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Environmental responses of phenology and allergenic pollen to recent climate change and urbanisation

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Life is like phenology..

Beautiful flower
- you emerge and you drop -
but thinking of this flower
will never stop.

ABSTRACT

Background and aims

Phenology, the science of recurring natural events, has attracted increasing interest in recent years and is also in the focus of this publication based PhD thesis entitled "Environmental responses of phenology and allergenic pollen to recent climate change and urbanisation".

Air temperature is the most important factor influencing plant phenology in temperate regions; thus past and future warming had and will have significant effects on onset dates. However, new questions emerge when extrapolating of this obviously robust statistical relationship outside the present data range or outside present environmental conditions.

Altitudinal and especially urbanisation gradients allow better prediction of future phenology from current information since urban areas with their urban heat island, drier conditions and stronger air pollution may serve as a proxy for future scenarios. Based on such more extreme situations, we aimed to investigate the influence of air temperature and other environmental factors; a challenging question since a few previous approaches revealed that most of the tested factors besides temperature lack sufficient explanatory power.

In general, plants located in urban areas characterised by warmer conditions tend to bloom earlier in spring, however, published results on the magnitude of the urban-rural difference in phenology are inconsistent. Our broad-scale analysis therefore included a large number of phenological observation sites along natural temperature gradients provided by different degrees of urbanisation and allowed an assessment of phenology-temperature relationships outside the established median conditions.

Existing descriptive urban phenology studies mostly failed to demonstrate the consequences and relevance of their findings. Therefore, we chose the phenology - human health nexus with allergic diseases since flowering behaviour of allergenic plants and pollen associated characteristics are considered to change with climate change and urbanisation. Thus, in our monitoring network the phenological behaviour of plants in urban-rural environments as well as along an altitudinal gradient was studied and pollen production and allergenicity was assessed.

The major questions that will be addressed in this PhD thesis are:

a) Which factors besides temperature also have an influence on phenology?
b) How adequate are phenological data to estimate local / regional temperature patterns?
c) What are the effects of climate change / urbanisation on pollen?

Material and Methods

Phenological observations were conducted within three consecutive years (2009-2011) in four different regions: along urbanisation gradients in Munich, Ingolstadt (Germany), and Campinas (Brazil) as well as along an altitudinal gradient in the Zugspitze region (Germany, Austria). The research focused on flowering or / and leaf unfolding of allergenic temperate trees (silver birch, horse chestnut), tropical trees (yellow trumpet tree, false Brazilwood, racehorse tree) and on allergenic grass species (cocksfoot, meadow foxtail). To ensure consistent methods observations were based on the BBCH-code (Meier 2001).

This study also incorporated long-term data (1951-2008) of various spring phenophases derived from the German Meteorological Service (DWD) in the greater area of Munich, Cologne and Frankfurt as well as southern Bavaria (Germany).

We installed a broad-scale observation network in Munich (*N* = 38) and Campinas (*N* = 31) and measured air temperature and humidity directly on site - at the stem of the observed trees. Further meteorological data derived from national meteorological services (DWD, IAC [Instituto Agronômico]).

Pollen sampling in the Alpine region (2008-2009) was achieved using three volumetric Burkard traps recording the number of pollen per cubic meter of air. Birch catkins from 40 trees at urban and rural sites in the Munich study area were sampled in 2010 and allergen associated factors such as Bet v 1, $PALM_{LTB4}$, $PALM_{PGE2}$ and adenosine content were analysed. Furthermore, monocyte-derived dendritic cells were isolated and cultured, and neutrophil migration assays and skin prick tests were performed. Additionally, pollen amount was measured in 2009. In 2010, foliar nutrient concentration of birch as well as site-specific O_3 and NO_2 levels were assessed.

The major statistical analyses included (partial) correlation analysis, linear / multiple regression, t-test, Mann-Whitney U-test, ANCOVA and discriminant analysis. Maps and geostatistical analyses were produced using GIS. The research incorporated *inter alia* a novel GIS-based regionalisation technique based on the lowest-method and the calculation of the urban index, a variable describing the degree of urbanisation.

Results and Discussion

Factors influencing phenology

Air temperature was the most important factor influencing phenological onset dates of birch (r^2 = 43.4 to 61.3 %) in Munich (Chapter 3). Also shown by high correlations (Chapter 2), onset dates of birch phenophases were suitable to describe the urban heat island of Munich. Our study was the first that analysed tropical tree phenology in urban and rural environments in detail (Chapter 2). In Campinas the relationship between temperature and phenology was only evident for one species - racehorse tree (Chapter 2). The finding that most phenophases of tropical trees were not responsive to temperature pointed out the relevance of humidity variables, especially demonstrated for leaf unfolding of yellow trumpet tree.

Other important variables triggering temperature and thus phenology in temperate regions were altitude (Chapter 4, 5 and 7), aspect (Chapter 7) and land use factors such as the extent of impervious surfaces and forests and the distance to urban areas (Chapter 4). Additionally, the urban index was found to be an adequate proxy for temperature conditions (Chapter 2). The inclusion of this continuous variable (Chapter 5) counteracted the limitations of a plain urban-rural dichotomy that was commonly used in urban phenology so far.

Another central result of our study was that nutritional status - especially the nutrients potassium (K), boron (B), zinc (Zn) and calcium (Ca) - was relevant for birch phenophases (particularly leaf unfolding) in Munich (Chapter 3). These nutrients are normally involved in cell extension, membrane function and stability. Inclusion of these nutrients in phenological models could increase the model fit by up to one fifth of the r^2 using temperature alone.

Estimation of local and regional temperature patterns

Flowering phenology of forsythia was found to be a useful proxy of regional climate variation arising from both natural and anthropogenic factors (Chapter 4). By introducing a new method in phenological research - the GIS-assisted regionalisation of phenological data using the statistical results of the discriminant analysis - we were able to estimate the temperature distribution at the regional scale (southern Bavaria). Without applying any meteorological data our selected land use variables were able to represent temperature conditions adequately.

Using several methods, data and regions of interest, this research demonstrated advanced phenological development in the city compared to rural areas. Phenological observations in spring 2011 in Munich revealed significant urban-rural differences in onset dates of birch ranging between -1.5 and -3.4 days (Chapter 2).

Land use differentiation from entirely rural to entirely urban in the greater area of Munich, Cologne and Frankfurt implied an advance of 2.6 to 7.6 days suggesting that urbanisation processes had and will have major implications on temperature and phenology (Chapter 5).

Our study was the first that analysed the effects of anomalous high spring temperatures on phenology at the micro- and mesoscale (Chapter 6). High temperatures, mainly warm spells, were more likely to result in simultaneous flowering in urban and rural environments; low temperatures resulted in a larger delay in phenological onset times. This was not only demonstrated within the field study in Munich and Ingolstadt (2009), but also using long-term phenological data (1951-2008) of the greater area of Munich.

Impacts on airborne pollen and aeroallergens

Analyses of grass flowering in the Alpine region (Chapter 7) demonstrated substantial responses to altitude (7 days $(100\ \mathrm{m})^{-1}$) and therefore to temperature (-10 days $(1\ ^{\circ}\mathrm{C})^{-1}$) indicating greater temperature sensitivity compared to birch phenophases (3 days $(100\ \mathrm{m})^{-1}$, -7 days $(1\ ^{\circ}\mathrm{C})^{-1}$). This suggests that further temperature increase will result in a remarkably earlier grass pollen season and, in turn, lead to major consequences for human health.

The comparison of phenological and aerobiological data in the Zugspitze region (Chapter 7) revealed a good agreement in the start of season dates, especially at lower altitudes. Therefore, local pollen emissions accounted most for the timing of the aerobiological start of the season. Airborne pollen counts at the highest and vegetation-free site (UFS) were particularly affected by long- or medium-range transport which even contributed to high and medically relevant pollen concentrations.

In our study areas, Munich and Ingolstadt, where we found synchronous flowering due to anomalous high spring temperatures in 2009 (Chapter 6), people suffering from allergy were probably affected by atmospheric pollen loads starting almost the same day - both in the city and the countryside. Symptoms were most likely strong during the beginning of the pollen season since a greater number of birches started to flower simultaneously. However, symptoms probably did not persist for an extended time span due to the rapid transition of phenological flowering phases (from beginning of flowering to full flowering).

Laboratory examination of birch pollen grains also revealed that the amount per catkin did not differ significantly between the city and the countryside which might be probably related to less pronounced urban-rural temperature differences (Chapter 6).

In 2010, pollen grains from birch trees exposed to elevated O_3 levels contained higher levels of Bet v 1 than pollen from lower O_3 exposed birch trees (Chapter 8). In addition, this pollen also induced higher neutrophil chemotaxis, was less potent in inhibiting the dendritic cell IL-12 response and induced enhanced wheal-and-flare reactions in skin prick tests. Urban areas (that are characterised by lower cumulative O_3 levels) might therefore facilitate *de novo* sensitisation by providing Th2 promoting signals; rural areas (that are characterised by higher cumulative O_3 levels) might intensify allergic reaction in already sensitised individuals.

Conclusions

This PhD thesis demonstrated several new techniques in phenological and pollen associated allergological research and highlighted important findings. Since urban areas provide an excellent means of assessing climate change impacts, we were able to simulate future plant development in spring. Therefore, we could show that a temperature increase of 1 °C already leads to a 3 day earlier flowering of birch. Warm spells during spring, which are expected to increase due to climate change, however, will result in simultaneous urban-rural flowering. Besides temperature, which constitutes the most important influential factor in phenology, we demonstrated the importance of nutritional status of plants. Tropical tree species were found to be especially responsive to humidity. Urbanisation / climate change also has an impact on the allergen content of birch pollen. Higher sensitisation rates in the city and stronger allergic symptoms in the countryside - a stunning result. However, not temperature was the responsible factor; but tropospheric ozone, which is expected to increase due to further climate change and urbanisation. Although seven publications were presented in this PhD thesis, there is still a need for further research.

KURZFASSUNG

Zielsetzungen

Die Phänologie als Wissenschaft von wiederkehrenden Ereignissen in der Natur hat in den vergangenen Jahren einen besonderen Aufschwung erfahren. Sie steht auch im Mittelpunkt dieser publikationsbasierten Dissertation mit dem Titel „Umweltreaktionen von Phänologie und allergenen Pollen auf rezente Klimaerwärmung und Urbanisierung“.

Der wichtigste Einflussfaktor auf die Pflanzenphänologie in den gemäßigten Breiten ist die Lufttemperatur. Aus diesem Grund hat die bisher beobachtete Klimaerwärmung phänologische Eintrittstermine bereits merklich verändert. Jedoch ergeben sich bei der Extrapolation dieser offensichtlich robusten statistischen Beziehung - außerhalb des derzeitigen Temperaturbereichs oder außerhalb der derzeitigen Umweltbedingungen - neue Fragestellungen.

Höhengradienten und insbesondere Urbanisierungsgradienten ermöglichen mit vorhandenen Informationen eine bessere Abschätzung der künftigen phänologischen Änderungen. Denn städtische Gebiete mit ihrer urbanen Wärmeinsel, trockeneren Bedingungen und stärkerer Luftverschmutzung können als Proxy für künftige Bedingungen dienen. Basierend auf derart „extremen“ Situationen wird in dieser Arbeit der Einfluss von Lufttemperatur und anderen Umwelteinflüssen untersucht. Dies ist eine besondere Herausforderung, da bisherige Studien gezeigt haben, dass die meisten neben der Temperatur getesteten Faktoren keine ausreichende Erklärungskraft in der Phänologie aufweisen.

Pflanzen blühen in der wärmeren Stadt im Frühling eher. Der sich daraus ergebende Stadt-Land-Unterschied ist jedoch nicht für alle Pflanzenarten und Regionen universell; das zeigen auch veröffentlichte Studien. Unsere großflächig angelegte Studie beinhaltet deshalb eine große Anzahl an phänologischen Untersuchungsstandorten entlang eines natürlichen Temperaturgradienten, der sich aus verschiedenen Urbanisierungsgraden ergibt. Dies erlaubt die Beziehung zwischen Phänologie und Temperatur auch außerhalb der bekannten durchschnittlichen Bedingungen festzustellen.

Existierende stadtphänologische Arbeiten sind oftmals nur deskriptiv und zeigen die Konsequenzen und die Relevanz ihrer Ergebnisse nicht oder nur ungenügend auf. Diese Dissertation hingegen stützt sich vor allem auf den Nexus Phänologie – menschliche Gesundheit / Allergie, da das phänologische Verhalten von allergenen Pflanzen und pollenassoziierten Eigenschaften im Rahmen der Klimaänderung und Urbanisierung markanten Veränderungen unterworfen ist. Daher wurde innerhalb unseres breit angelegten Beobachtungsnetzwerks das phänologische Verhalten von Pflanzen in urbanen und ruralen Gebieten sowie entlang eines Höhengradienten erfasst und die Pollenproduktion und Pollenallergenität bestimmt.

Die Hauptfragen, die in dieser Dissertation behandelt werden, lauten:

a) Welche Faktoren neben der Temperatur haben ebenso einen Einfluss auf die Pflanzenphänologie?
b) Wie geeignet sind phänologische Daten, um lokale und regionale Temperaturmuster abzuschätzen?
c) Welche Einflüsse hat die Klimaänderung / Urbanisierung auf Pollen?

Material und Methoden

Phänologische Beobachtungen wurden in drei aufeinander folgenden Jahren (2009-2011) in vier verschiedenen Regionen durchgeführt: entlang eines Urbanitätsgradienten in München und Ingolstadt (Deutschland) und Campinas (Brasilien) sowie entlang eines Höhengradienten in der Zugspitzregion (Deutschland, Österreich). Blüte und / oder Blattentwicklung von allergenen Bäumen der gemäßigten Breiten (Hängebirke, Rosskastanie), tropischen Bäumen (gelbe Jacaranda, Sibipiruna, Goldtrompetenbaum) und allergenen Gräsern (Wiesen-Fuchsschwanz, Wiesen-Knäuelgras) wurden beobachtet. Um konsistente Methoden zu gewährleisten, stützten sich die phänologischen Beobachtungen auf den BBCH-Code.

Zudem wurden lange Zeitreihen (1951-2008) von diversen Frühlingsphasen des Deutschen Wetterdienstes (DWD) im Großraum München, Köln, Frankfurt sowie in Südbayern (Deutschland) analysiert.

In dem eingerichteten großflächigen Beobachtungsnetzwerk in München (N = 38) und Campinas (N = 31) wurden Lufttemperatur und -feuchtigkeit direkt am Beobachtungsstandort gemessen - am Stamm der Untersuchungsbäume. Weitere meteorologische Daten stammen von nationalen Wetterdiensten (DWD, IAC [Instituto Agronômico]).

Die Pollenzählung im Alpenraum (2008-2009) erfolgte mit drei volumetrischen Burkhard-Fallen, die die Menge der Pollen pro Kubikmeter Luft erfassen. Um allergie-assoziierte Faktoren wie Bet v 1, $\mathrm{PALM}_{\mathrm{LTB4}}$, $\mathrm{PALM}_{\mathrm{PGE2}}$ und Adenosingehalt zu analysieren, wurden im Jahr 2010 Birkenkätzchen von 40 urbanen und ruralen Bäumen in München gesammelt. Aus den Birkenpollen wurden wässrige Pollenextrakte hergestellt, welche bei anschließenden Zellstimulationsversuchen (aus Monozyten generierten dendritischen Zellen) und bei Migrationsassays mit neutrophilen Granulozyten verwendet wurden. Daneben wurde die kutane Reaktion von Patienten mit Hilfe von Pricktests untersucht. Die Pollenmenge wurde zusätzlich im Jahr 2009 erfasst. Die Ermittlung von Blattnährstoffkonzentrationen der beprobten Birken und standortspezifische O_3- und NO_2-Konzentrationen erfolgten im darauf folgenden Jahr.

Statistische Analysen umfassen unter anderem (partielle) Korrelationsanalyse, lineare / multiple Regression, t-Test, Mann-Whitney U-Test, ANCOVA sowie Diskriminanzanalyse. Karten und geostatistische Analysen wurden mit GIS produziert. Die Studie beinhaltet unter anderem eine neuartige GIS gestützte Regionalisierungstechnik, basierend auf der Lowest-Methode, und die Berechnung des *urban index*, eine Variable, die den Urbanisierungsgrad beschreibt.

Ergebnisse und Diskussion

Einflussfaktoren in der Phänologie

Die Lufttemperatur stellte den wichtigsten Faktor dar, der die phänologischen Eintrittstermine der Birke in München beeinflusst (r^2 = 43.4 bis 61.3 %, Kapitel 3). Somit erwiesen sich die phänologischen Phasen der Birke als geeignet, um die städtische Wärmeinsel in München zu beschreiben. Dies wurde auch durch die hohen Korrelationen zwischen Phänologie und Temperatur gezeigt (Kapitel 2). In Campinas war diese Beziehung nur für eine Baumart deutlich: Sibipiruna (Kapitel 2). Die Tatsache, dass die meisten Phänophasen von tropischen Bäumen nicht auf Temperaturänderungen reagierten, deutet auf einen großen Einfluss von Feuchteparametern, was vor allem für die Blattentwicklung des Goldtrompetenbaums belegt wurde.

Weitere wichtige Faktoren, die die Temperatur und somit auch die Phänologie beeinflussten, waren Höhenlage (Kapitel 4, 5, 7), Exposition (Kapitel 7) und Landnutzungsvariablen, wie der Anteil an versiegelter Fläche und von Waldgebieten sowie die Distanz zu städtischen Gebieten (Kapitel 4). Zudem erwies sich der *urban index* als geeigneter Proxy für Temperaturbedingungen (Kapitel 2). Die Einbindung dieser kontinuierlichen Variablen (Kapitel 5) konnte die Einschränkungen einer einfachen urban-ruralen Dichotomie überwinden, die bisher in der Stadtphänologie angewandt wurde.

Ein weiteres zentrales Ergebnis dieser Forschungsarbeit war, dass der Nährstoffstatus - vor allem der Nährelemente Kalium (K), Bor (B), Zink (Zn) und Kalzium (Ca) - bedeutend für die Pflanzenentwicklung der Birke (v.a. Blattentfaltung) ist. Diese Nährelemente sind normalerweise für Zellvergrößerung, Membranfunktion und -stabilität verantwortlich. Der Einbezug dieser Nährstoffe in Modelle konnte das Bestimmtheitsmaß - im Vergleich zu nur auf Temperatur basierten Modellen - relativ um bis zu ein Fünftel erhöhen. Diese Studie war somit die erste, die den Einfluss von Nährstoffen auf die Pflanzenentwicklung auf lokaler Ebene dargelegt hat.

Beurteilung der lokalen und regionalen Temperaturverteilung

Die Blühphänologie der Forsythie stellte sich als nützlicher Proxy für regionale Klimaunterschiede heraus, die sowohl durch natürliche als auch durch anthropogene Faktoren hervorgerufen sein können (Kapitel 4). Durch die Einführung einer neuen Methode in der phänologischen Forschung - die GIS basierte Regionalisierung von phänologischen Daten unter Zuhilfenahme der statistischen Ergebnisse der Diskriminanzanalyse - war es möglich, die Temperaturverteilung auf regionaler Ebene (Südbayern) abzuschätzen. Ohne Einbeziehung irgendwelcher meteorologischen Daten konnte mit den ausgewählten Landnutzungsvariablen die Temperaturbedingungen adäquat repräsentiert werden.

Mit Hilfe von verschiedenen Methoden, Datensätzen und Untersuchungsgebieten zeigte diese Arbeit eine verfrühte phänologische Entwicklung in der Stadt gegenüber dem Land. Signifikante Stadt-Land-Unterschiede bei den Eintrittsterminen der Birke (-1,5 bis -3,4 Tage) konnten anhand von phänologischen Beobachtungen im Frühling 2011 in München errechnet werden (Kapitel 2). Landnutzungsänderungen von "vollständig rural" zu "vollständig urban" bewirkten eine Verfrühung von 2,6 bis 7,6 Tagen im Großraum München, Köln und Frankfurt (Kapitel 5). Dies deutet darauf hin, dass Urbanisierungsprozesse bedeutende Auswirkungen auf die Temperatur und Phänologie hatten und haben werden.

Diese Studie war auch die erste, die den Einfluss von ungewöhnlich hohen Frühlingstemperaturen auf die städtische Pflanzenentwicklung analysierte (Kapitel 6). Hohe Temperaturen, vor allem Hitzewellen, führten zu einer zeitgleichen Blüte in urbanen und ruralen Gebieten; niedrige Temperaturen dagegen führten zu einer Verzögerung der phänologischen Eintrittstermine. Dies wurde nicht nur in der Feldstudie in München und Ingolstadt (2009) gezeigt, sondern auch anhand von langen Zeitreihen (1951-2008) im Großraum München.

Einflüsse auf Pollen und Allergene

Die statistische Auswertung der Gräserblüte in der Alpenregion um Garmisch (Kapitel 7) zeigte beträchtliche Variationen mit der Höhelage (7 Tage $(100\ \mathrm{m})^{-1}$) und damit deutliche Temperaturreaktionen (-10 Tage $(1\ ^\circ\mathrm{C})^{-1}$). Die Blüte der Gräser erwies sich dabei als temperatursensitiver als die Blüte der Birke (3 Tage $(100\ \mathrm{m})^{-1}$, -7 Tage $(1\ ^\circ\mathrm{C})^{-1}$). Dies deutet darauf hin, dass ein weiterer Temperaturanstieg zu einer beträchtlich früheren Pollensaison der Gräser führt, was wiederum bedeutende Konsequenzen für die menschliche Gesundheit haben wird.

Der Vergleich zwischen phänologischen und aerobiologischen Daten (Kapitel 7) im Zugspitzgebiet zeigte eine gute Übereinstimmung hinsichtlich des Starts der Pollensaison, vor allem in Garmisch-Partenkirchen. Daher trug die lokale Pollenemission auch hauptsächlich zum aerobiologischen Beginn bei. Die Pollenzahlen auf dem höchsten und vegetationsfreien Standort (UFS) waren von atmosphärischem Pollentransport geprägt, was sogar zu hohen und medizinisch relevanten Pollenkonzentrationen führte.

In unseren Untersuchungsgebieten München und Ingolstadt, in denen wir eine zeitgleiche Blüte aufgrund der ungewöhnlich hohen Temperaturen im Frühling 2009 festgestellt haben (Kapitel 6), waren Allergiker - sowohl in der Stadt als auch auf dem Land - ab dem gleichen Zeitpunkt von atmosphärischer Pollenbelastung betroffen. Zu Beginn der Pollensaison waren die Beschwerden dabei wahrscheinlich stark, da eine größere Anzahl an Birken gleichzeitig Pollen freisetzte. Es ist jedoch wahrscheinlich, dass die Beschwerden auf Grund des schnellen Übergangs der phänologischen Phasen (von Beginn der Blüte bis hin zu Vollblüte) nicht lange Zeit anhielten. Laborauswertungen haben zudem gezeigt, dass sich die Pollenmenge pro Birkenkätzchen nicht signifikant zwischen Stadt und Land unterschied, was möglicherweise auf den gering ausgeprägten Temperaturunterschied zwischen urbanen und ruralen Standorten zurückzuführen ist.

Birkenpollen, die im Frühling 2010 höheren Ozonwerten ausgesetzt waren, enthielten mehr Bet v 1 (Hauptallergen der Birke, Kapitel 8). Daneben wirkten diese Pollen auch stärker chemotaktisch auf neutrophile Granulozyten, waren weniger wirksam bei der Hemmung der IL-12 Antwort der dendritischen Zellen und riefen eine verstärkte Quaddel- und Erythembildung bei Pricktests hervor. Birkenpollen aus städtischen Gebieten (die durch geringere kumulative Ozongehalte charakterisiert sind) begünstigen möglicherweise eine allergiefördernde Th2 gerichtete Immunitätslage (*i.e.* Sensibilisierung). Birkenpollen aus ländlichen Gebieten (die durch höhere kumulative Ozongehalte charakterisiert sind) hingegen, besitzen ein gesteigertes immunstimulatorisches Potential und könnten u.a. durch ihre erhöhte Allergenität eine verstärkte Symptomatik in bereits sensibilisierten Menschen hervorrufen.

Fazit

Diese Dissertation entwickelte neue methodische Ansätze in der Phänologie und Pollen basierten Allergologie und erbrachte damit wesentliche Resultate. Da Städte einen Blick in die Klimazukunft zulassen, war es uns möglich, mit der hier vorgestellten Forschungsarbeit den Frühling der Zukunft zu simulieren. Somit konnte gezeigt werden, dass bereits eine Temperaturerhöhung von 1 °C eine Verfrühung der Birkenblüte um 3 Tage bewirkt. Hitzeperioden im Frühling, die im Rahmen der Klimaänderung ebenfalls zunehmen werden, haben jedoch den Effekt, dass sich zwischen Stadt und Land eher eine zeitgleiche Blüte einstellen wird. Neben der Temperatur, die mit Abstand die Phänologie am meisten beeinflusst, konnte auch der Einfluss der Nährstoffversorgung der Pflanzen aufgezeigt werden. Für tropische Baumarten standen Feuchtevariablen im Vordergrund. Die Urbanisierung / Klimaänderung wirkt sich zudem auf den Allergengehalt der Birkenpollen aus. Höhere Sensibilisierungsraten in der Stadt und stärkere Symptomatik auf dem Land - ein frappierendes Ergebnis. Dabei ist es jedoch nicht die Lufttemperatur, die den entscheidenden Einfluss ausübt, sondern die troposphärische Konzentration von Ozon, das durch den fortschreitenden Klimawandel sowie durch weitere Urbanisierung künftig weiter ansteigen dürfte. Auch mit sieben Publikationen, die hier in den Rahmen dieser Dissertation eingebettet sind, ist der Forschungsbedarf lange noch nicht gedeckt.

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1 INTRODUCTION

1.1 From global to local climate change

1.1.1 Global climate change

As a result of human activities, atmospheric concentrations of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and dinitrogen monoxide (N_2O) have increased noticeably since the beginning of the Industrial Revolution. Most of the observed temperature increase since the 1950s is very likely attributable to the observed changes in the concentration of these trace gases (Stott *et al.* 2001, IPCC 2007a). CO_2 constitutes the most important driver of climate change (Hofmann *et al.* 2006, IPCC 2007a); its atmospheric concentration increased from a pre-industrial value of 280 ppm to 392 ppm in 2011 (NOAA 2012).

During the last century, the mean global air temperature has increased by 0.74 °C (± 0.18 °C) (IPCC 2007a). The global warming trend was especially high in the last four decades (Luterbacher *et al.* 2004, Hansen *et al.* 2006), and was estimated with 0.15-0.2 °C per decade (Hansen *et al.* 2010). Several years of the last two decades were among the warmest since the beginning of instrumental observations in the 1850s (IPCC 2007a, Hansen *et al.* 2010, Jones *et al.* 2011).

For the last decade of the 21st century, temperature will increase between 1.8 °C (1.0-2.9 °C) and 4.0 °C (2.4-6.4 °C), depending on the underlying scenario (IPCC 2007a). Besides, an increase in winter precipitation and a decrease in summer precipitation in the temperate region are expected (Christensen & Christensen 2007, IPCC 2007a). Due to the inertia of the climate system and feedback processes, the temperature increase could persist for centuries - even if the greenhouse gas concentrations do not further increase (IPCC 2007a, Solomon *et al.* 2009).

1.1.2 Urban climate change

Human activities do not only alter climate on the global scale *via* the emission of greenhouse gases; human activities also cause a much more localised phenomenon, the urban climate that is characterised by multiple environmental changes. The factors that are associated with urban climate are illustrated in Fig. 1.1.

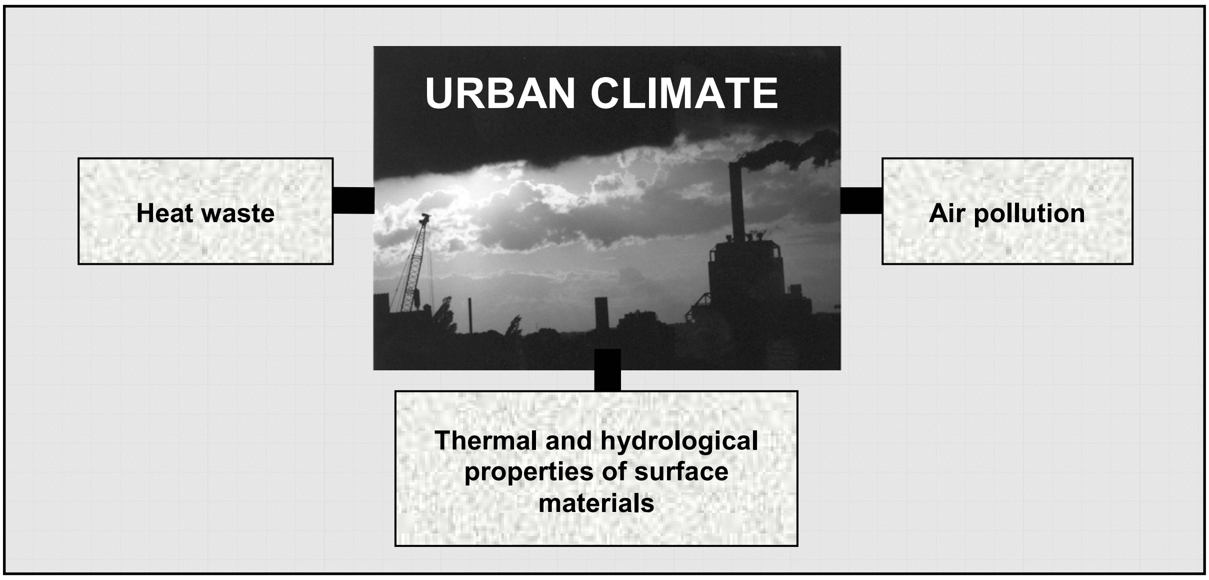

Fig. 1.1 Major characteristics of urban climate.

The major difference between urban and rural environments is their surface structure. Whereas natural or agricultural landscape is characterised by an abundance of vegetation and permeable soil, urban areas comprise only a few vegetation elements, but predominately highly compacted and impermeable surfaces (Landsberg 1981). These different surfaces also show distinctive differences in heat capacity and heat conductivity (Landsberg 1981, Kuttler 2004). Thermal properties of the urban surface material (e.g., concrete, asphalt) are characterised by low albedo. A high amount of the incident shortwave radiation is absorbed by the urban material and transformed into sensible heat. Particularly, three-dimensional constructions with tall multi-storey buildings, high building density and narrow streets support a high absorption rate (Oke 1987, Matzarakis 2001).

In addition, impervious surfaces impede precipitation to infiltrate into the soil. The lack of vegetated areas implies less evapotranspiration and therefore higher temperatures (Oke 1983, Landsberg 1981). Thus, the main reason for the temperature increase in the city is the replacement of natural environment not only by impermeable surfaces but also by built up areas.

Another factor that contributes to higher urban temperatures is the emission of sensible heat as well as latent and chemical generation of moisture associated with private and industrial energy consumption (Sailor 2011).

Urban air is modified by a drastically higher concentration of nitrogen oxides (NO_x) and carbon monoxide (CO) which function as ozone (O_3) precursors (Landsberg 1981). The trace gas sulphur dioxide (SO_2) is, however, decreasing throughout the world, with the exception of some Central American and Asian cities (Baldasano *et al.* 2003). Other air pollutants are e.g., non-methane hydrocarbons (NMHCs), volatile organic compounds (VOC) and aerosols such as soot and dust - especially particulate matter (PM) - which constitutes an important human health issue (Davidson *et al.* 2005). Major sources of pollutants are motor vehicles, power production, refineries and industries, incineration of waste and space heat (Landsberg 1981). In contrast, rural air is also characterised by a higher amount of decay products from plant material such as ammonia (NH_3), hydrogen sulphide (H_2S) (Landsberg 1981) and biogenic volatile organic compounds (BVOC) (Simpson & McPherson 2008) as well as by higher cumulative O_3 concentrations (Gregg *et al.* 2003).

Urban air pollutants do not only have an effect on air quality and therefore on human health (Davidson *et al.* 2005, Kampa & Castanas 2008) but also on urban energy fluxes (Landsberg 1981). Particulates scatter and absorb the incoming solar radiation leading to a reduction of global radiation and a higher amount of diffuse radiation (Landsberg 1981, Kuttler 2004). Due to absorption and reemission by infrared active gases and aerosols, the downward longwave atmospheric radiation is greater in urban areas (Kuttler 2004).

In summary, the most obvious result of urbanisation is the urban heat island (UHI), defined as the temperature difference between urban and rural areas (Landsberg 1981, Fezer 1995, Matzarakis 2001). The magnitude of the UHI strongly depends on the prevailing synoptic conditions: urban-rural temperature differences are greatest during clear and calm conditions and especially marked during night time and winter season (Landsberg 1981). Therefore, UHI is greater for minimum than for maximum temperatures (Baker *et al.* 2002, Mimet *et al.* 2009, Shustack *et al.* 2009). Moreover, the UHI depends on the size of a city and its building density (Landsberg 1981, Zhang *et al.* 2004), however, it is present in every town and city (Landsberg 1981). The urban heat island effect has been well documented for various cities in different parts of the world (Landsberg 1981, Bründl *et al.* 1987, Matzarakis 2001) ranging on average from +0.5 to +3.0 °C (Kuttler 2004). During specific weather conditions in winter the difference of minimum temperature can amount to +10 °C or even +15 °C (Kuttler 2004).

1.2 Phenology

1.2.1 Scopes of phenological applications

There is a great body of evidence that global climate change, especially the increase in temperature, has already implicated physical and biological systems across the globe (Parmesan & Yohe 2003, Root *et al.* 2003, IPCC 2007b, Rosenzweig *et al.* 2008). Plant phenology, the science of natural recurring events such as flowering, leaf unfolding, fruit ripening or leaf fall, can be used to track these changes associated with climate change (Walther *et al.* 2002, Badeck *et al.* 2004, Cleland *et al.* 2007, Luo *et al.* 2007). Particularly during the last two decades substantial phenological changes have been observed (Parmesan & Yohe 2003, Root *et al.* 2003, Menzel *et al.* 2006a). Based on the close relationship between temperate spring / summer phenology and temperature, changes in phenology can be related to changes in temperature (Sparks & Carey 1995, Menzel 1997, Menzel & Fabian 1999, Menzel *et al.* 2006a).

However, phenology is much older than its function of “footprint” of recent climate change. Knowledge about the time when fruits can be harvested was essential for survival; special phenological events provided a time base for recurring periods within one year (Defila & Jeanneret 2007, Demarée & Rutishauser 2009). A prominent example is the start of cherry flowering in Japan, recorded for more than 1300 years, that serves as the date when cherry flowering festivals are hosted (Aono & Kazui 2008). Such long-term phenological records are also helpful for estimating temperature conditions of periods when temperature measurements have not been available (Chuine *et al.* 2004). The field of application also spans agricultural and forest science and many other disciplines (Badeck *et al.* 2004). New emerging topics are climate feedbacks since phenology affects ecosystem productivity and gas exchanges with the atmosphere *via* photosynthesis induced annual carbon uptake in terrestrial ecosystems (Cleland *et al.* 2007). Moreover, phenology of allergic species plays an increasing role in medicine and human health (Traidl-Hoffmann *et al.* 2003), where proper predictions of pollen flights are required. Another essential field constitutes urban climatology that is explicitly described in 1.2.3.

1.2.2 Observed changes in phenology

Spring phenology

Trends in phenological onset dates were reported for various phenophases, both in plants and animals, and for numerous regions all across the world. Global meta-analyses showed a mean advance of spring phases by 2.3 and 5.1 days per decade, respectively (Parmesan & Yohe 2003, Root *et al.* 2003). Early phases tend to show greater advances than phases that occur later in the year. This was *inter alia* demonstrated by Fitter & Fitter (2002) and Sparks & Menzel (2002). Several authors (Rötzer *et al.* 2000, Defila & Clot 2003, Lu *et al.* 2006) attributed this behaviour to differences in temperature increase that was more pronounced in January to March and less pronounced in April and May, or to the characteristic of early flowering species requiring less forcing temperatures and being therefore more sensitive to temperature changes (Joeng *et al.* 2011). Moreover, annuals and insect pollinated species are reported to have more advanced than perennials and wind pollinated plants, probably related to differences in life history strategies (Fitter & Fitter 2002). A temperature response of 4.6 days per 1 °C (the advancement of a phenophase after a warming of 1 °C) was reported by Menzel *et al.* (2006a) for spring and summer phases in Europe. The authors could also demonstrate stronger temperature response rates for warmer countries and for earlier phenophases. The latter finding is also in accordance with Jeong *et al.* (2011) who reported stronger temperature responses in early spring flowering species.

Summer and autumn phenology

Phenological trends decrease in magnitude with time of the year: summer phenophases, e.g., fruit ripening, show a less pronounced advance than spring phenophases (Menzel *et al.* 2006a). In addition, crop phenology depends also on human management (Zhang *et al.* 2004) and thus experienced only a minor advancement in contrast to wild species (Menzel *et al.* 2006b). Changes in phenology are also reflected by a delay in autumn phenophases (e.g., Luo *et al.* 2007), albeit there are some aspects that impede the interpretation of these changes (Sparks & Menzel 2002). First, it is more difficult to define phenological autumn phases such as leaf colouring and leaf fall. Second, data of autumn phenology has not been collected to the same extend as data of spring phenology. Third, abrupt weather conditions (e.g., frost or heavy storm) may be influential. On the whole, these facts lead to an indistinct climate signal. According to Estrella & Menzel (2006) a warm late summer leads to later leaf colouring and a warm May and June to an acceleration of this autumn phenophase.

Vegetation period

The vegetation period is mostly prolonged due to the advance of spring phenophases. Menzel & Fabian (1999) reported a lengthening of the vegetation period in Europe since the beginning of the 1960s by 10.8 days using phenological data of the IPG (International Phenological Gardens) network. Defila & Clot (2003) calculated a prolongation of the vegetation period in Switzerland of 13.3 days within 50 years (1951-2000), also mostly influenced by earlier spring phenophases (-11.6 days). In contrast to ground based observations on individual species level, satellite derived data mirrors plant development on the community level (Badeck *et al.* 2004). Myneni *et al.* (1997) reported a lengthening of the growing season by 12 days for northern latitudes located between 45 °N and 70 °N using AVHRR derived NDVI (Normalised Difference Vegetation Index) in the period 1981 to 1991. These findings are also confirmed by analyses of the atmospheric CO_2 signal that showed an advance of the seasonal decrease in CO_2 uptake by 7 days compared to the 1960s which is related to earlier plant photosynthesis activity (Keeling *et al.* 1996).

1.2.3 Applied urban phenology

There are only a few, however essential, application fields of urban phenology, as depicted in Fig. 1.2 and described below.

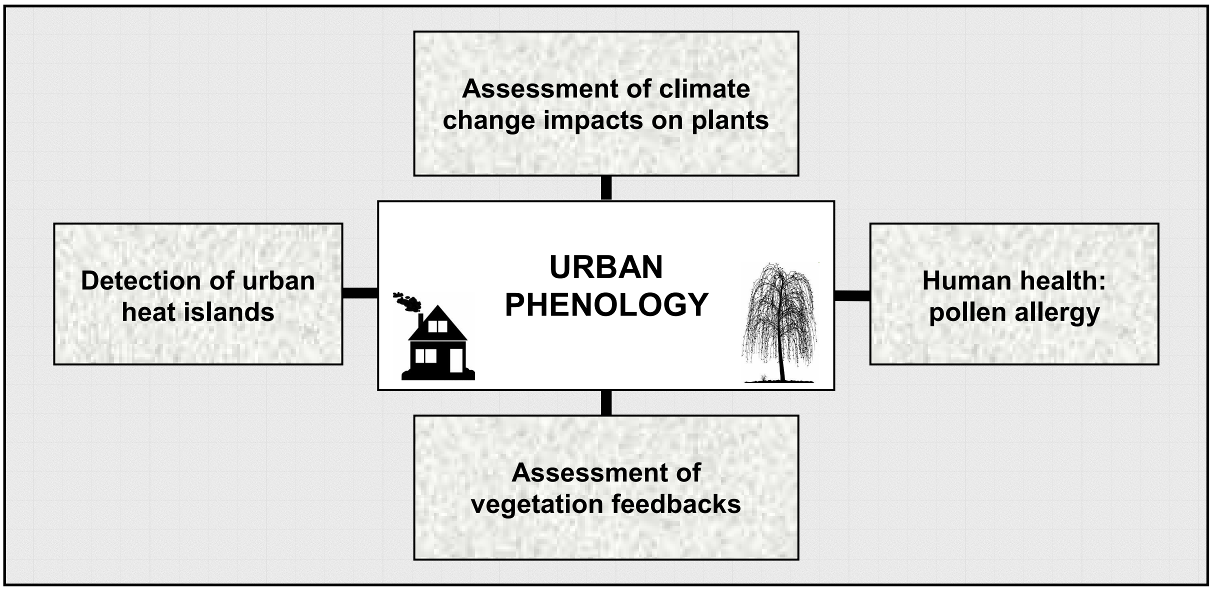

Fig. 1.2 Application fields of urban phenology.

(a) Detection of urban heat islands

Since temperature is well reflected in phenological data (Cannell & Smith 1986, White *et al.* 1997, Wielgolaski 1999, Menzel & Fabian 1999), phenological observations are suitable for the temporal and spatial analysis of the urban heat island (e.g., Omoto & Aono 1990, Bernhofer 1991, Lakatos & Gulyás 2003). Therefore, the primary idea of urban phenology was to detect and describe local temperature variations by inexpensive phenological observations (Bernhofer 1991, Matsumoto *et al.* 2009).

Phenological observations for assessing microclimatic conditions have a long tradition in temperate cities. Numerous studies exist on plant phenology in urban areas of Europe, North America and Asia (see Table 1.1) which were either broad-scale or long-term. Broad-scale studies cover a large region and are conducted in a single year or in only a few years (e.g., Mimet *et al.* 2009, Fukuoka & Matsumoto 2003, Lakatos & Gulyás 2003). Through the variety of temperature conditions in urban areas spatial patterns can be translated into temporal patterns. For special sampling campaigns (e.g., Henniges & Chmielewski 2006, Gazal *et al.* 2008) participating schools, students or other volunteers are recruited as phenological observers and the number of observation sites can be quite high. Long-term studies (e.g., Defila 1999, Rötzer & Sachweh 1995, Rötzer *et al.* 2000) - mostly based on phenological network data - focus on a longer time span using only a few observation sites where preferably always the same plant is observed.

Urban-rural differences

Spring phenology

A simple method to describe the urban heat island effect is the calculation of differences between the city and the countryside, both for temperature data (Landsberg 1981) and for phenological data (Rötzer & Sachweh 1995). Different species or phenophases within the same species respond differently to the urban heat island effect. Herbaceous plants are more sensitive to microclimatic variations than trees (Mimet *et al.* 2009). Furthermore, early phases are more sensitive to temperature (Fitter & Fitter 2002). Therefore, the effect of urban heat islands on phenology should also be greater for phases occurring early in the year (Shustack *et al.* 2009). This was also verified in urban studies by Rötzer & Sachweh (1995), Defila (1999) and Rötzer *et al.* (2000). The authors attributed the larger differences in early phenophases to the amplified urban heat island effect in winter - a phenomenon that is well documented in literature (Landsberg 1981).

Zhang *et al.* (2004) concluded that urban heat island effects on plant phenology are stronger in North America than in Europe or Asia; a fact probably related to the dense and vertical urban design in North American cities (Zhang *et al.* 2004, Bonnan 2002). Tropical cities have been mainly neglected so far. Urban phenology studies are not only rare; phenological behaviour of tropical trees is also not satisfyingly understood yet (Borchert *et al.* 2002, Singh & Kushwaha 2005). The only existing study by Gazal *et al.* (2008) reported earlier bud burst dates in the tropical city Bangkok (-23 days), but not in the other analysed cities Korat (+9 days) and Dakar (+9 days). Besides, they documented a high variation in onset dates of phenological phases in the tropics.

Autumn phenology

Whereas spring and summer phenophases show an earlier onset in temperate cities compared to the countryside, autumn phenophases are reported to provide ambiguous results. Defila (1999) did not find any differences for autumn phenophases in urban and rural sites of Zurich, Switzerland.

Table 1.1 Overview of urban phenology studies. F = flowering, BB = budburst, LU = leaf unfolding, LC = leaf colouring, LF = leaf fall, PL = planting, H = harvest; negative values in the column "Main results" refer to an earlier urban flowering; positive values to an advance in the countryside.

Author(s)	**Year**	**Survey area**	**Observed plants**	**Period**	**Main results**
Baumgartner	1952	Munich, Germany	16 spring / summer phenophases	1952	max. intra-urban differences of -9 to -37 days
Franken	1955	Hamburg, Germany	*Forsythia suspensa* (F)		up to -17 days
Zacharias	1972	Berlin, Germany	*Tilia* x *euchlora* (F)	1967	up to -10 days
Baumgartner *et al.*	1984	Munich, Germany	*Aesculus hippocastanum* (LU, F), *Tilia platyphyllos* (LU, F), *Forsythia viridissima* (LU, F), *Cornus mas* (LU, F)	1984	considerable small-scale differences, representative for thermal differences
Karsten	1986	Mannheim, Germany	*Robinia pseudoacacia* (LU, F), *Forsythia suspensa* (F), *Platanus acerifolia* (LU, F)	1978-1983	distinctive small-scale differences, phenology matches the microclimatic structure
Koch	1986	Vienna, Austria	*Prunus avium* (F), *Malus domestica* (F), *Prunus armeniaca* (F), *Pyrus communis* (F)	1973-1982	-10 days to -13 days
Omoto & Aono	1990	several Japanese cities	*Prunus yedoensis* (F)	1965-1983	-0.2 days to -7.8 days
Bernhofer	1991	Vienna, Austria	*Forsythia suspensa* (F, LU)	1988	-16 days to -29 days
Rötzer & Sachweh	1995	Munich, Germany	*Galanthus nivalis* (F), *Forsythia* sp. (F), *Prunus avium* (F), *Malus domestica* (F)	1951-1990	-5 to -18 days
Rötzer	1996	Munich, Nuremberg, Augsburg, Regensburg, Germany	8 phenological phases	1951-1980	spring: -3.5 days to -7.5 days, autumn: +3.8 days
Aono	1997	Osaka, Kyoto, Kobe, Japan	*Prunus yedoensis* (F)	~1950-1990	-1.4 days (1950s), -5.4 days (1980s) (Kobe) -3 days to -5 days (Osaka 1989)
Rötzer *et al.*	1997	Munich, Nuremberg, Augsburg, Regensburg, Germany	8 phenological phases	1961-1990	-3.5 days to -8.0 days
Sachweh & Rötzer	1997	Munich, Nuremberg, Augsburg, Regensburg, Germany	*Galanthus nivalis* (F), *Forsythia* sp. (F), *Prunus avium* (F), *Malus domestica* (F)	1951-1990	remarkably strong trend for *Galanthus nivalis* in urban areas (-7.3 days / decade)
Defila	1999	Zurich, Switzerland	28 phenophases	1986-1995	-1 to -2 weeks
Rötzer *et al.*	2000	10 central European cities	*Galanthus nivalis* (F), *Forsythia* sp. (F), *Prunus avium* (F), *Malus domestica* (F)	1951-1995	-1.5 days to -4 days stronger trends in rural areas (1980-1995)
Baker *et al.*	2002	Phoenix, USA	*Gossypium* (PL, H)	1997-2000	-14 days (PL), -22 days (H)

Author(s)	**Year**	**Survey area**	**Observed plants**	**Period**	**Main results**
White *et al.*	2002	Eastern USA	ecosystem level (AVHRR NDVI)	1990-1999	vegetation period +7.6 days; start of vegetation -5.7 days
Defila & Clot	2003	Geneva and Liestal, Zurich	*Aesculus hippocastanum* (BB), *Prunus avium* (F)	1808-2002 1894-2002	stronger urban trend (-2.4 days / decade) compared to rural trend (-0.8 days / decade)
Fukuoka & Matsumoto	2003	Kumagaya City, Japan	*Prunus yedoensis* (F), *Ginkgo biloba* (LC*), Acer palmatum* (LC)	2000-2001	-2 days (*Prunus*), +2 to 3 weeks (*Ginkgo*), +7 to 10 days (*Acer*)
Lakatos & Gulyás	2003	Debreczin and Szegedin, Hungary	*Forsythia suspensa* (F)	2003	-4 days to -8 days
Ziska *et al.*	2003	Baltimore, USA	*Ambrosia artemisiifolia* (F)	2000-2001	-3 days to -4 days
Primack *et al.*	2004	Boston, USA	372 records of herbarium specimen (37 genera)	1885-2003	more visible trend towards earlier flowering due to urban heat island effect
Zhang *et al.*	2004	North America, Europe, Asia	ecosystem level (MODIS data)	2001	start of vegetation 4-9 days earlier, end 2-16 days later (North America)
Fisher *et al.*	2006	Providence, Eastern USA	ecosystem level (Landsat TM, ETM+)	1984-2002	-5 days to -7 days earlier start of vegetation
Henniges & Chmielewski	2006	Berlin, Germany	11 spring phenophases	2006	-0.1 days to -7.1 days
Lavoie & Lachance	2006	Montreal, Quebec City, Canada	*Tussilago farfara* (F)	1918-2003	earlier flowering only noticeable in large urban areas
Lu *et al.*	2006	Beijing, China	*Prunus davidiana* (F), *Prunus armeniaca* (F), *Robinia pseudoacacia* (F), *Syringa oblata* (F)	1950-2004	-1.5 to -2.9 days / decade
Luo *et al.*	2007	Beijing, China	*Prunus davidiana*, *Hibiscus syriacus*, *Cercis chinensis* (all BB, F, LC, LF)	1962-2004	pronounced changes with time in response to climate change and UHI
Rötzer	2007	IPG, Europe	different tree and shrub species	1961-1998	-2.1 days (LU), -4.8 days (F), +4.8 days (LC), +5.4 days (LF)
Gazal *et al.*	2008	7 cities in Asia, Europe, Africa, North America	7 species (BB)	2005	-23 days to +9 days
Matsumoto *et al.*	2003	Tokyo, Japan	*Prunus yedoensis* (F)	2004	-5 days to -6 days
Mimet *et al.*	2009	Rennes, France	*Platanus acerifolia* (BB, F), *Prunus cerasus* (BB, F)	2005	urban-rural gradient in onset dates, influential factors: DTR, Tmin, ground cover types
Shustack *et al*.	2009	Central Ohio, USA	*Aesculus glabra* (LU), *Lonicera maackii* (LU), *Acer negundo* (LU)	2006	abundance of exotic species caused phenological urban-rural differences
Jeong *et al.*	2011	9 cities in South Korea	*Forsythia koreana, Rhododendron mucronulatum, Prunus yedoensis*, *Prunus persica (F)*	1954-2004	degree of advancement is roughly proportional to degree of urbanisation

Rötzer (2006) reported an advance of leaf colouring of horse chestnut in Bavarian cities by almost 4 days. IPG data, however, revealed that densely populated areas exhibited a delay of ca. 5 days for leaf colouring and leaf fall, respectively (Rötzer 2007). Fukuoka & Matsumoto (2003) found a two to three weeks later leaf colouring of gingko and a 7 to 10 days later leaf colouring of Japanese maple in urban areas of Japan.

Less densely built areas are linked to a delay in autumn onset dates due to temperature increase; plants in extreme densely built areas, however, have to experience stressful situations (increased pollution, soil water deficit) and react with earlier leaf fall (Rötzer 2007). The latter is also in accordance with Ziska *et al.* (2003) who reported earlier senescence for common ragweed in urban Baltimore, USA.

Vegetation period

Recent urban studies have not only applied *in situ* field measurements - referring to the species level - but also satellite derived data - referring to the ecosystem level. Implications of urban climate on the length of the vegetation period were assessed for the eastern United States by several authors. White et al. (2002) reported a prolongation of the vegetation period by ca. 8 days in urban areas compared to deciduous broadleaf forests in rural areas; the earlier greenup (ca. -6 days) contributed most to this difference. In addition, Zhang *et al.* (2004) demonstrated a prolongation of the vegetation period of about 15 days, whereas the start was recorded 7 days earlier in cities. According to Fisher *et al.* (2006) the vegetation period started 5-6 days earlier in urban areas of Providence.

However, differences in greenup across large areas should be interpreted with caution. Shustack *et al.* (2009) analysed leaf phenology of trees in 11 forests spanning an urban-rural gradient and found that predominately the abundance of exotic species promoted earlier greenup in urban forests.

(b) Assessment of climate change impacts on plants

Cities represent important study areas since their urban heat island effect and therefore higher temperature conditions allow an assessment of possible impacts of future climate change on plant development. Specifically, urban areas are seen as a proxy showing conditions that are expected for more rural environments in the near future (Ziska *et al.* 2003, Zhang *et al.* 2004, Luo *et al.* 2007, Mimet *et al.* 2009), since the magnitude of the UHI in many cities is comparable to future projected warming at the global or regional scale (1.8 to 4.0 °C, IPCC 2007a) (Baker *et al.* 2002). Therefore, phenological differences between the city and the countryside cannot only be used for the temporal and spatial analysis of the urban heat island effect; these differences also provide evidence regarding how phenology will respond to future temperature increase and to alterations of other environmental conditions typical for urban climate (see. 1.1.2).

The majority of studies on climate change impacts on phenology focused on non-urban ecosystems (Neil & Wu 2006). Here, it is necessary to distinguish between the effects of local urban warming and the more general characteristics of global climate change (Primack *et al.* 2009). The approach to use urban-rural gradients as an empirical analogue (Beggs 2004) is an excellent and low-cost alternative to open-top chambers, climate chambers and e.g., free-air CO_2 enrichments (Ziska *et al.* 2003).

Moreover, understanding the impact of the anthropogenic urban heat island effect on phenology allows a better assessment of the effect of climate change *per se* and counteracts incorrect interpretations of phenological data and trends (Defila 1999).

Temporal urban and rural trends

The analysis of temporal trends is a simple way to assess the consequences of climate change on phenology. Besides, the comparison of urban and rural trends enables a precise evaluation of phenological changes under different temperature conditions. Rötzer (1995) found more pronounced trends (1951-1990) in urban areas of Munich by analysing flowering data of snowdrop, forsythia, sweet cherry and apple - however only statistically significant for snowdrop. In addition, herbarium-based analyses by Primack *et al.* (2004) and Lavoie & Lachance (2006) demonstrated trends towards earlier flowering due to the urban heat island effect in Boston, USA, and noticeable earlier flowering dates in large urban areas of Montreal and Quebec City, Canada, respectively. Also Defila (1999), Defila & Clot (2003) and Rötzer & Sachweh (1995) detected stronger trends in urban phenological time series in Switzerland and Germany. In contrast, Rötzer *et al.* (2000) reported that trends (1980-1995) of spring phases were stronger in the countryside compared to the city (see also Table. 1.1).

Rötzer *et al.* (2000) argued that differences in trends are related to differences in urbanisation rates. Whether the trends are stronger in urban or rural areas may be determined by the degree of urban development, e.g., construction of buildings. In accordance, Joeng *et al.* (2011) revealed that the magnitude of advancement in spring phenology is roughly proportional to the degree of urbanisation in South Korea. The authors also reported more pronounced advances in spring phenology compared to Europe (Rötzer *et al.* 2000) and attributed this finding to a more severe urbanisation. Therefore, the socio-economic background is a major factor in interpreting phenological time series.

Some authors (e.g., Lu *et al.* 2006, Luo *et al.* 2007) only analysed phenological time series of urban areas but did not compare these results with rural stations. Therefore, temporal trends reported in these studies are the consequence of both large-scale climate warming and the effect of urban heat islands. Jeong *et al.* (2011), for example, did not include data of the respective countryside, but incorporated nine differently urbanised cities in South Korea to assess changes depending on the degree of urbanisation.

(c) Allergy and human health

Aeroallergens constitute an important human health issue. Climate change related effects could already be observed with respect to the pollen amount, pollen allergenicity, timing and duration of the pollen season, plant and pollen distribution (e.g., reviewed by Beggs 2004) and episodes of long-range transport (D’Amato & Cecchi 2008). Urban areas do not only offer a glance at future pollen related problems (Ziska *et al.* 2003). Several studies have demonstrated that urban dwellers (especially children) are predominately influenced by pollinosis incidence (e.g., Riedler *et al.* 2000, Bibi *et al.* 2002), probably due to westernised lifestyle, urbanisation-induced temperature increase and abundance of air pollutants (Braun-Fahrlander *et al.* 1999, D’Amato 2000, Ring *et al.* 2001).

Pollen season. Start and duration of the pollen season have already changed. With global climate change and local urban warming advancing phenology, an earlier start of the pollen season (and therefore onset time of pollinosis) has been reported (Emberlin *et al.* 1997, van Vliet *et al.* 2002, Ziska *et al.* 2003). A prolongation of the pollen season was mainly found for summer and late flowering species, especially for grass species (Emberlin 1994).

Pollen amount. Experimental studies (Ziska & Caulfield 2000, Ziska *et al.* 2003) revealed that the CO_2 concentration as well as temperature is positively correlated with the amount of pollen produced by common ragweed. Besides, studies based on long-term data of pollen traps underlined the fact that pollen amounts increased over time - probably as a result of temperature increase (e.g., Frei 1998, Rasmussen 2002, Frei & Gassner 2008).

Pollen allergenicity. Findings on changes in pollen allergenicity are contrasting. According to Ahlholm *et al.* (1998) and Hjelroos *et al.* (1995) Bet v 1, the major birch allergen, is elevated with higher temperatures. Conversely, Ziska *et al.* (2003) and Guedes *et al.* (2009) reported decreased allergen content of common ragweed and white goosefoot, respectively, under warmer conditions.

Air pollution. In polluted regions, especially in urban areas, airborne pollen does not merely constitute an allergen carrier; it also interacts with particulate air pollutants enhancing protein and allergen release and changing the morphology of the pollen surface (Behrendt & Becker 2001, Traidl-Hoffmann *et al.* 2003, Guedes *et al.* 2009). The interaction between particulate matter and pollen generates allergenic aerosols (Behrendt & Becker 2001) that can reach the peripheral airways and prompt asthma in sensitised individuals (D'Amato *et al.* 2007).

(d) Assessment of vegetation feedbacks

Divergences in flowering times between urban and rural areas may lead to reproductive isolation; especially with respect to plants that have a short flowering duration. A condensed or missing overlap of the flowering period of entomophilous plants may impact the sharing of pollinators and the time of pollen availability (Primack *et al.* 2009). Different responses of plant phenology between urban and rural environments could block or restrict gene flow among meta-population and meta-community in rural-urban transects. In addition, these different responses are also likely to accelerate species polarisation (Neil & Wu 2006).

Moreover, plant phenology also influences meteorology on the mesoscale *via* partitioning net radiation into sensible and latent fluxes (Fitzjarrald *et al.* 2001, Schwartz & Crawford 2001). A lengthening of the vegetation periods in urban areas (White *et al.* 2002, Zhang *et al.* 2004, Fisher *et al.* 2006) is linked to changes in evapotranspiration and albedo and will therefore affect near-surface climate (Jeong *et al.* 2011). An extended vegetation period also implies a longer time for the plants to be active, likely involving an increase in annual CO_2 uptake (Keeling et al. 1996). In addition, the effective deposition of e.g., O_3 and particulate matter constitutes a positive impact, especially during leaf season (Nowak *et al.* 2000, McDonald *et al.* 2007); however, the emission of allergenic pollen (D'Amato 2010) and biogenic volatile organic compounds (BVOC) involved in O_3 formation (Cortinovis *et al.* 2005, Simpson & McPherson 2011) are regarded as negative effects.

