normal [Nathan, 2007; Lakhani et al., 2012]. This leads to delay seeking medical attention and hence receiving an appropriate therapy, which worsen the already impaired physical and sociological functioning (impaired work or school performance) of allergic rhinitis sufferers [Lakhani et al., 2012].

1.3 ALLERGIC DISEASES ON THE RISE

Chronic non-communicable diseases

The burden of chronic non-communicable diseases (NCDs), such as heart disease, stroke, cancer, type II diabetes and respiratory diseases, has increased rapidly in most regions of the world, now accounting for 60% of the 58 million global annual deaths and 46% of the global burden of disease in disability-adjusted life years (DALYs), and is projected to increase further as life expectancy continues to improve [WHO, 2005]. From a global perspective, chronic diseases have been historically considered as a problem in high-income countries, while lower income countries focused on the major problems of communicable diseases and malnutrition. However, in all regions of the world except for sub-Saharan Africa, chronic NCDs are now the major causes of death [WHO, 2005; Kjellstrom et al., 2010].

"Allergy epidemic"

Allergic respiratory diseases, as part of the group of chronic NCDs, have also increased in both prevalence[1] and incidence[2] in the last decades, making the most important public health organizations (e.g., EEACI and WAO) talk of "allergy epidemic". Recent literature stressed the importance of dedicating more research in this field in order to identify the possible reasons of such a steep rise, still largely unknown [Beggs, 2004; Beggs and Bambrick, 2005; WHO, 2005; Frei and Gassner, 2008]. Among

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[1] Prevalence is a frequently used epidemiological measure, defined as the number of individuals affected by a particular disease (or a condition) at a particular time point by the number of individuals examined [Le and Boen, 1995].

[2] Incidence is another epidemiological measure that, differently from prevalence, evaluate the ratio between the number of new cases of a disease (or a condition) in a specified time period (usually a year) divided by the size of the population initially disease free [Le and Boen, 1995].

the proposed ones, the most credible seems to be the interaction between pollen allergens and air pollution [Bartra et al., 2007; Sofiev et al., 2009], the latter able to exacerbate the allergic reactions to pollen grains, but other factors have also been considered. One of the most interesting explanations of the phenomenon is the so-called "hygiene hypothesis": the westernized life style of the developed countries provides for intensive indoor cleaning, and this could expose sensitive subjects to the allergens present in detergents, as well as prevent the necessary contact with infectious agents in early childhood, facts that could trigger later problems of the immune system in not recognizing common substances as innocuous [WHO, 2005]. The modality of exposure to allergens are in fact considered of crucial importance for the development of allergic diseases.

Aerobiological literature

In recent years, phenological, biometeorological and aerobiological investigations on allergenic plants have gained greater importance because of the increasing prevalence of allergic diseases worldwide. Many of these studies were performed with the aim of building a phenological model that is able to forecast the start, duration and severity of the pollen season at the local level [Rodríguez-Rajo et al., 2004]; This is important to plan the start of antiallergic treatment before the beginning of pollination in order to optimize its effectiveness [Laaidi, 2001a; Torrigiani Malaspina et al., 2007; Sofiev and Bergmann, 2013].

Contribution of this research

But on the other hand, aerobiological research on wider geographical areas is largely lacking [Sofiev et al., 2009]. This is the reason why the presented research project has been focussed on more general aspects of the topic. The research contribution presented in the current thesis confirms an unequivocal increase in the quantities of airborne pollen over a large area (European scale) and a considerably extended time span (1977-2009), suggesting such an increase as a possible concomitant cause of the allergy rise [Ziello et al., 2012b, chapter four of this thesis]. In fact, a larger presence of pollen grains in the air inevitably leads to increase the probability of being exposed to pollen allergens, and hence of getting sensitized to pollen. Another important contribution in this direction is provided in Ziello et al. [2012a, chapter three of this thesis], in which the lengthening of the pollen season has been clearly inferred from the phenological observations analyzed. This last finding is of great clinical importance: a more extended pollen season is additionally considered a possible cause of the increasing rates of allergic diseases, due to a longer time of exposure to pollen allergens [Beggs, 2004; Beggs and Bambrick, 2005].

1.4 ALLERGENIC SPECIES IN EUROPE

Allergenic plants are usually very common, widespread species that produce pollen in high quantity and are mainly wind pollinated [Culley et al., 2002]. The most important allergenic pollen in Europe belongs to members of the families of Betulaceae, Poaceae, Asteraceae, Oleaceae and Urticaceae. As reported by D'Amato et al. [2007], Europe can be divided into five areas, each of them characterized by its own distribution of allergenic plants as follows:

- Arctic area - birch;
- Central area - deciduous forest, birch, ragweed;
- Eastern area - grasses, mugwort, ragweed;
- Mountain areas - grasses;
- Mediterranean area - *Parietaria*, olive trees, grasses, cypress.

Commonly, allergenic species are classified into three main groups: grasses, weeds and trees.

1.4.1 *Grasses*

Grasses (the Poaceae family) represent the most common cause of hay fever in Europe as well as in many parts of the world: an atmospheric airborne pollen concentration of 30 grains/m^3 is often regarded in the literature as the minimum required to trigger the pollinosis symptoms, even if such thresholds strongly depend on the subject (the more sensitive the patients, the lower the pollen counts) and on the location analyzed [Esch, 2004; D'Amato et al., 2007; Sofiev et al., 2009]. Generally, the classic peak pollination period for grasses is June; More specifically, the pollination period starts at the beginning of May and finishes at the end of July [Esch, 2004]. In southern Europe, the pollination period of grasses has advanced by about one month, due to higher temperature and milder climatic conditions, while in mountain areas is delayed by around two/three weeks [D'Amato et al., 2007; D'Amato and Cecchi, 2008; Emberlin, 2009]. Among the allergenic grasses that produce the highest pollen quantities are timothy (*Phleum pratense* L.), orchard grass (*Dactylis glomerata* L.), meadow foxtail (*Alopecurus pratensis* L.), and cultivated rye (*Secale cereale* L.), however all pollen produced by grasses show a very high level of cross reactivity.

1.4.2 *Trees*

More detailed is the classification of allergenic trees. They can be divided in turn into those belonging to the order of Fagales, those belonging to the Oleaceae family, and finally the cypress family (Cupressaceae).

In the order of Fagales, we find three main allergenic families: ordered by their clinical importance, the Betulaceae family, including birch (*Betula* genus) and alder (*Alnus* genus), the Corylaceae family, including hazel (*Corylus*), hornbeam (*Carpinus*) and hopbeam (*Ostrya*), and the Fagaceae family, to which oak (*Quercus*), beech (*Fagus*), and sweet chestnut (*Castanea*) belong. *Hazel and alder* Hazel and alder are the first to flower in the year (December to April), followed by birch, hornbeam and hopbeam. However, in the European mountainous areas, the peak alder season occurs later, between the end of May and mid-June [D'Amato et al., 2007]. *Birch* Concerning the birch, probably the most important allergenic tree from a clinical point of view, the pronounced sensitivity to temperature of this early-successional species causes geographical variations in the start of the pollen season, from the end of March in western Europe, or beginning of April to mid-April in central and eastern Europe, until the end of April or the end of May in northern Europe, where *Betula* pollen represents the most important cause of allergy. Temperature also is the driver of the extreme variability in the length of the flowering/pollen season for birch, which ranges from two to eight weeks, yearly alternating high pollen production and very low pollen presence [Sofiev et al., 2009]. *Oak, beech, sweet chestnut* The oak pollen season starts later in spring, shortly before that of beech, followed in June by the sweet chestnut season. Because of the phenomenon of cross reactivity, birch pollen-induced allergies can give symptoms that start during the pollen season of hazel and alder, peak concomitantly with the birch pollen season and decrease in intensity, finally disappearing, tracking the oak pollen season. In this way, a subject allergic to birch pollen presents respiratory issues up to the whole first half of year [D'Amato et al., 2007].

The allergenic species of Oleaceae are mainly three: olive (*Olea europea* L.), European ash (*Fraxinus excelsior* L.), and European privet (*Ligustrum vulgare* L.). Ash and privet cause allergies very rarely, differently from olive, which is instead one of the most clinically important species in the Mediterranean region. The main pollen season of olive is from April to June.

Olive

Last but not least, the Cupressaceae family includes the genus *Cupressus*, producing in winter an impressive amount of anemophilous pollen. This genus is considered to be responsible for a large part of the total annual amount of airborne pollen in the Mediterranean area. It seems very difficult to identify a reference time span for the cypress pollination season: this is due to the gradual mechanism of microsporophyllous maturation (from the bottom to the top of the flower), which influence the extreme length of the season (longer than one month) and, furthermore, because pollination shows a high variability year by year [Hidalgo et al., 2003; Torrigiani Malaspina et al., 2007].

Cypress

1.4.3 *Weeds*

The Urticaceae (nettle) family includes the allergenic genus *Parietaria* (pellittory), of which the most clinically important species are *Parietaria judaica* L. and *Parietaria officinalis* L., and whose pollen is considered as perennial (i.e., found all year round) due to its extremely long persistence in the atmosphere [D'Amato et al., 2007]. It principally grows along coastal Mediterranean areas, but can also be found up to the UK. Its extensive flowering period covers a time span ranging from March to November [Sofiev et al., 2009]. In particular, *P. judaica* flowers twice a year, with a longer pollen season from the beginning of spring until summer months, and a shorter one between the end of August and October [D'Amato et al., 2007].

Parietaria

Among the extremely large (more than 20000 species) Asteraceae family, also known as Compositae, the genera Ambrosia (ragweed) and Artemisia (mugwort) are the most involved in allergic diseases. In particular *Ambrosia artemisiifolia* L., a species native to North America and whose pollen represents a common allergen in the US, was first identified in Europe during the sixties and has invaded in the last decades large parts of eastern Europe (e.g., Hungary), spreading also in some parts of the Mediterranean area (e.g., northern Italy or Croatia) [Sofiev and Bergmann, 2013; D'Amato et al., 2007], with a marked preference for mid-latitude areas of continental climate. Mugwort

Ragweed and mugwort

seems to represent a minor problem in terms of amount of pollen produced, but it has to be noted that airborne pollen counts could to some extent underestimate the actual amount available at ground level. In fact, this pollen type could be too heavy to reach pollen traps, usually positioned at several meters above the ground [Sofiev et al., 2009].

1.5 ALLERGENIC SPECIES: DOES THE POLLINATION MODE MATTER?

Angiosperms and gymnosperms

Seed-bearing plants (spermatophytes) are taxonomically separated into two classes: angiosperms, whose ovules are enclosed in an ovary, and gymnosperms, in which the ovules are borne naked on the surface of the megasporophylls often arranged in cones. Among the previously cited allergenic species, all except cypress belong to the angiosperms, and this factor could play a key role in explaining, understanding then forecasting the phenology (the timing of cyclic life events like flowering or leaf fall) of such plants.

Wind pollination

Whilst the gymnosperms were originally wind-pollinated, anemophilous angiosperms switched from insect-pollinated ancestors to wind pollination, as inferred from anatomical considerations and most recent phylogenetic analyses [Dowding, 1987; Culley et al., 2002; Friedman and Barrett, 2008]. Wind pollination was previously thought to be less efficient than entomophily at mid- or long-range distances, also because anemophilous pollen is characterized by a shorter longevity [Regal, 1982; Dafni and Firmage, 2000; König and Ashley, 2003]. But the effectiveness of anemophily has recently been reconsidered in the attempt to understand the evolutionary choice of those angiosperms that, for at least 65 times, switched again to wind pollination during their evolutionary patterns, taking advantage of the different energetic balance that characterizes this reproductive system [Heinrich and Raven, 1972; Heinrich, 1975; Friedman and Barrett, 2009].

Main findings

The presented research [Ziello et al., 2012a, chapter three of this thesis] clearly established that, in recent years, wind-pollinated species tend to show larger advancements in first flowering dates than insect-pollinated ones. This surprising finding is explained in light of the peculiar ecological evolution of the wind-pollinated angiosperms, which could have finally selected species able to respond more efficiently and more rapidly than others to climate variability. Moreover, the mechanism that regulates the time of flowering and, hence, the pollination season, can be simpler in species that do not rely on biological vectors (insects, birds) for their reproductive success. In fact, such species do not experience any compulsory need for tracking the phenological behavior of pollination agents, which could potentially be stable under climatic changes or exhibit only minor changes, so the start of their reproductive phases is independent of this aspect. In addition, since the advancements of full flowering time of wind-pollinated species have been inferred as large as those of insect-pollinated species, it can be said that wind-pollinated species not only start flowering earlier than insect-pollinated ones, but have also lengthened the time in which they are in flower while insect-pollinated plants do not. This last finding is particularly important for public health: The plant group including the majority of allergenic plants responds to recent climate change extending its pollination period, i.e. the time span of the pollen release, with clear consequences for allergy sufferers.

1.6 PHENOLOGY AND ITS CONNECTIONS TO AEROBIOLOGY: NEW PERSPECTIVES FOR AN ANCIENT SCIENCE

What is phenology?

The word phenology derives from ancient greek words φαίνω (to show, to appear) and λόγος (study, reasoning). It indicates the science of seasonally recurring life-cycle stages in plants and animals, often referred to as phenological phases or phenophases. Phenological observations have a long tradition, starting probably among those primitive farming societies that first abandoned nomadism in favor of a more stable and sedentary lifestyle, which needed to note changes in vegetation and harvests according to the seasons; Unfortunately, little or no trace has remained of such early observations [Schwartz, 2003]. The great Mediterranean civilisations (Egypt, Mesopotamia), as well as those in Asia (China), have left signs of phenological observations made thousands of years ago [Schwartz, 2003], but the 17th century only has been witness of the important passage from descriptive phenology to modern scientific phenology, thanks to the work of White, Marsham and above all, Linnaeus and Reaumur [Puppi, 2007]. However, after a long period in which the practice of such science was neglected and its use very limited, it acquired a new strategic importance in light of recent climate change. In fact, phenological research has been one of the most successful tools in assessing the (sometimes dramatic) impact of changing climate on a large number of natural ecosystems [Rosenzweig et al., 2007].

Plant phenology

Especially plant phenology, being mainly influenced by temperature followed by moisture and daylight, has become an important benchmark to test the effects on vegetation of rising temperatures [Badeck et al., 2004; Menzel et al., 2006]. The scientific community has been able to assess a general trend for natural spring and summer events to advance [Menzel et al., 2006; Parmesan, 2006; Cleland et al., 2007; Ibáñez et al., 2010; Thackeray et al., 2010], as well as for autumn phenophases to delay [Jeong et al., 2011; Zeng et al., 2011] under climate change conditions. As a consequence, the vegetation period (in other words, the growing season) has increased in length for a large part of the areas and the species analyzed [Ibáñez et al., 2010; Jeong et al., 2011; Zeng et al., 2011]. Such evidence is, in principle, valid for every part of the planet, but in practice mostly tied to the Northern Hemisphere, where the majority of suitable phenological data is concentrated.

The importance of well-established networks

In order to investigate the spatial and temporal response to climate change or to reconstruct past climate, the analysis of data sets covering extensive areas and large time spans is essential. The early establishment of national and/or continental networks has been hence of fundamental importance for phenological research. In a recent review, Tooke and Battey [2010] reported the most important phenological networks and relative data sets, whose numerous analyses still continue being published [Primack and Miller-Rushing, 2012]. Among the others, the European phenological network established within the COST Action 725, titled "Establishing a European Phenological Data Platform for Climatological Applications", was the source of the data analyzed in two of the three publications collected in the present thesis.

Aerobiology and phenology

Also out of the context of the present thesis, the relationship between aerobiology and phenology is clear: the phenological phase of flowering is connected to the release of pollen. A plant in flower is, reasonably, a plant whose pollen is ready to be spread by the appropriate vector, according to a specific pollination mode. In the context of aerobiological research, the role of phenology is of primary importance and mostly, but not only (see for example Jochner et al. [2012]), related to the construction of phenological models to be nested in models of atmospheric transport for pollen level forecasting [Sofiev et al., 2006]. In fact, the use of phenological models can sensibly improve the reliability of pollen level forecasting, because it provides a process-based (more accurate) estimate of the timing in which allergenic plants will flower [Kasprzyk and Walanus, 2007], which add to the known relationships of pollen counts to meteorological factors [Sofiev and Bergmann, 2013]. In the presented research, the phenology of flowering dates was simply used as a proxy for likely changes in pollen release dates: Flowering dates are easier to obtain than the very expensive pollen levels from pollen traps, and represent a reasonable, even if not ideal, tool to estimate pollen counts [Estrella et al., 2006; Tormo-Molina et al., 2011].

1.7 GENERAL RESEARCH SUMMARY AND RESEARCH QUESTIONS

Background ideas

The general aim behind the research project presented here was to assess the actual changes on a large scale (at best on a European scale) in both intensity and duration of the pollen season, particularly focusing on allergenic species, with a further purpose to link these changes (if present) to the global processes that characterize climate change. Additionally, the purpose of not resorting to data obtained as model output was especially important: In contrast to data from phenological observations, modeled data are an additional source of (sometimes large) uncertainty, and they obviously decrease the statistical significance of final results. To achieve such an ambitious goal, the only approach suitable was to work with data sets as large as possible and derived from direct observations, including a variety of species as large as possible, and covering a geographical area as large as possible. But managing such extensive data sets imply several issues, such as the possibility of spatial autocorrelation, or that of including more (or less) predictors than needed when modeling the data (statistical flaws known as overfitting and underfitting, respectively). Moreover, since direct aerobiological measurements (i.e., pollen counts) are especially time- and human resources-consuming, requiring for the evaluation a number of intermediate steps not yet automated, these data are especially difficult to obtain and extremely expensive; Hence, phenological data from well-established European networks have been used to infer indirectly large-scale changes in the duration of the pollen season.

For such reasons the described research plan, necessary to investigate continental-scale changes, implied the consideration of more specific underlying topics. The need for analyzing a large set of species led to include in the analysis some correlates connected with plant traits, in an attempt to avoid neglecting factors important for describing species' phenology. And also, the large size of the geographical area of interest, which includes a number of highly elevated zones, made necessary an assessment of the influence of the altitude on both plant phenology and plant phenological trends.

Research questions

To summarize what has been reported above, the primary research questions that will be addressed are:

- Are there changes in the intensity of the pollen season (i.e., in the total amount of airborne pollen)?
- Are there changes in the duration of the pollen season?
- If present, are those changes somehow connected to recent climate change?

To address these questions in full, it was also necessary to answer the following two secondary research question:

- Does altitude a.s.l. influence phenology and phenological trends, and hence aerobiological data in turn?
- Do plant traits influence the phenological responses of vegetation to climate change? If so, which ones?

Main findings

The research questions have been answered through this thesis according to the following scheme:

- *Second chapter*: This publication [Ziello et al., 2009] analyzed the influence of altitude a.s.l. on first and full flowering dates of selected plant species of the Alpine region, over the years 1971-2000. More importantly, the effect of altitude on the temporal trends of the available phenological dates was also evaluated.
- *Third chapter*: This publication [Ziello et al., 2012a] assessed the influence of pollination system (wind versus insect pollination) and woodiness on the phenological trends of a large set of species, using a data set whose geographical scope covers a good part of Europe. The detection of differences between trends of first flowering dates and those of full flowering dates identified appreciable changes in the temporal length of the total (i.e., all-species) flowering period, hence indirectly of the period in which plants release pollen in the air.

- *Fourth chapter*: This publication [Ziello et al., 2012b], made possible by a European collaboration with those numerous aerobiologists who were willing to share direct pollen data (partially manipulated), allowed the first continental-scale multi-species investigation of changes in the amount of pollen in the air, over the years 1977-2009. This analysis, hence, evaluated the existence of trends in the intensity of the pollen season, over considerable time extension as well as over a large geographic area.

1.8 OUTLINE OF THIS THESIS

A more detailed description of the work presented in this thesis will be given in the following.

Chapter one

The first chapter is an introductory chapter. Some preliminary information is reported, as well as the present detailed outline of this thesis.

Chapter two

In the first publication [Ziello et al., 2009], presented as second chapter in this thesis, the influence of altitude a.s.l. on the phenology of a number of species widely distributed in Europe, some of them with allergenic relevance, was assessed. This analysis involves a large part of the Alps and surroundings, and was planned to test whether mountain areas, always considered healthier for hay-fever sufferers due to the low presence of airborne pollen, are experiencing, under climate change conditions, changes that could appreciably compromise this characteristic. The used data were collected within the COST (European COoperation in Science and Technology) action 725 "Establishing a European phenological data platform for climatological applications" (`http://www.cost725.org`). While a clear influence of altitude on the phenological dates examined was observed, and also quantified in terms of delay in days per 100 m altitude for specific phases and species, the analysis showed an almost total lack of any altitudinal effect on phenological trends. Therefore, this work has excluded the possibility of phenological advancements driven by altitude, even if it confirms the extreme phenological sensitivity of the whole region in terms of strong averaged trends. Moreover, the region was divided into different zones and the existence of latitudinal as well as longitudinal dependencies was evaluated. This last analysis showed that the influence of altitude on phenodates was more marked at the north than at the south of the Alpine arch, as could be inferred from the peculiar topography of the area under study, which influences local climatic conditions.

