Investigating and predicting atmospheric concentrations of allergenic fungal spores (*Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma*)

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Declaration

I hereby declare that the work presented in this thesis has been carried out by myself and does not incorporate any material previously submitted for another degree in any university.

To the best of my knowledge and belief, it does not contain any material previously written or published by another person, except where due reference is made in the text.

I am willing to make the thesis available for photocopy and loan, if it is accepted for the award of the degree.

/ Magdalena Sadyś /

Abstract

Air quality is an increasing concern of the European Union, local authorities, scientists and most of all inhabitants that become more aware of the quality of the surrounding environment. Bioaerosols may be consisted of various elements, and the most important are pollen grains, fungal spores, bacteria, viruses.

More than 100 genera of fungal spores have been identified as potential allergens that cause immunological response in susceptible individuals. *Alternaria* and *Cladosporium* have been recognised as the most important fungal species responsible for respiratory tract diseases, such as asthma, eczema, rhinitis and chronic sinusitis. While a lot of attention has been given to these fungal species, a limited number of studies can be found on *Didymella* and *Ganoderma*, although their allergenic properties were proved clinically.

Monitoring of allergenic fungal spore concentration in the air is therefore very important, and in particular at densely populated areas like Worcester, UK. In this thesis a five year spore data set was presented, which was collected using a 7-day volumetric spore trap, analysed with the aid of light microscopy, statistical tests and geographic information system techniques.

Although Kruskal-Wallis test detected statistically significant differences between annual concentrations of all examined fungal spore types, specific patterns in their distribution were also found. *Alternaria* spores were present in the air between mid-May/mid-June until September-October with peak occurring in August. *Cladosporium* sporulated between mid-May and October, with maximum concentration recorded in July. *Didymella* spores were seen from June/July up to September, while peaks were found in August. *Ganoderma* produced spores for 6 months (May-October), and maximum concentration could be found in September. With respect to diurnal fluctuations, *Alternaria* peaked between 22:00h and 23:00h, *Cladosporium* 13:00-15:00h, *Didymella* 04:00-05:00h and 22:00h-23:00h and *Ganoderma* from 03:00h to 06:00h.

Spatial analysis showed that sources of all fungal species were located in England, and there was no evidence for a long distance transport from the continent. The maximum concentration of spores was found several hours delayed in comparison to the approximate time of the spore release from the crops. This was in agreement with diurnal profiles of the spore concentration recorded in Worcester, UK. Spores of *Alternaria*, *Didymella* and *Ganoderma* revealed a regional origin, in contrast to *Cladosporium*, which sources were situated locally.

Hence, the weather conditions registered locally did not exhibit strong statistically significant correlations with fungal spore concentrations. This has had also an impact on the performance of the forecasting models. The best model was obtained for *Cladosporium* with 66% of the accuracy.

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Publications

Accepted articles

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O'Connor, D.J., Sadyś, M., Skjøth, C.A., Healy, D.A., Kennedy, R., Sodeau, J.R. (2014) Atmospheric concentrations of *Alternaria*, *Cladosporium*, *Ganoderma* and *Didymella* spores monitored in Cork, (Ireland) and Worcester, (England) during the summer of 2010. *Aerobiologia*, 30 (4), 397-411.

Sadyś M., Strzelczak, A., Grinn-Gofroń, A., Kennedy, R. (2015) Application of redundancy analysis for aerobiological data. *International Journal of Biometeorology*, 59(1), 25-36.

Sadyś, M., Skjøth, C.A., Kennedy, R. (2014) Determination of *Alternaria* spp. habitats using 7-day volumetric spore trap, Hybrid Single Particle Lagrangian Integrated Trajectory model and geographic information System. *Urban Climate*, DOI: 10.1016/j.uclim.2014.08.005.

Skjøth, C.A., Baker, P., Sadyś, M., Adams-Groom, B. (2014) Pollen from alder (*Alnus* sp.), birch (*Betula* sp.) and oak (*Quercus* sp.) in the UK originate from small woodlands. *Urban Climate*, DOI: 10.1016/j.uclim.2014.09.007.

List of acronyms

ANN	Artificial Neural Networks
ANOVA	Analysis of Variance
AP	Air Pressure
BAF	British Aerobiology Federation
CCA	Canonical Correspondence Analysis
CRAN	The Comprehensive R Archive Network
CV Error	Cross Validated Error
DCA	Detrended Correspondence Analysis
DPT	Dew point temperature
GIS	Geographic Information System
HYSPLIT	Hybrid Single Particle Lagrangian Integrated Trajectory
MLP	Multi Layer Perceptron
MRT	Multivariate Regression Tree
mvpart	Multivariate Partitioning
NPARU	National Pollen and Aerobiology Research Unit
RAIN	Rainfall
RAST	Radio-Allergo-Sorbent Test
RDA	Redundancy Analysis
RH	Relative humidity
SFI	Seasonal Fungal Index
SOP	Standard Operating Procedure
SPT	Skin Prick Test
TMA	Maximum temperature
TME	Mean temperature
TMI	Minimum temperature
UK	United Kingdom
UCT	Coordinated Universal Time
USA	United States of America
vegan	Community Ecology Package
VIF	Variance Inflation Factor
WD	Wind direction

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Chapter 1

Introduction

1.1. Air quality and bioaerosols

Majority of the living organisms undergo two the most important processes: nutrition and respiration. Regarding respiration an adult man on average takes 12 breaths and inhales approximately 0.05 m^3 of air per minute. This multiplied by 60 minutes and 24 hours results in 864 m^3 of air within a 24 hour period. Therefore air quality should be a major concern of all of us.

In the air there are many suspended small particles, both organic and in-organic. The former group is constituted of airborne pollen grains, fungal spores, algae, bacteria, viruses, plant debris. Fungal spores may form up to 45% of coarse particulate matter (Fröhlich-Nowoisky et al., 2009). Basidiospores and ascospores constitute between 40% and 100% of total fungi detected in air particulate matter (Després et al., 2007; Wu et al., 2003).

The impact of biological particles is often neglected in studies regarding physics and chemistry of the atmosphere (Elbert et al., 2007; Morris et al., 2011) including their impact on human health and the environment. Hence, little is known with respect to the origin, proportions and components of bioaerosols and several authors called for further investigations (Comtois and Isard, 1999; Després et al., 2007).

1.2. Allergy and asthma

More than 100 fungal genera were proved to be allergenic and to be a cause of respiratory tract diseases such as asthma, rhino-conjunctivitis, rhinitis etc. (Green et al., 2005). Typical examples of allergic responses are: sneezing, running noses, wheezing, tightness of chest, coughing and shortness of breath, urticaria,

angioedema and anaphylaxis (Kurup et al., 2000; Kurup et al., 2002), pruritus, nasal obstructions (Simon-Nobbe et al., 2008). Such allergic symptoms cause a considerable negative impact on the quality of human health (Bousquet et al., 2001).

Bouziane et al. (2005, 2006) found that fungal spores contain higher concentration of allergens than mycelia, while Green et al. (2003) proved that spore germination induced a greater production of allergens. Regardless of the viability, fungal spores are always allergenic as even autoclaved spores were found to contain allergens (Mitakakis et al., 2003).

The National Health Service (NHS) in the United Kingdom reported that 5.4 million of people were diagnosed to have asthma, while a treatment spent for those people cost NHS £ 1 billion annually (Asthma UK, 2013). Moreover, every single day 3 people die due to acute asthma in the UK (Asthma UK, 2013). This results in the highest number of reported asthma deaths across the Europe (Wolfe et al., 2011).

1.3. Fungal genera examined in this study

Out of numerous fungal genera spores of four genera were selected for a detailed investigation in this study, *i.e.*: *Alternaria* spp. (hereafter *Alternaria*), *Cladosporium* spp. (hereafter *Cladosporium*), *Didymella* spp. (hereafter *Didymella*) and *Ganoderma* spp. (hereafter *Ganoderma*), (Fig. 1.1). This selection was made upon the spore (1) allergenicity and (2) biology.

1.3.1. Allergenicity

Spores produced by *Alternaria* and *Cladosporium* (Fig. 1.1) out of all identified allergenic fungal spores are considered to be the most important and jointly they may constitute up to 93% of the total fungal spores found in the air (Ataygul et al., 2007). *A. alternata* has been studied the most intensively out of all *Alternaria* species and Alt a 1 was found to be the most important isolated allergen (Horner et al., 1995). *Alternaria* spores may be a cause of respiratory tract diseases, such as asthma or chronic sinusitis (Breitenbach and Simon-Nobbe,

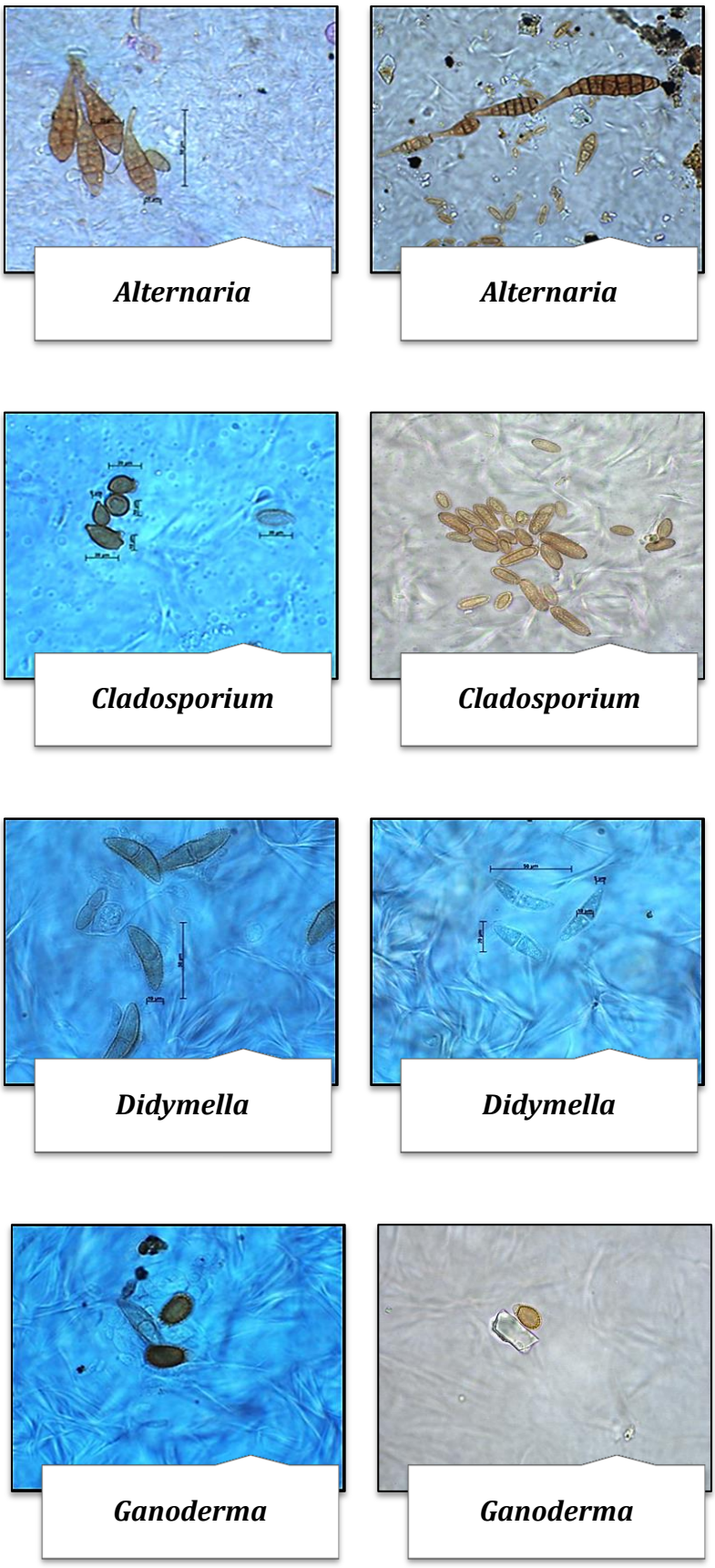


Fig. 1.1 Investigated fungal spores.

2002; St-Germain and Summerbell, 2011). *Cladosporium* spores as previously mentioned are highly allergenic and they cause pulmonary and cutaneous infections (Horner et al., 1995; Breitenbach and Simon-Nobbe, 2002). *C. herbarum* and *C. cladosporioides* have been studied the most intesively out of all *Cladosporium* species and the most important isolated allergens are Cla h 1 and Cla h 2 (Horner et al., 1995; Breitenbach and Simon-Nobbe, 2002).

Downs et al. (2001) summarized results of research, where sensitisation to *Alternaria* species has been studied in sample populations of children. The sensitisation varied between <1% in Austria to 50% in the USA. O'Hollaren et al. (1991) also reported that asthma can be a cause of death, and although this phenomenon is rare, a number of cases have increased in England, Denmark, United States, Australia and New Zealand. According to Zureik et al. (2002) overall sensitisation to fungal spores in the United Kingdom and Republic of Ireland was found to be the highest in comparison with Northern, Central and Southern Europe. Sensitisation to *Alternaria alternata* affected 17.6% of patients with diagnosed asthma, *Cladosporium herbarum* affecting 6.8% (Zureik et al., 2002). *Alternaria alternata* caused a greater sensitisation than birch or ragweed pollen in studied populations (Zureik et al., 2002). Lower results of sensitisation both to *Alternaria* and *Cladosporium* spores were reported by the European Community Respiratory Health Survey, which indicated respectively 4.4% and 2.3% of adults aged between 20 and 44 years to be allergic to the above mentioned fungal spores (Bousquet et al., 2007). It is notable that more than 11 300 adults were examined, and overall it was found that 35.6% of subjects were found to be atopic (Bousquet et al., 2007).

Frankland and Gregory (1973) found a link between high ascospore counts of *Didymella exitialis* (Fig. 1.1) in the air and asthmatic symptoms of two patients, who lived in the vicinity of barley fields. Further investigation revealed that 12 patients out of 100 showed sensitisation to *Didymella exitialis* extracts using skin prick tests (Frankland and Gregory, 1973). Subsequently Harries et al. (1985), Packe and Ayres (1985) and Alderman et al. (1986) confirmed that *Didymella exitialis* was responsible for the late summer asthma with an exception of Morrow Brown and Jackson (1985) findings, who could not find similar correlation in their

data. At present, *Didymella* spores are considered as one of the major allergenic taxa in the United Kingdom (Newson et al., 2000), which trigger respiratory tract diseases although additional examinations should be carried out regarding *Didymella* allergens.

A limited number of clinical studies, regarding sensitisation to *Ganoderma* spores (Fig. 1.1) and to basidiospores in general, was conducted in the United Kingdom (Herxheimer et al., 1966; Herxheimer et al., 1969; Hyde, 1972; Hyde, 1973; Jenkins et al., 1981), as well as other countries, *i.e.*: New Zealand (Hasnain et al., 1984; Hasnain et al., 1985; Cutten et al., 1988), United States (Lehrer et al., 1986; Sprenger et al., 1988; Horner et al., 1993; Horner et al., 1998), Canada (Tarlo et al., 1979; Vijay et al., 1991) and India (Singh et al., 1995). Although the clinical studies have already proved that *Ganoderma* spores cause allergic responses in susceptible individuals, *i.e.*: positive SPT and RAST reactions were observed (Herxheimer et al., 1969; Singh et al., 1995), no allergen has been yet extracted (Horner et al., 1995; Helbling et al., 2002).

Frankland and Davies (1965) estimated that the daily mean *Alternaria* spore concentration must be equal to or above 50 s m^{-3} in the air to trigger clinical symptoms of sensitised people in the UK. Different clinical thresholds have been established in other European countries, for instance 100 s m^{-3} in Croatia (Peternel et al., 2004), Spain (Rodríguez-Rajo et al., 2005) and Finland (Ranta and Pessi, 2006), while 80 s m^{-3} were observed in Poland (Rapiejko et al., 2004). The clinical threshold that has been estimated for *Cladosporium* spores in the air in the UK (Frankland and Davies, 1965) and Croatia (Peternel et al., 2004) is equal to 3000 s m^{-3}, while it is 2800 s m^{-3} in Poland (Rapiejko et al., 2004) and 4000 s m^{-3} in Finland (Ranta and Pessi, 2006). The concentration levels have not yet been established for other allergenic spore types including *Ganoderma* and *Didymella* spores.

1.3.2. Biology

Fungi successfully colonize various habitats due to their ability of different reproduction strategies, *i.e.*: sexual and asexual. As a result of sexual reproduction (meiosis) ascospores and basidiospores are produced. This stage is called as

teleomorph. Asexual reproduction is done via fragments of hyphae and spores obtained as a result of mitosis, in contrast to sexually produced spores, are called as *conidia* (Ingold, 1979). This stage is termed as *anamorph.* The life cycle of "perfect fungi" consists of both sexual and asexual stage; these fungi are then called as *holomorph.*

Fungi, which teleomorph is unknown are usually described as "imperfect". It is believed, that these species are anamorphs of either Ascomycetes or Basidiomycetes, though in most of the cases the links between their teleomorphs have not been proved yet (Ingold, 1979; Müller and Loeffler, 1987). Ingold (1979) suggested that these species were most probably Ascomycetes, which "*have lost the* ascus *stage completely*". Hence, in the recent taxonomic classifications Fungi Imperfecti, as an artificial division, was not included (Alexopoulos et al., 1996; Blackwell and Spatafora, 2004). Fungi Imperfecti (Ingold, 1979) are also known in the literature as Hyphomycetes (Ellis 1971; Ogden et al., 1974; Ellis, 2001), Deuteromycetes (Podbielowski et al., 1986; Müller and Loeffler, 1987; Sweykowska and Szweykowski, 2005), Mitosporic Fungi (Haines et al., 2003) or Conidial Fungi (Ingold, 1979; Lacey, 1981). Recently this name has been replaced by Anamorphic Fungi (Kirk et al., 2004). *Alternaria* and *Cladosporium* are common moulds, which belong to the artificial group of Anamorphic Fungi. *Didymella* represents Ascomycetes, while *Ganoderma* Basidiomycetes.

1.3.2.1. *Alternaria*

Alternaria is an anamorph of *Lewia* spp. with a worldwide distribution (Kirk et al., 2004), (Fig. 1.1). Approximately 300 species of this genus have been identified (Simmons, 2007), although some authors suggest that there are only 50 species (Rotem, 1994). This confusion derives from observed morphological changes of the same species while parasitizing different hosts (Rotem, 1994). There are many species among the *Alternaria* genus, which are pathogenic to host plants within a group of economically important crops (Thomma, 2003). In the temperate zone the highest yield losses are caused by an early blight of potato (Olanya et al., 2009), early blight of tomato (Chaerani and Voorrips, 2006; Vloutoglou and Kalogerakis, 2000), leaf blight of carrot (Boedo et al., 2010; Boedo

et al., 2012; Dugdale et al., 2000; Maude et al., 1992), purple blotch of onion (Meredith, 1966; Suheri and Price, 2000), purple blotch of leek (Gladders, 1981), leaf spot of bean (Russell and Brown, 1977), leaf spot of cabbage (Geeson and Browne, 1979; Valkonen and Koponen, 1990), leaf spot of linseed (Evans et al., 1997; Vloutoglou, 1994). The causal agents of these diseases are *Alternaria solani, Alternaria dauci, Alternaria porri, Alternaria alternata, Alternaria brassicicola, Alternaria raphani,* and *Alternaria linicola,* respectively.

Spores and mycelium of *Alternaria* can survive the winter in soil, in infected plant debris, tubers and seeds. Infected seeds sometimes develop together with fungus, and therefore on the seedlings it is possible to notice symptoms of infection such as collar rot or stem lesions (Laemmlen, 2001). More often it happens that mycelium first must store modicum of food, and afterwards it produces a tangle of aerial branches that divide into cells (this process is called sporulation). Maturation of these cells changes them into spores, with size and shape characteristic of specific species (Christensen, 1965). Spores are spread all over the plants by wind and with rain drops. They start germination whilst favourable weather conditions, *i.e.* when relative humidity is high. Host plants can be attacked directly through stomata or through wounds. Vigorous and sound tissues are more resistant to fungus penetration than old, weak, stressed or wounded tissues (Laemmlen, 2001).

1.3.2.2. *Cladosporium*

Similarly to *Alternaria*, *Cladosporium* (Link, 1816) has a worldwide distribution (Kirk et al., 2004), (Fig. 1.1). There are approximately 60 species in the *Cladosporium* genus. An anamorph of *Cladosporium* is *Davidiella* spp. (*Mycosphaerella* spp.). *Cladosporium* is mainly a secondary invader of dead plant debris and trees, e.g. beech wood (Gravesen, 1979). However, *Cladosporium fulvum* is a well-known pathogen of tomato, which causes tomato leaf mold (Jones et al., 1997). Not only leaves are infected by the mold, but also occassionally stems, petioles and fruits (Jones et al., 1997).

Colonies of *Cladosporium herbarum* are powdery - velvety in structure, and their colour may vary from olivaceous to brown. Conidiophores have smooth walls

and terminal and intercalary protuberances. Single or multi-celled conidia are formed at the end of the conidiophores in either simple or occassionally branched chains. Conidia germinate on moist surfaces resulting in lesions black in colour (König et al., 2009).

1.3.2.3. *Didymella*

The genus *Didymella* (Sacc., 1880) is a group of widespread saprophytic and pathogenic fungi and around 75 species have been described (Kirk et al., 2004), (Fig. 1.1). *Didymella* can be often found on the leaves of cereals, e.g. ascochyta leaf scorch caused by *D. exitialis* (Stefansson and Hallsson, 2011), vegetables, e.g. ascochyta blight of chickpea caused by *D. rabiei* (Trapero-Casas et al., 1996), fruits, e.g. spur blight on red raspberries caused by *D. applanata* (Burchill and Beever, 1975), and ornamental flowers, e.g. ray blight of chrysanthemum caused by *D. chrysanthemi* (Fox, 2005). Although the occurrence of *Didymella* has been reported in many countries around the world (e.g. Australia, New Zealand), this fungus is frequently observed in Europe, *inter alia* in the United Kingdom, where it grows on barley (*Hordeum vulagare*) and wheat (*Triticum sativum*), (Corden and Millington, 1994; Cromey et al., 2004; Frankland and Gregory, 1973; Jackson, 1984; Khan et al., 2013).

The class of Ascomycetes is characterized by production of sexual spores (ascospores) in sac-like structures called *asci*. A karyogamy followed by mitosis takes place inside the *ascus*. This results in formation of usually 8 haploid ascospores. The *pseudothecia* (fruiting bodies with two layer asci organised irregularly) and *pycnidia* (spherical structures resembling fruiting bodies, which produce conidia) of *Didymella* species start to be formed in autumn, while at the beginning of spring they usually reach maturity (Burchill and Beever, 1975). Ascospores release takes place from the end of winter until spring, and decrease completely by the beginning of summer (Gamliel-Atinsky et al., 2005). Once pseudothecia are depleted, no more ascospores are produced and the pseudothecial walls decompose (Gamliel-Atinsky et al., 2005). Symptoms of infection are apparent in a form of necrotic spots on host plant leaves, when concentration of airborne ascospores declines (Burchill and Beever, 1975).

Airborne ascospores constitute a primary inoculum source of disease, while conidia produced by pycnidia are considered to cause infection within a crop to a lesser degree (Khan et al., 2013; Trapero-Casas et al., 1996). However, how far viable *Didymella* ascospores can be transported with the air currents from the inoculum source has not been yet examined (Keinath, 2011).

1.3.2.4. *Ganoderma*

In contrast, *Ganoderma* is a common bracket fungus (Fig. 1.1), which infects both deciduous and coniferous trees, e.g. beech and oak (Gregory and Hirst, 1952; Pegler and Young, 1973), yew (Pegler and Young, 1973), hornbeam (Gibbs and Evans, 2000), chestnut (Rose, 2004), poplar, maple and ash (McKay, 2011). Pegler and Young (1973) identified and described in detail six species of *Ganoderma* found in the United Kingdom, *i.e.*: *G. applanatum*, *G. lucidum*, *G. adpersum*, *G. pfifferi*, *G. resinaceum* and *G. valesiacum*.

The class of Basidiomycetes is characterized by production of sexual spores (basidiospores) in aseptate, club-shaped cells called *basidia.* Each *basidium* produces usually four haploid spores. Airborne spores, if landed on wounded trunk or a branch, after germination may lead to formation of a perennial fruiting body - conk, although the details of this process are still somewhat unclear (Elliott and Broschat, 2000). A single *G. applanatum* basidiocarp can produce 30 billion spores per day, over a period of six months (Levetin, 1990).

1.4. Spatial and temporal distribution of fungal spores

1.4.1. Spatial analysis of fungal spore distribution

Traditionally an analysis of the spatial distribution of fungal spores was done by a comparison of spore load recorded at two (Palmas and Consetino, 1990) or more monitoring stations (Kasprzyk et al., 2013). Following this approach no article has been yet published in terms of aerobiological surveys conducted in the UK, with the exception of two abstracts printed in Conference Proceedings (Corden et al., 2003; Corden and Stępalska, 2006).

Recently, an analysis of the spatial distribution of airborne particles was performed using atmospheric models, such as HYbrid Single Particle Lagrangian

Integrated Trajectory model (HYSPLIT), (Stach et al., 2007) or California Puff model (CALPUFF), (Pfender et al., 2006) in combination with Geographic Information System (GIS) techniques. These lagrangian models were initially constructed to examine forward and backward trajectories of the air masses, but their application turned out to be wider. Although a great number of interesting studies have been published with regard to allergenic pollen, such as oak (Hernández-Ceballos et al., 2011), birch (Skjøth et al., 2009) or olive (Fernández-Rodríguez et al., 2014; Hernández-Ceballos et al., 2014a; 2014b), there are limited studies that have analysed the origin and transport of the allergenic fungal spores recorded in the air of urban areas (Isard et al., 2005; Sadyś et al., 2014; Skjøth et al., 2012).

1.4.2. Temporal fluctuations in fungal spore concentration

Application of certain microscopic counting techniques for airborne fungal spores does not always allow the investigation of hourly changes in distribution, e.g. the method of random fields commonly used in Finland (Käpylä and Penttinen, 1981). The necessity of studying diurnal variations in fungal spore concentration has been raised since it was noticed that a 12 transverse transects method for counting tapes from the Hirst volumetric spore trap may omit a sudden "burst of spores" (Lacey and West, 2006). These unexpected "clouds of fungal spores" may trigger allergic reactions in sensitised people, resulting in hospitalisation, or even death (Asthma UK, 2013).

Studying diurnal distribution of allergenic fungal spores, such as those of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* can determine when increased concentration of these spores may occur. Additionally weather conditions that are significantly associated with peak concentrations registered within 24 hour periods of time can be associated. Recorded one year hourly distributions could be constant or subject to yearly change at the same spatial location.

Knowing the time of highest concentration of allergenic fungal spores can help patients plan their activities and reduce their exposure to fungal spores to a minimum, while allowing sufferers to spend time outdoors.

1.5. Forecasting methods in the aerobiology

Forecasts for airborne concentrations of fungal spores play an important role in the timing of prophylactic medication and in maintaining compliance in treatments. They also help allergic people to plan their activities to avoid exposure to high atmospheric concentrations of fungal spores. The prediction of concentration of allergenic fungal spores, based on archive data records, has become a primary aim of many researchers. Studies dedicated to *Alternaria* spores were conducted using the following statistical techniques, (1) regression analysis (Hjelmroos, 1993; Angulo-Romero et al., 1999), (2) multiple regression models (Lyon et al., 1984; Mitakakis et al., 2001; Munuera Giner et al., 2001; Stennett and Beggs, 2004; Rodríguez-Rajo et al., 2005; Stępalska and Wołek, 2005; Jesús Aira et al., 2008; Grinn-Gofroń and Rapiejko, 2009; De Linares et al., 2010; Sabariego et al., 2012; Recio et al., 2012), (3) Auto Regressive Moving Average models (ARMA), (Katial et al., 1997), (4) Auto Regressive Integrated Moving Average models (ARIMA), (Escuredo et al., 2011), (5) Artificial Neural Networks (ANN), (Angelosante Bruno et al., 2007; Grinn-Gofroń and Strzelczak, 2008; Tomassetti et al., 2009; Astray et al., 2010; Tomassetti et al., 2013), (6) Multivariate Regression Trees (MRT), (Grinn-Gofroń and Strzelczak, 2009). Unconventional predictive models for *Alternaria* spores were produced by Iglesias et al. (2007) and Escuredo et al. (2011). For example, (1) a model of propitious days (P-days), (2) model of accumulation of disfavourable days (DD), (3) model of interrupted wet periods (IWP) using similarly archived spore and meteorological data.

However, the aerobiological studies dedicated to *Alternaria* in the UK, are limited to several investigations regarding the general monitoring of spore concentration in the air of selected cities (Derby, Cardiff) and descriptive analysis of the relationship between the presence of conidia and meteorological parameters (Hyde and Williams, 1946; Adams, 1964; Morrow Brown and Jackson, 1978; Corden and Millington, 2001; Corden et al., 2003). The first attempt of the prediction of concentrations of *Alternaria* spores in the UK was done by Corden et al. (2003), who used a multiple regression to examine 30 years of data.

The number of prediction models specifically produced to forecast concentration of airborne *Cladosporium* spores worldwide is limited (Grinn-Gofroń

and Strzelczak, 2008; Grinn-Gofroń and Strzelczak, 2009; Herrero and Zaldivar, 1997; Mediavilla Molina et al., 1998). Several methods have been used including (1) linear regression analysis, (2) multiple regression analysis (Herrero and Zaldivar, 1997; Mediavilla Molina et al., 1998; Troutt and Levetin, 2001), (3) ARMA (Stephen et al., 1990), 4) ANN (Grinn-Gofroń and Strzelczak, 2008, 2009). Similarly to *Alternaria*, studies with regard to *Cladosporium* spores performed in the UK were limited to the concentration levels and relationship with local weather parameters (Ainsworth, 1952; Harvey, 1967; Hollins et al., 2004). To date no numerical forecasting model has been produced for this taxon in the UK, as well as for *Didymella* and *Ganoderma* spores.

1.6. Gaps in general knowledge

Following gaps in knowledge in the aerobiology science were identified:

- Lack of aeromycological profile for a densely populated city such as Worcester, United Kingdom.
- Lack of spatial distribution analyses for any fungal spore type in the UK.
- Lack of extensive studies regarding diurnal distribution of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores in general and in the UK.
- Lack of forecasting models using advanced statistical techniques (such as ANN) produced for selected fungal spores in the UK.

1.7. Aims and objectives of this study

The overall aims of this study were to investigate atmospheric concentrations of the allergenic fungal spores of *Alternaria*, *Cladosporium*, *Didymella*, *Ganoderma* and to produce forecast models for predicting their concentrations in the air in Worcester, UK. This was accomplished by using fungal spore and meteorological data collected in Worcester, UK from 2006 to 2010, and by completing the following studies:

1) Analyse annual variations in fungal spore concentrations in the air in relation to selected meteorological variables. This was the foundation upon which the forecast models were constructed. Results of this examination

were presented in Chapter 3 of this study. A part of this chapter was published as a research article.

2) Analyse the diurnal variation in fungal spore concentrations in the air in relation to meteorological variables was also used in production of forecast models. Results of this examination were presented in Chapter 4 of this study.
3) Analyse spatial variation in fungal spore distribution in the air using back trajectory analysis. This was done to identify the major sources of the fungi investigated. Results of this examination were presented in Chapter 5 of this study. A part of obtained results was published as a research article.

   A comparison of aeromycological profiles between Worcester (England) and Cork (Ireland) within the British Isles turned out to be beyond the primary scope of this study. Hence, it was removed from the thesis and also published in a form of a research article.
4) Produce prediction models for each of the studied taxa (*Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma*) using ANN approach. Results of this analysis were presented in Chapter 6 of this study and Appendix VI.
5) All successfully published research articles (5) were enclosed in Appendix VII of this thesis.

Chapter 2

Materials and methods

2.1. Sampling

2.1.1. Air sampler used in the study

The seven day volumetric spore trap (Hirst, 1952) has been used to collect air samples during the study period (Fig. 2.1). The operating machine was manufactured by the Burkard Manufacturing Co. Ltd. company (Rickmansworth, Hertfordshire, UK).

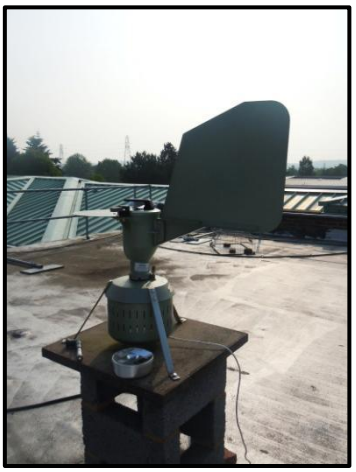


Fig. 2.1 The Hirst type sampler.

The 7-day volumetric spore trap works by impacting particles present in the air, directly on to "Melinex" tape, which is tightly fastened to a drum inside the sampler. The "Melinex" tape is covered by an adhesive medium, *i.e.*: a mixture of petroleum jelly and paraffin wax (Fig. 2.1). The pump located in the lower part of the sampler produces an air pressure with a suction power equal to 10 l of air per minute. The drum, which has a built-in clock mechanism, rotates with a speed of 2 mm per hour. The drum has a circumference equal to 40 cm, therefore it can sample for a period of one week.

2.1.2. Period of sampling

Aerobiological monitoring was conducted from 1st of January 2006 to 31st of December 2010. The whole data set consisted of over 1800 microscope slides, where each microscope slide corresponded to a single day. Occasionally, fungal spore counts were not recorded for a few days within the sampling period. Missing gaps were filled with spore counts from microscope slides prepared for pollen

grain examination, simultaneously collected using the same type of air sampler co-located with a spore trap. This was an acceptable decision, as previous studies have already proved that correlation coefficients between two Hirst type samplers at a distance equal to 1 m were 0.97 for fungal spore data and 0.94 for pollen data (Pedersen and Moseholm, 1993; Irdi et al., 2002).

2.1.3. Location of the sampler

Worcester is located in the West Midlands of England (Fig. 2.2). Worcestershire together with surrounding Herefordshire and Gloucestershire are considered agricultural counties, well-known for their orchards, cider and perry production.

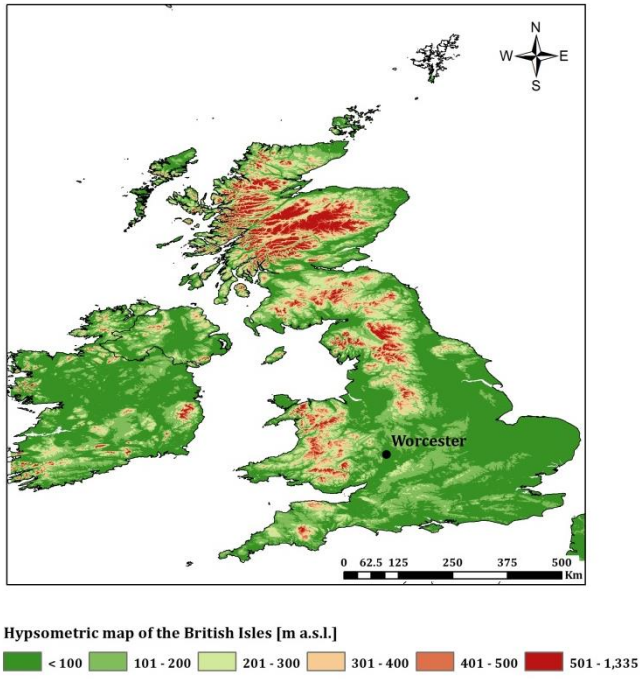


Fig. 2.2 Map of United Kingdom presenting the location of Worcester.

The town population of Worcester is estimated as 98800 inhabitants with a population density of 2969 people per km^2. The mean population density of England and Wales is around 371 people per km^2 (Rice, 2011). The river Severn, which is the longest river in Great Britain (220 mi), flows through the city centre and divides Worcester as an Eastern and Western part. The majority of the city is situated on the Eastern side of the river, but the University Campus is mainly located on the Western bank in St. John's district.

The climate of Worcester is temperate marine and characterised by mild winters and warm summers. The annual mean temperature of the city is around 9.5°C. The annual mean precipitation occurs around 669 mm and the sky is covered by cloud for 70% of the year (Cavan, 2004). There are only approximately 17 days with possible snow cover. The south westerly winds are the most frequent,

and the closest meteorological station is Birmingham Airport (Elmdon), (Met Office, 2012).

A 7-day volumetric spore trap was situated on the roof of the University of Worcester (52° 11' N, 2° 14' W) approximately 25 m above sea level and 10 m above ground level (Fig. 2.1).

2.1.4. Collection and processing of the air samples

Drums were prepared following the NPARU Standard Operation Procedure, which is described in Appendix I. Drums were changed every week on Thursday at 09:00h UTC. The procedure for drum replacement was described in detail in the NPARU SOP for changing drums in the Burkard, 7-day volumetric spore trap, which is described in Appendix II.

The procedure for mounting the preparation is defined in the protocol in Appendix III (NPARU SOP Mountant making for pollen and fungal spores). Appendix IV (NPARU SOP Slide preparation for pollen and fungal spores) provides information about the steps in the technical processing of the air samples.

2.1.5. Identification of fungal spores

The concentration of 21 types of fungal spores, *i.e.*: *Alternaria, Aspergillus* spp. / *Penicillium* spp. (hereafter *Aspergillus* / *Penicillium*), *Blumeria* spp. (hereafter *Blumeria*), *Botrytis* spp. (hereafter *Botrytis*), *Cladosporium*, coloured basidiospores, *Didymella, Drechslera* type (hereafter *Drechslera*), *Entomophthora* spp. (hereafter *Entomophthora*), *Epicoccum* spp. (hereafter *Epicoccum*), *Ganoderma, Leptosphaeria* type (hereafter *Leptosphaeria*), *Periconia* spp. (hereafter *Periconia*), *Pithomyces* spp. (hereafter *Pithomyces*), *Pleospora* spp. (hereafter *Pleospora*), *Polythrincium* spp. (hereafter *Polythrincium*), rusts, smuts, *Stemphylium* spp. (hereafter *Stemphylium*), *Tetraploa* spp. (hereafter *Tetraploa*) and *Torula* spp. (hereafter *Torula*) in the atmosphere of Worcester was measured during the study period. However, only four fungal spore types were selected for a detailed investigation in this thesis, *i.e.*: *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma*.

Alternaria spores may vary in shape from obclavate to ovoid, often compared to "tadpole" or "grenade" (Fig. 1.1). Size of the spores is approximately 7-13 µm x 20-70 µm, while the colour varies between olivaceous brown and brown. Surface texture is another variable feature as spores with smooth and rough (verrucose) walls can be found. Other key features of correct *Alternaria* spore identification are longitudinal wall surface and transverse septae (muriform), as well as often visible scar at the apical cell (Ellis, 1971; Haines et al., 2003).

Shape of the spores varies from cylindrical, through ellipsoidal and ovoid up to sub-spherical (Fig. 1.1). They are usually small in size (40-60 µm x 3-22 µm) from olivaceous to brown in colour, frequently observed in branched chains. Hence depending on the location, spores may have a shield or round shape at the ends, visible black scars of attachment points. Spores towards the end of chain are smaller, occasionally with up to 3 transverse walls. Surface of the wall may be either smooth or rough (verruculose or echinulate), (Ellis, 1971).

Didymella spores have a characteristic biconic shape and they are approximately 12-18 µm x 4-6 µm in size (Dennis, 1981; Fig. 1.1). Spores are both hyaline with smooth wall and yellowish in colour with rough wall surfaces. However, one septum in the middle is the most important feature that allows distinguishing this genus from the others (Dennis, 1981).