1.3 Background and objectives of research

The research conducted in this publication based PhD thesis was supported by the Deutsche Forschungsgemeinschaft (DFG, grant ME 179/3-1), the Bavarian State Ministry of the Environment and Public Health (StMUG, grant U-119) and partly by the EUROPA MÖBEL-Umweltstiftung. In this interdisciplinary project, we closely collaborated with the Center of Allergy and Environment (ZAUM) of the Technische Universität München. The research conducted in Brazil was supported by CAPES/PROBAL (360/11) and DAAD (German Academic Exchange Service, 50752579).

This PhD project had several aims. First, the factors that have an influence on phenological onset dates in temperate and tropical plant species were examined. Special regard was given to urban areas, since they reproduce the conditions and plant performances that are likely to occur in more rural environments in the near future due to climate change. Second, we tested whether regional or local temperature patterns (urban heat island effect) can be assessed using phenological data. Third, this study predominantly focused on allergenic plants and the alteration of their flowering behaviour and allergenicity. In the following section, a brief description of study design and data collected prepares the way for a detailed explanation of the objectives of this PhD thesis.

1.3.1 Data and study design

Phenology. Phenological observations were conducted within three consecutive years from spring 2009 to autumn 2011 in four different regions: along urbanisation gradients in Munich, Ingolstadt (Germany), and Campinas (Brazil) as well as along an altitudinal gradient in the Zugspitze region (Germany, Austria). To ensure consistent methods, observations were based on the BBCH-code (Meier 2001). The research focused on flowering or / and on leaf unfolding of allergenic temperate trees, tropical trees, and on allergenic grass species: *Betula pendula* Roth (silver birch), *Aesculus hippocastanum* L. (horse chestnut), *Tabebuia chrysotricha* (Mart. Ex DC.) Standl. (yellow trumpet tree), *Caesalpinia peltophoroides* Benth. (false Brazilwood), *Tipuana tipu* (Benth.) Kuntze (racehorse tree), *Dactylus glomerata* L. (cocksfoot) and *Alopecurus pratensis* L. (meadow foxtail). Moreover, this study is also based on extensive, standardised, terrestrial, species-specific phenological observations of the German Meteorological Service (DWD). Data for the period 1995-2008 for beginning of flowering of *Forsythia suspensa* (Thunb.) Vahl (forsythia) was utilised for Southern Bavaria, Germany. In addition, the study incorporated DWD data for the period 1951-2008 for spring phenophases of *Corylus avellana* L. (hazel), *Galanthus nivalis* L. (snowdrop), *Salix caprea* L. (goat willow), *Forsythia suspensa*, *Acer platanoides* L. (Norway maple), *Fagus sylvatica* L. (beech), *Malus domestica* Borkh. (apple), *Picea abies* (L.) Karst. (spruce) and *Syringa vulgaris* L. (lilac) located in the greater areas of Munich, Cologne and Frankfurt (Germany).

Meteorology and air pollutants. Meteorological data derived either from national meteorological services (DWD and IAC [Instituto Agronômico]) or from the installed broad-scale networks in Munich (2010-2011, N = 38) and Campinas (2011, N = 31) using air temperature and air humidity loggers (HOBO U23-001, Onset Computer Corporation, Southern MA, USA). Data of O_3 and NO_2 in spring 2010 was collected using passive samplers for seven days. The passive samplers then were photometrically analysed for NO_2 adsorption; O_3 samplers were analysed by Passam AG.

Pollen. Pollen in the Zugspitze region (2008-2009) was sampled with three Burkard volumetric traps (Hirst 1952) recording the number of pollen per cubic meter of air. Birch catkins from 40 trees at urban and rural sites in the Munich study area were collected in 2010. The isolated pollen was analysed for Bet v 1, $\mathrm{PALM_{LTB4}}$, $\mathrm{PALM_{PGE2}}$ and adenosine, monocyte-derived dendritic cells were isolated and cultured, and neutrophil migration assays were performed.

Foliar nutrients. Furthermore, foliar nutrient concentration of birch in Munich in 2010 was analysed using ICP-OES (Spectro, Kleve, Germany) and an elemental analyzer (Elementar, Hanau, Germany).

Land use and elevation data. Land use data were derived from the seamless vector database CORINE Land Cover, a product from the European Environment Agency (EEA 2000) and from the Brazilian National Institute for Space Research (INPE, Vieira *et al.* 2010). Additionally, the SRTM 90-m digital elevation model (DEM) (Jarvis *et al.* 2006) was used.

Statistical analyses and maps. Statistical analyses were conducted using R 2.9.2, SPSS 19.0 and SAS 9.2. The applied analyses included Kolmogorov-Smirnov-test, Pearson’s and Spearman’s correlation analyses, partial correlation analyses, linear regression and multiple linear regressions, Levene’s test, t-test, Mann-Whitney U-test, analysis of covariance (ANCOVA) and discriminant analysis. All maps and geostatistical analyses were produced using ArcGIS 9.3 and 10. The research incorporated *inter alia* a novel GIS-based regionalisation technique based on the lowest-method and the calculation of the urban index, a variable describing the degree of urbanisation.

1.3.2 Research questions

Within this PhD thesis the following major research questions will be addressed:

a) Which factors other than temperature have also an influence on phenology?
b) How adequate are phenological data to estimate local / regional temperature patterns?
c) What are the effects of climate change / urbanisation on pollen?

The next section concentrates on these questions by pointing out the research that already exists as well as their limitations. In order to identify research gaps, important topics that are still insufficiently explored will be addressed.

1.3.2.1 Factors influencing phenology

According to Defila & Clot (2001) different phenophases and plant species react differently to various environmental parameters. This statement seems to be contradictory to the common perception that temperate phenology is mainly influenced by air temperature (Schnelle 1955, Menzel & Fabian 1999, Wielgolaski 1999). This study explores a range of influential factors both in temperate and tropical phenology, clarifying and better elucidating the environmental responses of phenology.

Temperature. Air temperature is the most important environmental cue in many temperate and boreal regions (Schnelle 1955, Wielgolaski 1999) as well as in the temperate monsoon climate of China (Lu *et al.* 2006). This meteorological parameter is able to explain a huge amount of the variance in phenological onset dates from year to year (Menzel & Fabian 1999). In contrast, phenology of tropical trees is commonly considered to be water or light limited and yet the role of temperature is uncertain (Morellato *et al.* 2000, Borchert *et al.* 2002; Singh & Kushwaha 2005). In general, temperature does not always have the necessary resolution at the meso- and microscale, and interpolation methods often fail to lead to proper results (Ashcroft 2006). Moreover, we do not know much about the error we receive when using only temperature data of climate stations nearest to the phenological observation site. Therefore, the following approaches were pursued within this study:

- Measurement of air temperature in the immediate surrounding of the observed trees - directly on the stem.
- Exploration of the suitability of environmental variables that have a direct effect on temperature and therefore also on phenology, i.e., land use (mainly urbanisation) and altitude.

This study incorporated temperature data of a broad-scale observation network characterised by different degrees of urbanisation within the greater area of Munich and Campinas. Air temperature was measured using loggers directly on site. The following topics will be addressed:

- Which amount of variance in phenological onset dates of birch in Munich can by explained by mean temperature of the previous month(s)?
- Is there a difference in model fit when regarding only one observation year or two observation years?
- Are meteorological parameters related to air temperature also valuable explanatory variables in tropical phenology?

Land cover. Zhang *et al.* (2004) analysed satellite derived data from the northern hemisphere between 35° N and 70° N and investigated the response of vegetation phenology to temperature for several key land cover types. The authors found that spatial variation in phenology is associated with land cover type. These finding prompted to further investigate particular elements of land cover and their influence on phenology. Following issues are of major importance:

- Which land use variables have an influence on temperature and hence on phenology?
- Are these factors able to explain differences in phenology at the mesoscale?

Urban index. Phenological research is often limited by scarce observation sites within urban areas in existing phenological networks. Some urban phenology studies even dealt only with stations that are situated outside the urban core area (Rötzer 1995, Koch 1986). Moreover, the city as a whole is often compared with rural sites, neglecting any intra-urban variations. A plain urban-rural dichotomy may induce unexplainable and inconsistent results of differences in plant phenology. This study wants to account for the variations of climatic conditions related to urbanisation and address the topics:

- Does a continuous variable describing the degree of urbanisation improve phenological modelling and counteract the problems described above?
- Is there a correlation between the degree of urbanisation, phenology and temperature?
- If yes, is the urban index a good estimate for local temperature conditions?

Altitude. There are various variables that change with altitude. However, regarding phenology, the most important variable is air temperature that changes within a relatively short distance (-0.6 °C $(100\ \mathrm{m})^{-1}$, Barry 1981). Investigations of phenological behaviour in mountainous regions will increase the understanding of temperature responses and the ecological impacts of global change (Defila & Clot 2005; Ziello *et al.* 2009). Besides, urban agglomerations are often located in river valleys characterised by lower altitudes than their adjacent countryside. Hence, the interpretation of urban phenological time series is often impeded by altitudinal influences. This makes it necessary to include altitudinal effects in phenological models and to address the following issues:

- Is there an influence of altitude on phenology and are altitudinal gradients species' specific?
- Are plants in the mountains more sensitive to temperature and therefore show stronger responses to temperature increase associated with climate change?
- Are the temperature response rates in phenology calculated using temperature lapse rates similar to thermal responses reported in existing studies?

Aspect. Aspect is a further characteristic of mountainous regions contributing to substantial temperature differences (Scherrer *et al.* 2011) and therefore differences in phenology (Chen 1994). Subsequently, there is a need to address these topics:

- How large is the influence of aspect on flowering and leaf unfolding of birch?
- Is the influence of aspect on phenology even greater than altitudinal effects?

Further phenological triggers. Beside temperature, there exists an array of external and internal factors influencing phenology ranging from photoperiod (Leopold 1951, Levy & Dean 1998, Schaber & Badeck 2003), precipitation (Rathcke & Lacey 1985, Peñuelas *et al.* 2004, Wielgolaski 2001), chemical and physical soil characteristics (Wielgolaski 2001), diseases, pests, competition, and pollutants (Cape 2003, Honour *et al.* 2009) to individual genes (Baumgartner 1952), age (Rosenzweig *et al.* 2008), and size (Seiwa 1999, Augspurger & Bartlett 2003, Tomita & Seiwa 2004).

Air humidity. Precipitation can be an important trigger in plant phenology. This was found along a coastal-continental gradient in western Norway (Wielgolaski 2001), in seasonal tropical forests (Rathcke & Lacey 1985) and in Mediterranean forests (Peñuelas *et al.* 2004). Additionally, phenology in tropical trees is considered to be water limited (Borchert *et al.* 2002, Singh & Kushwaha 2005). The only study dealing with tropical urban phenology (Gazal *et al.* 2008) indicated the importance of humidity. Therefore, air humidity was measured at the observed trees in the study sites of Munich and Campinas to concentrate on these aspects:

- Does air humidity related variables (relative and absolute humidity) influence phenological onset dates in tropical urban phenology?
- Is there an effect of humidity on temperate urban phenology?

Foliar nutrient status. Existing studies incorporating nutrient analyses are predominantly related to agriculture, horticulture or forest science (Steiner *et al.* 2007, Yang *et al.* 2011, Weih & Karlsson 2001). Phenological studies often only analyse soil nutrition, either by manipulation experiments using fertilisers, or by the actual evaluation of the availability of a small number of nutrients in the soil (Wielgolaski 2001). Soil and air temperature have an effect on the activity of soil microorganisms, nutrient mineralisation, nutrient uptake and translocation rates (White & Haydock 1970, Fisher 1980, Marschner 1995). Conventional soil analyses do not account for the heterogeneous soil conditions, especially prevalent in urban areas. In addition, impervious surface in cities might entirely obstruct soil analyses. Therefore, it is reasonable to analyse foliar nutrient concentration that may be more supportive for the assessment of environmental conditions plants are exposed to and address these topics:

- Which nutrients are important for plant development of birch?
- Which amount of variance in onset dates can by explained by foliar nutrient concentration?
- Are there hints that foliar nutrient analysis is more adequate in phenological models than soil nutrition?

1.3.2.2 Estimation of temperature patterns

Regional scale. Temperature distribution is not uniform within regional scales but experiences alterations mostly by land cover (see previous sections). The identification of thermal hot spots and their influential factors at the regional scale is a basic requirement for further analyses at the local scale. Common methods for the regionalisation of phenological data utilise regression methods (Rötzer & Chmielewski 2001). Hence, this study explores:

- Can a new statistical approach be introduced in phenological research that uses the analytical result of the discriminant analysis to conduct a GIS-based regionalisation based on point wise phenological data at the regional scale?
- Is the application of land use data in phenological models a cheap and appropriate alternative for direct temperature measurements or inadequate interpolation methods?

Local scale. Existing literature (see Table 1.1) revealed phenological differences between the city and the countryside. However, inconstant findings provoke broad-scale analyses of urban-rural phenology that include a large number of observation sites along a natural temperature gradient provided by different degrees of urbanisation. The following topics are still insufficiently answered and need to be addressed:

- Are the observed urban-rural differences not only species-specific, but do they also vary from year to year?
- What are the consequences of extreme temperatures in spring on differences between urban and rural phenology?
- Is the temperature difference between the city and the countryside (UHI) a good explanatory variable for urban-rural differences in phenology?
- Which pattern in urban-rural phenology can be observed in tropical cities?

1.3.2.3 Effects of climate change and urbanisation on pollen

Most of the existing urban phenology studies are descriptive and failed to demonstrate the consequences and relevance of their findings. Therefore, also Neil & Wu (2006) claimed: “It is time that we move beyond descriptive studies that show differences in phenology between urban areas and surrounding rural environments.” Allergies are an important human health related issue. The estimated prevalence rate of pollen allergy in Europe amounts to 45 % among young adults (PID 2010). Start and length of the pollen season are considered to change with climate change and urbanisation. Since the severity of allergic diseases is additionally influenced by pollen amount and allergenicity (see also 1.2.3), it is essential to focus on a wide range of impact factors and changes associated with pollen.

Phenological observations of allergenic plants. Phenological observations of allergenic plants are indispensable for our understanding of potential future threats and the assessment of future impacts of climate change on the development and severity of allergies. There are several interesting aspects that will be addressed in this interdisciplinary study:

- How do flowering times (and therefore the onset of allergic diseases) change?
- Is there a prolongation of the flowering and pollen season?
- Which factors have an influence on the features of the flowering and pollen season?
- Do higher elevated regions in the Alpine region (and rural areas) show less airborne pollen and are therefore more beneficial for pollen allergic people?
- Can the pollen season of major allergenic species be adequately predicted by phenological observations?

Quantitative assessments of pollen production and pollen allergenicity. Urbanisation and climate change may alter pollen production or allergenicity. Their assessments allow profound conclusions on the severity of clinical symptoms. There have been some studies incorporating laboratory experiments, however, *in situ* data using urban-rural gradients as an experimental surrogate for climate change are rare (Ziska *et al.* 2003, Bryce *et al.* 2010) and also reported different findings, especially in terms of pollen allergenicity. Surprisingly, to date there exist no definition of *pollen allergenicity* (Behrendt *et al.* 2007). Birch pollen, however, does not only contain allergens (e.g., Bet v 1) but also bioactive substances such as pollen associated lipid mediators (PALMs) that exhibit a structural and functional similarity to leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) (Traidl-Hoffmann *et al.* 2003) and adenosine, recently identified as a potent immunregulatory substance (Gilles *et al.* 2011). The field study in Munich enabled the analysis of relatively modest gradients of temperature and pollutant concentrations and allows addressing the following questions:

- Are the effects of urbanisation and climate change on pollen amount and allergenicity not straightforward?
- What kind of differences in these pollen associated characteristics can be found?
- What are the possible environmental factors that contribute to these variations?

Functional and physiological relevance assessed by in vitro* and *in vivo experiments. *In vitro* experiments (e.g., cell assays) and *in vivo* experiments (e.g., prick tests) might support the functional and physiological / clinical relevance of the experimental findings derived from this interdisciplinary study.

- Do *in vitro* and *in vivo* experiments support the experimental findings?
- Which findings related to neutrophil chemotaxis can be observed?
- Does high- and low-ozone exposed pollen show different skin reactions?

1.4 Outline of thesis

After Chapter 1 with a general overview of global and local climate change, a short summary of observed changes on phenology, with a complete and comprehensive review of urban phenology, several research papers follow that further contribute to the understanding of urban phenology related to climate change and human health.

In total, research results of seven scientific papers; six with first authorship and one with co-authorship are included. Four of the papers are already peer-reviewed with three of them published (see Chapter 11).

Chapter 2, 3, 4, 5, 7 mainly relate to the research question a) (Which factors besides temperature also have an influence on phenology?), Chapter 2, 3, 4, 5, 6 to b) (How adequate are phenological data to estimate local / regional temperature patterns?) and Chapter 6, 7 and 8 to c) (What are the effects of climate change / urbanisation on pollen?).

The first publication (Chapter 2) "**Urban heat islands in tropical and temperate regions assessed by phenology**" (Jochner *et al.* 2012, submitted to Journal of Applied Ecology) examines whether differences in air temperature and humidity at the local scale can influence phenological onset dates in a temperate mid-latitude city (Munich) and a neotropical city (Campinas). We identify whether the two cities show distinctive urban heat island effects and explore the suitability of the selected phenophases and species to detect temperature variations. Dissimilarities and similarities were identified by an equivalent study design that is based on consistent phenological observation methods according to common practice (BBCH) and site-specific meteorological data.

The second publication (Chapter 3) "**Nutrient status - a missing factor in phenological research?**" (Jochner *et al.* 2012, submitted to Global Change Biology) deals with phenological onset dates of birch phenophases in the greater area of Munich. We do not only apply temperature data measured in the close proximity to the birches - directly on the stem - but also leaf nutrient concentration. We explore how representative birch phenophases detect differences in temperature at the local scale. Additionally, we examine the effect of nutrient concentration since this environmental variable has been neglected so far in phenological research.

The third publication (Chapter 4) "**The integration of plant phenology and land use data to create a GIS-assisted bioclimatic characterisation of Bavaria**" (Jochner *et al.* 2011, Plant Ecology and Diversity) uses a new statistical approach in phenological research. We apply discriminant analysis to determine important land use variables that have an influence on temperature and therefore on flowering onset of forsythia in Southern Bavaria, Germany. The analytical result of the discriminant analysis is used to conduct a GIS-based regionalisation beyond the known values of phenological onset dates at the regional scale. This bioclimatic characterisation illustrates differences in flowering dates and also regions that can be interpreted as "thermal hot spots" where flowering occurs first.

The fourth publication (Chapter 5) "**The influence of altitude and urbanisation on trends and mean dates in phenology (1980-2009)**" (Jochner *et al.* 2012, International Journal of Biometeorology) is based on phenological observations derived from the DWD and addresses urbanisation effects on plant development for three different study areas - Frankfurt, Cologne and Munich - and nine different phenological spring phases. We introduce a continuous variable (the urban index) to describe the degree of urbanisation and do not use a plain classification into "urban" and "rural". We do not only describe trends and mean dates during 1980-2009 but also apply multiple regression analyses incorporating urbanisation and altitudinal effects (both factors that have an influence on temperature and therefore on phenology) in order to how mean onset dates and their temporal trends are influenced by altitudinal or urbanisation effects.

The fifth publication (Chapter 6) “**Effects of extreme spring temperatures on urban phenology and pollen production: a case study in Munich and Ingolstadt**” (Jochner *et al.* 2011, Climate Research) incorporates first data of the vast field study in Munich and Ingolstadt. In 2009 the second highest April temperatures since the beginning of instrumental measurements were recorded in Munich. The effects of extreme spring temperatures on urban phenology have not been studied in detail so far. Therefore, the weather conditions of April 2009 were predestined for analysing the impacts of extreme temperatures in spring on differences in urban-rural phenology and variations in birch pollen amount. The utilisation of long-term phenological and meteorological data of the DWD allows further insights in the phenological behaviour of three different plants in the greater area of Munich over time and its meteorological influences.

The sixth publication (Chapter 7) “**Spatio-temporal investigation of flowering dates and pollen counts in the topographically complex Zugspitze area on the German-Austrian border**” (Jochner *et al.* 2012, Aerobiologia) uses a detailed regional set of phenological data obtained from a field survey in 2009 in the Alpine region. We examine flowering behaviour of the major allergenic species birch, cocksfoot and meadow foxtail along an altitudinal gradient (700 to 1700 m) and additionally evaluate aerobiological and meteorological data for different altitudinal levels for 2008 and 2009. We do not only address the question whether the pollen season of the analysed allergenic species can adequately predicted by phenological observations but also examine whether temperature and wind conditions alter the characteristics of the pollen season on different altitudes. Since mountain sites are regarded as retreats for pollen allergic people we examine whether this suggestion is straightforward or not.

The seventh publication (Chapter 8) “**Ozone impacts on the allergenicity of birch pollen**” (Beck, Jochner *et al.* 2012, in preparation for Nature) is focused on a quantitative assessment of pollen allergenicity of birch in Munich and its rural surroundings. This field study enables the analysis of relatively modest gradients of temperature and pollutant concentrations. We examine differences in pollen associated characteristics and relate them to environmental factors such as temperature, urban index, NO_2 and O_3. Allergenicity is not only assessed by the major birch allergen Bet v 1, but also by the amount of PALMs (LTB_4 and PGE_2) and adenosine. Cell assays demonstrate immune stimulatory and modulatory effects on the cells of the immune system. To further assess the physiological relevance of the measured allergen content we apply *in vivo* experiments to answer the question whether pollen from urban and rural birch trees shows differences in the human cutaneous immune response.

The general and summarising discussion (Chapter 9) highlights the significance of the results according to the emerging research questions and places them in the context of other work. All references are pooled in Chapter 10. The publications and the candidate’s individual contribution are listed in Chapter 11.

2 URBAN HEAT ISLANDS IN TROPICAL AND TEMPERATE REGIONS ASSESSED BY PHENOLOGY

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Abstract

1. Urban phenology is relevant for assessing heat islands effects and potential impacts of climate change on plants. Temperature is the main factor regulating plant development in moist / temperate regions. However, plants in tropical cities may respond to additional environmental cues such as water availability.

2. We examined whether differences in air temperature and humidity within an urban-rural gradient influence phenological onset dates of trees in a temperate mid-latitude city (Munich, Germany) and a city in the neotropics (Campinas, Brazil). (Dis-)similarities were identified by an equivalent study design incorporating consistent phenological observation methods and site-specific air temperature and humidity data.

3. We found that phenological onset dates for flowering and leaf unfolding of silver birch *Betula pendula* were suitable to describe the urban heat island of Munich. Correlation coefficients of mean temperature and onset dates of birch were significant and varied between -0.48 and -0.72.

4. However, phenological onset dates for yellow trumpet tree *Tabebuia chrysotricha*, false Brazilwood *Caesalpinia pluviosa* and rosewood *Tipuana tipu* in Campinas had greater standard deviations than temperate trees, and the magnitude and significance of correlation coefficients with temperature varied greatly among species and phenophases. Nevertheless, we found statistically significant relationships, especially for flowering and leaf unfolding phenophases of *Tipuana* and air temperature variables and for leaf unfolding phenophases of *Tabebuia* and humidity variables.

5. *Synthesis and applications*. Our findings revealed that the phenology of *Tipuana* was sensitive enough to detect urban heat island effects in the tropical city of Campinas and might therefore be a useful indicator of local temperature variations and in turn of global warming. Since *Tipuana* is widely used for urban arborisation in South America, we foresee many applications for monitoring heat island effects in the neotropics. Furthermore, the fact that most phenophases of *Caesalpina* and *Tabebuia* were not responsive to temperature increase has stressed the importance of humidity variables. We recommend the consideration of humidity to understand urban effects in tropical cities under climates with distinctive dry and wet seasons. Additional species and phenophases should be explored to identify the further potential of phenology for monitoring heat islands in tropical cities.

Keywords. BBCH, Brazil, Campinas, flowering, Germany, humidity, leaf unfolding, Munich, temperature, urban climate.

2.1 Introduction

Since Luke Howard (1833) published the first study about the urban heat island (UHI) in London, this phenomenon has gained growing interest among meteorologists (Kratzer 1937; Sundborg 1951; Parry 1956; Chandler 1964; Oke 1976; Landsberg 1981). The examination of the urban heat island effect and associated aspects is still relevant and indispensable, especially because an increasing percentage of the world population - particularly in the tropics - live in (mega-)cities (Grimm *et al.* 2008; DeFries *et al.* 2010) or are regular visitors to urban areas for *inter alia* occupational, social or recreational reasons.

The major cause of UHI development is the substitution of the natural environment through built up and sealed areas (Landsberg 1981). The material used in cities (e.g. concrete, asphalt) has a lower albedo and does not allow water to penetrate into the soil. Consequently, a high proportion of the incident shortwave radiation is absorbed and transformed into sensible heat (Landsberg 1981). Particularly the three-dimensional urban design with tall multistorey buildings, high building density and narrow streets promotes a high fraction of radiation being absorbed (Oke 1987; Landsberg 1981). In contrast, vegetated urban areas represent cooler spots through evaporation processes (Shustack *et al.* 2009). Another factor that contributes to the UHI is the anthropogenic emission of sensible heat as well as moisture associated with energy consumption (Sailor 2011). In addition, urban air pollutants affect urban energy fluxes (Landsberg 1981): Particulates scatter and absorb the incoming solar radiation leading to a reduction of global radiation and a higher amount of diffuse radiation; besides, absorption and reemission by infrared active gases and aerosols increases downward longwave atmospheric radiation.

In phenological research, cities represent important study areas since their warmer conditions allow assessing potential future impacts of climate change on plant development. Therefore, urban areas can be used as a surrogate or experimental treatment for future global warming (Ziska *et al.* 2003; Luo *et al.* 2007; Mimet *et al.* 2009). This application is related to one of the original purposes of urban phenology: detecting urban heat island effects. Phenological observations in urban areas for assessing microclimatic conditions have a long tradition in temperate regions. There are plenty of studies dealing with plant development in urban areas of Europe (e.g. Franken 1955; Zacharias 1972; Baumgartner *et al.* 1984; Karsten 1986; Bernhofer 1991; Lakatos & Gulyás 2003; Mimet *et al.* 2009; Jochner *et al.* 2011, 2012a), North America (e.g. White *et al.* 2002; Zhang *et al.* 2004; Fisher *et al.* 2006) and Asia (e.g. Omoto & Aono 1980; Lu *et al.* 2006; Luo *et al.* 2007; Jeong *et al.* 2011).

These studies clearly support that plants growing in temperate cities flower earlier than plants in rural areas due to higher local temperatures. However, urban phenology studies in tropical cities are rare (e.g. Gazal *et al.* 2008) and mostly absent in the neotropics. There is still a lack of understanding in how the phenology of tropical tree species is influenced by temperature, e.g., whether there is a temperature threshold for plant activity under warm tropical climates (Clark 2007; Cowell *et al.* 2008). Moreover, phenology in tropical trees is generally considered to be water or light limited (Morellato *et al.* 2000; Borchert *et al.* 2002; Singh & Kushwaha 2005; Staggemeier & Morellato 2011). There are suggestions that climate-driven models are not applicable for predicting plant phenology in the tropics (Borchert *et al.* 2005; Gazal *et al.* 2008), and, that phenology in tropical biomes may fail to be a useful indicator of global warming (Borchert *et al.* 2005). Does this in turn also apply for the estimation of the temperature distribution at the local scale?

This study incorporated one temperate mid-latitude city (Munich, Germany) and one tropical city (Campinas, Brazil) of almost the same size in which two broad-scale networks with air temperature and humidity directly at the observed trees were installed. Due to consistent phenological observation methods, equivalent study design and meteorological data collection, we were able to answer the major research questions:

1) Do the two cities show distinctive urban heat island effects?
2) How suitable are the selected phenophases and species for detecting differences in urban-rural temperature at the local scale?
3) Are the urban index, an estimate for the degree of urbanisation, as well as relative and absolute humidity also valuable explanatory variables in urban phenology?

2.2 Material and methods

Study area

Geographical location

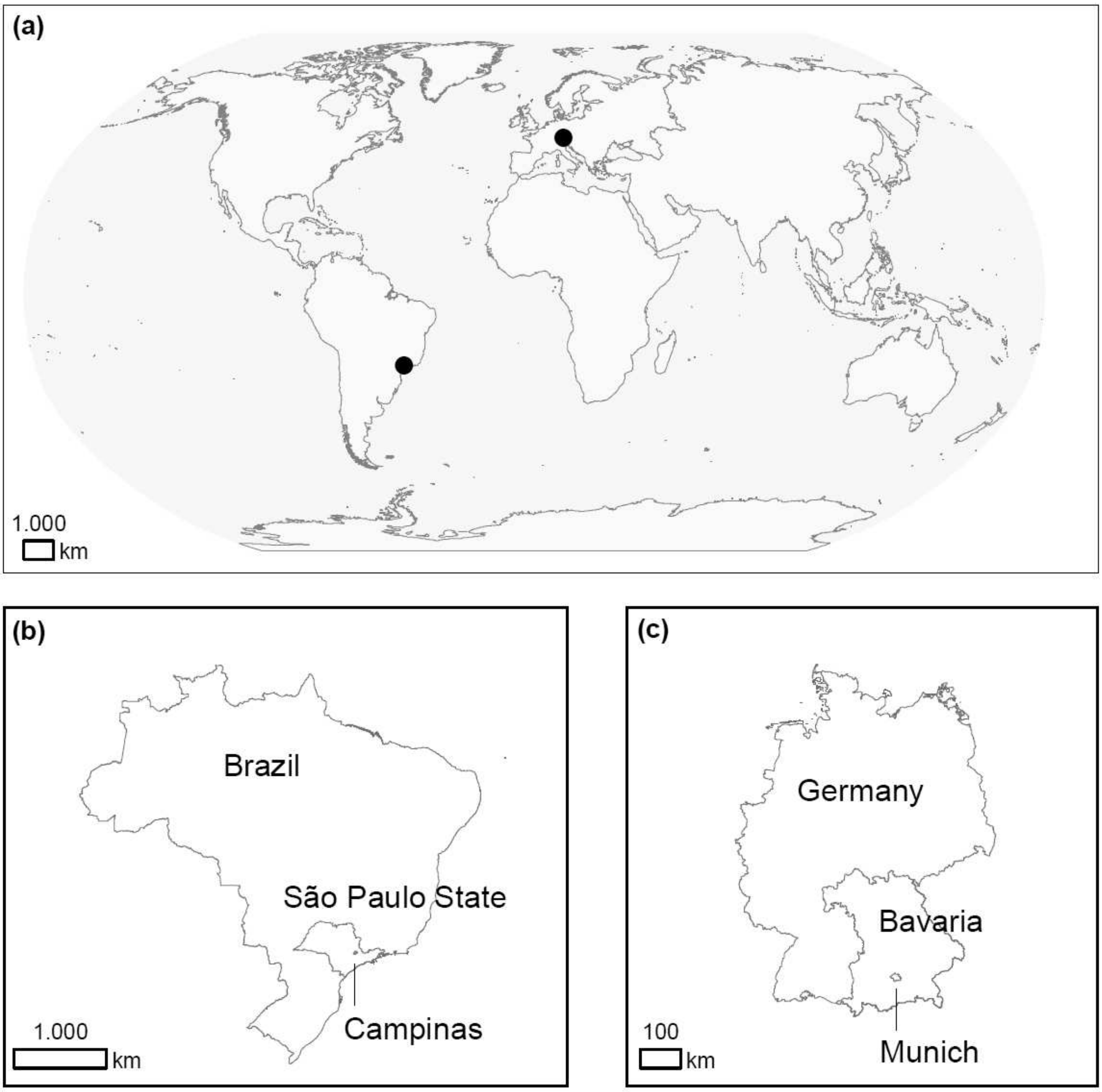


Fig. 2.1. Location of **(a)** the study sites (black dots) in Brazil and Germany, **(b)** Campinas (22°54' S, 47°3' W), São Paulo State, Brazil and **(c)** Munich (48°8' N, 11°35' E), Bavaria, Germany (country boundaries: ESRI 2011).

Campinas municipality (22°54’ S, 47°3’ W) is located in the state of São Paulo, Brazil (see Fig. 1), on a plateau at about 685 m altitude, near to the Serra do Japi in the Piracicaba River watershed. Munich (48°8’ N, 11°35’ E) is located in southern Bavaria, Germany (see Fig. 1), on the Isar river north of the Bavarian Alps at an altitude of around 515 m. The population size is 1.38 Mio (Munich) and 1.08 Mio inhabitants (Campinas), respectively. However, their city structure differs considerably. Whereas Campinas’ inner city is dominated by numerous multi-storey buildings, Munich’s architecture is characterised by only a few tall buildings higher than 100 m. Conversely, a number of green open areas can be found in both cities. For details of land use see Fig. 2.

Climate (1971-2000)

Campinas has a seasonal tropical climate characterised by a wet and warm season from October to March and a dry and cold season from April to September, and an annual mean precipitation of 1410 mm. During the dry season only 25 % of the annual rainfall is received and the average precipitation during the driest month August is 33 mm. Maximum mean monthly rainfall occurs in January (250 mm). Mean annual temperature is 21.9 °C (minimum in June: 18.4 °C, maximum in February: 24.6 °C) (data source: IAC, Instituto Agronômico).

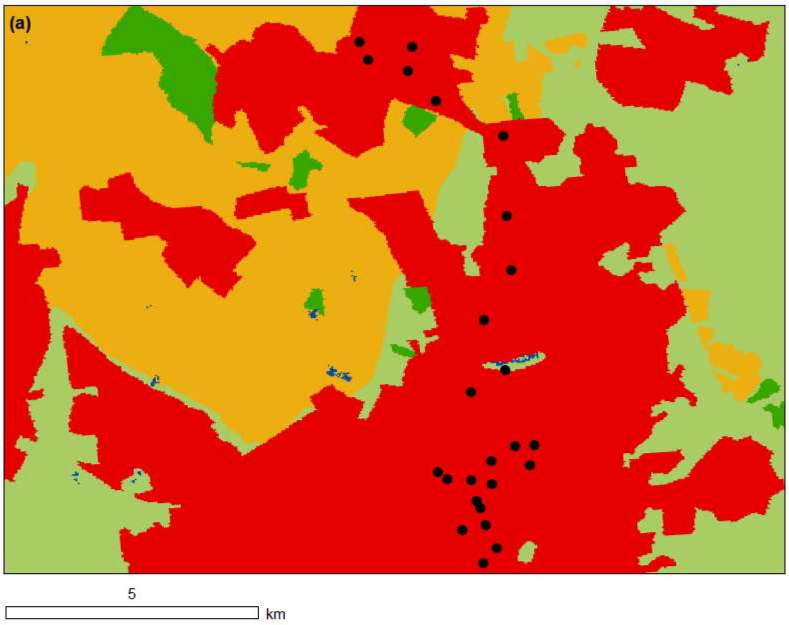
Munich is characterised by a warm temperate continental climate. The annual mean temperature is 9.5 °C with an average of 0.3 °C in the coldest month (January) and 18.9 °C in the warmest month (July). Annual precipitation average is 954 mm, with most of the rain occurring during summer with a maximum of 125 mm in July. The winter is dryer with a minimum of 46 mm in January (data source: DWD, German Meteorological Service).

Phenological observations

Selected plants

The Brazilian study was based on flowering and leaf unfolding of three different species (see circles in Fig. 2a). *Tabebuia chrysotricha* (Mart. Ex DC.) Standl., known as yellow trumpet tree or ipê, is a deciduous tropical tree species of the *Bignoniaceae* family and native of Brazil. It is the national flower of Brazil and often used as ornamental tree in urban settlements, parks and in the lining of streets (Souza *et al.* 2005). *Caesalpinia peltophoroides* Benth., also named sibipiruna or false Brazilwood, is a legume tree of the *Fabaceae* family. It originates from Brazil and is often used as ornamental tree in cities (Corte *et al.* 2008; Bueno *et al.* 2012). *Tipuana tipu* (Benth.) Kuntze, commonly named tipu tree or racehorse tree, is a large deciduous to semi-deciduous tree belonging to the *Fabaceae* family and originates from Argentina and Bolivia (South America). *Tipuana* is widely planted in urban areas, mainly in Southern Brazil (dos Santos Pereira *et al.* 2003). Since rural areas outside the borders of Campinas are mostly occupied by sugar cane or pasture, the prevalence of the three selected tropical trees is rather restricted to settlements.

For the German study area we selected leaf unfolding and flowering of *Betula pendula* Roth (silver birch) (see circles in Fig. 2b). It is a deciduous tree of the *Betulaceae* family and a pioneer plant that is widespread across Europe and can be frequently found both in urban and rural areas (Aas 2000). Due to the allergenicity of its pollen, the tree gained strong interest in studies related to phenology (Siljamo *et al.* 2008; Jochner *et al.* 2011), aerobiology (Emberlin *et al.* 2002; Jochner *et al.* 2012b) and medicine (Traidl-Hoffmann *et al.* 2003; Bryce *et al.* 2010).



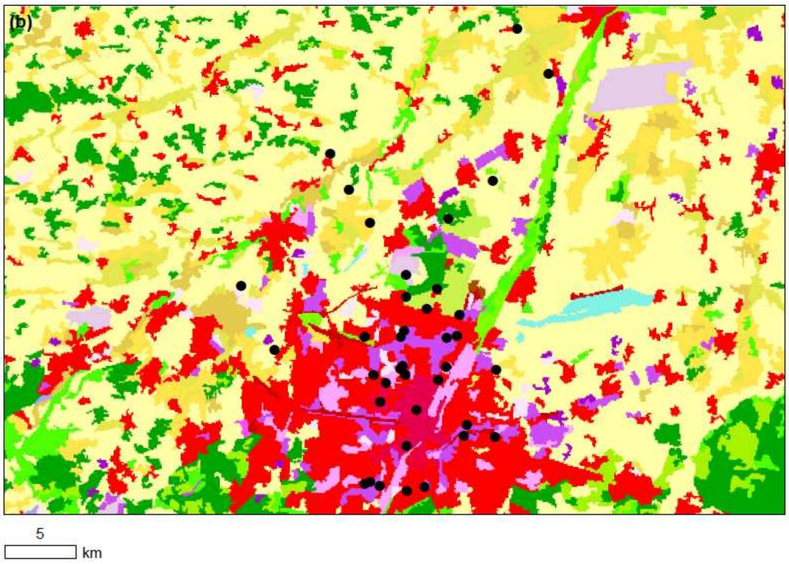


Fig. 2.2 The studied cities of **(a)** Campinas, background: land cover (INPE, Brazilian National Institute for Space Research, Vieira et al. 2010, see also: http://urlib.net/8JMKD3MGP7W/36QPBQ5), major classes: red = urban fabric, orange = sugar cane, light green: pasture, dark green: eucalyptus forest remnants; and **(b)** Munich, Germany, background: land cover (CORINE Land Cover 2006, EAA, 2010, see also: http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster for a complete legend), major classes: red = urban fabric, green = forest and pastures, yellow = arable land, blue = rivers, lakes. Black dots represent the sites within each city.

Observation methods

The observation sites in both cities were chosen along urban-rural gradients within a ~100-m total elevation range to avoid altitudinal and associated temperature effects on phenology. The selection of sites was mainly based on the occurrence of trees (2 to 9 individuals required) and depended on, e.g., the free accessibility or an authorization from the property owner. We observed 83 *Tabebuia* trees at 19 sites, 101 *Caesalpinia* trees at 28 sites and 68 *Tipuana* trees at 17 sites in Campinas (see Fig. 2a). In Munich, we observed 130 *Betula* trees at 38 sites (see Fig. 2b).

To ensure consistent phenological observation methods we used the BBCH-code (Meier 2001). Initially developed for development stages of agricultural plants by the **B**iologische Bundesanstalt, **B**undessortenamt and **CH**emical industry, the extended BBCH-scale for mono- and dicotyledonous plant species allows the assessment not only of principal growth stages (e.g., flowering, leaf unfolding), but also of short developmental steps (sub-stages, secondary growth stages) that are passed successively within the development process (e.g., bud shows green tip; inflorescence or flower buds visible). In the result section for clarity, however, we only present the data of BBCH 61 (beginning of flowering: 10 % of flowers open / emitting pollen), BBCH 65 (full flowering: > 50% of flowers open / emitting pollen, first petals falling), BBCH 10 (mouse-ear stage: green leaf tips 10 mm above the bud scales) and BBCH 15 (> 50 % leaves unfolded).

Observations were carried out by only one person per city in order to diminish subjective variations in assessing BBCH development stages and were repeated every third day. The observation period in Munich lasted from end of March to mid of April 2011, in Campinas from mid of July to beginning of October 2011.

Meteorological measurements

One individual tree per site (Campinas: n = 31, Munich: n = 38) was equipped with an air temperature and humidity sensor (HOBO U23-001, Onset Computer Corporation, Southern MA, USA). The loggers were mounted in a radiation shield at the northern (Munich) and southern (Campinas) side of the trunks at 3 m height to minimise theft or vandalism since almost all sites were communal or municipal and therefore open for the public. Both in Munich and Campinas, one logger was stolen and not replaced during the observation period. The direction of loggers in the study site of Campinas was not fully respected prior to end of September for the safety reasons described above. Thus, there might be still an influence of solar radiation on temperature during day, albeit loggers were placed in a radiation shield and only less than half of the loggers were not southern orientated. To avoid possible wrong interpretations, we incorporated mean night temperatures - that reflect temperature data in the absence of sunlight - in statistical analyses as well. Loggers in Campinas had been sequentially installed: 18 stations in August, 28 stations in September and further three loggers were only mounted in October.

Auxiliary data

Observation sites of the German study area were categorised into urban and rural according to CORINE Land Cover (CLC) 2006 data (EEA 2006) with a spatial resolution of 100 m. CLC data consists of 44 land cover classes which were grouped into five main categories (artificial surfaces, agricultural areas, forest and semi-natural areas, wetlands, water bodies). Using ArcGIS 10 (ESRI 2009, Redlands, CA, USA) we extracted particular elements of artificial surfaces (e.g., continuous and discontinuous urban fabric, industrial and commercial units) that are characterised by a high degree of impervious surfaces to create an “urban layer”. Since CLC data only cover European countries we applied land use data of the Brazilian National Institute for Space Research (INPE, Vieira et al. 2010) with a spatial resolution of 30 m for the study area of Campinas and extracted areas which were already classified as ”urban”.

According to Jochner et al. (2012a) we calculated an index describing the degree of urbanisation (ui = urban index) for each site using the proportion of urban land use within a radius of 2 km to ascertain the identification of vastly or thinly urbanised regions. Sites were classified as urban when the ui-values were greater than 0.5; sites with smaller values were regarded as rural.

Statistical methods

We selected daily mean and maximum temperature (Tmean and Tmax) as well as mean night temperatures (Tnight) that were calculated using only temperature data from 6:00 pm to 6:00 am (Campinas) and 7:00 pm to 7:00 am (Munich), respectively. The diurnal temperature range (DTR) was regarded as the difference between the maximum temperature and the minimum temperature of one day. Besides relative humidity (RH, [%]), we also incorporated absolute humidity (a, [g m^{-3}]) that was calculated using equations 1 to 3,

$$e = RH*E/100 \tag{1}$$

$$E=6.1078*EXP((17.0809*T)/(234.175+T)) \tag{2}$$

$$a = e /(0.00462*(273+T)) \tag{3}$$

where e = water vapour pressure [hPa], RH = relative humidity [%], E = saturation water vapour pressure [hPa], T = temperature [°C].

The overall range of meteorological variables (range) is the difference between the warmest and coldest / most humid and least humid station. Besides, we calculated differences between urban and rural areas for meteorological and phenological data. Positive temperature / moisture differences indicate higher temperatures / more humidity in the city. The urban heat island effect (UHI) refers to differences in urban and rural temperature. The phenological differences - called the urban phenology effect (UPE) - indicate an earlier onset in the city compared to the countryside by negative values and a delay in the city by positive values.

Air temperature and humidity data as well as phenological data were tested for statistically significant differences between urban and rural areas by means of a two sample t-test. We applied Pearson's correlation analyses to examine relationships between phenological onset dates, the urban index and meteorological variables. With respect to biological relevance, we selected temperature and humidity data of the months before mean onset of the respective phenophases and omitted meteorological data for the time after mean onset (e.g., see missing cells in Table 4).

2.3 Results

Temperature characteristics

Table 1 shows meteorological data for urban and rural areas of Campinas in August and September and Munich in March, 2011. The urban heat island effect on mean temperature in Campinas was small and not significant for both months. Also the overall range of mean monthly temperatures did not show significant variations in August (0.9 °C) and September (1.1 °C). In both months differences between urban and rural areas were only significant for mean night temperatures (0.7 and 0.6 °C, respectively) and DTR (-1.7 and -1.9 °C, respectively), whereas relative and absolute humidity showed significant differences between urban and rural areas only in September.

In contrast, we found distinctive differences in temperature conditions for Munich (Table 1). The urban heat island effect was significant with 1.0 °C for mean temperatures, 1.4 °C for night temperatures and -1.2 °C for DTR, -5.8 % for relative humidity and -0.1 g m^{-3} for absolute humidity. Only maximum temperature of March did not show a significant difference between the city and the countryside. Compared to the study area of Campinas, the ranges of mean temperature as well as night temperature were higher, reaching 2.5 and 3.8 °C, respectively.

Table 2.1 Mean (μ) temperature (°C), humidity variables and respective standard deviation (SD) for selected months in urban and rural areas in Campinas (August = 8, September = 9) and Munich (March = 3) in 2011. Tm = mean temperature, Tnight = mean temperature during night, Tmax = maximum temperature, DTR = diurnal temperature range, RH = relative humidity (%), a = absolute humidity (g m^{-3}); diff = urban-rural difference, range = maximum value minus minimum value in the study area, *P* = significance, bold values: significant urban-rural differences at the 5 % level, italic values: significant urban-rural differences at the 10 % level

CAMPINAS		**urban**	**rural**	**diff (p)**	**overall**	**range**
Tm8 (°C)	μ	20.7	20.5	0.2 (0.294)	20.6	0.9
	SD	0.3	0.2		0.3	
Tm9 (°C)	μ	21.6	21.6	0.0 (0.849)	21.6	1.1
	SD	0.3	0.3		0.3	
Tnight8 (°C)	μ	18.6	17.9	**0.7 (0.024)**	18.4	2.3
	SD	0.5	0.5		0.6	
Tnight9 (°C)	μ	19.2	18.6	**0.6 (0.003)**	19.0	2.1
	SD	0.4	0.5		0.5	
Tmax8 (°C)	μ	26.1	25.6	0.5 (0.110)	25.9	2.2
	SD	0.7	0.5		0.7	
Tmax9 (°C)	μ	27.1	26.7	*0.4 (0.088)*	27	2.2
	SD	0.4	0.7		0.6	
DTR8 (°C)	μ	11.3	13	**-1.7 (0.003)**	11.8	4.7
	SD	1.0	1.0		1.2	
DTR9 (°C)	μ	12.8	14.7	**-1.9 (0.000)**	13.4	5.1
	SD	0.9	1.1		1.3	
RH8 (%)	μ	62.3	64.2	-1.9 (0.132)	62.9	9.0
	SD	2.3	2.4		2.5	
RH9 (%)	μ	54.9	56.8	**-1.9 (0.017)**	55.5	7.9
	SD	1.8	1.8		1.9	
a8 (g m^{-3})	μ	10.7	10.9	-0.2 (0.209)	10.7	0.9
	SD	0.3	0.3		0.3	
a9 (g m^{-3})	μ	9.9	10.1	**-0.2 (0.011)**	9.9	0.9
	SD	0.2	0.2		0.3	
MUNICH		**urban**	**rural**	**diff (p)**	**overall**	**range**
Tm3 (°C)	μ	6.1	5.1	**1.0 (0.000)**	5.7	2.5
	SD	0.5	0.4		0.6	
Tnight3 (°C)	μ	4.0	2.6	**1.4 (0.000)**	3.6	3.8
	SD	0.7	0.6		0.9	
Tmax3 (°C)	μ	11.7	11.3	*0.4 (0.073)*	11.6	2.6
	SD	0.7	0.5		0.6	
DTR3 (°C)	μ	10.6	11.8	**-1.2 (0.000)**	11	4.6
	SD	0.2	0.3		1.1	
RH3 (%)	μ	71	76.8	**-5.8 (0.000)**	72.9	14.7
	SD	2.5	2.2		3.7	
a3 (g m^{-3})	μ	5.1	5.2	**-0.1 (0.004)**	5.2	0.5
	SD	0.1	0.1		0.1	

The correlations between the urban index and temperature / humidity variables are summarised in Table 2. For Campinas the highest correlations with urban index were revealed by DTR (*r* =~ -0.8) and mean night temperature (*r* =~ 0.75). Besides, maximum temperature and relative humidity as well as absolute humidity in September showed relatively strong correlations (all *r* > |0.5|). However, mean monthly temperatures were not adequately reflected by the urban index: the correlation coefficients were either low (August, September) or not significant (September).

The urban index in Munich was best correlated with relative humidity (*r* =-0.84), mean temperature (*r* = 0.8) and mean night temperatures (*r* = 0.77) in March. All other coefficients were significant as well.

Table 2.2 Pearson's correlation coefficients (*r*) for urban index (ui) and temperature and humidity variables for selected months in Campinas (August = 8, September = 9) and Munich (March = 3) in 2011. Tm = mean temperature, Tnight = mean temperature during night, Tmax = maximum temperature, DTR = diurnal temperature range, RH = relative humidity, a = absolute humidity; *n* = number of sites, *P* = significance, bold values: significant coefficients at the 5 % level, italic values: significant coefficients at the 10 % level

CAMPINAS		**Tm8**	**Tm9**	**Tnight8**	**Tnight9**	**DTR8**	**DTR9**	**Tmax8**	**Tmax9**
ui	*r*	**0.486**	0.191	**0.750**	**0.756**	**-0.825**	**-0.794**	**0.534**	**0.633**
	P	0.041	0.340	0.000	0.000	0.000	0.000	0.022	0.000
	n	18	27	18	27	18	27	18	27
CAMPINAS		**RH8**	**RH9**	**a8**	**a9**				
ui	*r*	**-0.599**	**-0.678**	*-0.463*	**-0.623**				
	P	0.009	0.000	0.053	0.001				
	n	18	27	18	27				
MUNICH		**Tm3**	**T3night**	**Tmax3**	**DTR3**	**RH3**	**a3**		
ui	*r*	**0.798**	**0.767**	**0.348**	**-0.567**	**-0.839**	**-0.560**		
	P	0.000	0.000	0.035	0.000	0.000	0.000		
	n	37	37	37	37	37	37		

Phenological onset dates in 2011

The mean onset dates of flowering and leaf unfolding phenophases of the four studied species in urban and rural areas are summarised in Table 3. The urban phenology effect (UPE) showed significant differences for all phenophases of birch in Munich and ranged between -1.5 days (> 50 % leaves unfolded) and -3.4 days (full flowering), indicating an earlier onset in urban areas. In Campinas, UPE of *Tabebuia* varied between -1.5 days (> 50 % leaves unfolded) and +9.3 days (beginning of flowering). However, these differences were not statistically significant. This also applied for *Caesalpinia* where differences ranged between +2.2 and +5.7 days. Significant results were only found for flowering onset of *Tipuana* (-19.7 days) and for > 50 % leaf unfolding of the same species (-10.5 days, significant at the 10 % level).

The results for Campinas showed large standard deviations (SD) with overall mean values ranging between 8.9 (*Caesalpinia*: mouse-ear stage) to 17.4 days (*Tipuana*: beginning of flowering) (Table 3). However, even trees within one site exhibited a large asynchrony in flowering and leaf unfolding: mean SD ranged between 12.1 and 15.7 days for *Tabebuia*, between 9.4 and 12.9 days for *Caesalpinia* and between 7.5 and 14.1 days for *Tipuana* (data not shown). The highest SD for one site (with *n* = 6) was 36.1 days for full flowering of *Tabebuia*. In contrast, the results for *Betula* in Munich were characterised by relatively low SD values ranging between 1.6 (mouse-ear stage) and 3.0 days (full flowering) (Table 3). Regarding single sites mean SD did not exceed 2.4 days for all analysed phenological phases (data not shown).

Explanatory variables in urban phenology

Correlation coefficients between phenological onset dates of the three selected species in Campinas and urban index, temperature and humidity variables are summarised in Table 4. We did not find any significant correlations for flowering phenophases of *Tabebuia* and the selected explanatory variables. However, leaf phenophases revealed some meaningful correlations with mean, nocturnal and maximum temperature in August (*r* > |-0.5|). The highest correlations were obtained with relative and absolute humidity in August (*r* > 0.7). Partial correlation analyses with mean temperature as control variable underlined the statically significant influence of humidity variables on leaf phenophases (all *r* > 0.65, data not shown). For *Caesalpinia* we did not find any significant correlation across all analysed environmental variables and months. In contrast, there were several significant relationships for flowering and leaf unfolding phenophases of *Tipuana*. Beginning of flowering was mostly influenced by diurnal temperature range ($r = 0.68$), mean night temperature and humidity related variables in September (all $r > |0.5|$). For full flowering the correlation with relative and absolute humidity was not that evident. We detected strong correlations for leaf unfolding phenophases and mean and nocturnal temperature (all $r > |-0.7|$). For almost all phenophases (except full flowering) statistically significant correlations with the urban index were greater than $r = -0.5$.

Table 2.3 Mean (μ) onset dates (DOY, day of the year), standard deviations (SD) and UPE (urban phenology effect, difference between urban and rural phenological onset dates in days) for flowering and leaf unfolding phenophases of *Tabebuia*, *Caesalpinia* and *Tipuana* in Campinas and *Betula* in Munich, 2011; BBCH 61: beginning of flowering, BBCH 65: full flowering, BBCH 10: mouse ear stage, BBCH 13: > 50 % of leaves unfolded, *n* = number of sites, *P* = significance, bold values: significant UPE values at the 5 % level, italic values: significant UPE values at the 10 % level

Species (City)		***n***	**BBCH 61**		**BBCH 65**		**BBCH 10**		**BBCH 11**	
			μ date	**SD**	**μ date**	**SD**	**μ date**	**SD**	**μ date**	**SD**
Tabebuia (Campinas)	overall	19	230.0 18.08.	13.0	242.7 31.08.	10.7	225.5 13.09.	13.3	271.8 29.09.	13.6
	urban	14	232.5 21.08.	13.8	244.0 01.09.	11.8	257.1 14.09.	14.5	271.4 28.09.	15.0
	rural	5	223.2 11.08.	7.9	239.1 27.08.	6.2	251.3 08.09.	9.5	272.9 30.09.	9.8
	UPE (*P*)	19	9.3 (0.178).		4.9 (0.394)		5.8 (0.426)		-1.5 (0.846)	
Caesalpinia (Campinas)	overall	28	246.2 22.09.	11.5	272.2 29.09.	10.6	240.1 28.08.	8.9	248.2 05.09.	10.4
	urban	19	265.7 23.09.	10.6	274.1 01.10.	8.8	240.8 29.08.	8.9	249.2 06.09.	10.5
	rural	9	261.2 18.09.	13.4	268.4 25.09.	13.5	238.6 27.08.	9.3	246.1 03.09.	10.6
	UPE (*P*)	28	4.5 (0.350)		5.7 (0.191)		2.2 (0.541)		3.1 (0.474)	
Tipuana (Campinas)	overall	17	264.9 22.09.	17.4	280.0 07.10.	14.6	235.0 23.08.	10.3	241.7 30.08.	10.9
	urban	12	259.1 16.09.	16.0	276.3 03.10.	14.4	241.2 29.08.	7.6	238.6 27.08.	10.7
	rural	5	278.8 06.10.	12.8	288.8 16.10.	11.9	232.4 20.08.	10.5	246.1 06.09.	8.0
	UPE (*P*)	17	**-19.7 (0.028)**		-12.5 (0.111)		-8.8 (0.108)		*-10.5 (0.068)*	
Betula (Munich)	overall	38	98.6 09.04.	2.1	101.0 11.04.	3.0	94.7 05.04.	1.6	101.0 11.04.	2.5
	urban	25	97.8 09.04.	1.5	99.8 10.04.	2.1	94.2 04.04.	1.3	100.5 11.04.	2.3
	rural	13	100.1 10.04.	2.3	103.2 13.04.	3.3	95.7 06.04.	1.8	102.0 12.04.	2.7
	UPE (*P*)	38	**-2.3 (0.000)**		**-3.4 (0.004)**		**-1.5 (0.008)**		*-1.5 (0.092)*	

Table 2.4 Pearson's correlation coefficients (r) for flowering and leaf unfolding phenophases of *Tabebuia*, *Caesalpinia* and *Tipuana* in Campinas and *Betula* in Munich, 2011, with ui = urban index, Tm = mean temperature, Tnight = mean temperature during night, Tmax = maximum temperature, DTR = diurnal temperature range, RH = relative humidity, a = absolute humidity, in March (= 3), August (= 8), September (= 9); BBCH 61: beginning of flowering, BBCH 65: full flowering, BBCH 10: mouse ear stage, BBCH 13: > 50 % of leaves unfolded *n* = number of sites, *P* = significance, bold values: significant coefficients at the 5 % level, italic values: significant coefficients at the 10 % level

Tabebuia		**ui**	**Tm8**	**Tnight8**	**Tmax8**	**DTR8**	**RH8**	**a8**	**Tm9**	**Tnight9**	**Tmax9**	**DTR9**	**RH9**	**a9**
BBCH61	*r*	0.284	-0.199	0.173	0.178	-0.192	0.012	-0.043						
	P	0.239	0.495	0.554	0.543	0.510	0.967	0.885						
	n	19	14	14	14	14	14	14						
BBCH65	*R*	0.162	-0.167	0.174	0.068	-0.200	0.081	0.077						
	P	0.507	0.569	0.553	0.819	0.493	0.783	0.795						
	n	19	14	14	14	14	14	14						
BBCH10	*R*	0.120	**-0.591**	*-0.533*	**-0.585**	0.234	**0.812**	**0.803**	-0.090	0.180	-0.322	0.164	0.168	0.152
	P	0.634	0.033	0.061	0.036	0.441	0.001	0.001	0.741	0.491	0.224	0.544	0.535	0.573
	N	18	13	13	13	13	13	13	16	17	16	16	16	16
BBCH13	*r*	-0.112	-0.455	**-0.557**	**-0.570**	0.371	**0.710**	**0.752**	0.046	0.187	-0.344	0.400	0.271	0.348
	P	0.647	0.102	0.038	0.033	0.192	0.004	0.002	0.860	0.474	0.192	0.125	0.309	0.186
	n	19	14	14	14	14	14	14	17	17	16	16	16	16
Caesalpinia		**ui**	**Tm8**	**Tnight8**	**Tmax8**	**DTR8**	**RH8**	**a8**	**Tm9**	**Tnight9**	**Tmax9**	**DTR9**	**RH9**	**a9**
BBCH61	*r*	0.215	-0.385	-0.060	0.014	-0.107	0.176	0.088	-0.061	-0.295	0.040	0.069	0.010	-0.049
	P	0.272	0.114	0.812	0.955	0.673	0.484	0.729	0.766	0.144	0.849	0.743	0.962	0.816
	n	28	18	18	18	18	18	18	26	26	25	25	25	25
BBCH65	*r*	0.273	-0.212	-0.020	0.176	-0.008	0.049	-0.042	-0.017	-0.293	0.131	0.041	-0.080	-0.152
	P	0.161	0.397	0.939	0.486	0.974	0.847	0.869	0.936	0.146	0.532	0.844	0.704	0.468
	n	28	18	18	18	18	18	18	26	26	25	25	25	25
BBCH10	*r*	0.109	-0.157	-0.052	0.164	0.071	-0.006	-0.100						
	P	0.582	0.534	0.837	0.515	0.780	0.982	0.692						
	n	28	18	18	18	18	18	18						
BBCH13	*r*	0.144	-0.203	-0.028	0.145	-0.004	0.001	-0.092						
	P	0.466	0.420	0.911	0.565	0.989	0.996	0.715						
	n	28	18	18	18	18	18	18						

Table 2.4 continued

Tipuana		**ui**	**Tm8**	**Tnight8**	**Tmax8**	**DTR8**	**RH8**	**a8**	**Tm9**	**Tnight9**	**Tmax9**	**DTR9**	**RH9**	**a9**
BBCH61	*r*	**-0.540**	-0.618	*-0.632*	-0.582	0.501	0.559	0.463	-0.114	**-0.578**	-0.365	**0.582**	**0.563**	**0.607**
	P	0.025	0.102	0.093	0.130	0.206	0.150	0.248	0.686	0.031	0.200	0.029	0.036	0.021
	n	17	8	8	8	8	8	8	15	14	14	14	14	14
BBCH65	*r*	-0.388	*-0.695*	*-0.624*	-0.550	0.385	0.545	0.393	-0.234	**-0.590**	-0.297	**0.548**	0.437	0.335
	P	0.124	0.056	0.098	0.158	0.347	0.163	0.335	0.401	0.026	0.302	0.042	0.118	0.241
	n	17	8	8	8	8	8	8	15	14	14	14	14	14
BBCH10	*r*	**-0.547**	**-0.727**	**-0.714**	*-0.638*	0.564	*0.674*	0.553						
	P	0.023	0.041	0.047	0.088	0.145	0.067	0.155						
	n	17	8	8	8	8	8	8						
BBCH13	*r*	**-0.561**	**-0.746**	**-0.779**	*-0.700*	*0.655*	**0.727**	0.612						
	P	0.019	0.034	0.023	0.053	0.078	0.041	0.107						
	n	17	8	8	8	8	8	8						
Betula		**ui**	**Tm3**	**Tnight3**	**Tmax3**	**DTR3**	**RH3**	**a3**						
BBCH61	*r*	**-0.574**	**-0.742**	**-0.752**	-0.163	**0.709**	**0.637**	0.124						
	P	0.000	0.000	0.000	0.336	0.000	0.000	0.465						
	n	38	37	37	37	37	37	37						
BBCH65	*r*	**-0.585**	**-0.739**	**-0.749**	-0.194	**0.679**	**0.673**	0.219						
	P	0.000	0.000	0.000	0.249	0.000	0.000	0.192						
	n	38	37	37	37	37	37	37						
BBCH10	*r*	**-0.404**	**-0.571**	**-0.609**	-0.030	**0.668**	**0.473**	0.031						
	P	0.012	0.000	0.000	0.861	0.000	0.003	0.855						
	n	38	37	37	37	37	37	37						
BBCH13	*r*	*-0.288*	**-0.482**	**-0.450**	-0.259	*0.324*	**0.412**	0.095						
	P	0.079	0.002	0.005	0.122	0.051	0.011	0.576						
	n	38	37	37	37	37	37	37						

Regarding birch spring development and the selected environmental variables, there was a high dependence on nocturnal and mean temperatures in March, especially for beginning of flowering and full flowering (all $r > |-0.7|$), as well as on diurnal temperature range (Table 4). In contrast, we did not find any significant correlations with maximum temperature and absolute humidity. The urban index was negatively correlated with onset dates; again, better for flowering phenophases ($r > |-0.57|$). Relative humidity was more correlated with flowering phenophases of birch than with leaf unfolding phenophases. This, however, is attributable to the high correlation between relative humidity and mean temperature ($r = -0.94$). Partial correlations with mean temperature as the control variable did not show any significant correlations instead (data not shown).