Chapter three

The second publication [Ziello et al., 2012a], presented as third chapter, was conceived to exploit the influence of plant traits on the phenological changes exhibited by plants and collected in an extensive European data set, very likely in the attempt to react to changing climate conditions (as already assessed over the same data set by Menzel et al. [2006]). The traits to study had to match to two basic criteria: i) they should have been connected to the allergenicity of species, in order to identify characteristics that could best correlate to this medical aspect and therefore allow an interpretation of the results from an allergological perspective, and ii) they had to be easy to assign to each species of a very large and diverse set, whose original composition ranges from aquatic to terrestrial species, including through agricultural crops and weeds. The choice has thus fallen on woodiness (woody versus non-woody) and pollination mode (insect versus wind). On the one hand, wind pollination is in fact a distinctive trait of allergenic species, which generally produce impressive quantities of light pollen grains and release them in the air, causing the exposure of sensitized subjects to huge amounts of allergenic bioaerosols. And on the other hand, woodiness ideally divides trees from grasses and weeds, which flower in substantially different periods of the year (late winter and spring for trees, summer and early autumn for grasses and weeds) and hence trigger hay-fever symptoms in diverse seasons. According to the standardization proposed by Cornelissen et al. [2003], pollination mode can be classified as a functional trait, woodiness as a Plant Functional Type (PFT), and flowering dates themselves as an ecological trait. By means of statistical techniques mainly novel in the context of phenological research, an advanced analysis of the influence of pollination, woodiness, and timing of onset on phenological trends was carried out. The analyzed trends were relative to first flowering and full flowering dates, and these two phases have been kept constantly separated in the performed calculations. The influence of pollination mode and woodiness on flowering (first flowering and full flowering) trends was assessed using weighted linear mixed models, with weights set to the precision inverse of the underlying regression (standard error) [Becker and Wu, 2007]. This was to account for the inhomogeneity of trend variances, because each trend in the data set was computed from a different linear regression (phenological dates on years) and was hence associated with a different standard error. Standard methods, such as t-test and linear regression, usually suitable for assessing statistical differences, assume independence of observations and variance homogeneity, which both cannot be assumed for this data set. In fact, in addition the sample cannot be seen as independent since observations come from different geographic locations, hence records from the same station are likely to be more similar to each other than records from different stations (phenomenon known as spatial autocorrelation); This specific flaw is generally negligible when the study area is limited, but in this case it is worth taking into account due to the considerable extent of the geographic range. These characteristics of the data were addressed by conducting the analysis in the framework of a Linear Mixed Model (LMM) [McCulloch et al., 2008]. As an important consequence, the statistical significance of the analyzed phenological trends is also accounted for in the analysis, which makes this a novel and innovative study in the field of phenological research. Statistical significance of results was assessed using 1,000 bootstrap samples [Efron and Tibshirani, 1993], and goodness of fit was calculated by means of an R^2 measure for mixed models based on the likelihood [Xu, 2003]. This was settled to adjust for data set size the probability to get statistically significant differences amount pollination modes and woodiness types, which could be biased due to the huge amount of entries the data set consists of (the probability to reject a hypothesis is, in fact, inversely proportional to the amount of data). The most important results show that wind-pollinated plants are more prone to advance their start of flowering dates than insect-pollinated species, and that in general first flowering phases are advancing more than full flowering ones, leading to a lengthening of the flowering period especially pronounced for wind-pollinated species. The phenological behavior of wind-pollinated species detected is in direct contrast with that found by Fitter and Fitter [2002], who reported totally different conclusions (insect-pollinated plants more advancing than wind-pollinated ones) in one of the best known published studies on the topic.

Chapter four

In the third publication [Ziello et al., 2012b], presented as fourth chapter, the basic idea was that of producing an aerobiological study unique of its kind for the time spans analyzed, number of species included, and size of geographical range covered. In fact, one of the major flaws of the nevertheless broad literature on aerobiology is the paucity of multi-location and/or multi-species studies; These geographical and ecological restrictions are inevitable for studies based on prediction models, the latter being tied to specific conditions due to the nature of the predictive mathematical tools, but of course not for works focussed on direct observations. The available literature, instead, usually proposes results valid mainly for single or a few localities, and for single or a few pollen taxa; But when this does not happen, in the rarely available more ambitious studies, the final results are often not consistent among the different localities and/or the different species analyzed [Sofiev et al., 2009]. For such reasons, aerobiological research suffers from the lack of more general results as well as of broader and more definitive conclusions. A secondary but not less tangible problem that contributes to this picture is the difficulty to obtain data of direct pollen counts, for the reasons previously discussed of high costs and experimental difficulties. In the presented research, the latter issue was solved i) starting a large collaboration among European scientists involved in this research area, and ii) deciding to focus on data already partially elaborated by the relative owners, instead of raw ones, so as to involve more actively the providers as co-authors. The use of partially elaborated data, specifically of *trends* in pollen counts, also allowed to neglect the problem of different quality levels and standards of raw measurements, resulting in a data set from 97 locations spread over 13 European countries. In this way, the study under consideration in this chapter could aim, with success, at assessing broad changes in the amount of pollen on the European scale, by means of very simple but cogent statistical analysis. The most important result was the detection of unequivocal increases in atmospheric pollen load for many taxa, even if the attribution of such changes to increased temperature, species' density, or geographical effects (latitude, longitude, altitude a.s.l.) did not lead to equally certain causes. Very interesting was the detection of higher increases in pollen amount in urban areas, compared to rural and semi-rural locations, which suggested the role of CO_2 and O_3, more largely present in urban environments, in triggering the observed phenomenon of increased pollen amounts.

Chapter five

Finally, in the last section presented as fifth chapter, the work done will be discussed in its entirety and its relations to all the most recent studies in literature will be extensively and carefully highlighted. Plus, key aspects of research will be elaborated in more depth.

2

INFLUENCE OF ALTITUDE ON PHENOLOGY OF SELECTED PLANT SPECIES IN THE ALPINE REGION (1971-2000)

Chiara Ziello[1], Nicole Estrella[1], Mariya Kostova[1], Elisabeth Koch[2], and Annette Menzel[1]

[1] Chair of Ecoclimatology, Technische Universität München,
Am Hochanger 13, 85354 Freising, Germany
[2] Department of Climatology, Zentralanstalt für Meteorologie
und Geodynamik, Hohe Warte 38, 1190 Wien, Austria

ABSTRACT

We present a quantitative and qualitative analysis of the influence of altitude on plant phenology over an area, delimited by 46 to 49°N latitude and 5 to 15°E longitude, that includes major parts of the Alpine region for the period 1971-2000. Our attention is focused on flowering of widely distributed plants in Europe, including some important allergenic species. We calculated the dependence of phenological mean dates on altitude for different phenophases as a linear regression. Results show a statistically significant linear dependence ($p < 0.0001$) for each phenophase, with regression coefficients that range from 0.92 ± 0.21 d $(100\ \text{m})^{-1}$ for beginning of flowering of Norway spruce, to 4.56 ± 0.21 d $(100\ \text{m})^{-1}$ for beginning of flowering of common hazel. Phenological temporal trends and thermal temporal trends show a less strong dependence on altitude, predominantly not significant. The most significant regression coefficients are negative for phenological trends (beginning of flowering of common alder, -0.065 ± 0.028 d yr^{-1} $(100\ \text{m})^{-1}$, $p = 0.0187$ and beginning of flowering of Norway spruce, -0.049 ± 0.020 d yr^{-1} $(100\ \text{m})^{-1}$, $p = 0.0167$) and positive for temperature trends (February, 0.0017°C yr^{-1} $(100\ \text{m})^{-1}$, $p < 0.0001$). The significant linear dependence of February temperature trends on altitude induces an inverse significant linear dependence for phenological trends of flowering of common

alder (occurring in March). The prevalence of negative mean phenological trends suggests a stronger advance of flowering phases at higher altitude. Finally, a regional analysis suggests a tendency towards a stronger altitudinal response in the northern than in the southern Alps.

2.1 INTRODUCTION

In the past, mountain regions were regarded as a healthy shelter for hay fever sufferers due to the low presence of airborne pollen, compared to plain regions [Michel et al., 1976]. Recently, however, highlands seem to be more strongly affected by climate change than other regions [Cannone et al., 2008]. Global warming could be a major cause of biodiversity change on mountain tops. A general rearrangement of the Alpine flora could result from an elevational shift of species range, following possible vulnerability of the resident species to invasion from newcomers [Walther et al., 2002]. Phenology, the science of recurrent seasonal natural events, can help scientists understand changes in plants of the Alpine region that are due to the influence of global warming, and phenological observations have gained an important role in assessing the impacts of climate change [Menzel, 2002].

Altitude drives phytophenological phases mainly by means of a temperature decrease of about 0.6°C every 100 m [Barry, 1981]. Although temperature can be considered the most important forcing factor in plant physiology [Defila and Clot, 2005], other effects concomitant with an altitude increase are: (1) progressive reduction of land area; (2) decrease of total atmospheric pressure and partial pressure of all the component gases (concentrations of O_2 and CO_2 are crucial for vegetation and are of vital importance for animal life); and (3) increment of solar radiation during clear sky conditions, with higher percentage of the UV-B component [Körner, 2007].

Several studies have been carried out to investigate the effects of altitude on phenological phases and their temporal trends in different zones of the Alpine region [Dittmar and Elling, 2006; Larcher, 2006]. However discrepancies in the results due to the different zones taken into account still need to be clarified [Defila and Clot, 2001; Studer et al., 2005]. Our main objective is therefore to gain a better comprehension of how phenophases and trends of allergenic plants depend on altitude. We study an extended fraction of the Alpine area with the aim of unambiguously pointing out (if present) such a dependence. Finally, to complete this geographical overview, we offer a brief analysis of the influence of land zones as a further forcing factor to identify other possible dependences.

2.2 MATERIALS AND METHODS

In the present study we used the data set collected within the European Cooperation in Science and Technology (COST) action 725 "Establishing a European phenological data platform for climatological applications" (`http://www.cost725.org`). Among this extensive dataset, comprising more than 125,000 digitally available observational series of various phases in 542 plant and 19 animal species from 21 European countries for 1971-2000 [Menzel et al., 2006; Rosenzweig et al., 2007], we selected widely distributed plants, including some important allergenic trees (e.g. *Corylus avellana* L. and *Alnus glutinosa* L.) and grasses (e.g. *Dactylis glomerata* L. and *Secale cereale* M.Bieb.) (Table 1). Monitoring of allergenic plants is of great importance in relation to climate change: modifications in range distribution and flowering (i.e. pollination) time for these species are important possible consequences of global warming. They affect the life quality of pollen-induced allergies sufferers, representing 10 to 20% of the total population of Europe [Huynen et al., 2003]. All phenological phases (see Table 1 for abbreviations) taken into account are related to different secondary stages of flowering, according to the extended BBCH-scale (Biologische Bundesanstalt, Bundessortenamt and CHemical industry). The variety of the considered phenophases is due to the multiple data provenance within the COST dataset, to which information converges from various phenological networks and hence from different monitoring schemes. We also used the E-Obs dataset from the EU-FP6 project ENSEMBLES for monthly mean temperature data in Europe [Haylock et al., 2008].

The study area (45°50′ to 49°N, 5 to 15°E) includes a good part of the Alps and their surroundings in Austria, Germany and Switzerland. In this territory are located 1,399 phenological stations: 948 in Germany, 277 in Austria, 152 in Switzerland and 22 International Phenological Gardens (IPGs), with altitude between 100 and 1,900 m above sea level (a.s.l.). Geographical position and relative altitude of each station are shown in Fig. 1. Temperature data were provided in a regular grid of resolution

Table 1: Phenological phases and plant species considered in this study.

Phase	Species (common name)
Full flowering FF	*Aesculus hippocastanum* L. (horse chestnut)
Beginning of flowering BF	*Alnus glutinosa* L. (common alder)
Beginning of flowering BF	*Corylus avellana* L. (common hazel)
Beginning of flowering BF	*Dactylis glomerata* L. (cocksfoot grass)
Beginning of flowering BF	*Picea abies* (L.) H. Karst. (Norway spruce)
First flowers open FFO	*Salix caprea* L. (goat willow)
Full flowering FF	*Sambucus nigra* L. (black elder)
First flowers open FFO	*Secale cereale* M. Bieb. (rye)
First flowers open FFO	*Tilia cordata* Mill. (small-leaved lime)
Full flowering FF	*Vitis vinifera* L. (common grape vine)

(0.5° latitude × 0.5° longitude) for a similar area, between 46 and 49°N and 5 and 15°E.

To calculate the average of phenological onset dates, we decided to select only time series with >15 yr of observations for the years 1971-2000. This threshold was considered high enough to make mean dates a better statistical estimate and low enough not to reject too many time series.

Temporal trends of phenodates (one for each station and each phenophase) were obtained as coefficients of linear regressions of phenological date (day of the year) versus year over the period 1971-2000. A negative value of a trend hence indicates an earlier occurrence of the respective phase, whereas a positive value represents a delay. On the other hand, temporal trends in monthly or seasonal mean temperature were calculated for every grid cell and plotted against years for the same period (1971-2000). In this case, if the coefficient of the linear regression is positive it is related to an increase in temperature, if it is negative to a decrease.

To better understand changes in behaviour of altitudinal gradients of both mean onset dates and phenological temporal trends that could depend on regional differences, we selected 7 different smaller areas inside that Alpine region previously indicated as the reference area of COST dataset (Fig. 2). Germany was divided into the Black Forest, Schwarzwald region (SW) and the region south of Danube and prealpine plateau (SDPP); Austria into the northern prealpine (NPR), alpine (AR) and southern prealpine regions (SPR); and Switzerland into the central (CR) and alpine regions (AR). GIS software was used to select the zones in which the calculation of altitudinal gradients was possible, discarding phenostations without corresponding sites at different altitudes.

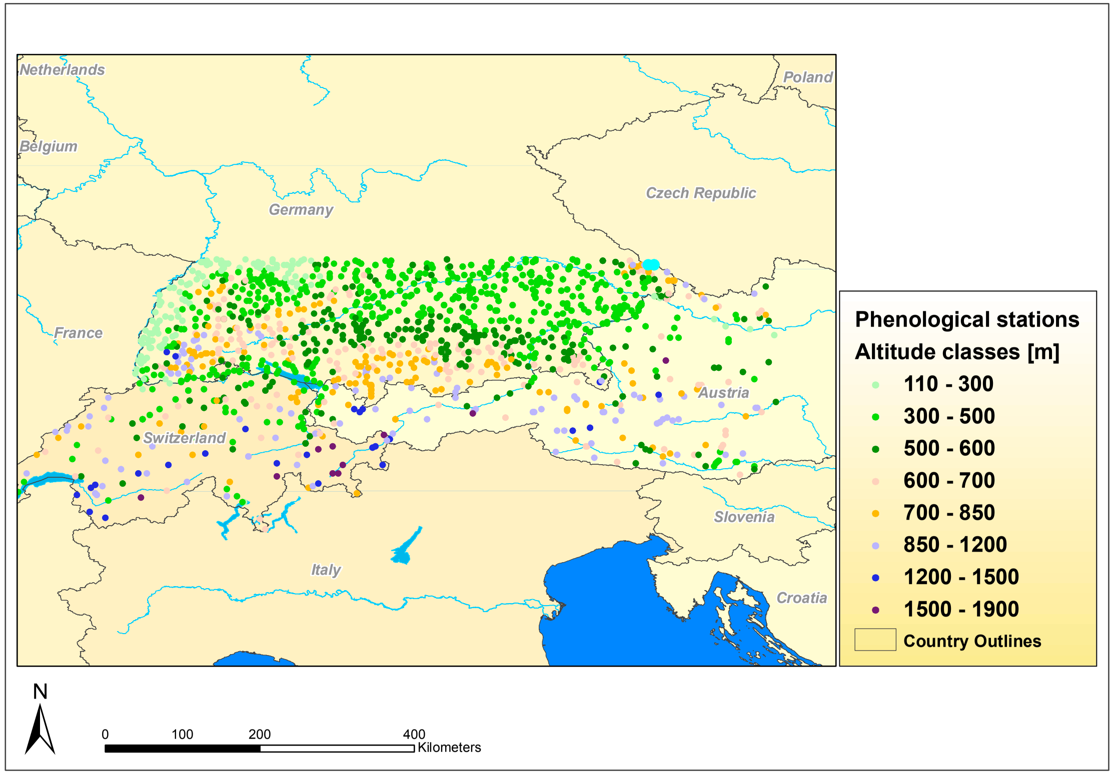

Figure 1: Geographical position and altitudes of phenological stations. Balancing station numbers per class without losing altitudinal representativeness resulted in unequal altitudinal ranges.

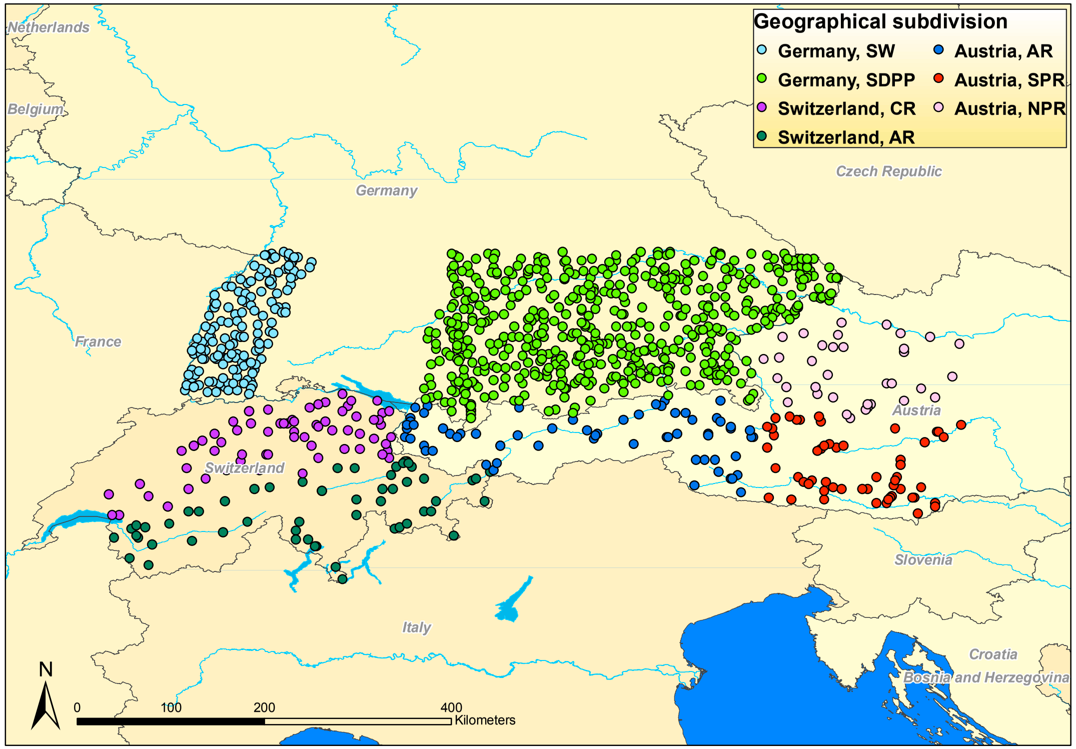

Figure 2: Geographical subdivision of the Alpine region originally defined in Fig. 1. Seven zones were selected to calculate altitudinal gradients by using GIS software. Germany (D) was divided into the Black Forest (Schwarzwald) region (SW) and the region south of Danube and prealpine plateau (SDPP); Austria (A) into the northern prealpine (NPR), alpine (AR) and southern prealpine regions (SPR); and Switzerland (CH) into the central (CR) and alpine regions (AR).

2.3 RESULTS AND DISCUSSION

Phenological mean dates were considered as a function of altitude of the station. Group representations in Fig. 3 show these altitudinal gradients (each series describes the behaviour of a different phenophase, and each point of a series represents a mean date of a single phenostation). A linear regression model has been used to fit these datasets, and the results are shown in Table 2.

The very high level of significance confirms for each series the existence of a linear dependence between phenological onset dates and altitude. The value of this altitude response (Table 2) ranges from 0.92 ± 0.21 d $(100\ \mathrm{m})^{-1}$ for Norway spruce BF to 4.56 ± 0.21 d $(100\ \mathrm{m})^{-1}$ for common hazel BF. These differences are probably due to the different sensitivity of species to changes in temperature. The value of R^2 is >0.50 for 6 out of 10 phenophases, indicating that variability of dates may be well explained by altitude dependence; in the remaining cases, a lower level of relevance (never <0.17) may have been caused by a restricted altitudinal range with respect to the potential range of the species.