Spores of *Ganoderma* have an ovoid or egg shape and they are approximately 8-13 x 4.5-8 µm in size (Pegler and Young, 1973; Fig. 1.1). The external wall of spores is hyaline, while the colour of internal wall varies from golden to dark brown. Both layers of the wall are connected by pillars, which may appear as dots. Spores have a flattened basal apiculus (Southworth, 1974).

Special training in fungal spore identification was taken by the author at the Basic Course on Aerobiology in Évora in Portugal (2009), basic and advanced training in Worcester, UK (2010) and training was given in Kraków, Poland (2011).

Fungal spores were identified visually using the light microscope (Nikon Eclipse E400) by the characteristic morphological features under x400 magnification by the author. However, sometimes to confirm the classification, spores were additionally observed under x1000 magnification. This method

allowed identification of fungal spores up to genus level. Although in very few cases it would be possible to identify spores also to the species level, for example spores of *Aspergillus niger* or *Epicoccum nigrum*. Several types of fungal spores were counted jointly because visual distinction was not possible between genera, *i.e.*: *Aspergillus* and *Penicillium*; or they were classified as an artificial "type", *i.e.*: *Drechslera* type and *Leptosphaeria* type or a "group", *i.e.* coloured basidiospores, rusts and smuts. This is a commonly accepted practice by many aerobiologists (Ogden et al., 1974; Southworth, 1974; Haines et al., 2003).

Correct identification of fungal spores requires a lot of experience and practice. Occasionally, when classifications became problematic several fungal spore atlases were used to determine affinity to a certain genus or group (Gregory, 1961; Barnett, 1967; Dennis, 1981; Ellis, 1971; Wilken-Jensen and Gravesen, 1984; Grant Smith, 1990; Ellis, 2001; Lacey and West, 2006; Ogden et al., 1974; Southworth, 1974). Material prepared for fungal spore workshops by Haines et al. (2003) and Gomes Câmara Camacho (2009) also proved to be very helpful.

2.1.6. Fungal spore counting methods

In aerobiological studies, the most commonly used techniques for microscopic counting are: the random field method (Käpylä and Penttinen, 1981), the tangential field method (Mandrioli, 1990), the longitudinal transects method (Domínguez-Vilches et al., 1992; Galán Soldevilla et al., 2007) and the transverse transects method (Emberlin et al., 1994; Lacey, 1995; Lacey and West, 2006). The precision of examination in relation to whole sampling area, as well as the differences between each counting method have been tested and discussed by several authors (Käpylä and Penttinen, 1981; Comtois et al., 1999; Sterling et al., 1999; Pessi, 2003; Cariñanos et al., 2000; Stępalska and Wołek, 2009a; Pessi and Kurkilahti, 2012; Cotos-Yáñez et al., 2013). The longitudinal transects method was additionally analyzed by Tormo Molina et al. (1996) and Irdi et al. (2002).

In this study fungal spore counts along one central longitudinal transect were taken, with an hourly division using a graticule with lines printed every 2 mm. Diurnal fluctuations could be omitted, if 12 transverse transects were counted, as sudden and short bursts of fungal spores were seen after summer

thunderstorms (Lacey and West, 2006). This method resulted in counting 3.86% of the examined area. The analysed surface increased by 3.05% compared to the previous method used by colleagues at NPARU in 2005, where fungal spores were enumerated within a graticule (0.50 x 0.50 mm) from every third field of view. Frenguelli (2003) suggested that the examined area should not be smaller than 10%-12%, in studies using pollen. No previous studies have reported a representative percentage of the examined area of tapes from Burkard traps for fungal spores.

2.1.6.1. Master and slave edges

Following the procedure described in "Airborne pollen and spores. A guide to trapping and counting" (Lacey, 1995), "master" and "slave" edges of the field of view have been chosen as shown in Fig. 2.3. This counting rule prevented an overestimation of number of counted fungal spores. All the fungal spores which were present or crossed fully or partly the "master" edge were taken into account. All the fungal spores which were present or crossed fully or partly the "slave" edge were omitted (Fig. 2.3).

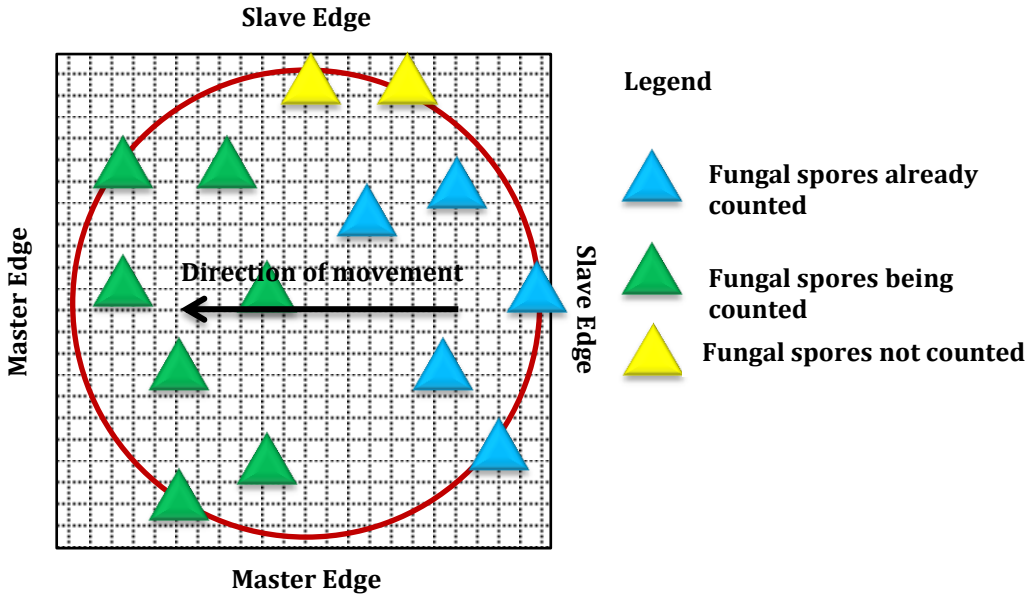


Fig. 2.3 Master and slave edges.

The importance of "master" and "slave" edge selection was described by Pedersen and Moseholm (1993), who calculated precision of reported pollen counts in Denmark. The difference between three experienced pollen counters varied by up to 25%, depending on whether they included partly visible pollen grains in the field of view of the microscope.

2.1.6.2. Correction Factor

To estimate concentration of biological particles collected on the adhesive tape within a 24 hour period or within 1 hour, it was necessary to calculate the correction factor. This was used to multiply the number of counted fungal spores to obtain their concentration (*i.e.*: number of spores per cubic meter of air).

The original width of the "Melinex" tape was 19 mm, and 48 mm length for 24 hour period (as the drum moved at 2 mm per hour). This would be a potential sampling area of the slide equal to 912 mm^2 (Fig. 2.4). However the orifice of the Hirst type sampler was narrower and it was 14 mm wide and 2 mm in length. Hence, the actual sampling area was equal to 672 mm^2 (Fig. 2.5).

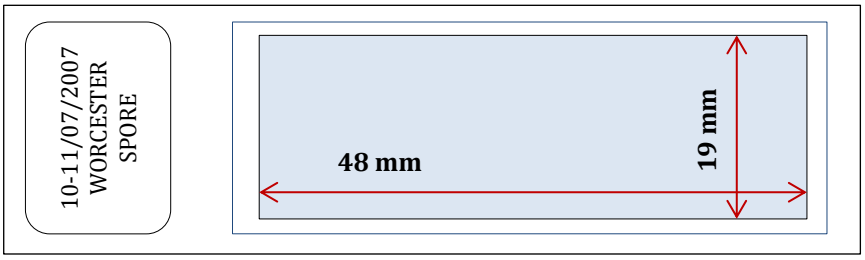


Fig. 2.4 Theoretical sampling area.

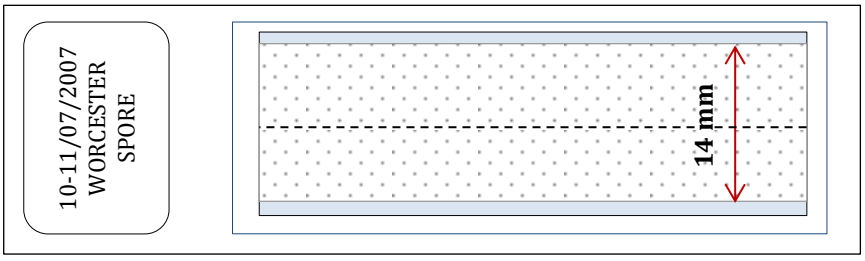


Fig. 2.5 Actual sampling area.

For the purpose of this study fungal spores were counted along one longitudinal transect, located in the central part of the sampling area (Fig. 2.5).

The second step was to measure the diameter of the field of view under the chosen magnification using a stage micrometer. The detailed description of this procedure can be found in "Airborne pollen and spores. A guide to trapping and counting" (Lacey, 1995). In this study, fungal spores were counted under x400 magnification, and the field of view of the microscope used (Nikon Eclipse E 400) was equal to 0.54 mm. This parameter was also included in the following equations (1-2).

The final step was to calculate the amount of air which was sampled and impacted on the collection tape. The Hirst trap sampled 10 l of air per minute. Therefore within a 24 hour period the total amount of sampled air was equal to 14.4 m^3 (24 hours x 60 min x 10 l). This parameter was then included in the following equations (1-2).

$$\text{Correction Factor} = \frac{14\ mm * 48\ mm}{(1 * 0.54\ mm * 48\ mm * 14.4\ m^3)} = 1.80\ \frac{1}{m^3} \quad (1)$$

To calculate a correction factor for an hourly spore collection, it was necessary to change the length of the examined area (2 mm of the tape was used).

$$\text{Correction Factor} = \frac{14\ mm * 48\ mm}{(1 * 0.54\ mm * 2\ mm * 14.4\ m^3)} = 43.22\ \frac{1}{m^3} \quad (2)$$

2.1.7. Data storage

The fungal spore counts were recorded on a worksheet, which was originally designed by Dr I. Bustos-Delgado and B. Adams-Groom, and then modified by the author (Appendix V – Fungal spore count sheet). All fungal spore count sheets were set in chronological order, and classified by the year of sampling. Folders have been stored in 075C EE room, at the NPARU, University of

Worcester. Furthermore, all the fungal spore data have been digitalised and stored safely at the NPARU's server (X-Drive) to enable access.

2.1.8. Weather data

The influences of maximum temperature, minimum temperature, mean temperature, dew point temperature, relative humidity, rainfall, air pressure and wind direction. The direction of the wind, which had been recorded in 16 cardinal directions, was re-calculated in a degree format using following formula: N=360, NNE=22.5, NE=45, ENE=67.5, E=90, ESE=112.5, SE=135, SSE=157.5, S=180, SSW=202.5, SW=225, WSW=247.5, W=270, WNW=292.5, NW=315, NNW=337.5. This change enabled further transformation of this parameter.

Weather data were obtained from the weather stations co-located with the air sampler at the University of Worcester, Edward Elgar building (52° 11' 48" N, 2° 14' 31" W). All meteorological parameters were recorded from 00:00h to 23:59h according to UTC local time.

2.2. Statistical analyses

2.2.1. Main spore seasons

Spore observation periods were calculated using the 90% method introduced by Nilsson and Persson (1981). The season started when 5% of accumulative sum of daily mean spore concentration was exceeded, and finished when 95% was surpassed. Calculating the season was applied to not extend artificially the length of fungal spore seasons, in case they were not frequently observed within the year (Jäger, 2003). Additionally this technique enabled the removal from the data days, when old spores could be present in the air due to re-suspension. The 90% method is the most commonly used technique with regard to the fungal spore analysis (Grinn-Gofroń and Strzelczak, 2008). Furthermore, fungal spore season for each investigated fungal spore type was subsequently understood here as a single "sample", using statistical terminology.

Seasonal Fungal Index (SFI) constituted a sum of the daily mean concentrations recorded within entire main spore season, calculated individually for each sampling year.

2.2.2. Distribution of the data

In this study, three statistical tests were used to examine normality of the data distribution, *i.e.*: Kolmogorov-Smirnov test, Kolmogorov-Smirnov test with Lilliefors correction and Shapiro-Wilk test. The following hypotheses were tested as H_0 there is no statistically significant difference between the distribution of the examined sample and a normal one, and H_A there is a statistically significant difference between the distribution of the examined sample and a normal one

All examined data referred to the *p* value ($p<0.05$). If value of *p* was greater or equal to 0.05, then the data was found to be normally distributed (the null hypothesis retained). As the normality of the fungal spore seasons was examined using three tests, the distribution of spores was understood as normal, when all three tests resulted in $p\geq0.05$.

An attempt of data normalisation was performed applying the following formula log(x+1). Analyses described above were conducted using Statistica (v. 12) software.

2.2.3. Relationships between spore occurrence and weather

2.2.3.1. Linear or non-linear

Detection of the linearity or lack thereof was conducted by visual inspection of the scatter graphs presented in Chapter 3 of this study, where investigated variables were plotted. If the relationship between fungal spore concentration and weather factors was linear, the data points would be symmetrically distributed around a diagonal line.

2.2.3.2. Correlation analysis

In this study, fungal spore counts collected in Worcester were shown to be non-normally distributed, and even application of the logarithmic transformation did not bring an expected normalisation of the data (Chapter 3). Hence, Spearman's rank correlation test was used to investigate the association between fungal spore concentration and selected meteorological parameters. Following hypotheses were examined, where: H_0 there is no statistically significant

correlation between X and Y, and H_A there is a statistically significant correlation between X and Y.

2.2.3.3. Redundancy analysis

Seasonal distributions of fungal spore concentrations varied between investigated fungal genera. To conduct an ordination analysis (ter Braak, 1995) for five months of observations of each studied year were selected (from May to September). However, prior to the ordination examination, fungal spore counts and eight meteorological parameters were normalized using log(x+1) transformation to minimalize the effect of outliers and to enable comparison of all variables, as each of them was measured using different units.

The relationship and co-linearity (redundancy) of independent variables was examined with the aid of a variance inflation factor (VIF) in canonical correspondence analysis (CCA), (Groß, 2003). Variance inflation factor helped identify those of the meteorological parameters were auto-correlated and which needed to be excluded from further investigation. It was assumed that significant co-linearity occurs, where VIF indicates value above 10 (Groß, 2003).

Subsequently, detrended correspondence analysis (DCA) was performed to explore the length of DCA first axis, which denominates the character of relationship between the presence of fungal spores in the air and optimal weather conditions following Obolewski and Strzelczak (2009). This association may be either monotonic or unimodal and hence it requires either redundancy or canonical correspondence analysis in the concluding examination. According to ter Braak (1995) in most of the ecological studies species responses to the environmental conditions are monotonic in their nature, and they are determined by values below 2.

In the end, an analysis of variance (ANOVA) test helped to denote statistically significant axes. CCA, DCA and RDA analyses were conducted using The Comprehensive R Archive Network (CRAN, R i.386 3.0.1., 2013), vegan (Oksanen et al., 2013) and permute (Simpson, 2013) packages and following functions: cca (Legendre and Legendre, 1998), decorana (Hill and Gauch, 1980; Oksanen and

Minchin, 1997), rda (ter Braak, 1986; Legendre and Legendre, 1998) and anova.cca (Legendre et al., 2011).

Results of RDA analysis were presented graphically on the biplots, where meteorological parameters were shown as arrows + their names, whereas fungal spore types were presented by their Latin names only. The significance of the correlation between weather factors and distribution of fungal spores depended on the length of the arrows; the longer arrow the stronger the correlation (ter Braak and Prentice, 1988). Vector of relationships (directly proportional or inversely proportional) were interpreted based on position of the spore types in terms of arrows ends. If spore types were closely located at the end of arrows the association was positively correlated. However, if the opposite occurred then correlation was negative (ter Braak and Prentice, 1988).

2.2.3.4. Multivariate Regression Tree (MRTs) analysis

MRTs were constructed using The Comprehensive R Archive Network (CRAN, R i.386 3.0.1., 2013), and Multivariate Partitioning (mvpart) package. Following De'ath and Fabricius (2000) and Grinn-Gofroń and Strzelczak (2009), to stabilise the Cross Validated Error (CV Error) 2000 multiple cross validations were applied. The final MRT models were selected based on the lowest obtained CV Error and *cp* value (level of the model complexity).

2.2.3.5. Circular statistics

Circular statistics was applied in order to examine relationship between fungal spore occurrence and both the local wind and air mass directions (Aradóttir et al., 1997; Borycka and Kasprzyk, 2014; Kasprzyk, 2006b; 2008). The analysis focused on monthly and annual variations in *Cladosporium* distribution. Obtained results were presented in Chapter 5. This included the following parameters and tests: mean direction, circular standard deviation, mean resultant length, skewness, kappa estimate, probability test of randomness, probability Rayleigh test of uniformity, Chi-square von Mises and probability Chi-square von Mises. The relationship between hourly mean *Cladosporium* spore concentration and wind direction (local wind and air mass), as well as association between air mass and

local wind direction, was examined using either both Spearman's rank and linear-circular association tests or a single test. The latter correlation test was performed using the cassociation module (Fisher, 1993) written by Suzanne J. Clark for GenStat. These statistical analyses were performed using Microsoft Excel (2010), GenStat (v. 17) and tools available in ArcGIS (v. 10) software.

2.2.4. Detection of the changes in the annual data

All investigated fungal spore seasons revealed non-normal distribution, therefore to examine the difference between annual sums of daily mean spore concentration the Kruskal-Wallis test was used (Chapter 3) following del Mar Trigo et al. (2000).

In this study five years of observations were examined, where a single year constituted an independent sample. The dependent variable was measured on the ordinal scale and it had a continuous character. Therefore, all assumptions of the Kruskal-Wallis test were fulfilled. The following hypotheses were tested, where H_0 there is no statistically significant difference between mean ranks of the examined years ($p \geq 0.05$), and samples come from the same population, and H_A there is a statistically significant difference between at least two mean ranks of the examined samples ($p < 0.05$), and samples do not come from the same population.

2.2.5. Detection of the changes in the diurnal data

2.2.5.1. Kruskal-Wallis test

The annual differences in diurnal distribution of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores were examined using the Kruskal-Wallis test (Chapter 4). The analysis was performed in the same way, as that described in Section 2.2.4 of this Chapter. The following hypotheses were tested, where H_0 there is no statistically significant difference in diurnal distribution of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores between investigated years of observation, and H_A there is a statistically significant difference in diurnal distribution of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores between the years of observation.

2.2.5.2. Sign test

In this study, the sign test was used twice. Firstly, to investigate whether there was any statistically significant difference in diurnal distribution of fungal spores during the days with no rainfall, and days when rainfall occurred (Stępalska and Wołek, 2009b). Secondly, to investigate impacts of different thresholds, which were applied for measurement of the fungal spore concentration.

2.2.5.3. Friedman test

This test was used to investigate whether there was or was no statistically significant difference in diurnal distribution of fungal spores during the days with various amounts of rainfall, relative humidity and maximum temperature that were recorded over a 24 hour period (Chapter 4).

2.2.6. Artificial Neural Networks (ANNs)

Artificial Neural Networks are computational type of models, which have an ability of learning, based on received examples, and predicting further time series with a fairly good accuracy (Galán, 2003). This kind of modelling is applied for an analysis of complex, non-linear relationships between variables (Scheifinger et al., 2013). ANNs have been already successfully employed in various disciplines of science (e.g. Burke, 1997; Shahin et al., 2001; Lek and Guegan, 2000; Khare and Nagendra, 2007), including aerobiology (e.g. Li and Flenley, 1999), where it is used mostly for forecasting concentration of allergenic and/or pathogenic biological agents, such as fungal spores and pollen grains (e.g. Sánchez-Mesa et al., 2002; Grinn-Gofroń and Strzelczak, 2008; Astray et al., 2010; Kasprzyk et al., 2011; Puc, 2012; Tomassetti et al., 2013).

The architecture of ANNs resembles a structure of human brain, and similarly consists of neurons; artificial neurons can be classified into three groups: input, hidden and output (Fig. 2.6). Input neurons represent all investigated variables, both dependent (e.g. spore or pollen concentration) and independent (e.g. rainfall, number of sunshine hours, presence or absence of thunderstorm). The output neuron is the predicted value of dependent variable, and in this case, it means a fungal spore concentration. The degree of model complexity (number of

hidden neurons) is equal to flexibility in learning, which is desired in modelling complicated relationships between variables (Sánchez-Mesa et al., 2005). Artificial neuron constitutes a linear function, although its activation function is non-linear in nature (Angelosante Bruno et al., 2007). Connections between artificial neurons, by analogue to biological neurons, represent dendrites and axons, and they are connected according to rule "everyone with everyone". "Synapse" in this context means a sum of signals, received by neuron from other neighbour neurons, which are subject to weighting. Finally, activation function is equal to the difference between weighted sum of entered signals and the threshold, what is an analogue of soma activity (Sánchez-Mesa et al., 2005).

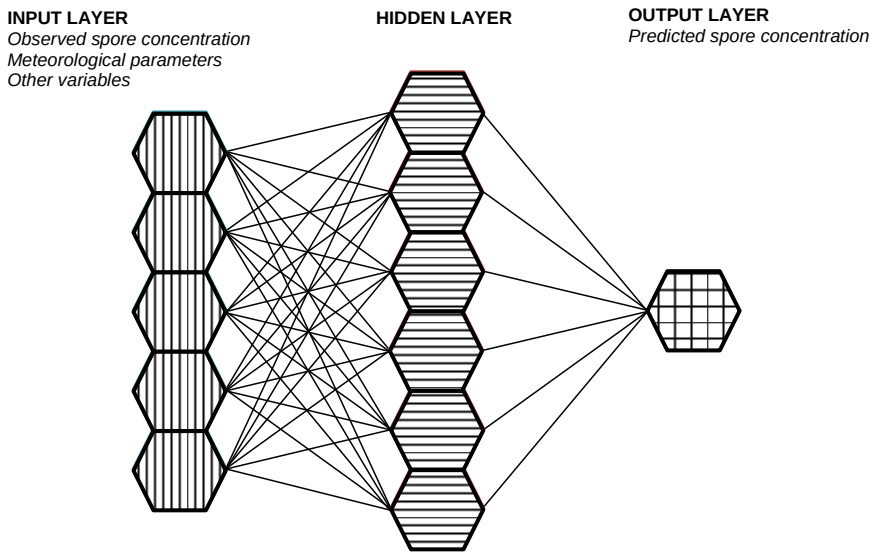


Fig. 2.6 Architecture of Artificial Neural Network used for forecasting spore concentration.

ANNs can be classified in several different ways depending on the dividing criterion, *inter alia*:

a) topology :
   - ❖ feed forwarded, e.g. Multi-Layer Perceptron (MLP)
   - ❖ feedback / bidirectional, e.g. Hopfield neural network

b) linearity:
   - ❖ linear models:
   - Adaptive Linear Neuron (ADALINE)

- Multiply Adaptive Linear Neuron (MADALINE)

❖ non-linear models:

- Multi Layer Perceptron (MLP) trained with back propagation algorithm
- Multi Layer Perceptron (MLP) trained with back propagation algorithm and conjugate gradient descent
- Radial Basis Function (RBF)

c) purpose of application:

❖ regression neural networks

❖ classification neural networks.

Construction of high quality neural network requires adequate selection of settings, including topology of model and its size, learning algorithm, and the size of learning, testing and validating subsets (Scheifinger et al., 2013). Choosing right criteria may be problematic for inexperienced researcher, therefore it is generally recommended to use an Automatic Problem Solver. In the newest version of Statistica software (Statsoft, v. 12) an Automatic Problem Solver designs and trains neural networks with back propagation only (Haykin, 1994; Fausett, 1994; Patterson, 1996), on the contrary to the previous versions, where models were additionally trained with conjugate gradient algorithms (Bishop, 1995). Small changes also touched the percentage of observations used for learning, validating and testing subsets, *i.e.*: 67% for 70%, 37% for 15% and 0% for 15% respectively. However, these settings can be easily changed, depending on the researcher's preferences. The number of hidden neurons is automatically adjusted to the number of input variables. Moreover, some authors (e.g. Grinn-Gofroń and Strzelczak, 2008; Kasprzyk et al., 2011) employ bootstrapping technique, which enables each case (observation) to be included simultaneously in all three data subsets; this way more representative learning, validating and testing collections can be created and usually higher quality neural models are being obtained.

2.2.6.1. Model parameterisations

The five year of study of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* airborne spores recorded in Worcester, UK were tested against weather parameters obtained simultaneously. A difference of time was used to examine the relationship between spore concentration and weather conditions. Spore counts were juxtaposed with meteorological parameters that were delayed for 1, 2 and 3 days.

These relationships were also analysed by application of the Spearman rank's test. The highest correlations were then selected and based on the obtained results mixed models were constructed. Therefore some variables could be day to day equal to the spore counts, while others could be delayed in time. If it happened, that no statistically significant correlation was found for a particular meteorological parameter, then this variable was excluded from further analysis.

In addition, the auto-correlation between independent variables was investigated. Models were constructed using either five or eight meteorological factors. The latter group consisted of the weather parameters, which did not show any parallel dependence. Selection was done based on the previously obtained results of the redundancy analysis. Out of four temperature parameters, the maximum temperature was indicated by the canonical correspondence analysis during three out of the five examined years of study (Chapter 3). Hence, the other three temperature parameters were discarded.

This analysis was used to investigate whether the spore counts were dependent on the local weather conditions. Overall, using all possible combinations, 11 forecasting models were produced for each examined fungal spore type. Their performance was then evaluated using several criteria, *i.e.* mean prediction power (mean of training, testing and validation values), highest *p* value (correlation between observed and predicted spore concentration), and correct prediction with regard to the established threshold values expressed in percentage, *i.e.*: 100 s m^{-3} (*Alternaria*), 3000 s m^{-3} (*Cladosporium*), 6000 s m^{-3} (*Didymella*) and 200 s m^{-3} (*Ganoderma*).

These models were produced with the aid of Statistica Statsoft (2012). Artificial Neural Networks were designed and trained using back propagation

(Haykin, 1994; Fausett, 1994) by selecting the Automatic Problem Solver. Bootstrap technique application allowed all observations in dataset equal chances to be included in three subsets, *i.e.* learning (70%), testing (15%) and validating (15%). Each time Multi Layer Perceptron regression networks were run 1000 times and only 1 of the best performing model was saved. Minimum number of hidden neurons was 4, while the maximum number of hidden neurons was limited to 25. All possible combinations with respect to the activation functions of the hidden neurons and output neurons were selected, *i.e.*: identity, logistic, tanh, exponential and sine. Results of the ANN analysis can be found in Chapter 6 of this study and Appendix VI.

2.3. Spatial analyses

2.3.1. Production of potential source maps

Two possible source maps were produced based on the Corine Land Cover 2000 data (European Commission, 2005), which presented the distribution of crops under rotation and pastures in the United Kingdom. The first map was constructed by a joint extraction of the non-irrigated arable land (code 211) and permanently irrigated arable land (code 212) following the procedure described by Skjøth et al. (2012). The second map contained only the areas occupied by the pastures (code 231).

Although the extraction was conducted for the entire European continent, only a part of the Western Europe that included the British Isles was used. Both source maps have been gridded to a tenth of the EMEP50 grid (http://www.emep.int/grid/griddescr.html), where each grid cell represented results expressed as percentage with a resolution of 5 x 5 km.

Maps presenting the topography of the United Kingdom, woodland cover and transport of the air masses for selected episodes were produced to analyse the spatial distribution of *Ganoderma* spores, were constructed by Dr Carsten A. Skjøth as a co-author of the article (Sadyś et al., 2014).

ArcGIS Arc Map software (v. 10) and Microsoft Office package (2010) were used to perform the spatial analysis the four spore types collected in Worcester, UK. Results are given in Chapter 5 of this study.

2.3.2. Back trajectory analysis

The back trajectories analysis was performed using the HYSPLIT (Hybrid Single Particle Lagrangian Integrated Trajectory) model available online at http://www.arl.noaa.gov/index.php (Draxler and Rolph, 2013; Rolph, 2013). This model has been produced and developed by the National Oceanic and Atmospheric Administration (NOAA) Air Resources Laboratory (ARL) in the United States. Global Data Analysis System (GDAS) meteorological data maintained by NOAA ARL were selected, while computing archive back trajectories.

Back trajectories of 48 hours with a time step of 1 hour at 500 m above ground level were calculated for selected days that were examined in detail. This procedure was previously described by Stach et al. (2007), and since then commonly applied in the aerobiological studies (Smith et al., 2008; Hernández-Ceballos et al., 2011; 2014a; 2014b; Fernández-Rodríguez et al., 2014).

The uncertainties related to the back trajectory analysis were discussed in Hernández-Ceballos et al. (2014a; 2014b), Skjøth et al. (2009), Makra et al. (2010), Šikoparija et al. (2011), Skjøth et al. (2012), Sadyś et al. (2014). No further uncertainties regarding the back trajectory analysis have been discussed herein, as this is beyond the scope of the thesis.

All results were extracted and downloaded from the website in a format that enabled their further analysis in ArcGIS Arc Map (v. 10) software. The height above ground level and the speed of the air masses was studied using several tools available in the programme. The percentage of the time, which air masses spent over particular areas, *i.e.*: a sea area, land areas, British Isles, UK and non-UK areas was also investigated. In addition, an analysis of the most dominant direction of the air masses was performed for *Cladosporium* and *Ganoderma* spores using a spatial resolution equal to 1 x 1 degree measured from centrally located Worcester. The results of the back trajectories analyses were presented and described in Chapter 5 of this study.

2.3.3. The weather synopsis

The description of the overall synoptic weather situation, which occurred during the examined days, was based upon the analysis of weather maps produced

by the UK Met Office. Due to exchange under the World Meteorological Organisation (WMO) Weather Watch Programme these maps were available online at http://www.wetterzentrale.de/topkarten/fsfaxsem.html. The synoptic weather situations were presented each time at 00:00h UTC.

Chapter 3

Annual variations in fungal spore distribution

3.1. Introduction

Twenty one fungal genera and groups in total were enumerated from the microscope slides collected from 2006 to 2010 in Worcester, UK. A detailed description of the sampling methods and statistical analysis was presented in Chapter 2. In this chapter only results for *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores were presented.

3.2. Results

3.2.1. Main spore seasons

Alternaria spores were present from mid-May – mid-June until the end of September – beginning of October in the air in Worcester (Table 3.1). The duration of the seasons varied from 107 (2009) to 141 days (2007). The shorter the season, the higher the maximum concentration observed. In contrast, severity of the *Alternaria* season measured with the aid of Seasonal Fungal Index (SFI) indicated that year 2006 as the most severe from a clinical point of view. The values of the maximum concentration of *Alternaria* spores exceeded a clinical threshold equal to 50 s m^{-3} for the UK. The examined years varied between 275 s m^{-3} (2007) and 644 s m^{-3} (2009). Peaks occurred mainly in the month of August, in the years 2007, 2008 and 2009.

Cladosporium characterised with quite short spore seasons in contrast to the other previously described fungi, as their duration varied from 111 to 186 days (Table 3.1). The daily peak values were approximately between 20000 and 47000, and they mainly occurred in July. In years 2006 and 2010, there were marked

differences in comparison to the remaining three years of observations. Both SFI values and daily maximum spore concentration were different for these two years.

Table 3.1 The characteristics of investigated fungal spore types in the air in Worcester, UK (2006-2010).

Taxon	Year	Period of occurrence	Duration	Daily peak value [s m^{-3}]	Date of daily peak	SFI[a]
Alternaria	2006	27.05-20.09	117	607	25.07	9297
	2007	19.05-06.10	141	275	05.08	6966
	2008	21.05-26.09	129	605	22.08	8092
	2009	24.06-08.10	107	644	08.08	8519
	2010	03.06-22.09	112	412	02.09	6022
Cladosporium	2006	14.06-02.10	111	36783	25.07	863607
	2007	27.04-29.10	186	19813	16.07	489363
	2008	20.05-12.10	146	22316	06.08	437171
	2009	23.05-28.10	159	23040	29.06	612198
	2010	26.05-29.09	127	46831	14.07	902136
Didymella	2006	27.06-24.09	90	2981	09.08	26976
	2007	17.06-22.08	67	9961	21.07	115817
	2008	08.07-09.09	64	19966	07.08	190186
	2009	11.07-20.09	72	11623	07.08	77793
	2010	13.07-17.09	67	2862	22.08	24185
Ganoderma	2006	02.06-29.10	150	376	11.09	19103
	2007	23.04-29.10	190	225	14.10	14947
	2008	10.05-17.10	161	281	18.09	15793
	2009	22.05-29.10	161	310	14.08	20612
	2010	25.05-14.10	143	254	22.09	16946

[a] SFI – Seasonal Fungal Index

Spore occurrence of *Didymella* ascospores covered the shortest periods of all twenty fungal taxa examined varying between 64 and 90 days (Table 3.1). Seasons started either at the end of June or at the beginning of July, and finished mainly in September each year. Although duration of the seasons was short, they were observed to be intensive as shown by SFI scores from 26000 to 190186. Years 2007 and 2008 had the greatest concentration of *Didymella* spores of the five year monitoring period in Worcester. The maximum spore concentration that was registered within 24 hours reached approximately 20000 s m^{-3}.

Observations of *Ganoderma* basidiospores were constant lasting five months, beginning in May and ending in October in each year (Table 3.1). Significantly greater quantities of *Ganoderma* spores were observed in 2006 and 2009. A bi-annual pattern of spore distribution characteristic for other coloured

basidiospores could not be detected. Daily maximum spore concentration varied from 225 s m^{-3} to 376 s m^{-3} and occurred mostly in September of each year.

3.2.2. Results of the descriptive statistics

The results of the descriptive statistical analyses included: arithmetic sample mean, 25^{th}, 50^{th} (median) and 75^{th} percentile, mode, sample standard deviation and sample variation as well as skewness and kurtosis values, which were calculated for all studied fungal spore taxa, for each individual year and for a joint five year period of study are shown in Table 3.2. Strong skewness of the data was detected for all investigated fungal spores, which can be read directly from the obtained skewness values ($\gamma_1 \neq 0$), as well as from the mean which was smaller than the double standard deviation.

Table 3.2 The results of the descriptive statistics.

Taxon	Year	$\bar{x}$	Q_1	Q_2	Q_3	Mo	s	s^2	γ_1	γ_2
Alternaria	2006	71.53	13.00	41.00	88.00	4.00	91.38	8350.23	2.64	10.08
	2007	44.66	5.00	20.00	56.00	2.00	60.65	3678.40	2.07	4.00
	2008	56.84	7.00	20.00	56.00	4.00	97.06	9420.25	3.02	10.49
	2009	72.60	14.00	36.00	74.00	multiple	110.87	12292.81	3.25	11.72
	2010	48.52	9.00	19.00	50.50	11.00	76.70	5883.33	2.79	8.32
	2006-10	58.06	9.00	23.00	63.00	multiple	88.33	7802.23	3.10	12.27
Cladosporium	2006	7017.37	2867.00	4910.00	9146.00	952.00	6239.49	38931205.87	2.35	7.10
	2007	2382.66	1010.00	1694.50	2984.00	multiple	2272.39	5163771.34	3.93	23.46
	2008	2698.05	1166.00	1879.50	3447.00	1676.00	2740.20	7508676.47	3.48	18.83
	2009	3484.22	1458.00	2362.00	4325.00	multiple	3547.46	12584449.08	2.85	10.36
	2010	6402.66	2450.00	4721.00	8118.00	no mode	6833.80	46700868.70	3.08	12.77
	2006-10	4102.28	1363.00	2608.00	4810.00	2608.00	4808.85	23124993.57	3.63	19.34
Didymella	2006	271.13	20.00	87.50	302.00	multiple	455.52	207494.43	3.15	13.66
	2007	1560.21	153.00	806.00	2023.00	74.00	2094.99	4388973.14	2.41	6.55
	2008	2694.02	334.00	1817.00	3940.00	multiple	3362.22	11304524.78	2.68	10.38
	2009	995.10	97.00	490.00	1539.00	multiple	1624.85	2640138.20	4.36	25.94
	2010	326.91	54.00	122.00	448.00	multiple	476.65	227191.08	2.95	11.70
	2006-10	1097.24	63.00	302.00	1235.00	11.00	2046.66	4188797.30	4.20	25.62
Ganoderma	2006	114.81	65.00	106.00	148.00	86.00	62.46	3901.83	0.90	1.16
	2007	71.19	38.00	58.00	97.00	49.00	46.23	2137.06	1.13	1.01
	2008	88.83	49.00	75.00	115.00	54.00	58.08	3372.78	1.25	1.42
	2009	115.95	63.00	109.00	158.00	multiple	64.70	4186.27	0.65	-0.05
	2010	106.76	59.00	99.00	140.00	multiple	57.14	3265.35	0.53	-0.47
	2006-10	97.87	50.00	86.00	133.00	54.00	60.10	3611.74	0.92	0.62

$\bar{x}$ (sample arithmetic mean); Q_1 (25^{th} percentile); Q_2 (50^{th} percentile or Median); Q_3 (75^{th} percentile); Mo (Mode); s (sample standard deviation); s^2 (variation); γ_1 (skewness); γ_2 (kurtosis).

3.2.2.1. Distribution of the data

The five year distribution of all investigated fungal genera was examined to detect the characteristics of the collected data. Histograms showed that spore data were positively skewed, and hence the null hypothesis was rejected (Fig. 3.1). The non-normality was confirmed by three independent tests for normality, *i.e.*: Kolmogorov-Smirnov test, Lilliefors correction to the Kolmogorov-Smirnov test and Shapiro-Wilk test (Fig. 3.1). Further analysis using non-parametric statistical techniques was applied.

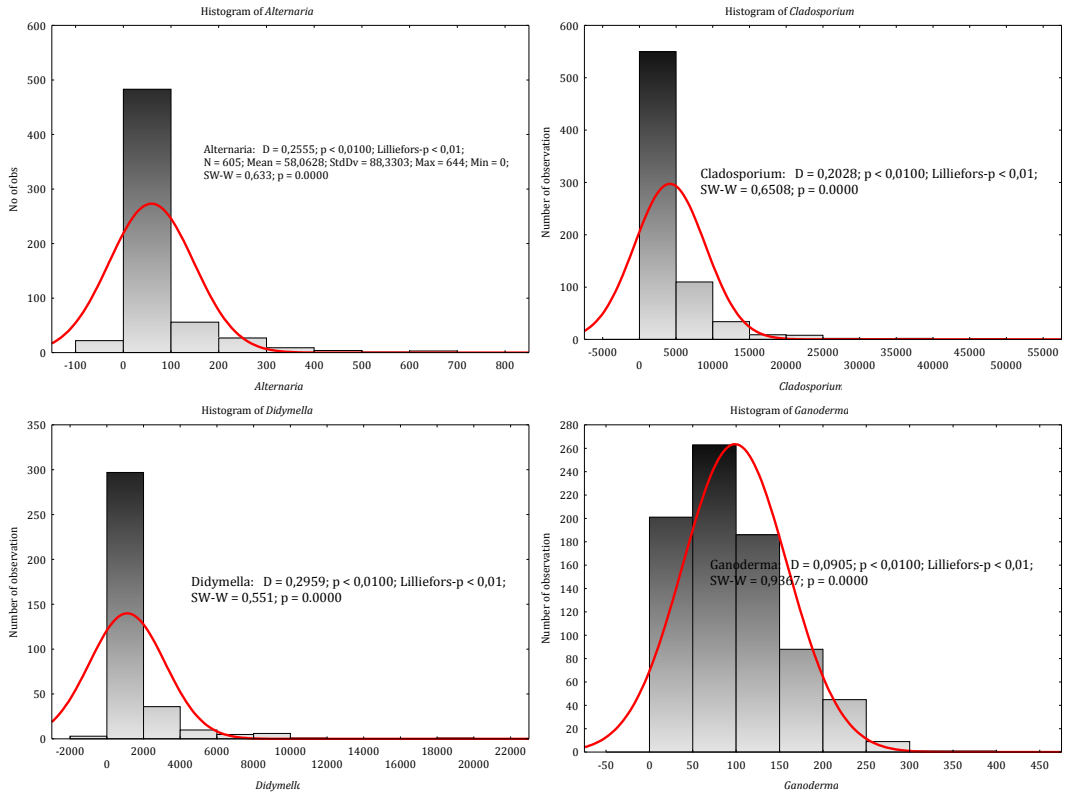


Fig. 3.1 Histograms showing distribution of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores with attached results of three normality tests.