2.4 Discussion

Urban heat island effect

We did not find any UHI in the tropical city of Campinas, contrasting with the more distinctive urban-rural temperature differences in Munich, showing a mean UHI of 1.0 °C. In addition, urban-rural differences were also more pronounced in Munich than Campinas for DTR, night temperatures and for the absolute temperature ranges between all sites. The fact that UHI is greater for minimum than for maximum temperatures is well documented in existing literature (Landsberg 1981; Baker et al. 2002; Mimet et al. 2009; Shustack et al. 2009), equally to our study for Campinas and Munich. Furthermore, relative and absolute humidity was significantly lower in the city of Munich compared to its rural environments (-5.8 % and -0.1 g m^{-3}, respectively). Campinas, however, was characterised by only a small urban-rural difference for relative and absolute humidity.

The question arises whether these negligible differences within the study area in Brazil are linked to the site selection criteria. Campinas most rural site exhibited an urban index (ui) of only 0.156 compared to 0.003 in Munich, with five additional sites having ui-values smaller than 0.010. Additionally, the maximum distance to the city centre was just 12.7 km in Campinas compared to 28.2 km in Munich. Therefore, we suggest, that the dominance of sites with high degree of urbanisation might have influenced temperature variations / ranges in the study area of Campinas. Also, the influence of the initial loggers' orientation on temperature measurements in Campinas cannot totally be ruled out (see Section Material and methods - Meteorological measurements).

In both cities the calculated urban index was a good estimate for local temperature and humidity conditions. The only exception was mean temperature in Campinas, that only showed a medium correlation in August ($r = 0.49$) and no significant correlation in September. Nevertheless, we propose that an easily computable urban index might be useful for a quick and inexpensive estimation of the spatial structure of urban heat islands.

Urban-rural differences in phenology

In contrast to small SDs of onset dates of *Betula* in Munich ranging between 1.6 (mouse-ear stage) and 3.0 days (> 50 % leaves unfolded), we observed high SDs in onset dates for the three selected species in Campinas ranging between 8.9 (*Caesalpinia*: mouse-ear stage) and 17.4 days (*Tipuana*: beginning of flowering). A high variation in onset dates of phenological phases in the tropics was also reported by Gazal *et al.* (2008) for three tropical cities in Asia and Africa, and for tropical wet and dry forest trees (Morellato *et al.* 2000; Borchert *et al.* 2005). The high within-species variation in tropical tree phenology might partly explain small urban-rural differences. We only found significant UHI related differences for *Tipuana* in Campinas, whereas almost all phenophases of *Tabebuia* and all of *Caesalpina* showed even positive urban-rural differences (albeit throughout not significant) that indicated an earlier onset in the countryside. Gazal *et al.* (2008) reported earlier bud burst dates only in the tropical city Bangkok (-23 days), but not in Korat (+ 9 days) and Dakar (+ 9 days). In addition, bud burst was delayed with increasing land surface temperature suggesting a low temperature sensitivity or other influencing environmental variables (e.g., humidity related variables).

In contrast to our findings regarding Campinas, we confirmed significant differences between onset dates for birch phenophases in Munich ranging between -1.5 days (mouse-ear stage) and -3.4 days (full flowering). An urban-rural comparison in the greater area of Berlin, Germany, conducted by Henniges & Chmielewski (2006) revealed also greater differences for birch flowering (-2.6 days) compared to birch leaf unfolding (-0.3 days). Hence, this result is in accordance with our finding: birch flowering phenophases are more responsive to temperature variations than leaf unfolding phenophases.

In Campinas, the vertical structure of the city is much more pronounced than in Munich. Zhang *et al.* (2004) concluded that urban heat island effects on plant phenology are stronger in North America than in Europe or Asia owing to the dense and vertical urban design in North American cities. However, this cannot be tested for our European and South American study sites since species were not identical. We only found a distinctive high difference of -10.5 to -19.7 days in *Tipuana* spring phenology that was much higher than the difference of -1.5 to -3.4 days in birch phenology.

Air temperature, humidity and the phenology of trees in urban areas

We demonstrated the suitability of birch phenophases in urban climatology applications by high correlations between phenological onset dates and DTR (exception > 50 % leaves unfolded). The diurnal temperature range is a good indicator for urban heat islands since it is smaller in the city due to the thermal energy storage of urban constructions (Landsberg 1981). Mimet *et al.* (2009) also reported that the DTR is best correlated with budburst onset dates of sour cherry in the city of Rennes, France. In general, UHI is greater for minimum temperatures (Baker *et al.* 2002; Mimet *et al.* 2009; Shustack *et al.* 2009). Therefore, we also found slightly stronger correlations with mean night temperature than with mean temperature (exception > 50 % leaves unfolded), however, no statistically significant correlation with maximum temperature. This is in accordance with Wielgolaski (1999) and Mimet *et al.* (2009) who stated that maximum temperature does not play an important role in phenological models. Furthermore, particularly for flowering phenophases of birch in Munich there was a strong and evident relationship between urban index and onset dates.

In Campinas, however, the significance and magnitude of the difference in onset dates varied considerably among species and phenophases. The most promising species for urban phenology applications was *Tipuana* through high correlations, especially with mean and nocturnal temperatures, and in addition, a significant relationship with the urban index for three of the four selected phenophases. *Caeasalpina* failed to show any significant correlations with the selected environmental variables and was hence not sensitive enough to demonstrate temperature variations at the local scale associated with the urban heat island effect. This also applied for flowering phenophases of *Tabebuia,* although leaf unfolding phenophases were particularly sensitive to humidity but also to air temperature.

The influence of air humidity in the study area of Munich was negligible since absolute humidity was not correlated with phenological onset dates of birch and partial correlation analyses with temperature as control variable showed no significant correlations with relative humidity.

On the other hand, our results suggest that humidity influences leaf phenology of *Tabebuia* and that small temperature variations, as detected in our tropical study area, did not produce any discernible differences in onset dates. However, there was no influence of humidity on the other phases and species. The only other study comparing urban and rural phenology of tropical tree species also pointed to the potential relevance of humidity (Gazal *et al.* 2008). Leafing phenology of tropical trees under seasonal climate is driven mostly by precipitation and also by non-climatic parameters such as leaf longevity, seasonal variation in water stress dependent on local soil water storage and increasing day length (Morellato *et al.* 2000; Borchert *et al.* 2005; Borchert 2008; Staggemeier & Morellato 2011). Therefore, the consideration of humidity variables is especially recommended to understand urban effects on tropical cities under climates with distinctive dry and wet seasons.

We propose that phenological observations of *Tipuana*, a tree species widely used for planting in South American cities, are useful for a quick and inexpensive estimation of the spatial structure of urban heat islands in neotropical cities. Facing the high number of different species in tropical biomes, the need for further investigations becomes particularly evident. In the temperate / moist regions of Europe, a smaller number of possible species has already been tested for their suitability in urban applications, and greater knowledge in this respect exists (Baumgartner 1952; Bernhofer 1991). Consequently, additional species and phenophases should be explored to further identify the potential of phenology to monitor heat islands in tropical cities.

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3 NUTRIENT STATUS - A MISSING FACTOR IN PHENOLOGICAL RESEARCH?

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Abstract

Temperature alone is able to explain a high percentage of the variance in phenological onset dates. However, the role of other environmental variables such as nutrient concentration is not yet adequately understood. Consequently, in the current study we examined the effects of both foliar nutrient concentration (reflecting the environmental conditions of the trees) and air temperature on spring phenology of *Betula pendula* Roth (silver birch) in Munich, Germany in 2010 and 2011.

We found that temperature was able to explain between 45 and 62 % of the variance in phenological onset dates. Partial correlation analyses revealed that potassium (K), boron (B), zinc (Zn) and calcium (Ca) were the most important nutrients influencing phenology; the higher their concentration the earlier the onset dates. The strongest correlation was between the beginning of leaf unfolding in 2011 and foliar K concentration ($r = -0.609$). Multiple regression analyses emphasised the importance of nutrients in phenological models based on air temperature. The increase in adjusted r^2 ranged between 1.7 % and 3.8 % for flowering phenophases and between 4.8 % and 9.8 % for leaf phenophases. Specifically, the incorporation of nutrients increased model fits (relatively) by up to one fifth of the r^2 using temperature alone. This was especially true for leaf phenophases (relative increase: + 9.1 % to 21.8 %).

In addition to the known influence of temperature on plant development, we demonstrated the importance of nutritional status at the mesoscale. The nutrients that correlated most strongly with onset dates (K, B, Zn and Ca) are all involved in cell extension, membrane function and stability. Since flushing of leaves is a turgor-driven process these nutrients are especially important and beneficial for leaf unfolding phenophases.

Keywords. *Betula pendula* Roth, birch, nutrients, Munich, phenology, temperature, urban heat island.

3.1 Introduction

Temperature is the most important driver of plant phenology in temperate and boreal regions (Menzel & Fabian, 1999; Wielgolaski, 1999). Using long-term phenological series, temperature is able to explain more than two thirds of the variance in onset dates (Menzel & Fabian, 1999). In contrast, studies conducted in a single year or in only a small number of years, such as those in urban areas, do not generally achieve such a good model fit (Lakatos & Gulyás, 2003; Matsumoto *et al.*, 2009).

Besides temperature, many other external and internal factors are reported to influence plant phenology, ranging from soil humidity, diseases, pests, competition, and pollutants to individual genes and plant age (Menzel, 1999). There have also been a few approaches to include other variables besides temperature in phenological models; however, most of the factors fail to be useful explanatory variables, since they do not contribute much to the explained variance (Sparks *et al.*, 1997).

Research on nutrients and their effect on the timing of phenological events are scarce. Some existing studies are predominantly related to agriculture (Dobermann & Cassman, 2002; Steiner *et al.*, 2007), horticulture (Reickenberg & Pritts, 1996, Yang *et al.*, 2011) and forest science (Sigurdsson, 2001; Weih & Karlsson, 2001). Moreover, they are often merely linked to seedlings and physiological parameters such as growth rates (Iivonen *et al.*, 2001; Weih & Karlsson, 2001). Another problem is that existing phenological studies often only analyse soil nutrition, either by manipulation experiments using fertilisers (Weih & Karlsson, 2001), or by analysing the actual availability of a small number of nutrients in the soil (Wielgolaski, 2001).

However, nutrient availability in the soil is not related to nutrient concentrations in the leaves in a simple way; there are some environmental cues influencing nutrient uptake and translocation rates. Variations in soil temperature modify the activity of soil microorganisms and nutrient mineralization (White & Haydock, 1970; Fisher, 1980; Marschner, 1995; Radizzani *et al.*, 2011). Through the effect of air temperature on transpiration, higher temperatures can support greater nutrient uptake and translocation rates (Marschner, 1995; Wielgolawski, 2001). Therefore, foliar nutrient concentrations may be valuable in the assessment of the environmental conditions that plants are exposed to. What is even more important is that traditional soil analyses may not account for heterogeneous soil conditions, especially in urban areas, where sealed surfaces, such as concrete and tarmac, impede access to the soil.

For these reasons, we decided to study the impact of nutritional status on phenology using foliar nutrient analyses for *Betula pendula* Roth (silver birch). We used an observation network (N = 34) characterised by different degrees of urbanisation within the greater area of Munich. In contrast to many other studies, air temperature was measured directly at the trees. To the best of our knowledge this study is the first dealing with the effects of foliar nutrient concentrations on phenological behaviour at the mesoscale. We address the question; what amount of variance in onset dates can by explained by (1) mean temperature during March and April and (2) additionally by foliar nutrient concentration?

3.2 Material and methods

Study area

Munich (48°8' N, 11°35' E) is located in southern Bavaria, Germany (see Fig. 3.1), on the Isar river north of the Bavarian Alps at an altitude of about 515 m a.s.l. We only used observation sites that were located on calcareous gravel, and were covered with loamy river sediments or calcareous low level moor.

According to Jochner et al. (2012) we calculated for each site an index describing the degree of urbanisation. This urban index is based on land use data derived from CORINE Land Cover (CLC) 2006 data (EEA, 2006) with a spatial resolution of 100 m. Particular elements of artificial surfaces (e.g. continuous and discontinuous urban fabric, industrial and commercial units) that are characterised by a high degree of impervious surfaces were extracted using ArcGIS 10 (ESRI 2009, Redlands, CA, USA). The proportion of urban land use was subsequently calculated within a 2 km-radius.

Phenological data

Phenological observations for flowering and leaf phenophases of birch (*Betula pendula* Roth) were carried out during spring 2010 and 2011 using the BBCH coding system (Meier, 2001). In this study we present the phenological data for BBCH 61 (beginning of flowering), BBCH

65 (full flowering), BBCH 10 (leaves at mouse-ear stage) and BBCH 11 (first leaves unfolded). Phenological onset dates of 2-6 trees were averaged for each site (in total 126 trees).

Temperature data

Temperature data were recorded at each site using sensors (HOBO U23-001, Onset Computer Corporation, Southern MA, USA) that were installed in a radiation shield on the northern side of birch trees at 3 m above ground level.

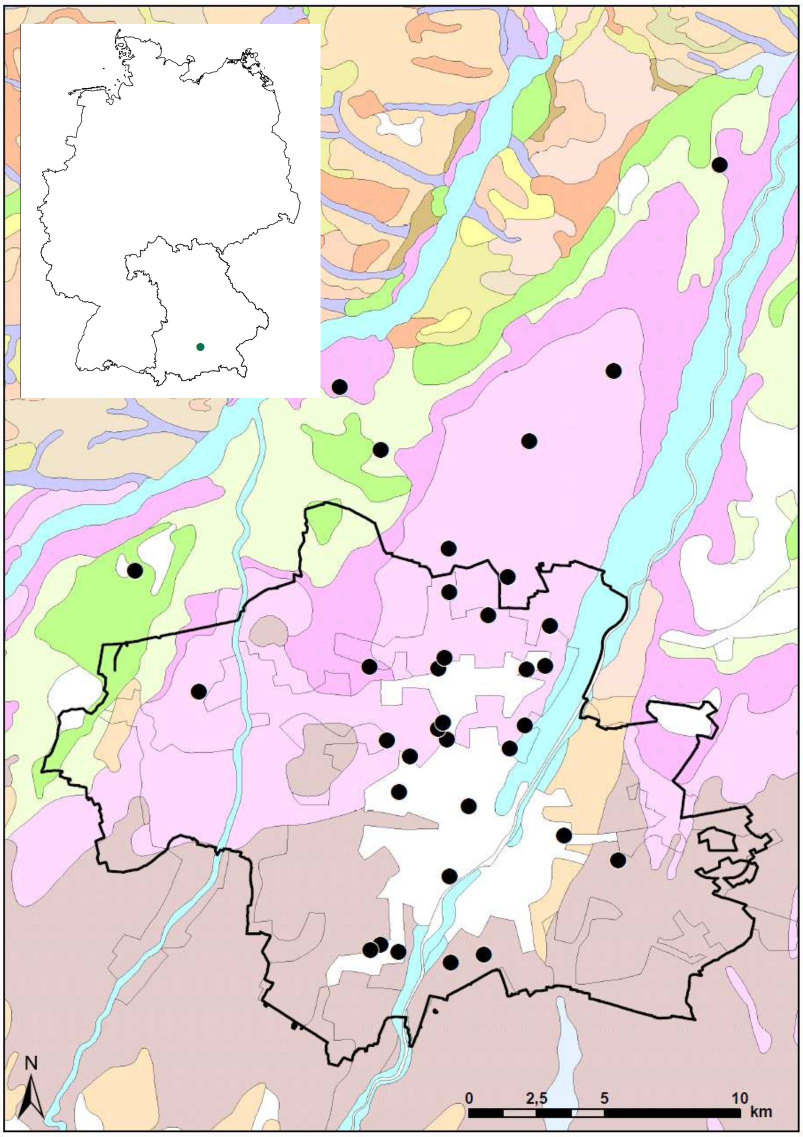

Fig. 3.1 Location of the selected birch sites (*N* = 34) in the greater area of Munich, Germany; city outline shown by the solid black line. Background: BK200 (Bodenübersichtskarte 1:200.000, Bayerisches Landesamt für Umwelt, 2012), **beige**: mainly Calcaric Regosols derived form deposited humic material above carbonatic gravel or building site rubble, **light pink**: Calcaric Regosols derived form carbonatic gravel, mostly covered with a shallow layer of marly or loamy river sediments, **dark pink:** mainly Calcaric Regosols very rich in humus derived form carbonatic gravel, mostly covered with a layer of marly or loamy river sediments, **brown**: mainly Luvisols and their transitional forms to Cambisols derived form carbonatic gravel covered with layers of loamy river sediments, **dark green**: mainly low-level moor, some of them calcareous, **light green**: mainly Humic Gleysols derived from fluvial marl over carbonatic gravel. For a complete legend see: www.geoportal.bayern.de. Inset: Location of the study area (dot) within Bavaria and Germany.

Assessment of nutritional status

Foliar samples were collected - according to common practice (Wellbrock *et al.*, 2006) - from different areas of the sun crown at the optimum time for sampling (end of July 2010) when leaves were fully developed and not yet affected by senescence or relocation of nutrients. 10 leaves from every tree at each location were used in a mixed sample to characterise conditions at the site. Adhering coarse particles were washed off by demineralised water. The leaves were dried (65 °C) for 2 days in an oven and subsequently milled. After digestion with HNO_3 (65 %) concentration of the elements phosphorus (P), potassium (K), sulphur (S), boron (B), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) was determined using ICP-OES (Spectro, Kleve, Germany). Total N was measured using an elemental analyzer (Elementar, Hanau, Germany). Nutritional status was assessed using the statistical method of Göttlein *et al.* (2011) based on the data for birch listed in the literature compilation of van den Burg (1985, 1990). We obtained nutrient threshold values for normal, excess and deficient nutrient supply.

Correlation and regression analyses

In order to reveal the relationship between phenological onset dates of birch and tissue nutrient content, we calculated partial correlations with the urban index as a control variable. Since this index is an estimate of the degree of urbanisation and therefore probably includes *inter alia* temperature and pollution effects, artificial correlations attributable to the site selection are eliminated. For example: The inner city of Munich is not only characterised by a greater amount of calcareous soil but also by higher temperatures compared to the northern countryside. A plain correlation analysis might therefore only reveal that the nutrient Ca is positively correlated with temperature and therefore negatively correlated with onset dates.

The nutrients with the highest, and most often significant, correlation with all phenological phases across the two study years were selected for subsequent multiple regression analysis. Linear regression analyses of phenological onset dates were used to identify the amount of variance explained (r^2) by mean temperature in March and April. In a further step we extended this model using the backward selection method to identify which nutrients were the most important explanatory variables in addition to temperature. For parsimony and in order to avoid overfitting we included only those nutrients which resulted in an increase in adjusted r^2 values.

3.3 Results

Variance explained by temperature

Table 3.1 Linear regression analyses of different phenophases for birch on mean March and April temperature in Munich in 2010 and 2011 (N = 34) and for 2010 and 2011 combined (N = 68). BBCH 61: beginning of flowering, BBCH 65: full flowering, BBCH 10: mouse-ear stage, BBCH 11: first leaves unfolded, model fit r^2, significance: all P <0.001

Year		BBCH 61	BBCH 65	BBCH 10	BBCH 11
2010	r^2	46.7	47.3	46.4	45.0
2011	r^2	62.5	61.5	53.9	54.7
2010+2011	r^2	87.0	88.1	63.4	84.6

Table 3.1 displays the model fits (r^2) for flowering and leaf unfolding dates of birch on mean March and April temperature data. All linear regression models were highly significant ($P \leq 0.001$). Values of r^2 in 2010 varied between 45 % and 47 % and were slightly higher for flowering phenophases than for leaf unfolding phenophases. This also applied for 2011, however, with higher r^2 values ranging between 54 % and 62 %. When data from both years

were combined in a regression analysis, larger r^2 values were obtained (87-88 % for flowering phases and 63-85 % for leaf unfolding phenophases).

Nutritional status

The threshold values obtained by the statistical evaluation of the van den Burg dataset for nutrient supply of birch are shown in Table 3.2. Following Göttlein *et al.* (2011) the dataset was corrected for outliers and the respective threshold values were only calculated if at least three data records were available. This is the reason why some cells in Table 3.2 are empty. For N the “thinning effect” which is well known in forest nutrition studies (Larcher, 1994) can be seen where the threshold value for extreme deficit is slightly higher than the threshold for trees showing a marked deficit. The dominance of shallow soils on calcareous gravel is reflected by the nutritional status of the trees. Most trees showed an excessive supply with Ca, and consequently the majority of trees were classified at the lower end and below the adequate range regarding the microelements Fe, Mn and Zn. Also for S and N most trees were classified as latent deficient. For K, P, Mg, Cu and B most trees fell within the range of adequate nutrition. As far as assessable by Table 3.2, extreme nutrient deficiencies were not found and only a few trees showed a marked deficiency for some elements.

Influence of nutrient concentrations on onset dates

Table 3.3 presents the partial correlation coefficients (after fitting urban index as a control variable) between phenological onset dates for birch and foliar nutrient concentration. The most important variables (according to the size of the correlation coefficients and their significance) were K, B, Zn and Ca. For K in particular the coefficients were always significant at the 5 % level and ranged between -0.421 and -0.609, indicating that higher concentrations of K were associated with earlier flowering and leaf unfolding of birch. This relationship also applied to B; higher concentrations leading to earlier phenophases. For B, the coefficients ranged between -0.322 and -0.536 and with the exception of mouse ear stage in 2011 ($P < 0.10$) all were significant at the 5 % level. Correlation coefficients for Zn were also negative, but were smaller and in two cases failed to reach even marginal significance ($P > 0.10$). A similar situation existed for Ca although four cases failed to reach even marginal significance ($P > 0.10$). A significant ($P < 0.05$) relationship between phenology and Fe and Mg was only detected in a single case each. For the other nutrients no significant correlations were found. Thus, we decided to use K, B, Zn and Ca in subsequent multiple regression analyses.

Influence of nutrient concentrations on onset dates in addition to temperature

In 2010 r^2 in regression models was always lower than in 2011 (Fig. 3.2). Apart from one exception (full flowering in 2011), K was the most important nutrient variable entering the linear model after fitting mean air temperature. Zn entered the regression model as the second variable for full flowering in 2011 but was only marginally significant ($P < 0.10$). For the mouse-ear stage in 2010 and the beginning of leaf unfolding in 2011 Zn was added as a third variable but was only highly significant for the latter. Ca was only included in the model for the beginning of leaf unfolding in 2010 ($P < 0.10$). The increase in adjusted r^2 was smaller for flowering phenophases (+1.7 % to 3.8 %) than for leaf phenophases (+4.8 % to 9.8 %) and was especially high for the leaf phenophases in 2010. In summary, the incorporation of nutrients increased model fits (relatively) by up to one fifth of the r^2 using temperature alone. This was especially true for leaf phenophases (relative increase: + 9.1 % to 21.8 %).

Table 3.2 Foliar threshold values of nutrient supply of birch (deficit, adequate and excess range) and number (*N*) of sites that fell into the respective category

Nutrient	**unit**	**Deficency range**						**Normal range**						**Surplus range**			
		extre-me	***N***	**deficency**	***N***	**latent**	***N***	**lower**	***N***	**central**	***N***	**upper**	***N***	**luxury**	***N***	**extreme**	***N***
N	mg/g	<19.4	0	<19.2	1	19.2-24.6	18	24.6-25.8	6	25.8-30.2	9	30.2-33.4	0	33.4-37.9	0	>37.9	0
P	mg/g			<1.3	1	1.3-1.9	6	1.9-2.4	3	2.4-3.5	20	3.5-4.2	4	4.2-5.3	0	>5.3	0
K	mg/g	<3.6	0	<5.4	1	5.4-7.2	4	7.2-8.9	10	8.9-11.9	14	11.9-13.1	4	13.1-31.0	1	>31.0	0
Ca	mg/g					<4.4	0	4.4-6.2	0	6.2-9.5	3	9.5-11.1	4	>11.1	27		
Mg	mg/g	<1.1	0	<1.2	0	1.2-2.0	2	2.0-2.5	5	2.5-3.5	14	3.5-4.1	11	>4.1	2		
S	mg/g			<1.4	2	1.4-1.9	20	1.9-2.0	7	2.0-2.6	5	2.6-3.1	0	>3.1	0		
Fe	µg/g					<85	32	85-99	2	99-144	0	144-175	0	>175	0		
Mn	µg/g	<12	0			<142	33	142-377	1	377-783	0	783-954	0	>954	0		
Cu	µg/g					<5	4	5-6	6	6-8	24	8-9	0	>9	0		
Zn	µg/g					<176	16	176-374	16	374-773	2	773-973	0	>973	0		
B	µg/g		0			<26	1	26-38	10	38-53	15	53-56	2	56-300	6	>300	0

Table 3.3 Partial correlation analyses with urban index as the control variable between different phenophases of birch in Munich 2010 and 2011 and foliar nutrient concentrations. BBCH 61: beginning of flowering, BBCH 65: full flowering, BBCH 10: mouse-ear stage, BBCH 11: first leaves unfolded, *r* correlation coefficient, *P* significance, bold / dark grey (italic / light grey) values indicate significant (marginally significant) correlations at the 5 % (10 %) level

	BBCH		**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Fe**	**Mn**	**Cu**	**Zn**	**B**
2010	**61**	*r*	0.069	-0.057	**-0.421**	-0.224	-0.098	-0.113	-0.042	0.056	-0.013	-0.288	**-0.396**
		P	0.703	0.752	0.015	0.210	0.587	0.531	0.818	0.756	0.942	0.104	0.023
	65	*r*	0.023	-0.129	**-0.473**	-0.281	-0.123	-0.115	-0.270	-0.080	-0.089	*-0.293*	**-0.429**
		P	0.898	0.475	0.005	0.113	0.495	0.524	0.128	0.657	0.623	0.098	0.013
	10	*r*	0.089	-0.071	**-0.547**	**-0.463**	-0.130	-0.076	**-0.420**	-0.053	-0.083	**-0.448**	**-0.487**
		P	0.621	0.694	0.001	0.007	0.472	0.675	0.015	0.768	0.644	0.009	0.004
	11	*r*	0.155	-0.140	**-0.510**	**-0.467**	-0.280	-0.063	-0.242	-0.026	0.023	**-0.401**	**-0.512**
		P	0.388	0.436	0.002	0.006	0.114	0.730	0.175	0.885	0.900	0.021	0.002
2011	**61**	*r*	-0.247	*-0.305*	**-0.605**	-0.269	-0.179	*-0.321*	-0.221	0.125	-0.220	*-0.295*	**-0.417**
		P	0.166	0.084	0.000	0.129	0.320	0.069	0.216	0.490	0.218	0.095	0.016
	65	*r*	-0.187	*-0.304*	**-0.511**	**-0.404**	**-0.377**	-0.232	*-0.343*	-0.036	-0.207	**-0.403**	**-0.407**
		P	0.298	0.086	0.002	0.020	0.030	0.193	0.051	0.842	0.248	0.020	0.019
	10	*r*	-0.074	-0.185	**-0.576**	-0.226	-0.038	-0.204	-0.200	0.174	-0.095	-0.220	*-0.322*
		P	0.683	0.301	0.000	0.206	0.835	0.255	0.265	0.332	0.599	0.218	0.068
	11	*r*	-0.087	-0.254	**-0.609**	**-0.405**	*-0.297*	-0.226	*-0.318*	-0.070	-0.129	**-0.487**	**-0.536**
		P	0.631	0.154	0.000	0.019	0.093	0.206	0.071	0.697	0.475	0.004	0.001

3.4 Discussion

Air temperature measured at the birches was able to explain between 43 and 61 % of the variance in onset dates in 2010 and 2011; similar to recent studies in urban phenology. For example, modelling of *Prunus yedoenis* (Yoshino cherry) flowering onset dates in Tokyo, Japan, by mean March temperature in 2004 revealed an r^2 value of 52 % (Matsumoto *et al.*, 2009). Lakatos & Gulyás (2003) reported an r^2 value of 42 % for onset dates of full flowering of *Forsythia suspensa* (forsythia) and urban heat island intensity in Debrecen, Hungary, in spring 2003. These relatively modest r^2 values may probably be related to smaller temperature variations between the selected sites within one observation year. Furthermore, the year 2010 had smaller r^2 values than 2011, which might be attributable to short-lasting weather conditions that could be better reflected in temperature variables at a finer temporal resolution than a calendar month (Jochner *et al.*, 2011). Only by analysing both years within a single regression analysis 63 to 88 % of the variance could be explained by air temperature.

In order to further explain variance in onset dates we analysed foliar nutrient concentrations. For N, Cu and Mn we did not find statistical relationships with phenological onset dates. For P and S only some very weak correlations were found, and for Mg and Fe only one significant correlation. All the elements with a higher number of significant partial correlations - K, B, Zn, Ca (Table 3.2) - have an influence, directly or indirectly, on cell extension as well as on membrane function and stability (Marschner, 1995).

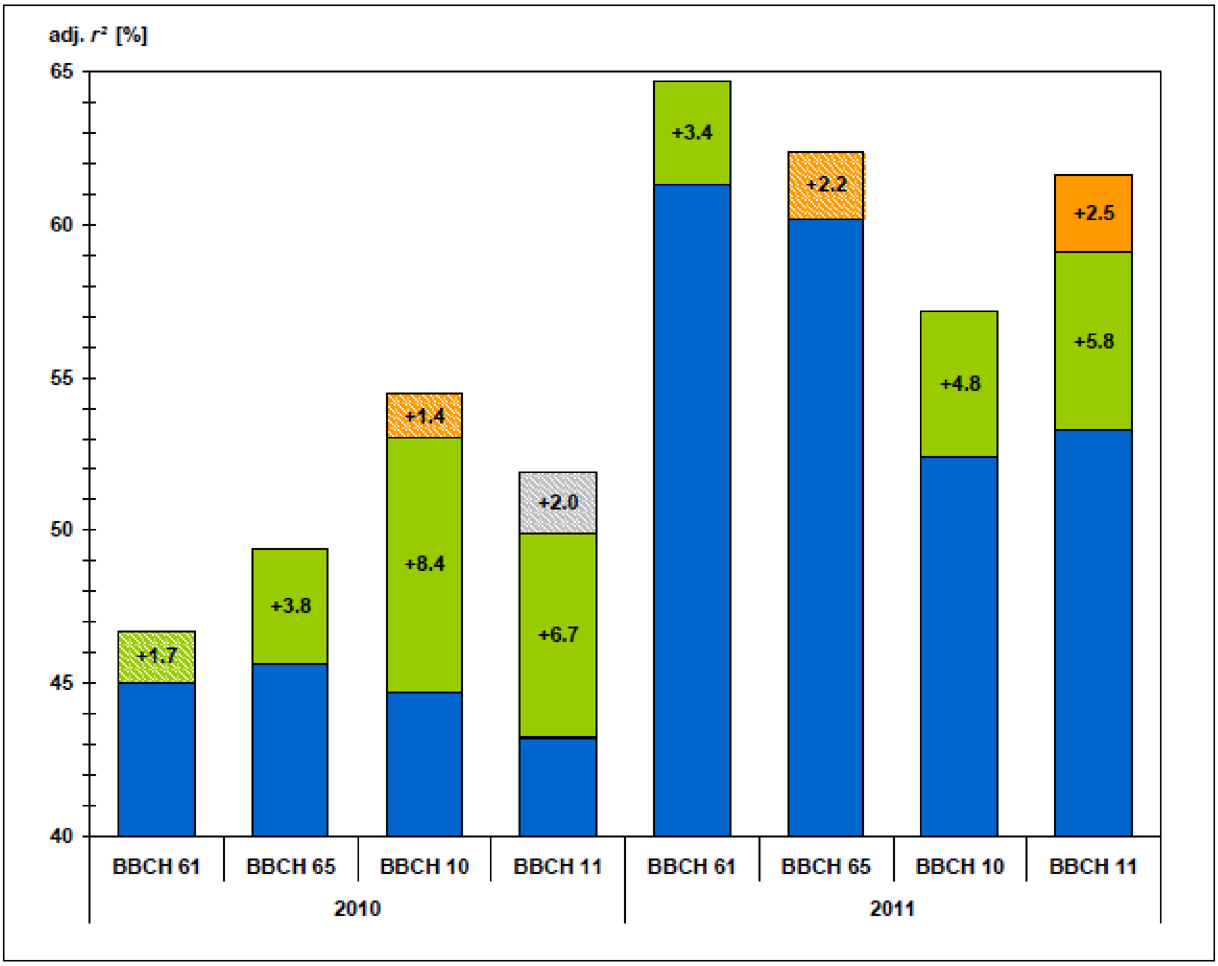

Fig. 3.2 Increase of adjusted r^2 values in regression models based on air temperature when adding nutrient variables (blue = mean temperature in March and April, green = foliar concentration of potassium (K), orange = foliar concentration of zinc (Zn), grey = foliar concentration of calcium (Ca)) for flowering (BBCH 61: beginning of flowering, BBCH 65: full flowering) and leaf unfolding (BBCH 10: mouse-ear stage, BBCH 11: first leaves unfolded) of birch in Munich 2010 and 2011. Variables were added until adjusted r^2 reached a maximum. Explanatory variables which were only significant at the 10 % level are shown dashed.

Their influence varied between years and between flowering and leaf unfolding phenophases. In all cases the observed relationship showed that an increase of the foliar nutrient concentrations of K, B, Zn and Ca was associated with an advance in phenological onset dates, with K being the nutrient having the highest influence on phenology, especially on leaf unfolding. K is characterised by a high mobility in plants and, in addition to other physiological functions, it contributes most to the osmotic potential of cells and tissues. Increasing the osmotic potential in the vacuoles is essential for cell extension (Marschner, 1995). In contrast to our findings where we could demonstrate that K had the strongest effect on phenological onset dates, Nord & Lynch (2009) proposed that deficiencies in K are less likely to affect the phenology or growth of plants. Flushing of leaves is a turgor-driven process that is initiated in spring by bud burst whereby a high amount of K might be supportive of rapid development. Catkins of birch, however, are already formed in late summer or early autumn, long before leaf expansion. With increasing temperature, anthers will extend and consequently pollen sacs will emit pollen whereas relative humidity plays an important role on the dehiscence of the sacs (Laaidi *et al.*, 2003). Thus, K should have a greater effect on leaf unfolding phenophases of birch than on flowering phenophases, as shown in our study. Note that these different phenophases occur within the same week (flowering earlier than leaf unfolding by an average of 2 days).

Zn deficiency results in growth depression, mainly inhibition of internode elongation and reduced leaf size (Marschner, 1995). The availability of Zn is limited in alkaline soils where deficiency of these elements can occur (Nord & Lynch, 2009). In our study about half of the investigated birch trees showed Zn deficiencies (according to Table 3.2). The observed correlation of Zn concentration and phenology confirms the beneficial role of Zn for functioning and growth of shoots. Although Zn and phenological onset dates of birch were strongly related according to the partial correlation analysis (Table 3.3), it was only selected three times in the multiple regression analysis: once in 2010 for mouse-ear stage, twice in 2011 for full flowering and beginning of leaf unfolding.

During cell elongation Ca stimulates the synthesis of cell wall precursors. Furthermore, Ca is of high importance for membrane stability and functionality (Marschner, 1995). Although a relationship between Ca and phenological phases was indicated by the partial correlation (Table 3.3) its importance in phenology at the mesoscale was only confirmed in a single case (leaf unfolding in 2010) using the multiple regression approach. The reason is that our study area is located on calcareous gravel so that plants do not have problems with Ca supply (see also Table 3.2).

B is involved in a number of metabolic pathways and plays a major role in cell wall biosynthesis and structure as well as plasma membrane integrity, with B deficiency in the shoot inducing symptoms similar to Zn deficiency. Both elements, Zn and B, have an influence on the metabolism of indole-3-acetic acid (IAA), a phytohormone which plays an important role in cell division and elongation (Marschner, 1995). The relationship of phenological onset dates of birch and the nutrient B became obvious in the partial regression analysis. However, B was always excluded from the multiple regression analyses.

Because the leaf concentration of K is correlated or marginally correlated with that of B (r = 0.600, $P \leq 0.001$), Ca (r = 0.332, P = 0.055) and Zn (r = 0.450, P = 0.008) K showed the highest predictive power in multiple regression analysis and in many cases prevented the other elements from being included in the models. However, at sites with different nutrient availability this behaviour needs not to be the case.

A literature search revealed conflicting findings on the effects of soil fertilisation on phenology. Roberntz (1999) and Amundson *et al.* (1995), for example, found no effect on bud burst of Norway and Red spruce growing on fertilised soils. However, Chandler & Dale (1990) reported an advancement of bud burst of fertilised Sitka spruces by three weeks. Earlier bud burst was also demonstrated by Amundson *et al.* (1995). In contrast, Wielgolaski (2001), who incorporated analyses of available soil nutrients without additional fertilisers, reported high levels of P, K, Mg and Ca in the soil that delayed plant development. This was especially true for early phenophases, but not for phases that occurred later in the season (e.g. cultivated plants) and strongly depended on high temperatures. Therefore, the author concluded that the influence of nutrients measured in the soil was moderated by climatic factors. This finding is also in agreement with Nord & Lynch (2009) who proposed that higher temperatures, *via* altering soil moisture, lead to higher mineralization rates and therefore affect nutrient availability. This confirms our suggestion that soil nutrient availability can not adequately reflect the physiological performance of a tree and supports the evaluation of foliar nutrient concentrations.

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4 THE INTEGRATION OF PLANT PHENOLOGY AND LAND USE DATA TO CREATE A GIS-ASSISTED BIOCLIMATIC CHARACTERISATION OF BAVARIA

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Abstract

Background: Since phenology is a good bio-indicator of temperature the latter is often used in phenological analyses. However, whilst meteorological data are difficult to interpolate from point measurements at the desired resolution, region-wide digital data, e.g. elevation models or land cover data, are usually readily available.

Aims: The potential of environmental variables, other than meteorological data, to create a bioclimatic classification of landscapes at the mesoscale was tested by the joint use of spatial data and the flowering dates of *Forsythia suspensa* at 70 phenological stations in southern Bavaria, Germany.

Methods: A linear discriminant analysis was carried out to identify relevant land use variables that were correlated with phenology and, using these results, to regionalise the observed flowering dates within the framework of a Geographic Information System.

Results: The generated map represented dates of onset at the regional scale, mostly influenced by the extent of impervious (hard, sealed) surfaces and forest, altitude and distance to urban areas. Thus, we overcame restrictions resulting from the difficulties of spatially interpolating available climatological data, and from the limited number of phenological datasets.

Conclusions: We demonstrated that the selected variables were capable of adequately modelling regional bioclimatic zonation, and that phenology was a useful proxy of regional climate variation arising from both natural and anthropogenic factors.

Keywords. Bioclimatic zonation, discriminant analysis, GIS, landscape phenology, land use, mesoscale, modelling, regionalisation.

4.1 Introduction

The growth of plants in mesic and temperate regions is predominantly controlled by air temperature, making the latter the most important variable in the analysis of phenological data. Temperature has been shown to be the most important variable influencing the onset of phenological dates in a large number of published studies (e.g. Schwartz and Reiter 2000; Fitter and Fitter 2002; Zhang et al. 2004; Menzel et al. 2006). Spring phenophases are particularly sensitive to temperature (Fitter and Fitter 2002). Another essential factor driving spring phenology in temperate regions and higher latitudes is photoperiod (Leopold 1951; Levy and Dean 1998). Its importance has been demonstrated in experimental studies (Saxe et al. 2001) and phenological models (Schaber and Badeck 2003); however, the influence of photoperiod is species-specific (Schaber and Badeck 2003; Badeck et al. 2004). While precipitation can play an important role in plant growth, e.g. along a coastal-continental gradient in hyperoceanic western Norway (Wielgolaski 2001), seasonal tropical forests (Rathcke and

Lacey 1985) and Mediterranean forests (Peñuelas et al. 2004), for mesic temperate regions, especially at the macroscale (e.g. Menzel et al. 2006), using temperature data alone to model seasonal plant development has shown to be sufficient.

Phenological maps (e.g. Ahas and Aasa 2001; Rötzer and Chmielewski 2001; Menzel et al. 2005) can be used to illustrate the movement (direction and speed) of seasonal progress (continuous phenological gradients) that allows an interpretation of greening-up across landscapes. The methods that have been used for such maps included spatial interpolation models (e.g. Ahas and Aasa 2001) or statistical models derived from multiple regression analyses (e.g. Rötzer and Chmielewski 2001; Menzel et al. 2005). In contrast to the above maps with continuous phenological gradients, the aim of our study was to determine and map bioclimatic zones that represent homogenous climatic characteristics. To this end, we tested the suitability of discriminant analysis and used non-meteorological spatial information for modelling the flowering onset of a test species, *Forsythia suspensa* (Thunb.) Vahl, a spring-flowering shrub. Since discriminant analysis is a new approach in phenological mapping, we demonstrate the suitability of this multivariate method not only to identify those variables which contribute most to differences among flowering dates, but also to use its statistical results for modelling the spatial distribution of areas of early, average or late flowering dates. As phenology is a good bio-indicator of temperature, we sought environmental variables that are known to be correlated with temperature and, therefore, also with phenological onset dates. These variables were derived from digital spatial data (elevation model and land use information) and managed by using a Geographic Information System (GIS) (ArcGIS 9.3, ESRI 2009). Our main motivation was to evaluate if the selected variables were able to represent mesoclimatic effects on phenology, and, in turn, overcome limitations resulting from (1) the difficulties of spatially interpolating available climatological data on a scale larger than the regional level and (2) the limited and spatially non-representative availability of most phenological datasets.

4.2 Material and methods

Study area and test species

This study focused on a 150 km x 150 km area (48.4 °N, 11.8 °E), centred around the city of Freising in southern Bavaria, Germany (Figure 4.1). We used a phenological dataset provided by the German Meteorological Service (DWD) containing onset dates of the flowering of *Forsythia suspensa* for the period of 1995-2008. *F. suspensa* is native to south-east Europe, but its planted distribution also spans southern Bavaria. It is widespread in urbanised areas, which, along with its temperature sensitivity, makes it a popular species in urban phenology and climatology studies (e.g. Bernhofer 1991; DWD 1991; Lakatos and Gulyás 2003).

Grouping variable and predictors

Data from 70 phenology stations were included in this study (Figure 4.1). In order to ensure representative mean values, only stations with at least 10 observations within the 14-year study period (1995-2008) were selected. The grouping variable *pheno* had three categories corresponding to early, average and late onset dates of flowering of *F. suspensa*. For each year the observed phenological dates, given as days of the year, at the 70 phenological stations were classified using the 33.3 and 66.6 percentiles as the upper limits for ‘early onset’ and ‘average onset’ categories, respectively. The final allocation of each station to one of the three categories was based on the most frequently occurring category across years.

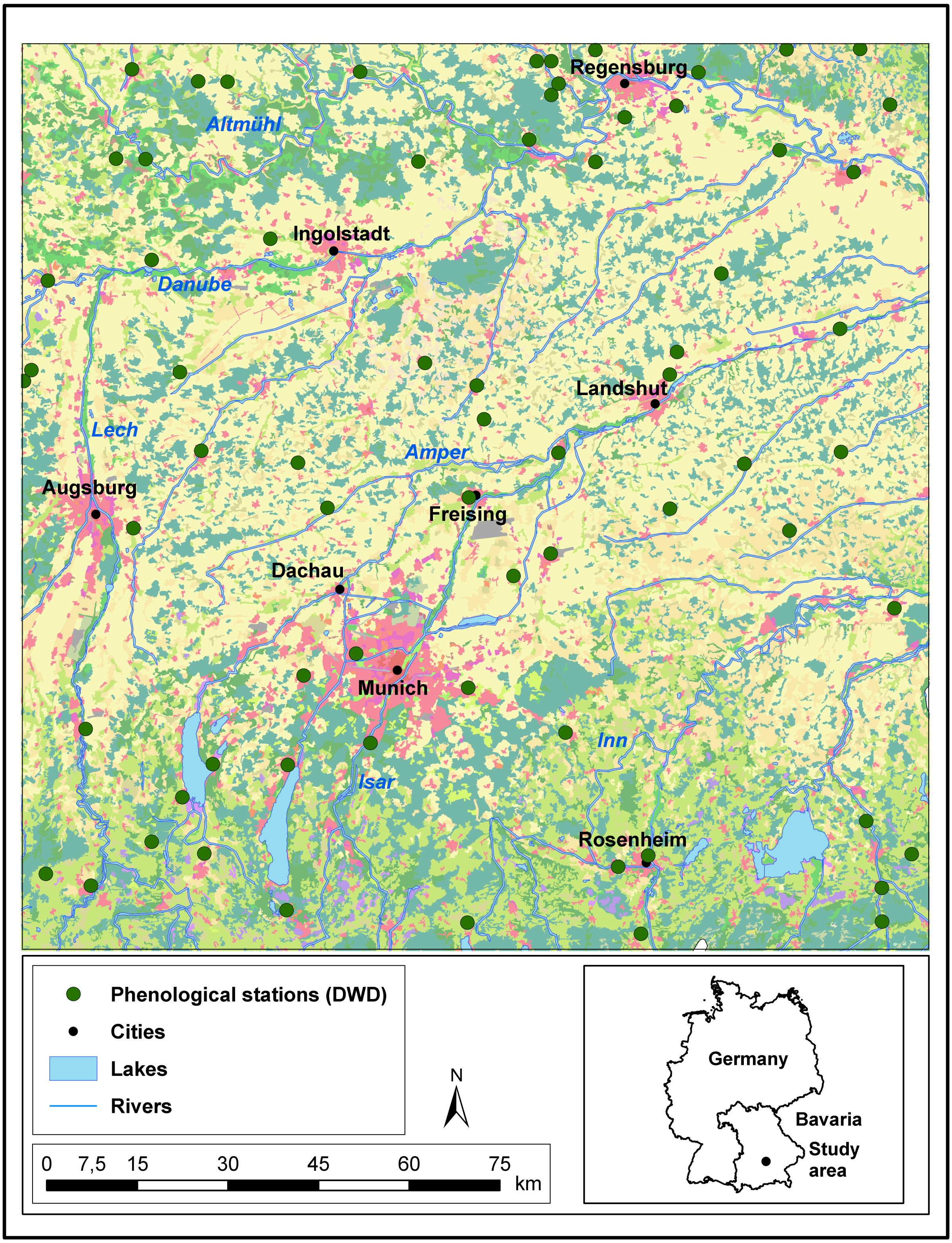

Figure 4.1 The 150 km × 150 km study area in southern Bavaria, Germany, centred on the city of Freising. Green circles: phenological stations (DWD, *n* = 70); background: CORINE land cover 2000 (EEA, 2000); major classes: red (urban fabric), green (forest and pasture), yellow (arable land), blue (rivers and lakes), for a complete legend see: http://dataservice.eea.europe.eu/dataservice/metadetails.asp?id=667; Inset: location of the study area (black dot) within Bavaria and Germany.

For 61 of the 70 stations, the most frequently observed onset category occurred in at least half of the observed years. This procedure was chosen in order to account for the temporal variability of climatic conditions from year to year (e.g. warm years vs. cool years). The group including the early onset of flowering of *F. suspensa* contained 26 stations, the average group 31 stations, and the late group 13 stations.

Potentially meaningful predictor (independent) variables were selected on a conceptual basis; those that represented conditions or processes known to be correlated with temperature and, therefore, phenology were sought, since they were most likely to vary significantly between different flowering date categories and could consequently improve discrimination (see Table 4.1).

Table 4.1 Predictors used in the discriminant analysis.

Predictors	**Description [unit]**	**Data source**
long	longitude [deg.]	DWD
lat	latitude [deg.]	DWD
alt	altitude [m a.m.s.l.]	derived from CORINE (EEA 2000)
seal	highly sealed soils within a radius of 2000 m [%]	derived from CORINE (EEA 2000)
dist_urban	distance to highly urbanised areas > 1 km² [km]	derived from CORINE (EEA 2000)
forest	forests within a radius of 2000 m [%]	derived from CORINE (EEA 2000)
dist_forest	distance to forests > 100 ha [km]	derived from CORINE (EEA 2000)
dist_water	distance to water bodies and rivers [km]	derived from CORINE (EEA 2000)

Elevation data for phenological stations (point values) were derived from the DWD dataset; while for the whole study area (raster), the SRTM 90-m digital elevation model (DEM) (Jarvis et al. 2006) was used. All other available data were prepared for the study area in a defined grid format of 200 m x 200 m. Land use data (see Figure 4.1) were derived from the seamless vector database CORINE Land Cover 2000 (CLC2000), a product from the European Environment Agency (EEA 2000).

The geographical locations of the phenological stations were not accurate since (1) the coordinates were given to degrees and minutes, (2) observations on individual plants were made with a radius of 1500-2000 m, and (3) the accuracy of recording station altitude was about 50 m. Thus, the exact locations of flowering records were not known. In order to account for this, the predictor variables were spatially averaged to represent the mean value within a radius of 2000 m around the coordinates of the phenological station.

Raster datasets for the variables *forest* and *seal*, representing the percentage of forest and impervious (hard, sealed) areas, respectively, were calculated using the CLC2000 dataset and a circular moving window with a radius of 2000 m (spatial analyst tools: neighbourhood, focal statistics). Focal statistics include any cells in processing the neighbourhood whose centre falls inside the radius (i.e. 2000 m) of the circle. The variable *seal* was defined as the percentage of highly sealed areas within a radius of 2000 m. It was assumed that land cover types such as continuous and discontinuous urban fabric as well as industrial and commercial units were associated with a high degree of impervious surfaces. An identical procedure was used for the variable *forest* (amount of forested areas within a radius of 2000 m). Urban areas, forests, and water bodies were selected from the CLC2000 data, and Euclidean distance grids were created (spatial analyst tools: distance, Euclidean distance) to derive the distance from each raster cell to the nearest forest >100 ha, urban area > 1 km² or water body (named *dist_forest*, *dist_urban*, *dist_water*, respectively). Cities with a continuous urban fabric greater than 1 km² included Munich, Augsburg and Regensburg (according to CLC2000; EEA 2000).

These predictor variables (*alt*, *seal*, *forest*, *dist_urban*, *dist_forest* and *dist_water)* together with the spatial coordinates longitude (*long*), latitude (*lat*) as well as altitude (*alt*) were tested for normality using the Kolmogorov-Smirnov test. Significant deviations from the normal distribution were found for the predictors *seal, dist_forest* and *dist_water*. The variables were used untransformed in these analyses since the suggested inverse transformation for L-shaped and positively skewed distributions (Tabachnick and Fidell 1989) did not lead to normality. However, discriminant analysis is reported to be relatively robust against violations of the assumption of normality (Lachenbruch and Goldstein 1979).

Discriminant analysis

Discriminant analysis (Fisher 1936) allocates a set of observations with *n* attributes (predictor variables) into two or more pre-defined classes (categorical grouping variables). The classification is achieved by means of a discriminant function (Equation (1)) that also allows the allocation of observations with an unknown group membership. For multiple group classification ($n > 2$), discriminant analysis is based on $n - 1$ discriminant functions.

$$d = \mathrm{b}_1\, x_1 + \mathrm{b}_2\, x_2 + \ldots\ \mathrm{b}_n\, x_n + \mathrm{a} \tag{1}$$

where x_1 to x_n are predictors, b_1 to b_n are discriminant coefficients, a is a constant and d is a discriminant or latent variable.

Discriminant analysis not only classifies cases but also assesses differences within and between groups, and the relative importance of the predictors in the classification. To produce a concise model and avoid overfitting, variables that did not contribute significantly to discriminating among the different groups were omitted.

Statistical significance of the discriminant model was tested by Wilks' Lambda (Λ), which measures the proportion of the total variance in the discriminant scores that is not explained by variations between groups:

$$\Lambda = W/(W+B) \tag{2}$$

where Λ is Wilks' Lambda, W is within groups sum-of-squares and B is between groups sum-of-squares.

The eigenvalue γ, also known as the characteristic or latent root, is a measure of the ratio of importance of the dimensions which classify cases of the grouping variable. It reflects the percentage of variance explained by the discriminant function(s) and indicates the relative discriminating power:

$$\gamma = W/B \tag{3}$$

where γ is eigenvalue, W is within groups sum-of-squares and B is between groups sum-of-squares. Smaller values of Wilks' Lambda signify a greater difference between the mean discriminant scores of the groups. The model discriminates significantly if the null hypothesis, that groups have the same mean discriminant function scores, can be rejected.

The association between the grouping variable and the discriminant function is shown by the canonical correlation, where a value close to unity indicates that the function discriminates well.

The results of the discriminant analysis are only valid for the used sample, but are essential for classifying new cases. The value of the discriminant functions can be further assessed by statistical cross-validation, where each case is classified in turn using a discriminant function excluding that case. For further information see e.g. Lachenbruch (1975), Lachenbruch and Goldstein (1979) and Backhaus (1990).

GIS-assisted regionalisation

The analytical results of the discriminant analysis were used to regionalise dates of onset of flowering of *F. suspensa*. A thematic map that modelled group categories (early, average, late date of onset) appropriate for forecasting and validation was developed. This approach is comparable to predictive mapping studies in vegetation science (e.g. Franklin 1995; Guisan and Zimmermann 2000; Miller et al. 2007), soil science (e.g. Scull et al. 2003) and geomorphology (e.g. Luoto and Hjort 2005; Marmion et al. 2009), where statistical relationships of spatial variables with the respective target variable (vegetation, soil, landform) are derived from point data and applied to a larger area. In our study we used phenological observation data (dependent variable) and additional spatial information such as altitude or land use data (predictor variables). The bioclimatic zonation was achieved from the predictor variables, available as raster-data.