Considering a temperature response of −1 to −5 d $°\mathrm{C}^{-1}$ for flowering phases in Europe [Menzel et al., 2006], under the influence of an altitudinal gradients of temperature of 0.6°C $(100\ \mathrm{m})^{-1}$ we should expect to observe an altitude response that ranges from 0.6 to 3.0 d $(100\ \mathrm{m})^{-1}$. Altitudinal gradients of phenodates are out of this range only for common hazel BF (4.56 ± 0.21 d $(100\ \mathrm{m})^{-1}$) and small-leaved lime FFO (4.03 ± 0.24 d $(100\ \mathrm{m})^{-1}$). Both seem to be triggered by relatively early onset dates in altitudes below 400 m a.s.l. The 2 phases with the lowest altitudinal gradients of onset dates, Norway spruce BF and common grape vine FF, are related to the only evergreen species and a warm-loving agricultural species, respectively. Anyway, there is the possibility that the inhomogeneous altitudinal distribution of phenostations results in biased gradients: this could be the case of horse chestnut and common grape vine, for which most of the stations are concentrated at the lower end of the altitudinal range (Fig. 3). In fact, regarding the calculation of the regression coefficients, this gives high weight to the less numerous elevated stations, which could be scarcely representative of their altitude due to the tendency of these species to be situated in thermally favourable places.

Temporal trends of phenodates, calculated for each phenological station, are reported for each phase as a function of altitude in Fig. 4. Results of a linear regression model are summarized in Table 3.

The analysis of altitudinal gradients of phenological temporal trends leads to less clear results in comparison to those of phenological gradients. Values (Table 3) range from 0.042 ± 0.022 (small-leaved lime FFO) to –0.065 ± 0.028 d yr^{-1} $(100\ m)^{-1}$ (common alder BF); however, these are statistical significant ($p < 0.05$) only for 3 phenophases (common alder BF, Norway spruce BF and black elder FF) and the relevance, as indicated by R^2, is low for every phase. In particular, R^2 never exceeds 0.075, reached for the dataset with the minimum number of experimental points (rye FFO). Moreover, the datasets with the largest number of stations involved (common hazel BF, 436; goat willow, 359) have the smallest R^2 value (0.0002 and 0.0012, respectively): this finding strongly suggests the lack of dependence between temporal trends and altitude.

However, negative values of altitudinal gradients of temporal trends occur more often (6 out of 10) than positive values, and among the negative coefficients are included the most significant ones (trends for common alder BF, $p = 0.0187$; Norway spruce BF, $p = 0.0167$). Moreover, mean values of trends are negative for each phenophase. These findings can be explained by a major sensitivity of highland plants to temperature changes, which leads to a stronger reaction to climate change [Defila and Clot, 2005].

Temporal trends in temperature are shown in Fig. 5. Here trends in monthly mean temperatures (January to May) and spring mean temperature (calculated as the mean of the March-May period) have been plotted against altitude, with each point representing the mean temperature of a grid cell (0.5° latitude × 0.5° longitude) with respect to the mean altitude of such a cell.

Table 2: Altitudinal gradients analysis of phenological mean dates across the Alpine region, based on 15+ year means during the period 1971-2000. Gradients have been calculated as coefficients of linear regression (±SE). BF: beginning of flowering; FFO: first flowers open; FF: full flowering; CH: Switzerland; D: Germany; A: Austria.

Phenological phase	Gradient (d per 100 m)	R^2	p	No. stations	Altitudinal range (m)	Country
Horse chestnut FF	3.35 ± 0.18	0.82	< 0.0001	77	300 – 1450	CH
Common alder BF	2.92 ± 0.28	0.30	< 0.0001	254	100 – 1000	D
Common hazel BF	4.56 ± 0.21	0.52	< 0.0001	436	100 – 1100	A, D
Cocksfoot grass BF	2.80 ± 0.41	0.64	< 0.0001	29	150 – 1100	A
Norway spruce BF	0.92 ± 0.21	0.17	< 0.0001	99	150 – 1200	A, D
Goat willow FFO	2.51 ± 0.16	0.42	< 0.0001	359	100 – 1000	D
Black elder FF	3.12 ± 0.21	0.71	< 0.0001	94	200 – 1650	CH
Rye FFO	3.22 ± 0.39	0.78	< 0.0001	21	150 – 900	A
Small-leaved lime FFO	4.03 ± 0.24	0.63	< 0.0001	164	100 – 900	D
Common grape vine FF	1.13 ± 0.26	0.24	< 0.0001	59	150 – 1000	CH

Table 3: Altitudinal gradient analysis of phenological temporal trends (1971-2000) in the Alpine region. Gradients have been calculated as coefficients of linear regression (±SE). BF: beginning of flowering; FFO: first flowers open; FF: full flowering.

Phenological phase	Gradient (d yr^{-1} per 100 m)	R^2	p	No. stations	Mean trend (±SE, d yr^{-1})
Horse chestnut FF	−0.0162 ± 0.0095	0.037	0.0943	77	−0.348 ± 0.024
Common alder BF	−0.065 ± 0.028	0.022	0.0187	254	−0.410 ± 0.046
Common hazel BF	0.006 ± 0.018	0.0002	0.7559	436	−0.543 ± 0.032
Cocksfoot grass BF	0.018 ± 0.021	0.026	0.4066	29	−0.382 ± 0.059
Norway spruce BF	−0.049 ± 0.020	0.058	0.0167	99	−0.090 ± 0.044
Goat willow FFO	−0.009 ± 0.014	0.0012	0.5210	359	−0.237 ± 0.025
Black elder FF	0.025 ± 0.011	0.053	0.0256	94	−0.490 ± 0.031
Rye FFO	−0.040 ± 0.032	0.075	0.2290	21	−0.399 ± 0.058
Small-leaved lime FFO	0.042 ± 0.022	0.023	0.0547	164	−0.189 ± 0.033
Common grape vine FF	−0.034 ± 0.020	0.048	0.0970	59	−0.418 ± 0.036

Additionally, temporal trends in temperature do not show a clear dependence on altitude (Fig. 5) except for February, when temperature trends show a significant and relevant dependence ($R^2 = 0.30$, $p < 0.0001$). Apropos of this, it is important to notice the effect of an altitudinal dependence of thermal trends in February on phenological trends of common alder BF, a phenophase that presents one of the best significance levels of linear regression ($p = 0.0187$, see Table 3). This is due to the greater sensitivity of species to the temperature of the month preceding their flowering time [Larcher, 2006; Menzel et al., 2006], that is March for Common Alder. Hence a stronger dependence of thermal trends on altitude in February induces a more significant dependence of phenological trends on altitude for common alder BF, occurring about 30 d later.

The same procedure and statistical analyses have been employed with reference to a division into zones of the Alpine region previously considered in its entirety. Table 4 reports altitudinal gradients analysis of onset dates. The decrease in observations for each phenophase following the geographical subdivision compromises the statistical quality. Plus, a comparison between the same phenophases among different zones can imply a comparison between datasets related to a different sample dimension and a different altitude range. Keeping in mind these considerations, it is still possible to undertake a systematic zone-by-zone analysis of altitudinal gradients of mean phenodates, by means of the most significant and relevant series.

There is agreement (see Table 4) between common hazel BF in Austria, NPR (5.45 ± 0.68 d $(100\text{ m})^{-1}$) and Germany, SW (5.43 ± 0.36 d $(100\text{ m})^{-1}$), whereas in Austria, SPR a smaller altitude response is observed (3.85 ± 0.87 d $(100\text{ m})^{-1}$). This is in accord with a difference in influence of altitude in Switzerland, between the southern part of the Alps (where an earlier onset of flowering phases occurs) and the northern part [Defila and Clot, 2005; Larcher, 2006]. As a further confirmation, the same behaviour has been found for black elder FF: a smaller gradient of phenodates in Switzerland, AR (2.70 ± 0.39 d $(100\text{ m})^{-1}$) than in CR (4.11 ± 0.42 d $(100\text{ m})^{-1}$). Involving only the northern part of the Alpine region, small-leaved lime FFO presents agreement between Germany, SDPP (2.66 ± 0.44 d $(100\text{ m})^{-1}$) and SW (3.43 ± 0.66 d $(100\text{ m})^{-1}$).

On the other hand, other phases present substantial agreement between northern and southern Alps. We observe such

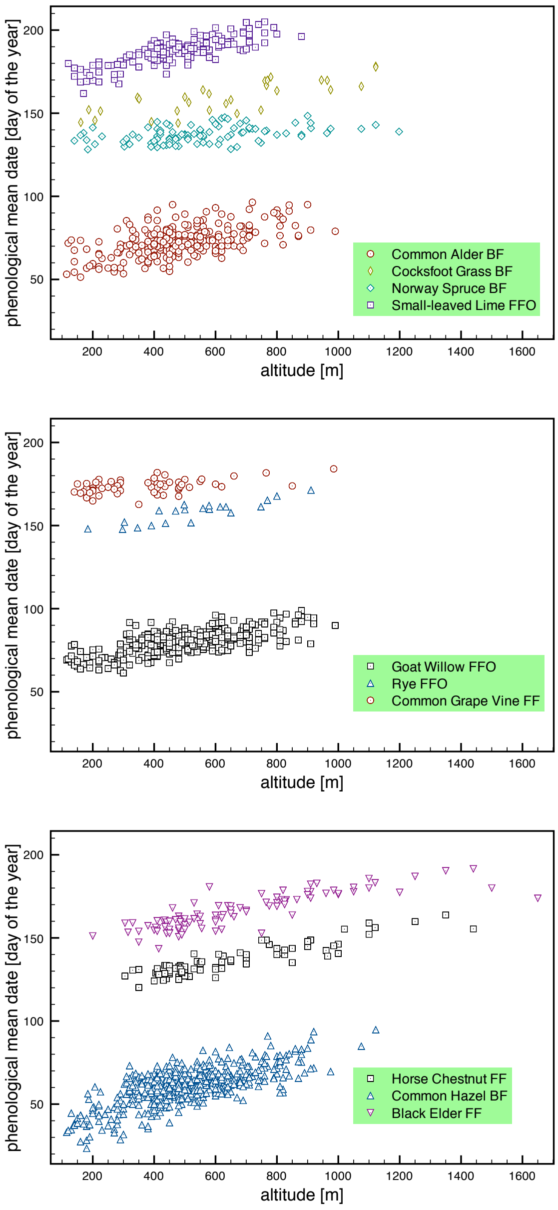

Figure 3: Phenological mean dates versus altitude, calculated in the Alpine region delimited by 45°50′ to 49°N, 5 to 15°E. Each altitudinal series is related to a different phenophase. Means are calculated over temporal series of more than 15 years. Table 2 shows results of a linear regression model used to fit these datasets. BF: beginning of flowering; FFO: first flowers open; FF: full flowering.

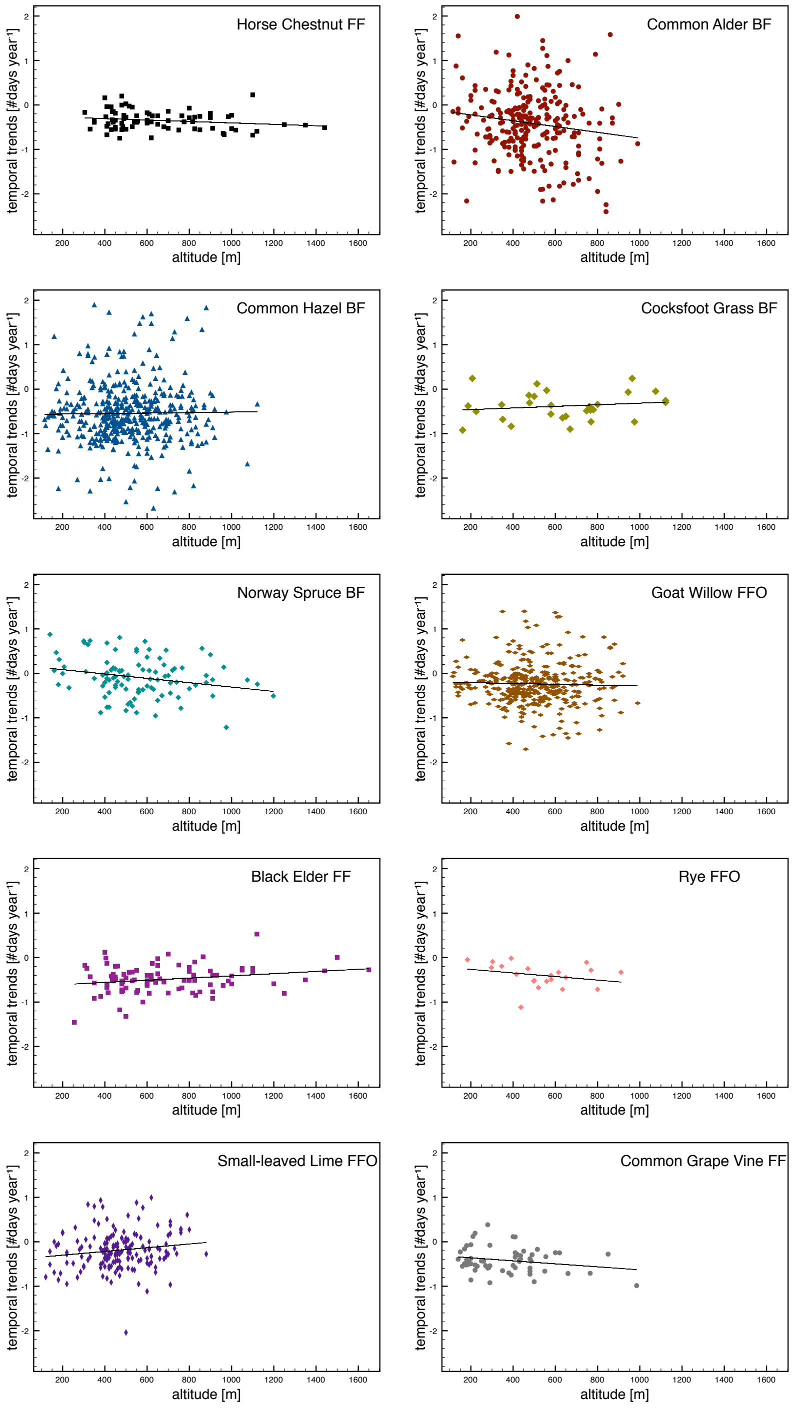

Figure 4: Temporal trends (1971-2000) of 15+ year phenological series versus altitude, together with their linear regressions, in the Alpine region delimited by 45°50′ to 49°N, 5 to 15°E. Results of linear regressions are reported in Table 3. BF: beginning of flowering; FFO: first flowers open; FF: full flowering.

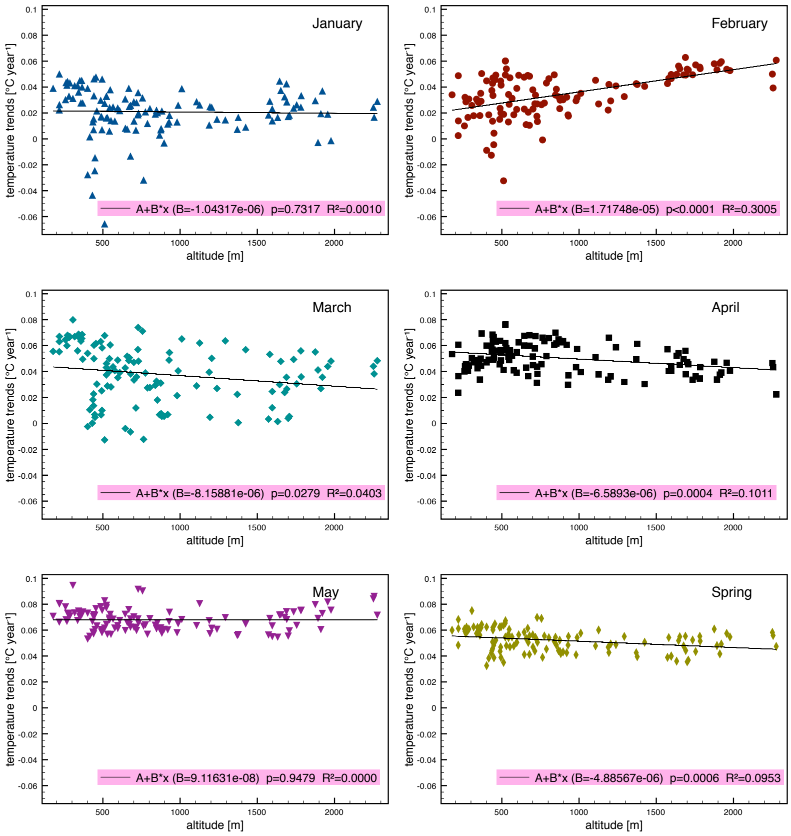

Figure 5: Temporal trends (1971-2000) of monthly mean temperatures and spring (March-May) mean temperature versus altitude in the Alpine region. Each point represents a 0.5° latitude × 0.5° longitude cell of a grid that covers an area delimited by 46 to 49°N and 5 to 15°E.

Table 4: Altitudinal gradients analysis of mean onset dates: geographical subdivision of data reported in Table 2. See Fig. 2 for a key to abbreviations for the different geographical zones. BF: beginning of flowering; FFO: first flowers open; FF: full flowering.

Area	Phenophase (No. stations)	Gradient (d per 100m)	R^2	p
Germany, SW	C. alder BF (34)	3.32 ± 0.51	0.57	< 0.001
	C. hazel BF (57)	5.43 ± 0.36	0.81	< 0.001
	N. spruce BF (10)	0.53 ± 0.29	0.29	0.1067
	G. willow FFO (52)	3.30 ± 0.23	0.80	< 0.001
	S.-l. lime FFO (19)	3.43 ± 0.66	0.62	< 0.001
	C. grape v. FF (19)	1.47 ± 0.98	0.12	0.1530
Germany, SDPP	C. alder BF (139)	1.48 ± 0.49	0.06	0.0030
	C. hazel BF (216)	2.12 ± 0.34	0.15	< 0.001
	N. spruce BF (46)	2.19 ± 0.44	0.36	< 0.001
	G. willow FFO (194)	0.61 ± 0.23	0.04	0.0084
	S.-l. lime FFO (91)	2.66 ± 0.44	0.29	< 0.001
Austria, NPR	C. hazel BF (12)	5.45 ± 0.68	0.86	< 0.001
	Cocksf. grass BF (7)	3.67 ± 0.68	0.85	0.0030
	Rye FFO (5)	3.74 ± 0.89	0.86	0.0245
Austria, AR	C. hazel BF (7)	2.6 ± 2.0	0.24	0.2616
	C. grass BF (7)	4.2 ± 1.1	0.75	0.0114
	N. spruce BF (6)	1.33 ± 0.80	0.41	0.1716
Austria, SPR	C. hazel BF (13)	3.85 ± 0.87	0.64	0.0010
	C. grass BF (7)	4.39 ± 0.79	0.86	0.0026
	N. spruce BF (6)	1.61 ± 0.50	0.72	0.0326
	Rye FFO (7)	2.99 ± 0.57	0.85	0.0032
Switzerland, CR	H. chestnut FF (38)	3.49 ± 0.33	0.75	< 0.001
	B. elder FF (46)	4.11 ± 0.42	0.68	< 0.001
	C. grape v. FF (11)	0.75 ± 0.72	0.11	0.3277
Switzerland, AR	H. chestnut FF (19)	3.54 ± 0.32	0.88	< 0.001
	B. elder FF (26)	2.70 ± 0.39	0.67	< 0.001
	C. grape v. FF (6)	2.79 ± 0.44	0.91	0.0031

behaviour (see Table 4) for horse chestnut FF between Switzerland, AR (3.54 ± 0.32 d (100 m)$^{-1}$) and CR (3.49 ± 0.33 d (100 m)$^{-1}$), as well as for Norway spruce BF between Austria, SPR (1.61 ± 0.50 d (100 m)$^{-1}$) and Germany, SDPP (2.19 ± 0.44 d (100 m)$^{-1}$), and cocksfoot grass BF among Austria, SPR (4.39 ± 0.79 d (100 m)$^{-1}$), NPR (3.67 ± 0.68 d (100 m)$^{-1}$) and AR (4.2 ± 1.1 d (100 m)$^{-1}$). Also rye FFO does not show a statistically significant difference between Austria, SPR (2.99 ± 0.57 d (100 m)$^{-1}$) and Austria, NPR (3.74 ± 0.89 d (100 m)$^{-1}$), even if these 2 areas involve both northern and southern parts of the Alps; but in this case, a restricted number of stations together with a particularly narrow altitude range (300 to 800 m in the north, 150 to 650 m in the south) may have statistically compromised the tendency towards a stronger altitudinal response to the north of the Alpine arch.

Regarding the geographical distribution of values of phenological temporal trends, the general lack of statistical significance does not allow for any systematic differences between zones to be identified. For this reason, such data have not been reported.

2.4 CONCLUSIONS

The temperature decrease due to the increase in geographical elevation causes a significant linear dependence on altitude of all the phenodates collected according to our selection criteria. A similar behaviour over the Alpine region, even if related to different phenophases and more restricted areas, has been reported in other studies [Studer et al., 2005; Dittmar and Elling, 2006; Larcher, 2006].

The mean temporal trends of all phases underline the strong response of spring phenology to warming in the recent decades. However, concerning altitudinal gradients of temporal trends, the low level of statistical significance does not permit the hypothesis of any dependence on altitude. On the contrary, it suggests that altitude does not influence phenological temporal trends, except for common alder BF and Norway spruce BF. The first of these 2 phenophases is influenced by the mean temperature of the month preceding its onset, February, the only month when temperature trends show a significant and consistently relevant linear dependence on altitude. The influence of the temperature of the preceding month on flowering phenodates has been detected in previous investigations [Larcher, 2006; Menzel et al., 2006], and the present study finds that the external dependence on altitude induces a direct connection between temporal trends in temperature and in phenology.