3.2.2.2. Linearity of the relationship between fungal spores and meteorological parameters

Matrix scatter plots indicated non-linear dependences between investigated fungal spore taxa and meteorological parameters (Fig. 3.2 a-d).

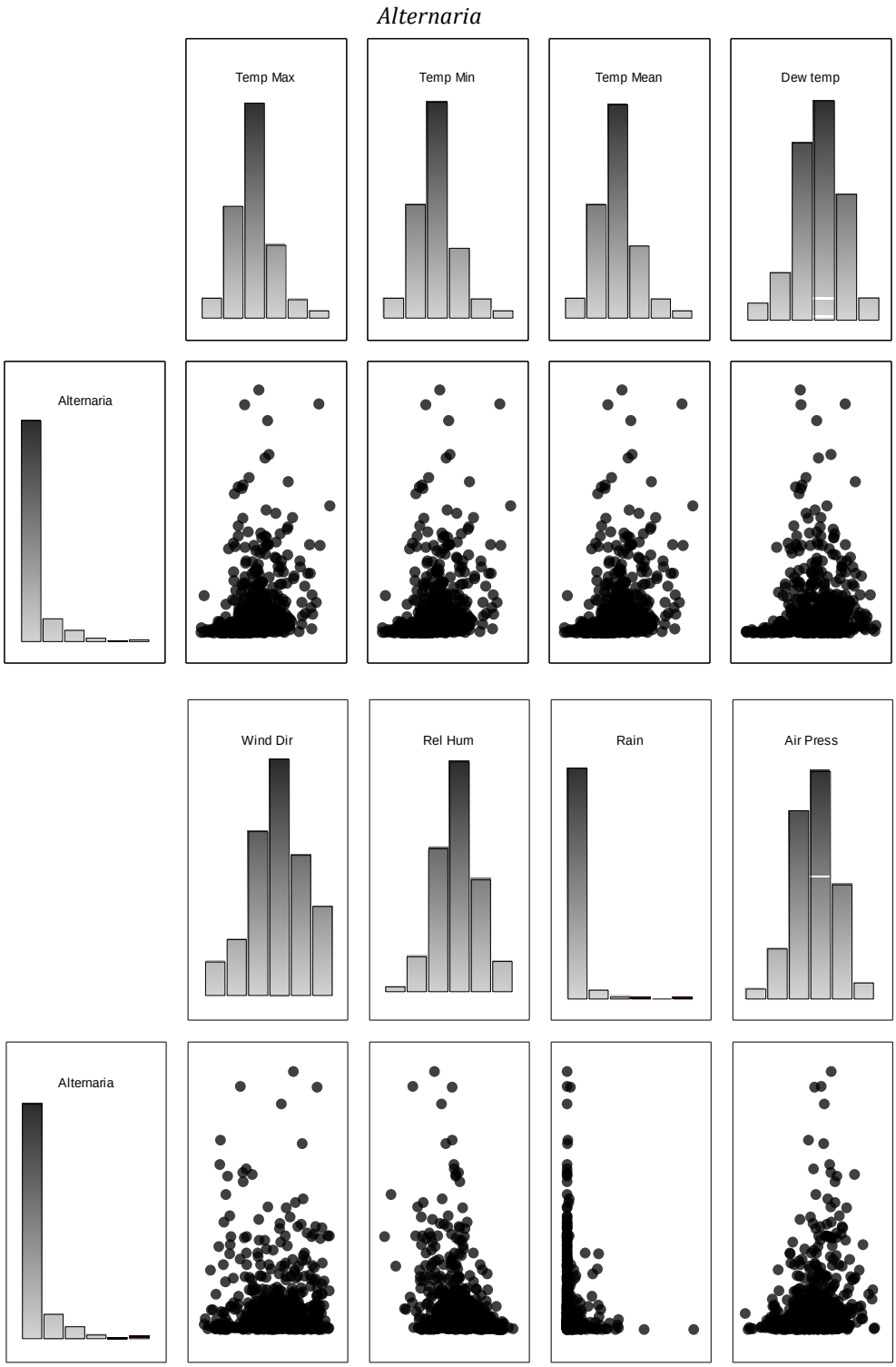


Fig.3.2a Matrix scatter plots between *Alternaria* spore concentration and meteorological parameters.

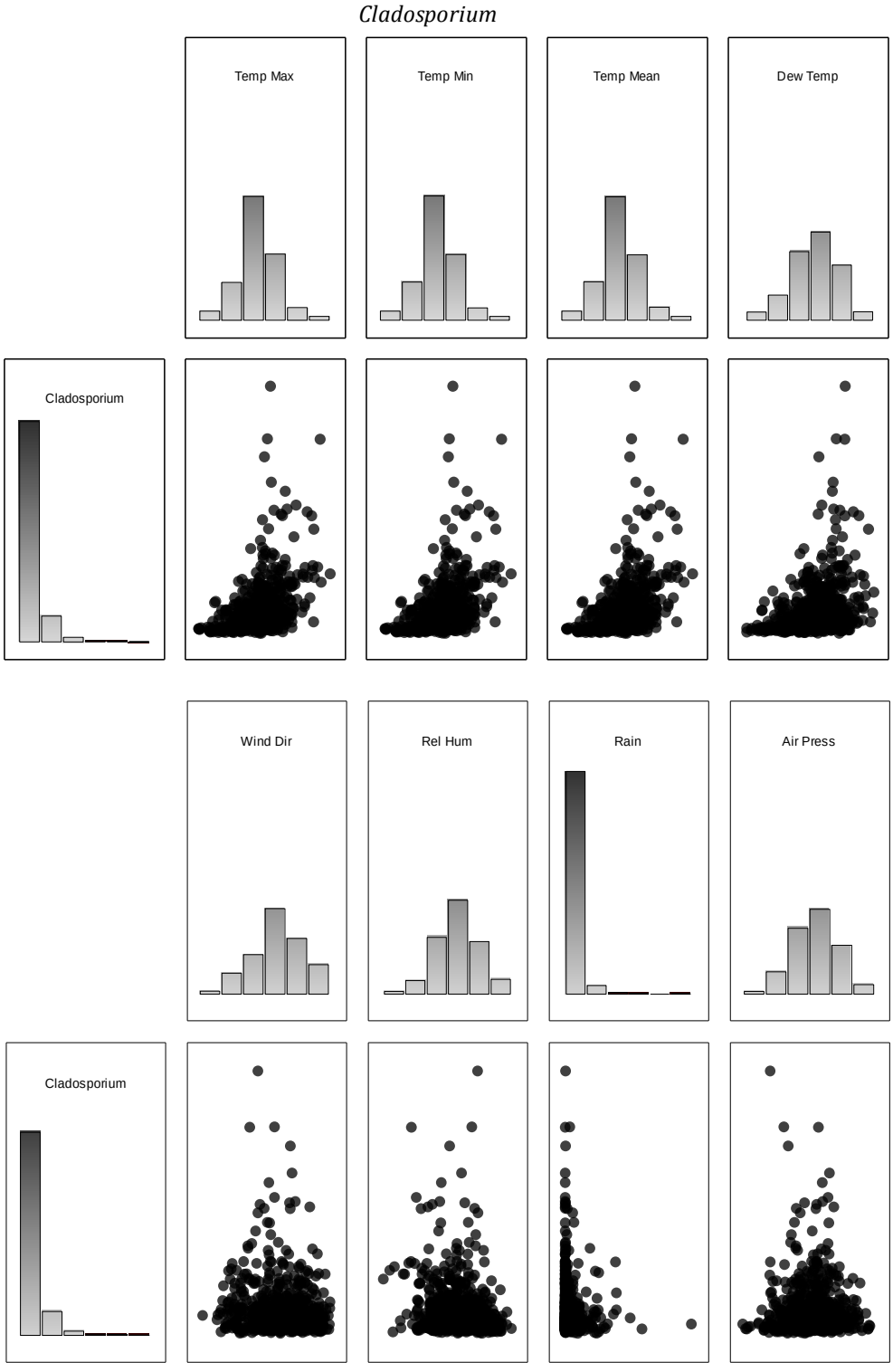


Fig. 3.2b Matrix scatter plots between *Cladosporium* spore concentration and meteorological parameters.

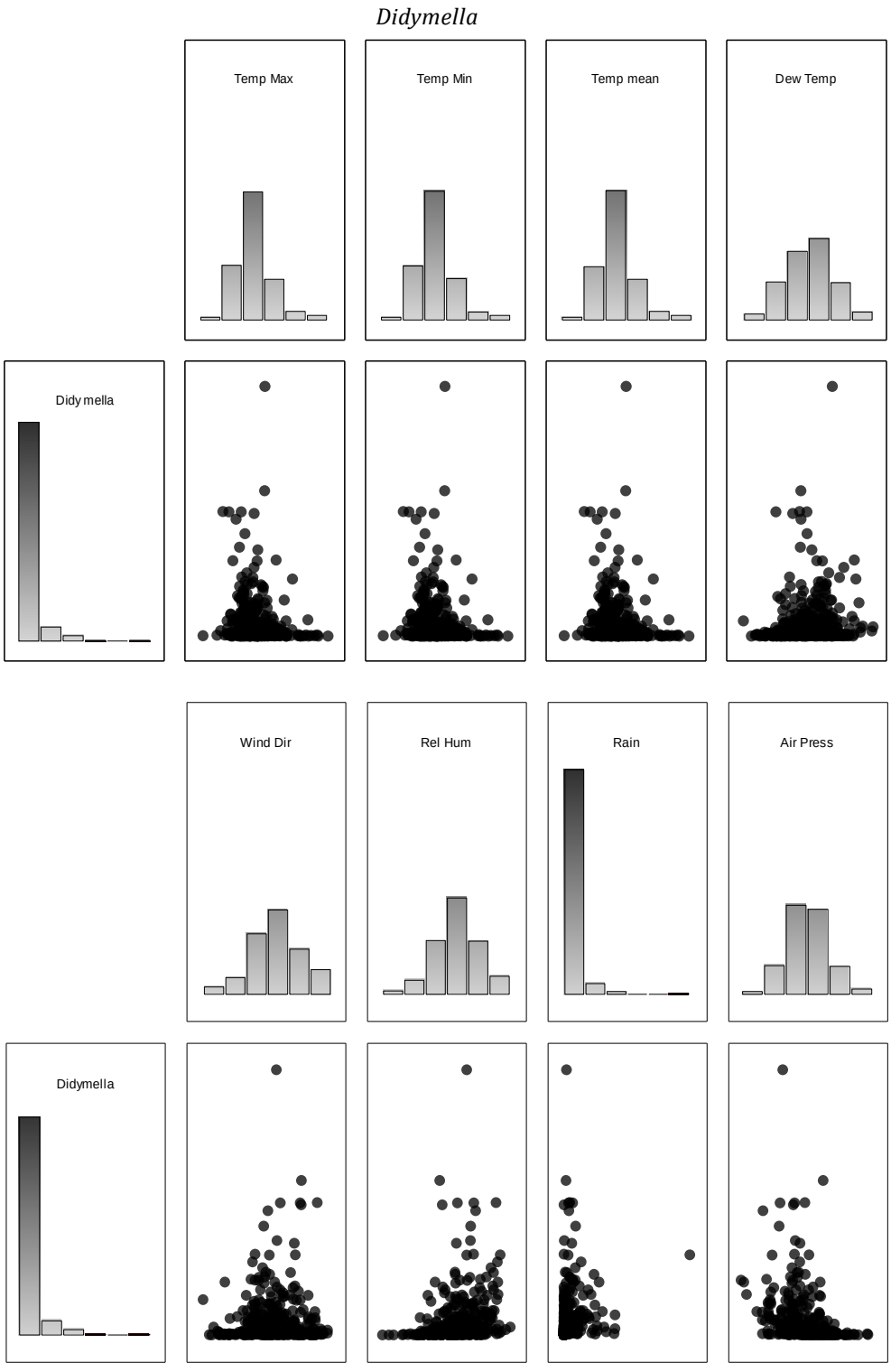


Fig. 3.2c Matrix scatter plots between *Didymella* spore concentration and meteorological parameters.

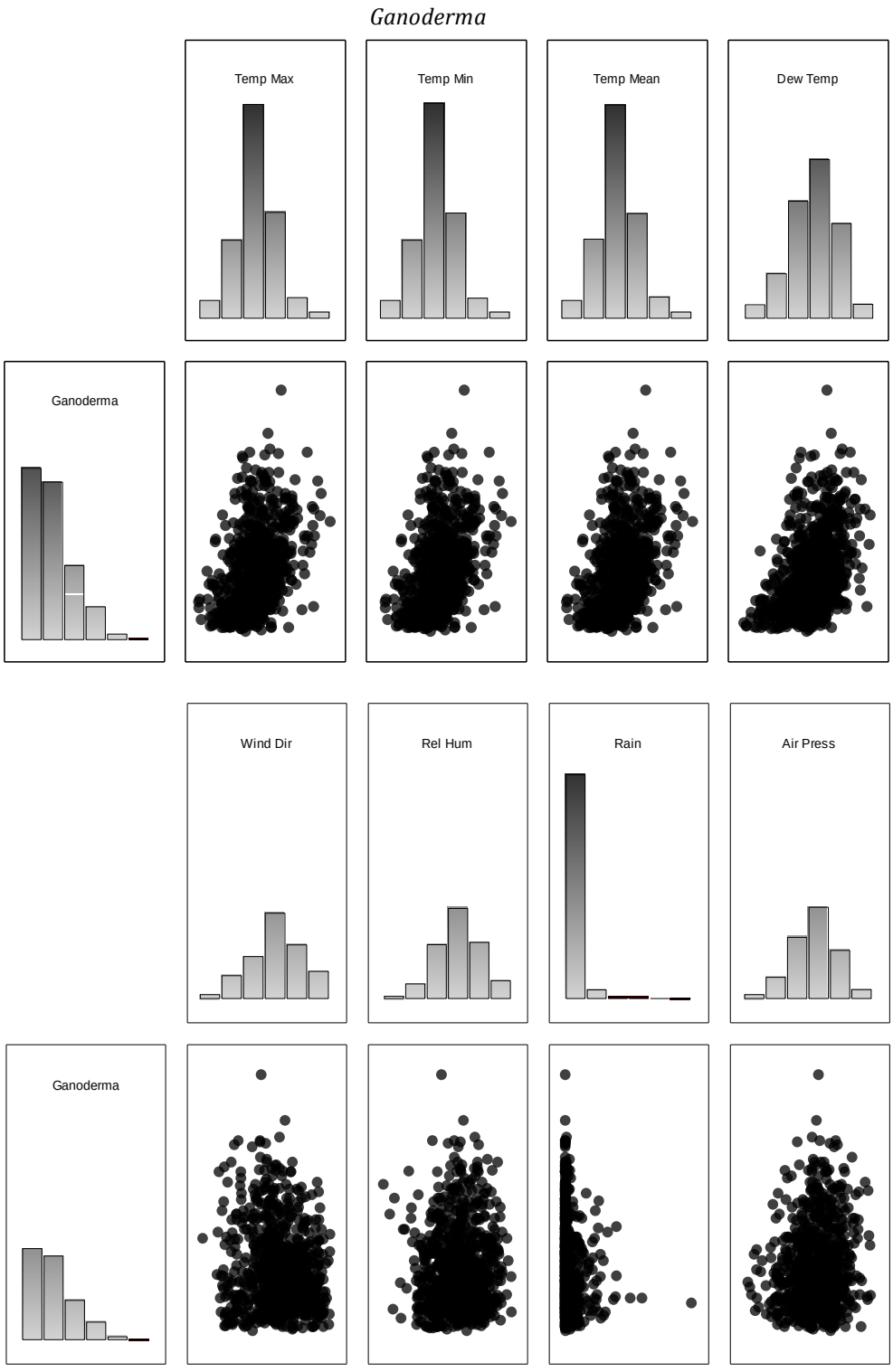


Fig. 3.2d Matrix scatter plots between *Ganoderma* spore concentration and meteorological parameters.

3.2.3. The annual variations in fungal spore distributions

The Kruskal-Wallis test was used to analyse whether there was any difference between individual fungal spore seasons within the investigated period of study (Table 3.3). Selection criteria of this statistical tool were described in detail in Chapter 2. Although various results were found for each of the examined fungal spore genus, *i.e.*: *Alternaria, Cladosporium*, *Didymella* and *Ganoderma*, annual fluctuations in fungal spore concentration were different obtaining a level of statistical significance of $p \leq 0.05$ (Table 3.3).

Table 3.3 The results of the Kruskal-Wallis test (2-tailed) for the comparison of annual variations in fungal spore distribution.

Taxon	**Year**	2006	2007	2008	2009	2010
Alternaria	2006		**0.01***	**0.03***	*ns*	*ns*
	2007	**0.01***		*ns*	**0.01***	*ns*
	2008	**0.03***	*ns*		**0.02***	*ns*
	2009	*ns*	**0.01***	**0.02***		**0.05***
	2010	*ns*	*ns*	*ns*	**0.05***	
Cladosporium	2006		**0.00***	**0.00***	**0.00***	*ns*
	2007	**0.00***		*ns*	**0.01***	**0.00***
	2008	**0.00***	*ns*		*ns*	**0.00***
	2009	**0.00***	**0.01***	*ns*		**0.00***
	2010	*ns*	**0.00***	**0.00***	**0.00***	
Didymella	2006		**0.00***	**0.00***	**0.00***	*ns*
	2007	**0.00***		*ns*	*ns*	**0.00***
	2008	**0.00***	*ns*		**0.01***	**0.00***
	2009	**0.00***	*ns*	**0.01***		**0.05***
	2010	*ns*	**0.00***	**0.00***	**0.05***	
Ganoderma	2006		**0.00***	**0.00***	*ns*	*ns*
	2007	**0.00***		**0.05***	**0.00***	**0.00***
	2008	**0.00***	**0.05***		**0.00***	**0.02***
	2009	*ns*	**0.00***	**0.00***		*ns*
	2010	*ns*	**0.00***	**0.02***	*ns*	

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

3.2.4. Contribution of spores to the total load

The distribution of fungal spores varied from year to year, as well as their contribution to the total load of identified and counted fungal genera in the air of sampling area (Table 3.4). Although a group of investigated genera was narrowed down to four fungal genera the differences between propitious weather conditions were still apparent (Table 3.4). *Cladosporium* spores, which contributed most significantly to total spore load between 47.25% and 75.29% reached its maximum concentration in 2010, while *Alternaria* and *Ganoderma* spores depicted in 2009. It was notable that *Didymella* spore contribution reached up to a maximum of 20.56% in 2008 (Table 3.4).

Table 3.4 Percentage shares of selected fungal spore types under investigation out of 20 measured taxa.

Taxon	2006	2007	2008	2009	2010	Mean
Alternaria	0.78	0.80	0.87	**0.88**	0.50	0.75
Cladosporium	72.70	55.89	47.25	63.24	**75.29**	64.10
Didymella	2.27	13.23	**20.56**	8.04	2.02	8.44
Ganoderma	1.61	1.71	1.71	**2.13**	1.41	1.70

The highest obtained values within five year period were distinguished in bold.

3.2.5. Relationship between spore occurrence and weather

3.2.5.1. Spearman's rank test results

Alternaria spores were mainly positively correlated with maximum, minimum and mean temperature (Table 3.5). A weaker relationship was found with dew point temperature. The closest relationships were observed in 2006 and 2009 using the Spearman's rank test, e.g.: with maximum temperature r_s=0.62 and r_s=0.60, respectively. A directly proportional influence on the spore concentration was also observed with wind direction in 2007, and an inversely proportional impact was observed for rainfall and relative humidity in 2006 and 2007. Minor statistically significant relationships were observed for air pressure in 2006 and 2009.

Distribution of *Cladosporium* spores was very similar to that already reported for *Alternaria* spores, although the dependence on the temperature was

more statistically significant (r_s=0.70 in 2006 and r_s=0.68 in 2009), (Table 3.5). Weak, negative correlations were also observed with rainfall in 2006 and 2010, and relative humidity in 2006.

Concentration of *Didymella* spores fluctuated in response to changes of relative humidity, dew point temperature and air pressure (Table 3.5). Vector of the latest dependency revealed to be negative. The most statistically significant correlations were noted in 2008 and 2010 year (e.g. r_s=0.56 and r_s=0.59 with relative humidity). The impact of other temperature parameters other than dew point temperature was insignificant for the majority of the investigated period of time (years 2006, 2007 and 2010). In addition, wind direction occasionally played a small role in the increased concentration of trapped *Didymella* spores in 2006 and 2007. In 2008 all examined weather parameters showed statistically significant correlations with the concentration of *Didymella* spores.

Ganoderma spores followed similar distribution patterns to those previously described for *Alternaria* and *Cladosporium* spores (Table 3.5). Overall, the major factor that affected *Ganoderma* spore concentrations was increasing temperature, and dew point temperature in particular (e.g. r_s=0.69 in 2009). Inversely proportional relationships were observed with rainfall and wind direction (e.g. r_s=0.35 and r_s= -0.23 respectively). Other environmental factors had a lesser influence, with respect to Spearman's rank coefficient values.

Table 3.5 The results of the Spearman's rank test between spore concentration and meteorological parameters from May to September 2006-2010.

Taxon	Year	TMA	TMI	TME	DPT	WD	RAIN	RH	AP
Alternaria	2006	**0.62***	**0.62***	**0.62***	**0.23***	*ns*	**-0.33***	**-0.45***	**0.17***
	2007	**0.54***	**0.54***	**0.54***	**0.20***	**0.33***	**-0.38***	**-0.33***	*ns*
	2008	**0.38***	**0.38***	**0.37***	**0.34***	*ns*	*Ns*	*ns*	**0.16***
	2009	**0.60***	**0.60***	**0.60***	**0.54***	*ns*	*Ns*	*ns*	*ns*
	2010	**0.44***	**0.44***	**0.44***	**0.36***	*ns*	*Ns*	*ns*	*ns*
Cladospo-rium	2006	**0.70***	**0.70***	**0.70***	**0.42***	*ns*	**-0.20***	**-0.33***	*ns*
	2007	**0.32***	**0.33***	**0.33***	**0.34***	*ns*	*Ns*	*ns*	*ns*
	2008	**0.36***	**0.35***	**0.35***	**0.32***	*ns*	*Ns*	*ns*	*ns*
	2009	**0.68***	**0.68***	**0.68***	**0.64***	*ns*	*Ns*	*ns*	*ns*
	2010	**0.61***	**0.61***	**0.61***	**0.45***	*ns*	**-0.16***	*ns*	*ns*
Didymella	2006	*ns*	*ns*	*ns*	**0.27***	**0.24***	**0.33***	**0.22***	**-0.33***
	2007	*ns*	*ns*	*ns*	**0.38***	*ns*	**0.40***	**0.34***	**-0.48***
	2008	**0.18***	**0.17***	**0.18***	**0.53***	**0.17***	**0.58***	**0.56***	**-0.58***
	2009	**0.40***	**0.40***	**0.40***	**0.59***	*ns*	**0.33***	**0.33***	**-0.38***
	2010	*ns*	*ns*	*ns*	**0.44***	*ns*	**0.41***	**0.59***	**-0.32***
Ganoder-ma	2006	**0.47***	**0.47***	**0.47***	**0.50***	**-0.23***	**-0.16***	*ns*	*ns*
	2007	**0.52***	**0.53***	**0.52***	**0.40***	**0.25***	**-0.35***	*ns*	**0.28***
	2008	**0.31***	**0.31***	**0.31***	**0.37***	*ns*	**-0.17***	*ns*	**0.30***
	2009	**0.66***	**0.66***	**0.66***	**0.69***	*ns*	*Ns*	*ns*	*ns*
	2010	**0.65***	**0.65***	**0.65***	**0.65***	*ns*	*Ns*	**0.18***	*ns*

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

3.2.5.2. Redundancy analysis

CCA detected high auto-correlation within examined weather factors. Hence it was necessary to remove each time from the data set, three out of four temperature parameters, as their VIF scored above 10. Dew point temperature and minimum temperature were included in data files, in 2006 and 2009 respectively, whereas in the remaining years observation of maximum temperature was analysed. After the applied reduction in number of used explanatory variables, other parameters obtained low redundancy (Table 3.6).

Table 3.6 Variance Inflation Factors for meteorological parameters after reduction.

Year	TMA	TMI	TME	DPT	WD	RH	RAIN	AP
2006	-----	-----	-----	1.11	1.04	1.74	1.47	1.21
2007	1.07	-----	-----	-----	1.12	1.65	1.88	1.29
2008	1.06	-----	-----	-----	1.02	1.62	1.73	1.42
2009	-----	1.09	-----	-----	1.01	1.51	1.74	1.34
2010	1.05	-----	-----	-----	1.08	1.61	1.78	1.23

DCA revealed that relationships between explanatory and investigated variables were monotonic in their nature for each studied year, as the length of

DCA first axis analysis resulted in values below 2. Therefore, redundancy analysis was chosen as a final examination tool.

RDA revealed, that in 2006 the first RDA axis explained 25% and second RDA axis 10% of the variance (Table 3.7). In the following two years, the amount of explained variance by the first and second RDA axis gradually increased obtaining dually 41% and 46% respectively. A slight fall was observed in 2009 and 2010, when 44% and 42% were determined by RDA 1 and RDA 2 axes. Other components were not important in the description of the variance or they did not achieve level of statistical significance (Table 3.7).

Table 3.7 Eigenvalues and their contribution to the variance.

Year	Value	Constrained Axes			
		RDA 1	**RDA 2**	**RDA 3**	**RDA 4**
2006	Eigen Value	**0.47***	**0.18***	*ns*	*Ns*
	Proportion explained	**0.25***	**0.10***	*ns*	*Ns*
	Cumulative proportion	**0.25***	**0.34***	*ns*	*Ns*
2007	Eigen value	**0.40***	**0.18***	*ns*	*Ns*
	Proportion explained	**0.28***	**0.13***	*ns*	*Ns*
	Cumulative proportion	**0.28***	**0.41***	*ns*	*Ns*
2008	Eigen value	**0.68***	**0.14***	*ns*	*Ns*
	Proportion explained	**0.38***	**0.08***	*ns*	*Ns*
	Cumulative proportion	**0.38***	**0.45***	*ns*	*Ns*
2009	Eigen value	**0.81***	**0.14***	*ns*	*Ns*
	Proportion explained	**0.38***	**0.06***	*ns*	*Ns*
	Cumulative proportion	**0.38***	**0.45***	*ns*	*Ns*
2010	Eigen value	**0.55***	**0.20***	*ns*	*Ns*
	Proportion explained	**0.31***	**0.11***	*ns*	*Ns*
	Cumulative proportion	**0.31***	**0.43***	*ns*	*Ns*

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

The ANOVA test revealed that during the studied period of time, the first two out of four RDA axes exceeded the threshold of statistical significance with *p* values below 0.01 in each examined year (Table 3.8). Biplot scores for constraining variables and species scores were therefore presented for selected axes in the supplementary materials section (Table 3.9 and Table 3.10, respectively).

Table 3.8 The results of ANOVA test.

Year	Axis	Degree of freedom	Chi square test		F	N. perm	Pr >(F)
2006	RDA1	1	0.47	55.16		199	**0.01***
	RDA2	1	0.18	21.58		199	**0.01***
	RDA3	1	0.02	2.73		2099	*Ns*
	RDA4	1	0.00	0.18		99	*Ns*
2007	RDA1	1	0.40	70.76		199	**0.01***
	RDA2	1	0.18	32.77		199	**0.01***
	RDA3	1	0.01	2.51		2299	*Ns*
	RDA4	1	0.01	1.38		99	*Ns*
2008	RDA1	1	0.68	102.67		199	**0.01***
	RDA2	1	0.14	21.33		199	**0.01***
	RDA3	1	0.01	0.97		99	*Ns*
	RDA4	1	0.00	0.15		99	*Ns*
2009	RDA1	1	0.81	101.78		199	**0.01***
	RDA2	1	0.14	17.13		199	**0.01***
	RDA3	1	0.01	0.79		99	*Ns*
	RDA4	1	0.00	0.02		99	*Ns*
2010	RDA1	1	0.55	81.55		199	**0.01***
	RDA2	1	0.20	29.58		199	**0.01***
	RDA3	1	0.01	1.05		99	*Ns*
	RDA4	1	0.00	0.15		99	*Ns*

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

The results of RDA analysis were described below and visually presented in Figures 3.3-3.7 for each of studied year individually.

Based on the length of the arrows, meteorological parameters could be classified in a following descending order of importance: relative humidity, dew point temperature, air pressure, rainfall and wind direction (Fig. 3.3).

A small dependence on the wind direction was only revealed for *Didymella* spores. The concentration of these spores in the air increased along with the degrees of the wind to certain extent. An influence of dew point temperature and high relative humidity revealed to be the most significantly affecting dispersal of *Didymella* spores. Changes in the pressure of the air were negatively correlated only with *Didymella*. Finally, *Alternaria* and *Cladosporium* spores constituted a

distinctive separate group, which revealed a directly proportional relationship with dew point temperature, and inversely proportional with rainfall and relative humidity.

Table 3.9 Biplot scores for constraining variables were presented for statistically significant axes only.

Year	Meteorological parameter	Axis			
		RDA 1	RDA 2	RDA 3	RDA 4
2006	Dew point temperature	0.71	-0.33	-----	-----
	Wind direction	0.05	-0.41	-----	-----
	Rainfall	-0.33	-0.62	-----	-----
	Relative humidity	-0.39	-0.67	-----	-----
	Air pressure	-0.06	0.66	-----	-----
2007	Maximum temperature	0.52	-0.61	-----	-----
	Wind direction	0.25	-0.31	-----	-----
	Rainfall	0.30	0.84	-----	-----
	Relative humidity	0.35	0.72	-----	-----
	Air pressure	-0.66	-0.42	-----	-----
2008	Maximum temperature	0.30	0.77	-----	-----
	Wind direction	0.35	-0.05	-----	-----
	Rainfall	0.64	-0.57	-----	-----
	Relative humidity	0.77	-0.14	-----	-----
	Air pressure	-0.73	0.28	-----	-----
2009	Minimum temperature	0.78	0.56	-----	-----
	Wind direction	0.20	-0.18	-----	-----
	Rainfall	0.20	-0.79	-----	-----
	Relative humidity	0.39	-0.64	-----	-----
	Air pressure	-0.18	0.72	-----	-----
2010	Maximum temperature	0.74	-0.62	-----	-----
	Wind direction	0.23	-0.11	-----	-----
	Rainfall	0.22	0.72	-----	-----
	Relative humidity	0.58	0.75	-----	-----
	Air pressure	-0.19	-0.38	-----	-----

The relative importance of weather conditions changed in 2007, and rainfall was demonstrated to be more important (Fig. 3.4). In 2007, the importance of rainfall was followed by relative humidity, then maximum temperature, air pressure and wind direction. However, similarly to the previous observations *Didymella* pores were the only types of spores, which were directly proportionally correlated with rainfall and high relative humidity conditions, and simultaneously inversely proportionally correlated with air pressure (Fig. 3.4). The concentration of *Cladosporium* was not affected entirely by changing weather conditions. The relationship with direction of the wind was demonstrated for *Alternaria* and

Ganoderma. Maximum temperature significantly influenced the abundance of *Alternaria* spores, although some influence was also shown on *Didymella*.

Table 3.10 Species scores were presented for statistically significant axes only.

Year	Taxon	Axis			
		RDA 1	RDA 2	RDA 3	RDA 4
2006	*Alternaria*	1.31	0.38	-----	-----
	Cladosporium	1.36	-0.01	-----	-----
	Didymella	0.39	-1.21	-----	-----
	Ganoderma	0.65	-0.03	-----	-----
2007	*Alternaria*	0.83	-1.15	-----	-----
	Cladosporium	0.25	-0.10	-----	-----
	Didymella	1.79	0.62	-----	-----
	Ganoderma	0.33	-0.42	-----	-----
2008	*Alternaria*	0.25	0.91	-----	-----
	Cladosporium	0.24	0.49	-----	-----
	Didymella	2.47	-0.17	-----	-----
	Ganoderma	0.16	0.44	-----	-----
2009	*Alternaria*	1.09	0.72	-----	-----
	Cladosporium	0.82	0.34	-----	-----
	Didymella	2.10	-0.63	-----	-----
	Ganoderma	0.75	0.34	-----	-----
2010	*Alternaria*	0.85	-0.59	-----	-----
	Cladosporium	0.82	-0.70	-----	-----
	Didymella	1.65	0.91	-----	-----
	Ganoderma	1.00	-0.42	-----	-----

Weather conditions in the following year 2008 were almost identical, with a significant influence of air pressure and maximum temperature (Fig. 3.5). *Didymella* spores were affected by relative humidity instead of rainfall. The maximum temperature once more had a significant impact on *Alternaria* spore distribution.

Year 2009 brought a re-shuffle of the weather, and minimum temperature became a major factor in this nearly semi-annual analysis (Fig. 3.6). *Ganoderma* spores usually closely matching direction of the prevailing winds remained under influence of temperature and their concentration increased along with it. *Alternaria* spore concentration was once more influenced significantly by temperature followed by *Cladosporium* and *Ganoderma* spores to some extent.

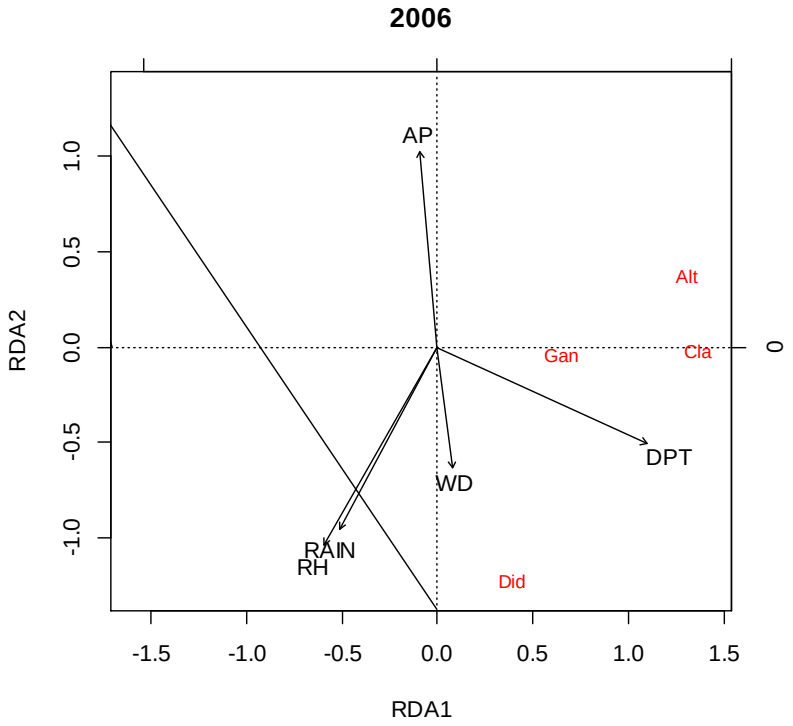


Fig. 3.3 The results of redundancy analysis showing airborne fungal spores and meteorological parameters from May to September 2006.

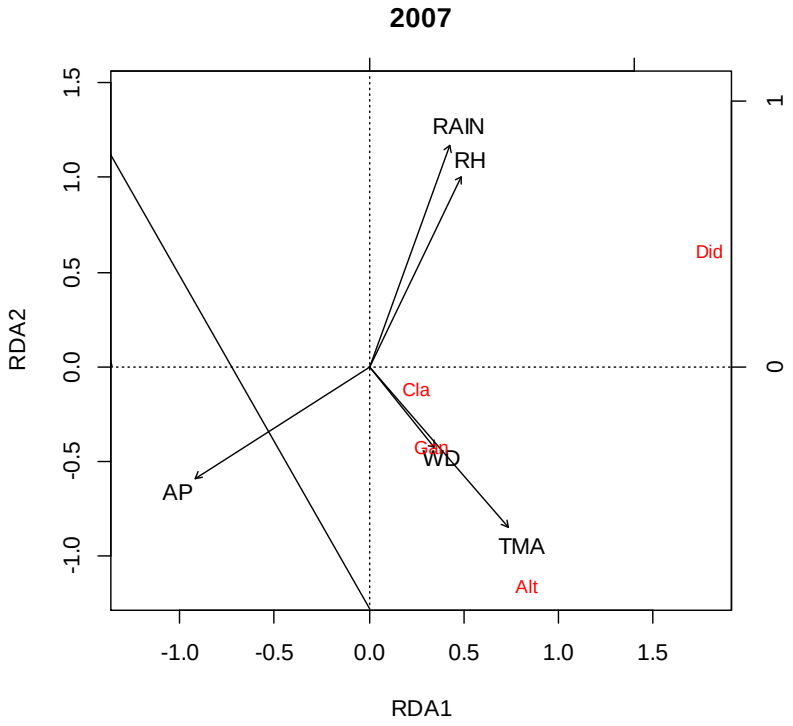


Fig. 3.4 The results of redundancy analysis showing airborne fungal spores and meteorological parameters from May to September 2007.

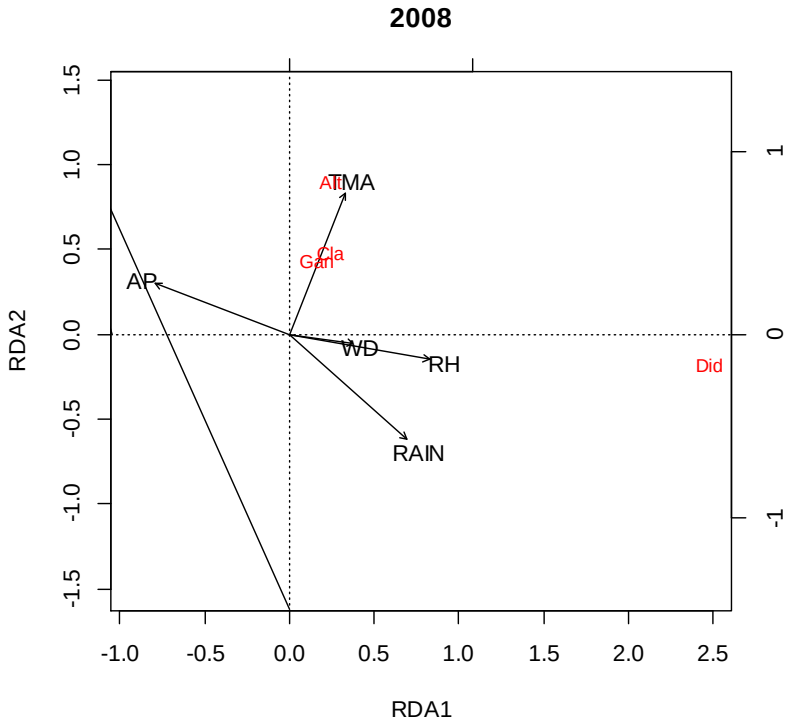


Fig. 3.5 The results of redundancy analysis showing airborne fungal spores and meteorological parameters from May to September 2008.