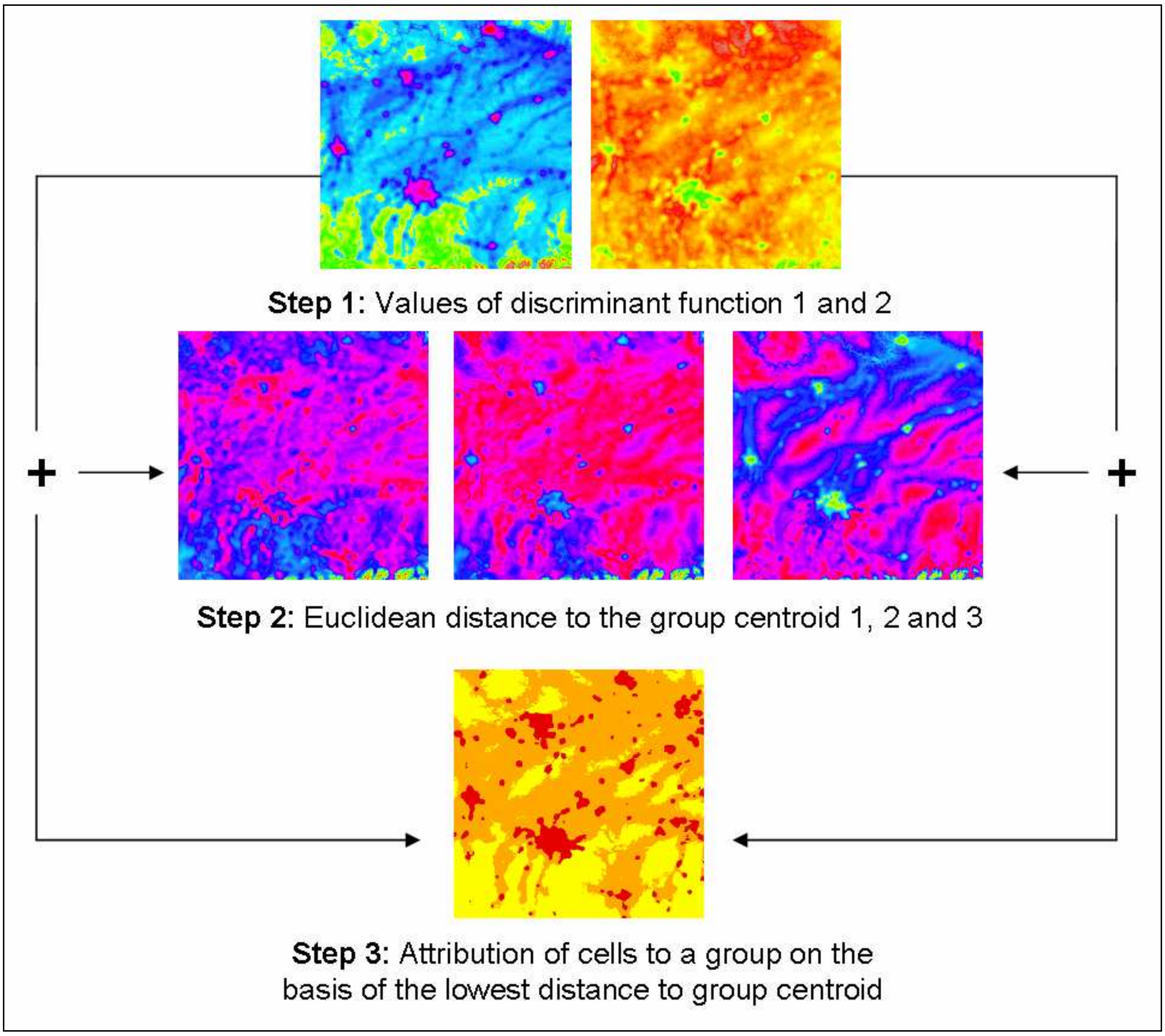

Figure 4.2 Using discriminant functions in a GIS framework to regionalise phenological group membership.

Using the values of the selected predictors at each phenological station, the discriminant analysis was carried out. In order to regionalise the phenological dates, the values of two (3 groups minus 1) discriminant functions, d_1 and d_2, were calculated for each raster cell (Figure 4.2, step 1). Phenological group membership (early, average, late onset) was assigned to each raster cell using a 'distance to group centroid' approach: three raster datasets containing the respective (Euclidean) distance of the function values to the centroids of the three phenological groups (Figure 4.2, step 2) were computed. Each raster cell was then allocated to the group whose centroid was closest to the respective values of d_1 and d_2 of the raster cell (Figure 4.2, step 3). The resulting raster map displayed the spatial distribution of early, average and late dates of onset of *F. suspensa* flowering.

4.3 Results

Correlations between variables were, in general, small (Table 4.2), but large correlations were found between *long* and *alt* (-0.786), and between *forest* and *dist_forest* (-0.534). The variables *long* and *lat* were excluded from further analyses, since their mean values did not differ significantly ($p < 0.1$) among the three groups (Table 4.3) and consequently did not contribute to the discrimination. The comparison of means (Table 4.3) showed differences between all groups for all other predictors with a small standard deviation in the majority of cases.

Table 4.2 Within-groups correlations for the predictor variables (bold indicates a correlation > |0.5|).

	lat	***alt***	***seal***	***dist_urban***	***forest***	***dist_forest***	***dist_water***
long	0.052	-0.786	-0.277	-0.372	-0.022	0.086	0.186
lat		-0.369	-0.029	0.266	0.105	0.104	-0.104
alt			0.222	0.160	0.079	-0.156	-0.066
seal				-0.132	-0.202	0.204	-0.195
dist_urban					0.003	-0.078	-0.087
forest						-0.534	-0.187
dist_forest							0.080

Table 4.3 Means (μ) and standard deviations (σ) of the predictor variables for the three groups (grouping variable *pheno* 1 = ‚early', 2 = 'average', 3 = 'late date of onset', *n* = number of cases, bold variable names indicate those that differed significantly ($P < 0.1$) between groups).

	***pheno* 1 (n=26)**		***pheno* 2 (n=21)**		***pheno* 3 (n=13)**		**overall (n=70)**	
	μ	**σ**	**μ**	**σ**	**μ**	**σ**	**μ**	**σ**
long [°]	48.39	0.35	48.55	0.43	48.47	0.61	48.48	0.44
lat [°]	11.60	0.56	11.81	0.54	11.88	0.78	11.75	0.60
alt [m a.m.s.l.]	457.38	61.71	463.71	91.74	589.23	120.45	484.67	100.50
seal [%]	28.76	19.62	13.04	14.93	4.17	4.51	17.23	18.17
dist_urban [km]	36.37	17.90	33.14	21.08	51.71	22.29	37.79	21.04
forest [%]	11.58	14.69	20.72	17.49	23.36	11.33	17.81	16.05
dist_forest [km]	1.72	1.47	1.15	1.33	0.65	0.35	1.27	1.32
dist_water [km]	2.27	2.37	2.23	2.13	4.15	3.67	2.60	2.63

Discriminant analysis

The estimation of the parameters of the two discriminant functions d_1 and d_2 resulted in:

$$d_1 = 0.008\,alt + 0.009\,forest + (-0.043)\,seal + 0.006\,dist\_urban + 0.097\,dist\_water + (-0.011)\,dist\_forest + (-3.888) \quad \textbf{(4a)}$$

$$d_2 = 0.003\,alt + (-0.014)\,forest + 0.041\,seal + 0.027\,dist\_urban + 0.172\,dist\_water + 0.016\,dist\_forest + (-3.511) \quad \textbf{(4b)}$$

The scatterplot (Figure 4.3) shows the values of the first and second discriminant functions for phenological stations and their group membership. The locations of group centroids indicated that function 1 separated groups 1 and 2 (early and average date of onset) from group 3 (late date of onset). The second function best separated group 2 (average) from groups 1 and 3 (early / late date of onset).

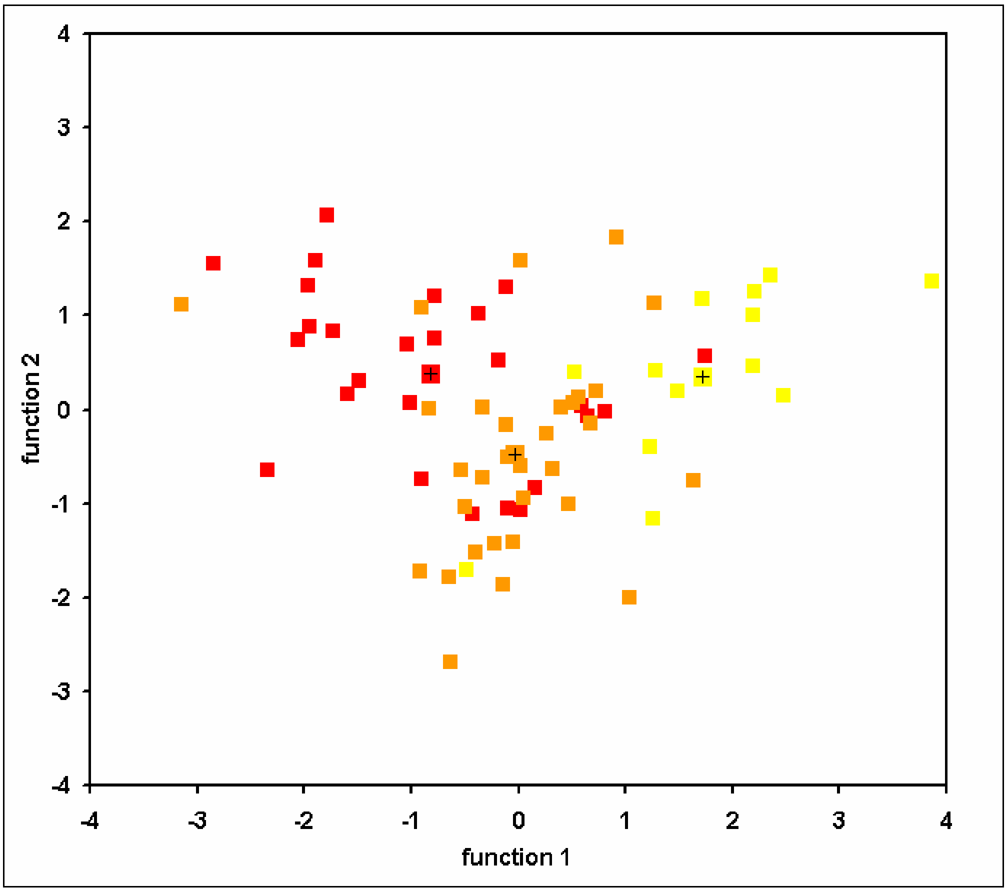

Figure 4.3 Scatterplot of discriminant values of function 1 and 2 for the phenological stations (*n* = 70) used in the analysis (red, early; orange, average; yellow, late onset of flowering) and corresponding group centroids (crosses).

Table 4.4 represents the structure matrix containing the correlations of the independent variables with the discriminant function values. The highest correlations with the first function were for *seal* (-0.608) and *alt* (0.595); other correlations were considerably smaller. The second function also correlated with *seal* and *alt*, but also with *dist_urban* (0.468) and *forest* (-0.414). Note that a bias might occur through collinearity between the variables, and for this reason the structure matrix should not be overinterpreted.

Table 4.4 Structure matrix: correlations of the predictor variables with the discriminant functions (bold text indicates the highest absolute correlation for each predictor).

Predictors	**Function 1**	**Function 2**
seal	**-0.608**	0.602
alt	**0.595**	0.465
dist_forest	**-0.329**	0.215
dist_water	**0.294**	0.288
dist_urban	0.306	**0.468**
forest	0.294	**-0.414**

Validation using statistical measures

The eigenvalue of the first function was 0.833 (Table 4.5), suggesting that the model had a good explanatory value compared to the second function (0.186).The first function explained 81.8% of the between-group variance. The canonical correlation of the first function was high (0.674) and indicated a good discrimination between the groups as well as a high explanatory power of the model. The canonical correlation of function 2 was somewhat smaller (0.396).

The value of 0.460 for Wilks' Lambda for all functions can be interpreted as a small within-groups variance, compared to the total variance (Table 4.6) and is highly significant. Wilks' Lambda for the second function was markedly greater (0.843) and only marginally significant ($P = 0.052$).

Table 4.5 Eigenvalues, per cent variances and canonical correlations of the discriminant functions.

Function	Eigenvalue	% of variance	Canonical correlation
1	0.833	81.8	0.674
2	0.186	18.2	0.396

Table 4.6 Wilks' Lambda and other test statistics for all discriminant functions (row 1) and for the second discriminant function only (row 2).

Test of function(s)	Wilks' Lambda	Chi-square	df	Significance
1 to 2	0.46	50.078	12	<0.001
2	0.843	10.987	5	0.052

Cross validation

The scatterplot (Figure 4.3) of the discriminant functions indicated that discrimination between groups 1 and 2 was less good and relied mostly on small differences in d_2. This was also reflected in the classification matrix (Table 4.7), showing absolute and relative frequencies of correctly and incorrectly predicted group memberships. Groups 1 and 3 were correctly classified in 65% and 69% of cases respectively, while 80% of group 2 were correctly allocated. In total, 72.9% of the cases were properly classified. A cross validation to better assess accuracy indicated 70% of all cases were correctly classified (Table 4.7). As random group allocation would have been expected to generate 19 to 44% proper allocation (prior probabilities from group sizes), the cross validation clearly confirmed the discriminating power of the model.

Table 4.7 Classification results derived from the entire dataset (72.9% of stations correctly classified) and by cross validation (70.0% of the stations correctly classified); grouping variable *pheno* 1, ‚early'; 2, 'average'; 3, 'late date of onset'. Correct classifications are shown in bold on the leading diagonal.

		Predicted group membership					
	pheno	**1**		**2**		**3**	
		Count	**%**	**Count**	**%**	**Count**	**%**
entire dataset	**1**	**17**	**65.4**	8	30.8	1	3.8
	2	4	12.9	**25**	**80.6**	2	6.5
	3	0	0	4	30.8	**9**	**69.2**
cross-validation	**1**	**16**	**61.5**	9	34.6	1	3.8
	2	4	12.9	**24**	**77.4**	3	9.7
	3	0	0	4	30.8	**9**	**69.2**

Based on the two discriminant functions, each raster cell was allocated to one of the three flowering groups (early, average, late) as shown in Figure 4.2. Figure 4.4 displays the spatial distribution of group membership. The areas where *F. suspensa* flowering was earliest are shown in red, followed by orange then yellow. For example, lower elevations in the larger valleys such as those of the Lech, Isar and Danube rivers generally exhibited earlier flowering than the higher areas in the foreland of the Alps (in the south of the map). The pattern also reflects land use, especially impervious surfaces, which was almost equally important and was highly correlated with the discriminant functions (Table 4.4). Larger cities such as Munich, Augsburg, Ingolstadt and Regensburg, as well as smaller towns such as Freising and Dachau are highlighted on the map, indicating an early flowering of *F. suspensa*. The red zone (early flowering) that spreads from the urban centres of Munich towards the north-east is characterised by several towns localised along Federal Highway (Bundesstraße) 11.

Regionalisation

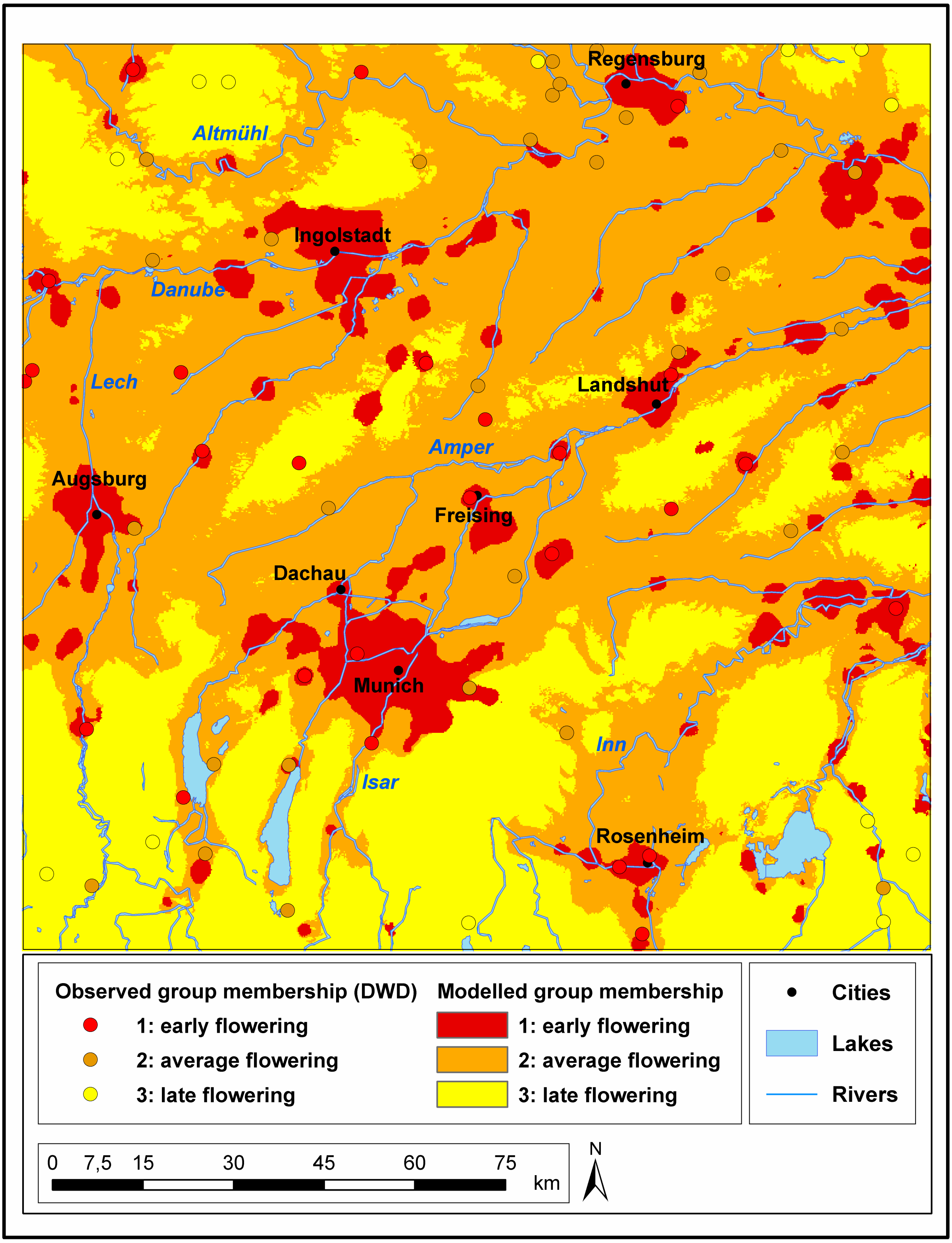

Figure 4.4 Modelled group membership of flowering of *Forsythia suspensa* (Thunb.) Vahl in southern Bavaria. Phenological stations (circles, n = 70) are coloured by their observed group membership (red, early; orange, average; yellow, late date of onset).

4.4 Discussion

Spatial phenological pattern and influencing factors

The spatial distribution of the factors most affecting the discriminant function is clearly reflected in Figure 4.4 For example, the variable *alt*, whose correlation with phenology is well documented (Rötzer and Chmielewski 2001; Ziello et al. 2009).

Impervious surfaces affect transpiration and lead to a change in radiation balance (Landsberg 1981), and were represented in the analysis by the variable *seal*. The distance to a city centre is important on the microscale as well as on the mesoscale, and is known to be correlated with phenological dates of onset (Zhang et al. 2004; Mimet et al. 2009). Therefore, urban areas, in particular urban heat islands, cause earlier occurrence of phenophases (Franken 1955; Karsten 1986; Koch 1972; Zacharias 1972; Bernhofer 1991; White et al. 2002; Zhang et al. 2004; Mimet et al. 2009). Such an effect could be clearly demonstrated in our analysis, where larger cities had an earlier onset of flowering of *F. suspensa*. However, this effect is also known in smaller towns, such as Freising, or Dachau (Figure 4.4; Rötzer 2007).

Lakes and rivers possess a high specific thermal capacity and hence reduce temperature amplitudes through their delayed warming and cooling (Fezer 1995). However, the variable *dist_water* did not have a marked effect on the onset of flowering in this study. In contrast, the variable *forest* appeared to be important. Forested areas may influence phenology in their vicinity through evapotranspiration and by providing shelter from wind (Adams 2007).

Scale dependency of predictor variables

Appropriate predictor variables may differ in respect to the scale used. None of the variables *long* (longitude) and *lat* (latitude) had sufficient discriminating power and were excluded from the analysis. Hence, the map shows no strict latitudinal gradient of phenology. A latitudinal gradient might only be expected for larger regions (e.g. Thompson and Clark 2006). In studies with a large spatial extent, for example Europe, latitude and longitude are often used in multiple regression analysis for regionalisation of flowering dates (Menzel et al. 2005). However, at the scale of our study, longitude (a proxy for distance to the ocean) and latitude (temperature decline toward the poles) (Rötzer and Chmielewski 2001) might not impact phenology.

Our study addressed the mesoscale. The variables *alt*, *seal*, *dist_urban*, *forest*, *dist_forest*, *dist_water* were selected since they were thought to be correlated with climatic conditions. The use of altitude can help to draw conclusions about the influence of variables that change with altitude in mountains, such as temperature, rainfall or solar radiation (Rötzer and Chmielewski 2001). The importance of variables describing urbanisation (e.g. built-up area index) has also been demonstrated for larger regions, for example for the state of Bavaria (Rötzer et al. 1997), and were proved to be essential in our study in terms of the variables *seal* and *dist_urban*.

Our selected variables are easily derived from digital data (such as remote sensing land cover datasets), and updates can be readily incorporated into datasets. Since climate data (such as temperature) are not easily interpolated from point measurements at the desired resolution (Ashcroft 2006), it is considered an advantage of the approach introduced in this study that such data were not included. One can consider additional variables known to influence phenology at the local scale. For example, slope, aspect (e.g. Chen 1994) or potential insolation (e.g. Chytrý and Tichý 1998) could easily be derived from a DEM. However, we chose not to include these because the exact location of plant observations was unknown and the values of both the grouping variable and the predictors could be given only for the area around the phenological stations where the plant individuals used for the phenological observations may be located (a circle of 2000 m radius for the DWD stations). As topography may vary greatly within such an area (e.g. locations with very different potential insolation may be contained therein), averaging the DEM derivatives over this area does not seem to make sense. The spatial scale on which they influence phenology is considered too small even for the 90 m DEM used in this study. In contrast, the average elevation within the 2000 m circle gives a more meaningful terrain classification reflecting the dependence of mean air temperatures on elevation at the local to regional scale.

The most important variables in our study according to Table 4.4 were *seal*, *alt*, *dist_urban* and *forest*. In particular, *forest* and *dist_urban*, providing information on distance to large urban areas, have so far been neglected in phenological maps. Plant phenology can be seen as a reflection of complex changes of environmental factors (Defila and Jeanneret 2007). Therefore, it can be assumed that phenology is also affected by other variables whose acquisition, recording or quantification was not possible in this study. In addition to the selected variables, there is an array of other factors which could potentially contribute to an improved distinction of group membership. These include environmental factors such as solar radiation, precipitation, mechanical, chemical and physical soil characteristics (Wielgolaski 2001), available nutrients, pests, fertilisation, competition and pollutants (e.g. Rusterholz and Erhard 1998; Cape 2003) as exogenous variables, as well as genetics, plant age, and vigour (Baumgartner 1952). For example, the increasing number of cloned species planted in numerous International Phenological Gardens (Schnelle and Volkert 1957) allows a comparison of environmental effects free from genetic variation among individuals. More complex models, including more numerous and/or more sophisticated predictor variables, could be suitable for predicting phenology at the local scale. With more variables, however, additional issues such as statistical overfitting arise. We argue that a parsimonious model with a relatively small number of easily accessed variables is adequate for the regional scale.

Discriminant analysis versus regression analysis

Our study showed that more than 70% of the stations were correctly allocated into flowering onset groups. Hence, our model demonstrates a high degree of accuracy and is appropriate for regionalisation. Using discriminant analysis to differentiate among three groups, the map differs methodically from phenological maps that have been produced for Europe (Rötzer and Chmielewski 2001, Menzel et al. 2005) or Bavaria (Rötzer et al. 1997) by using multiple regression models. The scale applied in this study is neither continent-wide nor focused on the microenvironment, but is rather intended to illustrate mesoscale effects on phenology. The phenological map, shown for comparison in Figure 4.5, was generated by Rötzer et al. (1997) using regression analysis. It illustrates the spatial distribution of onset dates (1961-90) for the flowering of *Syringa vulgaris* L. (lilac), another spring-flowering shrub. When compared with Figure 4.4, similar patterns of flowering phenology can be observed. However, the comparison of statistical approaches is only valid if the models are driven by the same data. Neither the phenological data (species, period), nor the independent (predictor) variables were exactly the same. Nevertheless, both maps (Figures 4.4-4.5) provide information on the spatial distribution of onset dates of spring phenophases in southern Bavaria, mostly influenced by climatic conditions.

Our study demonstrates that plant phenology is useful as an indicator of regional climate variations caused by both natural and human factors. The model used in this study is, however, not suitable to assess potential direct impacts of climatic change. If climatic changes affect the region of interest homogeneously, then the target variable, which is not absolute (days of the year) but a relative measure (early, average, late), is not expected to change. In the case of a climatic warming trend, all phenological phases may possibly shift towards earlier dates of onset.

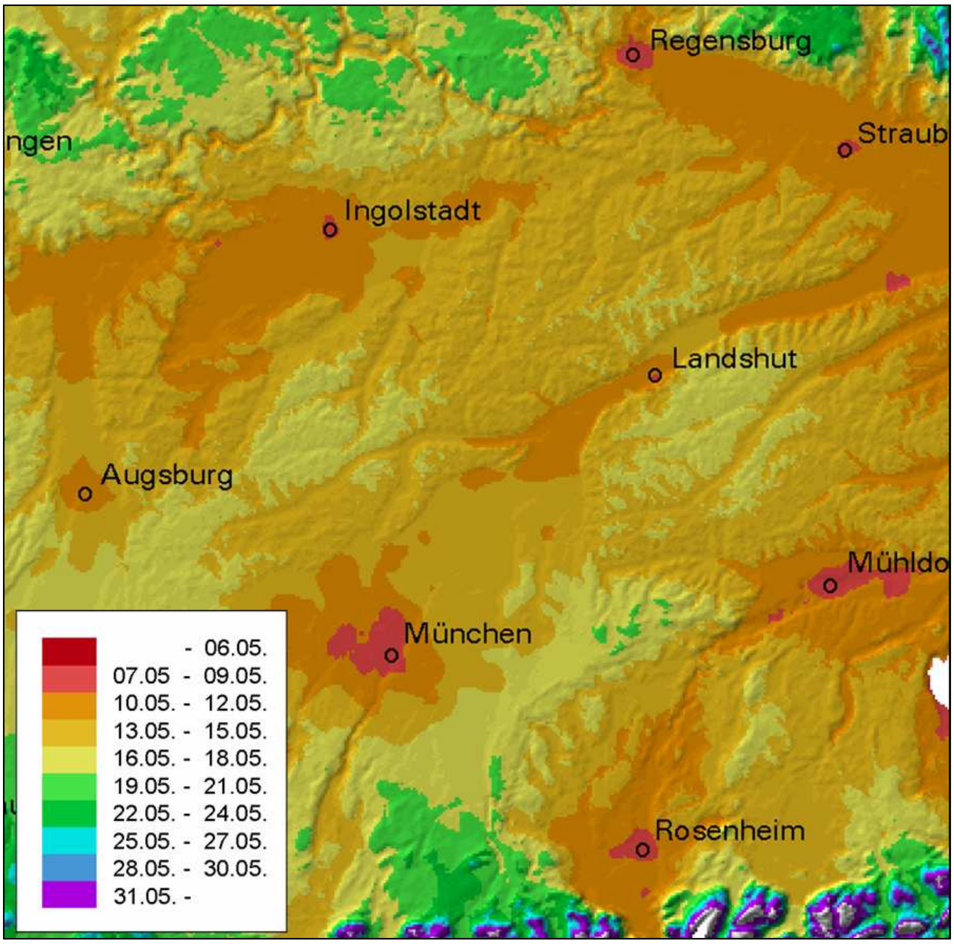

Figure 4.5 Phenological map of the onset of flowering of *Syringa vulgaris* L. (1961-90) prepared by Rötzer et al. (1997) using regression analysis. The original coverage of this map comprised the entire State of Bavaria.

4.5 Conclusion

Altitude, the proportion of impervious surfaces, the distance to highly urbanised areas, and the proportion of forested area were the most important predictors for phenological dates of onset in terms of their influence on mesoclimate. The phenological map (Figure 4.4), based on a novel method using discriminant analysis, generated a detailed bioclimatic zonation with the spatial pattern of early, average and comparatively late dates of onset for the flowering of *Forsythia suspensa*. This regionalisation made use of the association of the predictor variables with dates of onset recorded at 70 phenological stations. The approach presented in this study is also suitable for other areas, where predictor variables might have a higher or lower influence and other information could play an additional role (distance to the sea, latitude, etc). The underlying model provides opportunities for forecasting changes in phenology following land use change (but not climate change per se), since the predictor variables can easily be derived from, for example, updated remote sensing data.

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5 THE INFLUENCE OF ALTITUDE AND URBANISATION ON TRENDS AND MEAN DATES IN PHENOLOGY (1980-2009)

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Abstract

Long-term studies on urban phenology using network data are commonly limited by the small number of observation sites within city centres. Moreover, cities are often located on major rivers and consequently at lower altitudes than their rural surroundings. For these reasons, it is important (1) to go beyond a plain urban-rural comparison by taking the degree of urbanisation into account, and (2) to evaluate urbanisation and altitudinal effects simultaneously.

Temporal phenological trends (1980-2009) for nine phenological spring events centred on the German cities of Frankfurt, Cologne and Munich were analysed. Trends of phenological onset dates were negative (i.e. earlier onset in phenology) for 96% of the 808 time series and significantly negative for 56% of the total number. Mean trends for the nine phenological events ranged between -0.23 days year^{-1} for beech and -0.50 days year^{-1} for hazel. The dependence of these trends and of mean dates on altitude and on the degree of urbanisation was explored.

For mean dates we demonstrate an earlier phenological onset at lower altitude and with a higher degree of urbanisation: altitude effects were highly significant and ranged between 1.34 days (100 m)$^{-1}$ (beech) and 4.27 days (100 m)$^{-1}$ (hazel). Coefficients for the log-transformed urban index were statistically significant for five events and varied greatly between events (coefficients from -1.74 for spruce to -5.08 for hazel). For trends in phenology altitude was only significant for Norway maple, and no urban effects were significant. Hence, trends in phenology did not change significantly with higher altitudes / urbanised areas.

Keywords. Phenology; urban index; urban heat island; altitude; temporal trends; land use.

5.1 Introduction

Phenology, the science of recurring natural events, has attracted increasing interest in recent years. Several studies have been published whose main focus is the impact of global climate change on biological systems (Intergovernmental Panel on Climate Change 2007a, b; Parmesan and Yohe 2003; Root et al. 2003). With respect to the urban heat island effect, temperature in urban areas may serve as a proxy for future conditions and allow better prediction of future phenology from current information (Mimet et al. 2009; Neil and Wu 2006; Ziska et al. 2003). The urban climate is modified due to land use changes, such as the high amount of hard, sealed surfaces. This, together with the typical construction of urban buildings (e.g. skyscrapers), affects the radiation balance. Moreover, factors such as the lack of vegetation, heat waste and traffic contribute to higher temperatures (e.g. Kuttler 1998; Landsberg 1981; Matzarakis 2001; Oke 1987). Therefore, plants located in urban areas have to cope with warmer conditions compared to plants growing in rural areas. As a consequence, they tend to bloom earlier in spring. Terrestrial observational studies have been conducted in the German cities of Hamburg (Franken 1955), West Berlin (Zacharias 1972), Munich (Baumgartner et al. 1984) and Mannheim (Karsten 1986). These studies and those of other European cities, e.g. by Koch (1986), Bernhofer (1991), Defila and Clot (2003), Lakatos and Gulyás (2003) and Mimet et al. (2009) as well as studies in North America (e.g. White et al. 2002; Zhang et al. 2004) or Asia (e.g. Lu et al. 2006; Luo et al. 2007) confirm an earlier plant development in urban areas.

There are several ways to classify urban and rural sites. White et al. (2002) and Gazal et al. (2008) used satellite derived land cover classification, Rötzer et al. 2000 utilised *inter alia* present day and historical topographic maps and Zhang et al. (2004) not only employed MODIS NBAR spectral data but also nighttime light data and gridded population density data. Attempts to classify phenological sites by three levels of urbanisation were achieved by Pellissier et al. (2008) who used the proportion of six land use classes (surface water, woodland, cropland, grassland, roads, built-up areas) derived from digital orthophotos within 500 m x 500 m grid squares to obtain distinct urbanisation levels for the city of Rennes (France) by the use of principal components analysis (PCA). Their categorisation of urban, suburban and periurban sites was also adopted by Mimet et al. (2009) for the same study area. However, the classification of urban stations within existing phenological networks is not trivial due to the generally lower numbers of sites in very densely urbanised areas. Therefore, and in order to overcome the limited validity of a plain urban-rural dichotomy (that may induce unexplainable and inconsistent results in differences of plant phenology), continuous variables describing the extent of urbanisation are desirable. Rötzer et al. (1997), for example, calculated a building index on the basis of digital land use information that comprised a summation of raster elements with densely developed areas within a radius of 2.5 km. A further estimate of the degree of urbanisation was achieved by Shustack et al. (2009) who used orthophotos to evaluate the proportion of different cover types (forest, agriculture, mowed, paved, road) and the number of buildings within a radius of 1 km by means of a PCA. The first component of the PCA was defined as the index of urbanisation, where positive values indicated urban and negative values rural sites. Moreover, urban phenology is often affected by altitudinal influences on plant development since cities are frequently situated in major river valleys that feature lower altitudes than their rural surroundings. Therefore, it is reasonable to incorporate both urbanisation and altitudinal effects in phenological models.

In our study, we have decided not to use a classification based on the dichotomy “urban” and “rural”, but calculated a ratio scaled variable, the urban index (ui), which equates to the proportion of land associated with a high degree of impervious surfaces within a radius of 2 km. The phenological data used in this study are based on extensive, standardised, terrestrial, species-specific observations of the German Meteorological Service (DWD). We firstly describe trends and mean dates of nine spring phenophases during 1980-2009 centred on the cities of Frankfurt, Cologne and Munich. Subsequently, multiple regression analyses were conducted in order to answer the main questions of this study:

1) Is the mean date of onset of a specific phase a function of altitude and urbanisation (urban index), i.e. mean date~f(altitude, ui)?
2) Is the temporal trend in phenology of a specific phase a function of altitude and urbanisation (urban index), i.e. trend~f(altitude, ui)?

Although the urban heat island effect seems to be well documented by phenological data in the temperate zone, the pattern of urban-rural phenology deserves further investigation and principally, our urban index may better quantify the effects of land use changes on biological systems.

5.2 Material and methods

Study area

Areas selected for this study were centred within 50 km of three of the largest cities in Germany: Frankfurt, Cologne and Munich (Fig. 5.1; Table 5.1).

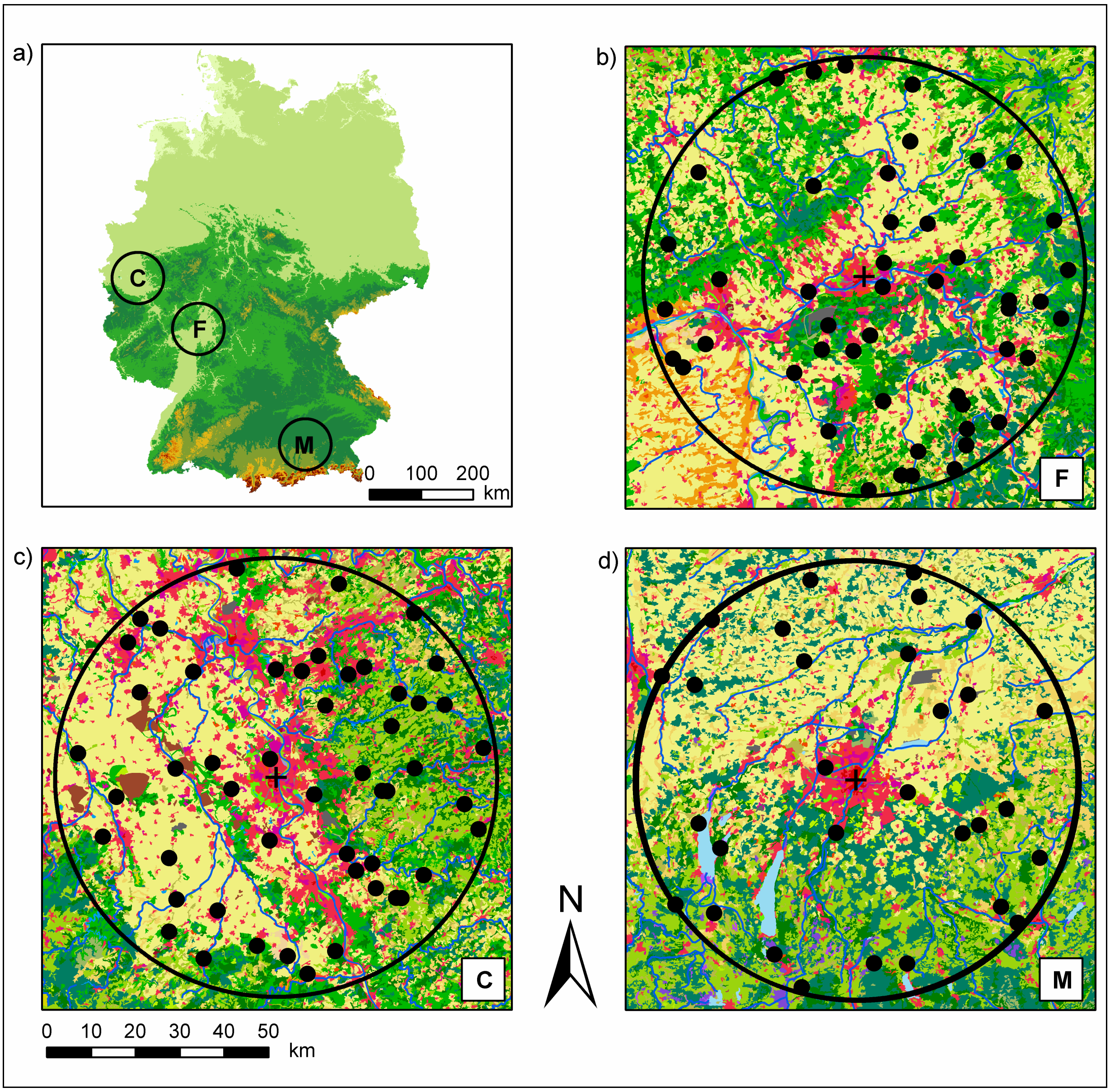

Fig. 5.1 a Locations of the three selected cities in Germany: *C* Cologne, *F* Frankfurt, *M* Munich; *background* digital elevation model (SRTM, Jarvis et al. 2006, see http://srtm.csi.cgiar.org/) **b-d** Survey area extending to 50 km: *black dots* phenological stations (DWD), *cross* city centres; *background* land cover (CORINE landcover 2000, EEA 2000); major classes: *red* urban fabric, *green* forest and pasture, *yellow* arable land, *blue* rivers and lakes; for complete legend see: http://dataservice.eea.europe.eu/dataservice/metadetails.asp?id=667

Urban index

We calculated an urban index (ui) based on CORINE Land Cover 2000 data (European Environment Agency 2000) using ArcGIS 9.3. This index reflects the proportion of predefined built up areas (e.g. continuous and discontinuous urban fabric, industrial or commercial units) within a radius of 2 km and thus can vary from 0 to 1; i.e. from a low (0) to a high (1) degree of urbanisation.

Table 5.1 Location of the centres of Frankfurt, Cologne and Munich, population size (2007) and characteristics of the phenological stations included: altitude [m a.s.l.], urban index, numbers (N) of phenological time series (1980-2009).

City	Location of the city centre			Population size	Altitude			Urban index			N
	Lat °N	Long °E	Alt		µ	Min	Max	µ	Min	Max	
F	50.12	8.69	112	652610	167	35	380	0.27	0	0.84	314
C	50.95	6.96	55	989766	192	90	460	0.22	0	0.65	300
M	48.14	11.58	528	1294608	526	420	730	0.19	0	0.76	194

The 2-km-radius was selected to ensure that mesoclimatic effects are covered by the index and extensive / thinly urbanised areas are identified as such. Therefore, the estimate for each phenological station concerning the degree of urbanisation could be obtained. Moreover, the geographical location of observed plants in the phenological dataset of the DWD is not exactly recorded, since the coordinates, specified in degrees and minutes, only indicate the centre of the observation area and phenological observers can search for individual plants of the respective species within a radius of 1.5 to 2 km around the coordinates of the phenological station.

Phenological data

Data for nine spring phenophases obtained from the phenological network of the DWD were selected: beginning of flowering of hazel (*Corylus avellana* L.), snowdrop (*Galanthus nivalis* L.), goat willow (*Salix caprea* L.), forsythia (*Forsythia suspensa* (Thunb.) Vahl), Norway maple (*Acer platanoides* L.), leaf unfolding of beech (*Fagus sylvatica* L.), flowering of apple (*Malus domestica* Borkh.), May sprout of spruce (*Picea abies* (L.) Karst.) and flowering of lilac (*Syringa vulgaris* L.).

These observations covered the 30-year period from 1980 to 2009 during which most of the recent temperature increase could be observed (Intergovernmental Panel on Climate Change 2007a). For inclusion, the only criterion was that phenological series had to contain more than 20 years of data. The number of stations per city differed according to the plants observed for each site. We could obtain a maximum number of 41 stations for Frankfurt, 45 for Cologne and 27 for Munich. The spatial distribution of the phenological stations is displayed in Fig. 5.1 b-d. In total, 808 phenological time series were obtained.

Mean onset dates and linear temporal trends (1980-2009)

Mean onset dates were calculated for each phenological time series (n=808) within the study period 1980-2009. Trends in phenological time series (1980-2009) were calculated by linear regression of date of onset on year for each series. Negative coefficients reflect an advance of the phenophase in days/year; positive values reflect a delay.

Multiple regression analyses

For each phenophase, mean onset dates were regarded as a function of altitude and urban index; i.e. mean date~f(altitude, ui) and submitted to a multiple regression analysis. The mean date of onset of the respective phenological phase was regressed on altitude and urban index; regression coefficients then equate to delays or advances expressed in days (100 m)$^{-1}$ or days ui^{-1} for altitude and urban index, respectively. Phenological trends (dependent variable) were also considered as a function of altitude and urban index (independent variables), i.e. trend~f(altitude, ui). Multiple regressions and interpretation of regression coefficients were as for mean date regressions in the previous paragraph. The cities do not differ significantly in their urban index values (P = 0.17). Consequently the use of the “altitude” term in our model incorporates both within- and between-city altitude effects. Thus, urban effects are assessed only after adjusting for both these effects.

All statistical analyses were conducted using SAS 9.2.

Transformation

Significant deviations from the normal distribution were found by the Kolmogorov-Smirnov test for the residuals resulting from the multiple regression analysis of flowering of *Salix caprea*. Therefore, the values of the urban index (which was also non-normally distributed) were converted using a $\log_{10}$-transformation for substantially positively skewed distributions (Tabachnick and Fidell 1989). Since values of the urban index can be zero, a constant was added that was calculated from the actual distribution of the data. According to Stahel (2008) we used the square of the first quantile of all values greater than zero divided by the third quantile (c=0.03363). The transformation was $\text{Lui}=\log_{10}(\text{ui} + 0.03363)$. All multiple regressions were recalculated using the transformed urban index (Lui) and none failed normality testing of residuals. These regression results are reported below.

5.3 Results

Linear temporal trends in phenology (1980-2009)

Table 5.2 comprises the linear temporal trends in phenology during the study period 1980-2009. Of 808 trends, 96 % were negative, and 56 % were significantly negative, pointing toward a clear and obvious advancement of phenological spring phases. The remaining positive trends (*n*=34) with two exceptions were not significant.

Table 5.2 Number of temporal linear trends in phenology (1980-2009) classified by direction and significance (sig $P \leq 0.05$, ns $P > 0.05$) for all phases with mean trend (days year^{-1}) and mean onset date (DOY), *SD* standard deviation, *FFD* first flowering date, *FLD* first leaf unfolding date, *MS* May sprout

Phase	**Number of trends**					**Mean trend days year^{-1} ± SD**	**Mean DOY ± SD**
	Total	**Negative trends (*n*=774)**		**Positive trends (*n*=34)**			
		sig	**ns**	**sig**	**ns**		
C. avellana FFD	102	42 (41%)	53	1	6	-0.50 ± 0.36	46.7 ± 10.8
G. nivalis FFD	111	53 (48 %)	49	0	9	-0.36 ± 0.29	50.3 ± 7.8
S. caprea FFD	105	36 (34 %)	63	0	6	-0.29 ± 0.24	76.4 ± 5.8
F. suspensa FFD	112	67 (60 %)	45	0	0	-0.44 ± 0.22	83.4 ± 7.3
A. platanoides FFD	71	52 (73 %)	18	0	1	-0.43 ± 0.26	100.8 ± 6.2
F. sylvatica FLD	78	47 (60 %)	26	1	4	-0.23 ± 0.17	115.5 ± 4.2
M. domestica FFD	55	37 (67 %)	18	0	0	-0.31 ± 0.21	117.0 ± 5.2
P. abies MS	78	51 (65 %)	23	0	4	-0.28 ± 0.18	123.6 ± 4.8
S. vulgaris FFD	96	68 (71 %)	26	0	2	-0.31 ± 0.20	125.4 ± 5.1
total	**808**	**453 (56 %)**	**321**	**2**	**32**	**-0.35**	**93.2**

The mean trends varied between -0.50 days year^{-1} for *Corylus avellana* and -0.23 days year^{-1} for *Fagus sylvatica*. The trends of each phase are plotted against their mean onset dates (1980-2009) in Fig. 5.2 and show that the more pronounced trends were generally detected for early phenophases and vice versa. However, the relationship between mean timing in spring and the respective advancement seems to be highly variable.

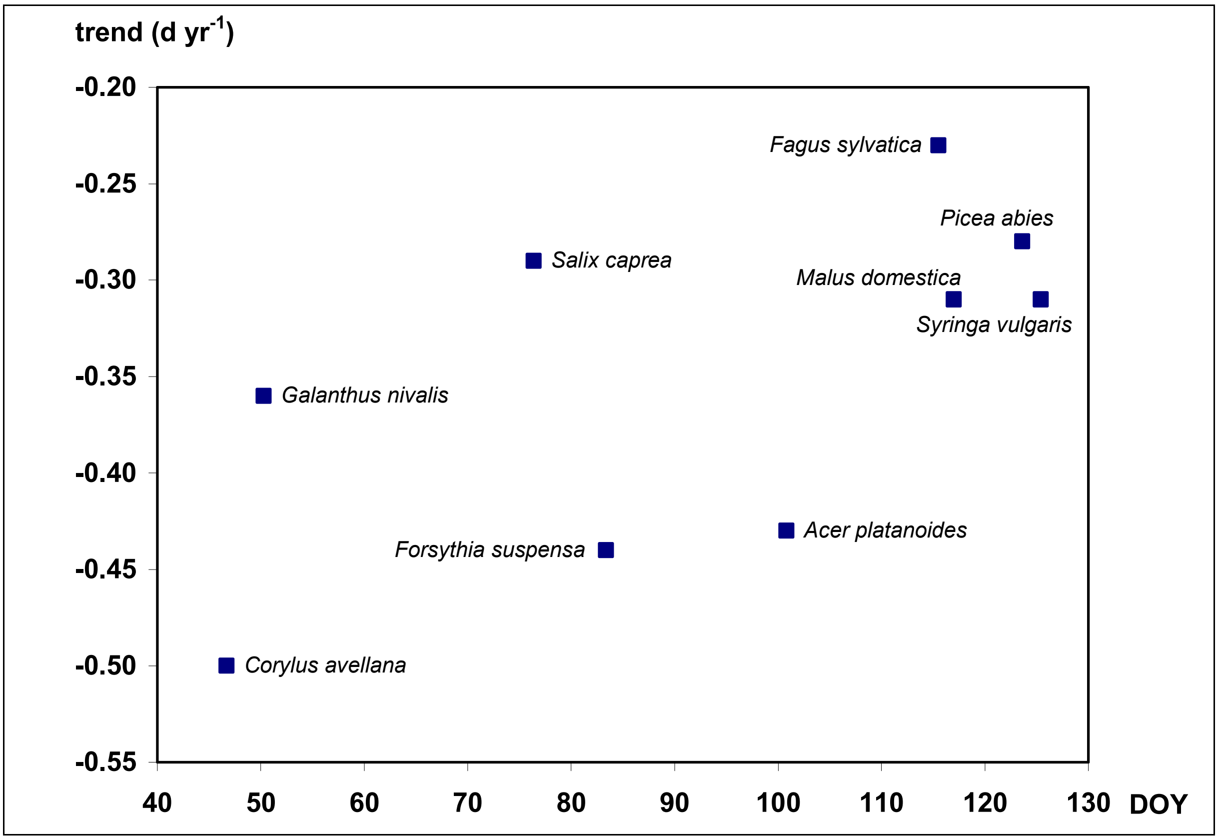

Fig. 5.2 Linear trends (days year^{-1}) for 1980-2009 versus mean onset dates (DOY) calculated for nine spring phenophases in the greater areas of three German cities (Frankfurt, Cologne and Munich)

Influence of altitude and urbanisation on mean onset dates

Mean onset dates, regressed on altitude and urbanisation are reported in Table 5.3. The model fits (R^2) ranged from 0.332 for *Salix caprea* to 0.710 for *Forsythia suspensa* indicating a medium to high degree of model accuracy. Coefficients for altitude were positive throughout and highly significant (all $P < 0.001$) indicating that mean onset dates are clearly influenced by the altitude of the stations. The highest coefficient was detected for *Corylus avellana* (4.27 days (100 m)$^{-1}$) and the lowest coefficient for *Fagus sylvatica* (1.34 days (100 m)$^{-1}$). Coefficients for the transformed urban index were negative throughout, ranging from -5.08 days Lui^{-1} for *Corylus avellana* to -1.74 days Lui^{-1} for *Picea abies.* These coefficients suggest that a transition from completely rural (ui = 0) to completely urban (ui = 1) equates to approximately 2.6- to 7.6-day advance in phenology. The coefficients were significant ($P \leq 0.05$) in five out of nine cases, i.e. for flowering of *Corylus avellana, Acer platanoides, Forsythia suspensa, Malus domestica* and *Syringa vulgaris*. Leaf unfolding of *Fagus sylvatica* was marginally significant at $P \leq 0.10$ and the remaining phases (flowering of *Galanthus nivalis*, *Salix caprea* and May sprout of *Picea abies*) slightly exceeded the 10 % level. The consistency in the direction of both coefficients of the multiple regressions confirmed an earlier onset with (1) lower altitude and (2) a higher degree of urbanisation.

Influence of altitude and urbanisation on linear trends

Table 5.4 shows the results of the multiple regression analyses for the nine selected spring phenophases of linear trends in phenology for each station (dependent variable) on altitude and urban index (independent variables). The models had small R^2 values ranging between 0.002 for *Galanthus nivalis* and 0.217 for *Acer platanoides*. The significance of most coefficients exceeded $P = 0.05$. Trends were influenced by altitude ($P < 0.001$) only for *Acer platanoides* and no urban index effects were significant ($P > 0.05$). The magnitude of the altitudinal coefficients was too small to recognise a distinct pattern and coefficients were only negative in 3 out of 9 cases; the coefficients for the transformed variable urban index ranged from -0.094 (*Corylus avellana*) to -0.079 days year^{-1} (Lui)$^{-1}$ (*Salix caprea*).

Table 5.3 Multiple regression analyses for nine spring phases in the greater area of Frankfurt, Cologne and Munich with mean onset date (1980-2009) as the dependent variable and altitude and (transformed) urban index as independent variables; *df* degrees of freedom, *FFD* first flowering date, *FLD* first leaf unfolding date, *MS* May sprout, *P* values: significant results ($P \leq 0.05$) in bold, marginally significant ($P \leq 0.1$) in italics.

Phase	**df**	**R²**	**Coefficient**		***P* value**	
			Altitude	**Urban index**	**Altitude**	**Urban index**
C. avellana FFD	99	0.537	4.27	-5.08	**<0.001**	**0.026**
G. nivalis FFD	108	0.498	3.03	-2.37	**<0.001**	0.152
S. caprea FFD	102	0.332	1.78	-2.36	**<0.001**	0.107
F. suspensa FFD	109	0.710	3.29	-3.44	**<0.001**	**0.003**
A. platanoides FFD	68	0.599	2.27	-3.49	**<0.001**	**0.019**
F. sylvatica FLD	75	0.436	1.34	-1.93	**<0.001**	*0.086*
M. domestica FFD	52	0.697	2.13	-3.96	**<0.001**	**0.003**
P. abies MS	75	0.490	1.73	-1.74	**<0.001**	0.154
S. vulgaris FFD	93	0.559	1.9	-3.84	**<0.001**	**0.001**

Table 5.4 Multiple regression analyses for nine spring phases in the greater area of Frankfurt, Cologne and Munich with linear trend in phenology (1980–2009) as the dependent variable and altitude and urban index as independent variables, d.f. = degrees of freedom, FFD = first flowering date, FLD = first leaf unfolding date, MS = May sprout, *P*-values in bold = significant results ($P \leq 0.05$)

Phase	**df**	**R²**	**Coefficient**		***P* value**	
			Altitude	**Urban index**	**Altitude**	**Urban index**
C. avellana FFD	99	0.008	-0.014	-0.094	0.541	0.402
G. nivalis FFD	108	0.002	0.004	0.037	0.809	0.667
S. caprea FFD	102	0.013	0.011	0.079	0.476	0.277
F. suspensa FFD	109	0.032	0.024	0.047	*0.060*	0.446
A. platanoides FFD	68	0.217	0.066	0.007	**<0.001**	0.935
F. sylvatica FLD	75	0.029	-0.011	-0.082	0.333	0.162
M. domestica FFD	52	0.018	0.015	0.073	0.407	0.427
P. abies MS	75	0.021	-0.013	-0.063	0.285	0.316
S. vulgaris FFD	93	0.008	0.001	0.056	0.930	0.393

5.4 Discussion

Linear temporal trends in phenology (1980-2009)

Our results showed remarkably clear trends towards earlier onset dates for all examined phenophases with 453 (56 %) of the analysed 808 series becoming significantly earlier (Table 5.2). Hence, phenological phases appeared to be affected by major changes - ranging between -0.23 days year^{-1} for *Fagus sylvatica* and -0.50 days year^{-1} for *Corylus avellana*, regardless of (1) the location with respect to altitude, and (2) the amount of urbanisation. On average, trends for early spring phenophases were largest and vice-versa (Fig. 5.2). This finding is in agreement with Fitter and Fitter (2002), Menzel et al. (2001), Rötzer et al. (2000)

and Sparks and Menzel (2002) who also found more pronounced advances in early spring phases. Rötzer et al. (2000) attributed this to increases in temperature in central Europe from 1961-90 for the months January-March and decreases for April. The less pronounced response of *Fagus sylvatica* is also confirmed by other studies (Kramer 1995; Menzel 1997; Menzel 2003) and attributed to its limited phenological plasticity (Vitasse et al. 2010).

Altitudinal and urbanisation gradients of phenological means and trends

Altitudinal gradients

Plant phenology can be monitored over a short distance along altitudinal and consequently thermal gradients (Defila and Clot 2005; Dittmar and Elling 2006; Guyon et al. 2010; Larcher 2006; Studer et al. 2005; Ziello et al. 2009). Therefore, mountainous regions are useful to describe wide-ranging characteristics in phenological behaviour. However, our study was not focused on mountainous regions and phenological sites only varied from 35 to 730 m a.s.l. (Table 5.1).

Trends for altitudinal dependence on phenological mean dates in our study were all significant and varied between 1.34 days (100 m)$^{-1}$ for flowering of *Fagus sylvatica* and 4.27 days (100 m)$^{-1}$ for flowering of *Corylus avellana* (Table 5.3). Taking an altitudinal gradient of temperature of -0.6°C (100 m)$^{-1}$ and a temperature response in phenology of -1 to -5 days °C^{-1} for flowering phases in Europe (Menzel et al. 2006), we would anticipate smaller altitudinal responses of between 0.6 and 3.0 days (100 m)$^{-1}$. In fact, altitudinal gradients calculated by Ziello et al. (2009) also exceeded these expectations by ranging between 0.9 days (100 m)$^{-1}$ for *Picea abies* and 4.6 days (100 m)$^{-1}$ for *Corylus avellana*. However, we could demonstrate that earlier phases, such as flowering of *Corylus avellana*, showed stronger altitudinal and therefore thermal responses. This is supported by Menzel et al. (2006) who found that early phases tended to have larger temperature responses.

Influences of altitude on trends were not significant with the exception of the altitude coefficient for *Acer platanoides* (Table 5.4). Studer et al. (2005) only found an altitudinal dependence of phenological trends in the northern, but not in the southern Alps. Our results are partly in agreement with the findings of Ziello et al. (2009) who demonstrated that trends from 7 of 10 phenological spring phases in the Alpine regions showed no statistical dependence on altitude and were associated with overall very low R^2 values. Nevertheless, the majority of altitudinal gradients calculated by Ziello et al. (2009) were negative, suggesting a more pronounced advancement in higher regions. Defila and Clot (2001) claimed that plants located at higher elevations were more sensitive and showed a stronger response to climate change since temperature is regarded as a limiting factor for plant growth and development. However, our study could not verify stronger trends for higher regions, although our sites were at considerably lower altitudes than those used by the authors cited above.

Urbanisation gradients

The dependency of mean dates on urbanisation, expressed by the (transformed) urban index, was significant for five of the nine selected phenophases and marginally significant for a further one (Table 5.3). Only for flowering of *Galanthus nivalis* and *Salix caprea* as well as May sprout of *Picea abies* were urban effects not proved statistically. However, coefficients for all phases were in the same direction. Land use changes from entirely rural (i.e. ui = 0) to entirely urban (i.e. ui = 1) imply, depending on the phenological phase, an advance of 2.6-7.6 days suggesting that urbanisation processes can lead to major changes in phenological onset dates. Our results confirm the findings of previous studies that showed cities generally having earlier phenological events than rural areas (e.g. Fukuoka and Matsumoto 2008; Lakatos and Gulyás 2003; Rötzer et al. 2000; White et al. 2002).

The spatial resolution of our response variables (i.e. phenological temporal trends and means), do not account for micro- but for mesoclimatic characteristics since the coordinates of the observation sites are not precise enough (recorded only in degrees and minutes) and observers are allowed to search for plants within a radius of 2 km. This might adversely affect the results for *Galanthus nivalis* since it is believed that herbaceous plants are more sensitive to microclimatic conditions (Mimet et al. 2009).

The fact that the largest differences in urban-rural phenology occur in early spring is well-known. Ten European cites were analysed by Rötzer et al. (2000), who found stronger urban-rural differences for early spring phenophases than for full-spring phases. However, in our study there was no clear relationship between the urban index coefficient of nine events and their associated mean onset dates, though some early spring phenophases exhibited large differences (e.g. -5.08 days Lui^{-1} for flowering of *Corylus avellana*).

A more pronounced advancement in urbanised areas could not be shown by our analysis (Table 5.4). However, Defila (1999) and Rötzer and Sachweh (1995) detected stronger trends in urban phenological time series in Switzerland and Germany, respectively. In contrast, the majority of phenological trends (1980-95) calculated by Rötzer et al. (2000) were larger in rural regions, and they argued that this fact was due to differences in urbanisation rates. Urban development, e.g. changes in hard surfaces (soil sealing), may determine if the trend is stronger or weaker in urban areas. These examples prove that the definition of a dichotomous variable with the categories urban and rural can not ultimately explain phenological behaviour. This may also be due to the fact that these studies used only a few phenological stations.