The lack of altitudinal dependence of phenological temporal trends is partially confirmed in Studer et al. [2005], in which a linear altitudinal dependence of phenological temporal trends was found in the northern but not in the southern Alps.

Finally, a systematic zone-by-zone analysis of altitudinal gradients of phenodates suggests a stronger tendency towards an altitudinal response of some phenophases in the northern part of the Alpine region than in the south. This result is supported by Larcher [2006], in which a discrepancy of up to 0.9 d (100 m)$^{-1}$ among altitudinal gradients of flowering onset dates of lilac (*Syringa vulgaris* L.) between the northern and the southern Alps was detected, and is also compatible with the general tendency towards an advance in flowering dates being more pronounced in the southern than in the northern Alps [Defila and Clot, 2005].

ACKNOWLEDGEMENTS

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3

FIRST FLOWERING OF WIND-POLLINATED SPECIES WITH THE GREATEST PHENOLOGICAL ADVANCES IN EUROPE

Chiara Ziello[1], Andreas Böck[2], Nicole Estrella[1,3], Donna Ankerst[2], and Annette Menzel[1,3]

1 Chair of Ecoclimatology, Technische Universität München;
Hans-Carl-von-Carlowitz-Platz 2, 85354
Freising-Weihenstephan, Germany
2 Biostatistics Center, Technische Universität München;
Maximus-von-Imhof-Forum 2, 85354 Freising-Weihenstephan,
Germany
3 Institute for Advanced Study, Technische Universität
München; Lichtenbergstrasse 2a, 85748 Garching, Germany

ABSTRACT

Increasing risk of pollinosis (hay fever) is one of the most anticipated consequences of climate change on human health. Wind-pollinated plants are representative of allergenic species because they include species with the highest capability of causing allergy-related diseases in humans. Therefore, changes in wind-pollinated species may reflect impacts of climate change on allergenic plants. In particular, flowering is one of the developmental stages most affected by climate change. This report specifically addresses changes in flowering dates that have occurred during the three decades 1971-2000 as a function of pollination mode and woodiness. The assessment is made using a phenological data set comprising trends of flowering dates of 29 species in 983 locations in Europe. Linear mixed models assessing the statistical significance of trends while adjusting for spatial correlation are used. The main results indicate for the first time that the onset of flowering of wind-pollinated plants advanced more than for insect-pollinated plants, while full flowering phases tended to advance less. These novel findings are contrary to the results of Fitter and Fitter [2002] for Oxfordshire,

who reported larger advances of insect-pollinated plants. Onset of flowering and full flowering of insect-pollinated species are more likely to advance for seasons early in the year; instead, wind-pollinated plants showed no dependence of trends on the season (first flowering) or a decreased advance of phases that are early in the year (full flowering). The effect of woodiness could not be unambiguously defined, but seems to be of minor importance. The presented findings suggest a lengthening of the flowering period in general, which might lead to an increasing time of exposure to airborne pollen of allergic subjects, with consequent likely increment in severity and incidence of allergic symptoms.

3.1 INTRODUCTION

Phenology, the science of naturally recurring events, has provided quantitative evidence of climate change impacts on ecosystems, indicating an increasing advancement of flowering phases in recent decades [Rosenzweig et al., 2007]. A stronger tendency for winter and spring phenological phases to advance, relative to summer phases, has been reported in the literature [Lu et al., 2006; Menzel et al., 2006]. Few studies have assessed the influence of plant traits on the response to global warming. A recent study in this direction reported a greater tendency towards temporal advancement among entomophilous (insect-pollinated) plants compared to anemophilous (wind-pollinated) species [Fitter and Fitter, 2002]. A few other multi-species studies and experiments have revealed considerable diversity in temperature sensitivity, either interspecific and temporal, by functional traits or life forms, or due to variations in community composition or functional diversity so far [Ogaya and Peñuelas, 2004; Cleland et al., 2006; Sherry et al., 2007; Bokhorst et al., 2008; Jentsch et al., 2009]. Regarding the often cited possible mismatch in ecosystem processes, e.g., in pollination, a recent study has shown that the phenology of bees has tracked the phenology of bee-pollinated plant species [Bartomeus et al., 2011].

Changes in the pollen season, particularly in its timing, duration, and intensity, are one of the most probable impacts of climate change [Huynen et al., 2003]. The threat this represents to human health is due to the expected increment to the already observed worldwide increase in the burden of pollen-related respiratory diseases [Beggs, 2004; D'Amato et al., 2007;

D'Amato and Cecchi, 2008]. Most of the scientific attention in this field has been dedicated to observing and forecasting the phenological behavior of single species characterized by a high allergenic effect, such as birch or ragweed [Laaidi, 2001b; Rasmussen, 2002; Wayne et al., 2002; Rogers et al., 2006]. Alternatively, this study assesses flowering trends and related seasonal variability among plant groups, ultimately leading to the identification of groups more likely to show changes in their phenology and, hence, more likely to increase the harmfulness to humans.

The studied species are all angiosperms. They are listed in Figure 6 ordered by mean flowering dates with their categorization according to pollination mode, woodiness and allergenicity. The disparity in the number of anemophilous and entomophilous species (8 versus 21) is due to the low percentage (~10%) of wind-pollinated species among the angiosperms. Note that all considered wind-pollinated species are allergenic. Even if allergenicity is a characteristic also present among insect-pollinated species, the pollen of anemophilous plants is considerably higher in amount and aggressiveness, at least for angiosperms. This aspect allows consideration of wind-pollinated species as representative of allergenic species, and the results of their monitoring can be used to reasonably estimate the consequences of climate change on allergic human subjects.

Woodiness is another trait connected to allergenicity. In fact, sensitized subjects are mostly allergic to grass (i.e., non-woody plants) pollen [Esch, 2004; Jäger, 2008], making it one of the most studied allergens in the literature, together maybe with the pollen produced by the plant genus *Ambrosia* [McLauchlan et al., 2010; Ziska et al., 2011]. This does not diminish the importance of the allergenic effect of some tree species, such as birch [D'Amato et al., 2007], whose pollen causes severe reactions among humans, particularly at northern latitudes where it is predominant.

3.2 MATERIAL AND METHODS

The analyzed phenological data consist of flowering records extracted from a more extended data set, which covers dates of diverse phenological phases for the years 1971-2000, and comprises more than 35,000 15^{+}-year-long series of flowering in central Europe [Menzel et al., 2006]. Most of these data are avail-

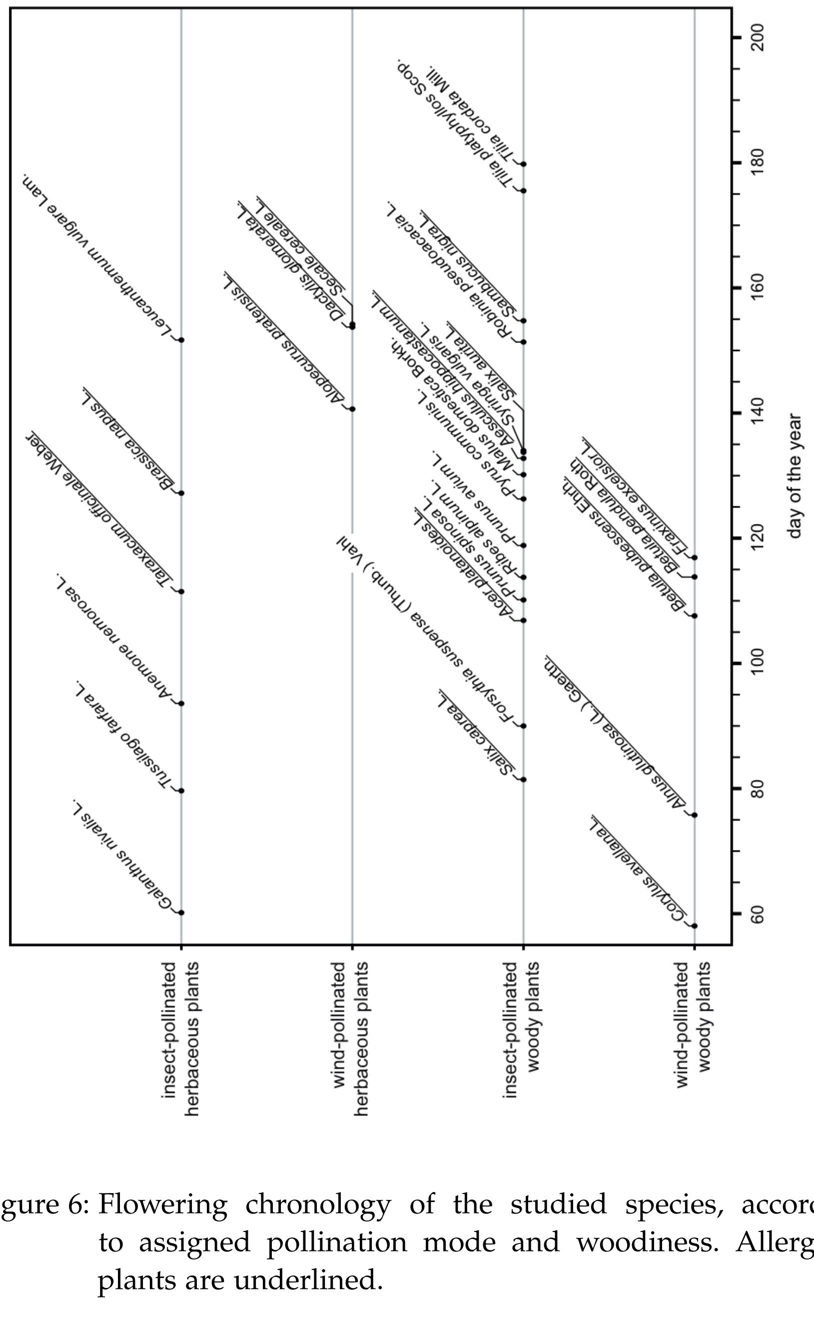

Figure 6: Flowering chronology of the studied species, according to assigned pollination mode and woodiness. Allergenic plants are underlined.

able at the COST (European COoperation in the field of Scientific and Technical research) database, collected within the now concluded COST Action 725 [Koch et al., 2009].

29 species that were represented in more than six stations in Europe, with records longer than 28 years during 1971-2000 were selected for analysis (Fig. 6). Each of the 5,971 series analyzed is denoted by species name, phenological phase (phenophase), mean phenological date (phenodates), temporal trend (estimated slope of the regression line fitting flowering dates on years) with relative standard error, significance of the regression, length of the time series and geographical coordinates of the observational station. Flowering phenophases available are 'first flower opens' and 'full flowering' (50% of flowers open). The size of the analyzed data set comprises 5,971 records based on over 170,000 single phenological observations, from 983 phenological stations, and the related geographic coverage spans a considerable part of Europe (list of countries by decreasing number of stations: Germany, Switzerland, Russia, Austria, Czech Republic, Slovenia, Latvia, Norway, United Kingdom, Croatia, Finland, Estonia, and Slovakia). The classification of allergenic plants follows the information available at `http://www.polleninfo.org`, the website of the EAN (European Aeroallergen Network, `https://ean.polleninfo.eu/Ean/`).

The influence of pollination mode and woodiness on flowering (first flowering and full flowering) trends was assessed using weighted linear mixed models, with weights set to the precision inverse of the underlying regression (standard error) [Becker and Wu, 2007]. Statistical significance of results was assessed using 1,000 bootstrap samples [Efron and Tibshirani, 1993], and goodness of fit was calculated by means of an R^2 measure for mixed models based on the likelihood [Xu, 2003]. Bootstrap samples were also presented in graphs to reflect uncertainty. Fixed effects considered were woodiness, pollination mode, and mean phenodate. A random effect for stations was included, which implies correlation between observations from the same station. More advanced spatial structures, such as the the exponential correlation structure [Pinheiro and Bates, 2000, page 230] that uses the coordinate information of stations, were also considered, but did not show any impact on the estimates of interest and therefore rejected. Altitude above sea level of stations was excluded as a fixed effect since it did not show significance, nor affected other estimates when included in the model, as similarly found in previous work [Ziello et al., 2009].

A series of model-based analyses were performed in duplicate for the outcome variables, first flowering trends (d yr^{-1}) and full flowering (d yr^{-1}). First univariable regressions of the effects of woodiness and pollination were performed. Then, the linear effect of mean date on trends was assessed separately by pollination mode and woodiness, and by combinations of pollination mode and woodiness. Finally, the linearity constraint of the mean date effect was relaxed via a spline approach to evaluate the robustness of the general conclusions made from assumption of a linear effect. Frayed ends of spline curves arise mainly from arbitrary extrapolation of the spline when bootstrap samples do not cover the whole time range, and should be used as natural limits for interpretation.

All calculations and graphics were performed using the statistical software R [R Development Core Team, 2010] with its mixed model library lme4 [Bates and Mächler, 2010] and bootstrap utilities boot function [Canty and Ripley, 2010].

3.3 RESULTS

Average flowering trends over all species and stations were negative for both pollination modes and woodiness types, indicating significantly earlier start ($p < 0.001$) of first and full flowering phases (see Table 5). First flowering times of wind-pollinated plants advanced –0.489 (± 0.019) d each year during the period 1971-2000; insect-pollinated plants significantly less ($p < 0.001$), –0.377 (± 0.003) d yr^{-1}. Full flowering phases of both pollination modes ($p = 0.11$) advanced approximately 0.3 d yr^{-1}. First flower opening phases of non-woody plants advanced –0.417 (± 0.003) d yr^{-1} compared to –0.279 (± 0.006) d yr^{-1} in woody plants. When comparing mean trends of first and full flowering (all significantly advancing, Table 5), for all plant groups except woody, the first flowering trend is larger than the respective full flowering one, leading to a longer flowering period, here defined as time between first and full flowering.

The linear effect of mean flowering date (day of year) on these time trends has been visualized in Fig. 7. For first flower opening phases of wind-pollinated plants there was no statistically significant relationship between trends and mean phenodate ($p = 0.81$). Full flowering phases revealed instead the expected pattern, with greater advances in the first part of the year ($p < 0.001$). Surprisingly, trends for insect-pollinated plants had the reverse association with mean phenodates, with

Table 5: Average temporal trends for first flower opening and full flowering phases, with significance of differences by pollination mode and woodiness.

Phenological phase	Plant group	Trend (d yr^{-1})	p-value
First flower opens	Wind-pollinated	-0.489 ± 0.019	< 0.001
	Insect-pollinated	-0.377 ± 0.003	
	Non-woody	-0.417 ± 0.003	< 0.001
	Woody	-0.279 ± 0.006	
Full flowering	Wind-pollinated	-0.312 ± 0.009	0.11
	Insect-pollinated	-0.337 ± 0.010	
	Non-woody	-0.317 ± 0.009	0.27
	Woody	-0.332 ± 0.011	

larger advances observed later in the year ($p < 0.001$). And woody and non-woody species exhibited the same unexpected pattern, full flowering for non-woody species being the only group with trends non-significantly dependent on mean phenodates ($p = 0.32$).

Separate models for every combination of pollination mode and woodiness have been reported in Fig. 8, except for first flower opening phases of wind-pollinated non-woody species and full flowering phases of wind-pollinated woody species, which had insufficient data. For insect-pollinated species, trends of both phenophases exhibited a similar pattern: negative linear dependence on the mean phenodate ($p < 0.001$). For first flower opening, woody plants indicated more advancement by mean phenodate compared to non-woody plants. Trends of wind-pollinated plants, instead, show no dependence on mean phenodates ($p = 0.87$) for first flower opening of woody species (whose lines from the model are located below both modelled lines of insect-pollinated species), and a significant linear dependence ($p < 0.001$), which was negative, for full flowering of non-woody species (whose modelled lines are mostly above both lines of insect-pollinated species).

Results of the regression with non-linear effects, generally confirming those of the linear models, are shown in Fig. 9. For first flower opening, modeled curves of wind-pollinated woody

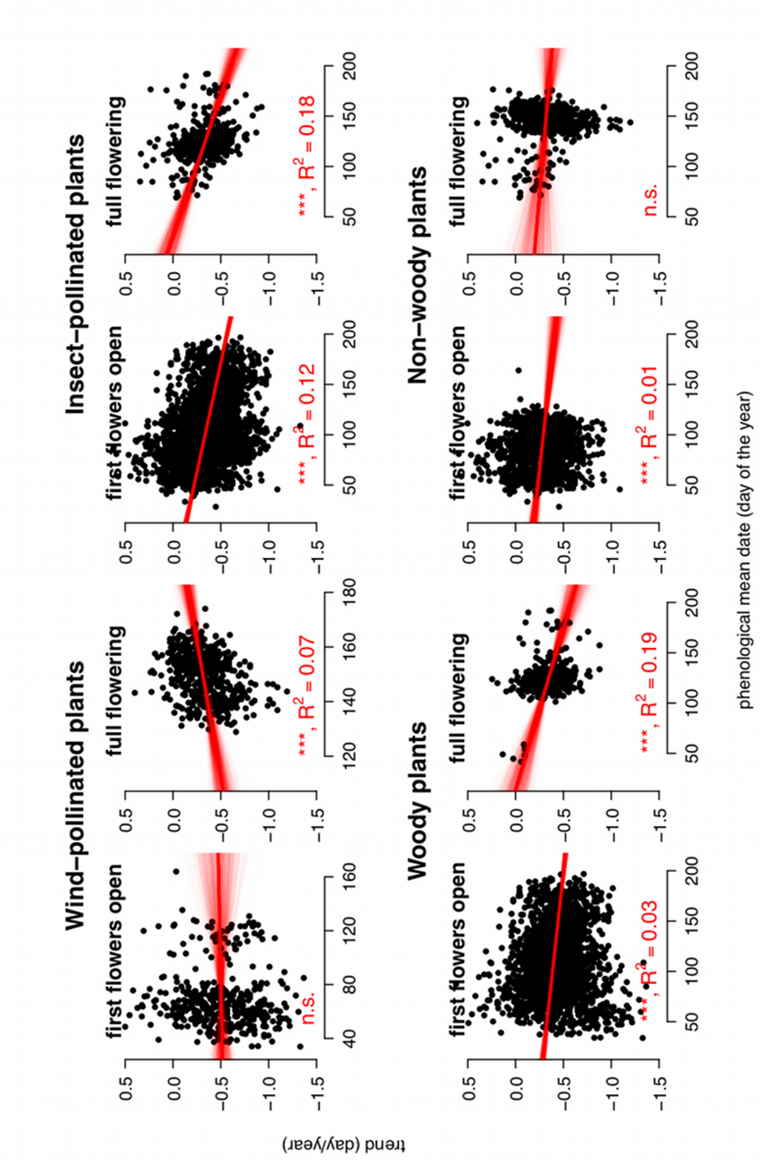

Figure 7: Long term time trends of flowering in days per year plotted against mean flowering dates, by pollination types (top) and woodiness (bottom), each group separated in turn by phenophase. Red lines show fit from a weighted linear mixed model, thick and thin lines show averaged and single bootstrap samples, respectively, the latter reflecting uncertainty. Significances (*** for $p < 0.001$, n.s. for not significant) of linear mean date effect are indicated, together with the relevance R^2.

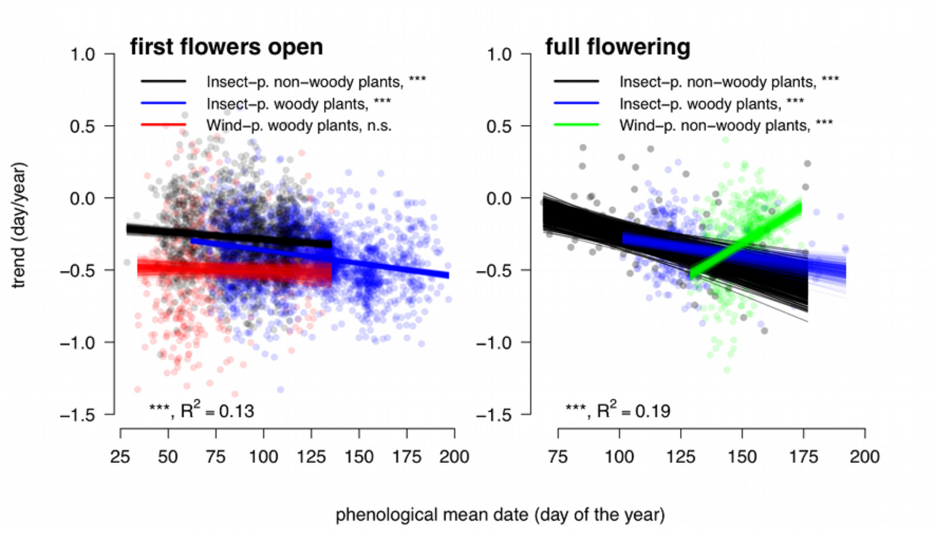

Figure 8: Long term time trends of flowering in days per year plotted against mean flowering date according to woodiness and pollination. Lines show bootstrap estimates, which reflect uncertainty.