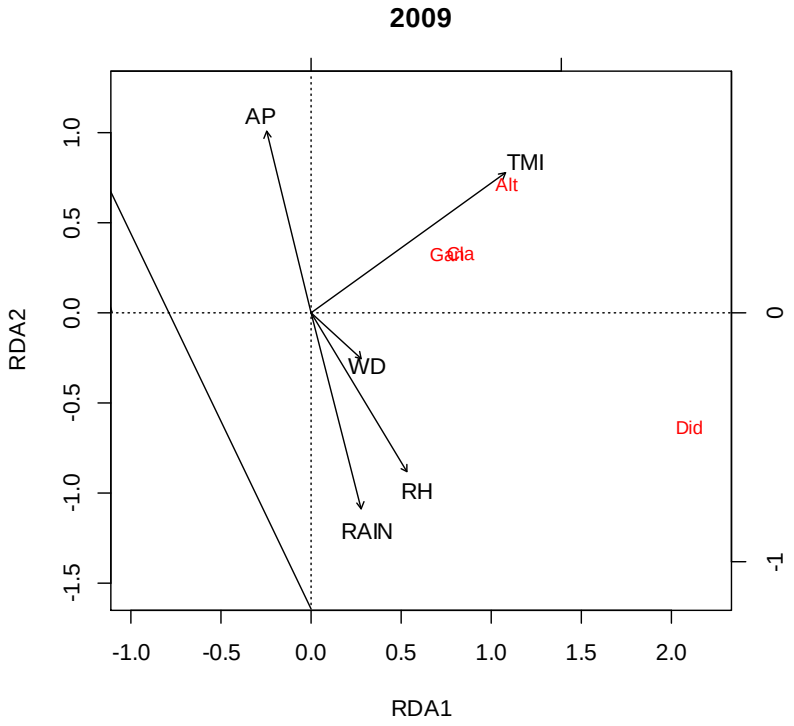


Fig. 3.6 The results of redundancy analysis showing airborne fungal spores and meteorological parameters from May to September 2009.

Finally 2010 was characterized by the dominance of the maximum temperature and the higher importance of the wind direction (Fig. 3.7). This had an impact on distribution of the fungal spores. *Cladosporium* had the strongest directly proportional correlation with maximum temperature, together with *Ganoderma* and *Alternaria* spores.

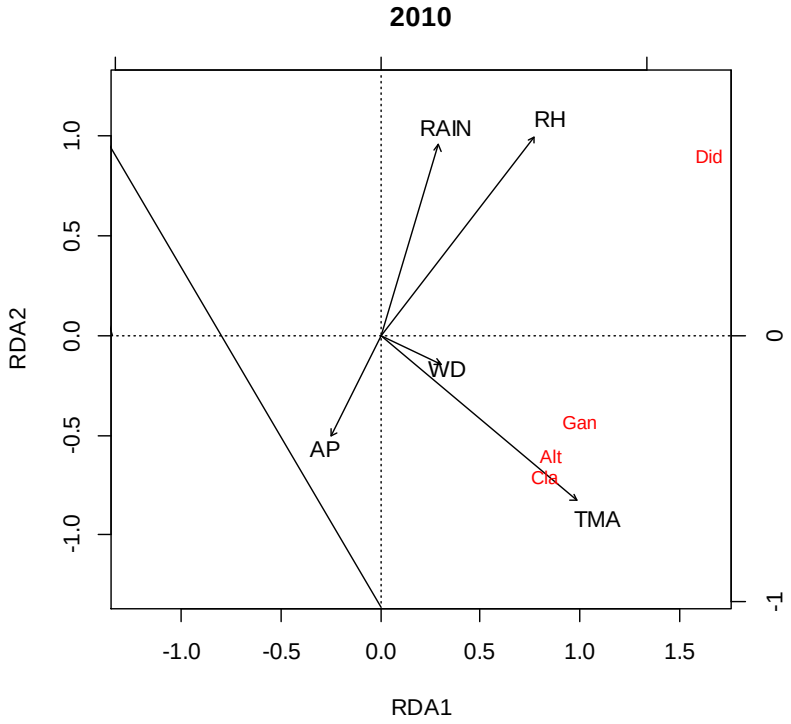


Fig. 3.7 The results of redundancy analysis showing airborne fungal spores and meteorological parameters from May to September 2010.

3.2.5.3. Multivariate Regression Tree analysis

The MRT analysis showed that the concentration of *Alternaria* spores was mostly dependent on the maximum temperature with values between 13.48-24.99°C (Fig. 3.8a). Four times maximum temperature was indicated as the most important parameter and in 2007 and 2009 as the only one, which had an impact on increased numbers of *Alternaria* spores in the air in Worcester, UK. The second important factor was found to be air pressure with a border value equal to 1022 hPa (Fig. 3.8a). Relative humidity varying between 62.02% and 76.37% was computed by decision trees as the third variable affecting *Alternaria* spore concentration in the air (Fig. 3.8a). The best annual MRT model was obtained for 2006 (CV Error 0.82) and the worst for 2010 (CV Error 1.38). The number of tree nodes varied as well, from 2 to 5 indicating simultaneously desired simplicity and lowest CV Error.

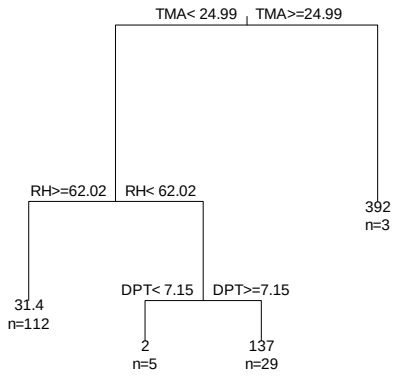
The concentration of *Cladosporium* spores was mostly dependent on the maximum temperature with values between 13.48-21.01°C according to the MRT analysis (Fig. 3.8b). Maximum temperature was indicated as the most important parameter with an exception for the year 2008, when dew point temperature had a greater impact on *Cladosporium* spore concentrations. The third important factor was found to be air pressure with values varying from 1005 hPa to 1017 hPa (Fig. 3.8b). There was an effect of minimum temperature in 2008, although it was of less importance than the above mentioned weather factors (Fig. 3.8b). The best annual MRT model was obtained for 2009 (CV Error 0.70) and the worst for 2007 (CV Error 1.55). The number of tree nodes varied as well, from 2 to 4 indicating simultaneously desired simplicity and lowest CV Error.

The MRT analysis showed that concentration of *Didymella* spores was very complex, as almost each year a different weather factor played a key role (Fig. 3.8c). However a dominance of increased relative humidity with a threshold equal to 89.18%, followed by the presence of light rain showers and decreased maximum temperature to 14.88°C positively affected the concentration of *Didymella* spores recorded in Worcester, UK (Fig. 3.8c). Additionally, it was worth noting that the wind blowing from West to the North direction was responsible for an increased

concentration of *Didymella* spores (3260-6660 s m^{-3}), (Fig. 3.8c). The best annual MRT model was obtained for 2008 (CV Error 0.96) and the worst for 2006 (CV Error 1.41). The number of tree nodes was almost constant (*n*=4), which helped to keep a CV Error below 2.

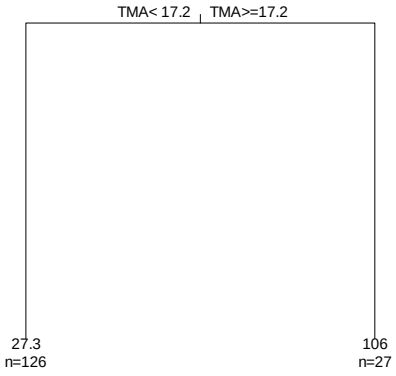
According to the MRT analysis *Ganoderma* spore concentration was affected to a similar extent by dew point temperature, air pressure and maximum temperature (Fig. 3.8d). Concentration of *Ganoderma* spores above 200 s m^{-3} was recorded in Worcester, UK when air pressure was equal to or above 1022 hPa and maximum temperature was found to be below 15.07°C taking as an example results obtained for 2008 year (Fig. 3.8d). An impact of the wind direction was also seen in 2006, 2007 and 2010, where MRT models indicated the prevalence of the wind arriving from the ESE - SW directions (Fig. 3.8d). The best annual MRT model was obtained for 2008 (CV Error 0.60) and the worst for 2006 (CV Error 0.81). The number of tree nodes varied from 3 to 6 indicating the lowest CV Error.

2006



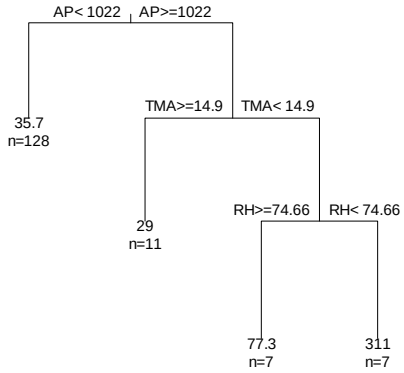
Error : 0.434 CV Error : 0.821 SE : 0.217

2007



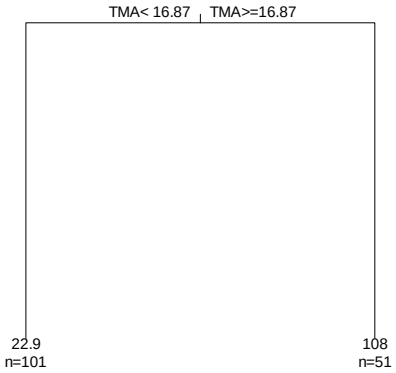
Error : 0.746 CV Error : 0.884 SE : 0.17

2008



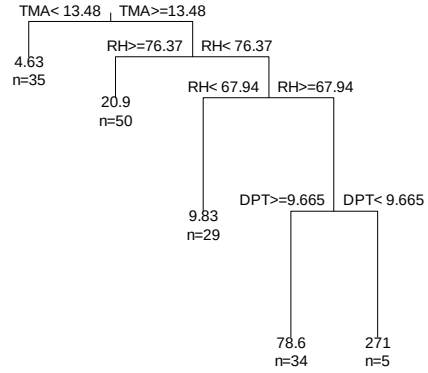
Error : 0.591 CV Error : 1.16 SE : 0.337

2009



Error : 0.829 CV Error : 1.13 SE : 0.358

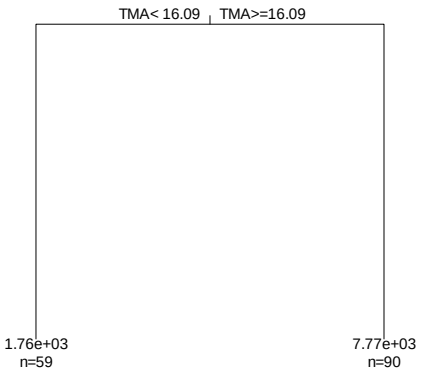
2010



Error : 0.44 CV Error : 1.38 SE : 0.367

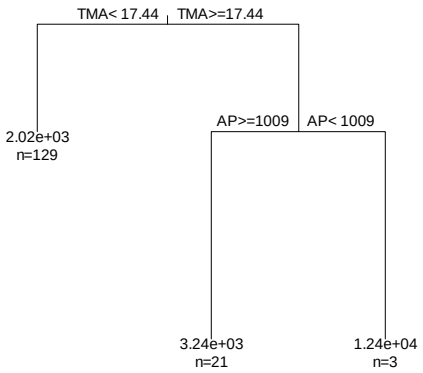
Fig. 3.8a Dependence of *Alternaria* spore concentrations on the meteorological parameters using Multivariate Regression Tree analysis from May to September 2006-2010.

2006



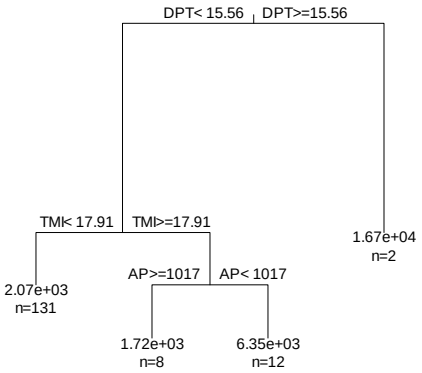
Error : 0.763 CV Error : 0.861 SE : 0.232

2007



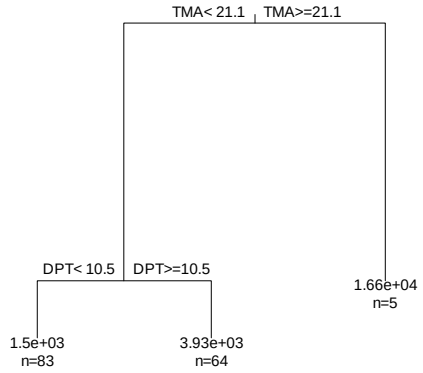
Error : 0.613 CV Error : 1.55 SE : 0.549

2008



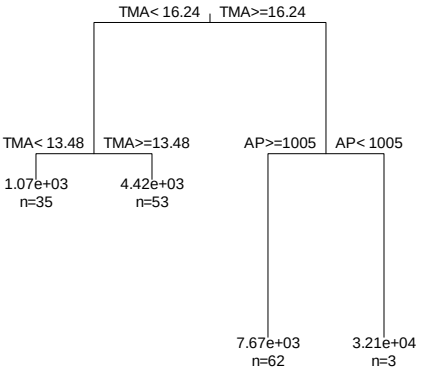
Error : 0.456 CV Error : 0.925 SE : 0.271

2009



Error : 0.378 CV Error : 0.698 SE : 0.181

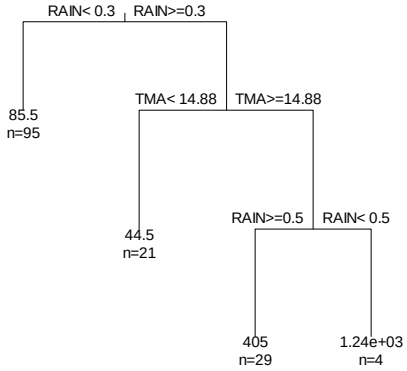
2010



Error : 0.55 CV Error : 0.939 SE : 0.256

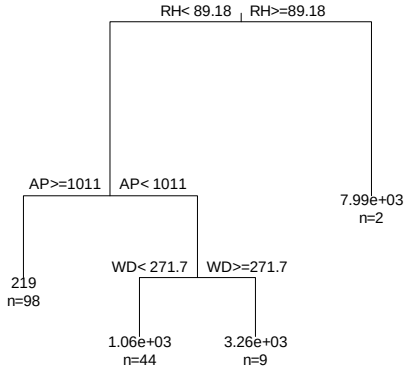
Fig. 3.8b Dependence of *Cladosporium* spore concentrations on the meteorological parameters using Multivariate Regression Tree analysis from May to September 2006-2010.

2006



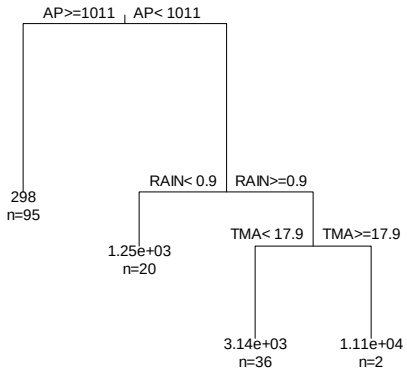
Error : 0.652 CV Error : 1.41 SE : 0.464

2007



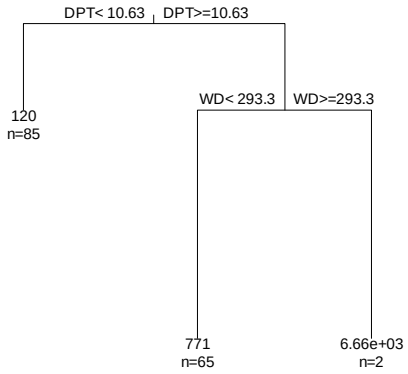
Error : 0.48 CV Error : 0.961 SE : 0.351

2008



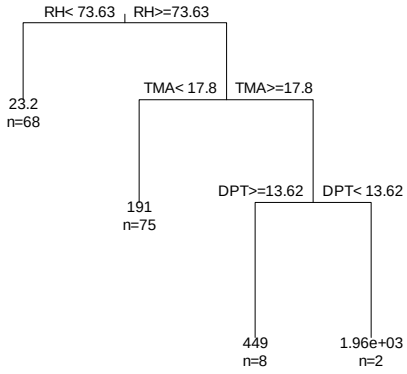
Error : 0.774 CV Error : 0.959 SE : 0.362

2009



Error : 0.578 CV Error : 1.24 SE : 0.604

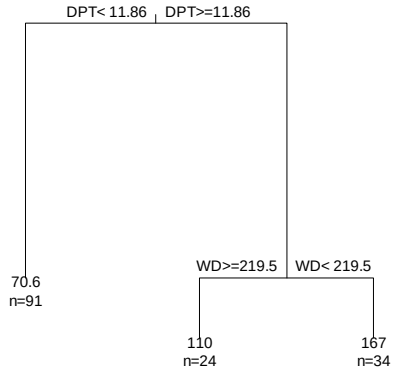
2010



Error : 0.545 CV Error : 1.07 SE : 0.435

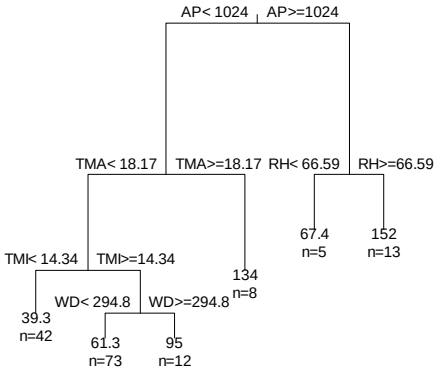
Fig. 3.8c Dependence of *Didymella* spore concentrations on the meteorological parameters using Multivariate Regression Tree analysis from May to September 2006-2010.

2006



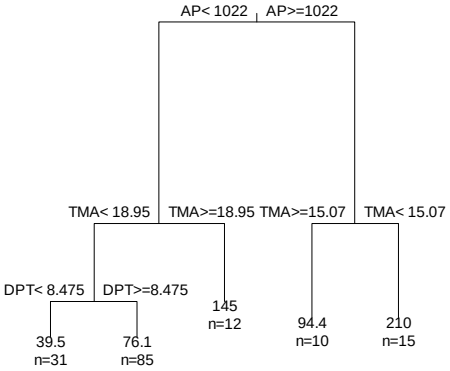
Error : 0.742 CV Error : 0.807 SE : 0.112

2007



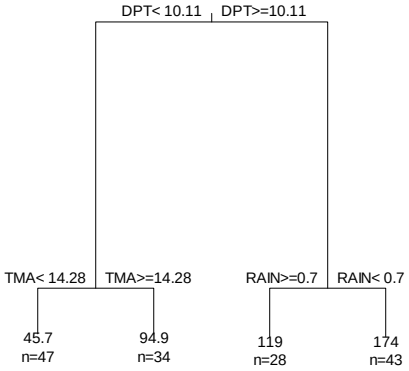
Error : 0.452 CV Error : 0.721 SE : 0.0893

2008



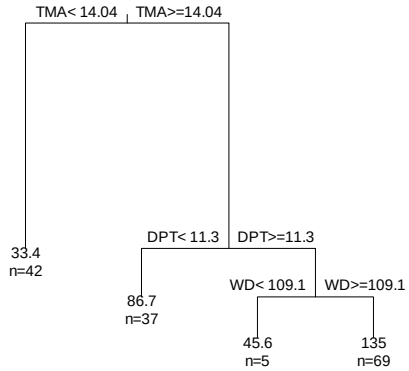
Error : 0.381 CV Error : 0.595 SE : 0.105

2009



Error : 0.497 CV Error : 0.703 SE : 0.0832

2010



Error : 0.497 CV Error : 0.709 SE : 0.0923

Fig. 3.8d Dependence of *Ganoderma* spore concentrations on the meteorological parameters using Multivariate Regression Tree analysis from May to September 2006-2010.

3.3. Discussion

3.3.1. *Alternaria*

Maximum concentration of *Alternaria* spores was recorded in 2009 could possibly be explained by dual influence of maximum temperature (r_s=0.60) and dew point temperature (r_s=0.54), (Table 3.5). Seeing that three years earlier maximum temperature achieved a slightly higher statistically significant correlation (r_s=0.62), but dew point temperature scored lower (r_s=0.23), (Table 3.5) overall the contribution of this genus to total load of spores was lower as well (Table 3.4). RDA analysis confirmed the impact of the former factor, as *Alternaria* spores were always closely located to arrows representing maximum and minimum temperatures (Fig. 3.4-3.7), but not the latter (Fig. 3.3). Li and Kendrick (1995) observed correlation between distribution of *Alternaria* and maximum temperature, during the growing season from May to October, however they have not included dew point temperature in their RDA analysis. Stennet and Beggs (2004) found a positive correlation (r_s=0.41, p<0.001) between occurrence of *Alternaria* spores in the air of Sydney and maximum dew point temperature, which in a rank of importance was preceded by mean and maximum temperature. This relationship was revealed to be even stronger, when a previous day value was examined (r_s=0.42, p< 0.001), (Stennet and Beggs, 2004). Nevertheless, dew point temperature is often being neglected in the environmental analysis of *Alternaria* spore distribution pattern (Grinn-Gofroń and Rapiejko, 2009; Recio et al., 2012). The impact of this parameter requires further statistical examination.

3.3.2. *Cladosporium*

The genus *Cladosporium* required different optimal weather conditions that would favour spore production, release and dispersal in comparison to other investigated fungal taxa as suggested by the results presented in Table 3.4. Although maximum concentration of *Cladosporium* spores was found in 2010 (Table 3.4), this year was not unusual with respect to Spearman's rank test results (Table 3.5). Moreover, it would be expected to observe higher spore counts in the year 2006 or 2009, where the values of coefficients for maximum, minimum and

mean temperatures varied between r_s=0.70 and r_s=0.68, and for dew point temperature r_s=0.42 and r_s=0.64 (Table 3.5). RDA analysis showed there was a lack of influence of any investigated meteorological parameters in 2007 (Fig. 3.4). Multiple regression analysis performed by Stępalska and Wołek (2005) confirmed the impact of the maximum temperature as the only explanatory variable for the post-peak period, however for the pre-peak period it indicated a joint influence of sunshine, relative humidity and rainfall. The same type of analysis conducted by Grinn-Gofroń and Rapiejko (2009) found mean temperature to be the main if not the only explanatory factor for fluctuations of *Cladosporium* spores in the air at three locations. It should be noted, that maximum temperature was not included in that study, therefore it could be possible that actually maximum temperature was the key factor there (Recio et al., 2012).

3.3.3. *Didymella*

The presence of rainfall and high relative humidity conditions favoured dispersal and increased spore counts in the air of *Didymella* ascospores. Air pressure had a negative impact on measurements of those spores. *Didymella* spores peaked in 2008 (Table 3.5), according to RDA analysis for Worcester and were mainly influenced by relative humidity between 2007 and 2010 (Fig. 3.4-3.7) and partly by dew point temperature in 2006 (Fig. 3.3). The Spearman's rank test suggested that rainfall was important. However relative humidity in 2006 and 2008 played a key role (Table 3.5). The importance of rainfall rather than relative humidity in relation to observed *Didymella* spore concentrations was also previously reported in Portugal at two sampling sites (Oliveira et al., 2009). Other studies indicated an influence of minimum temperature along with relative humidity using the Spearman's rank test and multiple regression analysis (Grinn-Gofroń and Mika, 2008; Stępalska and Wołek, 2005; Stępalska et al., 2012).

3.3.4. *Ganoderma*

Dependence on the temperature for the observation of *Ganoderma* spores was shown by both the Spearman's rank test (Table 3.5) and RDA analysis (Fig.

3.3-3.7). The highest concentration of *Ganoderma* spores was observed in 2009, where again the strongest correlations were with maximum, minimum and mean temperatures (r_s= 0.66), as well as with dew point temperature (r_s=0.69), (Table 3.5). The same results were obtained by Li and Kendrick (1995) using RDA analysis on data collected in Waterloo, Canada and using the Spearman's rank test for data sampled in Amares, Portugal by Oliveira et al. (2009) and in Szczecin, Poland by Grinn-Gofroń and Strzelczak (2011). Other weather parameters, although from time to time approached a statistical level of significance, exhibited minimal effect on *Ganoderma* spore variations in the air of Worcester (Table 3.5). Different results were found in Kraków, where in the pre-peak period multiple regression analysis indicated minimum temperature, relative humidity and sunshine as major factors. In the post-peak period minimum temperature and sunshine (Stępalska and Wołek, 2005), and in Porto, where minimum temperature was detected to be the most significant explanatory variable for *Ganoderma* spore observations using the Spearman's rank test (Oliveira et al., 2009).

3.4. Conclusions

Grouping fungi genera using a criterion of their response towards particular environmental factors has brought a lot of confusion. Seeing that observed species-environment reactions were different to that expected in certain cases, like results of Spearman's rank association test and significant spore count during specific years. Even application of one form of statistical examination, like redundancy analysis, showed various responses of the same fungal genus in relation to one explanatory variable. In other situations, both RDA analysis and Spearman's rank test could not provide definitive clear answer to the question which weather parameters influenced presence of individual fungal genera spores in the air. This study only showed a fraction of a number of environmental factors that could be potentially examined. Adaptation to the prevailing local environmental conditions by living organisms like fungi also encourages a change in their response to the same factors, such as maximum temperature and relative humidity. Different

responses would be then expected in different climatic zones for the same fungus, for example *Alternaria* or *Cladosporium* spores.

Although RDA analysis provided supplementary information about biology of the studied fungi in comparison with the results of Spearman's rank test, it has also shown its own limitations. Fungal spore types that exposed correlation with wind direction could not be directly linked with location of their sources. Similarly, no threshold of any other tested factor could be established, like the optimum maximum temperature for presence of *Alternaria* spores in the air. A more detailed RDA analysis could however be performed, if certain thresholds would be manually established.

Chapter 4

Diurnal variations in fungal spore distribution

4.1. Introduction

In this study the following questions were investigated: (a) the annual changes in diurnal pattern of selected fungal spores, (b) the diurnal fluctuations of fungal spores using a concentration threshold, (c) the influence of the rainfall on the diurnal distribution of fungal spores, (d) the changes in diurnal pattern of fungal spores in relation to the amount of rainfall, (e) the variations of the spore concentration within 24 hours as a response to the changes in relative humidity. The impact of the maximum temperature on the hourly changes in spore counts was also investigated. This analysis was carried out by plotting a number of graphs and applying appropriate statistical tests (*i.e.*: Kruskal-Wallis test, Sign test, Friedman test). Methods used in this study were described in detail in Chapter 2.

4.2. Results

4.2.1. Annual changes in diurnal pattern of examined fungal spores

Temporal variations of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores were shown in Fig. 4.1a-d for each of fungal spore season, regardless of the impact of rainfall. Distinct diurnal patterns for *Alternaria* spores were observed in 2008 and 2009 (Fig. 4.1a). The maximum concentration was recorded at 22:00h in both years reaching 249 s m^{-3} and 333 s m^{-3}, whilst the minimum concentration was found between 07:00h and 08:00h in the morning with spore counts equal to 71 s m^{-3} and 85 s m^{-3}, respectively. For *Cladosporium* and *Ganoderma* spores the most representative years were 2006 and 2010 (Fig. 4.1b, Fig. 4.1d). The maximum

concentrations of *Cladosporium* spores were observed at 15:00h and 13:00h exceeding 10000 s m^{-3} within an hour. The lowest concentrations were found at 09:00h and 07:00h decreasing to slightly below 3000 s m^{-3}. Unlike *Cladosporium*, *Ganoderma* ones were observed in greater concentrations early in the morning, *i.e.*: at 06:00h (2006) and 03:00h (2010) with counts slightly above 200 s m^{-3}. In contrast, during the afternoon hours (17:00h and 14:00h) half the numbers of *Ganoderma* spores per cubic metre of air were observed. *Didymella* spores were the only spore type studied, which exhibited a double peak pattern in air samples. The most *Didymella* spores were observed in years 2007 and 2008, although maximum spore counts were seen at different hours in these years (Fig. 4.1c). In 2007, maxima between 4200 s m^{-3} and 4600 s m^{-3} were found at 23:00h and 04:00h, whereas a year later peaks occurred at 22:00h and 05:00h with concentrations greater of 2000 s m^{-3} at both time periods. Significant decreases in *Didymella* spore concentrations oscillating around 400 s m^{-3} were found at 10:00h and 16:00h, respectively.

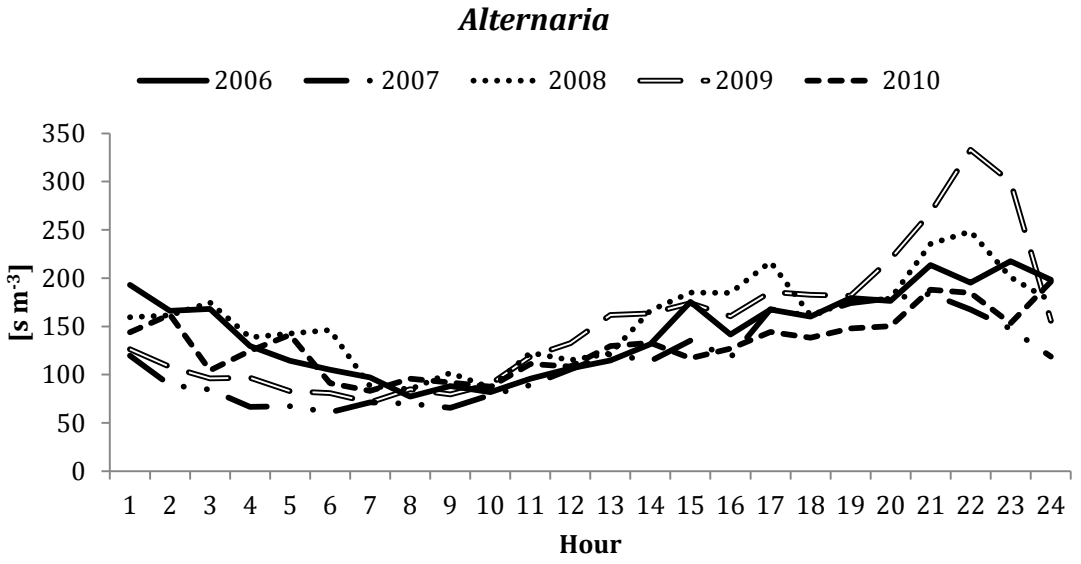


Fig. 4.1a The annual variations in hourly distribution of *Alternaria* spores for the years 2006-2010 in Worcester, UK.

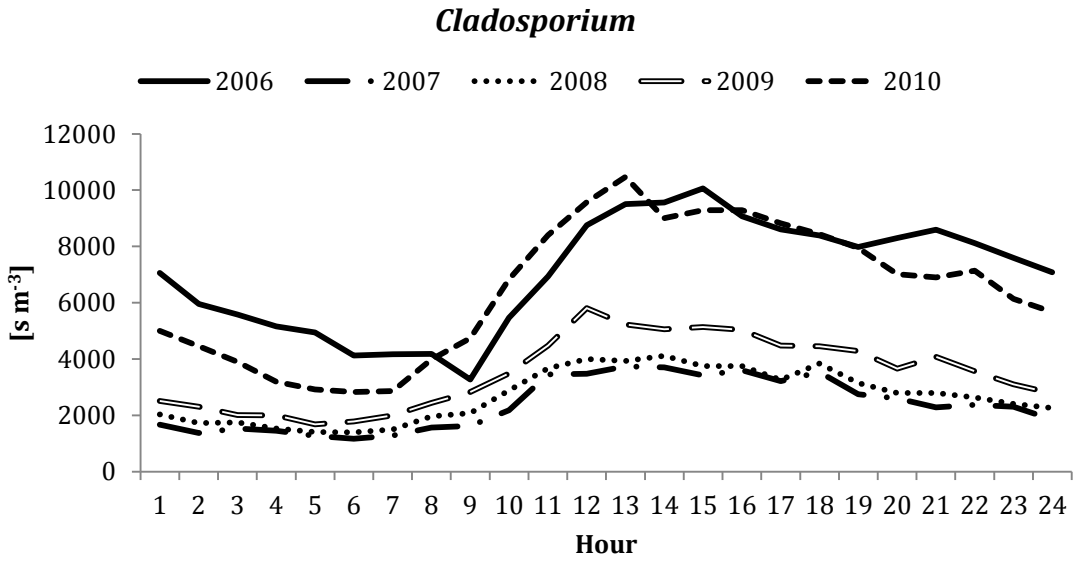


Fig. 4.1b The annual variations in hourly distribution of *Cladosporium* spores for the years 2006-2010 in Worcester, UK.

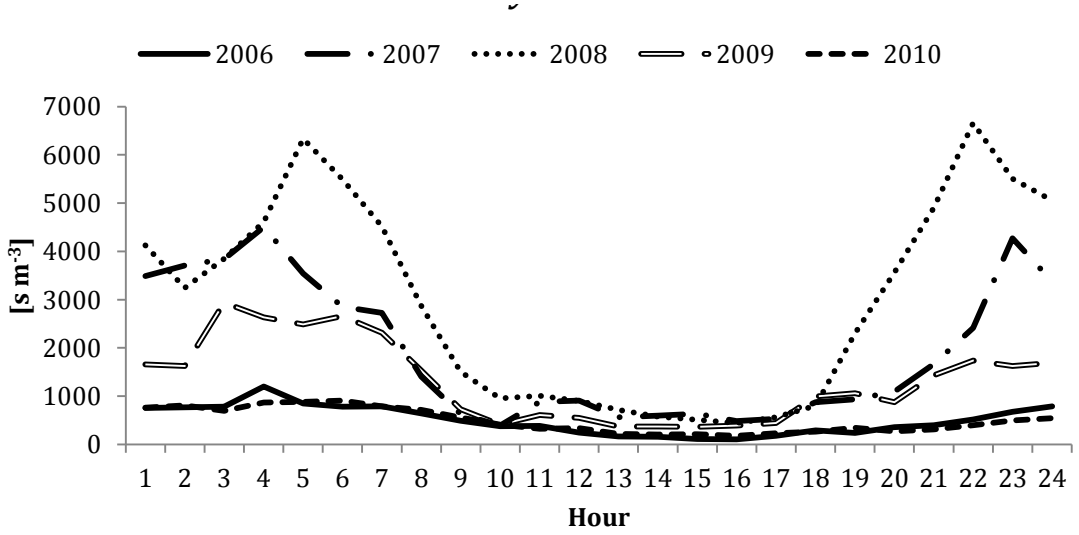


Fig. 4.1c The annual variations in hourly distribution of *Didymella* spores for the years 2006-2010 in Worcester, UK.

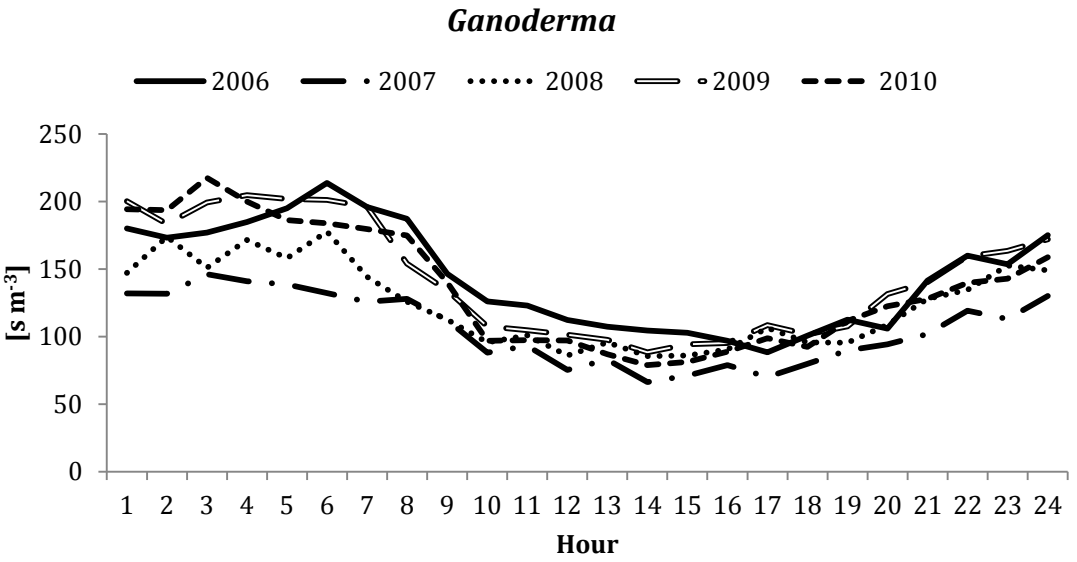


Fig. 4.1d The annual variations in hourly distribution of *Ganoderma* spores for the years 2006-2010 in Worcester, UK.

Table 4.1 The results of the Kruskal-Wallis test (2-tailed) for the comparison of annual changes in diurnal pattern of examined fungal spores.

Taxon	Year	2006	2007	2008	2009	2010
Alternaria	2006		*ns*	*ns*	*ns*	*ns*
	2007	*ns*		**0.02***	*ns*	*ns*
	2008	*ns*	**0.02***		*ns*	*ns*
	2009	*ns*	*ns*	*ns*		*ns*
	2010	*ns*	*ns*	*ns*	*ns*	
Cladosporium	2006		**0.00***	**0.00***	**0.00***	*ns*
	2007	**0.00***		*ns*	*ns*	**0.00***
	2008	**0.00***	*ns*		*ns*	**0.00***
	2009	**0.00***	*ns*	*ns*		**0.01***
	2010	*ns*	**0.00***	**0.00***	**0.01***	
Didymella	2006		**0.00***	**0.00***	**0.01***	*ns*
	2007	**0.00***		*ns*	*ns*	**0.00***
	2008	**0.00***	*ns*		*ns*	**0.00***
	2009	**0.01***	*ns*	*ns*		**0.01***
	2010	*ns*	**0.00***	**0.00***	**0.01***	
Ganoderma	2006		**0.01***	*ns*	*ns*	*ns*
	2007	**0.01***		*ns*	**0.01***	*ns*
	2008	*ns*	*ns*		*ns*	*ns*
	2009	*ns*	**0.01***	*ns*		*ns*
	2010	*ns*	*ns*	*ns*	*ns*	

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

The Kruskal-Wallis test detected statistically significant differences between diurnal profiles for all of the examined fungal species within 24 hours in the atmosphere of Worcester (Table 4.1). *Alternaria* spores showed the most constant profile, as only one difference between years 2007 and 2008 was found (Table 4.1). Slightly more variations in hourly distribution were observed with *Ganoderma* spores. Years 2006 and 2007, 2007 and 2009 were found to be different (Table 4.1). Furthermore, six out of ten combinations between diurnal profiles of *Cladosporium* and *Didymella* spores confirmed their uniqueness (Table 4.1).

4.2.2. Application of concentration thresholds

Several threshold values for fungal spore concentrations were examined to test stability of their diurnal pattern (Fig. 4.2a-d). For *Alternaria* and *Cladosporium* spores clinically established threshold values that caused an allergic response in sensitised people in the UK were 50 s m^{-3} and 3000 s m^{-3}, respectively (Corden and Millington, 2001), and their double values. No clinical threshold has been established yet for the other two fungal taxa under investigation. Thresholds have been chosen based on the most significant high spore counts registered in Worcester in the five years of monitoring. Both 200 s m^{-3} and 100 s m^{-3}, have been previously used by other authors as a *Ganoderma* threshold (e.g. Grinn-Gofroń and Mika, 2008; Sadyś et al., 2014), and 3000 s m^{-3} and 6000 s m^{-3} for *Didymella* spores.