Besides the fact that urban phenology in general is limited by the number of phenological stations established in the city centre, the location of the city at lower altitude leads to difficulties in analysing and interpreting urban phenology. Hence, without taking phenological lapse rates into account, urban-rural differences in phenology may be related not only to the urban heat island, but also to altitudinal differences. The selected cities for this study also show distinctive ranges of altitude within the study area (Table 5.1), with the urban centres at lower altitude. Therefore, the effect of the urban heat island might be enhanced due to the additional effect of altitude. Moreover, urban sprawl is not restricted to the adjacent areas of city cores. Figure 5.1 shows the pattern of continuous and discontinuous urban fabric (red colour). Consequently, the definition of urban as an area within a specified radius of the city centre seems to be inappropriate and justifies our approach to base comparisons on a variable that is derived from land use information - the urban index – simultaneously with altitude.

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6 EFFECTS OF EXTREME SPRING TEMPERATURES ON URBAN PHENOLOGY AND POLLEN PRODUCTION: A CASE STUDY IN MUNICH AND INGOLSTADT

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Abstract

Extreme temperatures have a notable effect on phenology – much greater than expected from the general rule that low temperatures lead to a later - and high temperatures to an earlier - onset of phenological phases. The latter phenomenon can be seen when comparing urban areas with their rural surroundings: plants flower earlier in cities due to the urban heat island effect that contributes to higher temperatures.

We investigated the effects of extreme temperatures on differences between urban and rural phenology and on human health (considering allergenic plants) in 2009 using phenological observations of flowering and leaf unfolding of birch *Betula pendula* Roth and flowering of horse chestnut *Aesculus hippocastanum* L. in the cities of Munich and Ingolstadt, Germany. Temperatures recorded during April 2009 were the second highest since records began in 1955 and led to rapid plant development whereas differences between urban and rural phenology were diminished. Laboratory examination of birch pollen grains revealed that the amount per catkin did not differ significantly between the city of Munich and the surrounding countryside.

Long-term observations (1951/1955 to 2008, German Meteorological Service) were used to study the differences in flowering onset times between Munich and its surroundings. We found that weather conditions lasting only a few days can influence phenological behaviour, especially at the micro- and mesoscale. High temperatures, mainly extreme warm spells, were more likely to result in simultaneous flowering in urban and rural environments; low temperatures resulted in a longer delay in phenological onset times for flowering in Munich.

Keywords. Phenology, extreme events, urban heat island, pollen amount, allergy, *Betula pendula*, temperature sums, Germany.

6.1 INTRODUCTION

Climate change is likely to have an impact not only on mean temperatures, but also on temperature variability (Schär et al. 2004). Extreme events (e.g. warm spells) are expected to increase in the future, both in frequency and intensity (IPCC 2007), and are likely to influence ecosystems more than any change in mean temperatures (Jentsch et al. 2007). Research in extreme event ecology is therefore increasing and further investigations in the field of phenology - human health interactions are still needed. Since temperature is the most important driver of plant phenology in temperate and moist regions, warm spells and their influence on plant development is of great interest. The heat wave during summer 2003 was a first opportunity to study such impacts (Fink et al. 2004, Meehl & Tebaldi 2004, Schär et al. 2004, Stott et al. 2004). The situation of 2003 is likely to be repeated: every second summer could be as warm as or even warmer by 2100 (Schär et al. 2004). Thus, we should learn from recent extremes and study their consequences which are likely to be more frequently experienced in the future.

As well as showing current extremes, cities can also be regarded as a mirror of future climate. There is plenty of evidence that plants located in urban areas flower earlier than those in the surrounding countryside (Franken 1955, Rötzer et al. 2000, White et al. 2002, Zhang et al. 2004, Mimet et al. 2009). A major feature of cities is the urban heat island effect (*UHI*) (Landsberg 1981, Oke 1987) that is associated with higher temperatures. The *UHI* can be observed throughout the year, but exhibits a daily and annual variability and is most distinctive during anticyclonic weather conditions with weak winds, clear skies and a marked diurnal temperature range (Oke 1987). The magnitude of the *UHI* also depends on the size of a city (Oke 1973). In general, the mean *UHI* varies between 1 and 3 °C (Landsberg 1981). In Munich, for example, this effect averages 1.9 °C and can even reach values of 8.2 °C (Matzarakis 2001). The temperature range of projected warming according to different scenarios is 1.8 to 4.0 °C by 2100 (IPCC 2007) and can therefore be compared with mean *UHI*. Consequently, studying urban ecosystems allows estimation of changes that may occur in the near future. Understanding phenological events is crucial for assessing the impacts of temperature increases on the life cycle of plants and thus their survival and distribution (Chuine 2010). Since 2008, more than half of the world population has lived in cities (Grimm et al. 2008), and these areas are thus fundamentally interesting.

There is strong evidence that people living in cities are more affected by pollinosis incidence than people living in the countryside - an effect that is probably related to urbanization, increased vehicle emissions and westernised lifestyle (Braun-Fahrlander et al. 1999, D'Amato 2000, Ring et al. 2001). Further features of urban phenology are that earlier onset dates for allergenic plants in the city imply earlier appearance of symptoms. Due to the temporal delay in flowering onset between the city and the countryside, wind transport may also transfer urban pollen to the countryside where the flowering season of the allergenic plant has not started. Subsequent to the end of urban flowering, pollen from the countryside can also be transported to the city, implying a protracted pollen season. Therefore, wind transport of airborne pollen is an important cause for unsynchronised starting dates in phenology and aerobiology (Estrella et al. 2006). In Europe, the incidence of asthma, allergic rhinitis, allergic conjunctivitis and eczema represents an important human health problem (WHO 2003). Pollen-related allergic diseases are influenced by generally earlier and longer pollen seasons, higher total pollen counts, stronger allergenicity and altered plant and pollen distribution (e.g. reviewed by Beggs 2004, Traidl-Hoffmann et al. 2009); thus, further investigations are needed to improve climate risk assessment.

We therefore aimed to explore the phenological behaviour of plants and the pollen production of *Betula pendula* Roth located in urban and rural areas in and around the Bavarian cities of Munich and Ingolstadt during the extreme warmth of April 2009. Long-term phenological and temperature data (1951/1955 to 2008) from the German Meteorological Service were used to study the reasons for diminished differences in onset dates between the city and the countryside. We hypothesised that higher temperature sums, i.e. warmer periods, lead to less pronounced differences in flowering phenology between the city and the countryside. Consequently, lower temperature sums, i.e. cooler periods, could imply a remarkable temporal delay between urban and rural flowering dates. We also hypothesised that the magnitude of the urban-rural temperature difference could have an influence on the differences in urban-rural phenology: smaller differences in temperature could also lead to smaller differences in phenological onset dates and vice versa. To describe the association between climate variability and allergenic disorders, we address the impacts of extreme warm periods on the phenological behaviour of (allergenic) plants and subsequently on human health.

6.2 MATERIAL AND METHODS

Study area

The regions of interest (Fig. 6.1, see Table 6.1) were two German cities located in the state of Bavaria, and their surroundings: Munich (48.14 °N, 11.58 °E, Fig. 6.1a) and Ingolstadt (48.77 °N, 11.43 °E, Fig. 6.1b). To examine urban and rural differences in phenology, the study areas were restricted to a maximum distance of 35 km from the city centres of Munich and Ingolstadt.

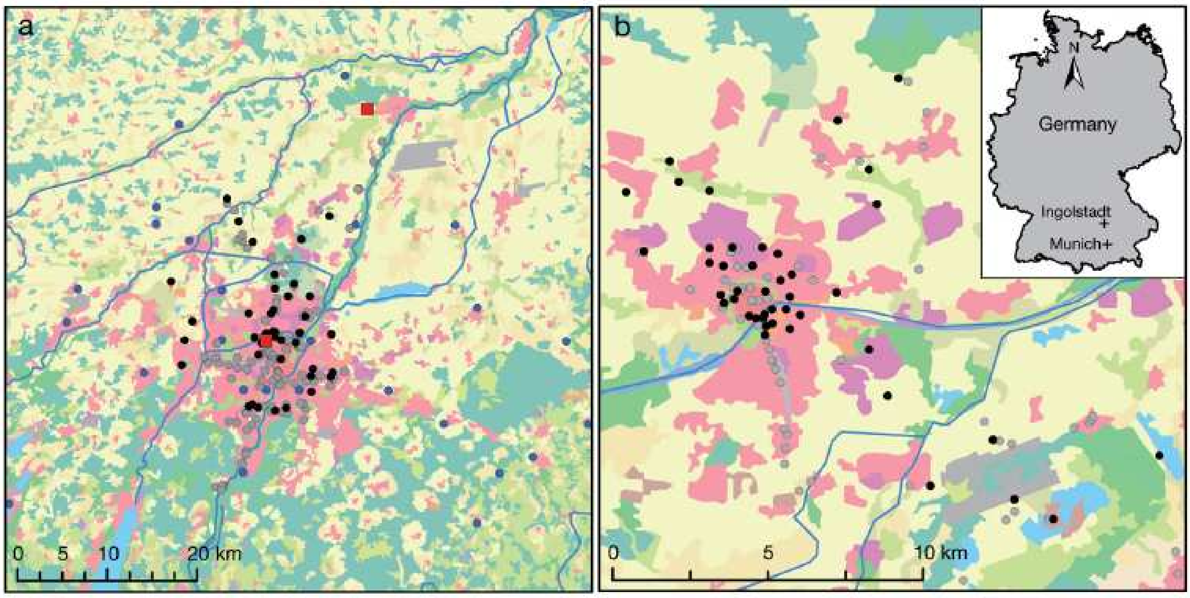

Fig. 6.1 Studied cities: (a) Munich and (b) Ingolstadt, located in southern Bavaria, Germany. (a) Red squares: German Meteorological Service (DWD) climate stations Munich City (urban) and Weihenstephan (rural); blue circles: long-term phenological observations. (a,b) Black and grey circles: field observations in 2009 for *Betula pendula* Roth (birch) and *Aesculus hippocastanum* L. (horse chestnut), respectively; background: CORINE Land Cover 2000 (EAA 2000), major classes: red = urban fabric, green = forest and pastures, yellow = arable land, blue = rivers, lakes (see: http://www.eea.europa.eu/themes/landuse/interactive/clc-download for a complete legend)

Table 6.1 Details of the cities of Munich and Ingolstadt. Values for latitude, longitude and altitude are for the city centre. No. of inhabitants are given for 2008. Land use: survey area. City: within city administrative boundaries

City	Lat [°N]	Long [°E]	Alt [m]	Inhabitants [no.]	Urban land use [%]	Rural land use [%]	City area [km²]	City urban land use [%]	City rural land use [%]
MUC	48.14	11.58	515	1,326,807	12.8	87.2	308.9	62.5	37.5
IN	48.77	11.43	370	123,925	4.9	95.1	133.4	26.7	73.3

The classification of sites as either urban or rural was based on CORINE Land Cover 2000 data (EEA 2000). Components of built up areas (e.g. continuous and discontinuous urban fabric, industrial or commercial units) were extracted from the land cover dataset to calculate the proportion of urban area within a radius of 2 km of the phenological recording stations. This radius was chosen to ensure that stations within a large built up area were identified as such. A radius of 2 km is also used by the DWD to define the observation area for their phenological stations. We denoted urban sites as those with at least 50% urban cover and the remainder as rural sites.

A subjective comparison of the observed sites in 2009 with our definition of urban and rural areas confirmed the classification, with agricultural areas, forests and natural areas being correctly classified as rural sites. Urban areas were conversely characterised by a high building density and a large proportion of impervious surfaces.

The characteristics of the two cities are summarised in Table 6.1. The amount of urban land use as specified above varied owing to the physical size of the city itself.

Phenological data

Phenological observations in 2009

In spring 2009, phenological observations were conducted according to the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) code (Meier 2001) for leaf unfolding and flowering of *Betula pendula* Roth (birch, see black circles in Fig. 6.1a,b) as well as for flowering of *Aesculus hippocastanum* L. (horse chestnut, see grey circles in Fig. 6.1a,b) in Munich and Ingolstadt.

Observations in each city were done by 2 to 4 trained recorders took place every third day. The observation period lasted from March 27 to May 8 (Table 6.2). In Ingolstadt, observations always started and ended a little bit earlier as phenological development stages were reached earlier than in Munich. Since both genetic variation among individuals and vigour influence phenology (Baumgartner 1952), dates for each phenological site were the mean from 2 to 6 plants per species. Moreover, we excluded trees of < 50 cm diameter as plant age alters phenological behaviour, i.e. older trees tend to have later onset dates in flowering and leaf unfolding than younger trees (Rosenzweig et al. 2008).

Table 6.2 Number of sites and trees per land use type for birch *Betula pendula* and horse chestnut *Aesculus hippocastanum* in Munich and Ingolstadt, and duration of the observation period. Urban (rural) = sites with > 50 % (< 50 %) urban land use within a 2 km radius

Species	City	Type	Sites	Trees	Observation period
Birch	**Munich**	Urban	28	66	04.04.2009–16.04.2009
		Rural	17	48	
		Total	**45**	**114**	
	Ingolstadt	Urban	28	85	27.03.2009–15.04.2009
		Rural	13	50	
		Total	**41**	**135**	
Horse chestnut	**Munich**	Urban	87	317	20.04.2009–08.05.2009
		Rural	26	74	
		Total	**113**	**391**	
	Ingolstadt	Urban	31	111	19.04.2009–04.05.2009
		Rural	31	85	
		Total	**62**	**196**	

We calculated descriptive statistics (mean, maximum and minimum date of onset, range, SD and SE) by city, species, phase, and land use (urban and rural).

We defined the difference in phenological onset dates between urban and rural areas as the urban phenology effect (*UPE*) since this deviation is supposed to be an expression of the *UHI*. Statistical comparison of means was done using two sample t-test unless the data were not normally distributed or failed the homogeneity of variance test (Levene's test), in which case a Mann-Whitney *U*-test was used.

Long-term phenological data (1951 to 2008)

Long-term observations of flowering (1951-2008) were provided by the DWD. We focused on 3 plants that are frequently found and observed in cities: *Corylus avellana* L. (hazel), *Forsythia suspensa* (Thunb.) Vahl (forsythia) and horse chestnut. We selected stations which were within a radius of 35 km from the city centre of Munich. To avoid problems with phenological lapse rates, the altitudes of phenological stations should be similar to the selected climate stations (Weihenstephan: 477 m, Munich City: 512 m). Therefore, only phenological stations ranging in altitude from 450 m to 560 m a.s.l. were chosen. Since observations within cities were sparse and often had gaps of observation years, we constructed composite time series using the R pheno package of Schaber (2003) from all available phenological data in urban areas. For rural areas, the time series were derived from those stations that had at least 15 observation years within the study period 1951-2008. This resulted in the following number of series (urban/rural): hazel (5/18), forsythia (5/19) and horse chestnut (5/20) (see blue circles in Fig. 6.1a).

Temperature data

Long-term temperature data (1955 to 2008)

The selected climate stations - Munich City (48.17 °N, 11.55 °E; urban station, 1955 to 2008) and Weihenstephan (48.4 °N, 11.7 °E; rural station, 1955 to 2008) (see red squares in Fig. 6.1a) - are operated by the DWD. In this study, the difference between the temperature measured at Munich City and that at Weihenstephan climate stations was defined as the *UHI* of Munich.

A common evaluation of extreme events focuses mostly on percentiles (e.g. 10th percentile), threshold, or duration indices (Alexander et al. 2006, IPCC 2007). Here we used the temperature deviation from the standard reference period 1961-90 of the respective station as a proxy for extremes of temperatures.

Correlation analyses incorporating these deviations and the *UHI* were used to identify relationships with the magnitude of the *UPE*.

Short-term temperature sums

Warm spells of relatively short duration may trigger *UPE* at a finer temporal resolution than a calendar month. Therefore, an additional analysis considering short-term temperature sums was calculated using the method of Ring et al. (1983):

$$Tsum = \sum_{i=1}^{n} Tmean - x \qquad \textbf{(1)}$$

where i = 1, 2, 3, .. , n, and n is the number of days that are considered, $Tmean = (Tmax + Tmin) / 2$ = mean daily temperature, $Tmax$ = daily maximum temperature, $Tmin$ = daily minimum temperature, x = base temperature, i.e. 0°; negative values of $Tmean$ were set to zero. Applying the zero method of Snyder et al. (1999), the base temperature was chosen to be zero for all phases, since this approach is believed to lead to quite reliable results.

The temperature sums were calculated for periods of different lengths (i.e. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 days before the beginning of flowering in the city of Munich). The mean region temperature sums (*Tsum*) were calculated using the daily temperatures averaged for Munich City and Weihenstephan.

The second summation was performed for the magnitude of *UHI*:

$$UHIsum = \sum_{i=1}^{n} UHI \qquad (2)$$

where *i* and n are as in Eq. (1), *UHI* = (*T urban* – *T rural*), *T urban* = mean daily urban temperature, *T rural* = mean daily rural temperature; negative values of *UHI* were set to zero.

These two temperature sums describing the short-term temperature conditions before urban flowering times of the plants were used for correlations with *UPE*.

Laboratory examination of pollen grains

Common methods for collecting airborne pollen include continuous volumetric pollen traps, e.g. the Burkhard trap or Hist type trap (see Giesecke et al. 2010 for a complete overview of the pollen trapping methods). Nevertheless, due to variable atmospheric conditions, one cannot be sure of the origin of the pollen. Moreover, the phenological development stage (e.g. start of flowering or full flowering) of the tree that released the pollen is not known. Therefore, we harvested catkins of birch in 2009 during the start of flowering and at full flowering. Catkins were dried for 24 hours and pollen was extracted by sieving catkins first with a 100 µm sieve followed by a 72 µm sieve. The amount of pollen per catkin was then weighed.

Due to rapid growth in April, it was hard to investigate the same number of birches in urban and rural areas of Munich and Ingolstadt. Therefore, meaningful analyses were available only for Munich: 10 samples for start of flowering (n: urban = 5, rural = 5); 26 samples for full flowering (n: urban = 12, rural = 14).

6.3 RESULTS

Field observations in 2009

Phenology of birch and horse chestnut

Table 6.3 shows the descriptive statistics of the observed phenophases in Munich and Ingolstadt in spring 2009. For leaf unfolding and flowering of birch the dates of onset occurred on average between 9 April (day of year DOY: 99) and 16 April (DOY: 106) in Munich and ~ 2 days earlier in Ingolstadt, which can be explained by the lower elevation (380 m) compared to Munich (515 m). The temporal delay between the start of flowering (BBCH 61) and full flowering (BBCH 65) was < 2 days, indicating rapid plant development in April 2009.

UPE for flowering and leaf unfolding of birch did not differ very much (*UPE* range: -1.1 days [flowering, BBCH 61, Ingolstadt] to +0.6 days [leaf unfolding, BBCH 11, Munich]). Furthermore, differences in Munich were not significant, although the differences for the start of flowering (*UPE*: -1.1 days, $p < 0.001$) and full flowering (*UPE*: -0.9 days, $p = 0.007$) in Ingolstadt were significant, albeit relatively small.

The start of flowering and full flowering of horse chestnut in Munich occurred from 23 April (DOY: 113, urban, BBCH 61) to 3 May (DOY: 123, rural, BBCH 65). In the city centre of Ingolstadt, first flowers had already opened on 21 April (DOY: 111) and the last day of full flowering was recorded on 29 April (DOY: 119) in the countryside.

In contrast to birch, differences in the mean dates of onset for the observed phenophases of horse chestnut were comparatively larger and ranged from -1.4 days (BBCH 61, Ingolstadt) to -3.0 days (BBCH 65, Munich). The *UPE* was greater for Munich than for Ingolstadt. Mean onset dates for flowering phenophases of horse chestnut for each city were significantly earlier than in their matching rural areas.

Table 6.3 Descriptive statistics and tests of equality of means for dates of leaf unfolding and flowering of birch *Betula pendula* Roth and flowering of horse chestnut *Aesculus hippocastanum* L. expressed in DOY (day of the year) in urban and rural areas of Munich and Ingolstadt. BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) codes - 11: first leaves unfolded, 61: start of flowering, 65: full flowering. *UPE*: urban phenology effect, difference between urban and rural phenological onset dates [days]; tests: t-test (for normally distributed data), Mann-Whitney (for data with non-normal distribution); significance (in UPE column): *** p < 0.001, ** p < 0.01, * p < 0.05, ns: not significant (p > 0.05)

Plant	City	BBCH	Type	n	Mean	Min	Max	SE	SD	*upe*	test
Birch	**MUC**	**11**	urban	28	101.9	99	106	0.3	1.5	0.6 ns	t-test
			rural	13	101.3	99	103	0.4	1.3		
		61	urban	28	101.2	99.2	104	0.2	1.1	0 ns	t-test
			rural	13	101.2	99.5	102.9	0.3	1		
		65	urban	28	102.8	100.5	105	0.2	0.9	0 ns	t-test
			rural	13	102.8	101.5	104.3	0.2	0.9		
	IN	**11**	urban	28	99.4	96.8	102	0.3	1.5	-0.1 ns	t-test
			rural	17	99.5	98.2	100.7	0.2	0.7		
		61	urban	28	98.8	97.5	100.5	0.2	0.9	-1.1 ***	t-test
			rural	17	99.9	97.8	101	0.2	1		
		65	urban	28	100.6	99	102	0.2	1	-0.9 **	t-test
			rural	17	101.5	99.5	103.5	0.3	1.1		
Horse chestnut	**MUC**	**61**	urban	69	113.4	110.8	120.5	0.3	2.4	-2.5 ***	t-test
			rural	24	115.8	111	120.9	0.6	2.7		
		65	urban	87	120	114.5	128	0.3	2.9	-3 ***	Mann-Whitney
			rural	26	123	117.5	128	0.7	3.5		
	IN	**61**	urban	24	110.6	109.8	112	0.1	0.7	-1.4 ***	t-test
			rural	31	112	110	115	0.3	1.5		
		65	urban	31	116.5	113	119	0.3	1.5	-2.9 ***	t-test
			rural	31	119.3	117	123	0.3	1.6		

Pollen amount

Table 6.4 shows the mean amount of pollen per birch catkin (g) for different development stages and urban and rural areas in Munich. For the urban areas, the amount of pollen during the start of flowering was slightly smaller than in rural areas (not significant). At full flowering, the differences were greater, with a higher amount of pollen per catkin in the city (not significant).

Table 6.4 Mean (± SD) amount of pollen per birch catkin (g) in April 2009 in urban and rural areas in Munich for different flowering phases (BBCH 61: start of flowering, BBCH 65: full flowering), ns: not significant (p > 0.05)

Phenophase	Urban	n	Rural	n	Difference	p
BBCH 61	0.013 ± 0.005	5	0.016 ± 0.013	5	-0.002	ns
BBCH 65	0.015 ± 0.004	12	0.007 ± 0.002	14	0.009	ns

Climatic conditions

Fig. 6.2 (left y-axis) shows the mean daily temperature from December 2008 to April 2009 for Munich City and the corresponding average for the standard reference period 1961-90. Temperatures for December 2008 to March 2009 were lower (by an average of 0.7°C) than the 1961 to 1990 mean for the same months. However, temperatures for April were 4.3°C higher and were the second highest April temperatures since the beginning of records in

1955. Therefore, this warm spell (especially during the first half of April 2009) with constantly high temperatures close to 15 °C, can be seen as an extreme event.

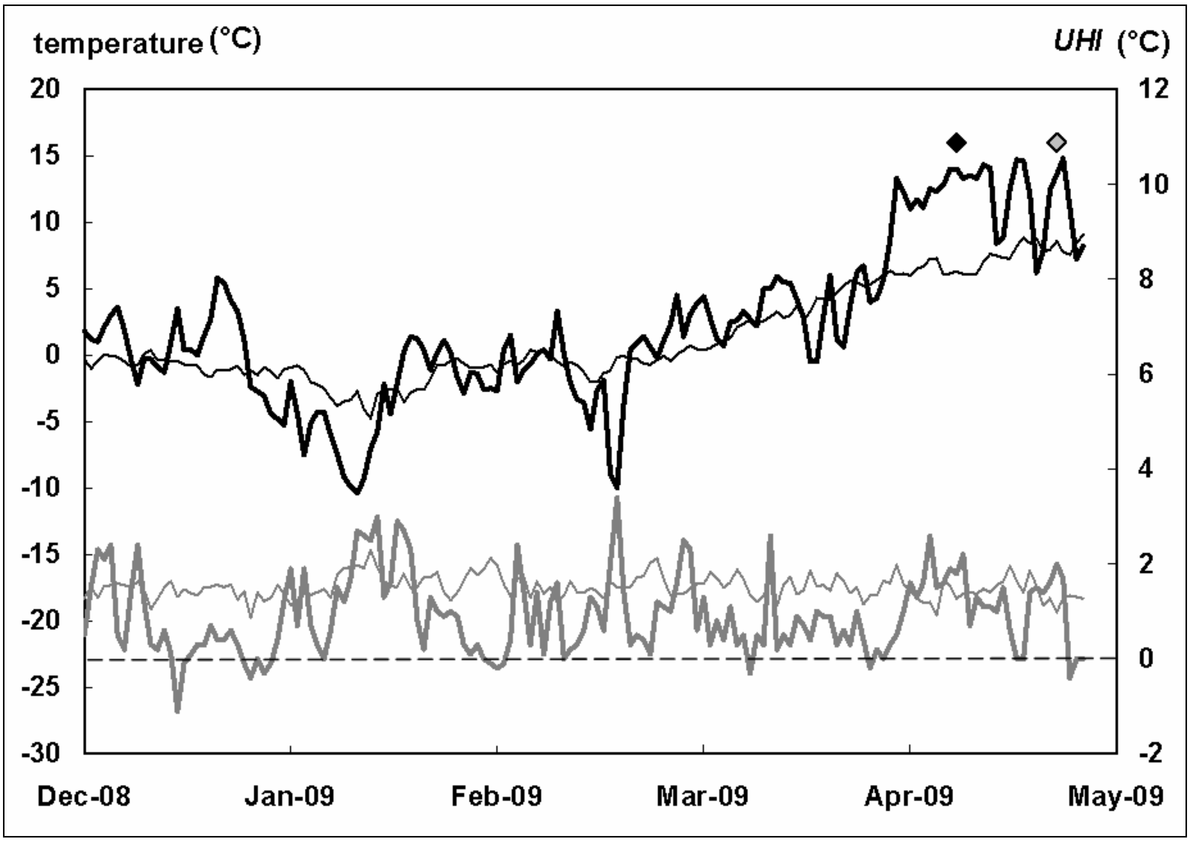

Fig. 6.2 Left axis (black curves): mean daily temperature at Munich City. Bold line: December 2008 to April 2009, thin line: standard reference period 1961 to 90. Right axis (grey curves): mean daily urban heat island effect (*UHI*, temperature difference between Munich City [urban] and Weihenstephan [rural]). Bold line: December 2008 to April 2009, thin line: standard reference period 1961 to 90, dashed line: *UHI* = 0. Diamonds: start of flowering (BBCH 61) for *Betula pendula* Roth (birch, black) and *Aesculus hippocastanum* L. (horse chestnut, grey) in the greater area of Munich in 2009.

For Munich the average yearly temperature difference between Munich City (urban) and Weihenstephan (rural) for 1961 to 90 was 1.6 °C, and was greater for minimum temperature (2.9 °C) than maximum temperatures (1.0 °C) (not shown). A less pronounced *UHI* effect was observed in Munich from December 2008 to April 2009 (mean = 0.9 °C) than during the reference period (1.5 °C). The highest deviation from the reference period was observed in December 2008: the *UHI* was only 0.6 °C, which was 0.8 °C smaller than the December 1961-90 average (1.4 °C).

Analyses of long-term phenological and temperature data

The urban phenology effect in Munich (1951 to 2008)

The mean differences in phenological onset between urban and rural areas (*UPE*) are shown in Table 6.5. Hazel had the most pronounced *UPE* for the period 1951 to 2008: first flowering averaged 4.7 days earlier in urban Munich. The smallest *UPE* was for flowering of forsythia (-3.5 days). The sub-period 1981-2008, when most of the recent climate warming occurred (IPCC 2007) had very similar *UPE* except for hazel where the mean *UPE* was -7.6 days. Nevertheless, temporal trends (*b*) of *UPE* were only significant for horse chestnut in 1981 to 2008, indicating a reduction in the difference between urban and rural dates.

Note that the temperature trends, e.g. of February to April, for Munich City (1955 to 2008, *b* = 0.039, p = 0.044) and Weihenstephan (1951 to 2008, *b* = 0.025, p = 0.006) did not differ significantly (p = 0.432), as tested using analysis of covariance (ANCOVA). The same was true for the period 1981 to 2008 (Munich City: *b* = 0.063, p = 0.071; Weihenstephan: *b* = 0.072, p = 0.039; ANCOVA: p = 0.844).

Table 6.5 Mean *UPE* (urban phenology effect, difference between urban and rural phenological onset dates) [days], temporal trend [b] of the *UPE* and R² for flowering of hazel *Corylus avellana*, forsythia *Forsythia suspensa* and horse chestnut *Aesculus hippocastanum* (1951-2008 and 1981 to 2008) in the greater area of Munich. *** p < 0.001, ns: not significant (p > 0.05)

Flowering of	period	Mean *upe*	SD	SE	b	R² (%)	P
Hazel	1951-2008	-4.71	10.91	1.53	-0.098	2.1	ns
	1981-2008	-7.63	13.50	2.88	0.240	1.8	ns
Forsythia	1951-2008	-3.47	4.46	0.64	0.014	0.3	ns
	1981-2008	-3.86	4.30	0.90	0.087	2.4	ns
Horse chestnut	1951-2008	-4.36	3.58	0.51	0.049	5.1	ns
	1981-2008	-3.82	3.50	0.75	0.319	50.2	***

Long-term temperature data

Correlation analyses of the *UPE* for flowering of hazel, forsythia and horse chestnut with the temperature difference between Munich City (urban) and Weihenstephan (rural) (*UHI*) are shown in Table 6.6. The results show that for single months and 2 or 3 months periods, no significant correlation between these variable existed.

Table 6.6 Correlations of *UPE* (urban phenology effect, difference between urban and rural phenological onset dates) [days] (1955-2008) for hazel *Corylus avellana (*n=47), forsythia *Forsythia suspensa* (n=45) and horse chestnut *Aesculus hippocastanum* (n=46) with *UHI* (temperature difference between Munich City and Weihenstephan) and *Tdev* (temperature deviation from the standard reference period 1961 to 90). ** p < 0.01, * p < 0.05, ns: not significant (p > 0.05)

	UHI c.f. UPE						Tdev c.f. UPE					
	Hazel		Forsythia		H. chestnut		Hazel		Forsythia		H. chestnut	
	r	P	r	P	r	P	r	P	r	P	r	P
Dec-Feb	-0.029	ns	-0.010	ns	-0.149	ns	-0.154	ns	-0.258	ns	0.240	ns
Jan-Mar	0.080	ns	0.025	ns	-0.153	ns	-0.121	ns	-0.149	ns	0.186	ns
Feb-Apr	0.252	ns	0.112	ns	-0.060	ns	-0.095	ns	-0.247	ns	0.217	ns
Jan-Feb	-0.039	ns	-0.081	ns	-0.215	ns	-0.148	ns	-0.229	ns	0.274	ns
Feb-Mar	0.214	ns	0.123	ns	-0.128	ns	-0.086	ns	-0.204	ns	0.107	ns
Mar-Apr	0.263	ns	0.151	ns	0.076	ns	-0.051	ns	-0.057	ns	0.167	ns
Dec	0.006	ns	0.136	ns	0.083	ns	-0.110	ns	-0.181	ns	0.059	ns
Jan	-0.138	ns	-0.122	ns	-0.111	ns	-0.123	ns	0.044	ns	0.225	ns
Feb	0.083	ns	-0.002	ns	-0.228	ns	-0.109	ns	**-0.326**	*	0.189	ns
Mar	0.248	ns	0.206	ns	0.052	ns	-0.030	ns	0.071	ns	-0.067	ns
Apr	0.158	ns	0.037	ns	0.065	ns	-0.046	ns	-0.197	ns	**0.381**	**

Similar findings were obtained from the correlations of *UPE*-values and the temperature differences from the 1961 to 90 mean for the selected climate stations (as a proxy of extreme temperatures) (see also Table 6.6). Only two significant correlations, for forsythia (February r = -0.329, p = 0.029) and horse chestnut (April r = 0.381, p = 0.009), were revealed. High temperatures in April were associated with a less pronounced *UPE* for horse chestnut. However, higher temperatures in February led to a more pronounced *UPE* for forsythia.

Short-term temperature sums

The results of short-term (1 to 50 days) temperature data indicated significant coefficients for *Tsum*, especially between 10 to 30 days before the first flowering in the city. The temporal pattern of the correlation coefficients is shown in Fig. 6.3 (black lines). Flowering of hazel (Fig. 6.3a) had the weakest relationships, with most of the coefficients being nonsignificant; however, they were significant although weak for *Tsum* during 20 to 25 days before urban flowering (highest $r = 0.345$, $p < 0.05$, *Tsum20*). For forsythia (Fig. 6.3b) and horse chestnut (Fig. 6.3c) the magnitude of the coefficients were notably higher and often significant, with respective maximum r values of 0.584 ($p \leq 0.001$) and 0.726 ($p \leq 0.001$) occurring around the 20th day before urban onset dates. The strongest correlation between *UPE* and *Tsum* was noted for horse chestnut at 20 days before urban flowering, as can be seen in Fig. 6.4: the higher the *Tsum* in the respective 20 day period, the less pronounced (close to 0 or even positive) was the *UPE*. The data of 2009 derived from the field study in Munich were added to Fig. 6.4 and are indicated by a diamond. For *UHI* sums the results (see Fig. 6.3, grey lines) are quite mixed: whereas no significant correlation was found for forsythia, the 15-day-period before urban onset of hazel flowering showed a significant but weak relationship with *UPE* ($r = -0.323$, $p \leq 0.05$). For horse chestnut, there were three significant coefficients ($p \leq 0.05$), with the highest values being calculated at 5 days before urban flowering (*UHIsum5*: $r = 0.343$, $p \leq 0.05$). For hazel the significant coefficient was negative, i.e. a higher difference in temperature between the urban and rural stations induced a more pronounced *UPE* (increasing negative values, Fig. 6.3a). In contrast, horse chestnut showed a positive relationship, i.e. a greater temperature difference led to a less pronounced difference in flowering times (i.e. *UPE* less negative of even positive).

6.4 DISCUSSION

Field observations in 2009

We considered April 2009 as an extreme event since it had the second highest temperature on record. This fact implied a rapid plant development, with trees flowering almost simultaneously and differences between urban and rural sites being reduced. This was especially true for the early spring phenophases of birch. The later spring phenophases of horse chestnut were not affected in this way. However, the observation data of 2009 shows a smaller *UPE* (compared to other years) with high temperature sums, e.g. for the 20 days before urban onset (see Fig. 6.4). Therefore, we suggest that differences in flowering dates between urban and rural environments are strongly affected by annual weather patterns.

Differences in urban-rural phenology (1951 to 2008)

The mean differences between urban and rural phenology (*UPE*, 1951 to 2008) showed earlier flowering of hazel, forsythia and horse chestnut in the city. However, temporal trends show that these differences did not change significantly, apart from a reduction in the *UPE* for horse chestnut in 1981 to 2008. The increases of temperature measured at the urban and rural stations did not differ significantly. With climate warming advancing spring phenology (Fitter & Fitter 2002, Parmesan & Yohe 2003, Root et al. 2003, Badeck et al. 2004, Menzel et al. 2006) in both urban and rural areas (see ANCOVA in Section 3.2.1), one could hypothesise that the *UPE* should be more or less stable as long as the *UHI* is not increasing. Consequently, we conclude that phenological differences between the city and its surroundings cannot adequately be explained by temperature means and their trends in spring. Weather conditions of only a short duration (extremes) may often be overlooked when using mean temperature data of a longer period (monthly or longer), although they can influence the timing of flowering (Jentsch et al. 2007).

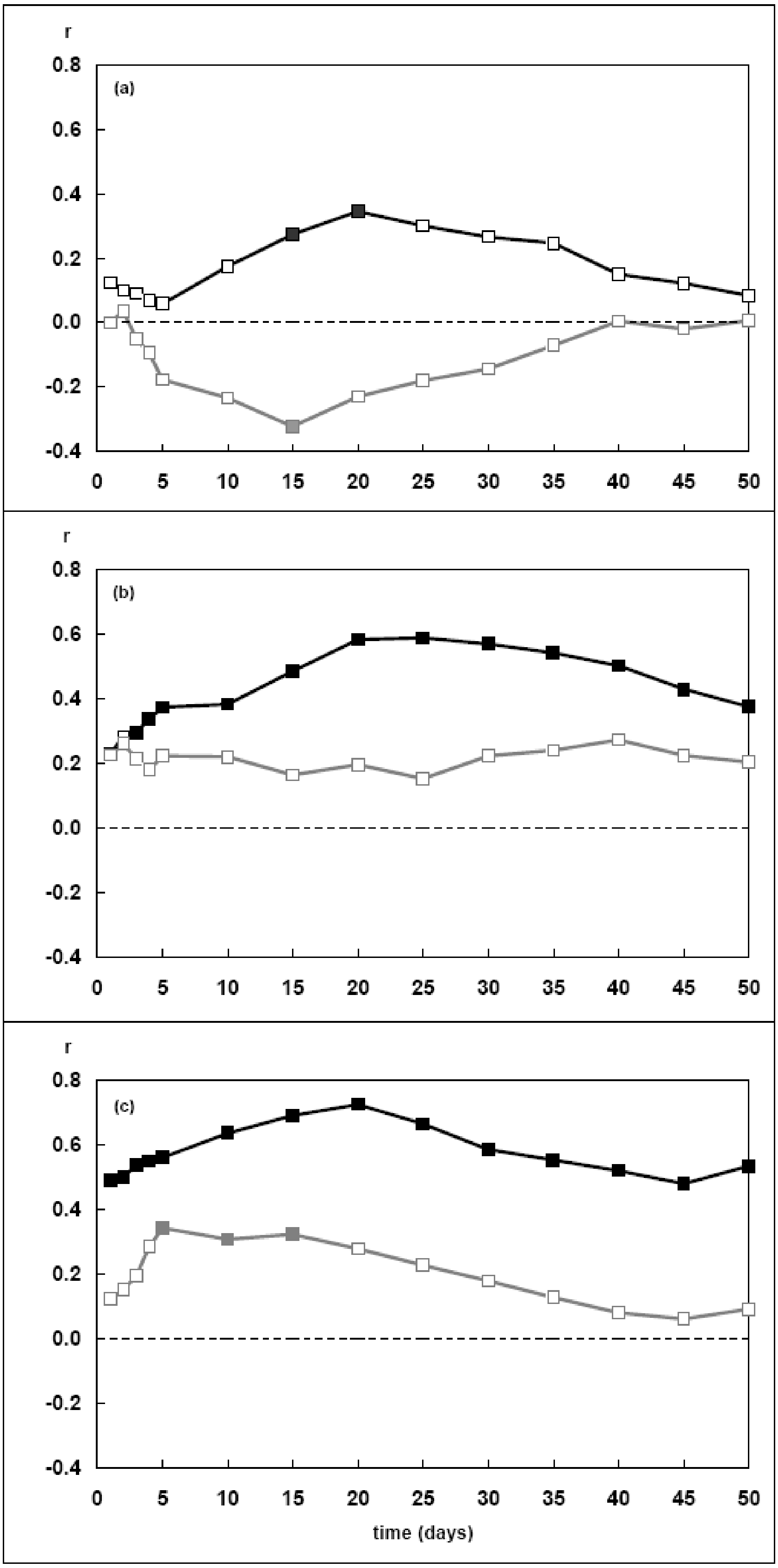

Fig. 6.3 (a) Temporal development (from 1 to 50 days before urban flowering) of correlation coefficients (r) of temperature variables (temperature sums [*Tsum*, black line] and urban heat island effect sums [*UHIsum*, grey line]) with *UPE* (urban phenology effect) for (a) hazel *Corylus avellana*, (b) forsythia *Forsythia suspensa* and (c) horse chestnut *Aesculus hippocastanum. UPE* is the difference between urban and rural phenological onset dates (in days). Filled squares: significant correlation coefficients ($p \leq 0.05$)

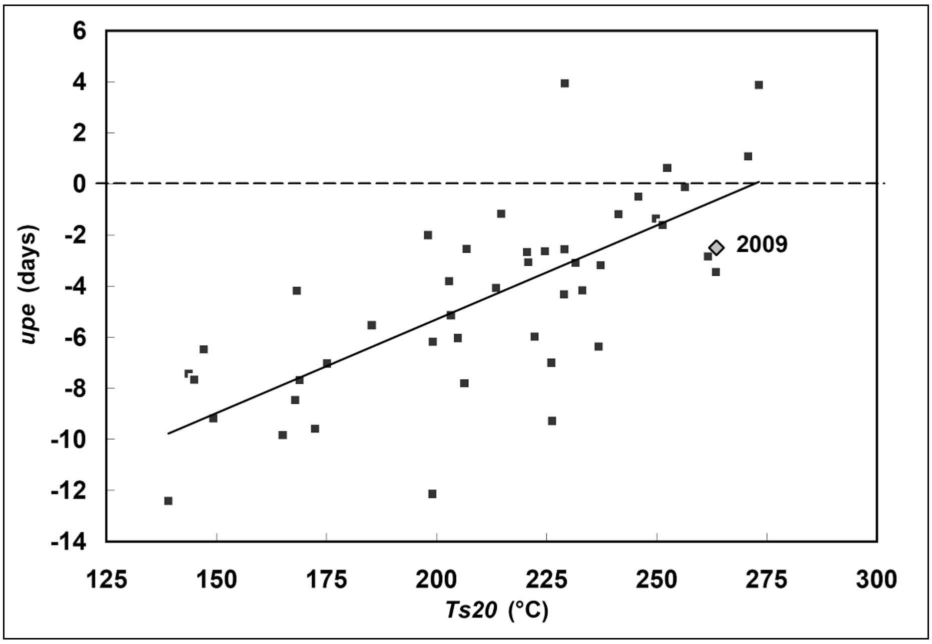

Fig. 6.4 Scatterplot of *UPE* (urban phenology effect; difference between urban and rural phenological onset dates in days) against temperature sums for a 20 day period before urban onset (*Tsum20*) for horse chestnut *Aesculus hippocastanum* in Munich (1955 to 2008). $R^2 = 0.5268$, regression equation: $y = 0.0735\ x – 20.001$.Grey diamond: data for the field study in 2009

Long-term vs. short term temperature data

The results of the phenological observations for April 2009 showed little difference in flowering dates (especially flowering and leaf unfolding of birch) between urban and rural areas of Munich and Ingolstadt. To evaluate these results, we explored the suitability of the *UHI* and the temperature deviations from 1961 to 90 in explaining variations in urban and rural flowering dates using DWD time series (1955 to 2008) with correlation analyses. However, a dependency of the variables calculated on a monthly or longer resolution could not be proven. To overcome the limitations of using crude monthly data, temperature sums of shorter periods (1 to 50 days before urban onset) were used for correlations with *UPE;* results showed that shorter periods are more suitable in explaining the temporal variation in urban and rural phenology. However, our results also indicated that temperature differences between the city and its rural surroundings are not sufficient to explain the variations in urban-rural phenology. The contrasting directions of the relationship between *UHI* and *UPE* for hazel (significant negative correlation coefficients) and for horse chestnut (significant positive correlation coefficients) suggest that differences in temperature may not fully explain temporal patterns in urban and rural phenology. Therefore, only the correlations between *UHI* and *UPE* for hazel showed the expected direction: the greater the temperature difference between the city and the countryside, the higher the difference in phenology (i.e. *UPE* values more negative). This could be attributed to the temperature sensitivity of plants flowering early in the year (Ahas et al. 2002; Fitter & Fitter 2002). One reason for the inconclusive results might be that the urban and rural temperatures respectively recorded at Munich City and Weihenstephan do not adequately reflect the variations within the sites used in this study. However, we specifically discarded stations located at higher elevations and thus suggest that the compact nature of the study area (within a maximum of 35 km from Munich city centre) should result in only minor latitudinal or longitudinal effects on temperature. In addition, we investigated gridded temperature data from the DWD to compare urban and rural temperature differences (results not shown) and found that the mean temperature data of the urban and rural sites matched the temperatures recorded at the two climate stations. It is thus more likely that short warm spells could be more explanatory than the *UHI* which is mainly affected by general atmospheric circulation patterns. Temperature sums provided often significant and larger correlations than *UHI*, especially at 20 to 25 days before urban onset dates. Therefore, we suggest that warm spells during this period could result in negligible differences in flowering phenology (*UPE* ~ 0) or even earlier flowering in the countryside (*UPE* > 0).

Subsequently, we suggest that weather conditions lasting only a few days can effectively influence phenology, especially at the micro- and mesoscale. Extremely high temperatures are more likely to result in simultaneous flowering in urban and rural environments. Low temperatures, e.g. cold spells, can contribute to a longer delay of phenological onset times in the countryside compared to the city.

Linkage to climate variability

In response to previous temperature extremes, the biological impacts of the heat wave of summer 2003 in the Swiss Alps were analysed by Jolly et al. (2005): Higher elevations were affected by increased growth and longer effective growing season length; however, lower elevations exhibited reverse impacts on plants, mainly due to an enhanced evaporative demand. Luterbacher et al. (2007) assessed the impacts of the exceptional warmth of autumn 2006 and winter 2007 in Europe. They observed that some plant species had a partial second flowering or an extended flowering season that lasted until the beginning of winter. Moreover, the first spring phases in 2007, e.g. flowering of snowdrop (*Galanthus nivalis* L.) or hazel, occurred distinctively earlier. Manipulation experiments conducted by Jentsch et al. (2009) showed that a 32 day period of drought advanced mid-flowering dates of grassland and heath species by 4 days on average and extended the flowering season by 4 days. Conversely, heavy rainfall led to a reduced flowering season. However, these studies focused on weather conditions prevalent in summer or autumn and winter, but not on atypical high spring temperatures that we explored in our study. Analyses of phenological onset dates of birch and horse chestnut in 2009 suggested that anomalous warm periods in spring could lead to synchrony in plant development between urban and rural areas, with various effects on pollen and human health.

Pollen and human health

Beside the consistency in phenological onset dates of birch, no significant variation in birch pollen amount per catkin could also be found between Munich and its surrounding countryside in 2009. Several studies reported a correlation between temperature and airborne pollen amount: Spieksma et al. (1995) found increased trends of annual sums of daily airborne pollen concentration in Basel, Leiden, London, Stockholm and Vienna. Rising birch pollen concentrations were also attributed to climate change by Frei & Gassner (1998) for Switzerland and Rasmussen (2002) for Denmark. Teranishi et al. (2000) demonstrated the existence of a significant relationship between mean temperatures of previous July and total pollen amount of Japanese cedar for urban areas of Japan. Ziska et al. (2003) found that higher atmospheric pollen amounts of the herbaceous plant common ragweed in North American urban areas were associated with increasing CO_2 concentrations and air temperatures, both of which are related to urbanization and climate change.

These findings suggest that higher temperature could be a primary cause of greater pollen concentrations. Small differences in temperatures (*UHI* ~ 0), as observed in winter 2008 and spring 2009 between urban and rural areas of Munich, would be expected to lead to an equality of pollen grain abundance. However, the method for sampling pollen was used for the first time in this study, thus the influence of the warm spell in April 2009 cannot be definitively assessed. Having pollen information of “normal” years with *UHI* and temperatures close to average values would allow a better understanding of the effect of extreme events on pollen production.

Since the development of both urban and rural catkins was rapid, pollen was released almost simultaneously in urban and rural Munich. The start of flowering in urban and rural sites in Ingolstadt only had a temporal lapse of 1.1 days. Therefore, allergic people in both the city and the countryside were affected starting almost the same day. Moreover, the temporal development from the start of flowering to full flowering was also rapid. Thus, the symptoms of birch allergy probably did not last long compared to other years when colder temperatures inhibit rapid development of catkins. In general, asthmatic and pollen allergic individuals can benefit from the countryside by being exposed later to pollen. However, when warm spells lead to urban-rural synchrony, people do not have the option to be less exposed in the nearby countryside. In contrast, during cold spells, there was a larger difference in onset dates between urban and rural areas. This would imply that people probably have the opportunity to spend pollen-free time in the countryside but would suffer for longer due to a steady development and transport of pollen in the air from the city to the countryside and vice versa.

Altered onset dates of flowering and pollen season durations of certain allergenic plants could enhance disorders, e.g. an overlapping with the peak of another allergenic plant (Doi et al. 2010) could result in strengthening of symptoms and a decrease of the allergy-free time. Earlier onsets of flowering could also imply interrupted pollen seasons due to colder and more humid weather conditions in late winter or early spring (D’Amato & Cecchi 2008), leading to perpetual minor and major ailments. Few studies focused on the impacts of extreme weather conditions: analyses of grass pollen production and airborne pollen loads during summer 2003 (Gehrig 2006) showed that the pollen season in Switzerland started remarkably early, but was also shorter than average. Allergic people experienced several days with high pollen concentration and therefore had more intense symptoms. In addition, the usually less affected higher regions of the Alps were also characterised by a more severe pollen season. In addition, precipitation plays an important role in pollen release. While dry conditions support the release of pollen from anthers, high humidity or rainfall events may interrupt this process (D’Amato & Cecchi 2008). However, Gehrig (2006) found that extremely long periods of negative water balance in southern Switzerland in 2003 led to exceptionally low concentrations of pollen from *Rumex* spp., *Urtica* spp. and *Artemisia* spp.

Climate variability is just one factor influencing the relationship between pollen and allergy but seems to have a great effect on allergic disorders, especially in terms of urban-rural comparison. Since allergies are prevalent worldwide and affect both young and old people, phenological observations of allergenic plants and the impacts of extremes are necessary to understand possible future risks and trends concerning this important human health issue. We have demonstrated homogeneity of flowering dates of allergenic plants due to warm spells at the micro- and mesoscale. For allergic people, the favourable consequence of shorter pollen seasons is contrasted with the loss of opportunity to escape to the countryside to experience a lower allergen load. Further investigations should examine a combination of pollen amounts produced by single trees and airborne pollen counts from populations, as this will make a valuable contribution for assessing human health impacts of climate change.

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7 SPATIO-TEMPORAL INVESTIGATION OF FLOWERING DATES AND POLLEN COUNTS IN THE TOPOGRAPHICALLY COMPLEX ZUGSPITZE AREA ON THE GERMAN-AUSTRIAN BORDER

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Abstract

Flowering behaviour of the major allergenic species *Betula pendula* Roth (silver birch), *Dactylis glomerata* L. (cocksfoot) and *Alopecurus pratensis* L. (meadow foxtail) was examined by phenological observations in 2009 along an altitudinal gradient (from 700 m up to 1700 m a.s.l.) in the topographically complex Zugspitze area on the German-Austrian border. The results were compared with pollen counts derived from pollen traps located at different altitudes (720 m, 1503 m, 2650 m a.s.l.).

Phenological onset dates showed a great dependence on altitude and on exposition. Altitudinal gradients of the two grass species showed a delay of between 6 and 7 days $(100\text{m})^{-1}$ and can be interpreted as a temperature response rate varying between -9 and -10 days $(1\ °\text{C})^{-1}$. For birch phenophases the altitudinal gradients were ca. 3 days $(100\text{m})^{-1}$; corresponding to temperature response rates of circa -7 days $(1\ °\text{C})^{-1}$. Northern and western exposed birch trees at the same altitude showed large differences in flowering dates (5 to 7 days).

A comparison of phenological and aerobiological data in the phenological survey area revealed good agreement in the start of season dates, especially at lower altitude. Therefore, it was local pollen emissions, not long- or medium-range pollen transport that accounted for the timing of the aerobiological start of the season. Pollen counts at the highest and vegetation-free site were particularly affected by medium-range transport.

More pronounced responses to altitude and therefore to temperature for the analysed grass species indicate a greater temperature sensitivity. This suggests that further temperature increase could result in a remarkably earlier grass pollen season and, in turn, lead to major consequences for human health. Particular wind patterns can contribute to high and medically relevant pollen concentrations even at high elevation sites, implying less favourable conditions for those people allergic to pollen.

Keywords. Phenology, aerobiology, temperature response, wind, medium-range transport, topography, altitude.

7.1 Introduction

Studying phenology in mountainous regions increases our understanding of temperature responses and the ecological impacts of global change because climatic - mainly temperature - and therefore phenological conditions change within a short distance due to altitude and topographical characteristics such as aspect and slope (Defila and Clot 2005; Ziello et al. 2009). With higher elevation, air temperature decreases by, on average, 0.6 °C $(100\text{ m})^{-1}$

(Barry 1981). Using this temperature lapse rate, altitudinal responses in phenology can be easily translated into thermal responses.

For people allergic to pollen, high mountain regions might be of particular interest since they are believed to have a more pollen-free environment: the amount of airborne pollen usually decreasing with increasing elevation (Clot et al. 1995; Gehrig and Peeters 2000). However, the opposite behaviour was reported by Frei (1997) who found a greater amount of allergenic pollen at a high elevation site (Gütsch 2300 m a.s.l.) compared to a lower elevation site (Davos 1600 m a.s.l.) in Switzerland. Moreover, mountainous areas are particularly sensitive to global warming. The Alpine region has experienced a more pronounced temperature increase compared to the adjacent lowlands (Beniston 2006) leading to a deterioration of potentially beneficial situations related to pollen. For Austria, Bortenschlager and Bortenschlager (2005) demonstrated that global warming not only affected the pollen season of lowland ecosystems, but at higher altitudes flowering periods also started earlier and lasted longer, and were associated with higher peak values and greater pollen production.

Several studies have revealed that the start of the airborne pollen season does not necessarily match with the beginning of local flowering. For example, Estrella et al. (2006) demonstrated that the start of the birch pollen season in Germany occurred on average 6 days earlier than local flowering, although the peak of the pollen season coincided with the mean flowering date. The mismatch of local phenological onset dates with the start of the pollen season can be partly explained by long- or medium-range transport of airborne pollen by moving air masses.

Short-range transport is limited to a horizontal distance of 1 km, whereas medium-range transport is defined as transport of air within 1 to 100 km, with long-range transport defined as longer distances (Rantio-Lehtimäki 1994). Usually, the maximum distance of long-range pollen transport is approximately 500 km (Rousseau et al. 2003; Van de Water et al. 2003). Therefore, pollen of species that are not established in a region could still be present in the air. This is of particular interest when pollen of allergenic plants, e.g. ragweed, is considered and when counts are high enough to induce sensitizations (Zauli et al. 2006; Cecchi et al. 2007). Cambon et al. (1992) and Rousseau et al. (2003) also found pollen of exotic taxa in Canada and Greenland, respectively, which originated at least 1000 km away. Even pollen transported 3000 km was found in the Arctic (Campbell et al. 1999). Regarding such long distances, pollen amounts are likely to remain below the plant species specific threshold at which symptoms are likely to occur and hence do not lead to major medically relevant consequences.

Our study was performed using a detailed regional set of phenological data obtained from a field survey in 2009 in the topographically complex Zugspitze area. We focussed on some of the most allergenic plants which have abundant pollen in the air during the flowering season, i.e. *Betula pendula* Roth (silver birch), *Dactylus glomerata* L. (cocksfoot) and *Alopecurus pratensis* L. (meadow foxtail). We also incorporated aerobiological and meteorological data for different altitudinal levels for 2008 and 2009 in the analysis.

Our central aims were:

- To analyse phenological data as a function of altitude and aspect.
- To use temperature lapse rates for assessing temperature response rates in phenology.
- To evaluate the spatial and temporal consistency between phenological and aerobiological data.
- To analyse the pollen content of air masses at the highest and vegetation-free pollen site affected by medium-range transport.

7.2 Material and methods

Study area

The phenological sites (Fig. 7.1) were located along an altitudinal gradient up to 1700 m a.s.l. in the topographically complex Zugspitze area, the highest mountain in Germany (2962 m a.s.l.), located in the Wetterstein Mountains in the Northern Limestone Alps. Phenological observations were recorded in Garmisch-Partenkirchen (720 m a.s.l.), northeast of the Zugspitze, and followed an altitudinal gradient up to the lake Eibsee (1000 m a.s.l.) and Ehrwald in Austria (1000 m a.s.l.), ending with the highest observation sites at 1200 m a.s.l. (Ehrwald Cable Car Station) and 1700 m a.s.l. (Hupfleitenjoch). At the lower sites, land use is mainly meadows and some small villages; spruce is the dominant tree species at higher altitudes. The tree line in the northern Alps is found at 1800 m a.s.l. (Ellenberg 1996).

Phenology

Phenological data and species characteristics

Phenological observations in 2009 were conducted according to the BBCH code (Meier 2001) and focused on flowering and leaf unfolding of silver birch, cocksfoot and meadow foxtail - one tree species and two grass species, respectively (Table 7.1, Fig. 7.1). Worldwide, grass pollen is the major cause of pollinosis. The most abundant grass pollen originates *inter alia* from cocksfoot and meadow foxtail (D’Amato et al. 2007). Additionally, these two grass species are monitored within the phenological network of the German Meteorological Service (DWD). The altitudinal limits of meadow foxtail and cocksfoot do not exceed 1500 m and 2000 m, respectively, in the northern Alps (DWD 1991). We also observed phenological phases of birch, since its pollen is the most allergenic tree pollen in northern, central, and eastern Europe (D’Amato et al. 2007). It is limited to below approximately 1800 m (DWD 1991); this altitude also corresponds to the tree line.

Table 7.1 Summary of analysed species and phenophases: number of individuals and sites, altitudinal range (m a.s.l), observation method according to BBCH (Meier 2001), PSD: method of pooled pre/post stage development (Cornelius et al. 2011), see text for details

species	common name	pheno-phases	No. of individuals / no. of sites	altitudinal range (m a.s.l.)	observation method (BBCH)
Betula pendula Roth	birch	flowering, leaf unfolding	115 / 26	696-1709	synchronous
Dactylis glomerata L.	cocksfoot	flowering	120 / 10	710-1233	PSD
Alopecurus pratensis L.	meadow foxtail	flowering	108 / 9	710-1233	PSD

Phenological observation methods and statistical analysis

Phenological observations were carried out in 2009 using two different methods: the synchronous method (Zacharias 1972) and the method of pooled pre/post stage development (PSD, Cornelius et al. 2011). For birch, the synchronous method with periodic observations on every third day was used. This method allows calculation of the onset date (day of year; DOY) for flowering / leaf unfolding and their respective sub-stages quite accurately. For grass we applied the PSD-method that is based on weekly recording of development indices and linear interpolation to estimate the onset dates of phenological stages. For further information about the PSD method see Cornelius et al. (2011).