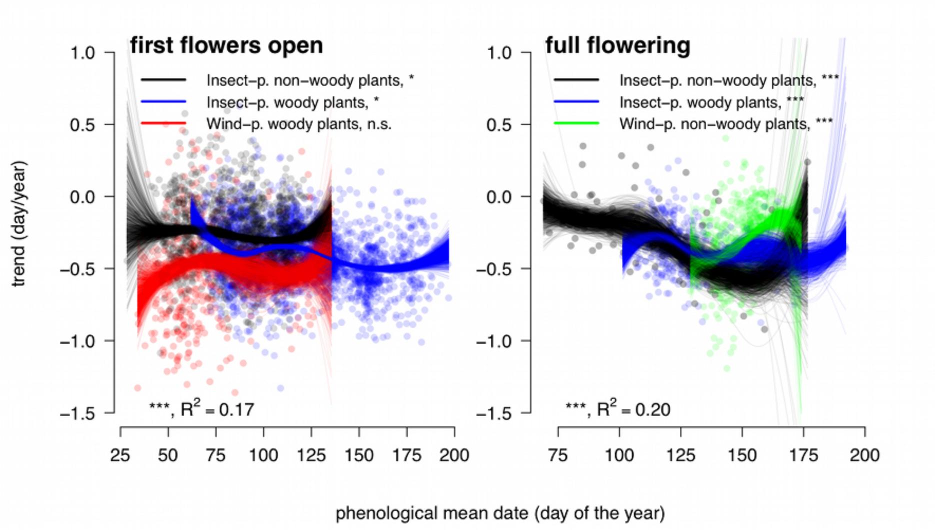

Figure 9: Long term time trends, fitted by flexible splines, of flowering in days per year plotted against mean flowering date according to woodiness and pollination. Individual lines show bootstrap estimates, which reflect uncertainty.

species showed that they exhibited more advances than for insect-pollinated woody species, which did not vary with phenodates ($p = 0.12$): the non-significant influence of phenological mean date on trends found in the previous analysis was hence not induced by too restrictive linearity assumptions. For the two remaining groups, a significant advancement of mean flowering dates was evidenced, where the size of advancement was significantly depending on phenological mean dates ($p < 0.05$). For full flowering, wind-pollinated non-woody species exhibited less advancement, depending on the season ($p < 0.001$), than insect-pollinated woody and non-woody plants, whose trends were in both cases depending on the season as well ($p < 0.001$).

3.4 DISCUSSION

3.4.1 *Observed changes in flowering*

In the present study, a general advancing trend of flowering phases, independent of pollination mode or woodiness, was found, confirming earlier reports of advancing flowering dates [Menzel et al., 2006; Rosenzweig et al., 2007]. However, from previous literature, one should expect a seasonal pattern with stronger advances of early-occurring phases [Lu et al., 2006; Menzel et al., 2006; Rosenzweig et al., 2007]. In the present analysis, the trends in Fig. 7 would be then negative, but the red line should increase indicating a decreasing trends by day of the year. Instead, for the majority of groups, our results did not match the patterns previously reported.

Since onset of flowering phases are advancing more than later occurring full flowering phases, the flowering period of all the combined species is therefore lengthening. Such a prolongation of flowering has only rarely been inferred from phenological ground observations, since typically only single phenophases such as the start of flowering are studied. In this sense, the present study represents a step forward since first and full flowering dates of numerous species have been analyzed and a prolongation of this flowering period has been inferred, which is of paramount importance for those allergic subjects that could likely experience a prolongation of their main suffering period. Due to the substantial lack of phenological data for the end of flowering, changes in the dates of this phase, which could directly assess the lengthening of the complete flowering period, can only be hypothesized. However, studies of direct pollen measurements have also reported longer pollen seasons [Rosenzweig et al., 2007], confirming the occurrence of longer flowering periods.

3.4.2 *Differentiation of trends by pollination mode*

Phases related to the onset of flowering of wind-pollinated species exhibited the greatest advances, providing evidence that the phenology of anemophilous species may be more strongly affected by climate change, even if showing the weakest changes by year among the analyzed groups (Fig. 7, 8). Compared to insect-pollinated species, wind-pollinated ones exhibited a larger prolongation of the flowering period, as inferred from the stronger advance of first flower opening phases compared to full flowering phases (Table 5, Fig. 9). It could hence also be inferred that the combined flowering period of all the species analyzed lengthened more for wind-pollinated than for insect-pollinated plants, which is a finding of high importance for pollen-associated allergic diseases.

Several studies have reported on differences in phenology and ecology between pollination modes (e.g., Rabinowitz et al., 1981, Bolmgren et al., 2003). In contrast to the findings of this study, Fitter and Fitter [2002] reported that, in a recent context of general and fast phenological changes, in Great Britain insect-pollinated species were more likely to flower early than wind-pollinated species. In addition to a different geographical area, this discrepancy could be due to different criteria for the selection of phenological series: They used records longer than 23 years in the periods 1954-2000, requiring at least 4 years in the decade 1991-2000. In the current study, series have been selected covering a shorter period (1971-2000) and were really complete (29 or 30 years out of 30). Hence, in this study the years 1991-2000 are much more represented and results may better mirror the effects of the pronounced warming of such a decade. This can be clearly seen in the magnitudes of changes: the median advances found by Fitter and Fitter are 6 to 3 days for 5 decades, probably equivalent to a trend of –0.1 to –0.2 d yr^{-1}. In the present study, the mean trends are all stronger than –0.3 d yr^{-1}, reaching almost –0.5 d yr^{-1}. There is also one more difference to Fitter and Fitter [2002]: In contrast to our findings that trends of insect-pollinated species are stronger later in the season, Fitter and Fitter reported that insect-pollinated species that flowered early were much more sensitive to warming than those that flowered later (see later in the discussion).

3.4.3 *Hypothesized reasons for stronger flowering responses of wind-pollinated species*

Wind pollination is a functional trait that can be preferentially found in specific geographical conditions, such as high altitudes and latitudes, in open vegetation structures such as savannah, in habitats presenting seasonal loss of leaves such as northern temperate deciduous forests, or in island floras [Whitehead, 1969; Regal, 1982; Ackerman, 2000]. Among the widespread angiosperms ($\sim 230,000$ plant species), around 18% of families are abiotically pollinated, and at least 10% of species are wind-pollinated [Ackerman, 2000; Friedman and Barrett, 2009]. All of the strongest allergenic species included in this study (e.g., birch, grasses) belong to this group.

A stronger advance in first flowering dates was found for wind-pollinated compared to insect-pollinated species. It can be hypothesized that in addition to their pollination syndrome, a set of characteristics that co-occur among plants using the same pollination agent, anemophilous angiosperms have inherited a more rapid adaptedness, in other words a major plasticity. Angiosperms in general show higher evolutionary rates since their first evolutionary stages than gymnosperms, having probably originated in an environment that favored rapid reproduction [Regal, 1982]. Fertilization periods, temporal gaps between pollination and consequent fertilization, are in fact known to be shorter in angiosperms than in gymnosperms [Williams, 2008]. The key to the huge success of angiosperms may be due to this rapidity, even if the reasons for their fast and wide-step radiation are still not completely understood. Within angiosperms, wind-pollinated species may have changed their pollination mode as a reaction to unfavorable environmental conditions, enabling more capability for responding to the variability of climate. This aptitude would make anemophilous angiosperms particularly sensitive to environmental changes, and thus a group of strong responders to global warming.

This enhanced sensitivity to warming is made more credible due to the absence of limiting factors, such as the availability of pollinators. Entomophilous plants could be less free to react to temperature variations because their pollinator strategies would not match those changes. Hence, they would be less likely to change their ecological internal clock.

3.4.4 *The effect of woodiness and time of the year*

This study revealed that onset of flowering of non-woody species advanced significantly more than of woody species (Table 5, $p < 0.001$) but there is no advancement for full flowering ($p = 0.27$). Similarly to wind-pollinated species, this would lead to infer a lenghtening of the flowering period. However, when considering the seasonal variation, the predominant effect of pollination mode over the trait of woodiness is clear. In fact, trends for woody and non-woody insect-pollinated species were quite similar and both clearly differed from the wind-pollinated woody or wind-pollinated non-woody groups (see Fig. 8 and 9).

In light of the results of this study, the dependence of the observed first flowering trends on the season seems to be more complex than previously reported. For entomophilous species the former finding of smaller advances of phases early occurring in the year is in contrast with the current study [Fitter and Fitter, 2002]. This difference in intra-annual patterns of changes could be due to differences in number of locations monitored (only one in Great Britain versus 983 in Europe), or to the accounting for the statistical significance of changes performed in this analysis.

3.5 CONCLUSION

By means of an extended data set of phenological flowering records, this study detected differences in phenological temporal trends due to pollination mode and woodiness, as well as the yearly pattern of trends. Results indicated a general tendency to a stronger advance in onset of flowering phases of wind-pollinated plants, a proxy for allergenic species. It can be excluded that this detected anemophily effect is uniquely due to the time of occurrence of the observed phenophases: In fact, anemophilous species exhibited a strong mean advance of flowering phases but the intra-annual variation of such trends did not depend on the date in which the relative phase occurred. The more pronounced advance for first flower opening phases of wind-pollinated plants, together with the less pronounced advance of full flowering phases, with respect to insect-pollinated ones, suggests the occurrence of a lengthening of the flowering season, i.e., the pollen season, of anemophilous species, at least for the analyzed species. According to these findings, under climate change conditions, sufferers from pollinosis are getting exposed for a longer period to one of the most aggressive allergens: the pollen produced by anemophilous species.

ACKNOWLEDGMENTS

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4

CHANGES TO AIRBORNE POLLEN COUNTS ACROSS EUROPE

Chiara Ziello,[1] Tim H. Sparks,[1,2,3] Nicole Estrella,[1,2] Jordina Belmonte,[4] Karl C. Bergmann,[5] Edith Bucher,[6] Maria Antonia Brighetti,[7] Athanasios Damialis,[8] Monique Detandt,[9] Carmen Galán,[10] Regula Gehrig,[11] Lukasz Grewling,[12] Adela M. Gutiérrez Bustillo,[13] Margrét Hallsdóttir,[14] Marie-Claire Kockhans-Bieda,[15] Concepción De Linares,[4] Dorota Myszkowska,[16] Anna Pàldy,[17] Adriana Sánchez,[1] Matthew Smith,[18] Michel Thibaudon,[19] Alessandro Travaglini,[7] Agnieszka Uruska,[20] Rosa M. Valencia-Barrera,[21] Despoina Vokou,[8] Reinhard Wachter,[22] Letty A. de Weger,[23] Annette Menzel[1,2]

1 Chair of Ecoclimatology, Technische Universität München, Freising-Weihenstephan, Germany
2 Institute for Advanced Study, Technische Universität München, Garching, Germany
3 Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland
4 Botany Unit and Institute of Environmental Science and Technology (ICTA), Universitat Autònoma de Barcelona, Bellaterra, Spain
5 Allergie-Centrum-Charité, Charité-Universitätsmedizin Berlin, Berlin, Germany
6 Laboratorio Biologico, Agenzia provinciale per l'ambiente, Laives (BZ), Italy
7 Dipartimento di Biologia, Università di Roma "Tor Vergata", Roma, Italy
8 Department of Ecology, School of Biology, Aristotle University, Thessaloniki, Greece
9 Section Mycology and Aerobiology, Scientific Institute of Public Health, Brussels, Belgium

10 Department of Botany, Ecology and Plant Physiology, University of Córdoba, Córdoba, Spain
11 Federal Office of Meteorology and Climatology MeteoSwiss, Zurich, Switzerland
12 Laboratory of Aeropalynology, Adam Mickiewicz University, Poznań, Poland
13 Department of Plant Biology II, Complutense University of Madrid, Madrid, Spain
14 Icelandic Institute of Natural History, Gardabær, Iceland
15 Station d'Aérobiologie, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg
16 Department of Clinical and Environmental Allergology, Jagiellonian University, Medical College, Kraków, Poland
17 Department of Biology, National Institute of Environmental Health, Budapest, Hungary
18 National Pollen and Aerobiology Research Unit, University of Worcester, Worcester, UK
19 Réseau National de Surveillance Aérobiologique, Brussieu, France
20 Laboratory of Palaeoecology and Archaeobotany, Gdańsk University, Gdańsk, Poland
21 Department of Biodiversity and Environmental Management, Universidad de León, León, Spain
22 Polleninformationsdienst Deutschland (PID), Ganderkesee, Germany
23 Department of Pulmonology, Leiden University Medical Center, Leiden, The Netherlands

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ABSTRACT

A progressive global increase in the burden of allergic diseases has affected the industrialized world over the last half century and has been reported in the literature. The clinical evidence reveals a general increase in both incidence and prevalence of respiratory diseases, such as allergic rhinitis (common hay fever) and asthma. Such phenomena may be related not only to air pollution and changes in lifestyle, but also to an actual increase in airborne quantities of allergenic pollen. Experimental enhancements of carbon dioxide (CO_2) have demonstrated changes in pollen amount and allergenicity, but this has rarely been shown in the wider environment. The present analysis of a continental-scale pollen data set reveals an increasing trend in the yearly amount of airborne pollen for many taxa in Europe, which is more pronounced in urban than semi-rural/rural areas. Climate change may contribute to these

changes, however increased temperatures do not appear to be a major influencing factor. Instead, we suggest the anthropogenic rise of atmospheric CO_2 levels may be influential.

4.1 INTRODUCTION

Many factors have been proposed to explain the 20th century increase [Beggs, 2004; Beggs and Bambrick, 2005; D'Amato et al., 2007; D'Amato and Cecchi, 2008] in the burden of allergic respiratory diseases, although the causes are still not fully understood [Reid and Gamble, 2009]. For example, air pollution can influence both allergens and allergic subjects in many ways, making the former more potent and increasing the immune reaction of the latter [Bartra et al., 2007]. However, these phenomena are insufficient to explain completely the increased rate of allergic diseases in humans [Bartra et al., 2007].

Plant phenology, the timing of life cycle events in vegetation (e.g. budburst, flowering), is generally sensitive to temperature [Menzel et al., 2006; Rosenzweig et al., 2007]. If not water-limited, it has responded strongly to global warming [Fitter and Fitter, 2002; Menzel et al., 2006]. Hence, it can be reasonably supposed that global change also affects pollen timing and production [Emberlin, 1994; Huynen et al., 2003]. These may contribute to the increasing trend in allergic diseases. However, single studies on pollen quantities in recent years have been inconclusive, e.g. inconsistent trends for five pollen types at five sites in Western Europe [Spieksma et al., 2003], or a more consistent increase for many taxa in Thessaloniki, Greece [Damialis et al., 2007].

Current aeropalynological research uses a number of different indicators to describe the pollen season (e.g., start and end dates, daily concentrations, timing of peak production). Past study results may have been influenced by the choice of indicator used [Jato et al., 2006]. In the present analysis of 1,221 European pollen time series at 97 stations (see Fig. 10), we focus on yearly trends of the annual pollen index (API), a quantity universally defined as the sum of average daily pollen concentrations over the year. The trends of API at each monitored location were normalized by the respective mean API. This normalized index allows a comparison across different provenances and microclimates within the large geographic range of species in Europe. Moreover, using this normalization, the different methodologies used to measure daily pollen concentrations are less likely to influence calculation or detection of temporal trends.

4.2 RESULTS AND DISCUSSION

4.2.1 *Trends in pollen counts*

Analyses showed that 724 (59%) APIs increased and 497 (41%) decreased. 271 (22% of the total) were statistically significant ($p < 0.05$), among which 171 (14% of the total) increased and 100 (8% of the total) decreased. In Fig. 11, annual changes in API are summarized for 23 families or genera chosen amongst important allergenic pollen types according to the sensitization and allergic symptoms of people living in specific regions, (e.g., *Alnus*, *Ambrosia*, *Artemisia*, *Betula*, *Corylus*, Cupressaceae, *Olea*, Poaceae), or from constantly important land-uses (e.g., *Fraxinus*, *Platanus*, *Carpinus*, *Castanea*, Pinaceae, *Plantago*, *Quercus*, *Rumex*).

For nine taxa, all with highly allergenic pollen, the indices increased significantly (Mann-Whitney test, $p < 0.05$), while only two taxa decreased significantly. API trends from tree species were, in general, larger than those from herbs and shrubs. In recent years, some tree taxa (e.g., Cupressaceae) have been extensively used as ornamental plants in cities, and hence their pollen trends could have been positively affected by urban planning. However, land-use changes in general (e.g., afforestation) may be too slow to explain increasing API in trees. The significant decreases for Chenopodiaceae and *Artemisia* could be possibly explained by intensification of weed control and less agricultural land set-aside in the context of increasing bioenergy demand. Analysis by countries (Fig. 12) also reveals a general increase in API, with 11 of 13 countries having median changes greater than zero, significant for five countries. The significant decrease for Spain, although the median trend is close to zero, is somewhat surprising, especially in light of a recent study reporting an increase in grass pollen in southern Spain [García-Mozo et al., 2010]. Our result for Spain is unlikely to be biased by a small sample size (215 series analyzed). Furthermore, our Spanish data cover a wider geographic range with varied water availability which may be more influential on API, particularly of grasses, in Spain compared to more northern countries.

A large variability in the API trends is evident, shown by the presence of large outliers in the boxplots of Fig. 11 and Fig. 12.

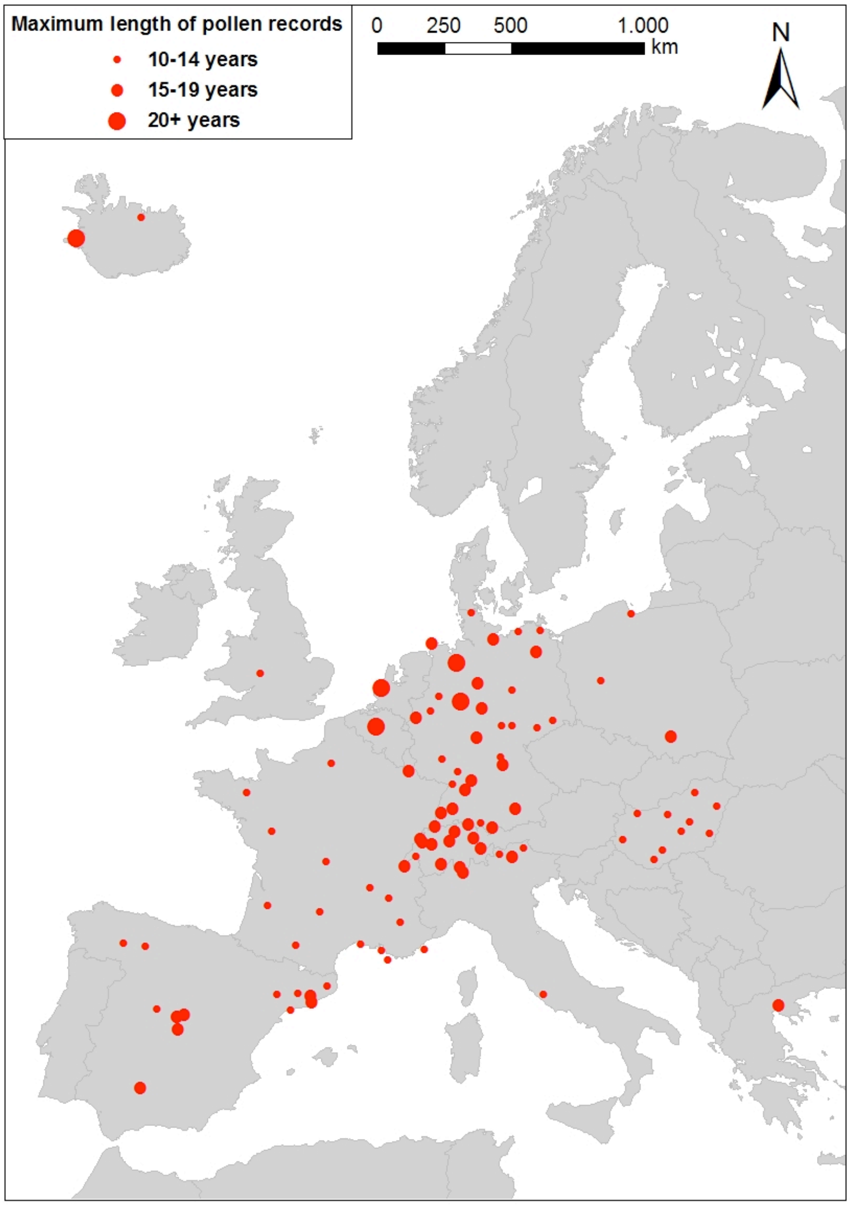

Figure 10: Locations of pollen sites. Each station has been indicated by a red circle. Symbol sizes are proportional to the temporal length of the local longest pollen record.