Table 4.2 The results of sign test for the comparison of changes in diurnal pattern of examined fungal spores using two different threshold values.

Taxon	Year	2006	2007	2008	2009	2010	2006-10
Alternaria	*z*	4.69	2.09	4.29	3.88	4.69	9.17
	p	**0.00***	**0.04***	**0.00***	**0.00***	**0.00***	**0.00***
Cladosporium	*z*	4.69	3.47	4.69	4.69	4.69	10.32
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Didymella	*z*	-------	2.25	3.47	1.43	-------	2.47
	p	-------	**0.02***	**0.00***	*ns*	-------	**0.01***
Ganoderma	*z*	4.69	3.47	3.47	4.69	4.27	9.59
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

Regardless of a threshold value that had been examined, the general pattern of diurnal distribution of all fungal spores was repeated (Fig. 4.2a-d). The higher the threshold, the clearer the pattern was observed with a sharp distinction between maximum and minimum hourly concentration of fungal spores. The time of all peak values, for *Alternaria*, *Cladosporium* and *Didymella* spores was constant, and it occurred at 22:00h, 13:00h and 05:00h, respectively. Maximum concentration of *Ganoderma* spores was found three hours earlier (at $n \geqslant 200$ s m^{-3} concentration threshold).

The results of the sign test confirmed the observed difference between diurnal patterns of *Ganoderma* spore concentrations for each year, as well as for a joint five year period (Table 4.2). Following statistically significant differences were also found for other fungal taxa under investigation (Table 4.2).

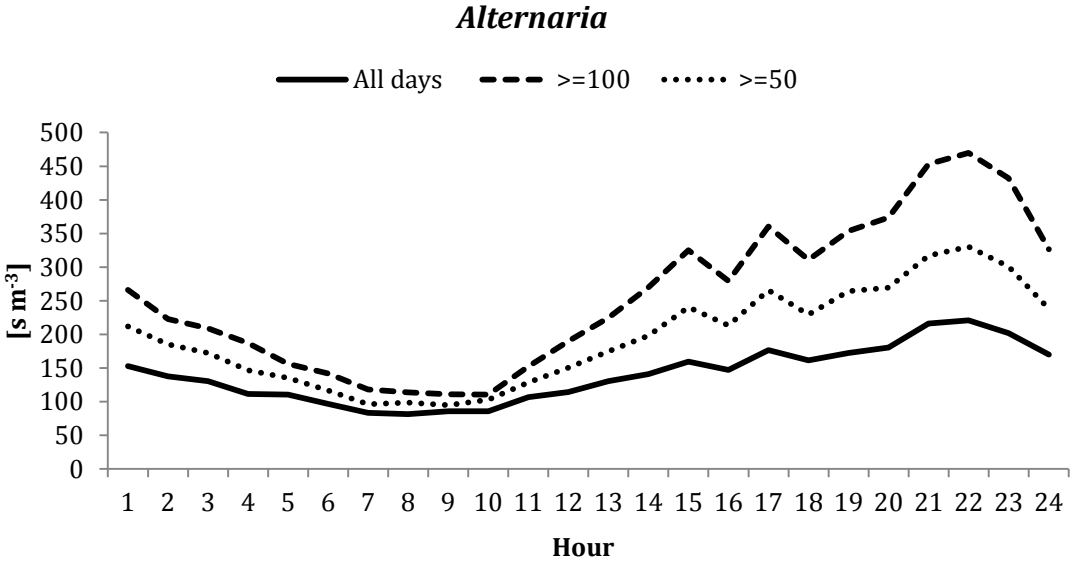


Fig. 4.2a The variations in hourly distribution of *Alternaria* spores at different concentration thresholds for the years 2006-2010 in Worcester, UK.

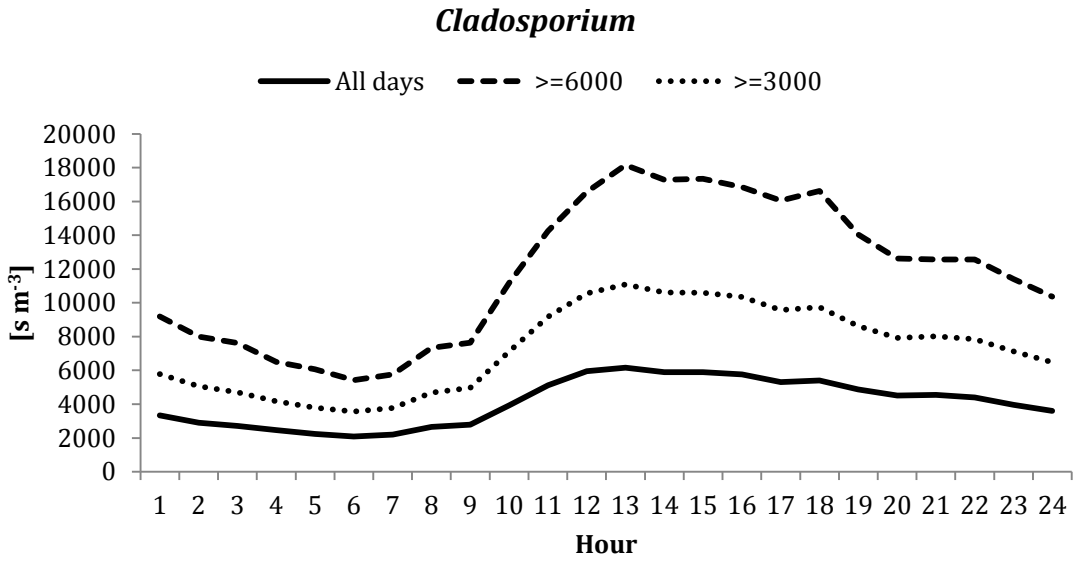


Fig. 4.2b The variations in hourly distribution of *Cladosporium* spores at different concentration thresholds for the years 2006-2010 in Worcester, UK.

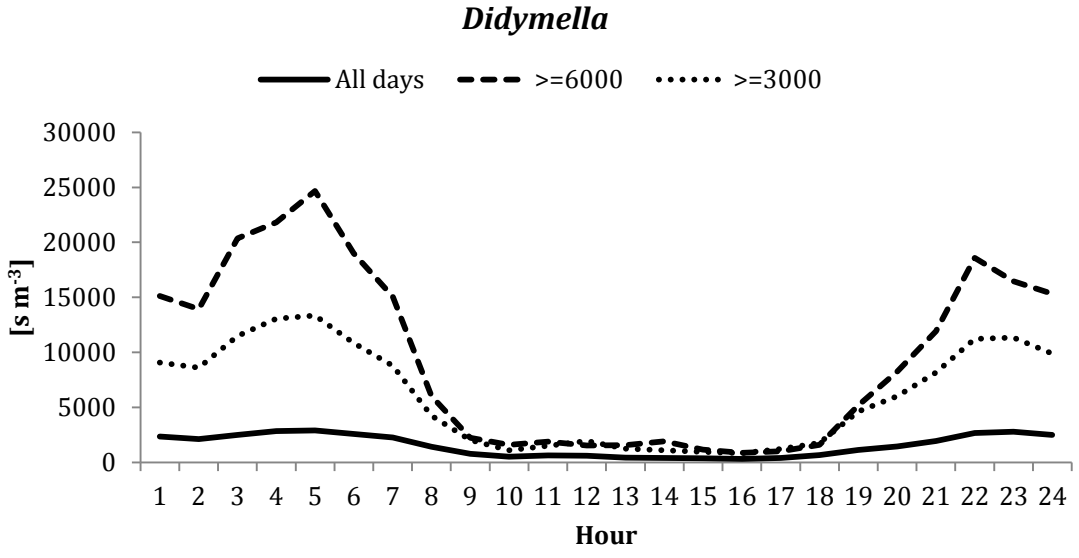


Fig. 4.2c The variations in hourly distribution of *Didymella* spores at different concentration thresholds for the years 2006-2010 in Worcester, UK.

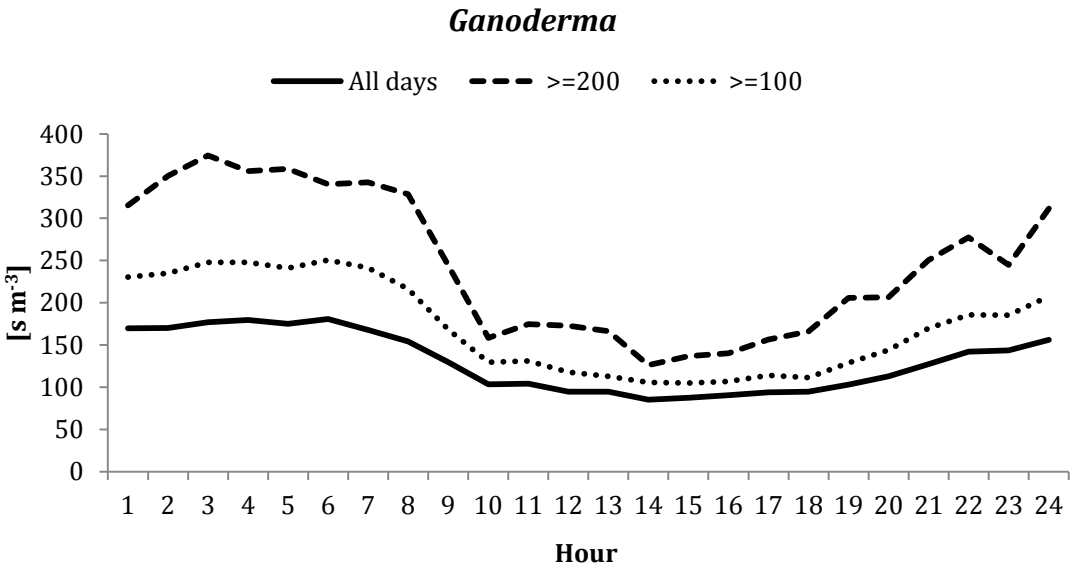


Fig. 4.2d The variations in hourly distribution of *Ganoderma* spores at different concentration thresholds for the years 2006-2010 in Worcester, UK.

No difference was found in diurnal distribution of *Didymella* spores in 2009, whereas no pattern at all could be distinguished in the years 2006 and 2010 by testing selected threshold values (Table 4.2).

4.2.3. Response to the presence of rainfall

A five year mean hourly distribution of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores measured in Worcester for all days, days with lack of rainfall ("dry days") and days with presence of a minimum of 0.20 mm of rainfall ("rainy days") was shown in Fig. 4.3a-d. A general trend in the distribution pattern of examined fungal spores was repeated, although at some time periods a significant reduction in fungal spore concentration was observed (Fig. 4.3a-d). The diurnal fluctuations of *Alternaria* spores was analysed as an example. Although the maximum concentration of *Alternaria* spores was constantly recorded at 22:00h, concentrations of spores decreased from 247 s m^{-3} during "dry days" to 172 s m^{-3} during "rainy days". In comparison to *Alternaria* spores and *Ganoderma* spores, *Cladosporium* ones revealed a slightly different response to the presence of rainfall (Fig. 4.3b). It was observed that 12 hours rainfall had a positive impact on *Cladosporium*, as its concentration increased significantly. This effect was observed

after 07:00h. Maximum spore concentration was also registered at 12:00h, an hour earlier in a comparison with "all days" and "dry days" distribution patterns, approaching 6400 s m^{-3}. Spores of *Didymella* increased significantly in air samples, when rainfall occurred compared to "dry days" (Fig. 4.3c).

The occurrence of a double peak pattern early in the morning and early at night was mainly found for "rainy days" and for "all days". The maximum concentration of *Didymella* spores was recorded at 05:00h and 23:00h, varying between 2800 s m^{-3} and 4000 s m^{-3}. A sudden fall in spore concentration was observed from 10:00h to 17:00h (Fig. 4.3c).

The sign test confirmed the differences between days without rainfall and days, where rainfall was present (≥0.20 mm), for three types of spores that were statistically significant (Table 4.3). The only exception was the distribution of *Cladosporium* spores, which remained unaffected, even after five years of data (Table 4.3).

The presence of rainfall significantly affected the diurnal fluctuations of *Didymella* spores in the air in Worcester, whereas *Ganoderma* spores in 2010 and *Alternaria* spores in 2006 and 2009 showed no difference in their concentration (Table 4.3).

Table 4.3 The results of sign test for the comparison of changes in diurnal pattern of examined fungal spores in response to presence of rainfall.

Taxon	Year	2006	2007	2008	2009	2010	2006-10
Alternaria	*z*	1.84	2.25	2.50	0.61	4.29	5.50
	p	*ns*	**0.02***	**0.01***	*ns*	**0.00***	**0.00***
Cladosporium	*z*	-0.20	1.43	1.02	0.61	0.20	0.27
	p	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Didymella	*z*	4.69	4.69	4.69	4.69	3.88	10.50
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Ganoderma	*z*	2.65	3.88	4.29	2.65	0.20	6.12
	p	**0.01***	**0.00***	**0.00***	**0.01***	*ns*	**0.00***

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

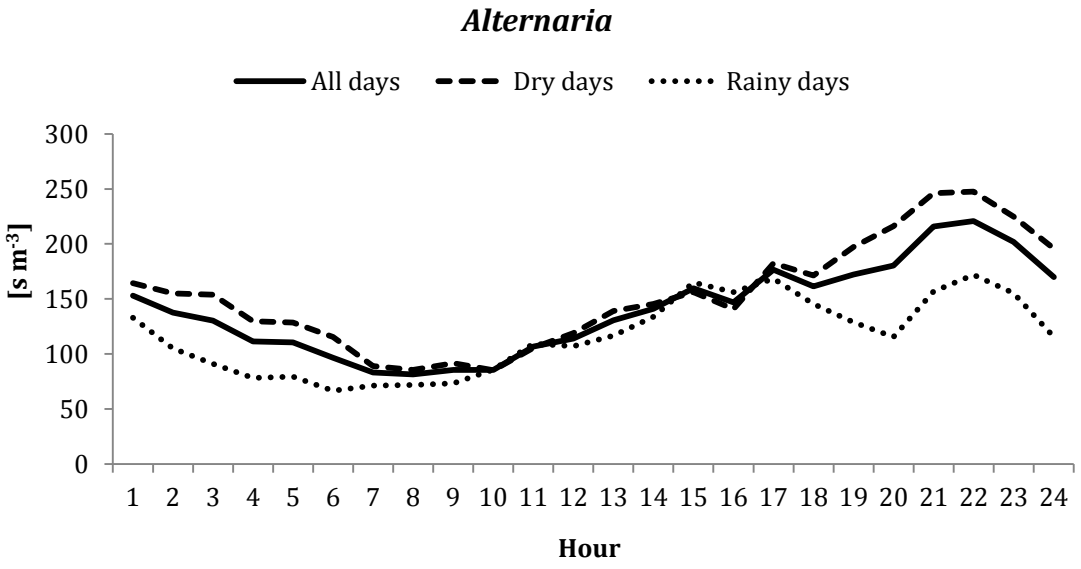


Fig. 4.3a A mean hourly distribution of *Alternaria* spores depending on presence or absence of rainfall for the years 2006-2010 in Worcester, UK.

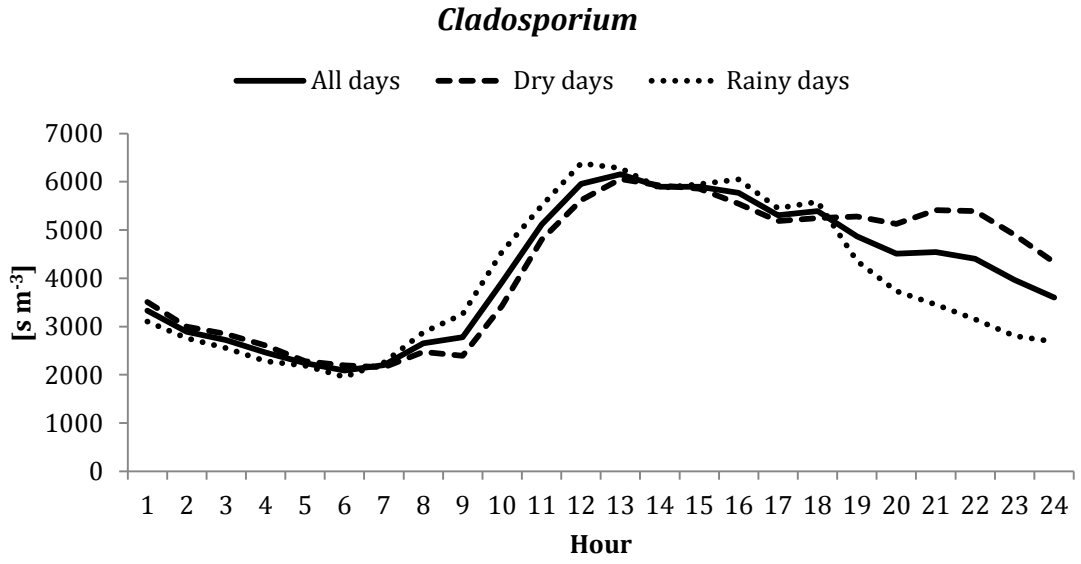


Fig. 4.3b A mean hourly distribution of *Cladosporium* spores depending on presence or absence of rainfall for the years 2006-2010 in Worcester, UK.

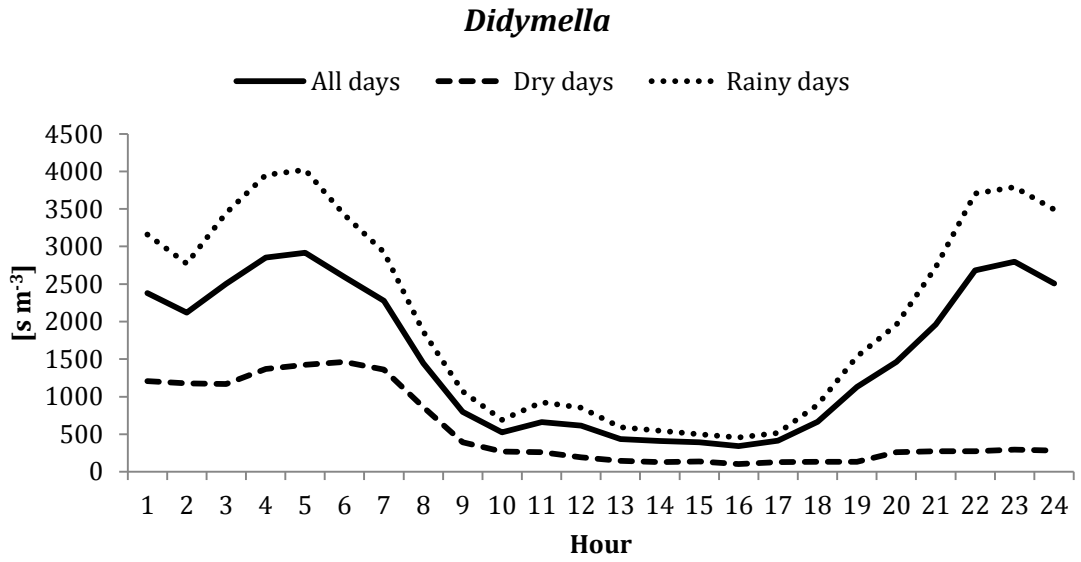


Fig. 4.3c A mean hourly distribution of *Didymella* spores depending on presence or absence of rainfall for the years 2006-2010 in Worcester, UK.

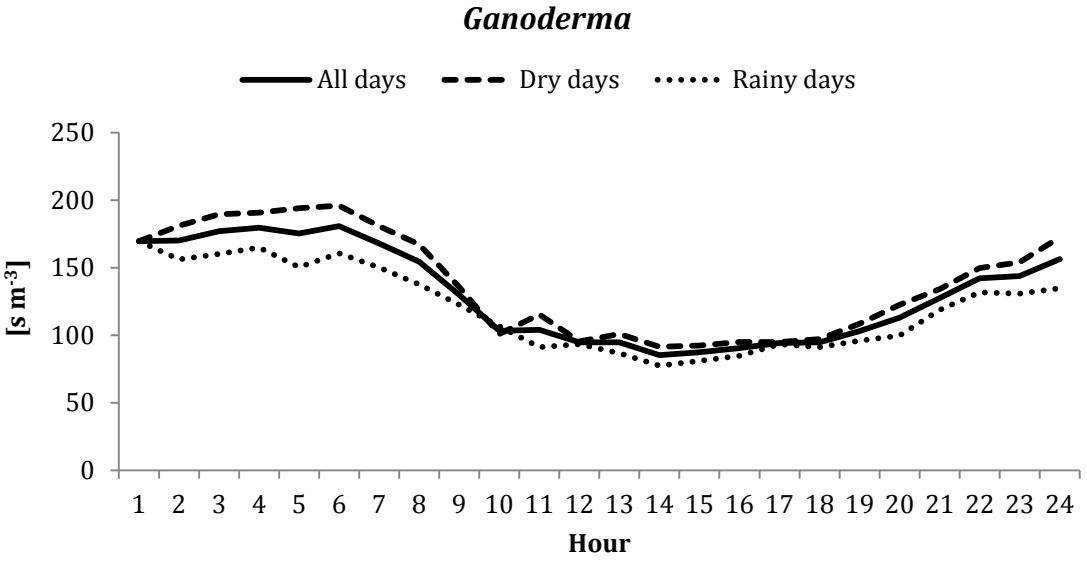


Fig. 4.3d A mean hourly distribution of *Ganoderma* spores depending on presence or absence of rainfall for the years 2006-2010 in Worcester, UK.

4.2.4. Response to various amounts of rainfall

The volume of rainfall that fell within 24 hours was examined, as observations made by the author during fungal spore counting suggested that a small amount of precipitation induced simultaneous presence of so-called "dry" (e.g. *Cladosporium* spores) and "wet" (e.g. *Didymella* spores) spores in greater quantities, in the air in Worcester. Figure 4.4a-d shows the impact of the amount of rainfall (n), classified within four categories: $0<n\leqslant5$ mm, $5<n\leqslant10$ mm, $10<n\leqslant15$ mm and $15<n\leqslant20$ mm on diurnal distribution of *Alternaria*, *Cladosporium*, *Ganoderma* and *Didymella* spores.

The highest hourly concentration of *Alternaria* spores (415 s m^{-3}) was recorded when the amount of rainfall occurring within 24 hours varied between 10< and ⩽15 mm (Fig. 4.4a). This observation was found at 22:00h. Similar amounts of rainfall favoured dispersal of *Didymella* spores, which peaked at 22:00h with a concentration approaching a maximum of 5700 s m^{-3} (Fig. 4.4c). Lower volumes of rainfall were beneficial for dispersal of *Cladosporium* spores ($0<n\leqslant5$ mm), which achieved the maximum trapped concentration (above 7000 s m^{-3}) at 12:00h (Fig. 4.4b).

Ganoderma spores required the highest amount of rainfall for spore dispersal ($15<n\leqslant20$ mm) out of all four examined fungal genera (Fig. 4.4d). The peak of *Ganoderma* spores was equal to 270 s m^{-3} and found at 17:00h (Fig. 4.4d).

The Friedman test confirmed that there was a statistically significant difference in diurnal distribution of *Alternaria*, *Cladosporium*, *Didymella* spores and occasionally *Ganoderma* spores depending on the amount of rainfall that occurred during a 24 hour period (Table 4.4). This association was observed for each of the examined years as well as for all five yearly sampling periods (Table 4.4). Exceptions were observed in years 2008 and 2010, where no statistically significance difference in *Ganoderma* spore diurnal profile was noted, whilst the various amounts of precipitation were recorded within 24 hours (Table 4.4).

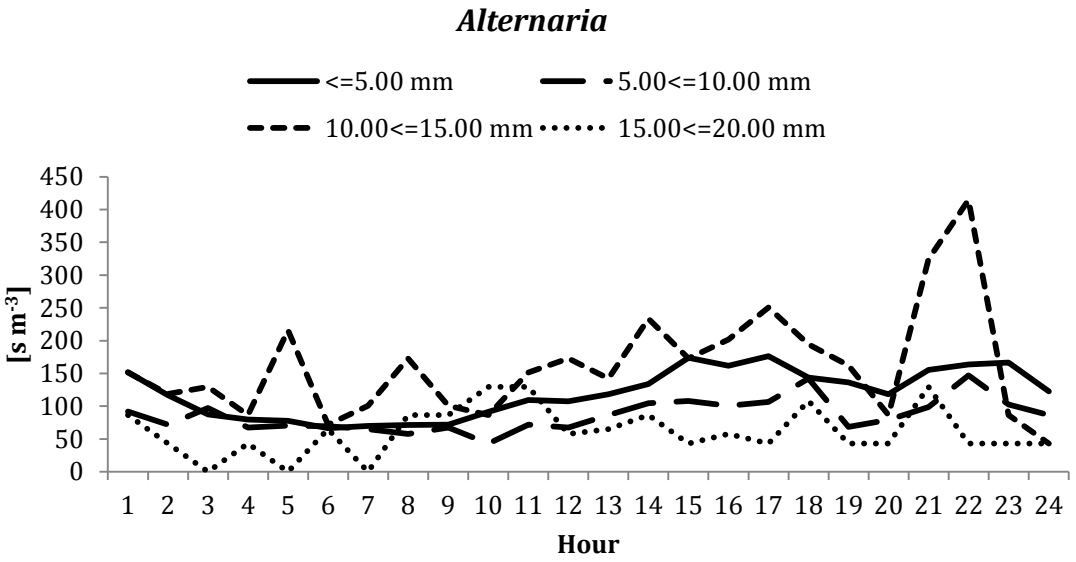


Fig. 4.4a A mean hourly distribution of *Alternaria* spores depending on the amount of rainfall registered within 24 hours for the years 2006-2010 in Worcester, UK.

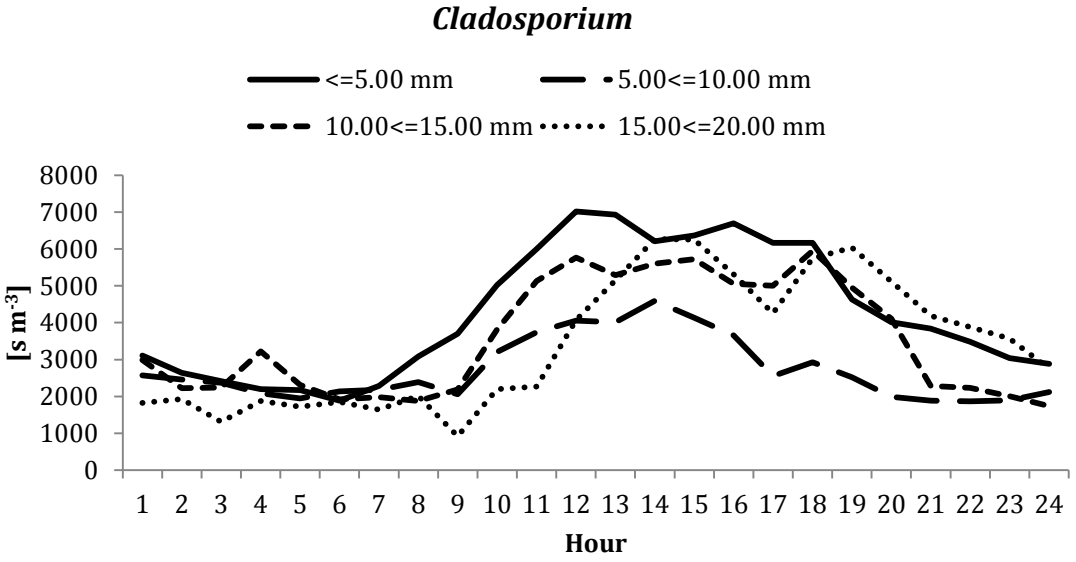


Fig. 4.4b A mean hourly distribution of *Cladosporium* spores depending on the amount of rainfall registered within 24 hours for the years 2006-2010 in Worcester, UK.

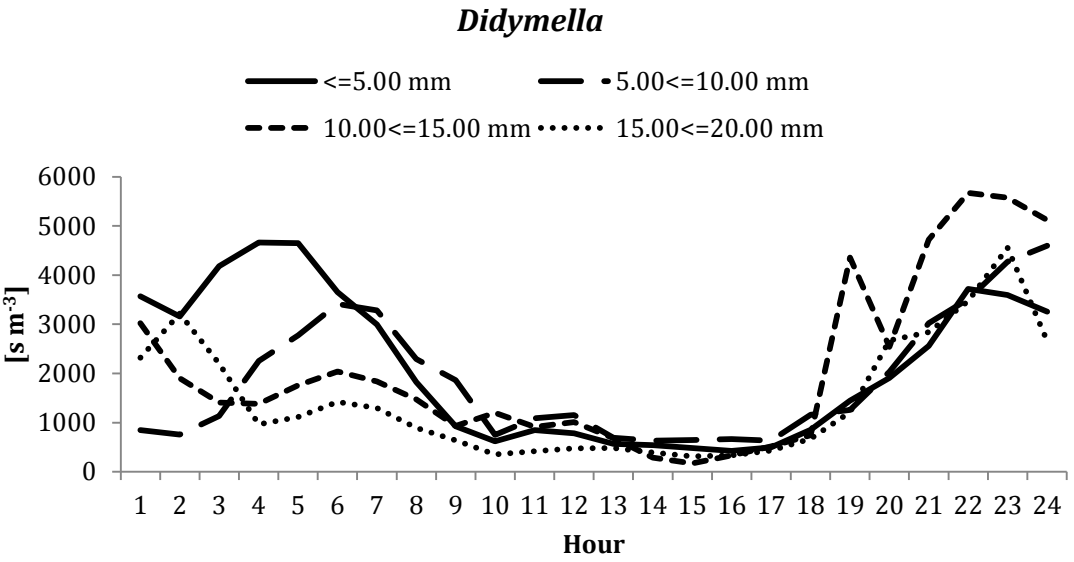


Fig. 4.4c A mean hourly distribution of *Didymella* spores depending on the amount of rainfall registered within 24 hours for the years 2006-2010 in Worcester, UK.

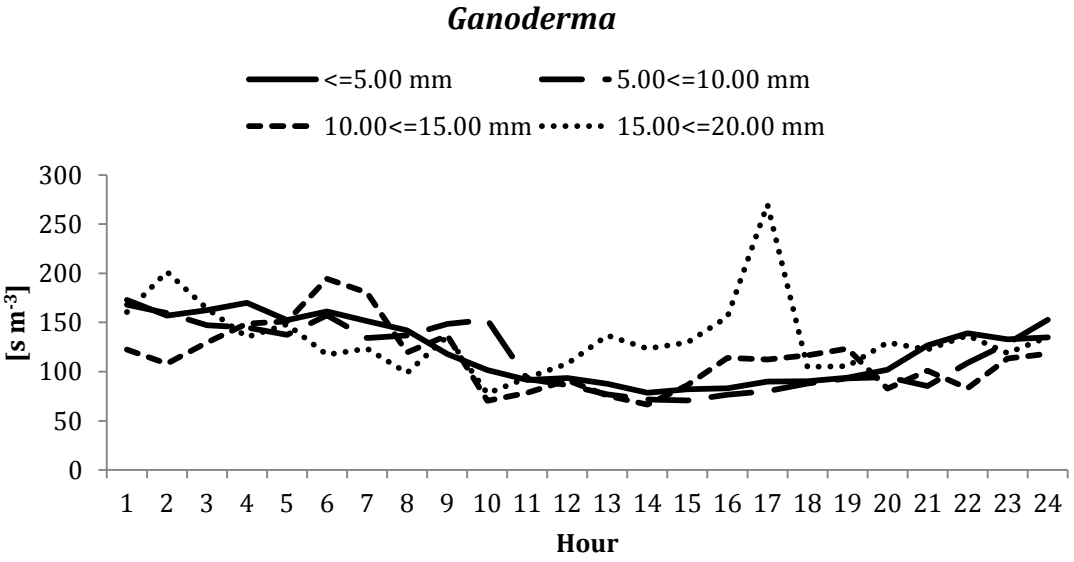


Fig. 4.4d A mean hourly distribution of *Ganoderma* spores depending on the amount of rainfall registered within 24 hours for the years 2006-2010 in Worcester, UK.

Table 4.4 The results of the Friedman test for the comparison of changes in diurnal pattern of examined fungal spores in response to various amount of rainfall recorded within 24 hours.

Taxon	Parameter	2006	2007	2008	2009	2010	2006-10
Alternaria	χ^2	42.21	47.55	41.58	38.90	27.20	134.92
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Cladosporium	χ^2	58.25	36.55	41.90	36.15	38.75	59.14
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Didymella	χ^2	47.41	18.20	8.85	34.55	25.37	45.17
	p	**0.00***	**0.00***	**0.03***	**0.00***	**0.00***	**0.00***
Ganoderma	χ^2	16.65	28.09	1.68	10.29	6.61	13.15
	p	**0.00***	**0.00***	*ns*	**0.02***	*ns*	**0.00***

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

4.2.5. Response to variations of relative humidity

The change in relative humidity was also investigated for its impact on hourly variations of *Alternaria, Cladosporium, Didymella* and *Ganoderma* spore concentration in the air in Worcester (Fig. 4.5a-d). The lowest relative humidity (*n*), which occurred within the study period, was 40%. To enable an easy comparison with impact of precipitation on diurnal pattern of selected fungal spores, relative humidity was divided into four groups: $40<n\leqslant55\%$, $55<n\leqslant70\%$, $70<n\leqslant85\%$ and $85<n\leqslant100\%$.

Three out of four fungi showed that drier conditions favoured spore dispersal, *i.e.*: where relative humidity varied between 40% and 55% (Fig. 4.5a-d and Fig. 4.5d). The maximum concentration of *Alternaria* spores was found at 02:00h, when spore counts were equal to 370 s m^{-3}. Similarly, early in the morning greater concentration of *Ganoderma* spores was observed. A peak occurred at 07:00h and the concentration reached 290 s m^{-3} (Fig. 4.2d). Although identical dry conditions were also required by *Cladosporium*, its peak was noted at 15:00h, when spore concentration above 11000 s m^{-3} was registered (Fig. 4.5b). In contrast, the most beneficial relative humidity for *Didymella* spores was found to be 85% to 100% (Fig. 4.5c). The spore concentration peaked at 23:00h with a count above 6500 s m^{-3}.

The Friedman test confirmed that there was a statistically significant difference in diurnal pattern of all examined fungal spore types associated with

relative humidity during 24 hour periods (Table 4.5). These relationships were observed in each sample year as well as for an averaged five year period (Table 4.5). The exception was found only for the year 2007, where no statistically significance difference in *Ganoderma* spore diurnal profile was noted (Table 4.5).

Table 4.5 The results of the Friedman test for the comparison of changes in diurnal pattern of examined fungal spores in response to the fluctuations of relative humidity.

Taxon	Parameter	2006	2007	2008	2009	2010	2006-10
Alternaria	χ^2	64.45	47.35	52.69	28.83	63.58	108.48
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Cladosporium	χ^2	68.00	13.55	29.75	30.05	67.25	60.32
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Didymella	χ^2	39.80	66.65	55.35	53.25	63.91	264.80
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Ganoderma	χ^2	23.15	6.92	16.41	12.50	51.65	40.01
	p	**0.00***	*ns*	**0.00***	**0.01***	**0.00***	**0.00***

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

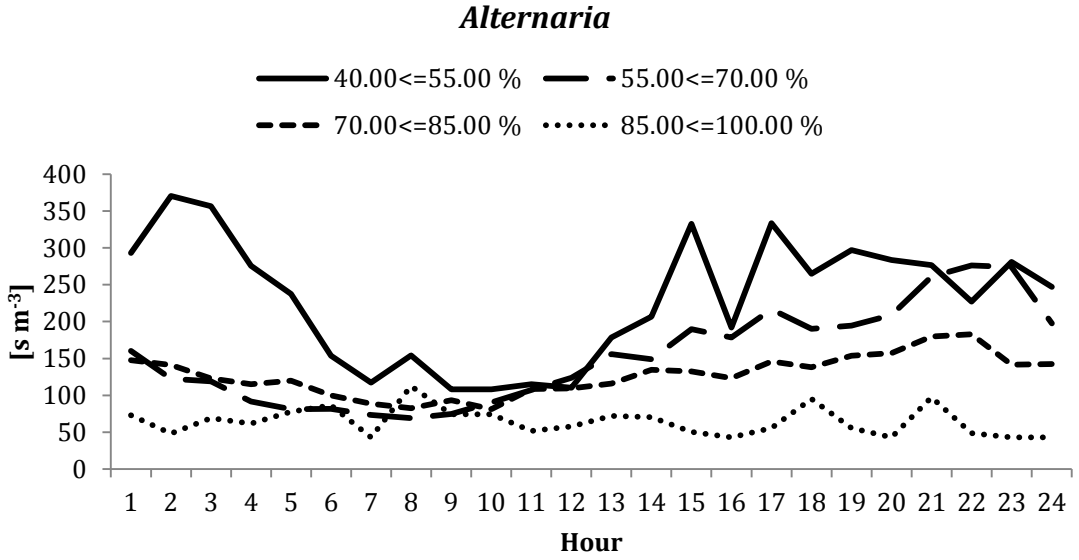


Fig. 4.5a A mean hourly distribution of *Alternaria* spores depending on the variations of relative humidity registered within 24 hours for the years 2006-2010 in Worcester, UK.

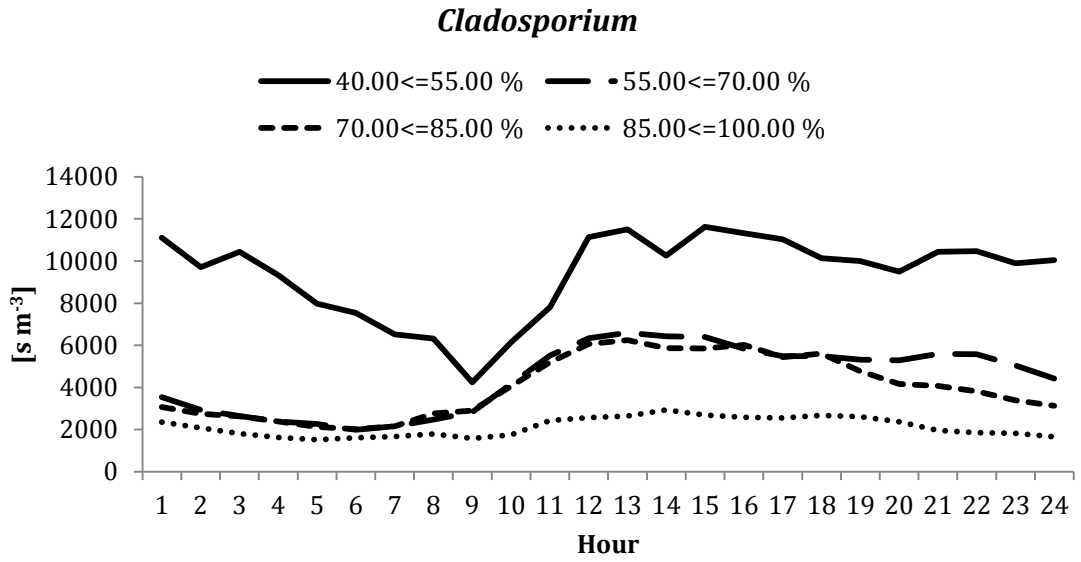


Fig. 4.5b A mean hourly distribution of *Cladosporium* spores depending on the variations of relative humidity registered within 24 hours for the years 2006-2010 in Worcester, UK.