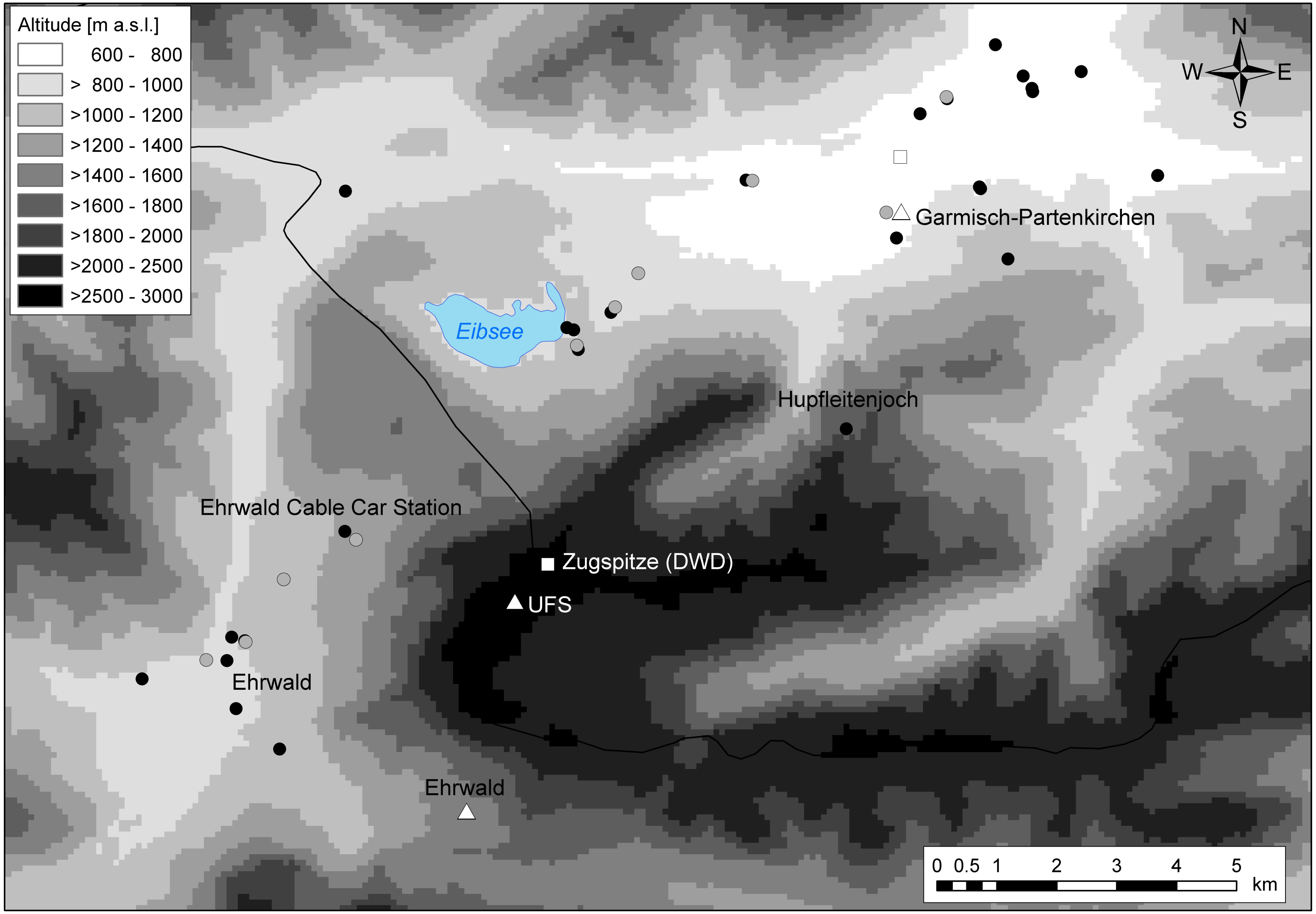


Fig. 7.1 Location of the study area, the Zugspitze region, located on the German-Austrian border (black line). Black dots: observation sites for birch (*n*=26), grey dots: observation sites for grass (*n*=10), white triangles: pollen sampling sites, white squares: German Weather Service (DWD) climate stations, background: SRTM 90 m digital elevation model (Jarvis et al. 2006)

We calculated the absolute first observed flowering date (FFD) and the mean date of beginning of flowering (MFD) for each site.

To determine altitudinal gradients, linear regressions were calculated with the mean onset date averaged for each site as the response and altitude as the explanatory variable. Positive coefficients indicate a delay of the phenophase in days $(100\ m)^{-1}$. Temperature response rates were calculated using the adiabatic lapse rates of air temperature that incorporate the mean daily temperatures at the climate station in Garmisch-Partenkirchen (719 m) and at the Schneefernerhaus (UFS, 2650 m), a research station directly below the summit of the Zugspitze (see Fig. 7.1). For birch phenophases we applied the mean temperature lapse rate of March and April in 2009 (-0.48 °C $(100\ m)^{-1}$) and for grass phenophases the mean of May and June 2009 (-0.68 °C $(100\ m)^{-1}$). Negative temperature response rates depict the advancement of a phenophase for a warming of 1 °C.

To evaluate the effects of aspect we selected different birch individuals located at almost the same altitude in Ehrwald (aspect: west, mean altitude: 998 m a.s.l.) and Eibsee (aspect: north, mean altitude: 990 m a.s.l.). To test whether the phenological onset dates differed between aspects we used a two sample t-test. Where data were non-normal, a Mann-Whitney U-test was used.

Aerobiology

Airborne pollen was collected using Burkard volumetric traps (Hirst 1952). The samplers were calibrated to handle an air flow of 10 $l\ min^{-1}$ to mimic human breath. Daily values were presented as number of pollen grains of *Betula* spp. (birch) and *Gramineae* (grass) per cubic metre of air. Note that *Gramineae* records consist of several grass species since it is not possible to distinguish between different grass species from the pollen that are captured on the slides. Pollen traps were located at three different sites: at 720 m a.s.l. in Garmisch-Partenkirchen, at 1503 m a.s.l. at the Ehrwald alp, and at 2650 m a.s.l. at the UFS (white triangles in Fig. 7.1). Straight-line distance between the pollen traps in Garmisch-Partenkirchen and UFS is ca. 9 km, between Garmisch-Partenkirchen and Ehrwald ca. 12 km and between Ehrwald and UFS ca. 4 km.

For birch we calculated the starting date of airborne pollen season using the method of Galán et al. (1995). This is defined as the date from which the cumulative sum of daily mean pollen concentration reaches 1% of the total annual sum. The end of the pollen season is in turn defined as the day when the cumulative sum reaches 99% of the total annual sum.

A common method for defining the pollen season of grass is the threshold 30 method (Sánchez Mesa et al. 2003). The start and the end dates of the pollen season are defined as the first and the last day, respectively, when pollen counts are ≥ 30 grains m^{-3}. However, in high alpine regions daily pollen counts are not as abundant as at lower altitude. Therefore, we decided to use a definition proposed by the German Pollen Information Service (PID, Polleninformationsdienst): on three consecutive days or on three days within one week the pollen concentration of grass pollen has to exceed 6 grains m^{-3} since this is the supposed threshold to induce medically relevant risks. In turn, we defined the end of the pollen season as when this criterion was last reached in the year.

Additionally, we determined the peak (maximum) of the daily pollen concentration and the total amount of pollen grains m^{-3} for each year and pollen station. Pollen sums were also available for the two halves of a day (0:00-12:00 and 12:00-24:00). Two-sample t-tests were used to identify significant differences in pollen amounts in these periods.

However, the data set is not complete because of temporary technical problems with the traps. For Ehrwald and UFS, continuous pollen trapping in 2009 started only after the beginning of the pollen season of birch. However, calculating the grass pollen season was not affected by the delayed start in sampling. Icebound instruments, dust or delayed replacement of sampling slides caused a few missing values for the traps at higher elevations (see Table 7.2). After September 29th 2009, snowfall inhibited sampling of grass pollen at the UFS. However, the pollen season was likely to have already ended by this date.

Table 7.2 Missing values of daily pollen counts during the flowering period for *Betula* spp. and *Gramineae* in 2008 and 2009 at Garmisch, Ehrwald and UFS

	Garmisch		Ehrwald		UFS	
	2008	**2009**	**2008**	**2009**	**2008**	**2009**
Betula	0	0	0	delayed start (12.05.); 0	3	delayed start (15.04.); 2
Gramineae	1	0	2	4	17	11

Aerobiology and phenology / meteorology

A comparison between pollen seasons defined from aerobiology and phenology was done for 2009 by visual interpretation of temporal pollen curves and phenological dates (FFD, MFD) for different altitudinal levels.

Besides altitudinal temperature lapse rates (see 2.2.2) we analysed the wind direction for 2008 and 2009 of two climate stations (white squares in Fig. 7.1) operated by the German Meteorological Service (DWD) which are located at Garmisch-Partenkirchen (719 m a.s.l.) and Zugspitze (2962 m a.s.l.) in order to examine associations between prevailing winds and airborne pollen amounts at the vegetation-free pollen station UFS.

Wind rose plots visualise not only the prevailing wind direction but also show the sum of pollen counts at the UFS station that were associated with specific wind directions. We did not restrict these analyses to the pollen season since it can be relatively short in duration at sites where no local vegetation exists. Therefore, we included the time period between the days of first and last occurrence of pollen at the UFS.

7.3 Results

Phenological data

Altitudinal trends 2009

Results of linear regression models for the selected phases for birch, cocksfoot and meadow foxtail are shown in Table 7.3. For all birch phases the altitudinal gradients were highly significant ($P < 0.001$) and ranged between 3.1 and 3.2 days delay per 100 m increase in altitude. High R^2-values (65.1 to 75.7%) confirm that a high fraction of the variation in onset dates can be explained by altitude.

For the two grass species, regression coefficients (6.3 to 7.1 days $(100\ \mathrm{m})^{-1}$) for the beginning of flowering and full flowering were even higher with R^2-values ranging between 71.8 and 83.5%. However, the latter results were based on only 9 and 10 samples, respectively. Accordingly, temperature responses varied between -6.5 days $(1\ °\mathrm{C})^{-1}$ and -6.7 days $(1\ °\mathrm{C})^{-1}$ for birch phenophases and between -9.3 days $(1\ °\mathrm{C})^{-1}$ and -10.4 days $(1\ °\mathrm{C})^{-1}$ for grass phenophases.

Table 7.3 Altitudinal gradients (days (100m)$^{-1}$) for selected phenophases of birch, cocksfoot and meadow foxtail in the Zugspitze region; *N* number of sites, *P* significance, *R²* goodness of fit

species	phenological phase	n	gradient (days (100m)$^{-1}$)	P	R² (%)
birch	leaf unfolding	26	3.1	<0.001	75.7
	beginning of flowering	25	3.2	<0.001	70.5
	full flowering	26	3.2	<0.001	65.1
cocksfoot	beginning of flowering	10	7.1	0.002	71.8
	full flowering	10	6.3	<0.001	83.5
meadow foxtail	beginning of flowering	9	6.8	0.001	82.9
	full flowering	9	6.4	0.001	81.1

Influence of aspect on single birch trees

Table 7.4 shows the effects of aspect on phenological phases of selected birch individuals located at Ehrwald (aspect: west) and Eibsee (aspect: north). The findings demonstrated that the onset dates of all phases were earlier at the Ehrwald site. The mean differences ranged between 5 days (leaf unfolding) and 7 days (full flowering); all were significant. In relation to altitudinal gradients and temperature response rates (Table 7.3) the effect of different aspects equates to an altitudinal difference of 161 m, 184 m and 213 m and a temperature difference of 0.8 °C, 0.9 °C and 1.0 °C for leaf unfolding, beginning of flowering and full flowering, respectively.

Table 7.4 Onset dates (DOY) for leaf unfolding, beginning of flowering and full flowering in 2009 for birch trees located on westerly (W) and northerly (N) exposed sites at Ehrwald (mean altitude: 998 m a.s.l.) and Eibsee (mean altitude: 990 m a.s.l.), respectively, and their temporal difference (days); *n* number of sites, *P* significance

	leaf unfolding	beginning of flowering	full flowering
Ehrwald (W); n=23	1 May (117.8)	20 April (110.5)	24 April (114.0)
Eibsee (N); n=17	6 May (122.8)	26 April (116.4)	1 May (120.8)
difference (days)	5	5.9	6.8
P	0.003	< 0.001	< 0.001
test	t-test	Mann-Whitney U-test	

Temperature data in 2008 and 2009

Table 7.5 lists the different monthly mean temperatures in 2008, 2009 and for the standard reference period 1971-2000 recorded at the DWD climate station at Garmisch-Partenkirchen.

Table 7.5 Mean temperatures (°C) measured at the climate station Garmisch-Partenkirchen (DWD) for 2008, 2009 and for the standard reference period 1971-2000

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
2008	0.2	4.5	2.7	6.6	13.6	16	16.2	16.4	11.4	8.4	2.9	-1.4	7.9
2009	-0.4	-1.4	2	10	13.8	14.3	16.8	17.6	13.9	7.6	4.5	-1.7	7.8
1971-2000	-2.5	-1.0	2.6	6.1	11.3	13.9	16.0	15.5	12.0	7.5	1.6	-1.7	6.8

The annual average of mean air temperature was only 0.1 °C higher in 2008 (7.9 °C) compared to 2009. However, single months revealed major difference between the analysed years: temperatures in January to March 2009 were colder than the corresponding temperatures of the previous year, especially in February (2008: 4.5 °C, 2009: -1.4°C). Particularly outstanding were the high temperatures in April 2009 (10 °C). June and October in 2009 were associated with lower temperatures than in 2008, however, all other months relevant for the grass pollen season (May, July, September) showed higher temperatures in 2009.

Aerobiological data

Table 7.6 summarizes the pollen seasons 2008 and 2009 for birch and grass at the three pollen stations. A comparison between the two study years revealed that the birch pollen season in Garmisch-Partenkirchen in 2009 was not only associated with very high April temperatures (+3.4 °C compared to 2008) but also started two weeks earlier (8th April) compared to the previous year (22nd April). However, the duration of the pollen season was almost identical (2008: 42 days; 2009: 40 days). The total amount of pollen was 8% higher in 2008 (about 1600 grains m^{-3}) than in 2009 (about 1480 grains m^{-3}).

The pollen traps located at Ehrwald and UFS were characterized by a slightly later start (by four days) of the birch pollen season in 2008 compared to the lower site in Garmisch-Partenkirchen but the end of the pollen season was only one day later. At the higher elevation sites, the total pollen amounts were only 40% (Ehrwald) and 24% (UFS) of the Garmisch-Partenkirchen value. Peak values were also reached later at the higher locations. The largest delay was at UFS, where the peak was eight days later than in the valley. The birch pollen season in Ehrwald and UFS between the two years could not be directly compared due to the later start of pollen trapping in 2009. However, there were indications that the pollen season duration in 2009 was longer at UFS (> 47 days), associated with a higher peak amount (ca. 80 grains m^{-3}) and recorded three weeks earlier compared to the previous year. The difference between the end of the season at UFS and in the valley may also have been greater in 2009 than in 2008.

Table 7.6 Features of the birch and grass pollen seasons in 2008 and 2009 for Garmisch, Ehrwald and UFS: start, end and duration of the pollen season, peak date and amount and total amount (grains m^{-3})

Species	station	2008					2009				
		start	end	days	peak	total	start	end	days	peak	total
Betula	**GAP**	22.04.	02.06.	42	27.04. 248	1602	08.04.	17.05.	40	16.04. 228	1481
	Ehrw	26.04.	03.06.	39	30.04. 126	643	< 12.05.	~ 26.05.	~ 15	<17.05. (>23)	> 75
	UFS	26.04.	03.06.	39	05.05. 68	392	< 15.04.	~ 31.05.	~ 47	<15.04. (>77)	> 344
Gramineae	**GAP**	23.05.	31.08.	101	10.06. 265	2645	10.05.	08.10.	152	11.06. 969	9535
	Ehrw	24.05.	11.08.	80	21.06. 32	621	13.05.	16.08.	96	14.07. 68	1249
	UFS	20.06.	4.08	46	23.06. 28	246	13.05.	01.08.	81	13.06. 40	399

The duration of the grass pollen season in Garmisch-Partenkirchen in 2009 was 50 % longer than in 2008 (101 days in 2008, 152 days in 2009) and lasted till the beginning of October. The 2009 grass pollen season was also associated with a 3.6 times higher total pollen amount (ca. 9540 grains m^{-3}). However, the location of the pollen trap was changed in 2009 when it was repositioned from a roof (10 m above ground level) to 2 m above ground level in a small meadow. For the higher elevation sites the pollen season duration and also the peak amounts were lower in both years than in the valley: total pollen amounts at Ehrwald and UFS were 24 % and 9 % of that in Garmisch-Partenkirchen in 2008 and 13 % and 4 % in 2009, respectively.

Comparison of season parameters determined from phenological and aerobiological data in 2009

Betula

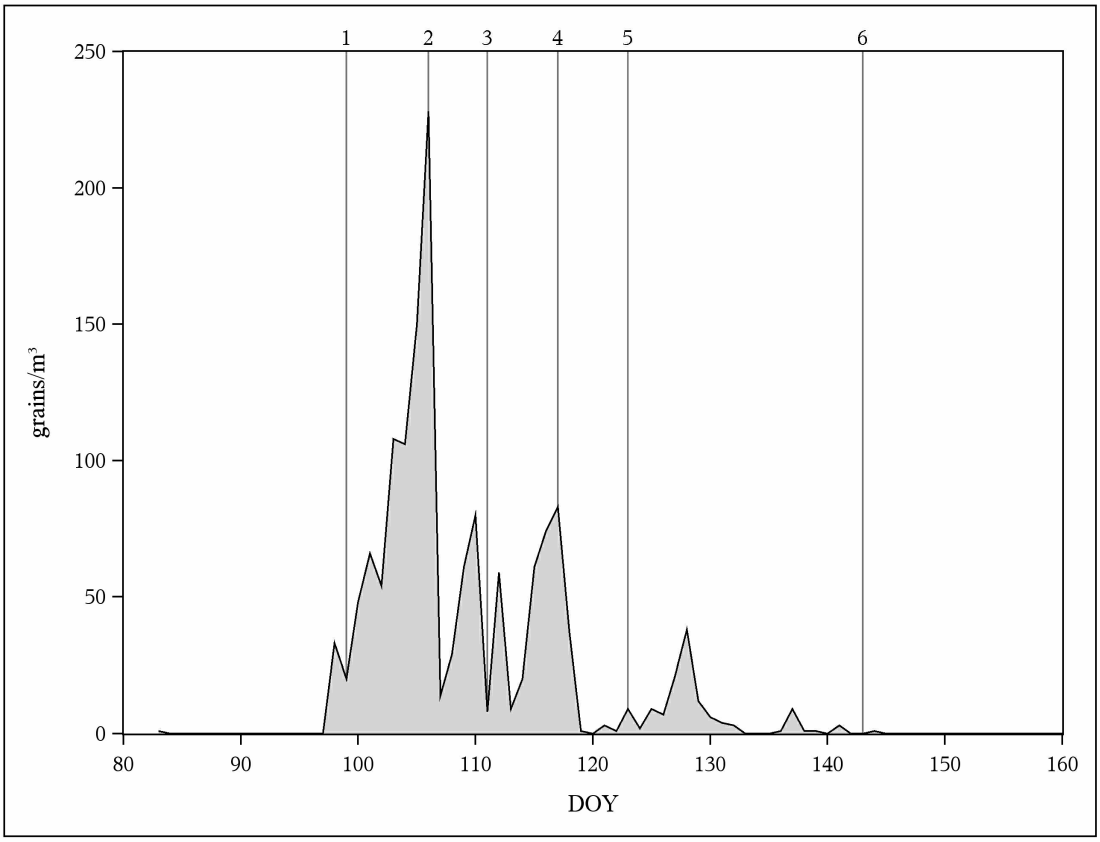
The relationship between phenology and airborne pollen counts can be explored in Fig. 7.2a in which the temporal course of birch pollen counts in Garmisch-Partenkirchen in 2009 and phenological onset dates for different altitudinal levels are shown. The first vertical line indicates the day when the first birch tree emitted pollen (FFD) in the valley of Garmisch-Partenkirchen (9th April). This date coincides very well with the start of the birch pollen season (8th April) calculated from aerobiological data. The second vertical line represents the mean date for the beginning of flowering (MFD) in Garmisch-Partenkirchen and exactly corresponds with the peak of the pollen season (16th April). The MFD in Ehrwald (line 3) and Eibsee (line 4) almost match a local peak observed at the Garmisch-Partenkirchen pollen station. The MFD of locations above 1200 m (lines 5 and 6) do not correspond directly with higher pollen levels in the valley.

Fig. 7.2b shows first and mean dates of the beginning of flowering (FFD and MFD) at different birch sites within the study area in 2009 compared with pollen amounts counted at Ehrwald and UFS. In contrast to Garmisch-Partenkirchen the start of trap recording in 2009 was delayed and thus pollen counts were not recorded before 15th April (UFS) or 12th May (Ehrwald), respectively. However, distinctive characteristics, especially for the UFS site, where no birches occur, can be derived. Within one week of the first flowering date and the start of the birch pollen season in Garmisch-Partenkirchen, pollen was transported to the 2650 m site. The corresponding daily pollen amount of 77 grains m^{-3} was the highest within the total pollen season of 2009, declining distinctively until 7th May. A few days after the MFD in Ehrwald (1200-1300 m), pollen amounts increased again, but did not again exceed 30 grains m^{-3}. The last small peak (8 grains m^{-3}) was observed on 29th May. The local peaks at Ehrwald matched those of UFS but were associated with higher pollen amounts.

Grass

The temporal course of grass pollen amount in Garmisch-Partenkirchen in 2009 is shown in Fig. 7.3a. For a comparison with phenology, we selected the mean dates for the beginning of flowering (MFD) for cocksfoot and meadow foxtail averaged for three of the lowest locations in the valley of Garmisch-Partenkirchen (700-800 m) and for two of the highest locations in Ehrwald (1100-1300 m). The start of the grass pollen season was on 10th May (Table 7.6) and therefore only two days before the MFD for meadow foxtail (vertical line 1) in the valley. During the next few days there was a continuous increase in pollen amounts and cocksfoot (2) started to flower two weeks after meadow foxtail in Garmisch-Partenkirchen. Almost three weeks later (16th June) the mean flowering date for meadow foxtail (3) occurred at the highest observation sites, however, pollen amounts had already decreased remarkably and did not again exceed 200 grains m^{-3}. The mean flowering date for cocksfoot (4) at high level sites was recorded on 30th June, but pollen grains remained under 50 grains m^{-3} until the end of the pollen season.

The temporal development of grass pollen amount in Ehrwald and at UFS in 2009 is shown in Fig. 7.3b. It can be seen that MFD in the valley for meadow foxtail (1) prompted an increase in pollen amounts, both in Ehrwald and at UFS, and that phenological dates of cocksfoot (2) corresponded well with local peaks. The onset of meadow foxtail (3) at higher altitudes did not seem to affect aerobiological data very much since it was followed by a decrease in pollen amounts. However, grass pollen amounts, especially for Ehrwald, were higher again after the mean flowering date of cocksfoot (4) at higher altitudes.



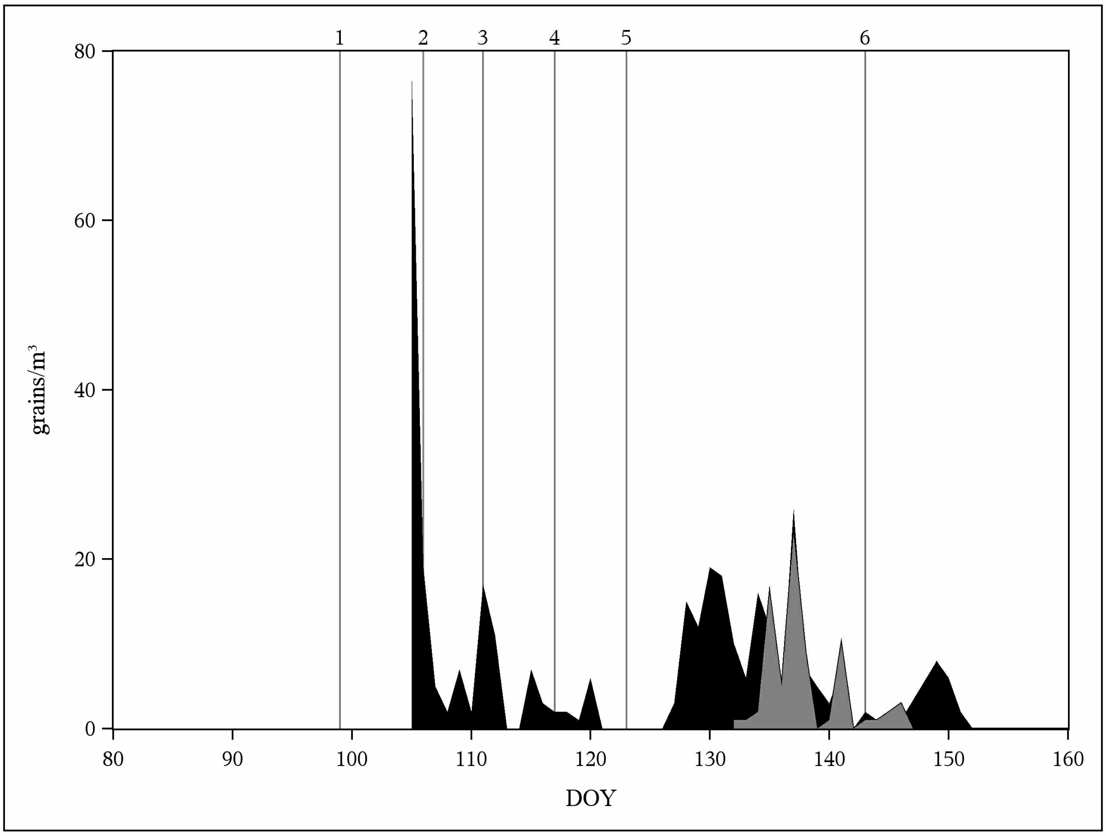
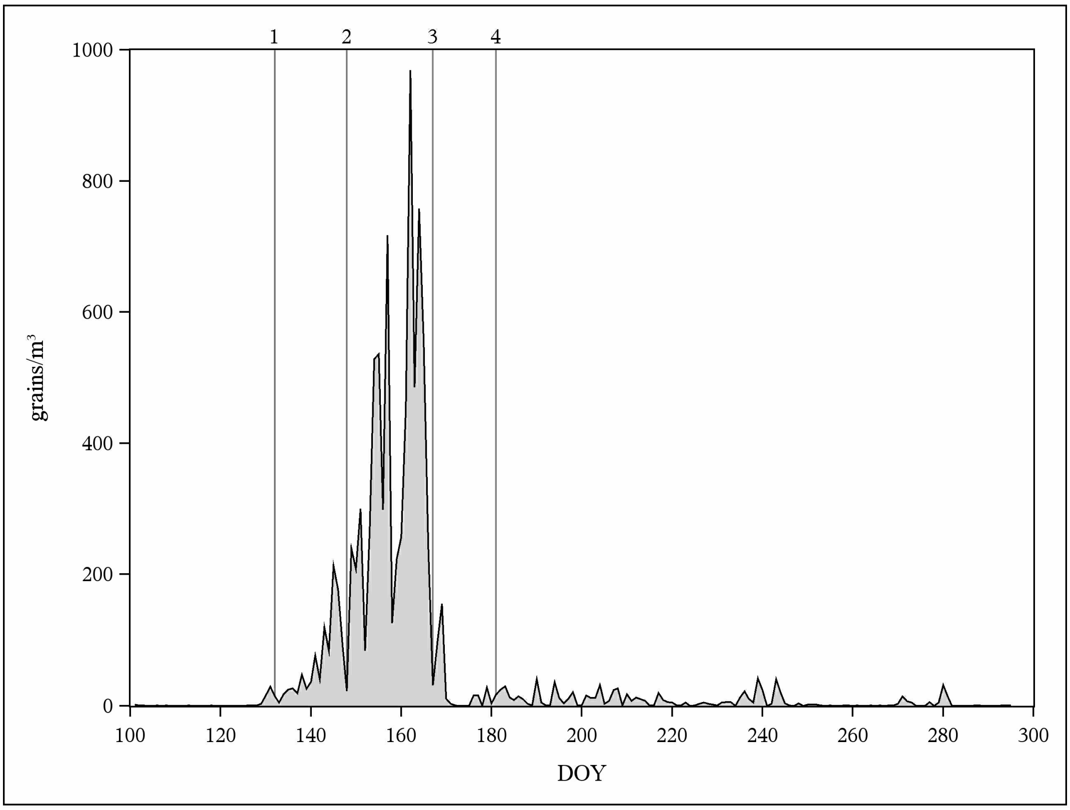


Fig. 7.2 Temporal course of birch pollen amount (grains m^{-3}) in 2009 for **a** Garmisch-Partenkirchen and **b** Ehrwald (grey) and UFS (black); vertical black lines (1-6) indicate the first flowering date (FFD, 1) and mean flowering date (MFD, 2-6) for different altitudinal ranges, *n* number of individuals: (**1**) FFD 9th April Garmisch-Partenkirchen (700-800 m, *n*=1); (**2**) MFD 16th April Garmisch-Partenkirchen (700-800 m, *n*=57); (**3**) MFD 21st April Ehrwald (900-1100 m, *n*=24); (**4**) MFD 27th April Eibsee (800-1020 m, *n*=24); (**5**) MFD 3rd May Ehrwald (1200-1300 m, *n*=5); (**6**) MFD 23rd May high level sites (1700 m, *n*=3)



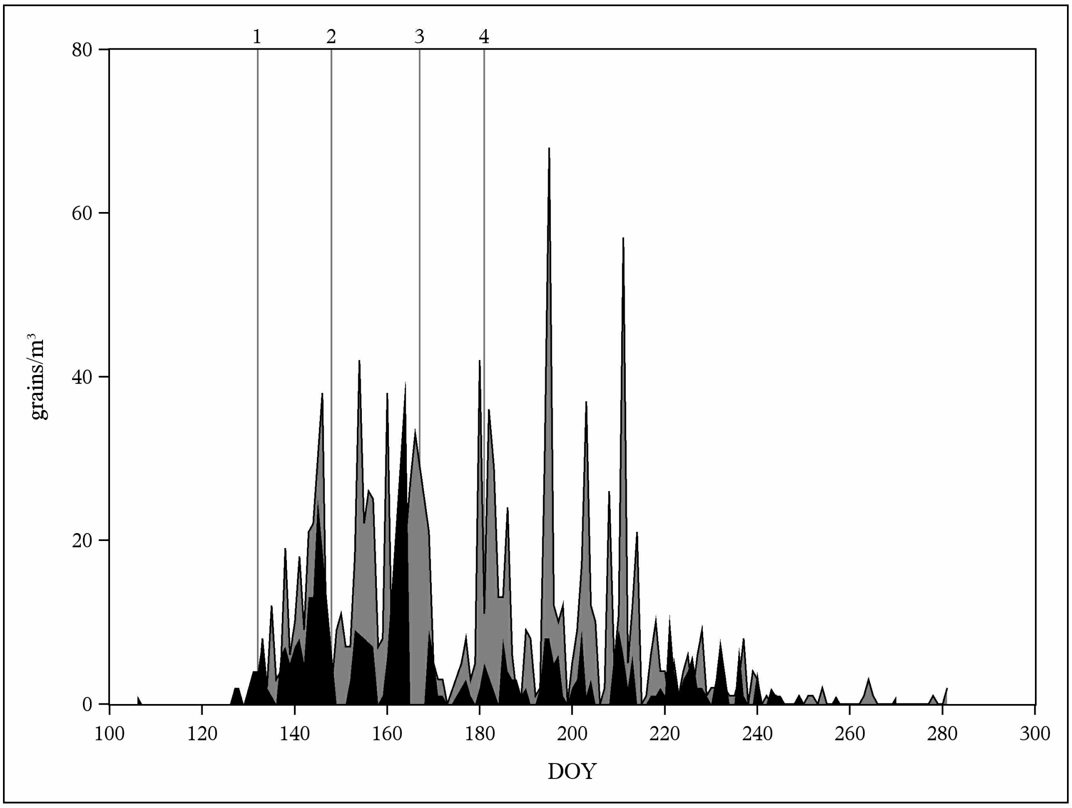


Fig. 7.3 Temporal course of grass pollen amount (grains m^{-3}) in **a** Garmisch-Partenkirchen and **b** Ehrwald (grey) and UFS (black) in 2009; vertical black lines (1-4) indicate mean flowering dates (MFD) for the lowest and the highest altitudinal level, n number of individuals: (**1**) meadow foxtail MFD 12th May Garmisch-Partenkirchen (700-800 m, *n*=3); (**2**) cocksfoot MFD 28th May Garmisch-Partenkirchen (700-800 m, *n*=3); (**3**) meadow foxtail MFD 16th June Ehrwald (1100-1300 m, *n*=2); (**4**) cocksfoot MFD 30th June Ehrwald (1100-1300 m, *n*=2)

Wind conditions and pollen amount at UFS in 2008 and 2009

Pollen that is released by the anthers of plants is suspended by turbulent vertical mixing and transported by wind (D'Amato et al. 2007). Wind is therefore a major factor that influences the dispersion of pollen after release into air (Laaidi 2001) and it is especially important for pollen traps located in vegetation-free areas, such as at UFS. Fig. 7.4 shows an example of the prevailing wind direction during the 2008 birch pollen season measured at the climate station in Garmisch-Partenkirchen (grey) and at the Zugspitze climate station (white).

The Zugspitze site is characterised by northerly, westerly and southerly / south easterly winds. Wind from the south can be attributed to the foehn, a wind system that results in higher temperatures on the northern slope of the Alps.

Garmisch-Partenkirchen is characterised by a mountain-valley wind system with daytime up-valley winds (NE, E) and nocturnal down-valley winds (SW, WSW). It can be assumed that this diurnal regime contributes to higher pollen amounts during the daytime since pollen from the valley is transported up to the high mountain site.

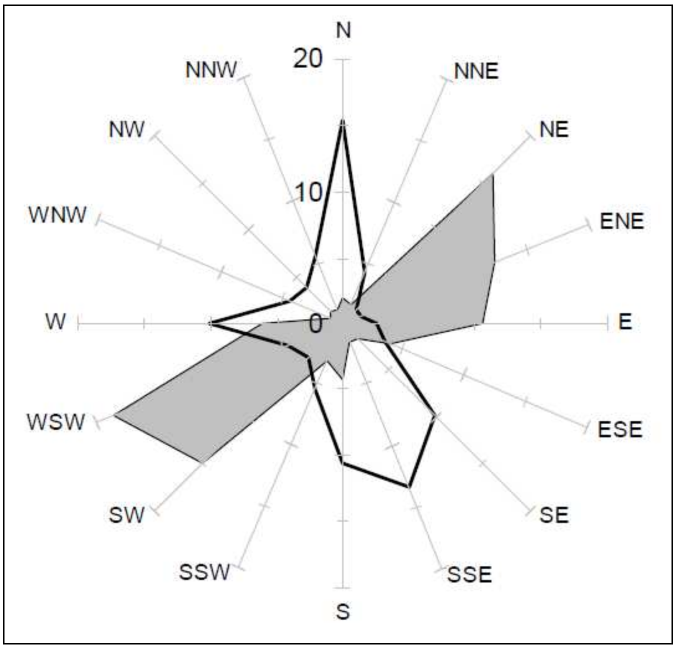


Fig. 7.4 Prevailing wind directions (hourly values) at Garmisch-Partenkirchen (grey) and Zugspitze (white) during pollen flight of birch 2008 at the Schneefernerhaus (UFS)

The necessary wind for this pollen transport originates from the northeast and accounted for almost 50% of the measured wind directions during 2008 and 2009 birch pollen seasons. Since diurnal pollen sums were only split at midnight and at noon, it is not possible to attribute the diurnal wind regime to these aerobiological data. Fig. 7.5 shows the prevailing wind directions for different times of the day and demonstrates that a separation at midnight and noon (Fig. 7.5a, b) does not coincide with the diurnal change of wind direction at Garmisch-Partenkirchen. A better discrimination is achieved when the wind direction is separated for the time before and after sunrise (Fig. 7.5c, d).

In Garmisch-Partenkirchen, pollen sums were higher during the 0:00 to 12:00 period with the only exception being birch pollen in 2009 (Table 7.7). In Ehrwald the picture was the reverse with most of pollen counted between 12:00-24:00, except for birch pollen in 2009. At the UFS most of the pollen was recorded between 12:00-24:00, however birch pollen in 2008 showed the opposite pattern. A t-test for comparison of means showed, however, that differences in pollen amounts for these two daily sub-periods were only significant for grass pollen amounts in Garmisch-Partenkirchen in 2009.

Table 7.7 Sums of birch and grass pollen amounts (grains m^{-3}) in 2008 and 2009 for the sites Garmisch, Ehrwald and UFS, divided into two daily sub-periods (0:00-12:00; 12:00-24:00). Bold p-value (t-test) indicates significant difference between the two time periods

species	station	2008			2009		
		0-12	12-24	p	0-12	12-24	p
Betula	GAP	816	776	0.901	650	831	0.512
	Ehrw	257	371	0.483	42	33	0.741
	UFS	219	187	0.701	96	248	0.091
Gramineae	GAP	1646	999	0.161	6388	3147	**0.048**
	Ehrw	274	347	0.226	528	730	0.116
	UFS	120	126	0.887	176	239	0.272

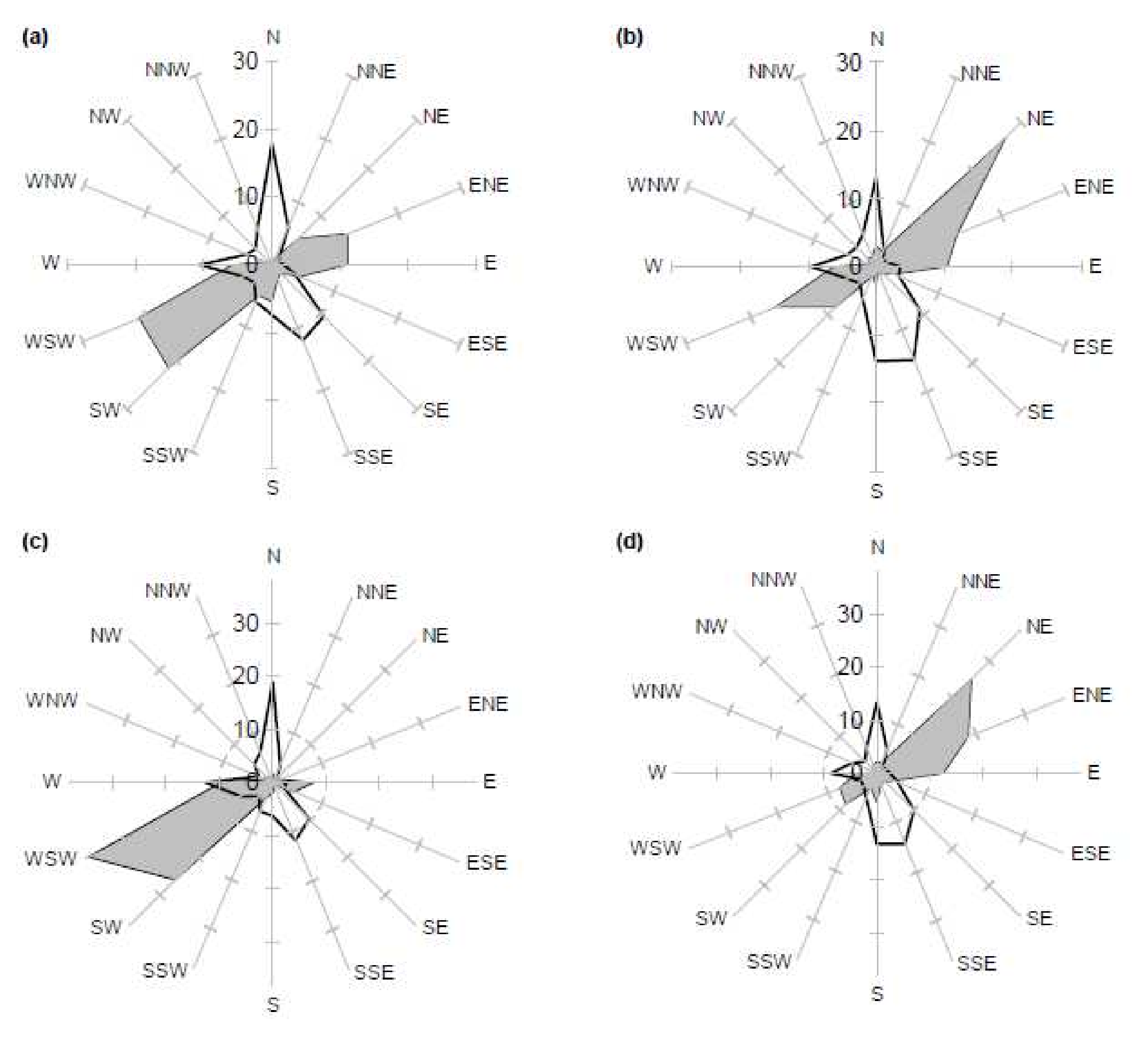


Fig. 7.5 Prevailing wind directions (hourly values) at Garmisch-Partenkirchen (grey) and Zugspitze (white) during pollen flight of birch 2008 at the Schneefernerhaus (UFS) separated for different times of the day; **a** 0:00-12:00, **b** 12:00-24:00, **c** 0:00-7:00 and 22:00-24:00, **d** 7:00-21:00

Fig. 7.6 a-d shows the sums (%) of birch and grass pollen in 2008 and 2009 that were transported with a specific wind direction. Since we only had two pollen counts per day, the data can merely be associated with the most frequent wind direction during the corresponding time period. Of the total birch pollen amount in 2008 recorded at the UFS, 40.9 % was associated with north easterly winds at Garmisch-Partenkirchen. Winds from the southwest and west-southwest contributed 11.3 and 33.5 % of the pollen amount, respectively. When wind at the Zugspitze came from the north 19.5 % of the pollen sum was recorded. South-easterly, south-southeasterly and westerly winds at the Zugspitze each contributed approximately 15 % of the pollen sum. In 2009 westerly winds at Garmisch-Partenkirchen contributed more than 60 % of the pollen amount at the UFS and southerly wind directions at the Zugspitze were associated with the highest percentage of pollen registered (41.4 %).

For grass pollen in 2008 the amount was almost equally distributed between winds from the northeast and southwest at Garmisch-Partenkirchen. About half of the pollen sums were associated with westerly winds at the Zugspitze. The highest percentage of pollen (26 %) in 2009 could be linked to westerly winds at the summit. Pollen sums were also higher at the UFS when northeasterly winds were prevalent in Garmisch-Partenkirchen.

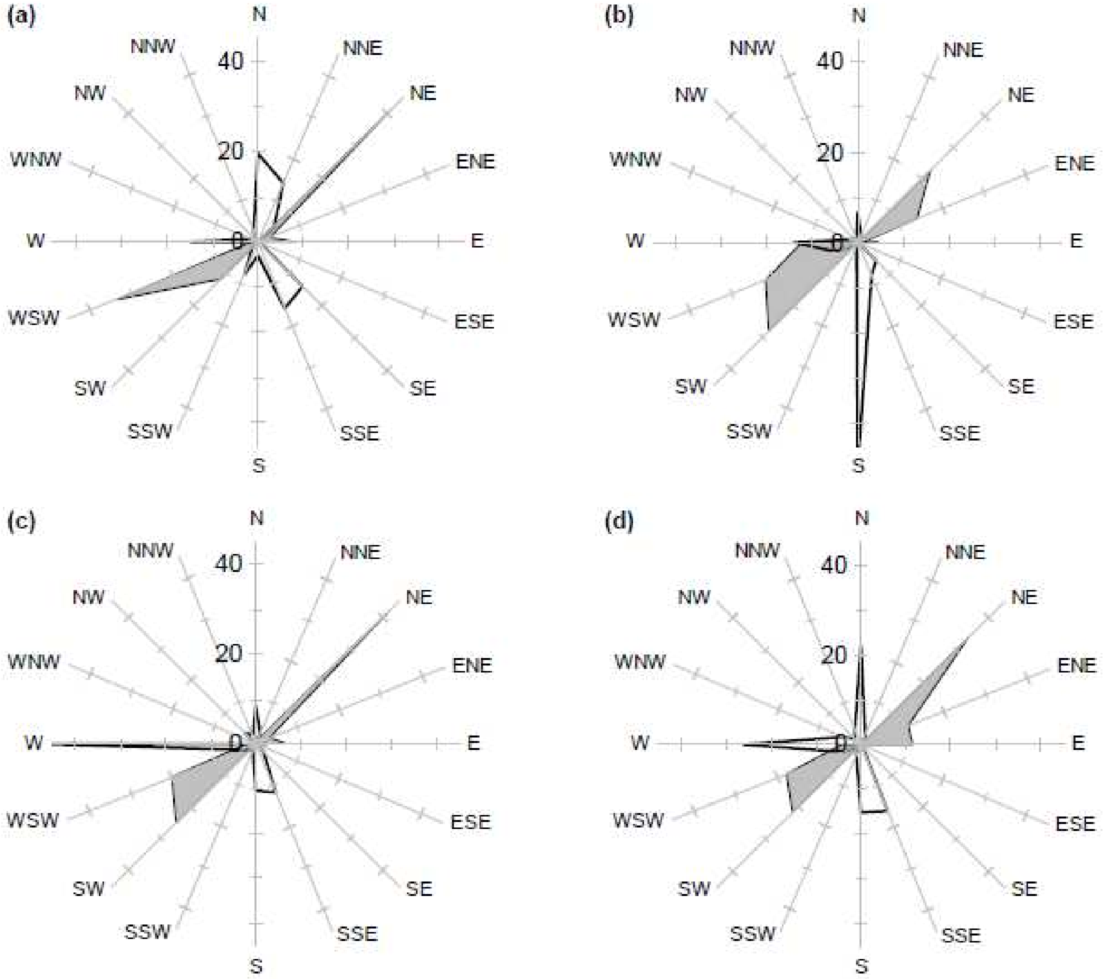


Fig. 7.6 Sums of pollen amount (%) associated with wind directions (modal values of two daily sub-periods, 0:00-12:00; 12:00-24:00) at Garmisch-Partenkirchen (grey) and Zugspitze (white) during pollen flight of **a** birch 2008, **b** birch 2009, **c** grass 2008 and **d** grass 2009 at the Schneefernerhaus (UFS)

7.4 Discussion

Influence of topography on phenology

Altitudinal gradients (Table 7.3) for all species and phases were statistically significant and associated with high R²-values indicating that altitude was able to explain a high percentage of the variation in phenological onset dates. The magnitudes of the regression coefficients were higher for grass phenophases (6.3 - 7.1 days (100 m)$^{-1}$) than for birch phenophases (3.1 - 3.2 days (100 m)$^{-1}$). Since temperature is the most important factor explaining phenological onset dates (Menzel and Fabian 1999; Badeck et al. 2004; Defila and Clot 2005) we conclude that the decrease in temperature with an increase in altitude is the main factor responsible for a delay of entry dates. Additionally, temperature response rates in phenology showed that phenological phases are delayed by almost 7 (birch) and 10 (grass) days per 1 °C decrease in temperature. Our findings suggest a discernible temperature sensitivity in the grasses and indicate that further temperature increases relating to climate change could lead to a notably earlier start of the grass pollen season, associated with major consequences for people suffering from pollen allergies.

Note that our study does not incorporate temperature data measured directly at the 36 phenological observation sites but temperature lapse rates calculated from two climate stations whereas the altitude of the highest situated station (2650 m) by far exceeds the altitude of our highest phenological observation site (1233 m). Besides, phenological observations were only conducted in one single year and should therefore be interpreted with caution. This makes it indispensable to compare our results with other studies. Altitudinal gradients for the Taunus hills, located in the Central German Uplands, were evaluated by Chen (1994). Regression coefficients for leaf unfolding of birch were 4 days (100 m)$^{-1}$. In addition, Rötzer and Chmielewski (2001) found a delay of 3 days (100 m)$^{-1}$ for the beginning of flowering of birch across Europe. For full flowering of cocksfoot and meadow foxtail Chen (1994) calculated a delay of only 2 days (100 m)$^{-1}$. Ziello et al. (2009) found an altitudinal gradient of about 4 days (100 m)$^{-1}$ for cocksfoot in the region of Garmisch-Partenkirchen. Whereas these values for birch almost match our findings, the values for the grass species are far lower. However, the study area of Chen (1994) only comprised a gradient from 100 m to 800 m.

Besides altitudinal effects, we also found discernible differences in phenology with different aspects (Table 7.4, 5 to 7 days). Chen (1994) also found that northern and southern exposed birches in the Taunus hills showed differences in onset dates up to 14 days. In our study, birches at the same altitudinal level but with different aspects revealed different onset times that equate to altitudinal / temperature differences ranging from 161 m / 0.8 °C for the beginning of leaf unfolding, to 184 m / 0.9 °C for the beginning of flowering and 213 m / 1.0 °C for full flowering. High temperature variations were also documented by Scherrer et al. (2011) who evaluated surface and soil temperatures in alpine landscapes and found substantial temperature variations of 2-3 °C related to slope exposure. Therefore, even locations at the same altitude can have noticeable differences in phenology and consequently in the timing of pollen release. Hence, being less exposed to pollen in high mountain altitudes is not only a question of elevation but also of aspect. However, our analysis only evaluated birch individuals of two different sites located at approximately the same altitude. Micrometeorological influences (e.g. proximity to forests or water bodies) that also alter flowering times (Jochner et al. 2011b) have not been evaluated here. Even though the database of grass (n=10) was not sufficiently large to allow a comparison of effects related to different aspects or microtopographical characteristics, phenology of grass species are believed to be strongly influenced by microclimate (Defila 2007) and might therefore show even higher differences than those that were detected for birch. The study of Scherrer et al. (2011), for example, not only found soil temperature differences for different slopes but also noticeable variations within slopes (3-4 °C) due to microtopography and even within a small plot (1 m²) resulting from plant cover effects.

Shifts in pollen seasons 2008 and 2009

The comparison of phenological and aerobiological data in Garmisch-Partenkirchen revealed a good agreement with respect to the start of the pollen season. Since phenology is responsive to the temperature of the preceding months (Menzel et al. 2006) this meteorological parameter is widely applied using mean temperatures or temperature sums in models for forecasting the start of the pollen season (e.g. Galán et al. 2001; Laaidi et al. 2003; Smith and Emberlin 2005). The two weeks earlier start of birch pollen season in 2009 compared to 2008 is probably related to the high mean April temperature (2009 10°C; 2008 6.6°C) that was among the six highest mean values since the start of temperature measurements in Garmisch-Partenkirchen in 1889. The average April temperature (1971-2000) was, however, only 6.1 °C, the highest value was recorded in 2007 (11.1 °C). As shown by Bruns (2009) and Jochner et al. (2011a), who described the phenological impacts of high temperatures in April 2009 for other parts of Germany, it can be assumed that the abrupt temperature increase in early April after a relatively cold winter and late spring also led to a much earlier and more rapid plant development in Garmisch-Partenkirchen, implying an earlier but also shorter birch pollen season compared to 2008. The same can be seen in the grass pollen season that also started almost two weeks earlier in 2009.

Synchronization between aerobiological and phenological data

Local emissions of birch pollen matched closely the timing of the start of the pollen season in 2009, defined by aerobiology. The peak of the pollen season also coincided with the mean flowering date of birch in the valley. Evidence that the pollen trap of Garmisch-Partenkirchen was affected by medium-range transport of pollen can be derived from Fig. 7.2 where the mean flowering dates of birch in Ehrwald and Eibsee, including Griesen, corresponded to local peaks of the temporal birch pollen concentration. Besides local influence, these peaks could be supplemented by a downward movement of pollen from higher locations. This movement can be attributed to the mountain-valley wind system that is characterised by down-valley winds during the night.

Pollen counts in Garmisch-Partenkirchen were not affected by mean onset dates of higher locations (> 1200 m, see Fig. 7.2a) where birch tree abundance and therefore pollen production was generally lower. This also indicates that the end of the pollen season can not be described and explained by phenological data as accurately as the start of the pollen season since a wide range of flowering times within a small area – as a consequence of the variety of temperature conditions characteristic of mountainous regions - leads to extended pollen emissions. Pollen originating from higher locations is transported later and over a longer distance to the valley of Garmisch-Partenkirchen but might be deposited earlier. Moreover, it is more difficult to predict the end of the pollen season since the effect of meteorological parameters on pollen emission and dispersion differ throughout the pollen season (Spieksma 1980; Vázquez et al. 2003). Sunshine and temperature are most important for growth and development before flowering occurs; sunshine, rainfall and relative humidity are relevant factors influencing pollen release and wind is the major factor responsible for pollen dispersal (Laaidi 2001). The time between the start and the peak of the pollen season is especially reliable for biological phenomena, e.g. pollen emission, and less affected by re-suspension of pollen that leads to an increase in pollen amounts (Vázquez et al. 2003).

Temporal delay between onset dates in phenology and aerobiology can partly be explained by long- or medium-range pollen transport (Estrella et al. 2006). The easiest way to detect long- or medium-range transport is to operate a pollen station, where no specimen of the considered species is located. This condition is satisfied for the pollen trap at the Schneefernerhaus (UFS), located at 2650 m a.s.l. We detected intermittently large amounts of birch and grass pollen that definitely do not originate from the near surroundings of the pollen station. At the UFS, birch pollen concentrations in 2008 and 2009 were about 25% of the amount that was measured in the valley of Garmisch-Partenkirchen. These high quantities may also be ascribed to the morphology of *Betula* spp. pollen: the almost spherical grains have a small diameter of 22 µm and belong to one of the furthest transported type of pollen grains (Sofiev et al. 2006). Estrella et al. (2006) reported that grass pollen is not as mobile as birch pollen. These grains mostly have a spheroid or ovoid shape and a diameter ranging roughly between 15 and 55 µm (Jäger 2008). We also only detected a smaller fraction of 9 % (2008) and 4% (2009) of the pollen at the UFS compared to the valley. However, the high amount of pollen in Garmisch-Partenkirchen in 2009 (ca. 9540 grains m^{-3}) can also be attributed to the location of the pollen trap since it was placed within a meadow in 2009 (2 m above ground level) and not on the roof of a building, as in 2008. First pollen grains at ground level might be up to 2 weeks earlier than at roof level (Rapiejko 1995). Therefore, not only the aerodynamic characteristics of pollen types but also the height of a plant relative to the trap can contribute to differences in pollen amounts.

The time interval between the date when the first birch pollen was emitted in the valley and the date when the first pollen grains were recorded at the UFS was less than one week; moreover the peak of the pollen amount at UFS was only one day before the local mean flowering date in Garmisch-Partenkirchen. Hence, pollen counts at the vegetation-free UFS are strongly related to phenological characteristics of the valley. All other local flowering dates at different altitudes were not so well reflected in the pollen data, suggesting that minor birch pollen emissions do not have a major effect on high mountain regions.

The time span between the aerobiological start of the grass pollen season and the mean flowering date of meadow foxtail for three locations in Garmisch-Partenkirchen was only 6 days, suggesting that the selected grass species is one of the earliest flowering ones and hence can be useful for predicting the start of the grass pollen season. Cocksfoot started to flower two weeks later and local pollen emission seems to contribute mainly to the pollen records. However, mean flowering dates for the grass species in higher regions (1100-1300 m) were relatively late (16th / 30th June) and did not account for an obvious increase in pollen amount in Garmisch-Partenkirchen. The mean flowering dates of the selected grass species meadow foxtail and cocksfoot in the valley of Garmisch-Partenkirchen also matched an increase in grass pollen at the higher elevated sites of Ehrwald and UFS (see Fig. 7.3b). Mean flowering dates of the two species at higher locations corresponded almost exactly with local peaks recorded at higher altitudinal levels, which was especially apparent for Ehrwald.

A minor inconsistency in phenological and aerobiological grass data has to be attributed to the species selected for observation. Whilst pollen of all *Gramineae* species is summarized in the aerobiological data we only observed phenological behaviour of cocksfoot and meadow foxtail. *Gramineae* records includes pollen from a larger number of species whose flowering periods overlap from spring to the end of summer (Jato et al. 2006). It is also evident that land use has a great impact on grass pollen concentrations. Therefore, it is obvious that the location of the pollen trap in Garmisch-Partenkirchen, surrounded by large areas occupied by meadows, led to a far higher pollen amount than in Ehrwald or at UFS.

Wind as an explanatory variable

Pollen concentrations at higher elevation sites are still detectable and often fluctuate in magnitude. This can be attributed to local weather conditions that are connected with specific wind directions. Jato et al. (2006) showed that pollen grains were predominantly abundant when turbulences keep wind-dispersed pollen in the air or when weather conditions provided low wind speeds where airborne pollen was transported at a very low velocity.

We only found diurnal variation in pollen amounts for *Gramineae* pollen in Garmisch-Partenkirchen in 2009 (Table 7.7). However, aerobiological data was only available twice a day for the time spans 0:00-12:00 and 12:00-24:00. Since birch shows its daily peak around midday and grass between 6:00 and 9:00 (Scheid and Bergmann 2004), it is evident that this separation is impractical for birch, but not for grass. Attributing wind directions to aerobiological data at this given temporal resolution does not fully allow an interpretation of medium-range transport. Phenological data at different altitudinal levels (see 3.4) is therefore more suitable to describe these characteristics.

7.5 Conclusions

Analyses of the flowering behaviour of major allergenic species along an altitudinal gradient in the Zugspitze area revealed a great dependence on altitude and on exposition. Grass species were found to be most responsive to altitude and therefore to temperature suggesting that climate change might have considerable effects on the start of the grass pollen season. Moreover, we found a good agreement in the start of phenological and aerobiological season dates, particularly at lower altitude, where local pollen emissions accounted most for the start of the season. In contrast, pollen amounts at the highest and vegetation-free site were particularly affected by medium-range transport, contributing to high and medically relevant pollen concentrations.

Besides the visual interpretation of temporal pollen curves in relation to phenological onset dates, there is a need for air mass back-trajectories that account for the daily atmospheric circulation pattern. However, the confirmation of these models is the weakest point, and that is why we need more direct observations of nature. This is especially true when considering mountainous and complex areas that are as small as in our study (Fig. 7.1, total area ~ 325 km², max. distance between pollen traps ~ 12 km). Back-trajectories also require a spatial grid of meteorological data which we do not have at the necessary resolution. Moreover, a finer temporal resolution of the aerobiological data would further allow for correlating meteorological data, especially wind data, with pollen and would enable a better interpretation and understanding of aerobiological processes.

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8 OZONE IMPACTS ON THE ALLERGENICITY OF BIRCH POLLEN

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Abstract

Background Evidence is compelling for a positive correlation between urbanization and prevalence of allergic sensitization and diseases. The reason for this association is not clear to date. Some data point to a pro-allergenic effect of anthropogenic factors on susceptible individuals.

Objective To evaluate how different factors of urbanization impact on pollen allergenicity.

Methods Catkins were sampled from birch trees from urban and rural sites in Munich, Germany. Pollen was isolated, NO_2 and O_3 exposure was measured and an index of urbanization was calculated for each sampling site. To estimate pollen allergenicity, Bet v 1 and pollen-associated lipid mediators (PALMs) were measured in aqueous pollen extracts. Neutrophil migration assays were performed to determine the immune stimulatory capacity of the pollen specimens. To assess the immune modulatory potential of the pollen specimens, their capacity to inhibit the dendritic cell interleukin-12 response was analyzed. *In vivo* allergenicity was assessed by skin prick tests in 5 human volunteers.