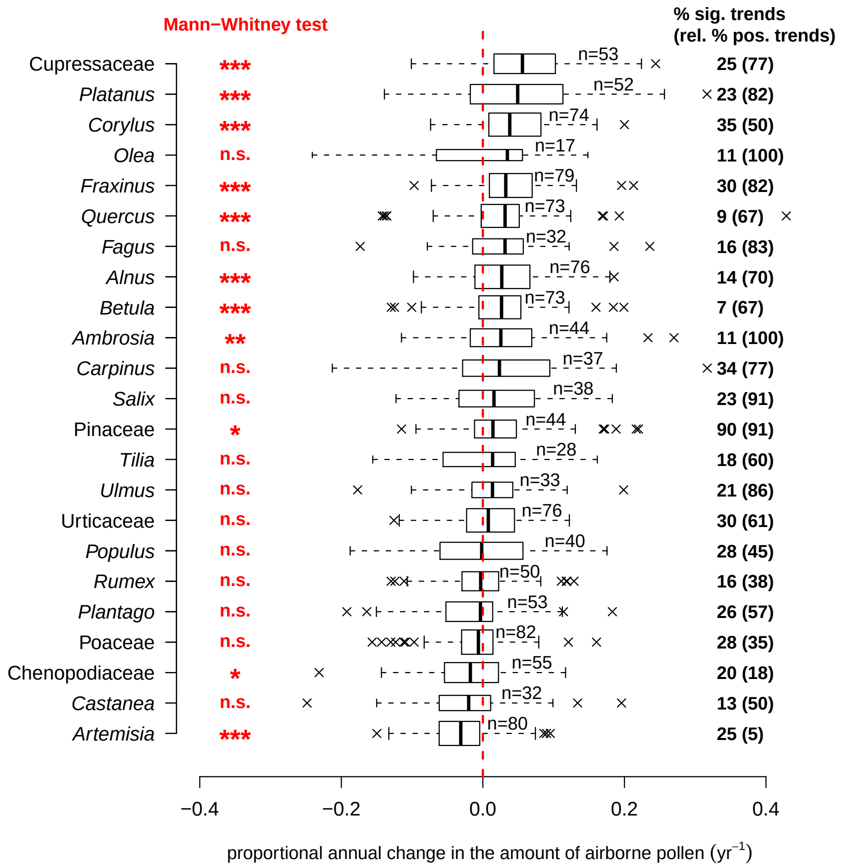

Figure 11: Trends of annual pollen index (API) by species. Boxplots show the proportional annual change of yearly pollen sums for the 23 pollen taxa analyzed (reasons for selection given in the main text). Medians are significantly different from zero (Mann-Whitney test, * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$, n.s.: $p > 0.05$) for 11 taxa. On the right, the percentages of significant trends are indicated for each taxon (of which the percentages of positive trends are given in parentheses). The height of the boxplot is related to sample size, taxa are arranged in decreasing order of their medians.

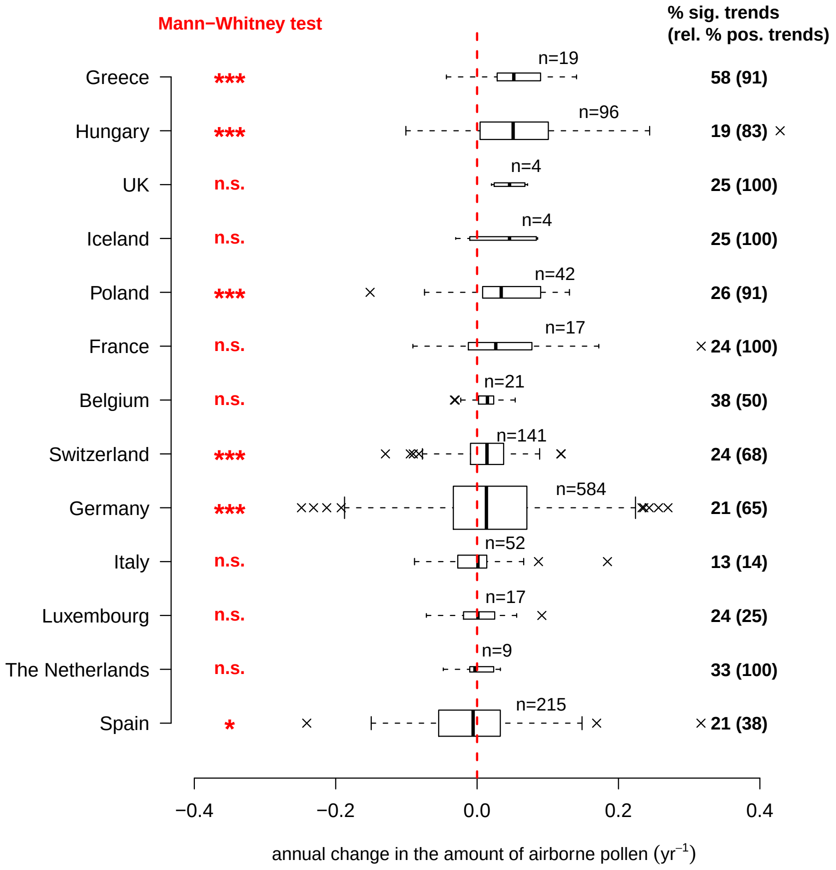

Figure 12: API trends by country. Boxplots show the proportional annual change of yearly pollen sums for 13 countries. Medians are significantly different from zero (Mann-Whitney test, * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$, n.s.: $p > 0.05$) for six countries. On the right, the percentages of significant trends are indicated for each country (of which the percentages of positive trends are given in parentheses). The height of the boxplot is related to sample size, countries are arranged in decreasing order of their medians.

Outliers may be caused by rainy weather during the pollen season reducing annual totals, favourable (warm and dry) weather, episodes of long-range transport, inherent inter-annual variation of pollen production (years of massive and synchronized pollen production by plants, the so-called masting behaviour), re-suspension phenomena (winds raising deposited pollen in the lower atmosphere), and abrupt changes in species density by local land management. Further research is needed to identify the relative importance of each of these factors.

4.2.2 *Considered drivers*

In an attempt to identify the causes of pollen increases, we tested the correlation between trends in API and trends of local mean temperature. As shown in Fig. 13, there was little evidence of correlation. This could be due to not matching exactly the lengths (10 to 28 years) and gaps of the pollen series with lengths of the temperature series (33 years). Only trends in *Betula* and *Carpinus* pollen amounts showed a significant but weak correlation, which was negative. *Betula* predominantly grows in mid to high latitudes at lower temperatures, and it has been hypothesized that an increase in temperature could limit its physiological performance [Emberlin et al., 2002], including the production of pollen [Clot, 2003]. The significant negative correlation between *Betula* pollen and temperature trends seems to support such a hypothesis.

Because consistent correlations between API trends and local temperature trends could not be demonstrated, we tested instead general relationships between mean API and mean local temperature. These are shown in Fig. 14. For many species regression lines were statistically significant. Except for three tree species this relationship was positive (i.e., more pollen at higher temperatures, indicating warmer southern sites or urban sites). In contrast, *Alnus*, *Betula*, and *Corylus* are tree genera more associated with high latitudes and low temperatures, thus their negative correlation of API with temperature could reflect the limited presence of these species at warmer sites. However, variation in the density of species will influence any API-temperature relationship. Therefore, we used the European Forest Data Centre (EFDAC) data set, which includes density information. These maps, the only ones available for Europe, display the species distribution in ha of tree cover per species at a 1 km resolution. For each taxa the respective tree species covers were determined by GIS within a radius of 10 km around each pollen station. Unfortunately, according to this data set, the majority of pollen sites was characterized by a complete absence of trees, due to the forest/non-forest GIS layer used that excluded human settlements and agricultural land. Thus, no hypothesis of linking API trends with temperature and density could be tested.

The environment in which the pollen was measured may influence results. In Fig. 15, boxplots of observed pollen trends in urban and in semi-rural/rural areas indicate a significant difference between these environments as well as an overall increase in pollen at urban sites (Mann-Whitney tests, $p < 0.05$). Urban environments are characterized not only by the "heat island" effect, but also by high levels of pollutants, such as NO_x, VOCs or particulates. Furthermore, higher atmospheric CO_2 concentrations are known to cause a general increase in vegetation biomass (at least temporarily), an increase in pollen production [Rogers et al., 2006; Wayne et al., 2002; Ziska et al., 2003, 2008, 2009], also shown in Free-Air CO_2 Enrichment (FACE) experiments [Darbah et al., 2008] and, probably, pollen allergenicity [Singer et al., 2005]. Therefore, it can be inferred that higher levels of CO_2, typical of urban areas, may cause a greater presence of airborne pollen in this environment. Lower tropospheric ozone (O_3) levels also characterize urban environments, due to higher concentration of nitrogen oxide (NO), which is involved in the breakdown of O_3. Because the effect of O_3 is to inhibit plant development [Darbah et al., 2008], enhanced plant growth in urban areas has already been reported [Gregg et al., 2003].

In addition, we tested the correlation of API and API trends (also by taxa) with latitude, longitude and altitude a.s.l. of the pollen stations, attempting to find geographical patterns in the observed changes in pollen amounts. However, no specific pattern could be detected, suggesting that regional differences in behaviour were small relative to background variability. Thus, possible biogeographical differences in behaviour are unlikely to have masked the overall reported trends here. We also tested for differences in API and its trends associated with specific plant traits, such as late-successional (e.g., *Fagus*) against early-successional taxa (e.g., *Betula*). Also in this case, no significant result could be found.

A delayed or missing fulfillment of the chilling requirement of plants for bud burst and thus flowering could play a key role

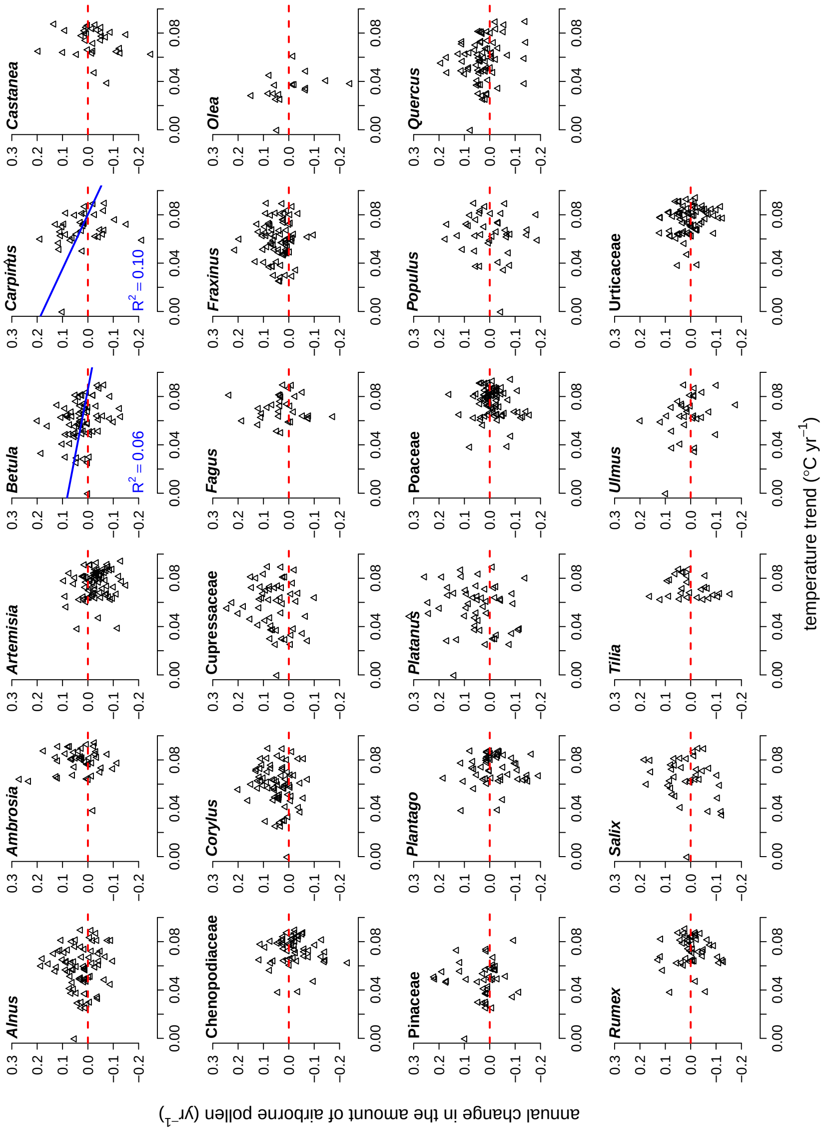

Figure 13: API trends against temperature trends by species. Proportional annual change of yearly pollen sums was plotted against local temperature trends for 23 pollen taxa. Temperature trends were calculated for each location for the mean temperature of two seasons, January to April (associated with the flowering of *Alnus*, *Betula*, *Carpinus*, *Corylus*, Cupressaceae, *Fagus*, *Fraxinus*, *Olea*, Pinaceae, *Platanus*, *Populus*, *Quercus*, *Salix*, and *Ulmus*) or April to August (related to *Ambrosia*, *Artemisia*, *Castanea*, Chenopodiaceae, *Plantago*, Poaceae, *Rumex*, *Tilia*, and *Urtica*), over the years 1977-2009. A regression line has been superimposed for *Betula*, the only statistically significant relationship.

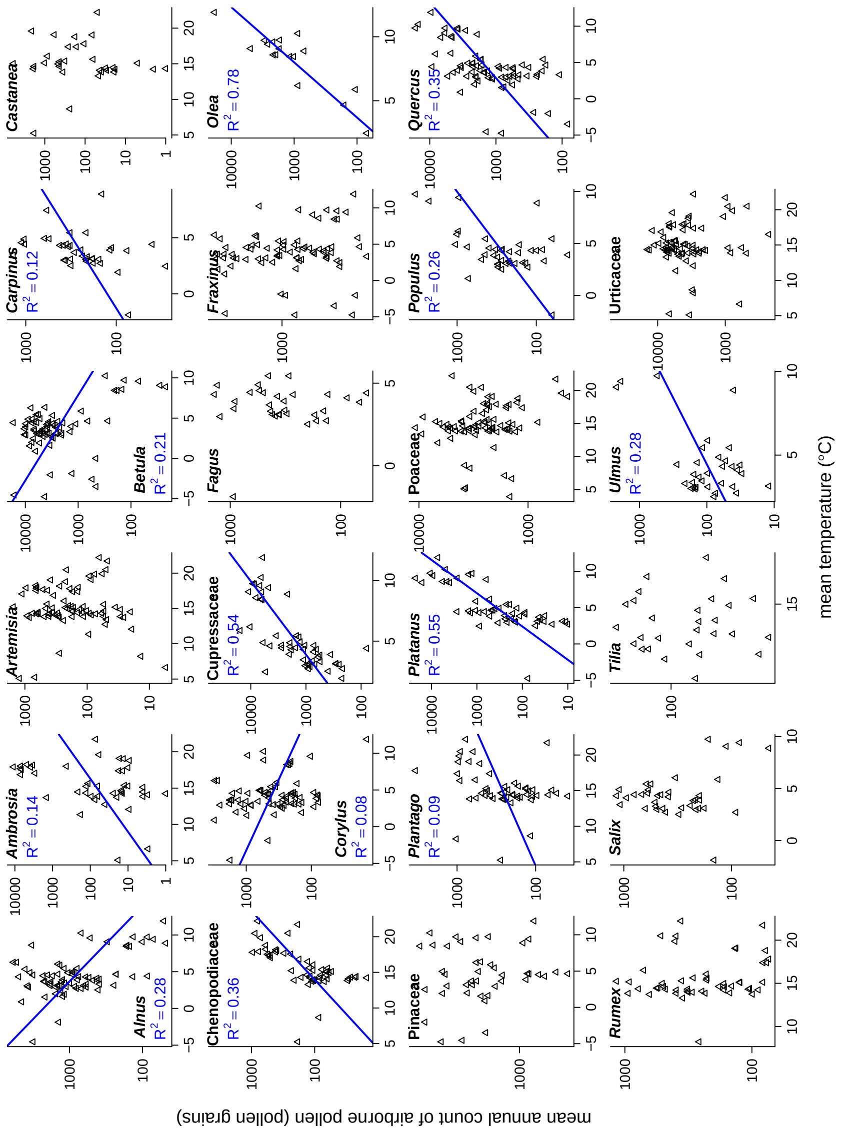

Figure 14: Mean API against mean local temperature. Log-scaled mean annual sum of airborne pollen was plotted against local mean temperature for 23 pollen taxa. Mean temperatures were calculated for two periods, January to April (associated with the flowering of *Alnus*, *Betula*, *Carpinus*, *Corylus*, Cupressaceae, *Fagus*, *Fraxinus*, *Olea*, Pinaceae, *Platanus*, *Populus*, *Quercus*, *Salix*, and *Ulmus*) or April to August (related to *Ambrosia*, *Artemisia*, *Castanea*, Chenopodiaceae, *Plantago*, Poaceae, *Rumex*, *Tilia*, and *Urtica*), over the period 1977-2009. Only significant regression lines are shown.

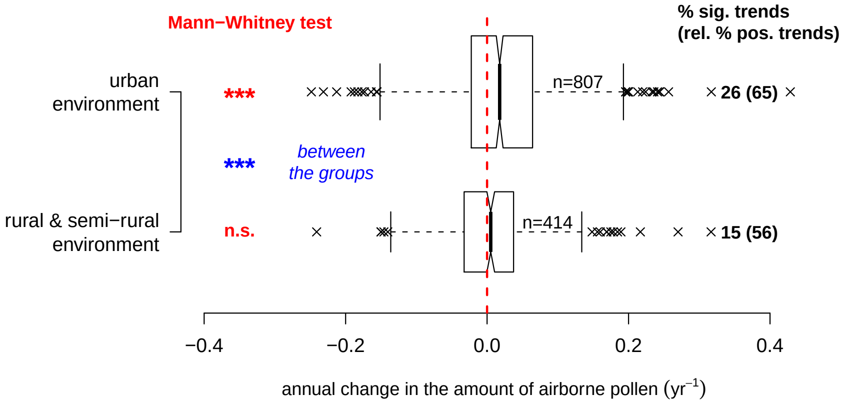

Figure 15: API trends by environment type. Boxplots show the proportional annual changes of yearly pollen sums for different environments. Mann-Whitney tests show a significant increase (median different from zero, $p < 0.05$) of airborne pollen in urban environments. The notches are calculated as $\pm$ 1.58 IQR $\times \sqrt{n}^{-1}$ and the height of each boxplot is related to sample size. On the right, the percentages of significant trends are indicated for each type of environment (of which the percentages of positive trends are given in parentheses).

under future scenarios of increasing winter temperatures. Even if not directly connected to the production of pollen, which is more sensitive to water availability, pre-flowering weather conditions (especially for herbs and grasses) [Smith et al., 2009], or weather conditions in the year preceding flowering (for some trees, such as birch) [Dahl and Strandhede, 1996], chilling temperatures may influence the timing of flowering in trees [Chuine et al., 1999; Emberlin et al., 2007]. A late or missing fulfillment of such a requirement may delay or, in the worst case, prevent flowering events, as hypothesized for fruit and nut trees [Petri and Berenhauser Leite, 2004; Luedeling et al., 2011]. As a consequence, length and intensity of the pollen season could be notably reduced, especially for species native to the Mediterranean area, where the greatest changes in winter temperature are expected for Europe [Parry et al., 2007].

4.2.3 *Conclusions*

Despite the lack of unequivocally identified drivers, it is evident that there is currently a clear tendency towards an increase in atmospheric pollen, including highly allergenic taxa. These trends could not be attributed to rising temperatures, but may be influenced by the anthropogenic increase of the greenhouse gas CO_2 as (experimental) studies suggest [Wayne et al., 2002; Ziska et al., 2003; Singer et al., 2005; Rogers et al., 2006; Darbah et al., 2008; Ziska et al., 2008, 2009]. More research is needed in this area because a further worldwide increase in atmospheric CO_2 is projected, e.g. by IPCC [Parry et al., 2007]. These changes may result in further increases in pollen amounts leading, in turn, to a greater exposure of humans to pollen allergens, with potentially serious consequences for public health.