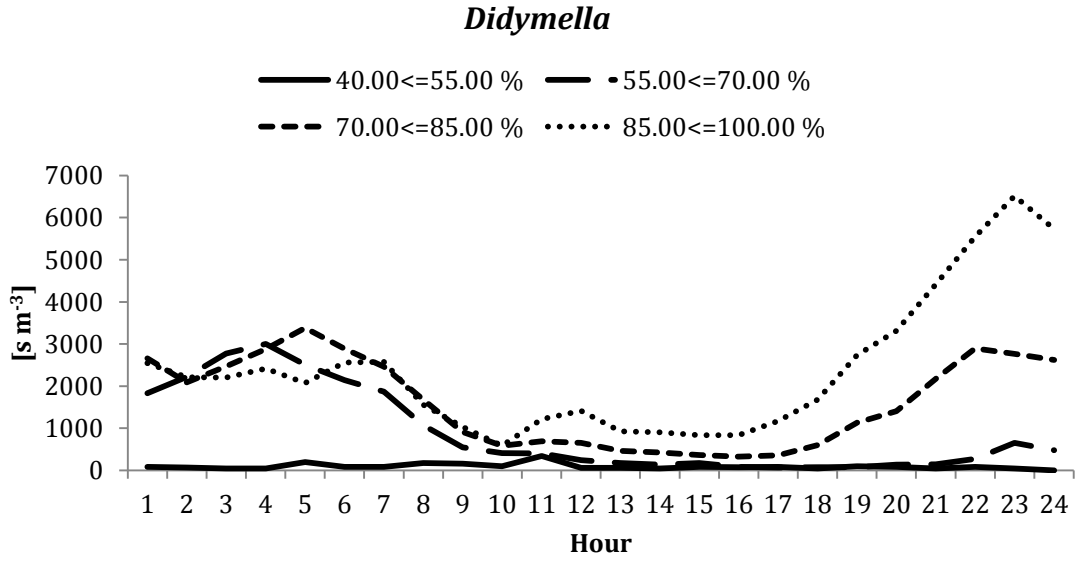


Fig. 4.5c A mean hourly distribution of *Didymella* spores depending on the variations of relative humidity registered within 24 hours for the years 2006-2010 in Worcester, UK.

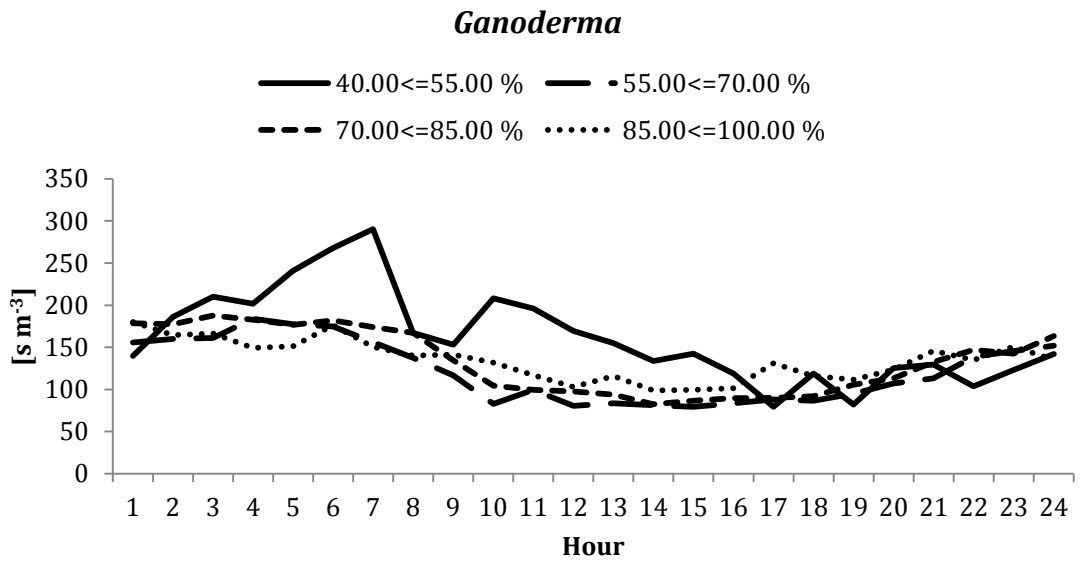


Fig. 4.5d A mean hourly distribution of *Ganoderma* spores depending on the variations of relative humidity registered within 24 hours for the years 2006-2010 in Worcester, UK.

4.2.6. Response to variations of maximum temperature

The maximum temperature was also included in this study (Fig. 4.6a-d). Four classes were identified: $0<n\leqslant10$°C, $10<n\leqslant15$°C, $15<n\leqslant20$°C and $20<n\leqslant25$°C, for this investigation.

The lowest maximum temperature, which varied from 0°C to 10°C had the most significant impact on the diurnal distribution of *Alternaria* spores (Fig. 4.6a). The maximum concentration of *Alternaria* spores (778 s m^{-3}) was recorded at 16:00h. *Didymella* spores were shown to be significantly affected by maximum temperature (Fig. 4.6c). A double peak pattern was observed, and a peak was recorded at 22:00h with a spore count of approximately 5000 s m^{-3}. Maximum temperatures between 20°C and 25°C were favourable for dispersal of *Cladosporium* and *Ganoderma* spores, although maximum spore concentrations were recorded at different times (Fig. 4.6b and Fig. 4.6d). *Cladosporium* was found in the greatest concentrations approximating 12000 s m^{-3} at 15:00h, whereas *Ganoderma* was recorded at a peak number at 06:00h, when the spore count was equal to 262 s m^{-3}.

A statistically significant difference between maximum temperature and diurnal distributions of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* for all individual years and five year averages was indicated by the Friedman test (Table 4.6).

Table 4.6 The results of the Friedman test for the comparison of changes in diurnal pattern of examined fungal spores in response to the changes in maximum temperature.

Taxon	Parameter	2006	2007	2008	2009	2010	2006-10
Alternaria	χ^2	54.34	56.64	57.15	24.29	58.21	170.99
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Cladosporium	χ^2	70.85	59.00	72.00	55.55	66.05	279.11
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Didymella	χ^2	43.85	64.97	61.35	51.95	37.18	218.73
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***
Ganoderma	χ^2	25.65	46.93	39.45	40.55	26.60	136.42
	p	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***	**0.00***

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

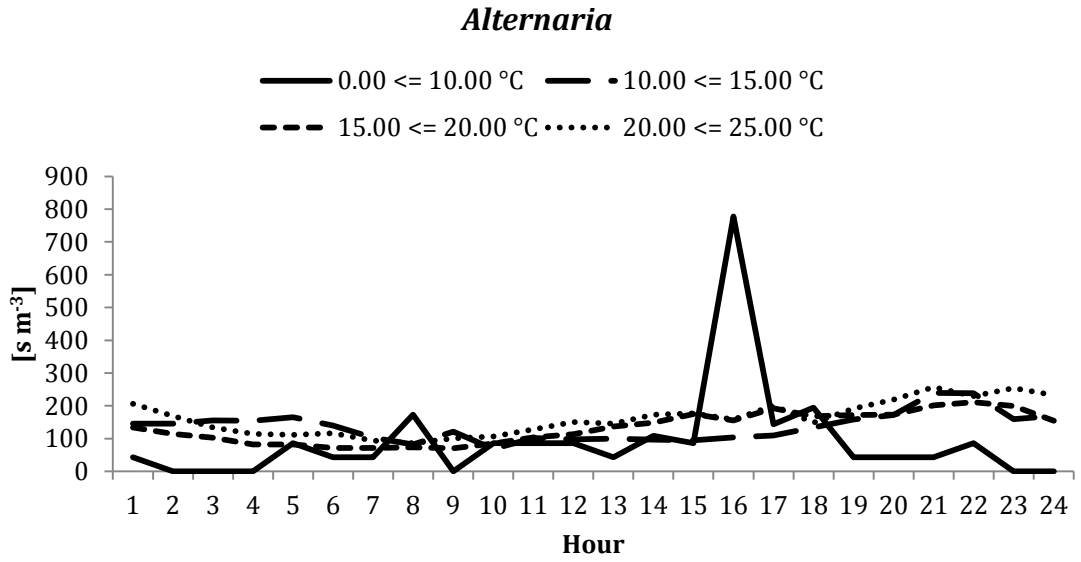


Fig. 4.6a A mean hourly distribution of *Alternaria* spores depending on the variations of maximum temperature registered within 24 hours for the years 2006-2010 in Worcester, UK.

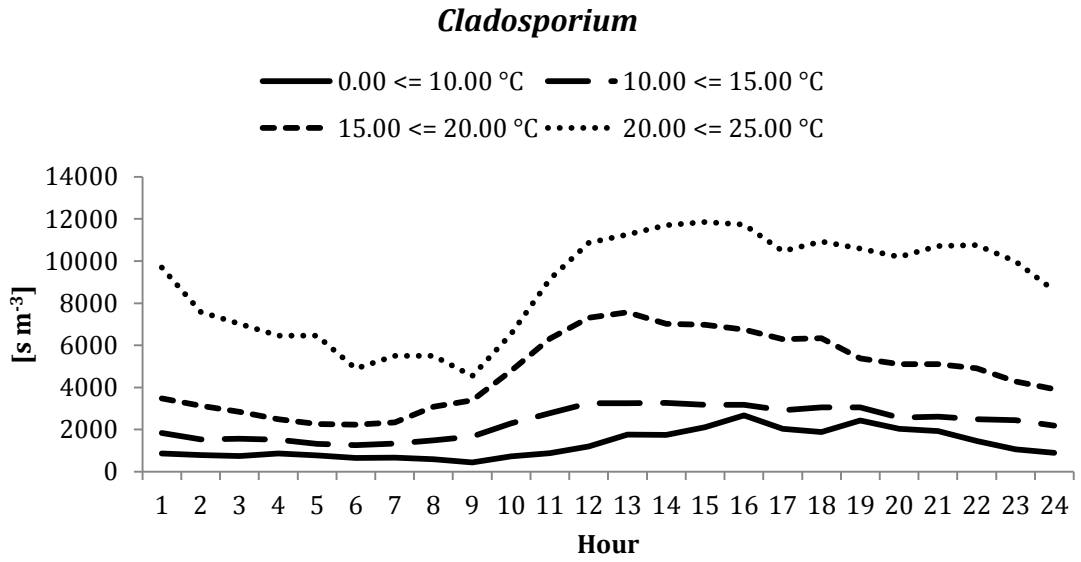


Fig. 4.6b A mean hourly distribution of *Cladosporium* spores depending on the variations of maximum temperature registered within 24 hours for the years 2006-2010 in Worcester, UK.

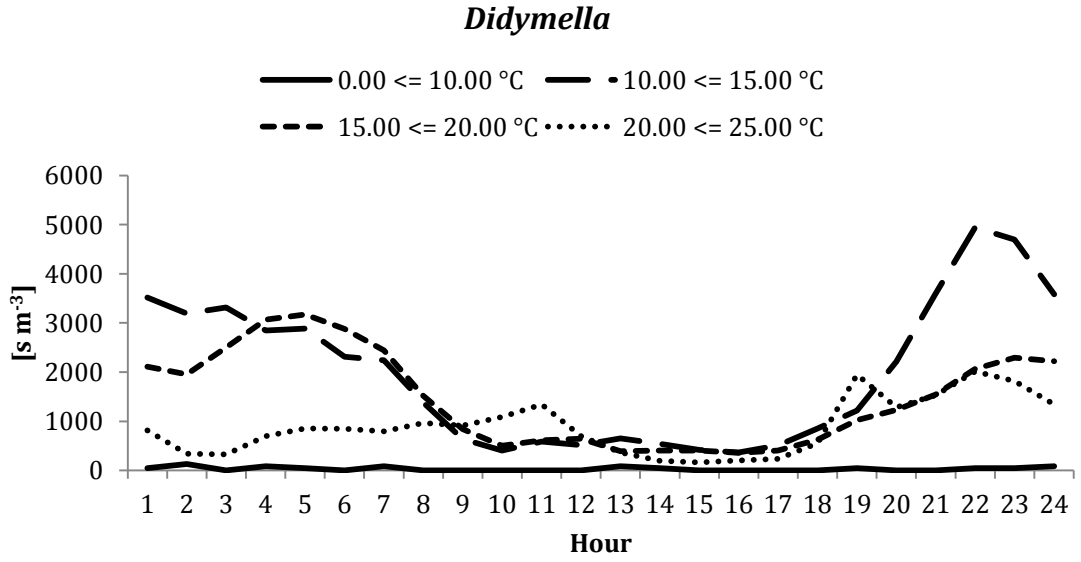


Fig. 4.6c A mean hourly distribution of *Didymella* spores depending on the variations of maximum temperature registered within 24 hours for the years 2006-2010 in Worcester, UK.

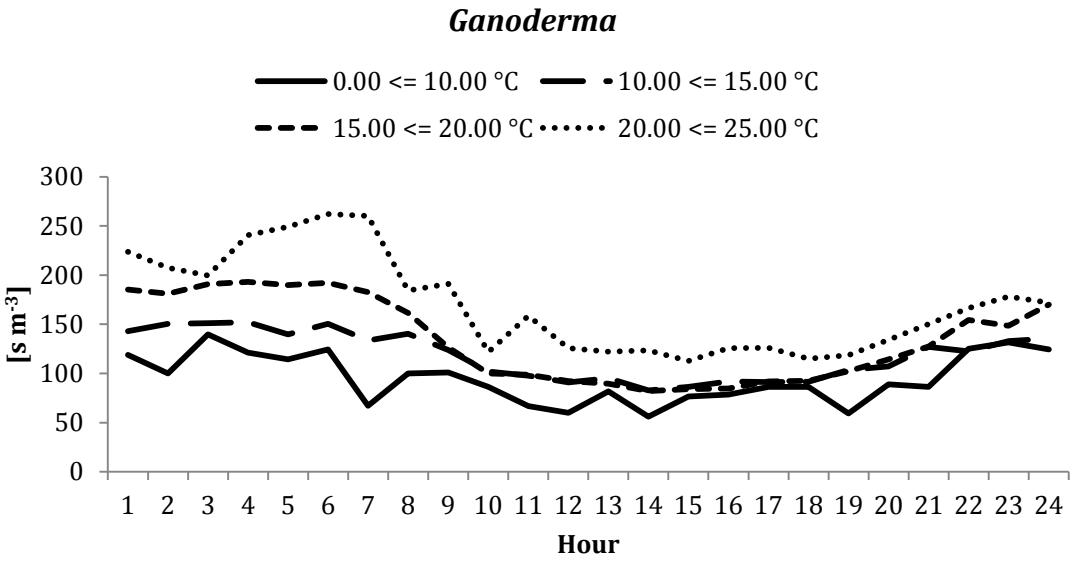


Fig. 4.6d A mean hourly distribution of *Ganoderma* spores depending on the variations of maximum temperature registered within 24 hours for the years 2006-2010 in Worcester, UK.

4.3. Discussion

4.3.1. *Alternaria*

The hourly distribution of *Alternaria* spores was quite constant, although annual differences were detected using the Kruskal-Wallis test (Table 4.1). Similar results were described by Munuera Giner et al. (2001), although these observations were not supported statistically. In Worcester, the most representative years for this taxon were 2008 and 2009 (Fig. 4.1a). In both years and throughout most of the analyses the maximum concentration of *Alternaria* spores occurred at 22:00h (Fig. 4.1a-4.4a), whereas in Derby, UK *Alternaria* peaked two hours earlier (Corden and Millington, 2001). In Valladolid, Spain maximum concentration was found at 00:00h (Sánchez Reyes et al., 2009), and in Murcia, Spain and Copenhagen, Denmark at 19:00h (Munuera Giner et al., 2001; Skjøth et al., 2012). The lowest concentration of *Alternaria* spores were recorded in Worcester between 07:00h and 08:00h, which was also observed in Murcia, Spain (Munuera Giner et al., 2001), whereas in Copenhagen it was observed between 05:00h and 07:00h (Skjøth et al., 2012). In Valladolid the lowest concentration

occurred at 08:00h and 17:00h (Sánchez Reyes et al., 2009). Spore counts were found to be above 50 s m^{-3}, which was sufficient to trigger respiratory problems (Fig. 4.1a).

All the quoted results were obtained at the sampling stations situated in urban zones (*i.e.*: Copenhagen, Derby, Murcia, Valladolid, Worcester). In contrast, the field study conducted at the Rothamsted Research Station, UK showed that *Alternaria linicola* spores achieved the maximum concentration of over 500 s m^{-3} between 12:00h and 13:00h (Vloutoglou, 1994). Monitoring early blight of potato caused by *Alternaria solani* at Pretoria, Republic of South Africa showed that conidia were trapped between 09:00h and 18:00h, with a peak recorded around 15:00h (van der Waals et al., 2003). *Alternaria brassicicola* measured at an experimental cabbage plot was found in the greatest concentration occurring at 10:00h (Chen et al., 2003). The mid-day pattern in *Alternaria* airborne spore concentrations was found for other *Alternaria* species, such as *A. dauci* (Langenberg et al., 1977) or *A. porri* (Meredith, 1966). This could suggest that the night time spore peaks recorded in the cities were a result of the horizontal transport of *Alternaria* spores in the air from the nearby sources during that time (Skjøth et al., 2012).

Different applied threshold values did not seem to have any impact on changes of diurnal distribution, although the sign test showed the presence of statistically significant disparities between diurnal profiles (Table 4.2). This type of analysis has not been previously reported.

The occurrence of rainfall on the trapping day was found to change the hourly distribution of *Alternaria* spores except in years 2006 and 2009, where no statistically significant difference was found (Table 4.3). Overall, a small reduction in the concentration was observed (Fig. 4.3a). These findings were in agreement with previously reported results by Stępalska and Wołek (2009b), who found statistically important differences between "dry" and "rainy" days for one individual year and a joint three year period. Vloutoglou (1994) reported that well-defined diurnal distribution of *Alternaria linicola* spores was seen only during the "dry days" in a linseed crop at the Rothamsted Research Station.

Low relative humidity, within a range of 40% to 55%, appeared to influence the amount of *Alternaria* trapped up to a maximum 370 s m^{-3} (Fig. 4.5a). This agreed with the outcomes of the Friedman test (Table 4.5). In Spain Maya-Manzano et al. (2012) found that distribution of *Alternaria* spores was significantly correlated with relative humidity, which would confirm the results obtained in the present study. Very dry conditions also had an influence by appearing to delay the maximum concentrations detected until 02:00h (Fig. 4.5a). The field observations of circadian distribution of various species of *Alternaria* reported that the maximum spore concentration occurred during the lowest relative humidity conditions (Chen et al., 2003; Langenberg et al., 1977; van der Waals et al., 2003). Meredith (1963) observed that decrease in relative humidity caused twisting of the conidial chains. Once this movement stopped after two or three seconds gas bubbles occurred, which subsequently lead to breaks of the chain and conidia liberation (Meredith, 1963). Changes in relative humidity either early in the morning or in the evening were found to influence the spore release by *Drechslera turcica*, *Alternaria alternaria* and several other fungal species, although a greater number of released conidia was seen when relative humidity was decreasing rather than increasing (Leach, 1975). Liberation of spores occurred both in darkness and brightness, while the infrared radiation alone triggered spore release at stable relative humidity (50%-65%), but not at fully saturated air (Leach, 1975).

However, a more significant impact than relative humidity had precipitation. A small amount of rainfall ($10<n\leqslant15$ mm) occurring within 24 hours could actually induce a concentration of *Alternaria* spores in the air in Worcester of up to 415 s m^{-3} (Fig. 4.4a). Observations from Spain also indicated an increasing trend in concentration of *Alternaria* spores, up to four days after the presence of precipitation (Maya-Manzano et al., 2012), although the authors in this study did not mention the amount of rainfall.

Optimum maximum temperatures of between 0°C and 10°C favoured the highest concentrations of *Alternaria* spores (778 s m^{-3}), and even accelerated the peak from 22:00h to 16:00h (Fig. 4.6a). No other study has examined the influence of maximum temperature on diurnal distribution of airborne *Alternaria* spores.

4.3.2. *Cladosporium*

Annual variations in diurnal profile of *Cladosporium* spores were found to be statistically significant as shown by the Kruskal-Wallis test (Table 4.1). The pattern was distinct for years 2006 and 2010 (Fig. 4.1b), where the maximum concentration of *Cladosporium* spores was found at 15:00h and 13:00h respectively, with spore counts exceeding 10000 s m^{-3}. The minimum concentration, below a clinical threshold equal to 3000 s m^{-3} (Frankland and Davies, 1965), was observed in the morning, between 07:00h and 09:00h (Fig. 4.1b). Previously, Adams (1964) reported, that in Cardiff, UK *Cladosporium* was seen at the greatest concentration between 12:00h and 15:00h, and the lowest concentration from 01:00h to 07:00h. However in these studies spore peaks were approaching 45000 s m^{-3} (Adams, 1964). The concentration of *Cladosporium* spores measured in the wheat field was found to be the greatest between 07:00h and 08:00h (Pady et al., 1969). Occasionally peaks were also observed at 03:00h (Pady et al., 1969).

The number of *Cladosporium* spores per cubic metre of air significantly increased during "rainy days", especially between 07:00h and 19:00h (Fig. 4.3b). Stępalska and Wołek (2009b) did not find any statistically significant difference between "dry" and "rainy" days, although a small positive influence of rainfall was seen between hours 14:00-19:00h and 00:00-08:00h.

Further examination of the amount of rainfall, revealed that 0 mm and 5 mm within 24 hour period was needed for *Cladosporium* spore release and dispersal. The diurnal pattern remained unchanged, as a peak was found at 12:00h (Fig. 4.4b). Results of the Friedman test confirmed that various amount of rainfall have had a different impact on diurnal distribution of *Cladosporium* spores (Table 4.4). Troutt and Levetin (2001) reported that 30 mm of rainfall within one hour (at 02:00h) was sufficient enough to reduce *Cladosporium* spore concentration from 3000 s m^{-3} to 0 s m^{-3}.

A significant impact on the trapped *Cladosporium* spore concentration was found for the relative humidity (Fig. 4.5b). Maximum concentration was registered three hours after a peak of relative humidity. The spore count increased by 36% in

comparison with propitious amount of rainfall. Changes in relative humidity affected the hourly variations of *Cladosporium* spores, and this was in agreement with the results obtained by the Friedman test (Table 4.5).

The effect of maximum temperature in Worcester were in agreement with results obtained by Troutt and Levetin (2001), who indicated that maximum temperature was the most important weather parameter that influenced hourly variations of *Cladosporium* spores in the air of Tulsa, US. Although application of multiple regression analysis explained hourly variation of *Cladosporium* spores by maximum temperature in 21%, but no range of the temperature was given by the authors (Troutt and Levetin, 2001).

4.3.3. *Didymella*

Didymella was the only taxon that exhibited a double peak pattern in Worcester, with peaks observed between 04:00h and 05:00h, and then again between 22:00h and 23:00h (Fig. 4.1c). However, the measurements taken in the chickpea field located in the vicinity of Córdoba, Spain did not show bimodal distribution in the presence of *Didymella rabiei* spores (Trapero-Casas et al., 1996). Maximum spore concentration was recorded between 14:00h and 16:00h (Trapero-Casas et al., 1996).

Application of *Cladosporium* threshold values helped to visualise a distinctive pattern of hourly variations in *Didymella* spore occurrence (Fig. 4.2c).

The presence of rainfall had the most significant impact on the diurnal distribution of hourly *Didymella* spore counts, while "dry days" did not show any specific pattern at all (Fig. 4.3c). A similar positive impact of rainfall was noted by Trapero-Casas et al. (1996), who caught 94% of airborne *Didymella rabiei* spores using a Hirst trap during "rainy days" in a five year study. In contrast, observations taken in Kraków, Poland showed a more distinctive diurnal pattern during "dry days" rather than in "rainy days" (Stępalska and Wołek, 2009b). Moreover, Harries et al. (1985) noted a clear diurnal pattern for both "dry" and "rainy" days in the hourly distribution of *Didymella* spores in London, UK. In Worcester, maximum concentrations were apparent, both at 05:00h and 23:00h hours (Fig. 4.3c),

whereas in Kraków a single peak occurred between 03:00h and 04:00h (Stępalska and Wołek, 2009b). The sign test confirmed the statistically significant difference between "dry" and "rainy" days for each individual year, as well as for a five year averaged period of time in Worcester (Table 4.3). The disparity between "dry" and "rainy" days was also found by Stępalska and Wołek (2009b), after a three year period of sampling (z=-2.75, p=0.03). A small increase in numbers of spore was also seen by Stępalska and Wołek (2009b) after dusk, from 17:00h to 00:00h during "rainy days".

Another analysis provided information that 10 mm to 15 mm of rainfall influenced the presence and concentration of *Didymella* spores in Worcester, although all variations in hourly distribution were statistically significant (Fig. 4.4c and Table 4.4). Harries et al. (1985) recorded a spore count of *Didymella exitialis* equal to 7000 s m^{-3} at the Rothamsted Research Station, when 4 mm of rain was recorded, four hours after the occurrence of a thunderstorm. However, Trapero-Casas et al. (1996) stated that discharge of *Didymella rabiei* ascospores was more dependent on the presence of rainfall rather than the amount of rain based on five years of observations.

A very strong association between high *Didymella* spore counts with relative humidity conditions between 85% and 100% were observed (Fig. 4.5c). Similarly von Wahl and Kersten (1991) reported an important correlation (r=0.40) between relative humidity above 80% and concentration of *Didymella* spores at a significance level of p<0.001. Richardson (1996) reported that a sudden increase in *Didymella* spore concentration (above 4000 s m^{-3} at 05:00h) in the air in Edinburgh, Scotland was explained by an increase in relative humidity up to 95%. The highest peak, which was recorded at the Worcester monitoring site, had a value equal to 6500 s m^{-3} at 23:00h (Fig. 4.5c). The Friedman test once more confirmed all the observed dependences (Table 4.5).

Maximum temperature without a time lag was revealed to be a less important factor influencing concentration of *Didymella* spores within 24 hour periods in Worcester. Significant *Didymella* spore concentrations were found to be associated with a temperature range of 10°C-15°C (Fig. 4.6c). This finding would

be in agreement with the observations from Edinburgh, where *Didymella* spore concentrations varying between 1001 and 5000 s m^{-3} were found at the temperature equal to 11°C (Richardson, 1996). Packe and Ayres (1985) suggested that higher temperatures, up to four days before the occurrence of the thunderstorm, could accelerate maturation of spores that then were ready to be released under higher relative humidity conditions. This was examined and further confirmed by Corden and Millington (1994) in Derby, UK, who determined a mean temperature value of 20°C to be important. The observations of infections of cucurbits caused by *Didymella bryoniae* in the greenhouses led to a conclusion that the optimum temperature for infection varied between 16°C and 24°C (Miller et al., no date). This was in agreement with results obtained by Corden and Millington (1994).

4.3.4. *Ganoderma*

Kasprzyk (2006a) measured the diurnal periodicity of *Ganoderma* spores in rural and urban areas. This study reported that *Ganoderma* spores peaked twice within 24 hours in a rural area, at 23:00h and then again at 03:00h. However the same peaks occurred in urban area at 03:00h and 07:00h. This pattern in diurnal distribution of *Ganoderma applanatum* has been reported by de Groot (1968), who took measurements in the middle of a forest. He observed three peaks of spore release, beginning at 21:00h, then at 02:00h and 06:00h, where each peak was smaller than the previous one. Late *Ganoderma* spore peaks in Worcester could be evidence for long distance transport up to several dozens of kilometres (Fig. 4.2d), depending on the speed of air masses movement that carried spores from their sources.

Although the overall pattern of hourly *Ganoderma* spore distribution remained constant, several changes have been observed during "rainy days" in comparison to "dry days" (Fig. 4.3d). The maximum concentration was found at 01:00h, five hours earlier than during the days without precipitation (Fig. 4.3d). The presence of rainfall had a negative impact on *Ganoderma* spore concentration, as reduced numbers of spores were found for each hour (Fig. 4.3d). The difference

between curves was confirmed by the results obtained by the sign test, with the exception of the data in 2010 (Table 4.3). The disparity between "dry" and "rainy" days was also found by Stępalska and Wołek (2009b), after three years of monitoring (z=2.13, p=0.03). Furthermore, in contrast to Worcester, precipitation in Kraków, Poland revealed a positive impact of rainfall on *Ganoderma* spores, and an increase in concentration was noted between 03:00h and 12:00h, as well as from 21:00h and 22:00h (Stępalska and Wołek, 2009b). Presence of rainfall did not affect the time when maximum concentration was observed, which was between 02:00h and 03:00h (Stępalska and Wołek, 2009b).

On the other hand, higher concentrations of *Ganoderma* spores were found, when relative humidity was recorded within the range of 40% to 55%. Other authors reported that peaks of *Ganoderma* spores were found at greater relative humidity, *i.e.*: 77% (McCracken, 1987) and 79% (Craig and Levetin, 2000).

Maximum temperature from 20°C to 25°C was found to have an influence on the changes in diurnal pattern of *Ganoderma* spores (Fig. 4.6d). Similar results were obtained by Craig and Levetin (2000), who observed spore peaks at a mean temperature of 22°C, in contrast to McCracken (1987), who found a peak at a lower mean temperature of 17°C.

4.4. Conclusions

Diurnal distributions of studied allergenic fungal spores, *i.e.*: *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* were fairly constant during five years of the aerobiological monitoring conducted in Worcester, UK. Several statistical tests, which were used in detailed examination, confirmed dependences between fungal spore concentration in the air and hourly variation as a response to changes of environmental factors (Table 4.7). Different fungal taxa revealed various optimal conditions for the spore release and dispersal processes (Table 4.7), such as *Didymella*, which was observed "to burst" on "rainy days" or *Alternaria*, which could accelerate the time of spore release, if the maximum temperature was very low. This information constitutes an important component of the knowledge about biology from which forecasting models will be constructed.

Table 4.7 The summary table.

Meteorological parameter	*Alternaria*	*Cladosporium*	*Didymella*	*Ganoderma*
Rainfall [mm]	$10 < n \leq 15$	$0 < n \leq 5$	$10 < n \leq 15$	$15 < n \leq 20$
Relative humidity [%]	$40 < n \leq 55$	$40 < n \leq 55$	$85 < n \leq 100$	$40 < n \leq 55$
Maximum temperature [°C]	$0 < n \leq 10$	$20 < n \leq 25$	$10 < n \leq 15$	$20 < n \leq 25$

Chapter 5

Spatial analyses in fungal spore distribution

5.1. *Alternaria*

5.1.1. Introduction

Alternaria spores constituted an important fraction of the total spore load registered in Worcester during the studied period. This has been described in more detail in Chapter 3. Methods used in this study were described in Chapter 2.

Less attention has been paid to fungi, which cause pathogenesis of vascular plants constituting substantial components of pastures and meadows, which are in fact another potential source of *Alternaria* (Scudamore and Livesey, 1998) in comparison to well-known crops under rotation (Boedo et al., 2012; Meredith, 1966; Olanya et al., 2009). Samples of maize and hay directly taken from the field showed that the greatest amounts of *Alternaria* mycotoxins derived from *Alternaria tenuissima* and *A. alternata* (Müller, 1992). The latter species was identified as a major pathogen of white clover (Zahid et al., 2002). Other studies indicated also the negative impact of *Alternaria alternata* on spotted knapweed (Stierle et al., 1988), St. John's wort and lovage (Frużyńska-Jóźwiak and Andrzejak, 2007). Furthermore, *Alternaria dianthus* was frequently isolated from dianthus (Mikulík et al., 2002), and *Alternaria nobilis* from soapwort (Garibaldi et al., 2013).

5.1.2. Results

5.1.2.1. Distribution of *Alternaria* spores

During a five year study conducted in Worcester (2006-2010) 100 days were observed where *Alternaria* spore concentration was equal to or above 100 s

m^{-3}. The highest daily mean spore concentration within the examined period of time was recorded on the 8th of August 2009 with a spore count equal to 644 s m^{-3}. This day required a more detailed analysis since it was observed that high *Alternaria* spore concentrations were recorded through six consecutive days oscillating around the major peak, *i.e.*: 133 s m^{-3}, 326 s m^{-3}, 644 s m^{-3}, 563 s m^{-3}, 101 s m^{-3} and 263 s m^{-3} respectively.

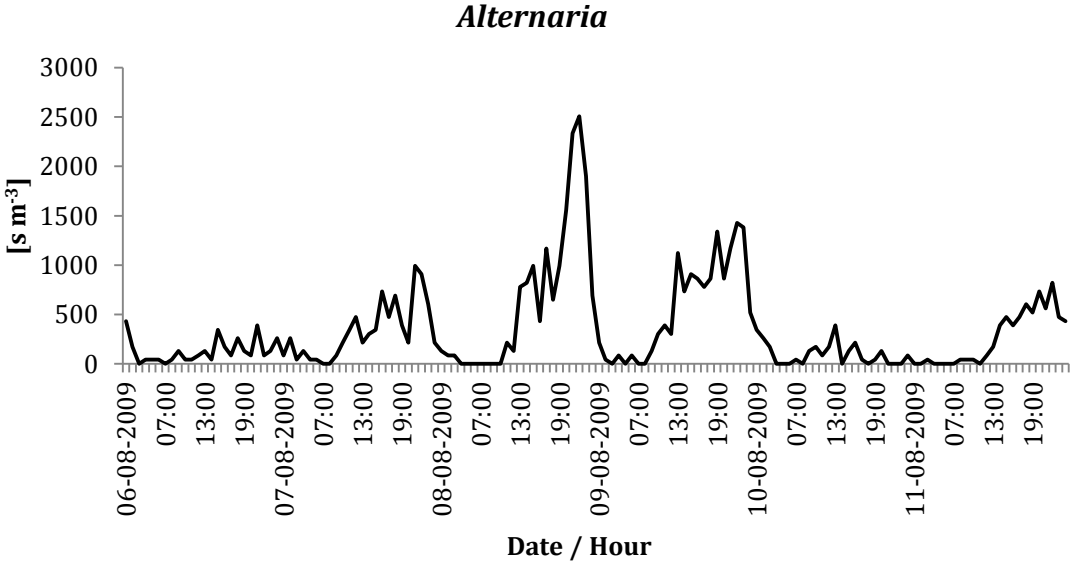


Fig. 5.1 Hourly variations in *Alternaria* spore distribution recorded between 6th and 11nd of August 2009 in Worcester, UK.

Throughout this episode, the maximum hourly spore concentration was observed mostly during the night hours, 01:00h with a spore count 432 s m^{-3} (6th of August 2009), 21:00h with a spore count 994 s m^{-3} (7th of August 2009), 22:00h with a spore count 2507 s m^{-3} (8th of August 2009), 22:00h with a spore count 1426 s m^{-3} (9th of August 2009), 13:00h with a spore count 389 s m^{-3} (10th of August 2009) and 22:00h with a spore count 821 s m^{-3} (11th of August 2009), (Fig. 5.1). The lowest *Alternaria* spore concentrations were found mostly between 03:00h and 08:00h in the morning (Fig. 5.1).

5.1.2.2. Source maps

Two source maps were produced based on the Corine Land Cover 2000 (EU, 2005). Non-irrigated arable land and permanently irrigated arable land were

extracted and jointly presented as "crops under rotation" following Skjøth et al. (2012). The distribution of crops under rotation in the United Kingdom was expressed as percentage with a 5 x 5 km grid. Second map showed the distribution of pastures in the United Kingdom, presented as a percentage within a 5 x 5 km grid. The crops under rotation source map showed that the density of the cultivated areas were found to be below 20% in each grid cell in almost all of Scotland, Wales, Northern Ireland, North West England and the city of London (Fig. 5.2). Similarly low surface cover of arable land was found in Ireland, especially in Ulster, Connaught and Munster regions (Fig. 5.2). In contrast, the largest densities of the crops under rotation varying between 80% and 100% were detected in East Midlands, East of England, the southern part of the Yorkshire and Humber, and in Northern France (Fig. 5.2). Minor sources of crops could also be seen in the North West England, West Midlands, South West England and the Leinster region in Ireland (Fig. 5.2). The pasture map showed that the density of the grasslands was found below 20% in each grid cell in almost all of Scotland, partly in England (East Midlands and East of Anglia), western coasts of Ireland (Connaught region), Northern Spain, Belgium and the majority of France (Fig. 5.3). In contrast, the largest densities of pastures varying between 80% and 100% were seen in majority of Ireland, Wales, England (West Midlands, South West England, North West England), and the Netherlands (Fig. 5.3). Minor sources were detected in France (Cotentin Peninsula, central France), (Fig. 5.3).

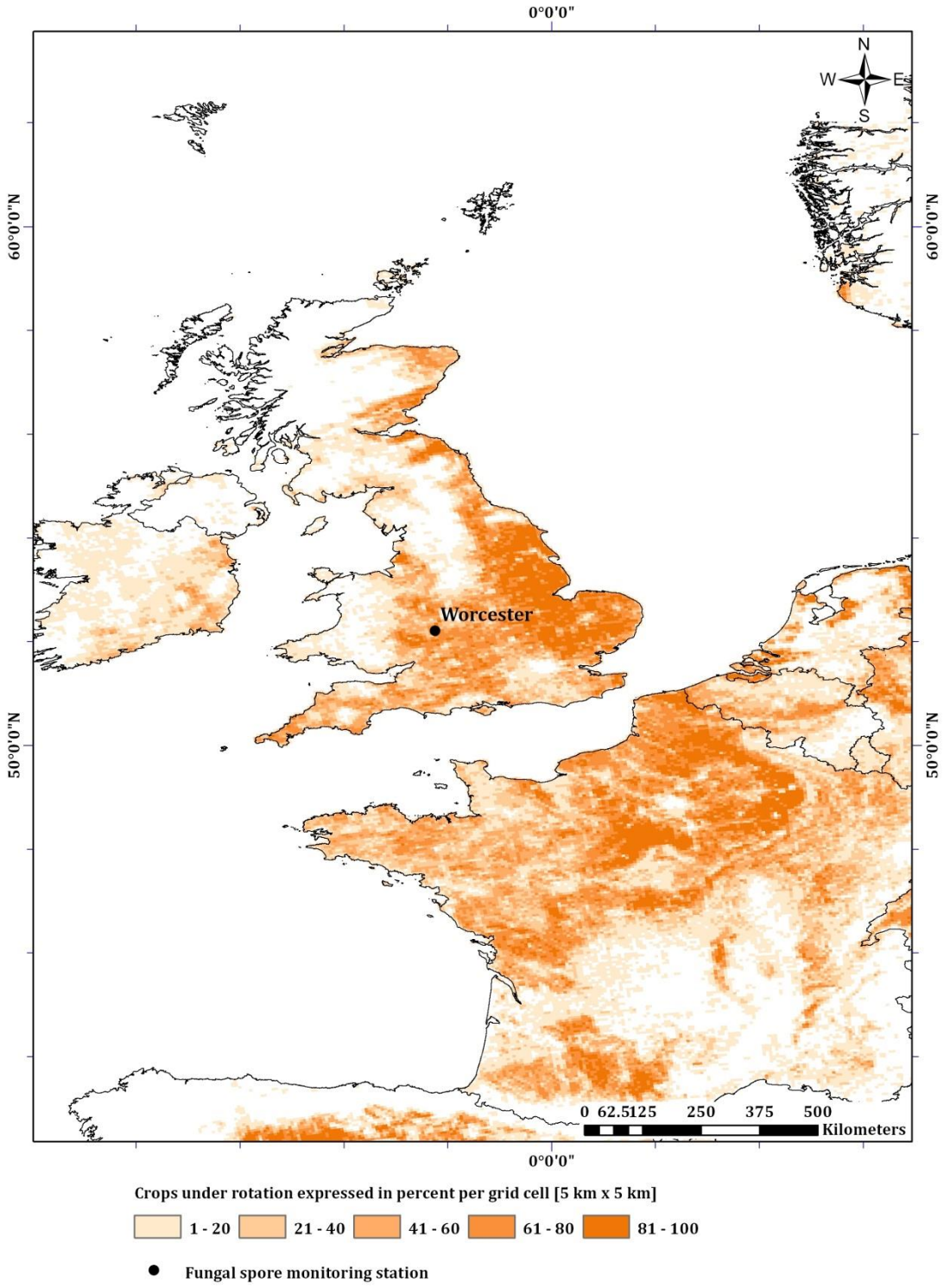


Fig. 5.2 The source map showing distribution of crops under rotation expressed in percent in 5 x 5 km grid cells and location of the aerobiological monitoring station.