Results The study revealed ozone as prominent environmental factor influencing the allergenicity of birch pollen. Enhanced allergenicity, as assessed in skin prick tests, was mirrored by enhanced allergen content. Beyond that, ozone induced changes in lipid composition and chemotactic and immune modulatory potential of the pollen.

Conclusion Increased immune modulatory potential of urban pollen specimens might indicate an elevated risk for allergic sensitization against birch pollen in the cities. In contrast, pollen collected from higher ozone exposed trees outside the city might be of higher allergenic potential in already sensitized patients.

Keywords. Environmental factors, urbanization, immune stimulation, lipid mediators, ozone, birch pollen.

8.1 Introduction

Epidemiological data show an increasing trend in allergies (Upton *et al.* 2000) leading to a major health problem in industrialized societies. Reasons discussed for this rising trend of allergic diseases include a westernized life style with diminished immune stimulation by severe and repeated infections (Radon *et al.* 2004, Strachan 1999) and rising anthropogenic air pollution (Behrendt *et al.* 1995, Kramer *et al.* 2000). Particularly, irritant gases and diesel exhaust particles have been shown to exert adjuvant or aggravating effects on sensitization and elucidation phases of allergic immune responses (Heinrich & Wichmann 2004, Diaz-Sanchez & Riedl 2005). Effects on cells of the immune system as well as epithelial barrier disruption are discussed (Ring *et al.* 2001). However, pollutants in ambient air do not only affect humans. In fact, they can also impact on the allergen-carrier itself, which, in the case of pollen allergy, is the pollen-producing plant. The question arises whether the observed increase in allergic diseases in the western world might in part be explained by modified allergenicity of pollen caused by global change. Extreme weather events such as warm spells are likely to influence our ecosystems more than mean temperatures changes (Jentsch *et al.* 2007). Climate extremes are often observed in cities, which can function as heat islands, and can thus be regarded as a mirror of future climate (Ziska *et al.* 2003). However, urbanization is not only characterized by higher temperatures, but also by lower wind speeds and higher levels of pollutants like particulate matter, CO_2 or NO_2. Some studies already addressed the question how pollutants affect the allergen carrier, showing that single pollutants strongly differ in their effects (Behrendt *et al.* 1997, Masuch *et al.* 1997).

The current study expands these observations by analyzing the allergen producer - the birch tree - in its natural environment. Hereby, we take into account the complex differences inherent to urban and rural environments such as different temperatures, air composition and humidity. Recent studies showed that pollen do not only liberate allergens, but also multiple non-allergenic compounds such as pollen-associated lipid mediators (PALMs), NADPH-oxidases and adenosine (reviewed in Gilles *et al.* 2012), which have been shown to exert immune modulatory or -stimulatory effects. For this reason, pollen content of PALMs and adenosine were included as study end point.

Herein, we provide first evidence that exposure of birch trees to elevated ozone levels modify both, the content of major allergen Bet v 1 and the ratio of immune stimulatory to immune modulatory PALMs. This is in line with more severe skin reactions of allergic patients to high ozone-exposed pollen. The differences in allergen and adjuvant lipid content might reflect differences in the potential of birch pollen for sensitization or aggravation of existing allergic disease. Long-term increases in urbanization and concomitant accumulation of tropospheric ozone might therefore bring about an aggravation of symptoms in pollen-allergic patients.

8.2 Methods

Sampling of birch pollen

Catkins were collected from birch trees located in central (n=20) or in the greater area of Munich (n=20). An index of urbanization was estimated for each of the locations (Fig. 8.6). Sampling of catkins took place during the birch flowering season in spring 2010. Developmental stages of birch catkins were assessed by an adopted and extended code of the BBCH (Biologische **B**undesanstalt, **B**undessortenamt und **Ch**emische Industrie) (Meier 2001). The code included 12 different developmental stages, starting with winter rest (stage 50) and ending with end of flowering (stage 69). The stages of collection were 60 (single catkins sporadically emit pollen) and 61 (10% of the catkins emit pollen). Immediately after collection, catkins were air-dried for 24 hours, counted and weighed and pollen was extracted by sieving. Pollen was stored at –80°C until further analyses.

Preparation of aqueous pollen extracts for Bet v 1, LTB_4 and PGE_2 ELISA

Aqueous pollen extracts (APEs) were prepared in 0.1 M NH_4HCO_3, pH 8.1, as previously described (Buters *et al.* 2008). For skin prick tests, APEs were prepared as described in Gilles *et al.* (2011). APE concentrations given in the figures refer to the elution of a given amount of pollen per mL of buffer (e.g. 1 mg pollen was eluted in 1 mL of buffer) before centrifugation. They do not refer to actual protein concentrations in the extracts.

Blood donors

Healthy, non-atopic blood donors without a history of allergic diseases were tested by RAST for common allergens including birch allergens. All subjects were tested negative and total IgE was <100 IU/ml. Volunteers did not take any medication for at least 15 days before blood sampling. The ethical committee of the Technical University of Munich approved the study, and volunteers were enrolled after written informed consent.

Isolation and culture of monocyte-derived dendritic cells

Monocyte-derived dendritic cells (moDCs) were cultured from human peripheral blood monocytes as described (Gilles *et al.* 2009). Immature DCs were harvested on day 5 followed by stimulation with LPS plus high and low ozone APE. After 24 h, supernatants were collected, and IL-12p70 was measured by ELISA (BD Pharmingen, Heidelberg, Germany). Viability of the cells after 24 h of culture was tested by propidium iodide incorporation and subsequent FACS analysis. Viability was not decreased by any of the conditions.

Neutrophil migration assays

The chemotactic activity of aqueous pollen extracts from high and low ozone exposed pollen was evaluated by measuring neutrophil migration through a 5µm pore polycarbonate membrane in a 96-well plate (ChemoTx Disposable Chemotaxis System). Neutrophils were isolated from peripheral blood as described (Traidl-Hoffmann *et al.* 2002). APEs were pipetted into the bottom chamber, neutrophils were suspended in complete RPMI 0.5% BSA at $1x10^6$ cells/ml and added to the top of the membrane. After 1 h of incubation at 37°C with 5% CO_2, the cell suspension was removed, the plate centrifuged and cells that had transmigrated into the lower chamber were recovered and counted with a FACSCalibur (Becton Dickinson, Heidelberg, Germany).

Bet v 1, PGE_2 and LTB_4 ELISA

Bet v 1 levels were determined by sandwich ELISA as described (Buters *et al.* 2008). Bet v 1-specific antibodies MAK 2E10G6G7 and 4B10D10F8 were kindly provided by Joachim Ganzer, Allergopharma, Reinbek/Hamburg, Germany. Concentrations of the eicosanoid-like PALMs in aqueous pollen extracts were measured by commercially available enzyme immunoassays for prostaglandin E_2 and leukotriene B_4 (GE Healthcare, Germany) according to the supplier's protocol.

Passive sampling of NO_2 and O_3, measurement of adenosine in pollen extracts, and calculation of urban index

For detailed information, see the Methods section of this article's Online Repository.

Skin prick tests

Birch allergic patients (n=5) were pricked on their forearms with APE (10mg/mL) prepared of low-ozone and high-ozone pollen samples (n=2 each). Wheal and flare sizes were measured after 15 min.

Statistics

Unpaired t-test was used for Gaussian populations to determine statistically significant differences between samples. For non-Gaussian populations, non-parametric Mann-Whitney test was applied. The correlation coefficients (r^2) and 95% confidence intervals were calculated using the Pearson approach for Gaussian populations and the Spearman approach for non-parametric correlation (Graph Pad Prism 5, San Diego, CA, USA). P values of 0.05 or less were considered significant (**: $p<0.01$, ***: $p<0.001$).

8.3 Results

Higher ozone exposure of trees corresponds to higher pollen Bet v 1 content

We first examined the correlation between the exposure of trees to anthropogenic environmental parameters (ambient NO_2 and O_3, temperature, urbanization index) and the expression of allergen (Bet v 1) in the pollen. There was no significant difference between Bet v 1 contents in pollen specimens from trees specified as “rural” and “urban” (Fig. 8.1a). Likewise, no correlation was seen between Bet v 1 content and the calculated urbanization index (Fig. 8.1b). However, a positive correlation was observed between Bet v 1 content and O_3 levels (Fig. 8.1c). In contrast, pollen Bet v 1 content was not correlated with ambient NO_2 levels (Fig. 8.1d).

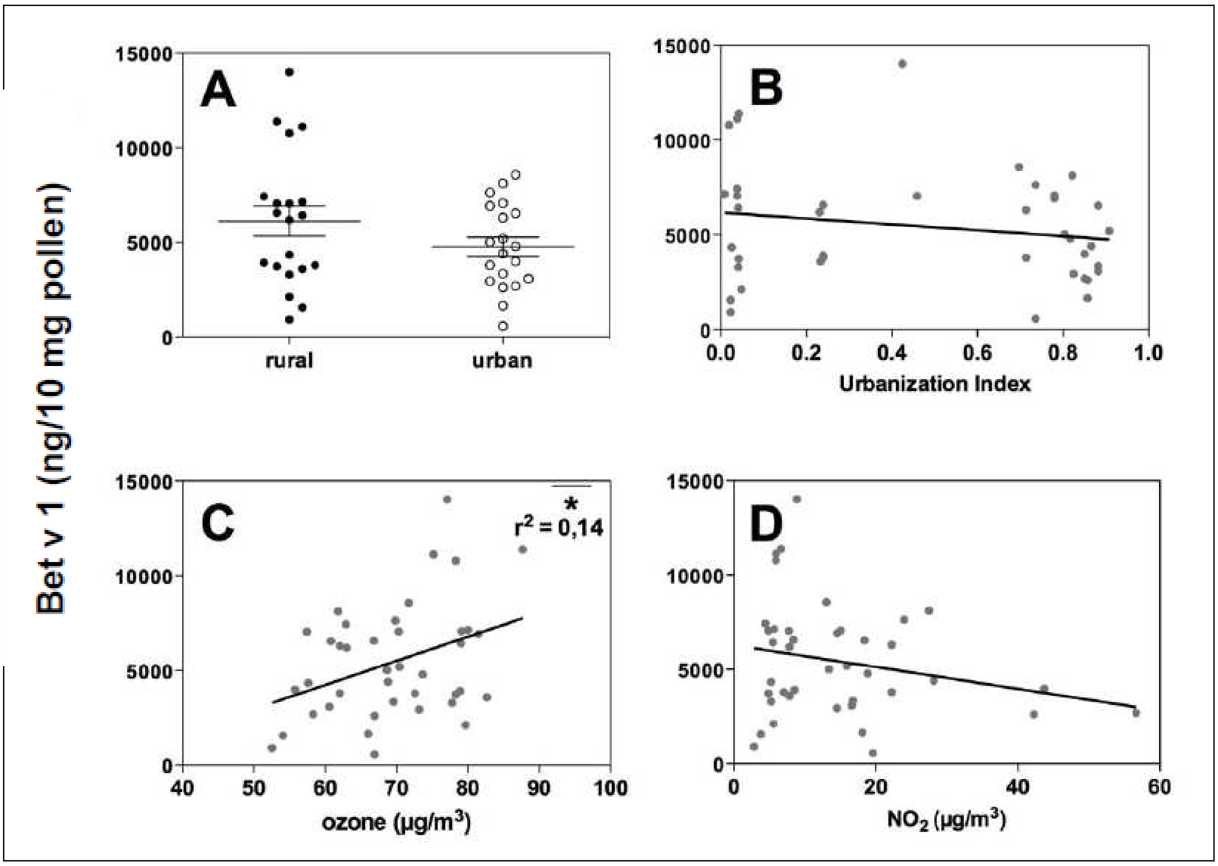

Fig. 8.1 Bet v 1 content of birch pollen in trees exposed to different, urbanization-related environmental conditions. **A**: Bet v 1 content did not differ in pollen sampled from urban and rural trees. **B**: No significant correlation was seen between pollen Bet v 1 content and the calculated urbanization index. **C**: Pollen Bet v 1 content was negatively correlated ozone levels in ambient air. **D**: No significant correlation showed between Bet v 1 and ambient NO_2.

A low urbanization index corresponds to a high ratio of $PALM_{LTB4}$ to $PALM_{PGE2}$

To determine immune stimulatory and -modulatory potential of the birch pollen specimens, we analyzed the content of PALMs in the different pollen extracts. Prostaglandin E_2-like PALMs, termed $PALM_{PGE2}$, harbor the immune modulatory PALMs (Traidl-Hoffmann *et al.* 2009, Behrendt *et al.* 2001). The content of $PALM_{PGE2}$, as assessed by ELISA, did not differ between pollen specimens from urban and rural sites (Fig. 8.2a). Likewise, no correlation was observed between the content of $PALM_{PGE2}$ and the calculated urbanization index (Fig.

8.2b). The LTB_4-like PALMs harbor the chemotactic, immune stimulatory PALMs (Traidl-Hofmann *et al.* 2002). There was a trend towards increased $PALM_{LTB4}$ content in extracts from rural as compared to urban pollen specimens (Fig. 8.2c), but the difference was not statistically significant (p=0.06). A similar, non-significant trend was observed correlating $PALM_{LTB4}$ content and UI (r^2=-0.049, p=0.10; Fig. 8.2d). However, the ratio of $PALM_{LTB4}$ to $PALM_{PGE2}$ differed significantly between pollen from urban and rural trees (Fig. 8.2e), and a low UI corresponded to a higher $PALM_{LTB4}/PALM_{PGE2}$ ratio. We also determined the content of adenosine in pollen, recently shown to promote the differentiation of regulatory T cells by dendritic cells *in vitro* (Gilles *et al.* 2011), differs among birch trees grown under urban or rural conditions. There was no correlation of adenosine concentrations and UI or urbanization-related factors such as ambient NO_2/O_3 or air temperature (data not shown).

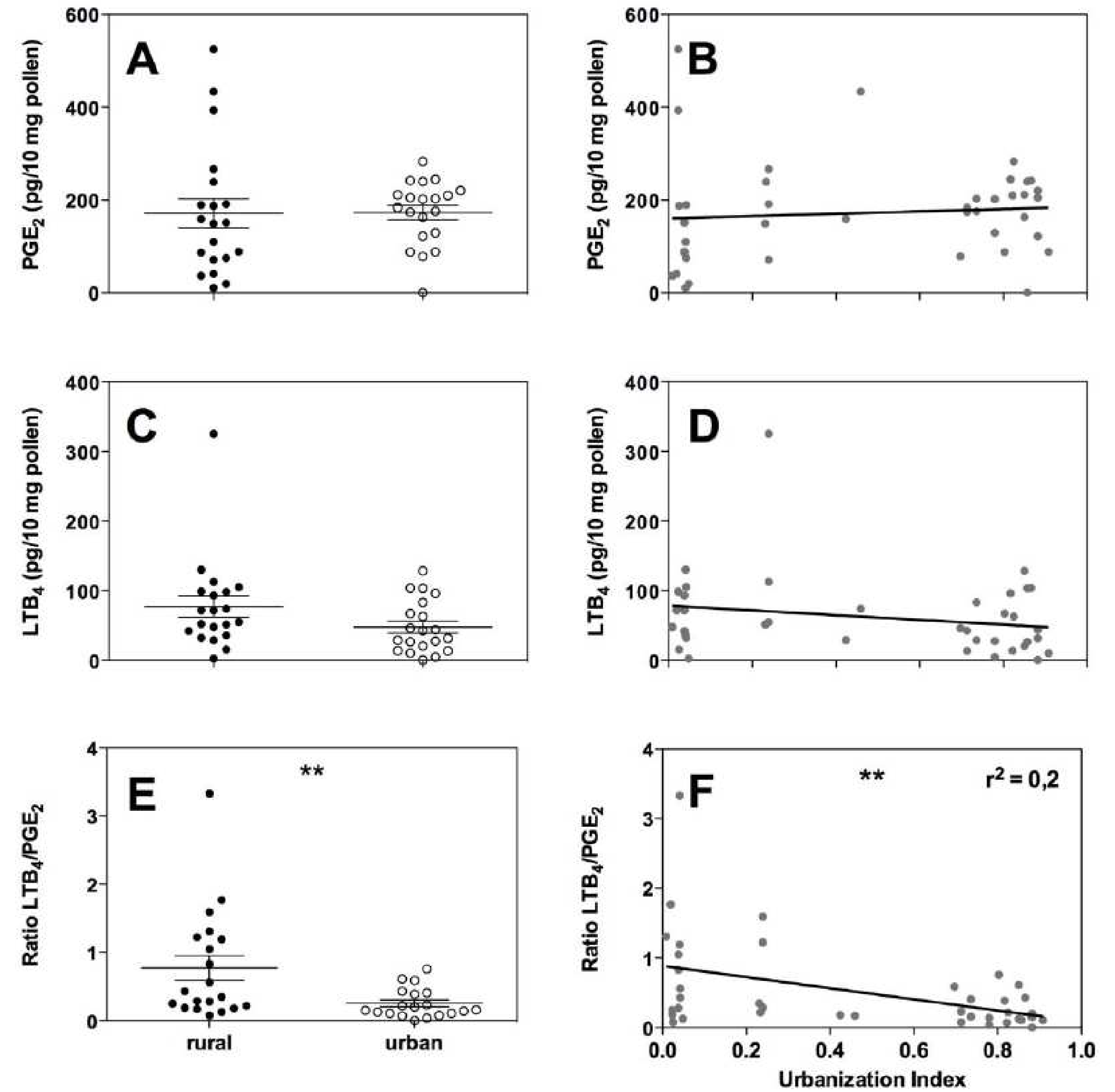

Fig. 8.2 Content of PALMs in pollen samples from urban and rural birch trees. **A**: $PALM_{PGE2}$ contents did not differ in pollen from urban and rural trees. **B**: No correlation was seen between $PALM_{PGE2}$ and urbanization index. **C**: $PALM_{LTB4}$ content was slightly enhanced in pollen sampled from rural trees. **D**: $PALM_{LTB4}$ did not show any significant correlation to urbanization index. **E**: The ratio of $PALM_{LTB4}/PALM_{PGE2}$ was significantly higher in pollen from rural compared to urban trees. **F**: The ratio of $PALM_{LTB4}/PALM_{PGE2}$ is significantly correlated with urbanization index.

Elevated ozone levels are associated with lower contents of $PALM_{PGE2}$ **and higher** *$PALM_{LTB4}/PALM_{PGE2}$-ratio*

No correlation was observed between $PALM_{PGE2}$ contents and ambient NO_2 (Fig. 8.3a). Instead, the $PALM_{PGE2}$ content was negatively correlated with ambient O_3 levels (Fig. 8.3b). $PALM_{LTB4}$ contents were not correlated either with NO_2 or O_3 levels (Fig. 8.3c, d). Finally, a higher $PALM_{LTB4}/PALM_{PGE2}$ ratio was negatively correlated with NO_2 and positively correlated with O_3 levels (Fig. 8.3e, f).

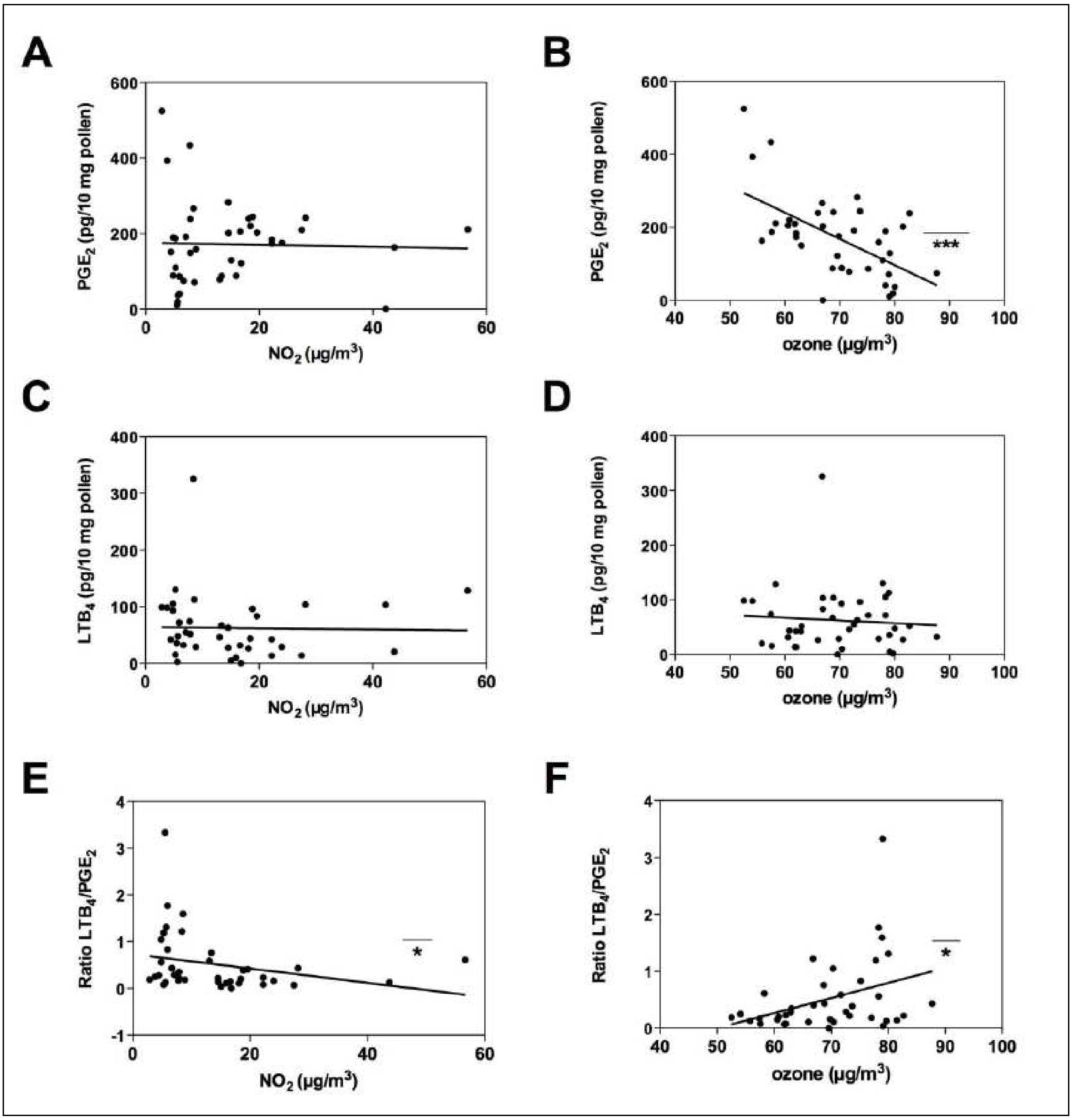

Fig. 8.3 Content of PALMs in pollen of trees exposed to different ambient gas concentrations. $PALM_{PGE2}$ content did not show any correlation with ambient NO_2 levels (**A**) but were significantly correlated with ozone (**B**). $PALM_{LTB4}$ content did not show any correlation with NO_2 (**C**) or ozone levels (**D**). The ratio of $PALM_{LTB4}/PALM_{PGE2}$ showed a negative correlation with NO_2 (**E**) and a positive correlation with ozone (**F**).

High-ozone pollen induces enhanced neutrophil chemotaxis but is less potent in inhibiting dendritic cell IL-12 production

To determine whether the observed correlation of O_3 exposure with an elevated $PALM_{LTB4}/PALM_{PGE2}$ ratio is paralleled by enhanced immune stimulatory potential of the pollen, neutrophil migration towards the different APEs was assessed. Here, pollen samples from high ozone-exposed trees induced significantly stronger neutrophil chemotaxis than pollen from low ozone-exposed trees (Fig. 8.4a). To test the immune modulatory capacity of low and high ozone-exposed pollen, monocyte-derived dendritic cells were stimulated with LPS in the presence and absence of APE, and IL-12 was measured in the supernatants. As shown in Fig. 8.4b, pollen from high ozone-exposed trees were less potent inhibitors of the moDC's IL-12 response than low ozone-exposed pollen (Fig. 8.4b).

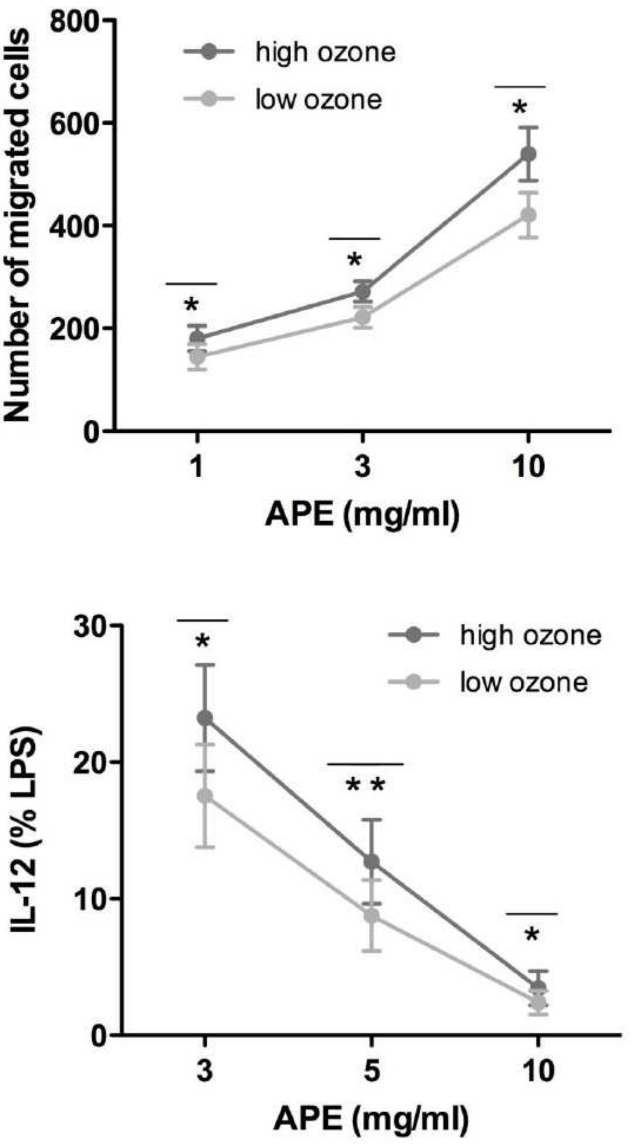

Fig. 8.4 Immune stimulatory versus immune modulatory potential of high and low-ozone pollen samples. Aqueous extracts of birch pollen sampled from high ozone-exposed trees (n=3) induce stronger neutrophil chemotaxis compared to low ozone pollen (n=3) (**A**). In contrast, birch pollen from low ozone-exposed trees is more potent than high-ozone pollen in inhibiting the LPS-induced release of IL-12p70 from human monocyte-derived dendritic cells (**B**). APE = aqueous pollen extract. *: $p<0.05$.

High-ozone pollen induces stronger allergic immune responses in the skin than low-ozone pollen

To test for clinical relevance of enhanced Bet v 1 levels, $PALM_{LTB4}/PALM_{PGE2}$ ratio and neutrophil chemotaxis, birch pollen allergic patients were subjected to skin prick tests with APEs prepared from different pollen specimens. Wheal and flare sizes were significantly larger when patients were pricked with APEs prepared from pollen from high ozone-exposed trees (Fig. 8.5a, b) going in line with higher Bet v 1 content of high-ozone pollen. Also, a significant positive correlation showed between wheal/flare size and ozone exposure of the pollen (Fig. 8.5c, d).

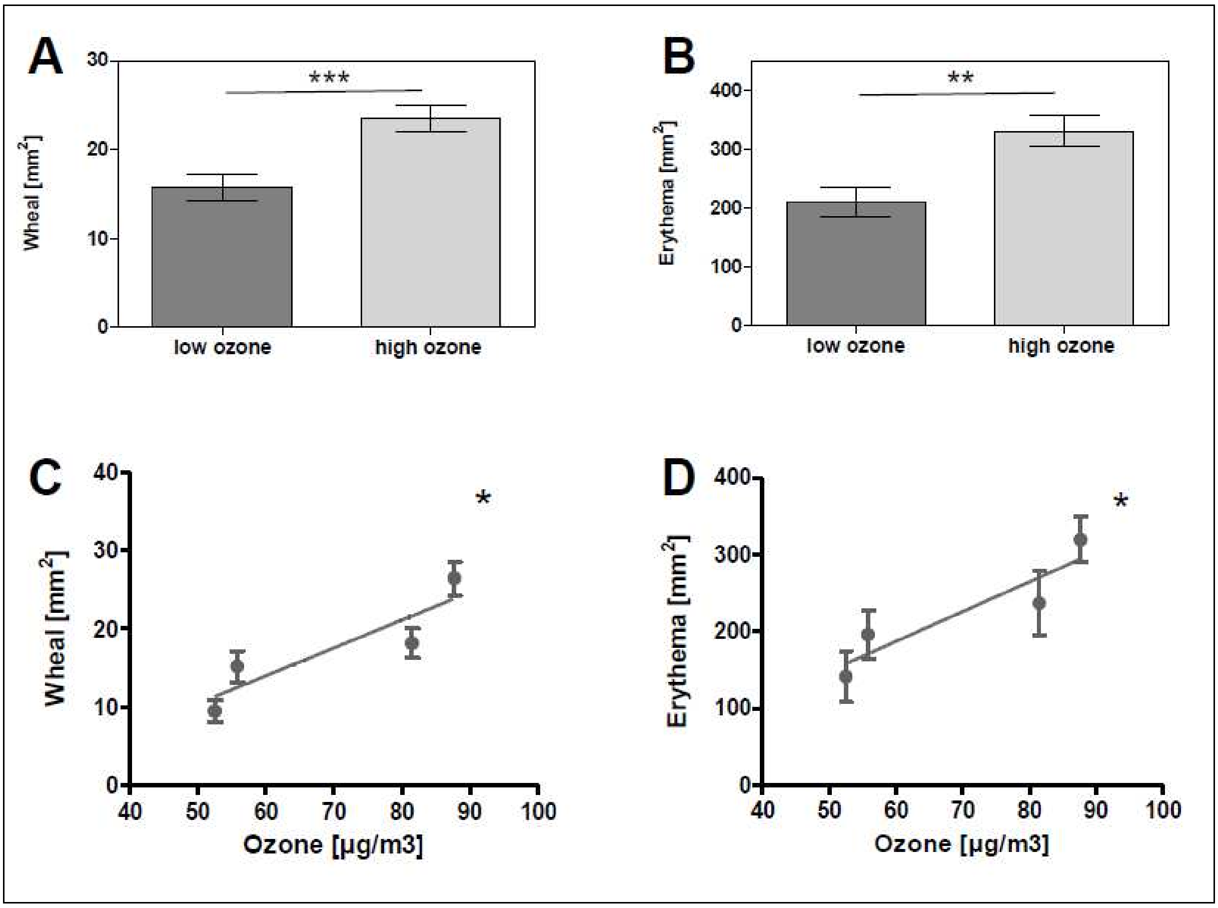

Fig. 8.5 Differences in the cutaneous immune response towards pollen from high and low-ozone exposed birch trees. High-ozone pollen induced larger wheals (**A**) and flare (**B**) in skin prick tests compared to low-ozone pollen. Pollen ozone exposure showed a positive correlation with wheal (**C**) and flare size (**D**) in prick tests. *: $p<0.05$. Number of two urban and two rural pollen samples were each tested on 5 different patients.

8.4 Discussion

The present study analyzes for the first time how anthropogenic factors related to urbanization influence the allergenicity of pollen sampled from naturally exposed birch trees. Moreover, we explicitly follow a holistic approach of allergenicity addressing not only allergen, but also of adjuvant, non-allergenic components released from pollen. Our data suggest ozone exposition as important factor influencing the allergenicity of birch pollen.

A central finding of our study is that birch trees exposed to elevated ambient ozone produce pollen that release higher levels of Bet v 1. Recent studies suggest that exposure to elevated ozone can lead to an increased allergen content in grass species (Masuch *et al.* 1997, Eckl-

Dorna *et al.* 2010). In these studies, plants were exposed to defined ozone concentrations by aeration in closed-top cabinets of a greenhouse or in open air. In our study, trees were subjected to pollen sampling in their natural environment and under natural exposure conditions. Ozone levels were moderately elevated for rural as compared to urban trees (Fig. 8.8). This might indicate that the trend towards higher Bet v 1 contents of pollen from rural trees is, at least in part, due to differences in ambient ozone. Confounders might be higher urban temperatures and NO_2. Ozone exposure of birch trees was not only associated with increased allergen content in pollen but also with an altered composition of PALMs. These lipids deriving from linolenic and linoleic acids have been shown to exert immune modulatory as well as -stimulatory activities *in vitro* and *in vivo* (Traidl-Hofmann *et al.* 2002, Plotz *et al.* 2004, Traidl-Hoffmann *et al.* 2005, Gutermuth *et al.* 2007, Mariani *et al.* 2007, Bryce *et al.* 2010, Gilles *et al.* 2010). Herein, no significant differences in $PALM_{LTB4}$ and $PALM_{PGE2}$ content were observed between birch pollen collected from urban and rural sites. This seems to be in contrast to a recent study (Behrendt *et al.* 2002). However, this observation is most likely explained by the fact that in the present study, pollen sampling was carried out at distinct maturation stages of both urban and rural catkins. The content of PALMs in pollen grains differs profoundly depending on maturation (Fig. 8.7). One parameter influencing catkin maturation is temperature, which differs between urban and rural environments. Likewise, Bet v 1 content was shown to depend strongly on pollen maturation (Buters *et al.* 2010). These results were reproduced by the current study (Fig. 8.7).

Immune stimulatory PALMs, such as phyto-octadecadienoic and phyto-octadecatrienoic acids, induce LTB_4 receptor-dependent chemotaxis and activation of neutrophils and eosinophils *in vitro* (Traidl-Hoffmann *et al.* 2002, Plotz *et al.* 2004). Among the immune modulatory PALMs are plant isoprostanes identified as E_1-phytoprostanes. E_1-phytoprostanes inhibit the IL-12 response in maturing DCs by inhibiting NFκB and a mechanism dependent on peroxisome proliferator-activated receptor (PPAR)-γ (Gilles *et al.* 2009, Traidl-Hoffmann *et al.* 2005), finally licensing DCs to differentiate naïve $CD4^+$ T cells into Th2 cells (Traidl-Hoffmann *et al.* 2005).

In plants, which lack arachidonic acid, all eicosanoid-like lipids are synthesized from α-linolenic acid. Phyto-octadecandienes, e.g., 13-hydroxy-octadecadienoic acid (13-HODE), have been shown to attract neutrophils *in vitro* (Bryce *et al.* 2010) and are synthesized either by a lipoxygenase (LOX)-catalyzed reaction or by autoxidation (Feussner *et al.* 1998). E_1-phytoprostanes, in contrast, are formed solely by non-enzymatic, free radical-catalyzed reactions (Imbusch & Mueller 2000). Elevated ozone levels showed a highly significant negative correlation with pollen contents of $PALM_{PGE2}$, bringing about an increase in the $PALM_{LTB4}/PALM_{PGE2}$ ratio. As evident from the literature, lipoxygenase can be activated by ozone (Feussner *et al.* 1998). The observed correlation of a high $PALM_{LTB4}/PALM_{PGE2}$ ratio with elevated ozone exposure might therefore be due to increased LOX activation by elevated ozone exposure.

The functional relevance of the increased $PALM_{LTB4}/PALM_{PGE2}$ ratio in high ozone APEs was confirmed by *in vitro* neutrophil chemotaxis. In agreement with a functional dichotomy of LTB_4- and PGE_2-like PALMs, APEs of high ozone pollen were significantly more chemotactic for neutrophils than extracts of low ozone pollen. As there was no correlation between ozone and the pollen content of $PALM_{LTB4}$, we hypothesize that other, as yet unknown substances might be involved. On the other hand, PGE_2 is known to inhibit multiple cellular signaling mechanisms involved in neutrophil chemotaxis (Armstrong 1995). Consequently, $PALM_{PGE2}$ might compromise the chemotactic potential of pollen, and this inhibitory activity might be lacking in high ozone pollen containing less $PALM_{PGE2}$. In contrast, low ozone APEs were significantly more efficient in inhibiting dendritic cell IL-12 secretion. The differences in IL-12 inhibition between high and low ozone pollen were most evident at high APE concentrations (3-10mg/mL). These doses can be estimated to correspond to E_1-phytoprostanes at concentrations of 0.05 - 0.5 µmol/L. At lower APE concentrations, E_1-phytoprostanes no longer contribute significantly to IL-12 inhibition, and other substances, including adenosine, become more prominent. Therefore, the observed differences in IL-12 inhibition are most likely due to differences in $\text{PALM}_{\text{PGE2}}$. It is likely that besides E_1-phytoprostanes, other, as yet unidentified immune modulatory PALMs play a role. Our results are a first hint towards differences in immune modulatory and -stimulatory potential of pollen from urban and rural sites. It is tempting to speculate that the former might facilitate *de novo* sensitization by providing Th2 promoting signals, while the latter might aggravate allergic responses in already sensitized individuals by recruiting inflammatory cells into exposed tissues. Finally, the correlation of elevated ozone exposure with increased pollen Bet v 1 and altered PALM composition was mirrored by enhanced reactivity of allergic patients in skin prick tests. This result is most likely explained by increased levels of Bet v 1 in high ozone pollen. However, it cannot be ruled out that the decrease in $\text{PALM}_{\text{PGE2}}$ in high ozone pollen contributes to the aggravation of skin reactions towards allergen. In this respect it is notable that inhibition of prostaglandin synthesis by indomethacin lead to augmented wheals in skin prick tests to cow dander and other allergens (Saarinen *et al.* 2001).

In summary, urbanization-related, anthropogenic environmental factors can influence birch trees to produce pollen with altered allergenic potential. Our study emphasizes the correlation of ozone exposure to the pollen content of allergen and non-allergenic, immune stimulatory and -modulatory PALMs. It is likely that future increases in urbanization and anthropogenic air pollutants such as NO_x will further enhance the local accumulation of tropospheric ozone. As indicated by this study, ozone might, apart from direct adverse impacts on human health, bring about increases in allergic symptoms *via* its impact on the allergen carrier.

8.5 Supplementary material

Passive sampling method for nitrogen dioxide (NO_2) determination

The nitrogen dioxide concentration was measured at the sites according to Palmes' principle (Palmes 1976). Shortly stainless steal meshes were immersed in a triethanolamine-aceton mixture and were air-dried for 10 minutes. Three coated meshes are brought into an air-tight tube. On the day of sampling the tubes were opened on site and the opening time was noted. NO_2 binds to the coated meshes by forming a triethanolamine-NO_2-complex. After one week the passive samplers were closed and end of sampling was noted. NO_2 adsorption was determined photometrically.

Passive sampling method for ozone (O_3) determination

Passive samplers for O_3 were provided and analysed by PASSAM AG, Switzerland. The passive samplers were opened and the time of opening was recorded. After a sampling period of 7 days the passive sampler were closed air-tight and end time point of sampling was recorded. Samplers were analyzed by Passam AG.

Calculation of urbanization index

An urbanization index (UI) based on CORINE Land Cover2000 data (European Environment Agency 2000) using ArcGIS 9.3 was calculated. This index reflects the proportion of predefined built up areas (e.g. continuous and discontinuous urban fabric, industrial or commercial units) within a radius of 2 km and thus can vary from 0 to 1; i.e. from a low (0) to a high (1) degree of urbanization. For details see Jochner et al. (2012).

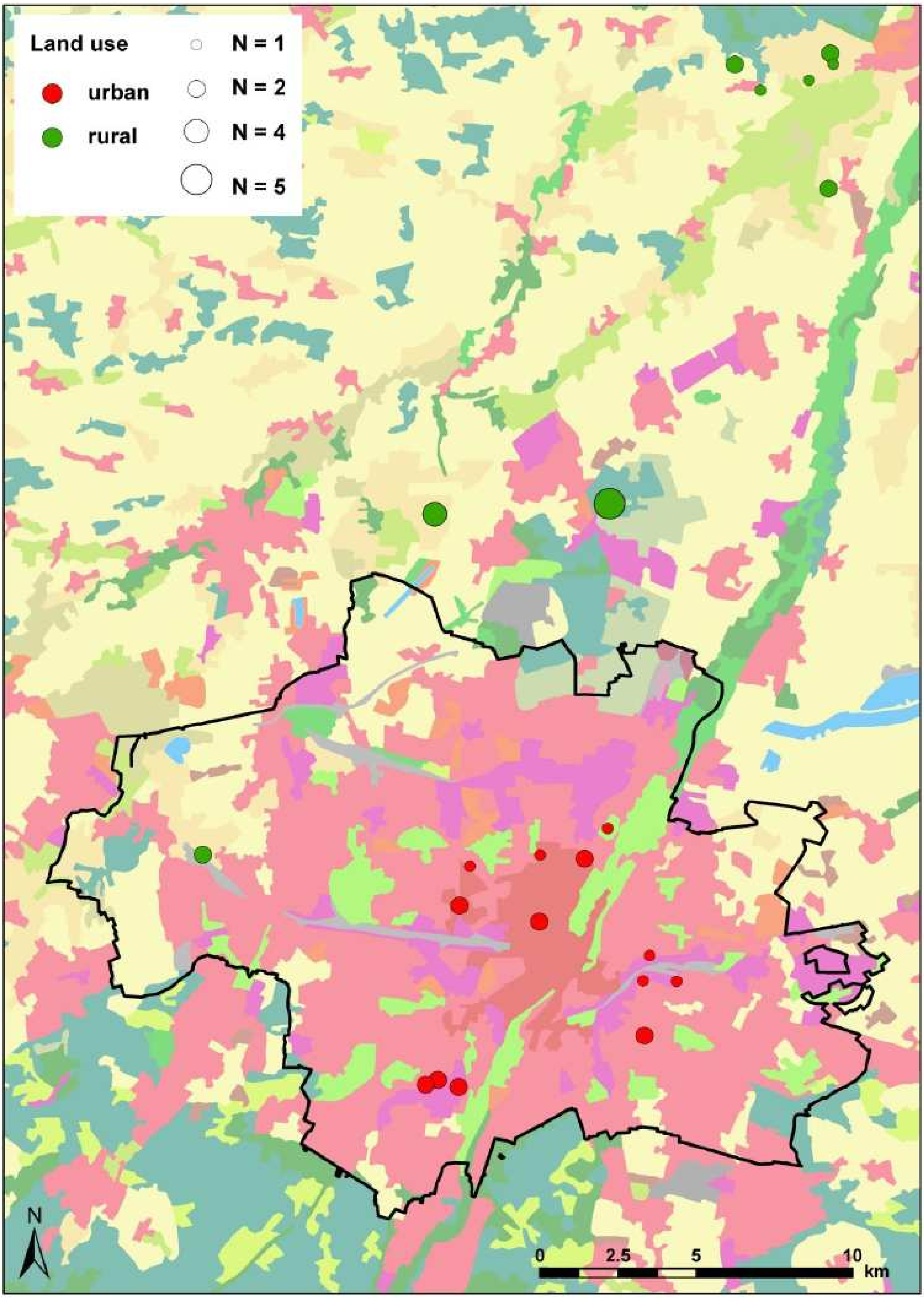

Fig. 8.6 Locations of pollen sampling. Birch pollen were sampled during the birch flowering season of 2010. Red dots represent urban trees and green dots represent rural trees. Background: CORINE Land Cover 2000 (EEA 2000), major classes: red = urban fabric, green = forest and pastures, yellow = arable land, blue = rivers, lakes (see www.eea.europa.eu/themes/landuse/interactive/clc-download for a complete legend)

Quantisation of adenosine in aqueous birch pollen extracts

Adenosine standards (Sigma Aldrich, Taufkirchen, Germany) and aqueous pollen extracts were subjected to ultra performance liquid chromatography (UPLC) with subsequent photodiode array detection as previously described (Gilles et al. 2011). Briefly, ion chromatograms were extracted at specific m/z per second values. Adenosine was detected as its protonated form (m/z of 268.103). The retention time of adenosine was 2.55 minutes.

Temperature measurements

15 birch trees were provided with loggers for air temperature (HOBO U23-001, Onset Computer Corporation, Southern MA, USA). The devices were fixed in a radiation shield at the northern side of the trees in 3 m height. Air temperatures were recorded every 10 minutes, and daily temperature means were calculated. Temperature data were acquired between July 1st and May 5th 2010.

Supplementary results

Pollen content of Bet v 1 and PALMs depends on catkin maturation state.

Catkins of different maturation stages were collected at different time points from the same tree and classified according to a BBCH. Pollen were isolated from the catkins and APEs were prepared. APEs were then analyzed for the presence of Bet v 1 and PALMs. The content of Bet v 1 peaked at maturation stages 60-61. Inversely to Bet v 1, levels of $PALM_{LTB4}$ and $PALM_{PGE2}$ were high in pollen from immature catkins and decreased during maturation. A concentration minimum of PALMs corresponded to a maximum in Bet v 1 (Fig. 8.7)

Urban trees are exposed to higher ambient NO_2 and higher average air temperatures.

Trees classified as “rural” were exposed to comparable ambient ozone concentrations as trees classified as “urban” (Fig. 8.8, A; p=0.19). Concurrently, trees classified as “urban” were exposed to significantly higher ambient NO_2 levels (Fig. 8.8, B) and higher average air temperatures (Fig. 8.8, C; p<0.0001). The Urbanization Indices (UI) for the birch trees chosen for pollen sampling differed significantly, a low UI corresponding to “rural” trees and a high UI corresponding to “urban” trees (Fig. 8.8, D; p<0.0001).

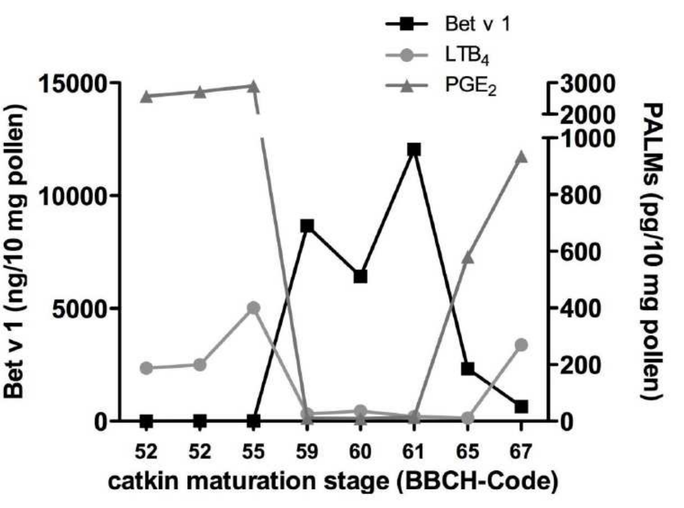

Fig. 8.7 Influence of catkin maturation on allergenic potential of pollen. A: The pollen Bet v 1 content is strongly dependent on the maturation stage of catkin and peaks between the BBCH-Codes 60 and 61. Additionally, the content of PALMs and Bet v 1 varies significantly depending on the catkin maturation stage.

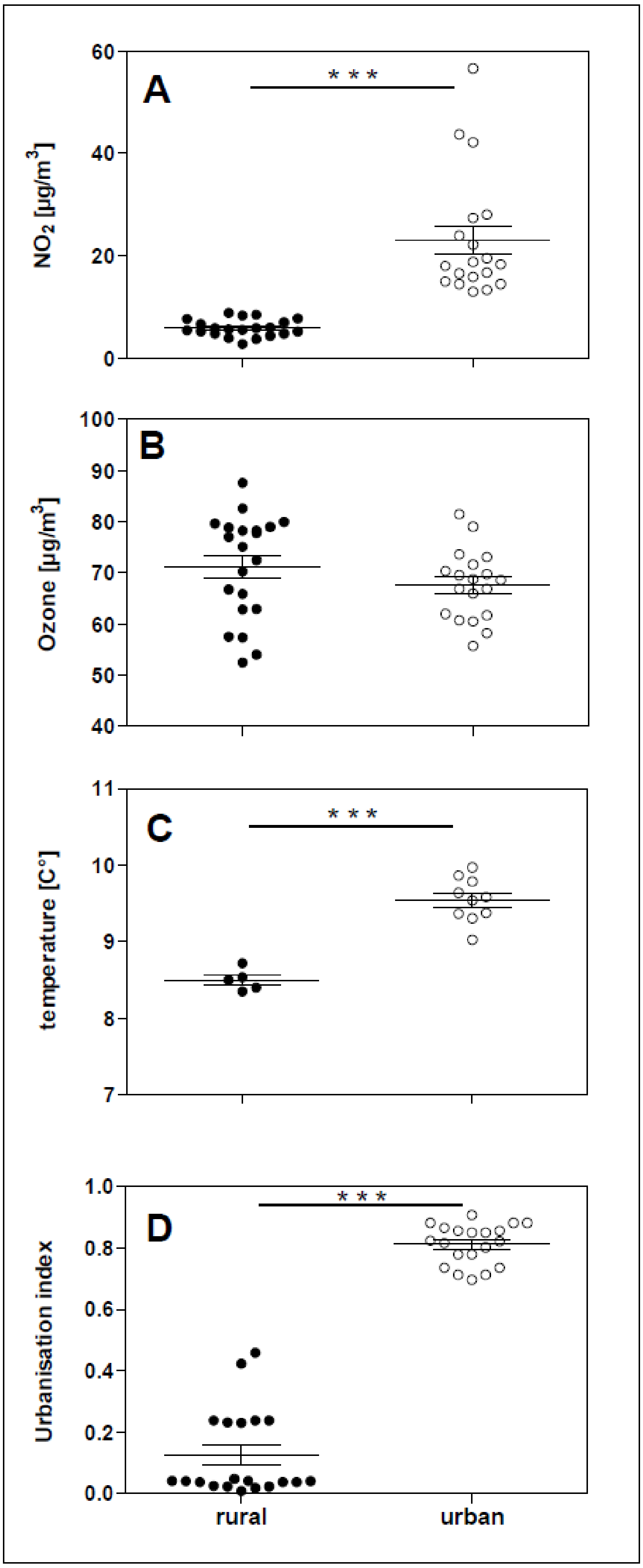

Fig. 8.8 Comparison of ambient gas concentrations, temperature and an urbanization Index at urban and rural trees. Rural trees were exposed to slightly elevated ambient ozone concentrations in comparison to urban trees (**A**). Concurrently, NO_2 exposition (**B**) and average temperature (**C**) were significant lower at rural compared to urban trees. The Urbanization Indices (UI) for the chosen birch trees differ significantly (**D**). *: $p<0.05$, **: $p<0.01$, ***: $p< 0.001$.

9 GENERAL AND SUMMARISING DISCUSSION

9.1 Factors influencing phenology

9.1.1 Temperature

In our detailed study areas, Munich and Campinas, temperature was measured directly on the stem of the observed trees. Therefore, it was possible to evaluate the variance in phenological onset dates that can be explained by temperature (Chapter 2 and 3).

Temperate phenology

Existing studies that analysed long-term phenological time series demonstrated that temperature, the most important factor influencing temperate phenology (Menzel & Fabian 1999, Wielgolaski 1999), is able to explain more than two third of the variance in phenological onset dates (Menzel & Fabian 1999). This seems to be in contrast with relative modest r^2 values of broad-scale urban studies that cover only a single observation year: Matsumoto *et al.* (2009) reported an r^2 value of 51.8 % derived from a linear model incorporating onset dates of cherry flowering in Tokyo, Japan, and mean March temperature in 2004. In addition, Lakatos & Gulyás (2003) calculated an r^2 value of 41.2 % for onset dates of full flowering of forsythia and urban heat island intensity in Debrecen, Hungary, in spring 2003.

We found that mean air temperature in March and April explained between 43.4 and 61.3 % of the variance in onset dates of birch phenophases in 2010 and 2011 (Chapter 3) whereas flowering phenophases showed higher r^2 value than leaf phenophases. However, when analysing both years within one single model, r^2 values increased and ranged between 63.4 % and 88.1 %. This is due to the fact that temperature variations among all sites are smaller within one year compared to several years that exhibit different weather and therefore temperature conditions. In fact, March 2011, for example, only showed a difference of 2.5 °C between the coldest and warmest observation site in Munich (Chapter 2). Besides, short-lasting weather conditions (e.g., warm spells) are better reflected by temperature variables of a finer temporal resolution than only a calendar month (Chapter 6); thus, they might increase r^2 of urban phenological models. Chapter 2 also demonstrated that the diurnal temperature range (DTR) as well as mean night temperature (Tnight) were useful variables in phenological research. They explained a great amount of the variance in onset dates of birch phenophases, whereas Tnight often and DTR sometimes showed even higher correlations with phenology than mean temperatures. This fact might be attributable to common characteristics of the urban climate. In general, DTR is smaller in urban areas compared to the natural environment since the material used for buildings and streets alters thermal energy storage (Landsberg 1981). Thus, DTR is an excellent measure for urban heat islands. This was also confirmed by Mimet *et al.* (2009): DTR was best correlated with budburst onset dates of sour cherry in the greater area of Rennes, France. In addition, the urban heat island is most pronounced during night time (Landsberg 1981, Baker *et al.* 2002; Mimet *et al.* 2009; Shustack *et al.* 2009) which implies that night temperature is able to describe urban excess heat adequately. On the contrary, maximum temperature was found to be not of general importance (Chapter 2). This was also demonstrated by Wielgolaski (1999) and Mimet *et al.* (2009).

Tropical phenology

Phenology in tropical trees is not satisfyingly understood yet, and the role of temperature is uncertain (Borchert *et al.* 2002, Singh & Kushwaha 2005). For instance, bud burst is even delayed with increasing land surface temperature (Gazal *et al.* 2008). Some authors claim that tropical phenology is an inadequate indicator of global warming (Borchert *et al.* 2005), since tree phenology is also influenced by other parameters such as leaf longevity, precipitation, ground water availability, seasonal variation in water stress and increasing day length (Borchert 1994, Eamus & Prior 2001, Borchert *et al.* 2005, Do *et al.* 2005, Singh & Kushwaha 2005).

Our study was the first that analysed urban phenology in tropical regions in detail. In contrary to previous literature on phenology in temperate regions, considerable variations of significance and magnitude of correlations between the selected tropical species / phenophases and air temperature were found in Campinas (Chapter 2). *Caeasalpina* (false Brazilwood) failed to show any significant correlations with the selected temperature variables and was hence not sensitive enough to detect temperature variations, similarly to flowering phenophases of *Tabebuia* (yellow trumpet tree*)*. In contrast, phenological onset dates of *Tipuana* (racehorse tree) showed high correlations with air temperature, especially for leaf unfolding phenophases and mean (r = -0.746, r^2 = 55.7) and nocturnal (r = -0.779, r^2 = 60.7) temperature data. These significant and relevant relationships are therefore comparable to the recent findings of urban phenology studies in temperate regions (Lakatos & Gulyás 2003, Matsumoto *et al.* 2009) and to the results derived from correlation analyses and linear regressions in Munich (Chapter 2 and Chapter 3).

Land use

The discriminant analysis (Chapter 4) incorporated flowering dates of forsythia in southern Bavaria and various explanatory variables such as altitude, geographical coordinates and land use variables, i.e., the amount of forest and impervious (hard, sealed) areas within a radius of 2 km, the distance to the nearest forest (> 100 ha), urban area (> 1 km²) and water body. These land use variables were selected since they were expected to be correlated with climatic conditions. In particular, impervious surfaces which are especially important in terms of urban areas have an effect on transpiration and therefore modify radiation balance and temperature conditions (Landsberg 1981). The proximity to the centre of large urban areas is also central since it is known to be correlated with phenological onset dates (Zhang *et al.* 2004; Fisher *et al.* 2006, Mimet *et al.* 2009). By providing shelter from wind and evapotranspiration processes (Adams 2007) forests may influence phenological behaviour in their surrounding area (Shustack *et al.* 2009). In addition, water bodies possess a high specific thermal capacity and hence reduce temperature amplitudes through delayed warming and cooling (Fezer 1995).

The factors that had the most important influence on phenological onset dates - according to the correlations of the independent variables with the discriminant function values - were altitude (see next section), the proportion of impervious (hard, sealed) surfaces, the distance to highly urbanised areas, and the proportion of forested area. The bioclimatic map that was produced using the analytical result of this multivariate approach reflected the most important land use variables clearly. Particularly, urban areas, e.g., of Munich, Augsburg, Ingolstadt and Regensburg, but also smaller cities such as Freising and Dachau exhibited an early flowering of forsythia and underlined the importance of the amount of impervious surfaces and the distance to large agglomerations. Early flowering was also demonstrated for the Federal Highway (Bundesstraße) 11, a connection line between the inner city of Munich towards the northeast, which is characterised by several towns next to this main street. Variables describing the degree of urbanisation (e.g., built-up area index) have been used for the generation of phenological maps for the state of Bavaria (Rötzer *et al.* 1997). However, the amount of forest and the distance to forests and to large urban agglomerations have been neglected in phenological maps so far. Therefore, this study added essential influential land use variables having an effect on temperature and, in turn, on phenology. Moreover, the amount of impervious surfaces was found to be an adequate variable in phenological research and provoked more detailed evaluations of the influence of urbanisation on phenology (Chapter 5, 2 and 3).

Urbanisation

Since most of the research in urban phenology is based on a plain urban-rural dichotomy (Rötzer *et al.* 2000, Gazal *et al.* 2008, Mimet *et al.* 2009), the explanatory value of these studies is arguable. Many authors (e.g., Franken 1955, Baumgartner *et al.* 1984, Bernhofer 1991) reported substantial phenological differences within the city. Bernhofer (1991) for example, showed that forsythia flowered 20 days earlier in the inner city of Vienna compared to a nearby park. Furthermore, the effect of the urban heat island does not abruptly stop at the city boarder. Zhang *et al.* (2004) demonstrated that the urban heat island effect extends beyond the concentration of developed land, whereas a substantial influence on phenology can be observed up to 10 km beyond the edge of urban land cover. Much more detailed information can therefore be received using a continuous variable describing the degree of urbanisation which can be related to continuous flowering dates along an urban-rural gradient.

Several authors claimed that geographic distance from the urban core may be a good predictor of phenological changes associated with urbanisation. The relationship between phenological onset dates and distance to the city centre was regarded as linear function by Mimet *et al.* (2009). Fisher *et al.* (2006), instead, found a power relationship between distance to the urban core of Providence (Rhode Island) and satellite derived onset dates. Furthermore, Zhang *et al.* (2004) presented an exponential fit between onset dates derived from satellite data and distance to the urban core. In addition, Gazal *et al.* (2008) also reported a dependence of the distance to the urban centre and onset dates for budburst in temperate cities, however only significant in one of four analysed cities. The importance of the proximity to urban areas was also detected in our study by the multivariate discriminant analysis in Chapter 4.

Nevertheless, the distance alone might not be sufficient as explanatory variable in univariate phenological models since urban cities do not always show a decrease in urbanisation with increasing remoteness (Jochner 2008). A more sophisticated approach was e.g., achieved by Rötzer *et al.* (1997), who calculated a building index based on digital land use information that comprised a summation of raster elements with densely developed areas within a 2.5 km radius.