4.3 MATERIALS AND METHODS

The analyzed data set consists of 1221 pollen time series at 97 locations in 13 European countries from 23 pollen taxa (see Fig. 10). Not every species was present in every location. Series length ranged from 10 to 28 years in the period 1977 to 2009. In Fig. 16, the longest local monitored periods are reported (short time gaps, occurring for few locations, have been omitted for clarity). Temporal trends of API were calculated as slopes from linear regression on time (years) and were normalized (i.e.,

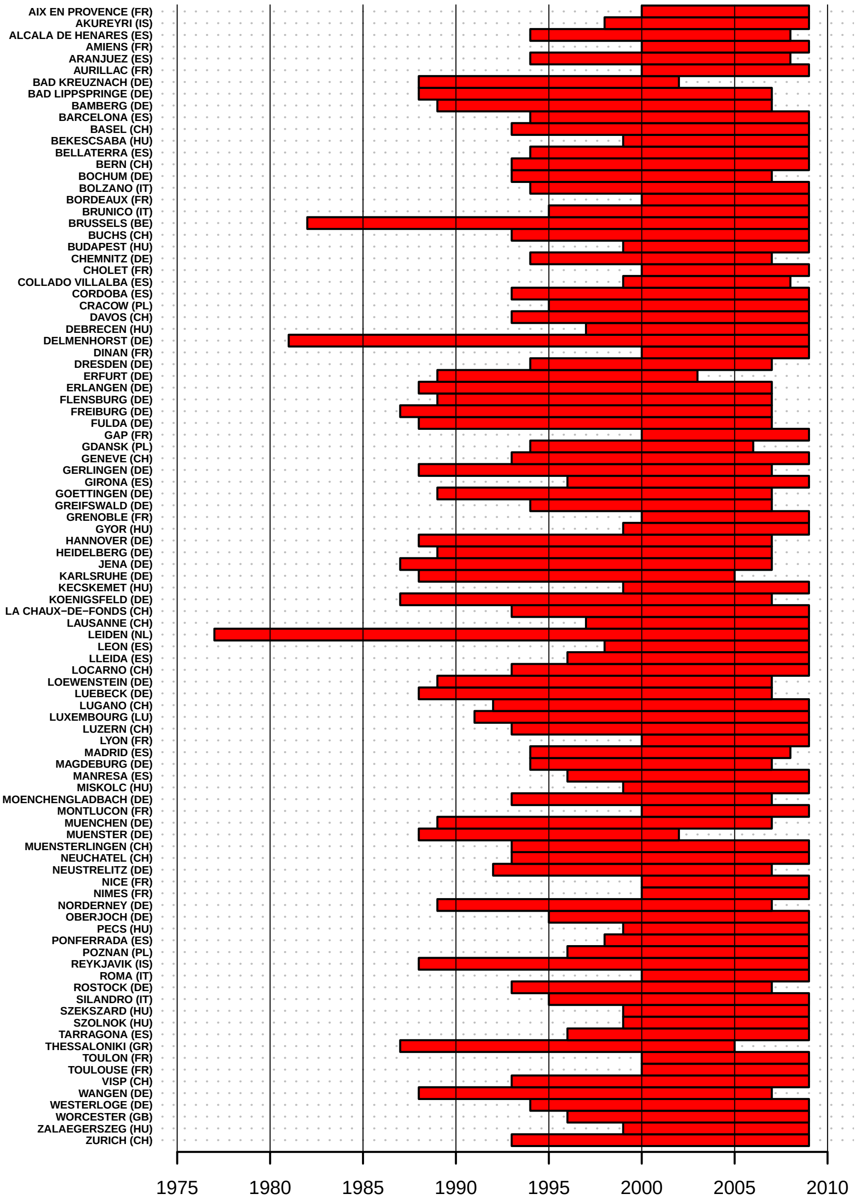

Figure 16: Maximum duration of pollen series by location. The local longest monitored period is shown as a red bar for each of the 97 locations considered. Missing years, occurring in few cases, have been omitted for clarity.

converted to proportional change per year) by dividing by the mean local API.

Trends in temperature were calculated over the years 1977-2009 for two seasons, January to April or April to August, associated with different species according to their flowering period. For each pollen station, temperature data of the respective grid cell of the ENSEMBLE project data were used [Haylock et al., 2008]. The temperature data from the ENSEMBLE data set, available at www.ensembles-eu.org, are based on a geographical grid of resolution 0.5°latitude × 0.5°longitude.

The statistical software R version 2.11.1 was used for both statistical analyses and to generate figures [R Development Core Team, 2010].

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5

GENERAL DISCUSSION

5.1 STRENGTH POINTS OF RESEARCH PROJECT

The attempt of the presented research project was to assess at a large geographical and temporal scale, as well as for a large number of allergenic species, the changes in a common natural event such as pollen release and/or the connected occurrence of flowering. To achieve such an ambitious plan, it has been decided to follow some main points, which have notably contributed to the novelty of results and the originality of analytical procedures:

- The use of large spatial data sets of phenodates and respective trends, as well as data of airborne pollen amounts, involving locations from good part of Europe;
- The consequent eventual accounting for the spatial autocorrelation of phenological data, made essential by the dimensions of considered geographical coverage;
- The use of only direct phenological observations, without use of data obtained as output of mathematical models, or to phenological observations derived from any kind of field manipulation experiments;
- The use of multi-species palynological and phenological data sets, less suitable to produce species-specific results but allowing for comparisons among species or species' traits, also promoting the discussion about species-independent phenological or palynological changes;
- The development of a novel technique in order to achieve results accounting for the statistical significance normally associated with phenological trends (as already explained in chapter one of this thesis).

Such points have been crucial in order to increase the general value of the presented research, and to make the relative obtained results more reliable. The reasons of the importance of the first three ones, neither reported in other sections nor of intuitive identification, needs to be further discussed and will be explained in the following.

5.1.1 *Working with large data sets: the assessment of large-scale impacts of climate change*

Long-term phenological series

Humans have long been interested in the seasonal cycles of nature. The very first "historical" phenological records have originated from the observation of local natural events of ecological or cultural interest. For example, the observation and recording of the timing of yearly recurrent natural events is an ancient practice that was undoubtedly important to the success of early gatherers and farmers [Post and Inouye, 2008]. But the study of phenology eventually expanded beyond its practical origins. Its purpose has ranged from documenting nature's patterns, to investigating the mechanisms behind phenological events, to exploiting applications in agriculture, and most recently to understanding the ecological consequences of climate change [Post and Inouye, 2008; Puppi, 2007]. The longest record of this kind is for the flowering of cherry trees in Kyoto, Japan: the observations that compose this world-famous phenological series stretch back over 13 centuries [Arakawa, 1955; Aono and Kazui, 2008]. Such long-term records of phenological dates allow an assessment of local climatic changes by reconstructing the environmental conditions that influenced the natural event under exam. In fact, the temporal length of phenological observations is the key parameter in order to investigate relevant changes in timing of phenophases and their relation to past and recent climate [Aono and Kazui, 2008].

Spatial coverage

Nevertheless, long-term records, which can commonly involve also more than one species, are generally from single localities or restricted areas. The evaluation of climate change impacts deriving from such observations is, then, inevitably tied to a specific geographical zone, and can be only theoretically extended to different areas, moreover under strict conditions. This is one of the reasons why, in recent years, many efforts have been dedicated to the creation of observational networks able to cover geographical areas as large as possible, at national scale or continental scale. Another important reason behind the promoting of phenological networks is the need for standardizing phenological observations: This is of great importance in order to make a comparison between data from different sources possible. In fact, the establishment of a common agreement on phenophases to monitor and protocols of monitoring lays the basis for a possible use of data from networks for large-scaled research.

What is a metadatabase?

Within phenological networks, not only a phenological database but also the relative metadatabase is usually available. A metadatabase (sometimes called a metabase or metadata repository) is a database for storing metadata (data that describe data) for a specific purpose. In other words, a metadatabase might include metadata about all configuration information in a system gathered from a number of sources. The use of such auxiliary data is crucial in the detection of climate change effects on vegetation: The availability of an additional information allows an exploitation of the connections between phenological changes and climate changing conditions, often confirming the attribution of changing phenodates to increasing temperatures.

Important phenological networks

Among extant phenological networks, two important recent examples are worth mentioning: The European Phenology Network (EPN, available under the web address `http://www.pik-potsdam.de/~rachimow/epn/`), which connects national networks of several European countries [van Vliet et al., 2003], and the federal USA National Phenology Network (USA-NPN, available at `http://www.usanpn.org`) [Betancourt et al., 2005]. The EPN also includes data from the International Phenological Gardens (IPG, accessible at `http://www.agrar.hu-berlin.de/fakultaet/departments/dntw/agrarmet/phaenologie/ipg/ipg_allg-e`), which are a European and individual network within the Phenology Study Group of the International Society of Biometeorology (ISB, available at `http://biometeorology.org/`).

IPGs

The project behind the IPG network aims, from its foundation in 1957, at doing large-scale and standardized phenological observations, focussed on excluding genetic variability from phenological responses to changes in climate. To achieve this scope, observations from a total of 89 gardens are made in similar conditions for 21 cloned species, in 19 European countries. In the vicinity of every phenological garden a meteorological station is located, whose data are provided so as to integrate the information of the phenological database (metadata).

Data used

In two of the three publications included in the present thesis [Ziello et al., 2009, 2012a, chapter two and chapter three of this thesis respectively], phenological data collected within the COST action 725, which have reference to the EPN, have been used. Such extremely wide set of phenological data for temporal length, spatial coverage and variety of monitored species allowed for a comprehensive and solid interpretation of phenological changes on the European scale. The final publication [Ziello et al., 2012b, chapter four of this thesis] is, instead, based on a considerably large data set of temporal trends of pollen counts, calculated and provided by the numerous coauthors who participated in the project of such large-scaled analysis. However, the respective raw pollen data are available under the European Aeroallergen Network pollen database (EAN, the portal is available under the address `https://ean.polleninfo.eu/Ean/` while the informative portal, with the list of monitored locations and respective contact people included in the database, is available under `http://www.polleninfo.org/`). But in this case, a problem of direct accessibility is evident, since "such data are owned by the participants and are only available under specific agreements", as reported in the EAN webpages. Only thanks to the active participation of several scientist was it possible to assemble, describe, and analyze the changes in pollen trends for a large number of locations spread all over Europe and many pollen *taxa*, publishing the first study of this kind in the whole aerobiology literature.

5.1.2 *Accounting for spatial autocorrelation*

Tobler's First Law of Geography

"Everything is related to everything else, but near things are more related than distant things [Tobler, 1970]". This is the simple but elegant form in which Waldo Tobler expressed his First Law of Geography (mostly known as Tobler's First Law, TFL). To rephrase it trying to go beyond this charmingly informal expression, one could state, for example, that for every geographic variable (a function of location $z = f(x)$) there exists some distance d below which covariance is monotonically increasing, or that there exists at least one scale for which spatial autocorrelation is positive [Goodchild, 2004]. However, the concept expressed by the TFL is essentially synonymous with the concept of spatial dependence that forms the foundation of spatial analysis.

Spatial dependence

In applications of statistics, spatial dependence is the existence of statistical dependence in a collection of random variables or a collection of time series of random variables, each of which is associated with a different geographical location. Spatial dependence is of importance in applications where it is reasonable to postulate the existence of a corresponding set of random variables at locations that have not been included in a sample. Thus, any atmospheric observation can be measured only at a set of specific locations, and such measurements can be considered as outcomes of random variables, but such observables clearly also occur at other locations and would again be random. As with other types of statistical dependence, the presence of spatial dependence generally leads to estimates of an average value from a sample being less accurate than had the samples been independent, although, if negative dependence exists, a sample average can represent a better estimate than in the independent case [de Knegt et al., 2009]. In other words, spatial dependence is the covariation of properties within geographic space: Characteristics at proximal locations appear to be correlated, either positively or negatively. Spatial dependence leads to the spatial autocorrelation problem in statistics since this violates standard statistical techniques that assume independence among observations. For example, regression analyses that do not compensate for spatial dependence can have unstable parameter estimates and yield unreliable significance tests. Spatial regression models capture these relationships and do not suffer from these weaknesses. It is also appropriate to view spatial dependence as a source of information rather than something to be corrected [de Knegt et al., 2009].

Spatial autocorrelation

Spatial autocorrelation statistics measure and analyze the degree of dependence among observations in a geographic space. Classic spatial autocorrelation statistics include Moran's I, Geary's C, Getis's G and the standard deviational ellipse. These statistics require measuring a spatial weights matrix that reflects the intensity of the geographic relationship between observations in a neighborhood, e.g., the distances between neighbors, the lengths of shared border, or whether they fall into a specified directional class such as "west". Classic spatial autocorrelation statistics compare the spatial weights to the covariance relationship at pairs of locations. Spatial autocorrelation, which is more positive than expected from random, indicates the clustering of similar values across geographic space, while significant negative spatial autocorrelation indicates that neighboring values are more dissimilar than expected by chance, suggesting a spatial pattern similar to a chess board [Graham and Fingleton, 1985].

Spatial regression methods

Spatial regression methods capture spatial dependence in regression analysis, avoiding statistical problems such as unstable parameters and unreliable significance tests, as well as providing information on spatial relationships among the variables involved. Depending on the specific technique, spatial dependence can enter the regression model as relationships between the independent variables and the dependent, between the dependent variables and a spatial lag of itself, or in the error terms [Graham and Fingleton, 1989].

In the presented research...

Within the presented research, the problem of spatial autocorrelation was approached using different solutions, depending on the case under exam.

- In Ziello et al. [2009, chapter two of this thesis], since a weak significance of latitudinal and longitudinal dependence in both phenological data and respective phenological trends over the Alpine area was detected, it was decided to proceed introducing in calculations a coarser spatial resolution of geographical coordinates. Instead of using spatial coordinates as predictors of spatial regressions, as previously calculated, the original zone was divided in several smaller areas, and respective mean values of phenological observables had been compared among them. The analysis yielded to the detection of some geographical differences in phenological mean dates. Above all, altitudinal gradients were found to be less pronounced at the south of the Alpine arch than at its north. In other words, the delay in flowering time caused by decreased temperature in higher altitudes was shorter per altitude unit, even if this was not detected for all species analyzed.

- In Ziello et al. [2012a, chapter three of this thesis], the spatial correlation was included in the calculations (weighted linear mixed models) as a random effect for locations, which implies correlation between observations from the same station. Such a solution was adopted after detecting the lack of a structured spatial dependence in the data, likely due to the scarce homogeneity of spatial distribution of phenological stations. Some areas, in fact, presented a high number of stations, while others very few. The use of a random effect for locations allowed for the accounting of spatial coordinates in the calculations, without which the analysis of such a large geographical data set would have been damaged in terms of reliability of results.

- The analyses presented in Ziello et al. [2012b, chapter four of this thesis] are, differently from the other two published studies, almost merely descriptive. The reason for such an approach is the novelty of a study of this kind in the aerobiology literature, so primary importance was given to the presentation of the data as a picture of the allergological situation in Europe under climate change conditions. In such a context, the spatial interpretation of results was considered as outside the scope of study. Nonetheless, spatial coordinates (latitude, longitude and altitude a.s.l.) have been used as predictors of pollen trends in an attempt to explain the remarkable increase detected in airborne pollen quantities. But unfortunately, such an attempt did not lead to any statistically significant result.

5.1.3 *Observational data vs. data from manipulation experiments or data modeling*

Impact of projected climate

In phenological research there are a large number of studies assessing the impact of likely future climate conditions, already referred to in chapter one of this thesis, on vegetation. Besides increased temperature and changes in atmospheric gaseous composition, special attention has been dedicated to extreme weather events like cold or warm spells, excessive rainfall, or droughts, which are projected to become more frequent under climate change conditions [Jentsch and Beierkuhnlein, 2008]. These kinds of research can be performed via mathematical modeling, or manipulating field (or greenhouse) conditions in many ways, depending on the hypotheses to be tested.

Manipulation experiments

In particular, by means of experimental manipulations it is possible to simulate the effect of extreme events such as, for example, water stress (droughts), or above average rainfall (irrigation), as well as those of increased concentrations of atmospheric CO_2 or O_3 (see, for example, Darbah et al. [2008] and Jentsch et al. [2009]). In particular the responses of plants to elevated CO_2 concentrations have been extensively studied over the last decade by a variety of techniques including open-top chambers, controlled environment chambers, free-air CO_2

enrichment technologies and by using a natural CO_2 spring [Wayne et al., 2002; Ziska et al., 2003; Rogers et al., 2006; Darbah et al., 2008; Ziska et al., 2008, 2009]. Manipulations that allowed the study of the effects of increased soil or air temperatures are also feasible, like others that exploit simulated changes (mainly increases) in nitrogen (N) deposition (see, for example, Cleland et al. [2006]).

The risk of underestimating real phenological responses

All these experimental procedures account for the bulk of published research confirming the great sensitivity of phenology to temperature, which is considered the most important forcing factor driving phenophases such as flowering or leafing. Nonetheless, a recent study on the reliability of warming experiments, relative to direct observations over time or space, casts a shadow on the (critical but little-tested) assumption that plant responses to experimental warming match long-term responses to global warming [Wolkovich et al., 2012a]. Wolkovich and colleagues, after comparing 50 experimental and observational studies available in the literature and quantifying sensitivities to temperature variability, came to the surprising conclusion that warming experiments did not replicate that observed in the natural environment. They reported that short-term warming experiments, commonly used to forecast changes in phenological responses at small scales, tend to underpredict such changes. Advances in the timing of flowering and leafing are underestimated by 8.5-fold and 4.0-fold, respectively, compared with long-term observations: In fact, warming experiments showed advances in flowering or leafing time of less than 1 day to 1.6 days per temperature degree rise, while in the observational studies the plants advanced 5 to 6 days per degree. Moreover, the well-known greater sensitivity to temperature of plants flowering earliest in spring is only detected via direct phenological observations. Such a phenological feature of early flowering plants, also reported in large-scale studies [Menzel et al., 2006], is totally absent among results obtained by means of experimental manipulations. As Wolkovich and colleagues suggested, this discrepancy could be a result of the artifacts from warming experiments, or confounding environmental factors from observations. But doubtlessly, in the light of the findings reported by Wolkovich et al. [2012a], studies evaluating the spontaneous responses of natural environments to climatic changes acquire a new importance in the attempt of understanding and predicting phenological changes [Rutishauser et al., 2012].

Data from modeling

The use of phenological data obtained as model output is also commonly used in phenological research, especially in aerobiological studies [Sofiev and Bergmann, 2013]. But also in this case, the decision of using such data could lead to some statistical flaws, relative to observational data. As previously said in chapter one of this thesis, differently from data from phenological observations, modeled data are an additional source of statistical uncertainty, and their use could obviously decrease the statistical significance of final results.

The choice for observational data

In the context of the considerations expressed above, the general findings of the research project presented in the current thesis, which are obtained by statistical elaborations of observational data (phenological mean dates and phenological trends as well as trends in pollen counts), acquire an additional value. The choice of using direct phenological observations, instead of similar data from field manipulation experiments or mathematical modeling, allows for a greater reliability of final results.

5.2 KEY RESEARCH FINDINGS

Among the results produced within the research project presented in the current thesis, some are more essential than others. They have more important consequences, present more general perspectives and lead to wider conclusions. The general character of such findings, in other words their likely wide validity outside the presented specific studies, makes them worth having separate discussion subsections in the following.

5.2.1 *Independence of phenological (and palynological) trends on altitude a.s.l.*

Alps and climate change

In allergology, there is a common assumption whose validity could, under climate change, be in question: Mountain areas are more suitable than lower regions for hay fever sufferers, due to the low presence of airborne pollen [Michel et al., 1976; Jochner et al., 2012; Sofiev and Bergmann, 2013]. This statement is still considered as true, even though highlands are getting heavily affected by climate change, more than other regions [Rosenzweig et al., 2007; Cannone et al., 2008; Cornelius et al., 2013]. If, in fact, over the past 100 years global annual mean temperatures have increased by about 0.7°C [Solomon et al., 2007], a much stronger temperature increase of about 2°C has been detected in the Alpine region [Auer et al., 2007]. This dramatic spread in temperature increases could potentially change the actual picture of highlands as areas with a diminished atmospheric content of bioaerosols, relative to lowland regions [Confalonieri et al., 2007; Jochner et al., 2012].

Consequences of altitudinal increments

Generally, altitude above sea level influences plant phenology in many ways. As reported, for example, by Barry [1981] or Körner [2007], the altitude increase is associated with:

- A progressive reduction of land area;
- A decrease of total atmospheric pressure and of the partial pressure of all the component gases;
- An increment of solar radiation at clear sky conditions, as well as a higher percentage of the UV-B component;
- A temperature decrease of about 0.6 °C every 100 m, whereas temperature is considered as being the most important forcing factor able to drive plants' physiology [Defila and Clot, 2005; Rosenzweig et al., 2007; Wolkovich et al., 2012a].

The vulnerability of alpine ecosystems

As a dramatic consequence of global change, an incremented warming of mountain areas could cause biodiversity change. Elevational shifts of species' altitudinal range could lead to a general rearrangement of the species composition of vegetation, also favoring an increased vulnerability of the old species to invasion from pioneer or weed species [Walther et al., 2002; Cornelius et al., 2013]. Monitoring Alpine species' phenology means taking the pulse of this special and delicate natural environment. There is still a need to understand if global climate change will be able to modify, momentarily or permanently, such a delicate ecosystem as humans currently know it so far. Several studies have been carried out to investigate the effects of altitude on phenological phases and their temporal trends in different zones of the Alpine region, comparing results at different altitudes, and also using experimental field manipulations [Defila and Clot, 2001; Studer et al., 2005; Dittmar and Elling, 2006; Larcher, 2006; Jochner et al., 2012; Cornelius et al., 2013]. However, discrepancies in the conclusions need to be clarified. This is the reason why the results of the study presented in chapter two are so important to understand the mechanisms behind the pronounced sensitivity of mountain zones to climatic influences.