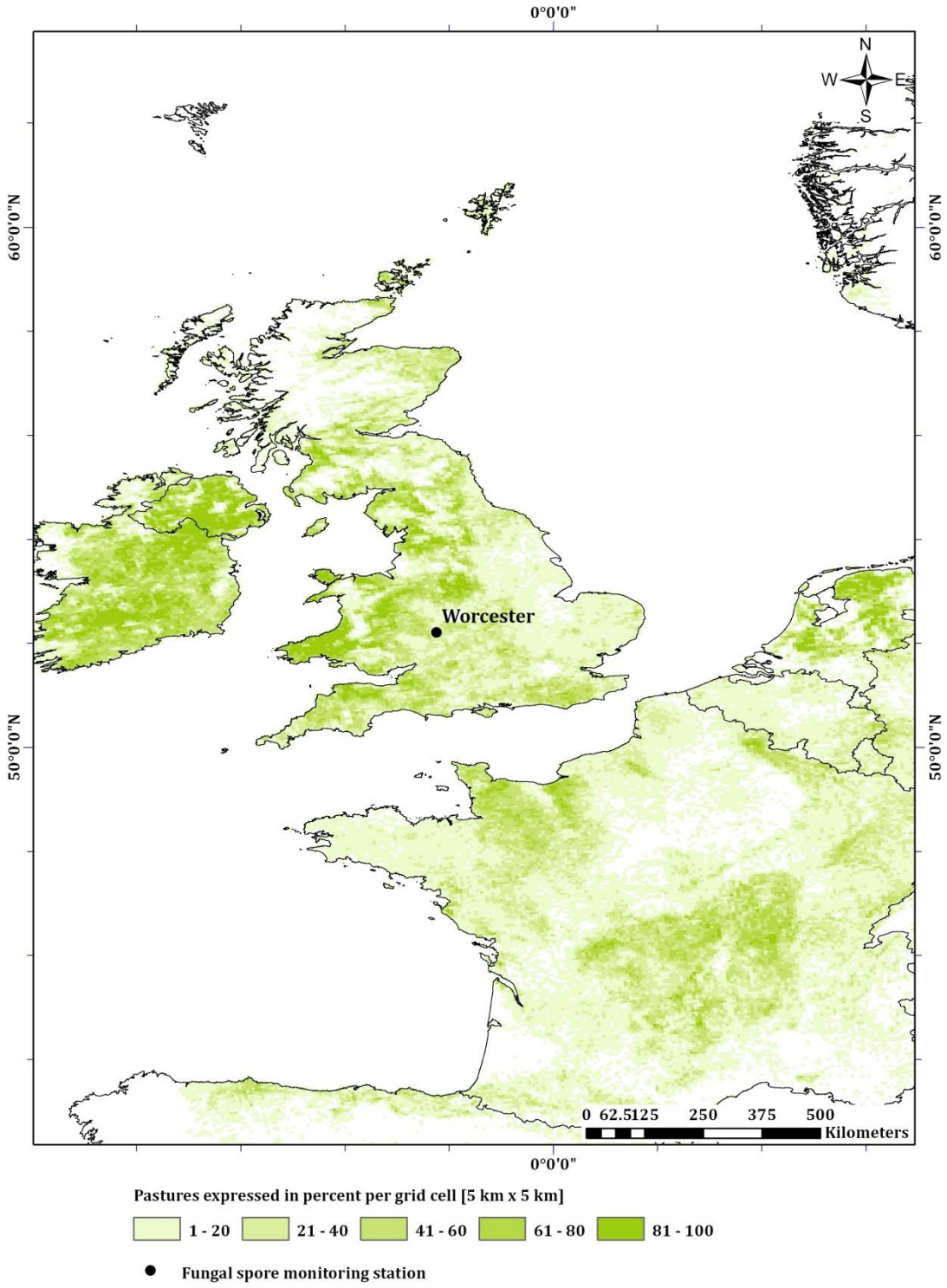


Fig. 5.3 The source map showing distribution of pastures expressed in percent in 5 x 5 km grid cells and location of the aerobiological monitoring station.

5.1.2.3. Weather synopsis

In Worcester during this period the mean temperature varied between 17°C and 20°C (Fig. 5.4). Rainfall was recorded only on the 7th of August 2009 and on the 10th of August 2009, when 1 mm and 0.60 mm of rain fell respectively. The relative humidity fluctuated accordingly, and the driest conditions were recorded on a peak day, the 8th of August 2009 (63%), while the most humid conditions were observed two days later (80%), (Fig. 5.4).

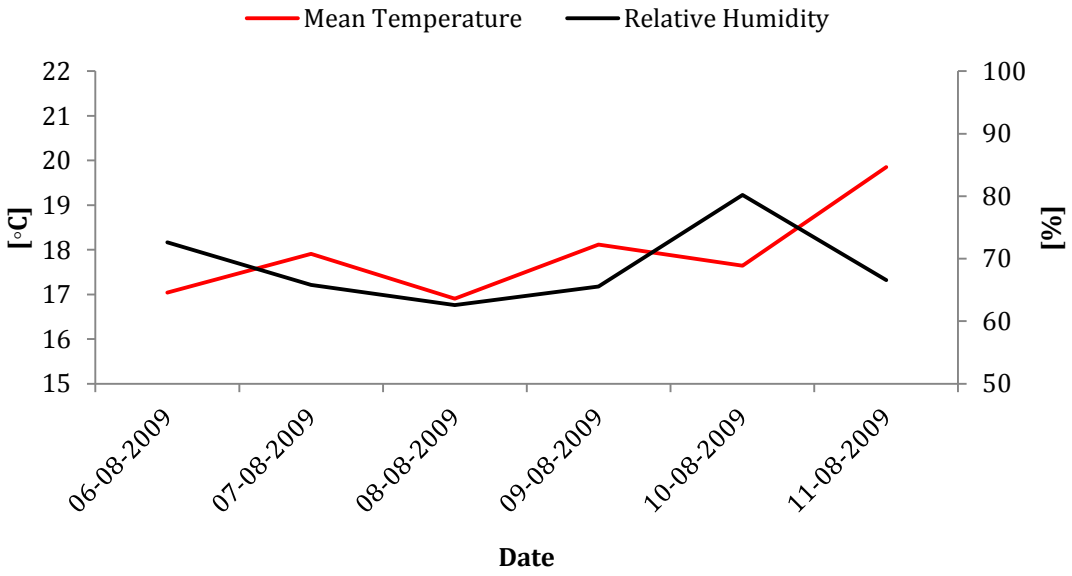


Fig. 5.4 Changes in mean temperature and relative humidity recorded between 6th and 11th of August 2009 in Worcester, UK.

Since the 5th of August 2009 Great Britain was under influence of a strong low pressure system, which was centred on the North Atlantic Ocean (982 hPa), between Iceland and Ireland. On the 6th of August 2009 a formation of a high pressure system was located on the western coast of Spain on the Atlantic Ocean (1024 hPa). At the same time it was possible to see a movement of another high pressure system from the Scandinavian Peninsula towards United Kingdom. The following day brought a slightly improvement in the weather, as England became in the sphere of influence of two high pressure systems. The weather remained almost unchanged on the 8th of August 2009. Subsequently a new low pressure

system started migrating towards British Isles, which had originated on the previous day by the western coast of Ireland (1010 hPa). Within the following 24 hours this low pressure system covered fully Ireland, Scotland and North England. However, simultaneously another high pressure system slowly shifted along the Spanish and French coastline, and approached southern England on the 10th of August 2009. On the 11th of August 2009 this high pressure system was then pushed out by new low pressure system coming over North Atlantic Ocean (1004 hPa). This system fully covered the British Isles on the next day.

5.1.2.4. Back trajectories

During the examined period of time, between 6th and 11th of August 2009, air masses spent from 46.85% to 85.29% of the time passing over the sea areas (Table 5.1). Time that was spent above the land areas was entirely limited to the territory of the British Isles, therefore no contamination was found that could originate from the continental Europe (Table 5.1). On two days, 6th and 9th of August 2009, the air masses that reached Worcester were exclusively moving above Wales and England (Fig. 5.5 and Fig. 5.8). In four cases it was observed that the air masses spent a minor fraction of time over Ireland, and this varied from 2.38% (10th of August 2009) to 18.28% (11th of August 2009), (Table 5.1). The overall direction of the air masses fell within the range between South-West and North (Fig. 5.5-Fig. 5.10), although day to day variations were also seen.

On the 6th of August 2009 back trajectories indicated that the air masses originated from above the Atlantic Ocean waters and approached Worcester from the South-West to North-West direction passing over mainly Wales and West Midlands (Fig. 5.5).

The dominant height of the air masses varied between 500 m and 1000 m above ground level (Fig. 5.5 a). Possible pasture sources of *Alternaria* spores were located in South Wales within 24 hours of the air masses transport (346 km/214 mi), (Fig. 5.5). Possible crop sources of *Alternaria* were located in Shropshire, Worcestershire and Herefordshire, within 6-12 hours of the air masses transport (43-130 km/26-80 mi), (Fig. 5.5).

Table 5.1 Location of the hourly trajectories from 6th to 11th of August 2009 (*n*=6).

Date	Area	Number of points	Percentage[a]
6 Aug 2009	Overall	1176	100.00
	Sea areas	796	67.69
	Land areas	380	32.31
	British Isles	380	32.31
	United Kingdom	380	32.31
7 Aug 2009	Overall	1176	100.00
	Sea areas	591	50.26
	Land areas	585	49.74
	British Isles	585	49.74
	United Kingdom	474	40.31
8 Aug 2009	Overall	1176	100.00
	Sea areas	736	62.59
	Land areas	440	37.41
	British Isles	440	37.41
	United Kingdom	321	27.30
9 Aug 2009	Overall	1176	100.00
	Sea areas	764	64.97
	Land areas	412	35.03
	British Isles	412	35.03
	United Kingdom	412	35.03
10 Aug 2009	Overall	1176	100.00
	Sea areas	1003	85.29
	Land areas	173	14.71
	British Isles	173	14.71
	United Kingdom	145	12.33
11 Aug 2009	Overall	1176	100.00
	Sea areas	551	46.85
	Land areas	625	53.15
	British Isles	625	53.15
	United Kingdom	410	34.86

[a] Percentage of the total number of back trajectory points.

According to the back-trajectories on the 7th of August 2009, the air masses arrived in Worcester from the North-West and North directions (316°-360°), (Fig. 5.6). The most dominant height of the air masses varied from 500 m to 1000 m above ground level, although a fraction of the air masses was also seen at greater heights (Fig. 5.6 a). The possible location of the crops, which would constitute a source of *Alternaria* spores, was found between 173 km and 194 km away from Worcester in the North West England (107-120 mi). The possible locations of pastures, which would constitute a source of *Alternaria* spores, were found in Shropshire and Staffordshire, between 43 km and 130 km away from Worcester (26-80 mi).

On the 8th of August 2009 back trajectories indicated that the air masses originated from above the Atlantic Ocean waters and approached Worcester from the West direction passing over southern Ireland, Wales and West Midlands (Fig. 5.7). The dominant height of the air masses varied between 500 m and 1000 m above ground level (Fig. 5.7 a). The air masses that travelled above Ireland were located at the greater heights, while passing over the Irish Sea their height was below 500 m above ground level (Fig. 5.7 a). Possible pasture sources of *Alternaria* spores were located in South Wales within maximum of 18 hours of the air masses transport (259 km/160 mi), (Fig. 5.7). Possible crop sources of *Alternaria* spores were located in Worcestershire and Herefordshire, within 6-12 hours of the air masses transport (65-130 km/40-80 mi), (Fig. 5.7).

According to the back-trajectories on the 9th of August 2009, the air masses arrived in Worcester from the South-West direction (225°), (Fig. 5.8). The most dominant height of the air masses varied from 500 m to 1000 m above ground level, although a minor fraction of the air masses was also seen at elevated and lower heights (Fig. 5.8 a). The possible location of the crops, which would constitute a source of *Alternaria* spores, was found in three counties: Worcestershire, Herefordshire and Gloucestershire, within a range of 65-130 km distance from Worcester (40-80 mi). The possible locations of pastures, which would constitute a source of *Alternaria* spores, were found in Pembrokeshire and Carmarthenshire, at a maximum of 194 km distance from Worcester (120 mi).

On the 10th of August 2009 back trajectories indicated that the air masses originated from above the Atlantic Ocean waters and approached Worcester from the South-West to North-West directions passing over Ireland, entirely Wales and West Midlands (Fig. 5.9). The dominant height of the air masses varied between 500 m and 1000 m above ground level (Fig. 5.9 a).

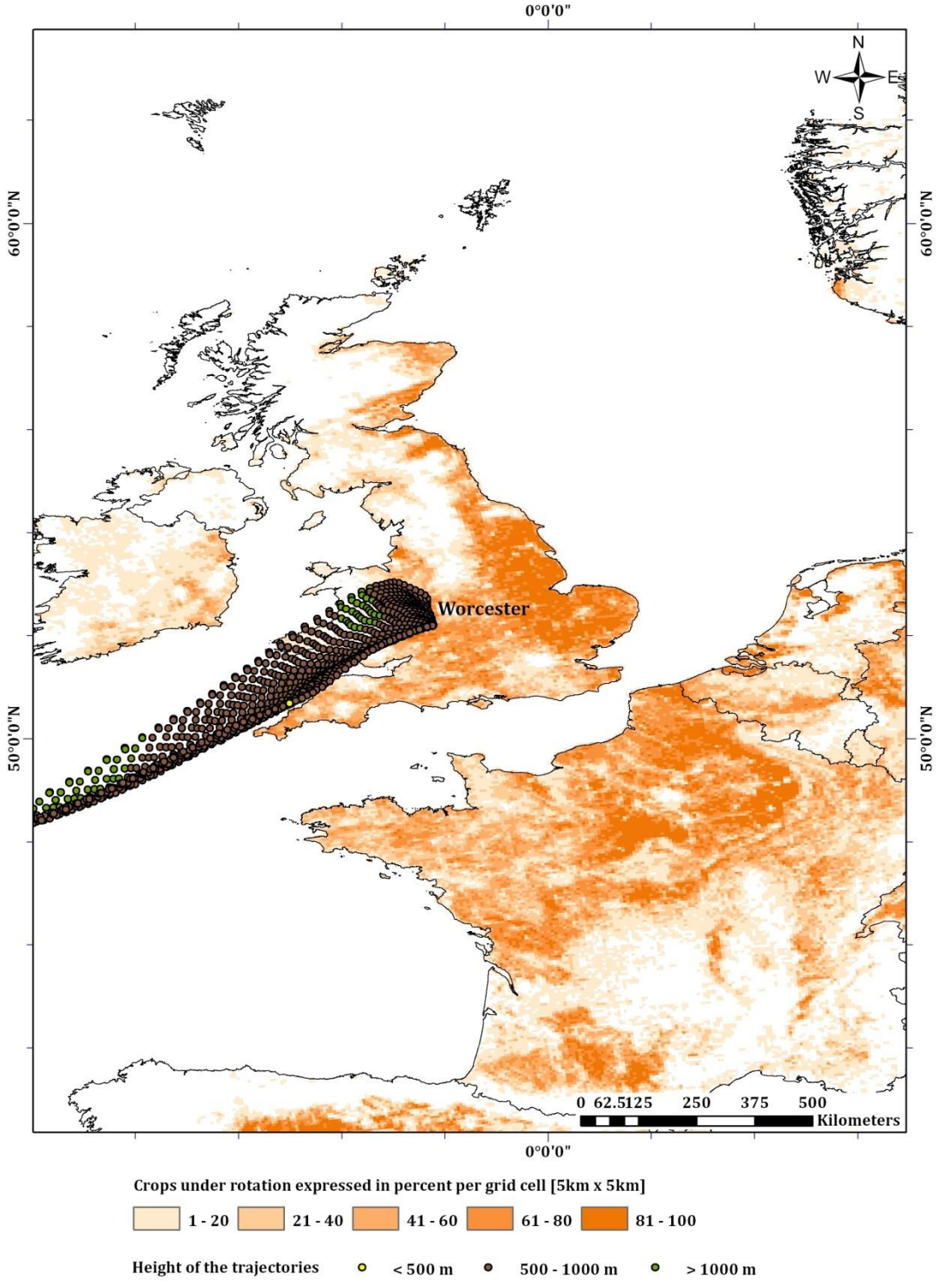


Fig. 5.5a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 6th of August 2009 (*n*=24).

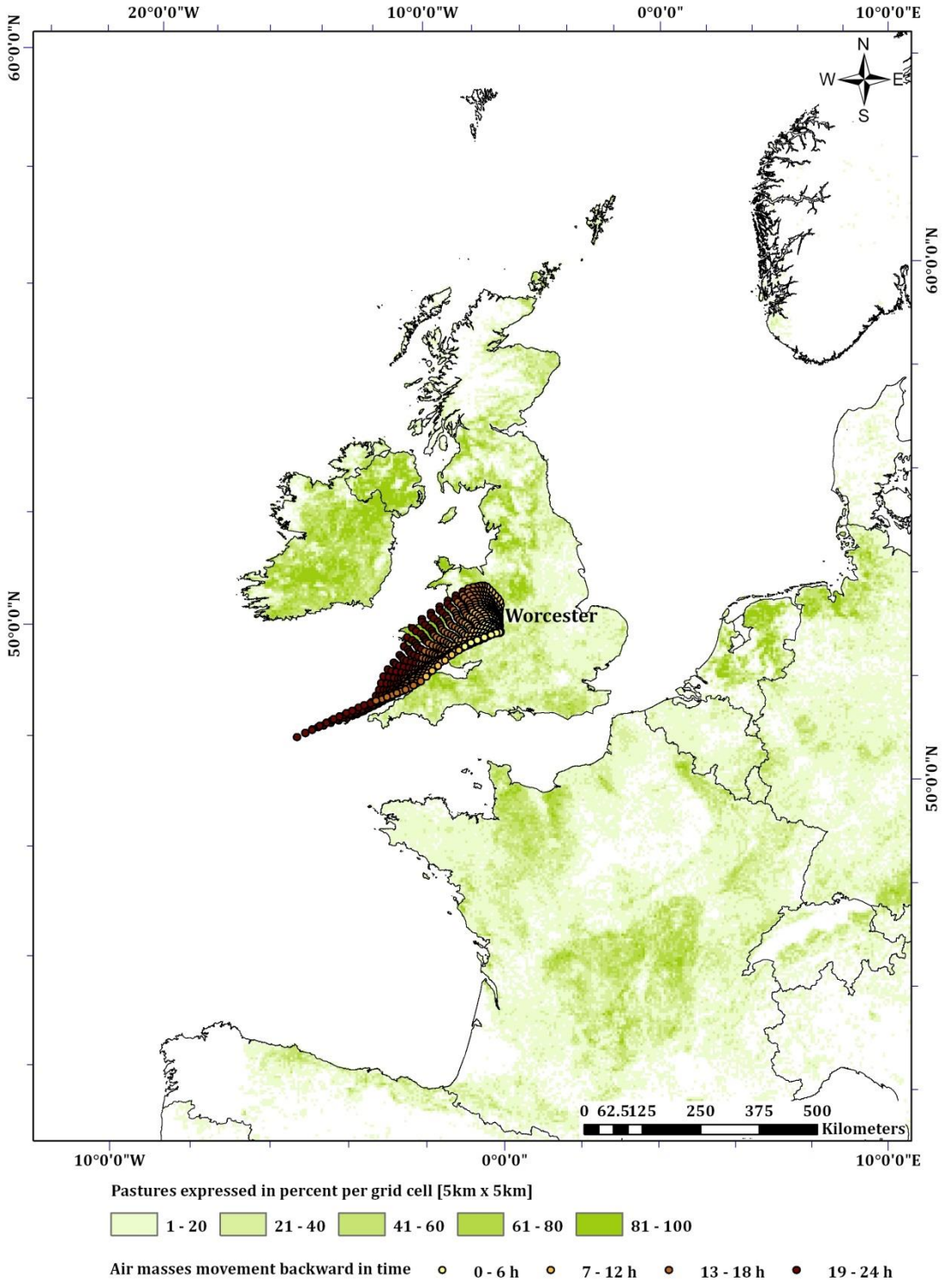


Fig. 5.5b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 6th of August 2009 (*n*=24).

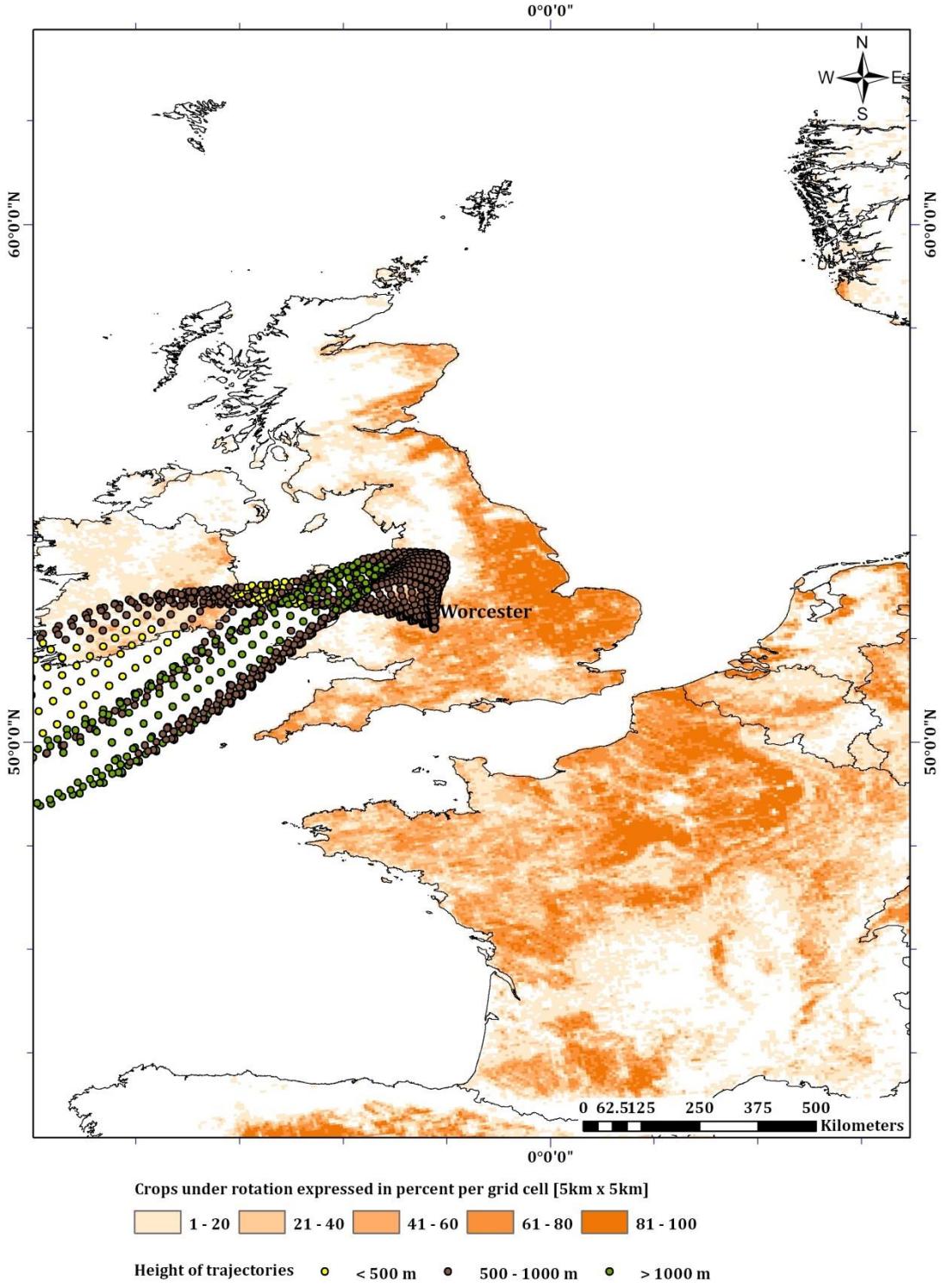


Fig. 5.6a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 7th of August 2009 (*n*=24).

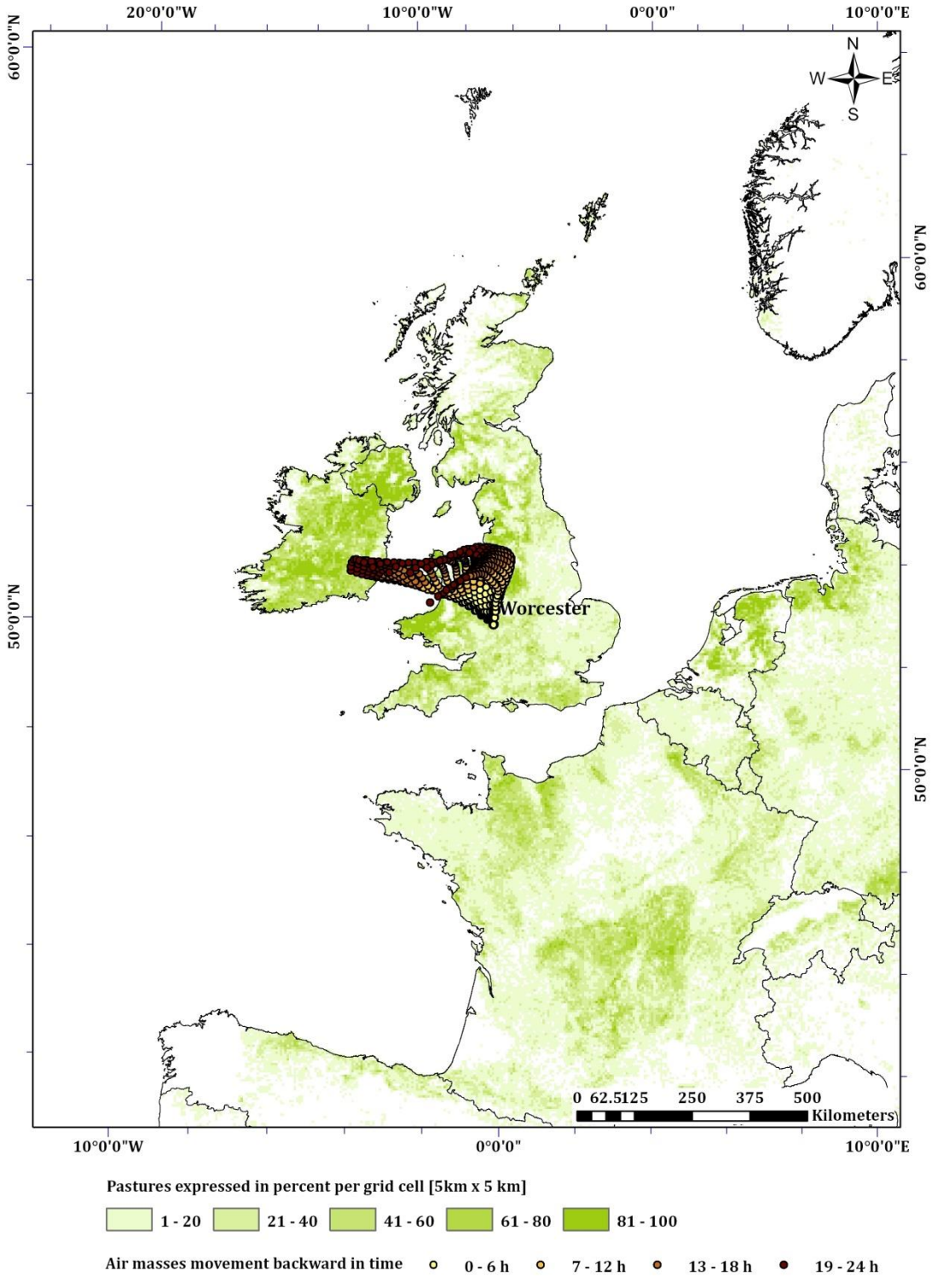


Fig. 5.6b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 7th of August 2009 (*n*=24).

The air masses that travelled above the southern coast of Ireland, Irish Sea and South West England were located at lower heights (Fig. 5.9 a). Possible pasture sources of *Alternaria* were located in Wales (Anglesey, Snowdonia, Ceredigion and Pembrokeshire) and England (Shropshire, Worcestershire) within a maximum of 12 hours of the air masses transport (389 km/241 mi), (Fig. 5.9). Possible crop sources of *Alternaria* were located in Worcestershire, Herefordshire, Gloucestershire and Cornwall, within 6-12 hours of the air masses transport (216-389 km/134-241 mi), (Fig. 5.9).

According to the back-trajectories on the 11th of August 2009, the air masses arrived in Worcester from the West to North-West directions (270°-315°), (Fig. 5.10). The most dominant height of the air masses varied from 500 m to 1000 m above ground level, although a minor fraction of the air masses was also seen at greater and lower heights (Fig. 5.10 a). The possible location of the crops, which would constitute a source of *Alternaria* spores, was found in North West England, West Midlands and South East Ireland, within a range of 130-259 km away from Worcester (80-160 mi). The possible locations of pastures, which would constitute a source of *Alternaria* spores, were found in North Wales, Northern Ireland and the rest of Ireland, within a range of 259-518 km away from Worcester (160-321 mi).

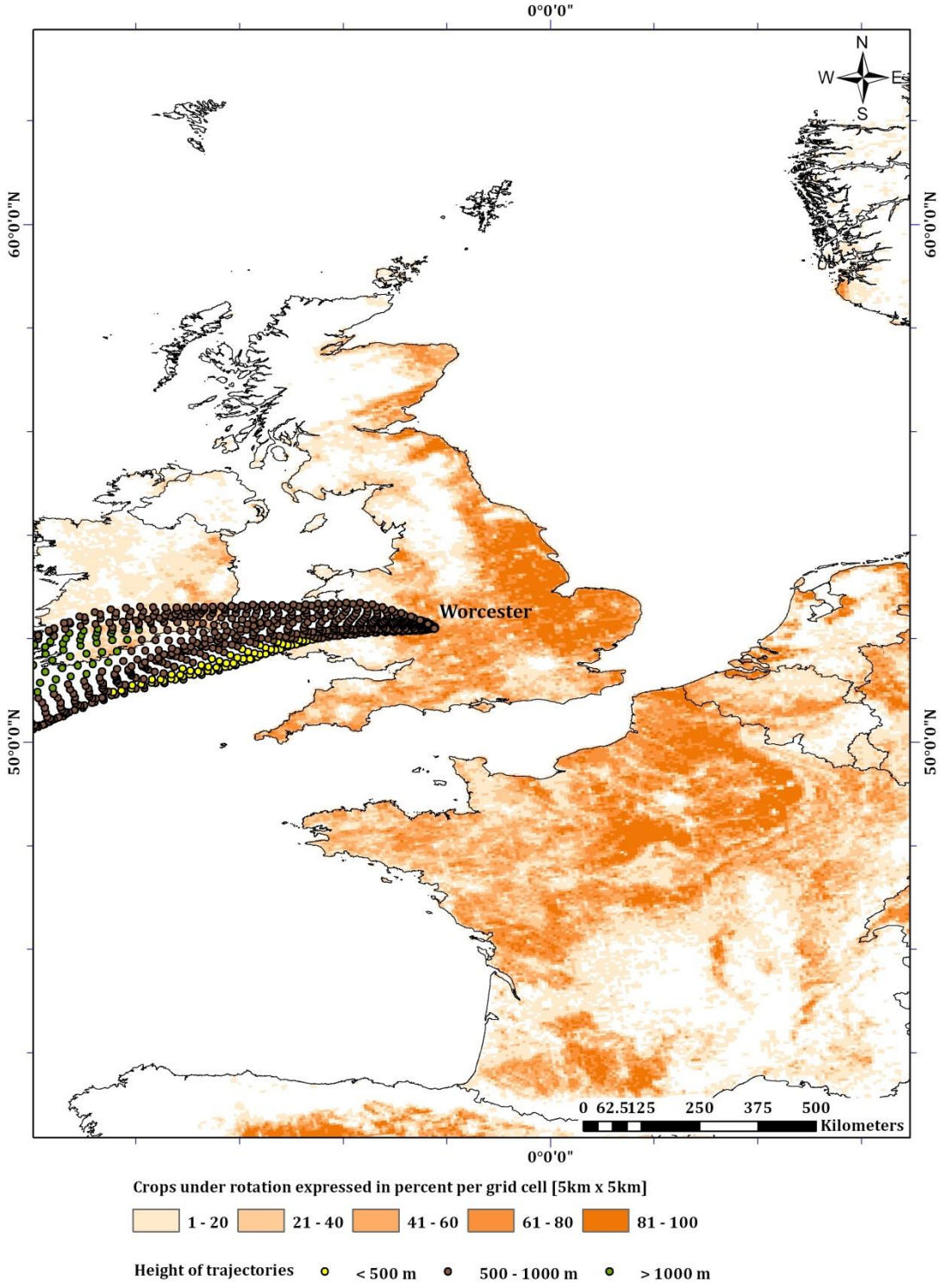


Fig. 5.7a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 8th of August 2009 (*n*=24).

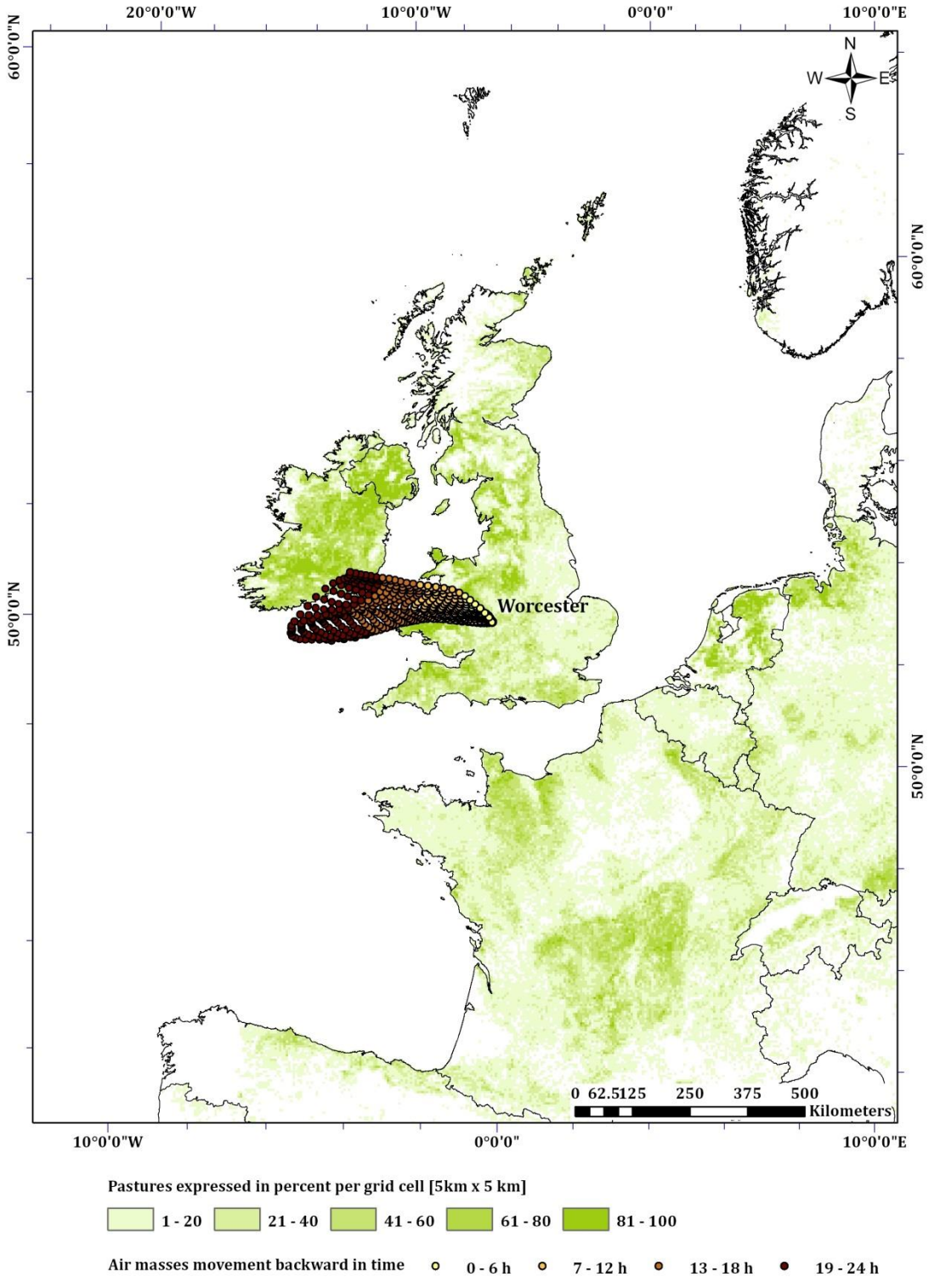


Fig. 5.7b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 8th of August 2009 (*n*=24).

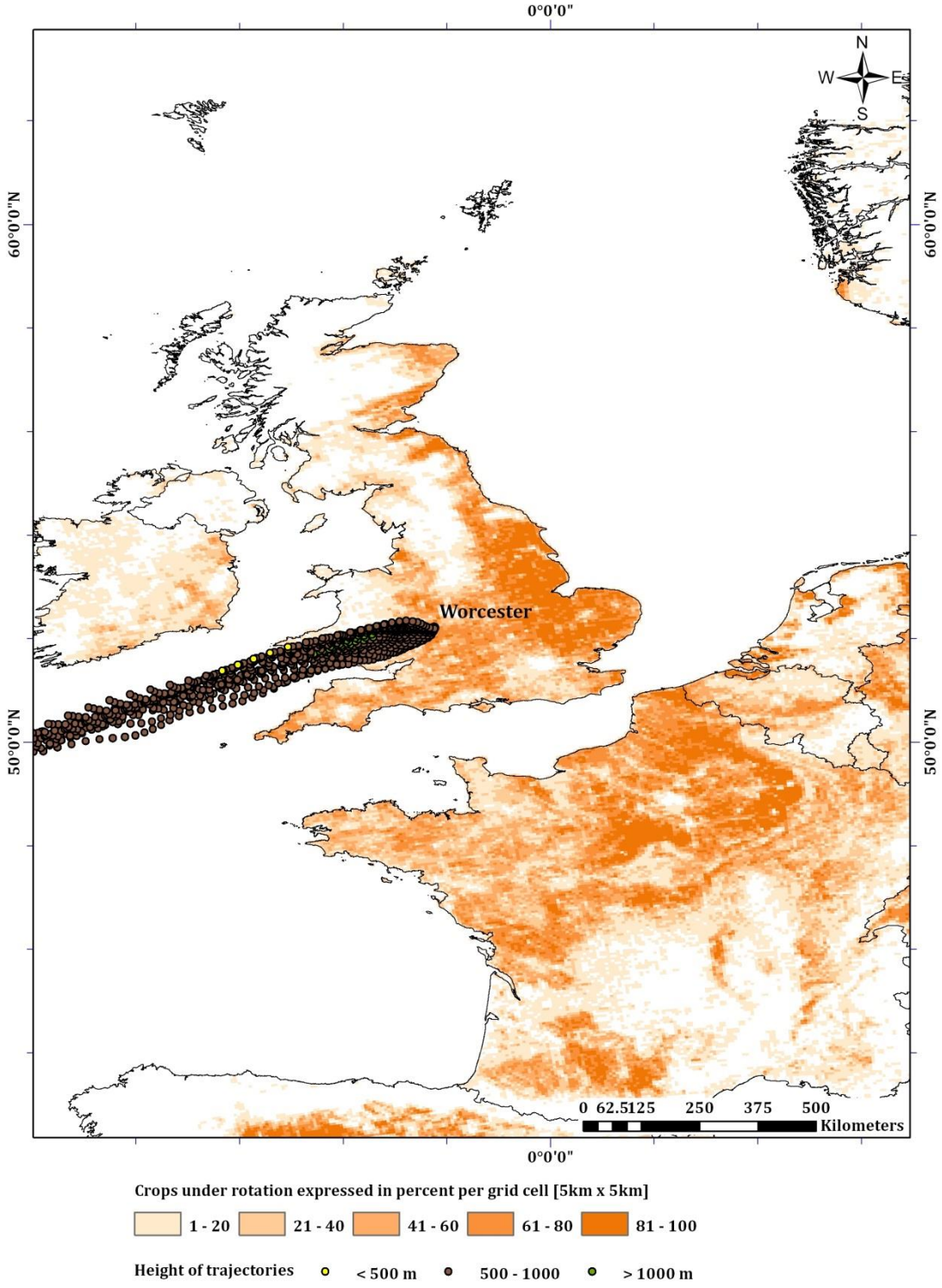


Fig. 5.8a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 9th of August 2009 (*n*=24).