We therefore developed an index (Chapter 5) that reflects the degree of urbanisation using land use data and GIS. The calculated index [0; 1] includes the percentage of predefined built up areas (e.g., continuous and discontinuous urban fabric, industrial or commercial units) within a radius of 2 km. Correlation analyses (Chapter 2) showed - both in Munich and in tropical Campinas - that the calculated urban index explained a high amount of the variance in temperature data. In Munich, the urban index was best correlated with relative humidity (r = -0.84), mean temperature (r = 0.8) and mean night temperatures (r = 0.77) in March. In addition, all other correlations with the urban index and meteorological parameters were significant as well. The highest correlations with urban index in Campinas were demonstrated for the diurnal temperature range (r = -0.8) and mean night temperatures (r = 0.75). Only mean monthly temperatures were not adequately reflected by the calculated urban index.

Significant correlations between the urban index and flowering and leaf unfolding phenophases could be revealed for birch in Munich in 2011 (r = -0.288 to -0.585, Chapter 2). In addition, also the phenophases of *Tipuana* in Campinas showed a significant relationship with the urban index (r = -0.338 to -0.561). *Tipuana* was additionally the only species that showed meaningful correlations with temperature variables. Therefore, it can be assumed that species or phenophases which are sensitive to temperature variations might also correlate with the urban index. The correlations of phenological onset dates with the urban index were, however, less pronounced compared to site-specific temperature data.

Analyses of long-term phenological data (1951-2008) for the cities Frankfurt, Cologne and Munich (Chapter 5) showed that the mean onset date of a specific phenophase is not only influenced by altitude (see next section) but also by urbanisation. Regression coefficients for all nine selected spring phenophases were in the same direction: the higher the urban index - and therefore the degree of urbanisation - the earlier the phenological onset dates. These analyses showed that a land use change from completely rural (urban index = 0) to urban (urban index = 1) implies - depending on the phenological phase - an advance of -2.6 to -7.6 days.

In literature manifold approaches to classify urban and rural sites can be found. Commonly, satellite derived land cover classifications are utilised to achieve a categorisation of those sites (White *et al.* 2002, Gazal *et al.* 2008). But also present day and historical topographic maps (Rötzer *et al.* 2000), MODIS NBAR spectral data, night time light data and gridded population density (Zhang *et al.* 2004) can be used. A categorisation of urban, suburban and periurban sites for the city of Rennes (France) was performed by Pellissier *et al.* (2008) using digital orthophotos within 500 m x 500 m grid squares to calculate the proportion of various land use classes (surface water, woodland, cropland, grassland, roads, built-up areas) by means of principal components analysis (PCA). This categorisation in three different levels of urbanisation was also implemented by Mimet *et al.* (2009). A further estimate of the degree of urbanisation was achieved by Shustack *et al.* (2009) who used orthophotos to evaluate the proportion of different cover types (forest, agriculture, mowed, paved, road) and the number of buildings within a radius of 1 km by means of a PCA. The first component of the PCA was defined as the index of urbanisation, where positive values indicated urban and negative values rural sites. In our study (Chapter 6), we denoted urban sites as those with at least 50 % urban cover (ui ≥ 0.5) and the remainder as rural sites. A personal evaluation of the observation sites confirmed the classification, with agricultural areas, forests and natural areas being classified as rural sites. Conversely, urban areas were characterised by a high building density and a large amount of impervious surfaces.

Altitude

Since air temperature is the most important environmental factor explaining phenological onset dates (Menzel & Fabian 1999, Badeck *et al.* 2004; Defila & Clot 2005 and also Chapter 2 and 3) we propose that variations in phenological onset dates along altitudinal gradients are predominantly attributable to altitudinal temperature decrease. We demonstrated that phenological onset dates showed a great dependence on altitude in Chapter 4, 5 and 7.

Within the discriminant analysis (Chapter 4), altitude was among the most important predictors for beginning of flowering of forsythia in terms of its influence on the mesoclimate. Our analysis revealed high correlations between the variable altitude and the values of the discriminant function. In addition, the influence of altitude on phenology was also evidently reflected in the generated bioclimatic map: in general, lower elevated areas, e.g., the river valleys of Lech, Isar and Danube, were characterised by an earlier flowering compared to the higher areas in the foreland of the Alps.

In Chapter 5 we utilised long-term observations of nine different spring phenophases in the greater area of Frankfurt, Cologne and Munich in order to demonstrate *inter alia* altitudinal effects on phenological onset dates. The regression coefficients for altitude in the linear models were all positive and highly significant. Temperature lapse rates allow translating altitudinal responses in phenology into thermal responses. A conversion of the altitudinal gradient of temperature (-0.6 °C $(100\ m)^{-1}$, Barry 1991) and the temperature response in phenology (-1 to -5 days $°C^{-1}$, Menzel *et al.* 2006a) for flowering phases in Europe, suggests altitudinal responses between 0.6 and 3.0 days $(100\ m)^{-1}$. The coefficients calculated in our study ranged between 4.3 days $(100\ m)^{-1}$ for beginning of flowering of hazel and 1.3 days $(100\ m)^{-1}$ for leaf unfolding of beech and are therefore higher than expected. Higher coefficients (up to 4.6 days $(100\ m)^{-1}$) were also found by Ziello *et al.* (2009) in the Alps. We could demonstrate that earlier phases such as flowering of hazel showed more pronounced altitudinal and consequently thermal responses. This was also reported by Rötzer (1997), Rötzer *et al.* (2000), Sparks & Menzel (2002) and Jeong *et al.* (2011).

Since temperature is a limiting factor for plant growth and development in higher elevations, Defila & Clot (2001) stated that plants growing in the mountains are more sensitive and show stronger responses to temperature increase associated with climate change. There have been a few studies that analysed the altitudinal dependence of phenological trends: Studer *et al.* (2005) confirmed a dependence of trends for the northern, however, not for the southern Alps. And Ziello *et al.* (2009) demonstrated that only three (out of ten) phenological spring phases in the Alpine region showed a statistical significant dependence of their trends on altitude. The majority of coefficients were negative, pointing to a more pronounced advancement in higher regions. In our study we found that temporal trends in phenology were only significantly influenced by altitude for Norway maple. The magnitude of the altitudinal coefficients was negative only in one third of the cases and too small to identify a profound pattern. Therefore, we could not confirm published findings of more pronounced trends associated with higher elevated areas, even though our locations were at noticeably lower altitudes.

In the Zugspitze region, located at the German-Austrian boarder, we were able to evaluate a much larger altitudinal gradient (700 to 1700 m, Chapter 7). All altitudinal gradients for the selected phenophases of the two grass species cocksfoot and meadow foxtail and for birch were statistically significant and associated with high r^2 values (65.1 to 83.5). For birch phenophases altitudinal gradients were ca. 3 days $(100\ m)^{-1}$ and related to temperature response rates of ca. -7 days $(1\ °C)^{-1}$. These results were similar to other studies on birch phenophases: regression coefficients calculated by Chen (1994) for leaf unfolding in the Taunus hills (Central German Uplands) were 4 days $(100\ m)^{-1}$, Rötzer & Chmielewski (2001) reported a delay of 3 days $(100\ m)^{-1}$ for the beginning of flowering across Europe. Altitudinal gradients for the grass species in our study were even higher, showing a delay of 6 to 7 days $(100\ m)^{-1}$. Their temperature response rates varied between -9 and -10 days $(1\ °C)^{-1}$. Chen (1994), whose study area only comprised an altitudinal range from 100 m to 800 m, calculated a delay of about 2 days $(100\ m)^{-1}$ for full flowering of cocksfoot and meadow foxtail. Ziello *et al.* (2009) reported a regression coefficient of ca. 4 days $(100\ m)^{-1}$ for cocksfoot in the region of Garmisch-Partenkirchen. Our results, however, suggest a more pronounced temperature sensitivity, especially for cocksfoot and meadow foxtail. Further temperature increase could therefore imply a remarkably advance of the grass pollen season which results in major consequences for pollen allergic people (see also Chapter 9.3).

Aspect

Our analyses revealed discernible differences in onset dates of 5 to 7 days for northern (Eibsee) and western (Ehrwald) exposed birch trees at the same altitude (~ 1.000 m) in the Zugspitze region (Chapter 7). These variations mirrored altitudinal / temperature differences ranging from 161 m / 0.8 °C for the beginning of leaf unfolding, to 184 m / 0.9 °C for the beginning of flowering and 213 m / 1.0 °C for full flowering. These findings point to the fact that differences in aspect induce stronger phenological shifts than an increase in altitude of 100 m.

Southern exposed trees have not been observed, however, it can be supposed that the differences in onset dates would be considerably higher. Chen (1994), for example, found that northern and southern exposed birches in the Taunus hills showed differences in onset dates up to 14 days. Therefore, the morphology of the landscape, especially in mountainous regions, is inevitable for the analysis and interpretation of phenological data (see also Chapter 5).

The microclimatological complexity of mountain areas is also documented by Scherrer *et al.* (2011) who evaluated surface and soil temperatures in alpine landscapes and found substantial temperature variations of 2 to 3 °C related to slope exposure. Soil temperature differed not only for different slopes but also noticeably varied within slopes (3 to 4 °C) due to microtopography and even within a small plot (1 m^2) resulting from plant cover effects.

9.1.2 Air humidity

In general, air humidity is closely related to air temperature: the higher the temperature, the lower the relative humidity (Häckel 1999). In the study area of Munich relative humidity and mean temperature was highly correlated (r = -0.94) in March 2011 (Chapter 2). This correlation makes it difficult to disentangle their effects on phenological onset dates. However, using partial correlations with mean temperature as control variable, no significant correlation between phenology and air humidity could be revealed. Therefore, it can be assumed that air humidity does not play an important role in the greater area of Munich which is characterised as moist and temperate.

In tropical Campinas, however, humidity was found to influence leaf phenology of *Tabebuia* more than variables related to air temperature (Chapter 2). However, influences of humidity on other phases and species in the tropical study area could not been detected. The potential relevance of humidity in tropical phenology was also addressed by Gazal *et al.* (2008). Inconsistent effects of air humidity in our study, however, point to the fact that its influence is species-specific and might vary greatly among phenophases.

9.1.3 Foliar nutrients

In order to further explain variance in onset dates, we analysed foliar nutrient concentrations of birch in Munich (Chapter 3). Our study was the first that analysed phenological behaviour in urban areas as a function of foliar nutritional status. Its influence varied between years and between flowering and leaf unfolding phenophases. The elements that showed a high number of significant correlations were potassium (K), zinc (Zn), boron (B) and calcium (Ca); an increase in the concentrations advanced phenological onset dates. In contrast, we did not find statistical relationships of phenological onset dates with nutrient concentration of nitrogen (N), copper (Cu) and manganese (Mn) and only some weak correlations for phosphorus (P), sulphur (S), magnesium (Mg) and iron (Fe). The increase in adjusted r^2 ranged between 1.7 % and 3.8 % for flowering phenophases and between 4.8 % and 9.8 % for leaf phenophases of birch. Explicitly, the inclusion of nutrient variables increased model fits (relatively) by up to one fifth of the r^2 using air temperature alone.

The elements that were shown to have the most relevant effect on phenology - K, Zn, B and Ca - influence cell extension as well as membrane function and stability (Marschner 1995). K was the nutrient that had the strongest influence on phenology, especially on leaf unfolding. This macronutrient is characterised by a high mobility in plants, and, besides other physiological functions it contributes most to the osmotic potential of cells and tissues. An increased osmotic potential in the vacuoles is essential for cell extension (Marschner 1995). Flushing of leaves is a turgor-driven process that is initiated in spring by bud break, whereby a high amount of K might be supportive for a rapid development. Catkins of birch, however, are already formed in late summer or early autumn, long before leaf expansion. Nord & Lynch (2009) proposed that deficiencies in K are less likely to affect the phenology or growth of plants. However, as shown in our study, K had indeed an enormous effect on phenology. Zn deficiency leads to growth depression, mostly visible in a decreased internode elongation and reduction of leaf size (Marschner 1995). Our analyses revealed high correlations of Zn concentration and phenological onset dates of birch and therefore proved the favourable effect of Zn on functioning and growth of shoots. A further important element was B. It is implicated in several metabolic pathways and is of importance for cell wall biosynthesis and structure as well as for plasma membrane integrity. B deficiency in the shoot induces symptoms that are similar to Zn deficiency. Both elements, Zn and B, have an influence on the metabolism of the phytohormone indole-3-acetic acid (IAA) that is required for cell division and elongation (Marschner 1995). The macronutrient Ca does not only stimulate the synthesis of cell wall precursors during cell elongation but also plays an important role in membrane stability and functionality (Marscher 1995). However, since the Munich study area is located on calcareous gravel, the birches do not experience a Ca deficit. This might be the reason for less predictive power of Ca in the multiple regression analyses. The dominant role of K might be due to correlations of K with B ($r = 0.600$, $P \leq 0.001$), Ca ($r = 0.332$, $P = 0.055$) and Zn ($r = 0.450$, $P = 0.008$).

For the greater area of Munich, using foliar K concentration alone is sufficient to explain phenological variations. This, however, might not be the case at sites with different soil conditions and nutrient availability. Higher temperatures - *via* altering soil moisture - lead to higher mineralisation rates and therefore affect nutrient availability (Marschner 1995, Nord & Lynch 2009). This is probably the reason for inconsistent findings of the effect of nutrients on phenology and confirms our suggestion that soil nutrient availability cannot really reflect the physiological performance of a tree adequately: Roberntz (1999) and Amundson *et al.* (1995) found no effect on bud break of Norway and Red spruce growing on fertilised soils. In contrast, Chandler & Dale (1990) and Amundson *et al.* (1995) reported an advancement of bud break of fertilised Sitka spruces. In Wielgolaski's (2001) study high levels of P, K, Mg and Ca in the soil delayed plant development of early phenophases, but not of late phases which strongly depend on high temperatures. Therefore, foliar nutrient concentration is regarded as a more adequate measure for the evaluation of environmental conditions birches have to deal with.

9.2 Estimation of temperature patterns at the regional and local scale

9.2.1 Regional bioclimatic characterisation

The bioclimatic characterisation (Chapter 4) was based on the association between predictor variables (mostly land use) and flowering onset dates of forsythia at 70 phenological stations across southern Bavaria. More than two third of the phenological stations were correctly categorised into three different flowering onset groups. Hence, the multivariate model derived from the discriminant analysis revealed a high degree of accuracy and is suitable for regionalisation of phenological data at the mesoscale.

By introducing a new method in phenological research - the GIS-assisted regionalisation of phenological data using the statistical results of the discriminant analysis - we were able to estimate temperature patterns at the regional scale. The regionalisation of phenological data could identify "thermal hot spots" that were linked to urban areas. Flowering phenology of forsythia is proposed to be a useful indicator of regional climate variations caused by both natural and human factors.

Without using any meteorological data our selected and calculated land use variables (i.e., altitude, proportion of sealed / forested areas, and distance to urbanised areas) were able to mirror temperature conditions adequately. Since land use variables can be easily derived from highly resolved digital data (and their updates) this method is a cheap and suitable alternative to commonly used interpolated temperature data based on insufficient resolutions (Ashcroft 2006). This new approach introduced in this study showed not only the potential for forecasting changes in phenology following land use change but is also a useful alternative for multiple regression models which are common methods for regionalisation of phenological data (Rötzer *et al.* 1997, Rötzer & Chmielewski 2001, Menzel *et al.* 2005).

9.2.2 Detection of urban heat island effects

Using several methods, data and regions of interest, this research confirmed major findings of previous studies (e.g., Aono 1997, Lakatos & Gulyás 2003, Rötzer *et al.* 2000, Zhang *et al.* 2004, see also Table 1.1) that demonstrated that cities generally experience earlier phenological events than rural areas (Chapter 2, 3, 4, 5 and 6).

Temperate phenology

Long-term phenological data (1951-2008) for the cities Frankfurt, Cologne and Munich (Chapter 5) showed that urbanisation processes imply considerable phenological changes: considering a complete land use change from urban to rural we expect - depending on the phenological phase - an advance of -2.6 to -7.6 days. There are many studies demonstrating that the largest urban-rural differences in phenology occur in early spring (e.g., Sparks & Menzel 2002). Though some early spring phenophases exhibited large urban-rural differences in this study (e.g., -7.6 days for flowering of hazel), no consistent relationship between the urban index coefficient and their associated mean onset dates could be found.

The vast and detailed field study in the greater area of Munich in spring 2011 (Chapter 2) confirmed significant urban-rural differences in onset dates for birch phenophases ranging between -1.5 days (mouse-ear stage) and -3.4 days (full flowering). These results are comparable to recent findings for the greater area of Berlin, Germany (Henniges & Chmielewski 2006) with more pronounced urban-rural differences for beginning of flowering (-2.6 days) than for leaf unfolding (-0.3 days). Additionally, in Chapter 7, stronger effects of altitude and aspect (and therefore of temperature) were found for flowering phenophases than for leaf unfolding phenophases in the Zugspitze region. These results suggest that flowering of birch is more responsive to temperature variations than leaf unfolding and thus more valuable for the detection of urban heat islands.

Using long-term data for flowering of hazel, forsythia and horse chestnut in the greater area of Munich (Chapter 6), we demonstrated earlier onset dates in the city of Munich that in its rural surroundings. These differences, called the urban phenology effect (UPE), ranged between -3.5 days (flowering of forsythia) and -4.7 days (flowering of hazel) for the period 1951-2008 and between -3.8 days (flowering of horse chestnut) and -7.6 days (flowering of hazel) for the period 1981-2008. The UPE was therefore most pronounced for the earliest phase, flowering of hazel, underlining the temperature sensitivity of early phases.

An increase of the UPE might result from an expansion of the urban heat island (Rötzer 1995). In our study we demonstrated that climate change advanced phenological spring events both in urban and rural areas of Munich - without a difference in urban and rural temperature trends - suggesting that the UPE must be more or less stable. Indeed, urban-rural differences in phenological onset dates did not significantly change over time - with the only exception of horse chestnut: here, a reduction of the urban-rural difference in the period 1981-2008 was revealed. This finding indicates that urban-rural differences in phenology cannot ultimately be described by spring time temperatures and their respective temporal trends.

Influence of warm spells on urban-rural phenology

Differences in temperature between the city and the countryside cannot entirely explain differences in phenology. The prevailing weather conditions shortly before flowering can mask the UHI effect on phenology. Baumgartner (1952) already proposed that intra-urban differences in flowering dates are more pronounced when spring temperatures rise gradually. However, no qualitative assessment in respect to this suggestion has been done so far. Our study was the first that revealed that warm spells are more likely to result in simultaneous flowering and cold spells rather contribute to a larger delay between urban and rural onset dates.

Weather conditions lasting only a few days (i.e., warm or cold spells) can already effectively influence phenological behaviour at the local scale (Chapter 6). The warm temperature conditions in April 2009 (+ 4.3 °C compared to the standard reference period 1961 to 1990) allowed studying the effects of anomalous high spring temperatures on plant development. We could demonstrate synchrony in phenological onset dates, especially for birch, in the city of Munich and Ingolstadt and their adjacent rural environments. A further examination of long-term data of the DWD supported our suggestion (Chapter 6). The higher the temperature before onset of urban flowering the less pronounced the difference in phenological onset dates between the city and the countryside.

However, we could not find any significant relationships with UPE and UHI on a monthly or longer basis. In addition, correlations with UPE and temperature deviations from the standard reference period 1961 to 1990 did only indicate two significant relationships, however weak and with contrasting directions of the correlation. Therefore, we conclude that only a finer temporal resolution than a calendar month (as shown with temperature sums and UHI sums) is capable of explaining urban-rural differences in phenology. We demonstrated that temperature sums were best correlated with urban-rural differences in phenology, whereas UHI sums showed less pronounced and also contrasting relationships. Especially for forsythia and horse chestnut, the correlation between temperature sums and UPE was high with maximum values of $r = 0.584$ and $r = 0.726$ occurring around the 20th day before urban flowering onset dates.

Temporal urban and rural trends

In our study (Chapter 5) we did not find any urban effect on trends in phenological time series. Existing studies incorporating differences in urban and rural trends equally revealed no decisive conclusion. Rötzer *et al.* (2000) reported that phenological trends (1980-1990) of spring phases were stronger in the countryside compared to the city (e.g., urban trends for pre-spring phases: 13.9 days per decade, rural: 15.3 days per decade). More pronounced urban trends were noticed by Rötzer (1995), Defila (1999), Defila & Clot (2003) and Rötzer & Sachweh (1995) analysing long-term data of spring flowering dates in Germany and Switzerland. This is also in accordance with Primack *et al.* (2004) and Lavoie & Lachance (2006) who detected stronger trends using herbarium data in cities of North America and Canada. These inconsistent findings might be attributable to differences in urbanisation rates (Rötzer *et al.* 2000) which should be regarded in further studies.

Tropical phenology

Phenological onset dates of the three selected species in Campinas were characterised by much higher standard deviations (8.9 to 17.4 days) compared to birch in Munich (Chapter 2). This is in line with Gazal *et al.* (2008), Morellato *et al.* (2000) and Borchert *et al.* (2005) who also demonstrated high within-species variation in tropical trees. In turn, mostly non-significant urban-rural differences in Campinas might be partly attributable to this feature of tropical phenology. Significant differences were only demonstrated for *Tipuana* and ranged between -10.5 and -19.7 days. Nearly all analysed phenophases of the other two tropical trees *Tabebuia* and *Caesalpina* showed even positive urban-rural differences (i.e., delayed urban onset, although not significant).

The only study published on urban phenology in tropical cities of Asia and Africa by Gazal *et al.* (2008) reported earlier bud burst dates in urban Bangkok (-23 days), but later urban onset dates for Korat (+ 9 days) and Dakar (+ 9 days). Probably a low temperature sensitivity of tropical tree phenology and other environmental factors might exert a further influence. In our study, we found that humidity related variables such as relative or absolute air humidity also affect the timing of phenological events in the tropics (see Chapter 9.1.2).

Urban heat island effects on plant phenology were reported to be more pronounced in North America than in Europe and Asia due to the dense and vertical urban design in North American cities (Zhang *et al.* 2004). Regarding our study regions, Campinas is characterised by numerous tall multi-storey buildings; in Munich, however, the vertical structure is less pronounced with only a few buildings taller than 100 m. A direct comparison between urban-rural differences in Munich and Campinas is not feasible since we did not analyse the same species. Nevertheless, significant differences were by far higher (-10.5 to -19.7 days) in Campinas than in Munich (-1.5 to -3.4 days).

9.3 Impacts on airborne pollen and aeroallergens

Allergies constitute an important human health issue since they are widespread across the globe (D’Amato *et al.* 2007) and are of further importance with regard to the increasing numbers of sensitisation and enhanced severity of symptoms (WHO 2003, D’Amato *et al.* 2010). The WHO (2003) stated: “The impact of climate change on the incidence, prevalence, distribution and severity of allergic disorders is unclear.” Therefore, it is essential to focus new research on a wide range of impact factors and changes associated with pollen:

(1) Phenological observations of allergenic plants
(2) Quantitative assessments of the pollen production and pollen allergenicity
(3) Functional and physiological relevance assessed by *in vitro* and *in vivo* experiments

The findings derived from our interdisciplinary study and their practical and medical relevance are discussed point-by-point below.

9.3.1 Phenological observations of allergenic plants

Observed changes

An indication that flowering dates have already experienced major changes was given in Chapter 5 where temporal phenological trends (1980-2009) for nine phenological spring events centred on the German cities of Frankfurt, Cologne and Munich were analysed. The investigated species included one of the most allergenic species that flowers in early spring, i.e., common hazel, and some other species that are reported to induce allergies such as goat willow. The calculated temporal trends of phenological onset dates were negative (i.e., earlier onset in phenology) for 96 % of the 808 time series and significantly negative for 56 % of the total number. Mean trends ranged between -2.3 days per decade for beech and -5.0 days per decade for hazel. Flowering of hazel, an early phenophase, showed the strongest temporal advance.

Changes in the start and the duration of the flowering period of allergenic plants may result in disorders in terms of human health. Since hazel now emits its pollen 15 days earlier compared to 1980 this implies a much earlier occurrence of symptoms for allergic people. Moreover, flowering periods of different allergenic species will overlap (Doi *et al.* 2010) and could result in a reduction of the allergy-free time and hence in reinforced ailments for pollen allergic people. In general, interrupted pollen seasons and therefore perpetual minor and major ailments could be the result of very early flowering onset dates, since weather in late winter and early spring are often connected with adverse (colder and more humid) conditions (D'Amato & Cecchi 2008).

Temperature sensitivity assessed by altitudinal responses

The analysis of flowering dates of allergenic species in the Zugspitze region in 2009 (Chapter 7) reported remarkably higher altitudinal trends for grass phenophases (6.3 to 7.1 days (100 m)$^{-1}$) compared to birch phenophases (3.1 to 3.2 days (100 m)$^{-1}$). Calculated temperature response rates in phenology showed that phenological phases advanced by almost 7 days (birch) and 10 days (grass) per 1 °C increase in temperature. In summary, this suggests that the analysed grass species, meadow foxtail and cocksfoot, are more responsive to temperature variations. Albeit, this study did not incorporate long-term phenological data, neither of meadow foxtail and cocksfoot, nor of silver birch, it is assumed that a further increase in temperature due to climate change implies noticeable changes in phenology, especially for grass flowering.

Consequences of urban-rural (a)synchrony

In April 2009 a rapid development of birch catkins both in the city and the countryside due to anomalous high temperatures could be observed (Chapter 6). Consequently, urban-rural differences diminished: onset dates for flowering of birch were recorded on the same day in urban areas of Munich and its rural surroundings, and only had a time lag of 1 day in urban and rural areas of Ingolstadt. The time span of 1 to 2 days between beginning of flowering and full flowering was extremely short. Thus, phenological behaviour in spring 2009 revealed valuable insights into some important possible consequences of such extreme warm spells:

- Urban and rural dwellers suffering from allergy were probably affected by atmospheric pollen loads starting almost the same day.
- Symptoms were probably strong during the beginning of the pollen season since a higher quantity of birch trees started to flower simultaneously.
- However, symptoms probably did not persist for an extended time span due to the rapid transition of phenological flowering phases (from beginning of flowering to full flowering).

Shifts in characteristics of the pollen season

There is evidence that recent temperature increase not only affects the starts of the pollen season (Emberlin *et al.* 1997, van Vliet *et al.* 2002, Ziska *et al.* 2003) but also the duration of the pollen season (Emberlin 1994, Gehrig 2006). This research did not incorporate long-term pollen data, however, in Chapter 7, two consecutive years of pollen sampling in the Zugspitze region were analysed.

The birch pollen season in 2009 started two weeks earlier compared to 2008. This is most likely attributable to differences in temperature conditions in April 2009 (10°C) and 2008 (6.6°C). The temperatures of April 2009 in Garmisch-Partenkirchen were among the six highest since the start of temperature measurements in 1889. Therefore, the steep increase in temperature in early April led to a much earlier and more rapid plant development in the study area. This was also shown by Bruns (2009) for spring phenophases in Germany and the field study of 2009 in Munich and Ingolstadt (Chapter 6), where a prompt onset of birch flowering both in urban and rural areas was observed. These anomalous high temperatures provoked not only a remarkably earlier start of the pollen season in Garmisch-Partenkirchen (Chapter 7) but also a shorter birch pollen season compared to 2008. Nevertheless, the duration of the pollen season was almost identical in both analysed years, and the total pollen amount was 8 % higher in 2008 compared to 2009.

The grass pollen season in Garmisch-Partenkirchen started roughly two weeks earlier and was 50 % longer in 2009 than in 2008 and associated with a 3.6 times higher total pollen amount. However, this might not only be attributable to different weather conditions but also to differences in pollen trap location since the trap was located on a roof (10 m above ground level) in 2008 and in a small meadow (2 m above ground) in 2009. It has to be noted that high temperatures do not inevitably increase pollen season length. Gehrig (2006), for example, related a shortened pollen season in Switzerland to the extreme temperatures in summer 2003.

Long- and medium-range transport

The evaluation of phenological and aerobiological data (Chapter 7) demonstrated a good agreement in the start of season dates in 2009, especially in the valley of Garmisch-Partenkirchen. Local birch flowering closely corresponded to the start of the pollen season, defined by aerobiology. Moreover, the peak of the birch pollen season matched with the mean flowering date in the valley. The analysis of flowering of meadow foxtail and the aerobiological start of the grass pollen season revealed a temporal mismatch of less than one week. Cocksfoot, however, flowered two weeks later and seemed less adequate for predicting the start of the grass pollen season.

The end of the pollen season could not be described and explained by phenological data as precisely as the aerobiological start. The variety of temperature conditions which are characteristic for mountainous regions (Scherrer *et al.* 2011) imply different flowering onset dates at different altitudinal levels and aspects. Besides, the effect of meteorological parameters on pollen emission and dispersion changes with progress of the season (Spieksma 1980; Vázquez *et al.* 2003), and only the time between the start and the peak of the pollen season is particularly reliable for biological phenomena such as pollen emission, and not affected by re-suspension of pollen (Vázquez *et al.* 2003).

Temporal delay between phenological and aerobiological onset dates can partly be explained by long- or medium-range pollen transport (Estrella *et al.* 2006). Pollen released from the anthers of plants is suspended by turbulences and transported by wind. Pollen reaches the free atmosphere with deep convection and can subsequently be transported by air masses of synoptic systems (D’Amato *et al.* 2007). According to Rantio-Lehtimäki (1994) short-range transport of pollen is restricted to a horizontal distance of 1 km, medium-range transport up to 100 km, and long-range transport exceeds this horizontal threshold.

Long- or medium-range pollen transport did not account much for the aerobiological start of the birch pollen season in Garmisch-Partenkirchen. However, the analysis of phenological and aerobiological data revealed impacts of medium-range transport, related to the mountain-valley wind system that is characterised by down-valley winds during the night (Barry 1981). Higher locations, where plant abundance and pollen production is generally lower (Markgraf 1980, Gehrig & Peeters 2000), are most suitable to detect long- or medium-range transport. The pollen season at the highest pollen trap (UFS) started only one week later compared to the valley. In addition, the peak of the pollen amount at UFS closely matched the local mean flowering date in the valley suggesting that pollen counts at higher altitudes are strongly related to phenological behaviour of plants growing at lower altitudes.

Besides medium- and long-range transport, inconsistencies in phenological and aerobiological grass data can be attributed to the observed species. Phenological observations in the Zugspitze region only included flowering behaviour of two grass species, cocksfoot and meadow foxtail; however, pollen of all *Gramineae* species whose flowering periods overlap from spring to the end of summer is summarised in the aerobiological data derived from pollen traps (Jato *et al.* 2006).

Retreats for pollen allergic people

Commonly, the amount of pollen decreases with increasing altitude (Markgraf 1980, Frei 1997, Clot *et al.* 1995) since mountains are characterised by reduced plant abundance (Gehrig & Peeters 2000). In addition, due to altitudinal temperature decrease, the growing season in mountainous regions starts later and is shorter compared to the lowlands, causing a reduced pollen production (Markgraf 1980, Gehrig & Peeters 2000). However, greater amount of allergenic pollen at a high elevation site (Gütsch 2300 m) compared to a lower elevation site (Davos 1600 m) in Switzerland was reported by Frei (1997).

Mountainous areas are especially sensitive to global warming. The Alpine region has experienced a more pronounced temperature increase compared to the adjacent lowlands (Beniston 2006); thus, a decline of the favourable characteristics related to pollen might be expected. There is already evidence that higher regions are also strongly affected by the impacts of climate change on pollen amount. According to Bortenschlager & Bortenschlager (2005) global warming induced changes in elevated regions of Austria led to an advanced start and delayed end of the flowering period, to higher peak values and greater pollen production. Gehrig (2006) observed during the exceptional warm summer 2003 several days with high and allergologically relevant pollen concentration.

In this study (Chapter 7) remarkably large amounts of birch and grass pollen in higher elevated areas of the Northern Alps were found in 2008 and 2009 probably due to distinctive wind patterns forming less favourable conditions for pollen allergic patients.

At UFS, intermittently high amounts of birch pollen were found in 2008 and 2009 (~ 25 % of the amount measured in the valley). This observation is party attributable to the spherical morphology and small diameter of birch pollen which makes it to one of the most mobile pollen grains (Sofiev *et al.* 2006, Estrella *et al.* 2006). Grass pollen, however, is not that mobile and characterised by a spheroid or ovoid shape and a diameter ranging between 15 and 55 µm (Estrella *et al.* 2006, Jäger 2008). Therefore, at the UFS only a smaller fraction of the total grass pollen amount recorded at Garmisch-Partenkirchen was found (9 % in 2008, 4% in 2009). Since fewer days (compared to the valley) exhibited medically relevant amounts of pollen, it can be suggested that higher altitudes are more beneficial for pollen allergic people but might not *per se* be denoted as retreats. Pollen abundance is not a linear function of altitude. There is a range of factors that influence the amount of pollen: local vegetation, land use, topography, exposition, wind patterns and mesoscale pollen transport (Gehrig & Peeters 2000, D'Amato *et al.* 2007). In addition, as Chapter 7 demonstrated, even sites at the same altitudinal level can have considerable differences in phenological onset dates and subsequently in the timing of pollen release. Therefore, the amount of pollen in mountainous regions is not only a question of altitude but also of aspect.

According to Spieksma (1980) pollen grains are transported through the air in more or less discrete clouds and considerable time and distance are required before a homogeneous distribution is reached. The urban-rural comparison (Chapter 6) showed that warm spells during spring do not lead to later pollen exposure in the rural regions and allergic people could not benefit from a (limited) opportunity to spend pollen-free time in the countryside. We might expect an urban-rural synchrony or asynchrony depending on the actual weather conditions.

Therefore, at this time it is not straightforward to recommend the countryside without restriction as possible region that is longer pollen-free. This is also supported by another fact. Pollen is not ultimately connected to its region of origin since pollen grains are involved in atmospheric transport (D’Amato 2010). In the case of pronounced urban-rural differences in phenological onset dates, pollen allergic people would experience symptoms for a longer time period due to a steady development and transport of pollen in the atmosphere from the urban to the rural settlements and *vice versa*.

9.3.2 Quantitative assessments of pollen production and allergenicity

Pollen amount

Silver birch, an anemophilous plant, is a major source of pollinosis across Europe (D’Amato *et al.* 2007) and therefore most suitable for this survey. The field observations in the greater area of Munich in 2009 (Chapter 6) showed only a minor temperature difference between the city and its rural surroundings. In addition, no significant urban-rural difference for flowering and leaf phenophases as well as for the amount of pollen per birch catkin was found.

There is evidence from long-term aerobiological data (Spieksma *et al.* 1995, Frei & Gassner 1998, Rasmussen 2002) and experimental studies using urban-rural gradients as surrogate for climate change (Ziska et al. 2003) that temperature increases the atmospheric pollen amount of various plants. These results support the presented suggestion in Chapter 6 that small differences in the UHI might also be the cause of equality in pollen grains.

However, a small number of studies do not support the finding that the relationship between temperature and pollen amount is straightforward. Gehrig (2006) found that extended periods of negative water balance implicated unusual small pollen loads of *Rumex* spp., *Urtica* spp. and *Artemisia* spp. in southern Switzerland in 2003. According to Emberlin *et al.* (1999) urban air pollution induces more stress on plants that may result in decreased pollen production. This is in agreement with Guedes *et al.* (2009) who reported a decreased production of pollen from white goosefoot in a polluted urban environment (Porto, Portugal).

Using pollen counts of installed pollen traps implies difficulties in interpreting the data since medium- and long-range transport as well as the micro-location (amount of species in the nearer surrounding) can contribute to much higher / lower pollen amounts. These limitations were also mentioned by Monn *et al.* (1999) explaining the lacking urban-rural difference of pollen counts of birch and grass species in different regions in Switzerland.

Pollen allergenicity

Chapter 8 did not only incorporate the analysis of the major birch allergen, Bet v 1, but also of adjuvant, non-allergenic components released from pollen, i.e., PALMs and adenosine, and examined how anthropogenic factors related to urbanisation affect the allergenicity of birch pollen sampled along an urban-rural gradient.

Bet v 1

There was a tendency towards higher Bet v 1 concentrations in rural areas (albeit not significant). O_3 constituted the most important environmental factor influencing the major birch allergen content. However, no other environmental parameter, e.g., NO_2 concentration or urban index, had a significant effect on Bet v 1. Since the urban index is highly correlated with temperature (Chapter 2), we did not find any temperature effect on Bet v 1 concentration. Pollen allergenicity assessed by Bet v 1 content of mountain birch pollen was found to be increased when they were exposed to higher temperatures (Ahlholm *et al.* 1998). This finding is in accordance with Hjelmroos *et al.* (1995) who demonstrated that antigenic proteins and allergens per unit weight of extracted silver birch protein were higher when the pollen derived from a catkin that was harvested from the south side of the tree. Recent reviews on allergens (Beggs 2004, Beggs & Bambrick 2006, Jianan *et al.* 2007) neglected opposite findings. For instance, pollen allergenicity of common ragweed pollen (Amb a 1) was enhanced at sites with lower temperatures (Ziska *et al.* 2003). In addition, Guedes *et al.* (2009) reported decreased concentrations of total soluble proteins in urban pollen of white goosefoot in Porto, Portugal, compared to rural areas. These studies using the urban-rural comparison as an empirical analogue indicated future expected impacts of climate change on pollen.

There is already evidence that allergen content of grass species was enhanced subsequent to O_3 fumigation experiments (Masuch *et al.* 1995, Eckl-Dorna *et al.* 2010). In our study, however, a relative modest O_3 variation with slightly higher amounts in the countryside was measured, indicating that a more pronounced range of O_3 levels might even imply greater changes in Bet v 1 content. The effect of multiple anthropogenic environmental changes on the growth of cottonwood in New York and its urban surroundings was studied by Gregg *et al.* (2003). Here, higher cumulative O_3 exposure in rural sites was found to be the only factor that attributed to the decreased plant growth in rural areas. The authors declared: “These findings are in contrast to […] the pervasive perception that rural environments are safe havens from urban pollutant emissions.” This statement, therefore, goes in line with the results derived within our interdisciplinary study.

Ongoing global change might have an influence on tropospheric O_3 concentration. Firstly, NO_x that is needed for the photochemical O_3 reaction (Landsberg 1991) might increase with further urbanisation leading to higher O_3 production. Subsequently, O_3 is transported to the adjacent rural areas where, however, NO_x is lacking to destruct O_3 in night time reactions. Secondly, O_3 concentrations are predominant during anticyclonic weather conditions in summer and can reach extremely high values during heat waves (Menzel *et al.* 2004). With climate change increasing the probability and length of warm spells (Schär *et al.* 2004) we might expect higher O_3 concentrations in the future which could, in turn, affect pollen allergenicity (assessed by Bet v 1) and human health. Thirdly, it was demonstrated that higher temperatures in general, induce higher tropospheric O_3 concentrations (IPCC 2007a).

Adenosine

Adenosine promotes the differentiation of regulatory T cells by dendritic cells *in vitro* (Gilles *et al.* 2011). This study (Chapter 8) did not reveal a significant difference in adenosine content between urban and rural pollen. In addition, the analysed environmental variables, air temperature, urban index, NO_2 and O_3 concentration, were not related to differences in adenosine which was, however, recently identified as a potent immunregulatory substance (Gilles *et al.* 2011).

PALMs

Pollen associated lipid mediators (PALMs) are allergen independent immunmodulatory factors that can contribute to an initiation, manifestation or aggravation of allergic inflammation (Traidl-Hoffmann *et al.* 2003). These lipids derive from linolenic and linoleic acids and have been reported to evoke immune modulatory (prostaglandin E_2-like PALMs, $PALM_{PGE2}$) and immune stimulatory (leukotriene B_4-like PALMs, $PALM_{LTB4}$) effects both *in vitro* and *in vivo* (Traidl-Hoffmann *et al.* 2002, Bryce *et al.* 2010, Gilles *et al.* 2010).

In contrast to Behrendt *et al.* (2002), this study (Chapter 8) could not prove a significant difference in the content of $PALM_{LTB4}$ and $PALM_{PGE2}$ between urban and rural sites. The timing of pollen sampling and therefore the maturation stage of pollen has a discernible influence on Bet v 1 and PALMs concentration. Different sampling methods used in the study by Behrendt *et al.* (2002) and this interdisciplinary research might have caused these different findings. We sampled birch pollen at distinct maturation stages of both urban and rural catkins. Behrendt *et al.* (2002), however, did not regard maturation stages but collected urban and rural birch catkins within the same day where differences in maturation stages - attributable to differences in urban-rural temperatures - were most likely to affect allergen and PALM content.

The $PALM_{LTB4}/PALM_{PGE2}$ ratio was significantly increased in rural areas. Furthermore, O_3 was shown to be negatively correlated with the content of $PALM_{PGE2}$ and positively correlated with the $PALM_{LTB4}/PALM_{PGE2}$ ratio. $PALM_{LTB4}$ is formed by lipoxygenase (LOX)-catalysed reaction or by autoxidation (Feussner *et al.* 1998). $PALM_{PGE2}$, however, is exclusively formed by autoxidation (Imbusch & Mueller 2000). Since lipoxygenase could be shown to be activated by O_3 (Maccarrone *et al.* 1992), it can be assumed that an increased LOX activation was probably related to increased O_3 exposure. This might, in turn, be a major reason for the observed urban-rural differences in $PALM_{LTB4}/PALM_{PGE2}$ ratio in our study.

9.3.3 Functional and physiological relevance

Immune stimulatory capacity

We demonstrated the importance of the tropospheric O_3 by *in vitro* neutrophil chemotaxis (Chapter 8). Neutrophil migration assays revealed that high ozone-exposed pollen induced significantly stronger neutrophil chemotaxis than low ozone-exposed pollen. However, since no significant correlation between O_3 and $PALM_{LTB4}$, was found, other substances might be involved. In addition, high-ozone exposed pollen was characterised by low levels of $PALM_{PGE2}$ which, in turn, inhibits multiple cellular signaling mechanisms involved in neutrophil chemotaxis (Armstrong *et al.* 1995). It can be assumed that a more pronounced neutrophil chemotaxis observed in high-ozone exposed pollen might be in part attributable to the lack of $PALM_{PGE2}$. Rural areas (that are characterised by higher ozone levels) might intensify allergic reactions in already sensitised individuals by recruiting inflammatory cells into exposed tissues.

Immune modulatory capacity

Monocyte-derived dendritic cells (moDCs) which were stimulated with LPS in the presence and absence of aqueous pollen extract (APE) demonstrated differences in immune modulatory capacity between low and high ozone-exposed pollen (Chapter 8). It was demonstrated - most evident at high APE concentrations - that low ozone-exposed pollen inhibited the moDC's IL-12 response more than high ozone-exposed pollen. This finding might be probably related to differences in $PALM_{PGE2}$. Urban areas (that are characterised by lower ozone levels) might therefore facilitate *de novo* sensitisation by providing Th2 promoting signals.

Skin prick tests

To test the *in vivo* allergenicity and thus the clinical and physiological relevance of enhanced Bet v 1 levels and $PALM_{LTB4}/PALM_{PGE2}$ ratio, skin prick tests using APEs from high- and low-ozone exposed pollen were applied. It was demonstrated that high-ozone pollen induced a more pronounced allergic immune responses since wheal and flare sizes were significantly larger. In addition, a significant and positive correlation between wheal and flare size and O_3 exposure of the pollen was found. The aggravation of skin reactions was probably induced by increased levels of Bet v 1 that were linked to high-ozone exposed pollen. In addition, the less pronounced amount of $PALM_{PGE2}$ in high-ozone exposed pollen might also be responsible for the increased reactivity of allergic patients in skin prick tests. Since high-ozone levels are related to rural environments, allergic reaction in already sensitised individuals might be more severe for people living in the countryside.

9. 4 Further research recommendations

Tropical phenology

Our study was the first that analysed urban phenology in tropical regions in detail (Chapter 2). We could show that one out of the three selected tropical tree species (*Tipuana*) seems to be suitable in urban phenology studies. However, the huge amount of potential plants that could be monitored in tropical cities makes it indispensable to further explore additional species and phenophases. Moreover, the urban index appears to be a useful measure for a quick and inexpensive estimation of temperature variations, especially in urban areas, both in temperate as well as in tropical cities. Our results additionally suggest the consideration of humidity to understand tropical phenology. Leafing phenology of tropical trees under seasonal climate is also known to be influenced by precipitation and edaphic factors such as ground water availability and seasonal variation of soil water storage as well as leaf longevity and increasing day length (Borchert *et al.* 2005, Borchert 2008). However, the acquisition, recording or quantification of these variables was not possible within this study. In future research, these parameters should also be implemented since they might be helpful predictors in tropical phenology.

Nutrient analyses

To our knowledge this study was also the first dealing with the effects of foliar nutrient concentrations on the phenological behaviour at the mesoscale (Chapter 3). We were able to highlight the importance of nutritional status in phenological models. The analysis of foliar nutrients is considered to be an adequate and thus recommended measure for the assessment of environmental conditions, trees are exposed to. However, we did not make an explicit comparison between soil and foliar nutrients. This question should be explored in further research. Moreover, it is recommended to extend the analysis to a wider range of plant species, soil conditions, and regions to obtain more general information about the influential character of single nutrients.

Weather extremes

The special weather situation of April 2009 gave us the opportunity to study the responses of plant phenology on such events and we have highlighted synchrony of flowering dates of plant species (Chapter 6). In a recent review paper about urban phenology, Neil & Wu (2006) asked: “Will global warming magnify urban climate change or mask them?” The frequency and intensity of warm spells are likely to increase in the future (Schär *et al.* 2004, IPCC 2007a). The occurrence of these extreme events is expected to have greater impacts on terrestrial ecosystems than any change in mean temperatures (Jentsch *et al.* 2007). Therefore, it can be assumed that warm spells in spring might lead to smaller urban-rural differences in phenology. However, there is still a need for further investigating phenological behaviour in the future to assess the validity of the results derived from our field study.

Long-range transport of airborne pollen

There have been suggestions of increasing episodes of long-range transport of allergenic pollen (Cecchi *et al.* 2006, Cecchi *et al.* 2007) attributable to changes in circulation patterns (D’Amato & Cecchi 2008) resulting in variations in the start, peak and end of the pollen season (PID 2010). In addition, pollen of allergenic plants that are not native to a region could be prevailing in the atmosphere and induce sensitisations (Zauli *et al.* 2006; Cecchi *et al.* 2007). In our study increasing episodes of long-range transport could not be verified since pollen sampling was only carried out in two study years (Chapter 7). Moreover, the quantity of other / new pollen besides birch and grass species could not be addressed. Further research is needed to adequately investigate these important pollen related issues.

Urban and rural pollen amount

We found no difference for the amount of pollen per birch catkin between urban and rural areas of Munich in spring 2009, probably related to a less pronounced UHI (Chapter 6). Since the method of pollen sampling by harvesting catkins was newly introduced in our study, we were not able to compare the results with other years, especially with years that exhibit different weather conditions (e.g., more pronounced UHI, less extreme spring temperatures).

There is a need to further investigate pollen concentration in urban environments since existing studies analysing urban-rural differences or within-urban differences in pollen amounts are not spatial inclusive and comprehensive (e.g,. Cariñanos *et al.* 2002, Kasprzyk 2006, Šikoparija *et al.* 2006, Rodríguez-Rajo *et al.* 2010). Considerable differences in pollen amounts at vertical and horizontal distances of a few meters were found in many studies (Raynor *et al.* 1973, Raynor *et al.* 1975, Spieksma *et al.* 2000). Pollen is not utterly mixed in the air and the influence of local vegetation can be substantial (Raynor *et al.* 1975, Emberlin & Norris-Hill 1991, Frenz 2000). Subsequently, further investigations of pollen amounts produced by single trees and airborne pollen counts from populations are needed. And this requires a dense network incorporating phenological observations, pollen production of single trees as well as aerobiological pollen counts derived from pollen traps. This combined approach would be an important contribution for the evaluation of human health impacts of global and local climate change.

Pollen allergenicity

Another important field, further research should focus on, is the alteration of pollen allergenicity due to climate change / urbanisation. Some authors (Hjelmroos *et al.* 1995, Ahlholm *et al.* 1998) reported an increase in allergen content of birch under warmer conditions. This is in contrast to our study (Chapter 8), as well as to the findings of Ziska *et al.* (2003) and Guedes *et al.* (2009). Instead, we found that O_3 was the only environmental variable that had an influence on Bet v 1. This provokes further research on this interesting topic that is linked to contradictory findings. O_3 fumigations simulating a greater range of ozone levels will give further insights in the relationship between allergenicity and climate change.

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11 PUBLICATION LIST

Reviewed publications

1. **Jochner** S, Beck I, Behrendt H, Traidl-Hoffmann C, Menzel A (2011) Effects of extreme spring temperatures on urban phenology and pollen production: a case study in Munich and Ingolstadt. Climate Research, 49, 101-112.
2. **Jochner** S, Heckmann T, Becht M, Menzel A (2011). The integration of plant phenology and land use data to create a GIS-assisted bioclimatic characterisation of Bavaria, Germany. Plant Ecology and Diversity, 4, 91-101.
3. **Jochner** S, Sparks TH, Estrella N, Menzel A (2012) The influence of altitude and urbanisation on tends and mean dates in phenology (1980-2009). International Journal of Biometeorology, 56, 387-394.
4. **Jochner** S, Ziello C, Böck A, Estrella N, Buters J, Weichenmeier I, Behrendt H, Menzel A (2012) Spatio-temporal investigation of flowering dates and pollen counts in the topographically complex Zugspitze area on the German-Austrian border. Aerobiology, doi: 10.1007/s10453-012-9255-1.

Submitted publications

5. **Jochner** S, Alves-Eigenheer M, Menzel A, Morellato LPC (2012) Urban heat islands in tropical and temperate regions assessed by phenology. Submitted to Journal of Applied Ecology.
6. **Jochner** S, Göttlein A, Menzel A (2012) Nutrient status - the missing factor in phenological research. Submitted to Global Change Biology.

Publications in preparation

7. Beck I, **Jochner** S, Gilles S, McIntyre M, Buters J, Schidt-Weber C, Behrendt H, Ring J, Menzel A, Traidl-Hoffmann C (2012) Impact of ozone on the allergenicity of birch pollen. In preparation for Nature.

Other publications

8. **Jochner** S (2008) Untersuchungen zur Phänologie urbaner und ruraler Gebiete in Deutschland. Unpublished diploma thesis. Katholische Universität Eichstätt-Ingolstadt.
9. Ziello C, Estrella N, **Jochner** S, Menzel A (2011) Altitude does not influence plant phenological trends in the Alpine region. Ed. Environmental Research Station Schneefernerhaus (UFS). Scientific Results 2009/2010, 51-52.

Conference Proceedings and Abstracts

Oral presentations

10. **Jochner** S, Estrella N, Menzel A (2009) Urban heat island effects on temporal trends and response rates of spring phenology - An analysis for the three German cities of Frankfurt, Cologne and Munich. COST 725: International Conference on Scope and current limits of linking phenology and climatology. 10th-12th March 2009 in Geisenheim, Germany.

11. **Jochner** S, Menzel A (2009) Extreme spring events and phenological onset times in Germany. GFÖ 2009. Dimensions of ecology: from global change to molecular ecology. 14th-18th September 2009 in Bayreuth, Germany.
12. **Jochner** S, Heckmann T, Becht M, Menzel A (2010) GIS-assisted regionalisation of phenological data in Southern Bavaria using discriminant analysis. International Conference on Phenology: Plant Ecology and Diversity, 7th-9th April 2010 in Edinburgh, Scotland.
13. **Jochner** S, Menzel A (2010) Impacts of extreme temperatures on urban-rural phenology. Phenology 2010: Climate change impacts and adaptation. 14th-16th June 2010 in Dublin, Ireland.
14. **Jochner** S, Ziello C, Böck A, Estrella N, Buters J, Weichenmeier I, Behrendt H, Menzel A (2011) Spatio-temporal investigations of flowering dates and pollen counts in the topographically complex Zuspitze area. Geophysical Research Abstracts 13, EGU General Assembly 2011, Vienna, Austria.
15. Beck I, **Jochner** S, Gilles S, Menzel A, Ring J, Behrendt H, Schmidt-Weber C, Traidl-Hoffmann C (2011) Allergenicity and pollen characteristics of birch trees in urban and rural environments. 6. Deutscher Allergiekongress. 8th-10th September 2011 in Wiesbaden, Germany.
16. **Jochner** S, Beck I, Estrella N, Behrendt H, Traidl-Hoffmann C, Menzel A (2011) Do cities modify flowering times and pollen? A case study from Germany (2008-2011). 9th International Congress of Biometeorology. 4th-8th December 2011 in Auckland, New Zealand.
17. Beck I, **Jochner** S, Gilles S, Mareike M, Buters J, Menzel A, Ring J, Behrendt H, Schmidt-Weber C, Traidl-Hoffmann C (2012) Impact of environmental factors on the allergenicity of birch pollen. 24. Mainzer Allergie-Workshop 2012. 22th-23th March 2012 in Mainz, Germany.

Invited talks

18. **Jochner** S, Ziello C, Böck A, Estrella N, Buters J, Weichenmeier I, Behrendt H, Menzel A (2011) Spatio-temporal investigations of flowering dates and pollen counts in the topographically complex Zuspitze area. PEP725 workshop, ZAMG (Zentralanstalt für Meteorologie und Geodynamik), Vienna, Austria.

Poster presentations

19. **Jochner** S, Menzel A (2010) Effects of extreme spring temperatures on phenology: a case study from Munich and Ingolstadt. Geophysical Research Abstracts 12, EGU General Assembly 2010, Vienna, Austria.
20. **Jochner** S, Heckmann T, Becht M, Menzel A (2010) GIS-assisted regionalisation of phenological data in Southern Bavaria using discriminant analysis. Geographical Research Abstracts 12, EGU General Assembly 2010, Vienna, Austria.
21. **Jochner** S, Heckmann T, Becht M, Menzel A (2010) GIS-gestützte Regionalisierung von phänologischen Daten in Südbayern mittels Diskriminanzanalyse. DACH Meterologentagung 2010, 20th-24th September 2010 in Bonn, Germany.
22. **Jochner** S, Menzel A (2010) Auswirkungen von extremen Temperaturen auf temporale Variationen urbaner und ruraler Eintrittstermine. DACH Meterologentagung 2010, 20th-24th September 2010 in Bonn, Germany.

Candidate's individual contribution

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

Isabelle Beck was in charge of the collection, preparation and weighting of the birch pollen and passed us the raw data. Annette Menzel contributed with suggestions for statistical analyses, corrections and proof reading. Most of the work (**95 %)**, ranging from data collection (phenological observations, long-term data), statistical analyses and the writing of the manuscript, was done by myself.

2. **Jochner** S, Heckmann T, Becht M, Menzel A (2011). The integration of plant phenology and land use data to create a GIS-assisted bioclimatic characterisation of Bavaria, Germany. Plant Ecology and Diversity, 4, 91-101.

Discussions with Tobias Heckmann led to the idea of this paper. He helped me with statistical analyses and GIS. Tobias, along with Annette Menzel, contributed with corrections and proof reading. Most of the work (**80 %)**, ranging from data collection (long-term phenological data, land use data), statistical analyses, GIS analyses and the writing of the manuscript, was done by myself.

3. **Jochner** S, Sparks TH, Estrella N, Menzel A (2012) The influence of altitude and urbanisation on tends and mean dates in phenology (1980-2009). International Journal of Biometeorology, 56, 387-394.

Tim Sparks and Annette Menzel suggested ideas for the statistical analyses. Nicole Estrella assisted with the collection of long-term phenological data. All of them contributed with corrections and proof reading. Most of the work (**90 %)**, ranging from statistical analyses, map drawing and the writing of the manuscript, was done by myself.

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

Andreas Böck conducted all the phenological observations for his Diploma thesis and passed us the data. Chiara Ziello contributed with fruitful discussions, ideas, corrections and proof reading. Jeroen Buters, Ingrid Weichenmeier and Heidrun Behrendt passed us the pollen data. Nicole Estrella and Annette Menzel contributed with suggestions for statistical analyses, corrections and proof reading. Most of the work (**70 %)**, ranging from statistical analyses, map drawing and the writing of the manuscript, was done by myself.

5. **Jochner** S, Alves-Eigenheer M, Menzel A, Morellato LPC (2012) Urban heat islands in tropical and temperate regions assessed by phenology. Submitted to Journal of Applied Ecology.

Milene Alves-Eigenheer was in charge for the study realisation in Brazil. She selected observation sites and trees, installed temperature and humidity sensors on site and conducted the phenological observations. She passed me both phenological and meteorological raw data. Patricía Morellato and Annette Menzel contributed with suggestions for statistical analyses, corrections and proof reading. Most of the work (**80 %)**, ranging from data preparation, statistical analyses, map drawing and the writing of the manuscript, was done by myself. In addition, I conducted the phenological observations in Munich and was in charge of the meteorological network. I also visited the Brazilian study site, trained Milene in the field in order to improve consistency of study design. I taught Milene the handling with phenological data and GIS.

6. **Jochner** S, Göttlein A, Menzel A (2012) Nutrient status - the missing factor in phenological research. Submitted to Global Change Biology.

Axel Göttlein and Annette Menzel contributed with discussions, suggestions for statistical analyses, corrections and proof reading. Axel Göttlein and his staff additionally advised me how to prepare leaf samples for nutrient analyses that were conducted by technical assistants. Most of the work (**80 %)**, ranging from data preparation, statistical analyses, map drawing and the writing of the manuscript, was done by myself. I conducted the phenological observations in Munich and was in charge of the meteorological network.

7. Beck I, **Jochner** S, Gilles S, McIntyre M, Buters J, Schidt-Weber C, Behrendt H, Ring J, Menzel A, Traidl-Hoffmann C (2012) Impact of ozone on the allergenicity of birch pollen. In preparation for Nature.

Isabelle Beck, Claudia Traidl-Hoffmann, Heidrun Behrendt and others were in charge of the whole study. Isabelle Beck designed the experimental study and performed all laboratory work. I contributed with processed meteorological data, did a lot of statistical analyses (e.g., partial correlation analyses) and processed a map of the study area. Isabelle Beck and I both helped each other with our field work. Annette Menzel and I contributed with corrections and proof reading. In total, I carried out **20 %** of the work.

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My co-authors involved in this work: Prof. Dr. Annette Menzel, Prof. Dr. Claudia Traidl-Hoffmann, Isabelle Beck, Prof. Dr. Tim H. Sparks, Dr. Nicole Estrella, Chiara Ziello, Andreas Böck, Prof. Dr. Leonor Patrícia C. Morellato, Milene Alves-Eigenheer, Prof. Dr. Axel Göttlein, Dr. Tobias Heckmann, Prof. Dr. Michael Becht, Prof. Dr. Jeroen Buters, Dr. Ingrid Weichenmeier, Prof. Dr. Heidrun Behrendt, Prof. Dr. Carsten Schmidt-Weber, Prof. Dr. Johannes Ring, Dr. Stefanie Gilles and Dr. Mareike McIntyre.

My diligent students Stephanie Meurer, Andreas Böck and Alessandro Haas who wrote their Master, Bachelor or Diploma thesis within this research as well as Doris Rottler, Holm Seifert, Katharina Katheder, Daniela Schröder, Sonja Gubo, Yvonne Munich and Rainer Behrendt for participating in practical work and phenological observations.

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Particularly, I thank my significant other, friend and partner - Chris - for his patience and guidance, for his loving and more. For his constant support and believing in me; not only as a scientist.

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

„**Environmental responses of phenology and allergenic pollen to recent climate change and urbanisation**“

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.

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