Main findings

By means of a multi-species data set including phenological stations all over the Alpine arch and wide surroundings, Ziello et al. [2009] established not only the expected progressive delay of flowering dates with altitude, but also a somewhat surprising lack of altitudinal dependence of phenological trends. In other words, the advancing trend of flowering dates is not affected by geographical altitude; In fact, phenological trends at low altitude are not statistically different from those at high altitude. Moreover, every additional attempt of detecting smaller areas with increased phenological sensitivity has failed, confirming an hypothesis of mountain areas as a whole region with high sensitivity. Such a completely new result reported in chapter two has also been confirmed in the successive study Ziello et al. [2012a, chapter three of this thesis]. In the context of a less restricted geographical analysis, involving stations from a larger part of central Europe, phenological trends did not show any altitudinal effect with no exceptions for species or species' traits. In fact, altitude above sea level of phenological stations was excluded as a fixed effect since it did not show statistical significance, nor affected other estimates when included in the model. Interestingly, trends in pollen counts from all over Europe analyzed in Ziello et al. [2012b, chapter four of this thesis] also did not exhibit any dependence on altitude. The latter result could imply a deeper than expected connection between phenological and palynological trends, which could be investigated in further research. On this direction, a recent paper [Charalampopoulos et al., 2013] reports the lack of altitudinal influence on pollen production rates of several species along the altitudinal gradient of Mt Olympos, in Greece, suggesting such a production "not responsive to environmental changes associated with elevation". However, relative to results presented in chapter three and chapter four, it gets difficult to detect altitude as a general predictor when enlarging the geographical area which phenological and palynological data are referred to. This is due to the introduction of a greater uncertainty, which generally hides the influence of some variables, such as altitude, that are more influential at local level.

5.2.2 *Pollination modes: their effect on climate-driven phenological changes*

Research background

It is pretty clear that increasing temperatures are causing the well-known advancements of flowering time and, in general, spring and summer phenological phases in plants (see the short review reported in § 1.6). And clear cause-effects have also been established, especially after several (even if not very numerous) publications about the influence of some plant traits, which have been thought as responsible for the variability of species responses to climate change [Fitter and Fitter, 2002; Ogaya and Peñuelas, 2004; Cleland et al., 2006; Sherry et al., 2007; Bokhorst et al., 2008; Jentsch et al., 2009]. In particular, pollination by wind was interpreted by Fitter and Fitter [2002] as a factor able to cause a limited resilience to climatic conditions, relative to insect pollination. Their conclusion was a consequence of smaller flowering advancements detected among plants presenting such a trait. This influential study heavily affected the research on this specific point in the following years, preventing the topic to be object of subsequent critical phenological investigations or, in general, discussed further. The publication by Fitter and Fitter [2002] likely promoted a research focused on insect pollination, considered more endangered under climate change; In fact, there is still ongoing debate on the effects of current and projected climate change conditions on the robustness of the plant-pollinator mutualism [Memmott et al., 2007; Hegland et al., 2009; Donnelly et al., 2011; Rafferty and Ives, 2011; Bartomeus et al., 2013; Burkle et al., 2013; Garibaldi et al., 2013; Tylianakis, 2013]. In conclusion: The major part of the literature on connections between pollination mode and climate takes for granted the greater phenological flexibility of insect-pollinated species. This is the reason why the (nevertheless crucial) problems of declines in insect pollinators and temporal plant-pollinator mismatches are those mainly investigated in recent years.

Main findings

In the picture described above, the findings of the research presented in the current thesis acquire crucial importance and wide breath. The results published in Ziello et al. [2012a, chapter three of this thesis] completely reverse the finding by Fitter and Fitter on wind pollination, with a very good reliability given by a more widespread data set analyzed and the development of novel and more complete statistical analyses, compared to the influential publication of 2002. Wind pollination is clearly identified as a key ecological trait in predicting responses to climatic changes: It allows for a more rapid phenological advancing of flowering phases and causes a lengthening of the flowering period, relative to plants relying on insects for their reproductive process. The interpretation given is focussed on the absence of any need to track the phenology of insect pollinators, which could in turn be affected by climate. In fact, these plants could not be influenced by factors, like pollinators, potentially able to limit the range of their climate-driven phenological changes. A partial confirm of such an hypothesis comes from Rafferty and Ives [2011]. They found, studying the however appreciable phenological response of insect-pollinated species to climate change [Bartomeus et al., 2011], that "five of six species with historically advanced flowering received more visits [from pollinators] when flowering was experimentally advanced, whereas seven of eight species with historically unchanged flowering received fewer visits when flowering earlier. This pattern suggests that species unconstrained by pollinators have advanced their flowering, whereas species constrained by pollinators have not".

Allergological perspectives

Last but not least, the findings reported in Ziello et al. [2012a] have a deep allergological relevance: Wind-pollinated species, the most dangerous for sufferers from pollinosis, are increasing pollen production and are lengthening their pollen season. This sounds like a serious alarm for public health that cannot be neglected by health care systems.

5.2.3 *The detection of appreciable increases in pollen count levels all over Europe: the role of urban areas*

Limits of current pollen literature

As already mentioned in chapter one of this thesis, aerobiological literature is constituted mainly by local and few-taxon (often mono-taxon) studies [Sofiev and Bergmann, 2013]. This is a consequence of the high costs of palynological data, which makes difficult the collaboration among different research groups, as well as a natural limit imposed by the numerical models used to forecast or describe airborne pollen levels. But generally, these studies identify the effects of recent climatic changes with good agreement [Beggs, 2010].

Pollen and climate change

The pollen season starts on average 10 days earlier and is longer than 50 years ago [Feehan et al., 2009]. A recent warming is associated with an earlier onset [Frei, 2008; Rodríguez-Rajo et al., 2011], an earlier end date [Stach et al., 2007; Recio et al., 2010], a longer pollen season [Stach et al., 2007; Ariano et al., 2010], an increase in the total annual pollen load [Ariano et al., 2010; Cristofori et al., 2010], furthermore an increase of patient number sensitized to pollen throughout the year [Ariano et al., 2010; Beggs, 2010].

Precursor studies

Recently, a comprehensive spectrum including a good coverage (at least seven taxa) of the regional pollen flora was only analyzed in a limited number of studies to the author's knowledge, namely in Clot [2003] (25 plant taxa), Gonzalo-Garijo et al. [2006] (48 plant taxa), Damialis et al. [2007] (16 plant taxa), Cristofori et al. [2010] (63 plant taxa), Pérez-Badia et al. [2010] (seven plant taxa), Tormo-Molina et al. [2010] (seven plant taxa), Makra et al. [2011] (19 plant taxa), Myszkowska et al. [2011] (15 plant taxa), Melgar et al. [2012] (34 plant taxa) and Deák et al. [2013] (eight plant taxa). An overall analysis of the pollen season characteristics for a given source area provides a more reliable picture of the climate sensitivity for each taxon studied based on their different optimum environmental conditions. On the other hand, recent multi-location (commonly, mono-taxon) studies are more numerous but very limited in both spatial and pollen taxa coverage: Also to the author's knowledge, from the year 2000 on one can list Emberlin et al. [2000] (12 locations), Weryszko-Chmielewska et al. [2001] (three locations, three plant taxa), Emberlin et al. [2002] (six locations), Rodríguez-Rajo et al. [2003] (two locations), Spieksma et al. [2003] (five locations, five plant taxa), Rodríguez-Rajo et al. [2004] (two locations), Rodríguez-Rajo et al. [2006] (four locations), Tedeschini et al. [2006] (four locations), Rieux et al. [2008] (18 locations, eight taxa), Stach et al. [2008] (four locations), Alcázar et al. [2009] (two locations, four plant taxa), Recio et al. [2009] (two locations), Sánchez-Reyes et al. [2009] (two locations), Smith et al. [2009] (13 locations), García-Mozo et al. [2010] (eight locations), Aira et al. [2011] (four locations, six plant taxa), Ziska et al. [2011] (10 locations), DellaValle et al. [2012] (14 locations, four plant taxa), Grewling et al. [2012] (13 locations), Newnham et al. [2013] (three locations), and Jato et al. [2013] (three locations, three plant taxa). As a consequence of the considerations expressed above, it is possible to infer the urgent need of a wide-breadth study evaluating the current situation of airborne pollen in Europe. The study by Ziello et al. [2012b], reported in chapter four, provides such a missing overview by analyzing 23 plant taxa over 97 locations.

Main findings

This publication carefully describes the unquestionable trend for airborne pollen to increase, all over Europe, for the majority of taxa and locations analyzed. In such a context, it also reports the interesting finding about a more pronounced percentage of high pollen trends in urban areas, probably due to some well-known phenomena characterizing these compromised environments. In fact, urban environments are characterized not only by the "heat island" effect, but also by high levels of pollutants, such as NO_x, VOCs or particulates. Furthermore, higher atmospheric CO_2 concentrations are known to cause a general increase in vegetation biomass, an increase in pollen production and pollen allergenicity [Wayne et al., 2002; Ziska et al., 2003; Singer et al., 2005; Rogers et al., 2006; Darbah et al., 2008; Ziska et al., 2008, 2009; Ziska and Beggs, 2012]. Therefore, the interpretation given is that higher levels of CO_2, typical of urban areas, may cause a greater presence of airborne pollen in this environment. Lower tropospheric O_3 levels also characterize urban environments, due to higher concentration of NO, which is involved in the breakdown of O_3. Because the effect of O_3 is to inhibit plant development [Darbah et al., 2008], enhanced plant growth in urban areas has already been reported [Gregg et al., 2003]. This means that in cities, to which population naturally converges and where hence tends to concentrate, some concomitant environmental factors could promote the increase of pollen in the atmosphere. Considering the increasing number of people that develop allergic diseases especially among city dwellers [Motta et al., 2006; D'Amato et al., 2007; Beggs, 2010], this situation potentially represents a worldwide emergency.

5.3 GENERAL CONCLUSIONS

A wide multidisciplinary scientific community is currently working on interactions between climate change, plant phenology and aerobiology of pollen grains in order to finally achieve more efficient and updated tools to help treat allergic diseases. In such a context, the research carried on within the presented project provides several original contributions of a certain relevance, not only from an allergological perspective.

Starting from the assumption that flowering and pollen release are intimately connected phases of plants' life, in a first part the presented research uses flowering phenology as a proxy for the pollen season. The goal is to investigate the changes undergone by allergenic species under global warming conditions performing large-scale analyses. Thanks to the large spatial and temporal coverage of phenological networks, the adopted procedures for data selection and statistical modeling allows us to go beyond the usual limits imposed by the traditional methodologies of aerobiological research, and leads to novel results. The most important findings can be summarized as i) the detection of scarce dependence of phenological trends on altitude a.s.l. in the Alpine region, which means that the impact of climate change is largely appreciable in the whole area but can not be differentiated by altitude, and ii) the more pronounced phenological advancement of wind-pollinated species, to which the major part of allergenic species belong, finally leading to a longer flowering season and a consequent likely increment in pollen exposure.

In a second and last part, the current research project aims to assess the effects induced in recent years by climate change on pollen directly by means of airborne pollen counts. After collecting the Europe's largest pollen data set for spatial coverage ever analyzed in the aerobiological literature, the research highlighted an appreciable overall increase in pollen amounts, especially pronounced in urban areas.

Besides ecological consequences, the results of the current research project suggest a likely exacerbation of allergic diseases, which have however been on the increase during the last half century due to causes only partially explained. Hence, it is important to continue studying the changes of the pollen season, with the aim of providing an always more precise picture about airborne pollen under climate change. The hope is that of contributing to treat and mitigate pollinosis symptoms, providing useful information to improve everyday life conditions for more than 400 million sufferers from allergic diseases worldwide.

5.4 OUTLOOK

Since the costs of pollen data are very high, one of the goals of the current research was to offer reliable results on changes in the pollen season bypassing the problem of collecting or buying such data. This was successfully done in a first step, using phenological data instead of palynological data, and also later, asking a large number of potential pollen data providers for European data already partially manipulated. But being in the position to access a full daily information on airborne pollen, instead of only annual trends of pollen amount, would mean having a more complete information to analyze.

It would then be possible to couple daily pollen concentrations, phenological information and weather data from the same location (or from close sites), and perform very accurate local or regional analyses of changes in pollen amount per taxon. This way, it could be feasible to give a more precise picture of each European country provided with an appropriate number of phenological, palynological and meteorological stations. Moreover, it would also be possible to consider the effective clinical threshold levels (i.e., the minimal atmospherical content of pollen that triggers allergic symptoms), not possible with the collected data. This means the ability to identify the days in which the quantities of airborne pollen actually exceed such thresholds, becoming actually able to cause allergic reactions in predisposed subjects. Focussing on the study of these "above-threshold" days would allow a more effective description of pollen patterns under climate change, ignoring less important information from a medical perspective.

For the reasons expressed above, the possibility of accessing daily counts of airborne pollen worldwide is of crucial importance. Data sharing represents a common need and a unique resource for science, especially for the global change community [Wolkovich et al., 2012b]: Aerobiological research is no exception.

There is a compelling need to keep on monitoring airborne pollen levels, and consequently the health status of pollinosis sufferers worldwide that could be endangered by global change. In fact, climate conditions are changing at a very elevated rhythm: For example, as recently (May 9th, 2013) detected at the observatory of Manua Loa, Hawaii, the daily mean concentration of CO_2 in the atmosphere surpassed 400 parts per million (ppm) for the first time since measurements began in 1958 (the rate of increase has accelerated since the measurements started, from about 0.7 ppm per year in the late 1950s to 2.1 ppm per year during the last 10 years)[1]. In light of this finding, and also considering actual and projected changes in the frequency of extreme events and in global mean temperature, it could be hypothesized that *r*-selected species, to which wind-pollinated plants generally belong, could be favored by such unstable climatic conditions relative to *K*-selected species[2]. Since,

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[1] To give a context for a correct interpretation of such an exceptional finding, before the Industrial Revolution (19th century) global average CO_2 was about 280 ppm. During the last 800,000 years, CO_2 fluctuated between about 180 ppm during ice ages and 280 ppm during interglacial warm periods. Today's rate of increase is more than 100 times faster than the increase that occurred when the last ice age ended.

[2] According to the theoretical r/K-selection model, where r is the intrinsic rate of natural increase in population size and K is the carrying capacity of a population, different species evolve different life-history strategies spanning

as already reported in different sections of this thesis (§ 1.5, chapter three, and §§ 5.2.2), among wind-pollinated plants are the most allergenic species to humans, the dangerous influence that unstable environmental conditions could potentially exercise on public health becomes evident, also from an allergological perspective.

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a continuum between these two selective forces. An *r*-selected species is one that has high birth rates, low levels of parental investment, and high rates of mortality before individuals reach maturity. Evolution favors high rates of fecundity in *r*-selected species. In contrast, a *K*-selected species has low rates of fecundity, high levels of parental investment in the young, and low rates of mortality as individuals mature.

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R. Gehrig, Ł. Grewling, A.M. Gutiérrez Bustillo, M. Hallsdóttir, M.-C. Kockhans-Bieda, C. De Linares, D. Myszkowska, A. Pàldy, A. Sánchez, M. Smith, M. Thibaudon, A. Travaglini, A. Uruska, R.M. Valencia-Barrera, D. Vokou, R. Wachter, L.A. de Weger, and A. Menzel. Changes to airborne pollen counts across Europe. *PLoS ONE*, 7(4):e34076, 2012b. (Cited on pages vi, vii, 8, 17, 19, 74, 77, 81, and 84.)

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COLOPHON

This PhD thesis was typeset using LaTeX and the typographical look-and-feel `classicthesis` developed by André Miede (`http://code.google.com/p/classicthesis/`), on an Apple MacBook 13.3 inch computer (2008). The style was inspired by Robert Bringhurst's seminal book on typography *"The Elements of Typographic Style"*. Programs used to generate the contents of the thesis included:

- SAS 9.1 statistical package on a Microsoft Windows environment to perform calculations presented in Chapter 2;
- PLOT 0.999 for generating figures presented in Chapter 2;
- R 10.2 statistical package and the editor JGR to perform calculations and generate figures presented in Chapter 3 and 4;

Other programs that were frequently used were the numerical sheet of NeoOffice and the suite OpenOffice especially for collecting the data for the analysis presented in Chapter 4, PS2EPS+ for converting Postscript files to Encapsulated Postscript on the Macintosh, the GIMP for modifying pictures generated by R, and Fetch 3.0.1 for FTP transfer of data from to and from the computer at the Chair.

ERKLÄRUNG

Ich erkläre an Eides statt, dass ich die der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Promotionsprüfung vorgelegten Arbeit mit dem Titel

Influence of recent global change on the pollen season in Europe

am Fachgebiet für Ökoklimatologie unter der Anleitung und Betreuung durch Prof. Dr. Annette Menzel ohne sonstige Hilfe erstellt und bei der Abfassung nur die gemäß § 6 Abs. 5 angegebenen Hilfsmittel benutzt habe.

Ich habe die Dissertation in keinem anderen Prüfungsverfahren als Prüfungsleistung vorgelegt.

Ich habe den angestrebten Doktorgrad noch nicht erworben und bin nicht in einem früheren Promotionsverfahren für den angestrebten Doktorgrad endgültig gescheitert.

Die Promotionsordnung der Technischen Universität München ist mir bekannt.

Freising, 29.07.2013

Chiara Ziello

CHIARA ZIELLO

PERSONAL INFORMATION

birthplace	Rome, Italy
birthdate	July 22nd, 1979
citizenship	Italian
marital status	Married, one child
email	✉ ziello@wzw.tum.de
website	🖱 http://oekoklimatologie.wzw.tum.de/
phone	☎ +49-8161-714746 · FAX +49-8161-714740

EDUCATION

July 2008-Present TU München, Freising, Germany *Ph.D. studies*

Ph.D. studies in progress · Chair of Ecoclimatology.
Graduation expected by December 2013.
Thesis: *Influence of Recent Global Change on the Pollen Season in Europe*.
Advisor: Prof. Dr. Annette Menzel

December 2007 Sapienza - Università di Roma, Italy *Master Degree in Physics*

'Laurea' Degree in Physics · Physics Department.
Marks: 105/110.
Thesis: *Study of Biologically Effective UV Radiation (Vitamin D Production and Erythema) as a Function of Atmospheric Parameters*.
Advisors: Dr. Anna Maria Siani & Dr. Marco Cacciani

High school diploma

July 1998 Liceo classico ‘Cornelio Tacito’, Roma, Italy

Esame di Maturità Classica. Marks: 60/60.

Piano studies

September 1997 Conservatorio di Musica ‘Luisa D’Annunzio’, Pescara, Italy

‘Compimento Inferiore di Pianoforte’ Exam · Musical instrument: Piano. Marks: 7.80/10.

Music theory studies

June 1995 Conservatorio di Musica ‘Licinio Refice’, Frosinone, Italy

‘Teoria, Solfeggio e Dettato Musicale’ Licence. Marks: 9/10.

WORK EXPERIENCE

July 2008-December 2011 Research assistant, CHAIR OF ECOCLIMATOLOGY, TU München

Research assistantship

Main research: Alpine Phenology, Phenological modeling, Particle Dispersion Modeling, Trend Analysis, Changes in Pollen Seasonality.
Research project: U119 ‘Impacts of Climate on Pollen Season and Distribution in the Alpine Region’.
References: Prof. Dr. Annette MENZEL · ☎ +49-8161-714740 · ✉ brigitte.fleischner@wzw.tum.de
Dr. Nicole ESTRELLA · ☎ +49-8161-714744 · ✉ estrella@wzw.tum.de

COMPUTER SKILLS

Operative Systems MICROSOFT WINDOWS: 98/NT/2000/XP/Vista/7

APPLE MAC OS X: 10.3/10.4/10.5/10.6

Programming Languages FORTRAN 77, FORTRAN 90, R, SAS®, LaTeX, UNIX

LANGUAGES

ITALIAN · Mothertongue
ENGLISH · Advanced (conversationally fluent)
FRENCH · Basic (simple words and phrases only)
GERMAN · Basic (simple words and phrases only)

INTERNATIONAL CONFERENCES AND WORKSHOPS

10-12 March 2009 COST Action 725 Final Conference

'Establishing a European Phenological Data Platform for Climatological Applications', held in Geisenheim, Germany · Poster presentation

14-18 September 2009 GFOE 2009

'Dimensions of Ecology', held in Bayreuth, Germany · Poster presentation

25 January 2010 Forstliche Klimawandelforschung

held in Freising, Germany · Poster presentation

2-7 May 2010 EGU, General Assembly 2010

held in Vienna, Austria · Poster presentation

20-21 May 2010 Erste Wissenschaftliche Tagung UFS

held in Iffeldorf, Germany · Oral presentation

14-17 June 2010 Phenology 2010: Climate Change Impacts and Adaptation

held in Dublin, Ireland · Oral presentation

23 September 2010 Phenology in a Changing World: Exploring Rhythms in Nature

held in Trento, Italy

3-8 April 2011 EGU, General Assembly 2011

held in Vienna, Austria · Oral presentation by Prof. Dr. Annette Menzel · Oral presentation by Dr. Susanne Jochner

INTERNATIONAL RESEARCH ACTIVITY

2-15 November 2008 Short Term Scientific Mission

supported by COST Action 725 'Establishing a European Phenological Data Platform for Climatological Applications', at Fondazione Mach (former Istituto Agrario San Michele all'Adige), Trento, Italy.

11 November 2008 Talk as Invited Speaker

at Fondazione Mach, Trento, Italy.

publications

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