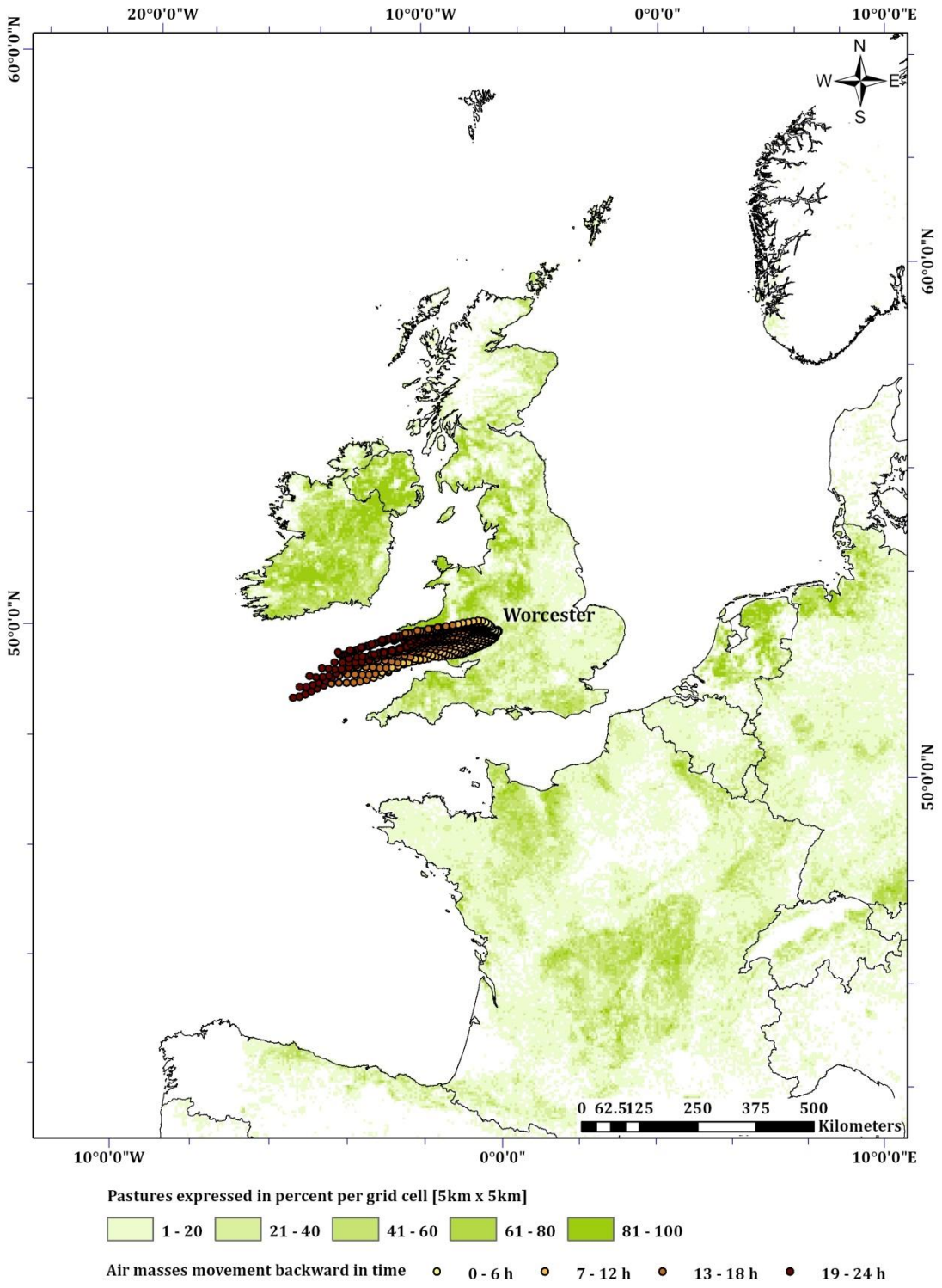


Fig. 5.8b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 9th of August 2009 (*n*=24).

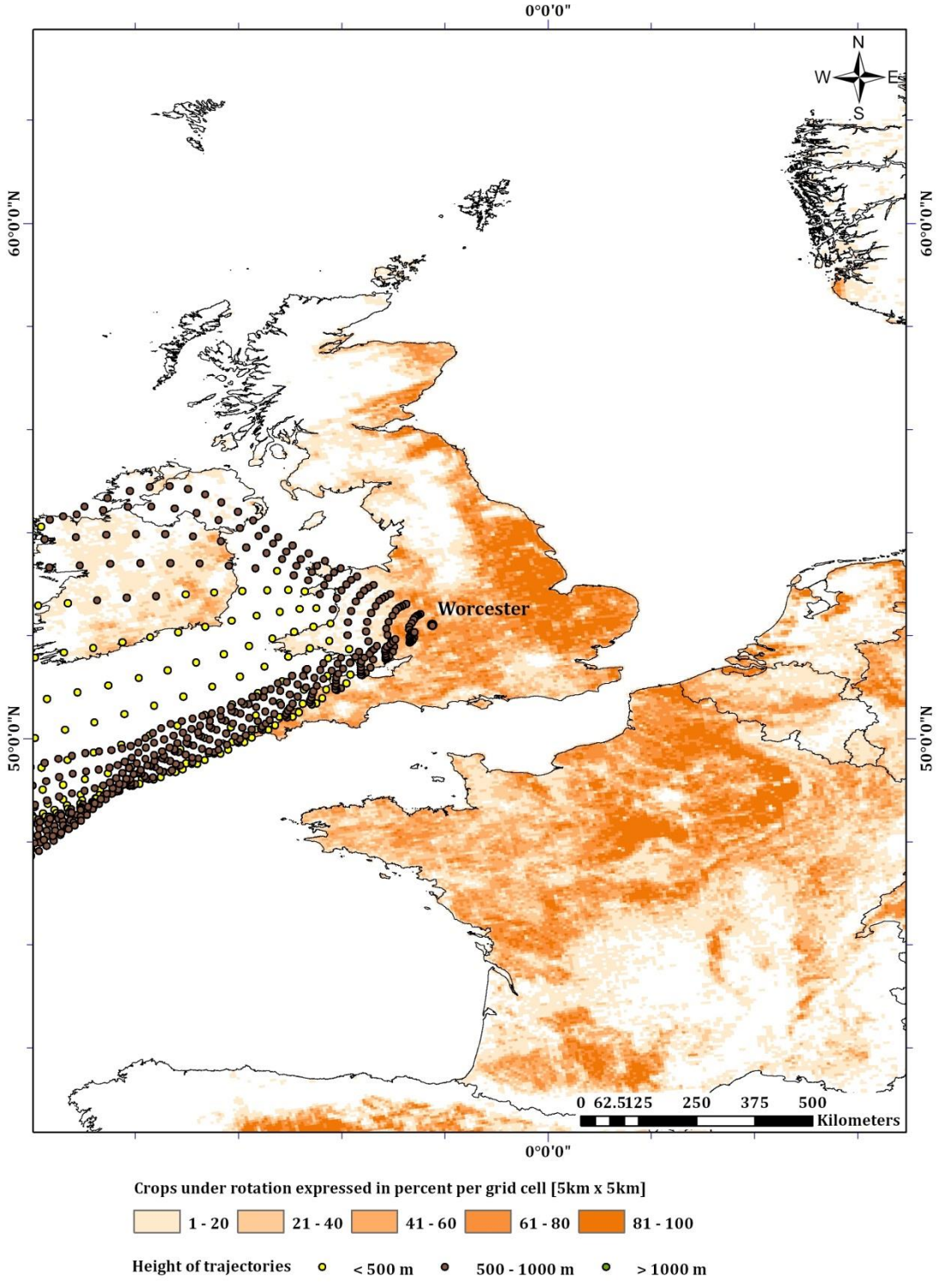


Fig. 5.9a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 10th of August 2009 (*n*=24).

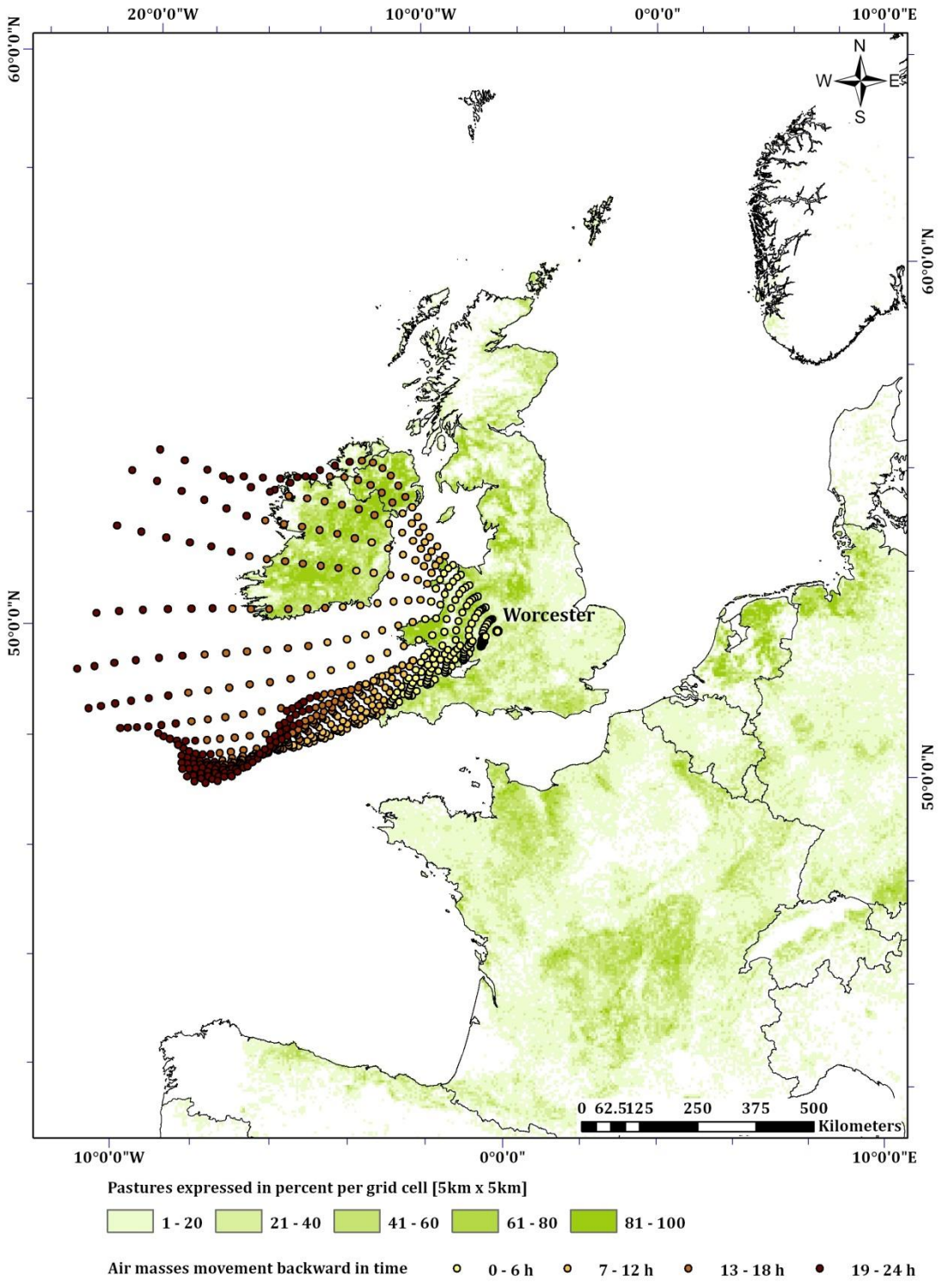


Fig. 5.9b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 10th of August 2009 (*n*=24).

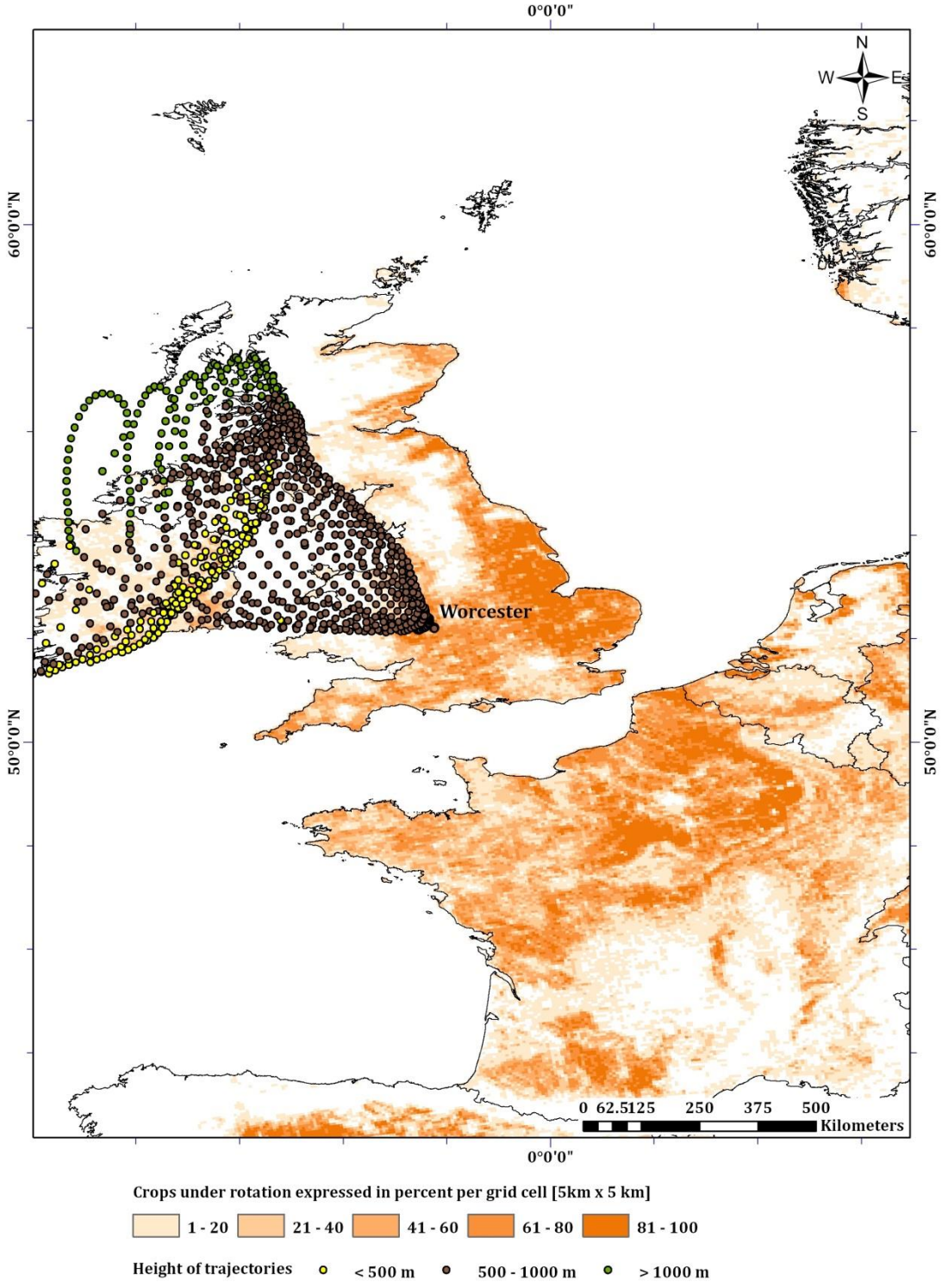


Fig. 5.10a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 11th of August 2009 (*n*=24).

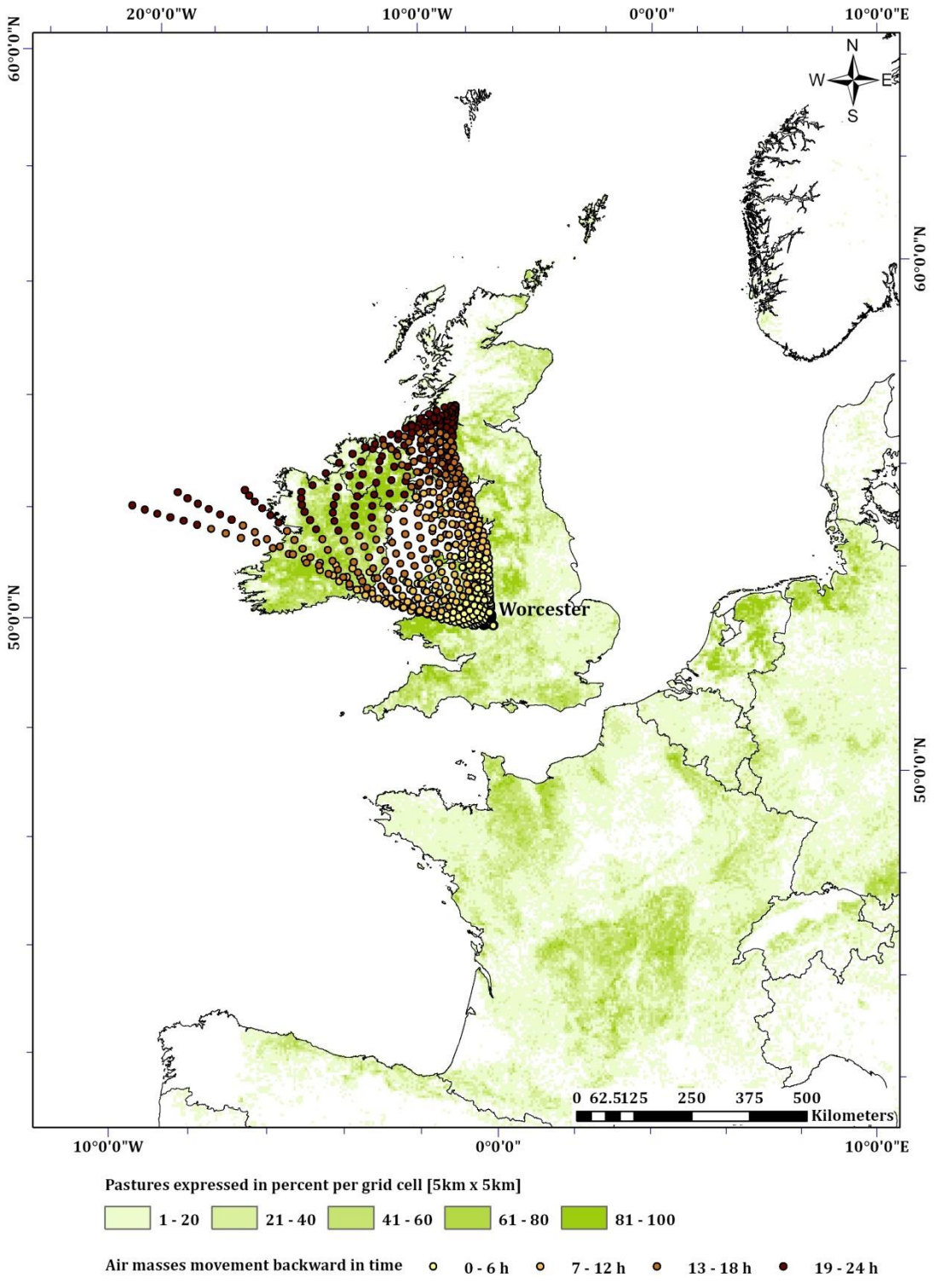


Fig. 5.10b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 11th of August 2009 (*n*=24).

Mean speed of the air masses within 6 hours, 12 hours, 18 hours and 24 hours before they reached Worcester along with corresponding mean concentration of *Alternaria* spores are visually presented in the Fig. 5.11. The highest concentration of *Alternaria* spores was seen, when within the periods 18-24 hours mean speed of the air masses varied between 3 m s^{-1} and 4 m s^{-1} (Fig. 5.11). The distance between the air sampler in Worcester and the possible sources of *Alternaria* spores could be within a 259-432 km range (160-268 mi). An increase of the mean speed of the air masses up to 10 m s^{-1} reduced significantly the mean concentration of *Alternaria* spores (Fig. 5.5).

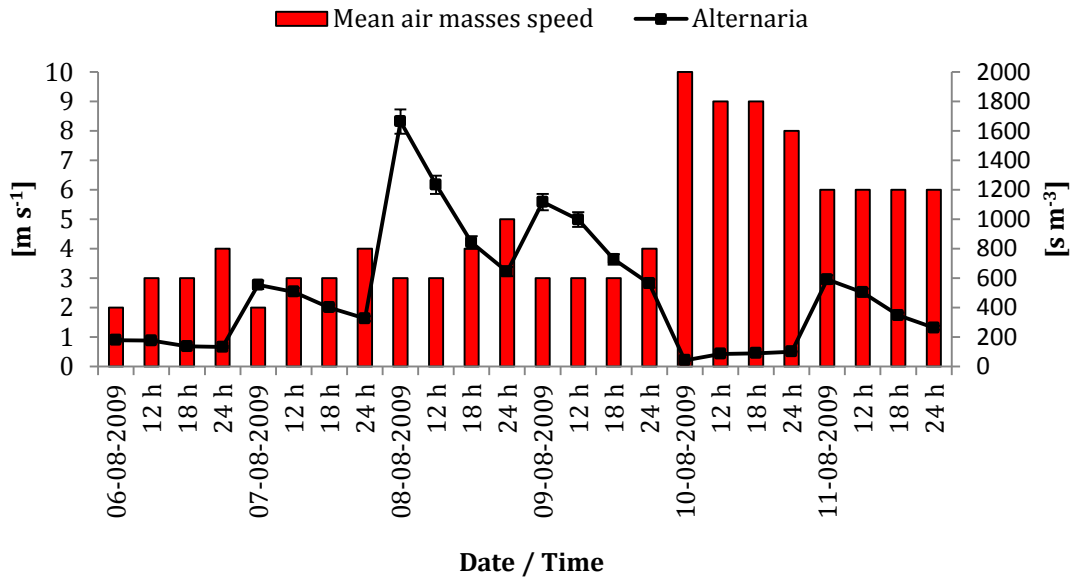


Fig. 5.11 The mean speed of the air masses within the last 6 hours to 24 hours before they reached Worcester, UK with corresponding mean concentration of *Alternaria* spores.

5.1.3. Discussion and conclusions

Within the examined period of time, *i.e.*: between the 6th and 11th of August 2009, no evidence for the long distance transport of *Alternaria* spores from the continent was detected.

The air masses revealed a fairly constant direction. The overall direction of the air masses fell within the range from South-West to North (Fig. 5.5-Fig. 5.10).

During the two highest spore count days (8th and 9th of August 2009) the most dominant air masses came from the West and South-West directions. These back trajectories indicated that the most important sources of *Alternaria* spores were located in the West Midlands of England, in the counties Herefordshire, Worcestershire and Gloucestershire.

Interestingly, the distance between Worcester and the potential sources of the fungus, which was calculated upon the air masses speed, was found to be 65-130 km/40-80 mi away and 6-12 hours of the air masses transport. The source maps that were used in this study showed that within this distance and direction, it was most likely that *Alternaria* spores originated from the crops under rotation rather than pasture.

However, a complete negation of pasture land contribution to the total recorded spore count would be a fault. The closest located grasslands to Worcester were found mainly within the distance of 43-346 km (26-214 mi) and 6-24 hours of the air masses transport. A minor impact of the pasture sources in Ireland might be possible.

The most likely species that could come from the pasture areas would be *Alternaria alternata*, which attacks equally annual plants, e.g. St. John's wort (Frużyńska-Jóźwiak and Andrzejak, 2007), biennial plants, e.g. lovage (Frużyńska-Jóźwiak and Andrzejak, 2007) and perennial plants, e.g. white clover (Zahid al., 2002). This hypothesis can be supported by the results of the experimental study conducted by Schafer and Kotanen (2004). Out of eight tested fungal species, *Alternaria alternata* is considered to be the most virulent, as its presence was detected in 88% of collected seeds. The most susceptible meadow plants were found to be *Danthonia spicata* and *Poa pratensis*.

Taking into consideration the time, when *Alternaria* spore concentrations were found at the greatest quantities, *i.e.*: at 22:00h on the 8th, 9th and 11th of August 2009, it could be concluded that the real time of spore release took place between 10:00h and 16:00h. This would be the evidence for a previously stated hypothesis that the night spore peaks recorded in the cities were a result of the horizontal transport of *Alternaria* spores in the air from the nearby sources during

that time (Skjøth et al., 2012). For more details please see the discussion in Chapter 4 dedicated to *Alternaria* spores (Diurnal variations in fungal spore distribution).

The elevated concentrations of *Alternaria* spores were recorded in Worcester, when the mean speed of the air masses varied between 3 m s^{-1} and 4 m s^{-1} (Fig. 5.11). Similar results were obtained in Denmark, where the back-trajectories analysis showed that the mean velocity of the air masses varied from 1 m s^{-1} to 6 m s^{-1} (Skjøth et al., 2012). No other study has so far employed atmospheric modelling to investigate the spatial distribution of *Alternaria* spores. Hereof, it was necessary to refer also to the measurements of the local wind speed taken directly from the crop fields, where infection of *Alternaria* was detected.

The measurement of the wind velocity and its variations was a subject of interest of many authors as initially it was believed that *Alternaria* spores were detached from the lesions of infected plants by a physical force only (Aylor and Day, 1976). This matter has been then examined under both the lab and field conditions (Strandberg, 1977). Later on, it was shown that although wind velocity played a key role, it was not the sole factor that enabled discharge of *Alternaria* spores (Lyon et al., 1984). Meredith (1966) analysed in detail the biology of *Alternaria porri,* an important pathogen of onion crops. He suggested that some species of *Alternaria* may in fact have an active mechanism of spore release in contrast to investigated *A. porri.* However according to his observations the greatest concentrations of *A. porri* spores were seen at the mean wind speed equal to 7 m s^{-1} (Meredith, 1966). A sudden decrease in spore concentration was observed during the days when either the mean wind speed had lowered to 3 m s^{-1} or it calmed down completely (Meredith, 1966). In contrast, mean wind speed between 2 m s^{-1} and 3 m s^{-1} was found to be significantly associated with greater quantities of *Alternaria dauci* spores in the air (Strandberg, 1977).

Summarizing, whether the mean speed of the air masses or the local wind was used in the analysis of *Alternaria* spore distribution, similar results were obtained. The mean speed of the wind velocity was not greater than 7 m s^{-1}, although the differences were found for different species of *Alternaria*. In the

aerobiology science, it is commonly believed that the Hirst type sampler provides a representative data with regard to the pollen and spore concentration up to the distance equal to 30 km away from the sampling site (Belmonte et al., 2008; Skjøth et al., 2010). If location of the potential sources of either pollen or spores was detected at the further distance, then it would be more relevant to include the mean speed of the air masses velocity above the mean speed of the local wind in to the analysis.

5.2. *Cladosporium* conidia

5.2.1. Introduction

Cladosporium spores constituted the most important fraction of the total spore load registered in Worcester during the studied period of time. This has been described in more detail in Chapter 3. Methods used in this study were described in Chapter 2.

During a five year study conducted in Worcester (2006-2010) there were 131 days, when *Cladosporium* spore concentrations ≥ 6000 s m^{-3} were observed. These days were selected for further investigation that aimed to detect whether the annual and monthly patterns of the air masses direction (cluster of 24 trajectories) was matching the annual and monthly patterns of the local wind direction (hourly mean), and hourly mean concentration of *Cladosporium* spores recorded in Worcester, UK.

5.2.2. Results

5.2.2.1. Source map

The general map presenting the land cover of the United Kingdom and location of the Worcester sampling site was produced based on the Corine Land Cover 2000 (EU, 2005). The only modification, which was applied, constituted the addition of a rectangle with the side equal to 1 x 1 degree (Fig. 5.12). The central point of the Fig. 5.12 covered the location of Worcester. The rectangle was then divided into eight equal parts representing major wind directions to enable enumeration of the back trajectory points. This layer has been prepared by Dr

Carsten Ambelas Skjøth for the analysis of *Ganoderma* spores described in Sadyś et al. (2014a).

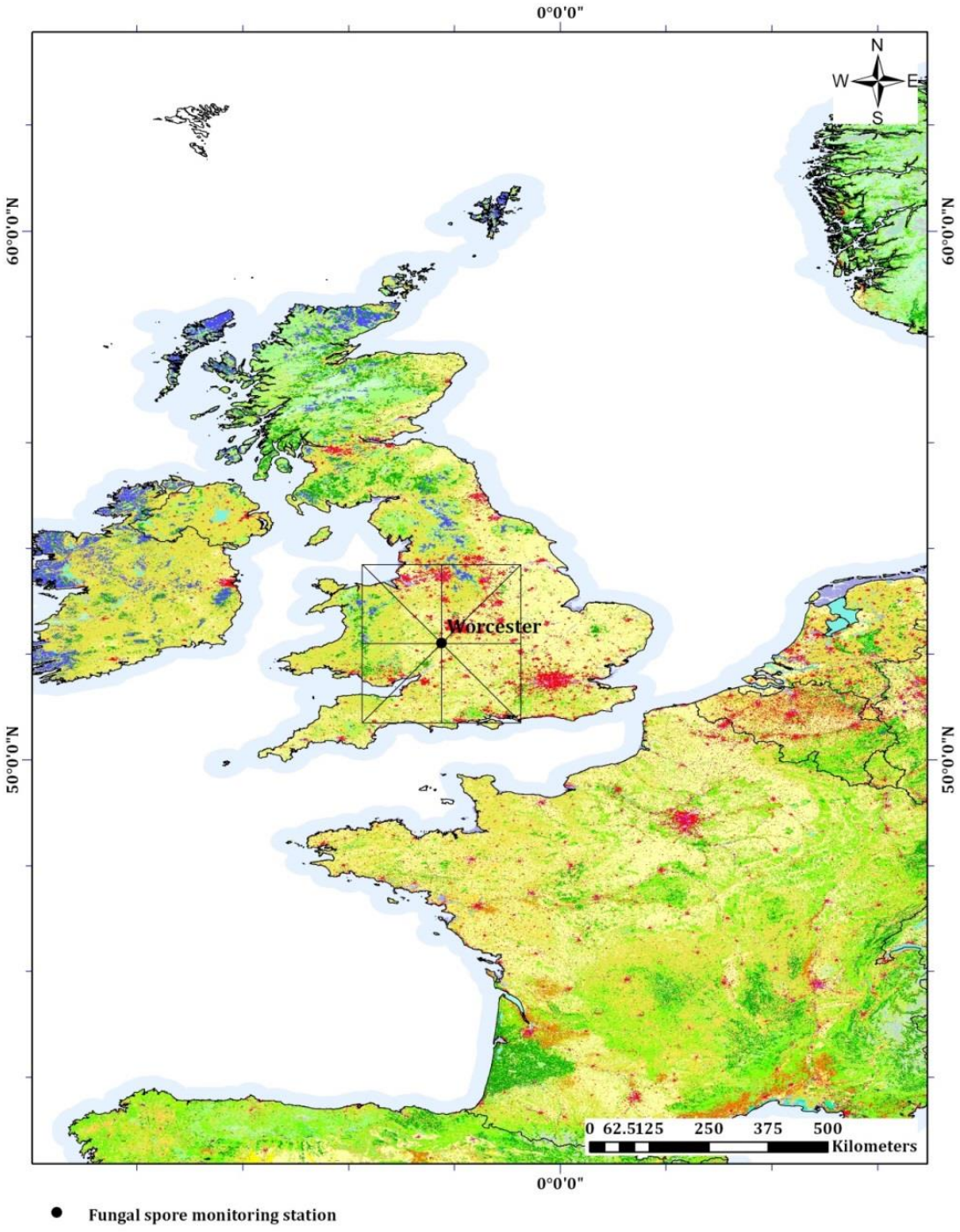


Fig. 5.12 Map showing the land cover of the United Kingdom along with location of Worcester and 1 x 1 degree box.

5.2.2.2. Annual distribution of *Cladosporium* spores

Cladosporium was characterised with quite short spore seasons, as their duration varied from 111 to 186 days (Table 5.2). The daily peak values were approximately between 20000 s m^{-3} and 47000 s m^{-3} and they mainly occurred in July. In 2006 and 2010, there were marked differences in comparison with the remaining three years of observations. Both SFI values and daily maximum spore concentration were different for these two years. The number of high spore count days (≥ 6000 s m^{-3}) varied annually (Table 5.2). The largest number of high spore count days was found in 2006 (*n*=47) and 2010 (*n*=44), while the lowest was detected in 2007 (*n*=8). The intra-diurnal distribution of *Cladosporium* spores showed that the highest concentrations were obtained between 08:00h and 18:00h for all individual years (Fig. 5.13).

Table 5.2 The characteristics of *Cladosporium* spore seasons in Worcester (England) during the period 2006-2010.

	2006	2007	2008	2009	2010
Period of occurrence	14.06-02.10	27.04-29.10	20.05-12.10	23.05-28.10	26.05-29.09
Duration [*n*]	111	186	146	159	127
Daily peak value [s m^{-3}]	36783	19813	22316	23040	46831
Date of daily peak	25.07	16.07	06.08	29.06	14.07
Mean [s m^{-3}]	7017.37	2382.66	2698.05	3484.22	6402.66
Median [s m^{-3}]	4910.00	1694.50	1879.50	2362.00	4721.00
Mode [s m^{-3}]	952.00	multiple	1676.00	multiple	no mode
SD[a] [s m^{-3}]	6239.49	2272.39	2740.20	3547.46	6833.80
SFI[b]	863607	489363	437171	612198	902136
High spore count days [*n*]	47	8	11	21	44
Contribution h.s.d. [%]	42.34	4.30	7.53	13.21	34.65

[a] SD – Standard deviation
[b] SFI – Seasonal Fungal Index

The largest differences between day/night concentrations were obtained in 2007 and 2008, while the smallest differences were seen for 2006, mainly due to higher night-time concentrations compared.

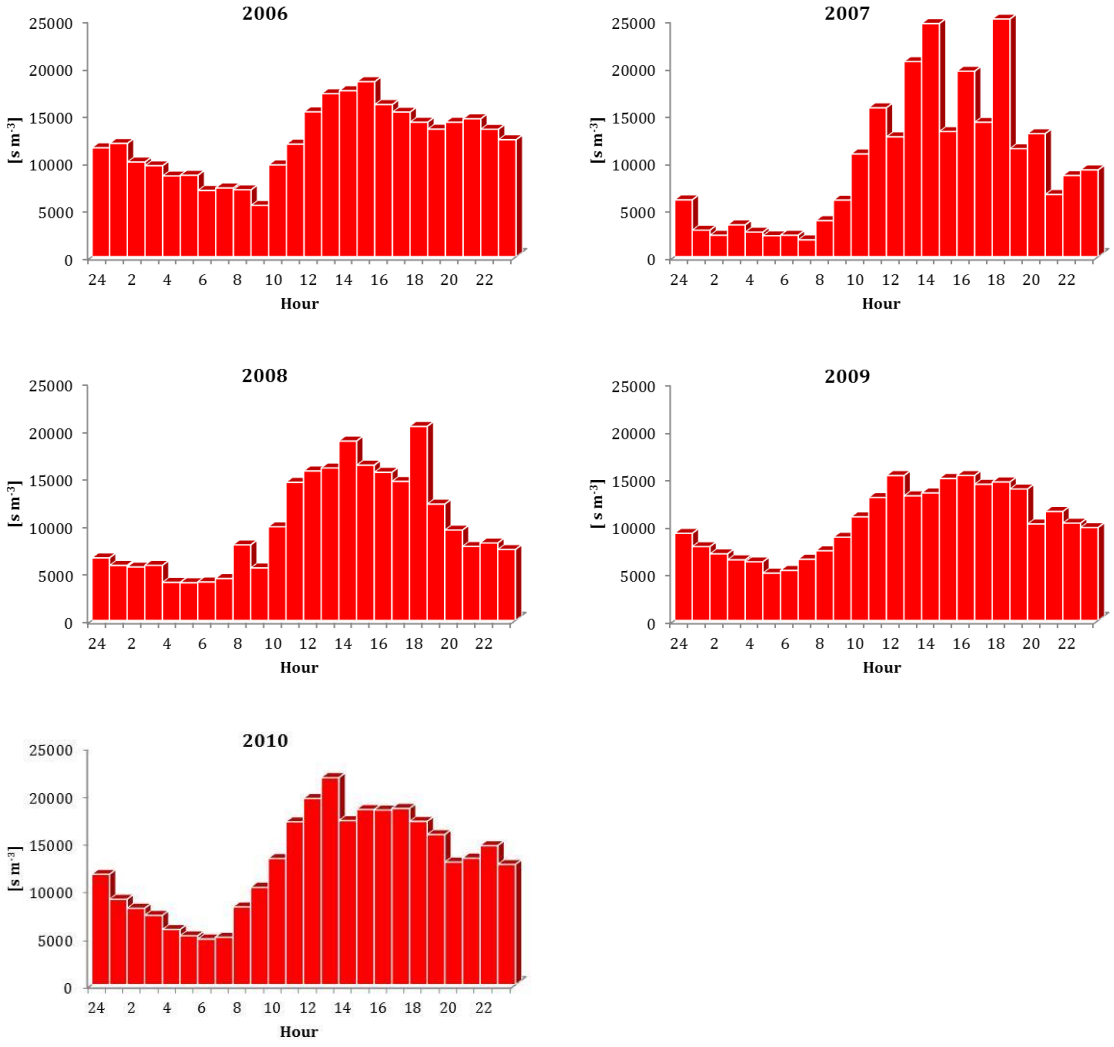


Fig. 5.13. Annual variation in hourly concentration of *Cladosporium* conidia during high spore count days (≥ 6000 s m^{-3}), (*n*=131).

5.2.2.3. Dependence of *Cladosporium* on wind direction

The Spearman's rank test (Table 5.3) showed that the relationship between hourly mean spore concentration and local wind direction was inversely proportional, and in each examined year it reached the level of statistical significance ($p \leq 0.05$). The highest correlation coefficient of r_s= -0.27 was found in 2007 (Table 5.3). With regard to air mass, the relationship with spore concentration also turned out to be inversely proportional (Table 5.3). The highest correlation coefficient value arose in 2009 (r_s= -0.39). No statistically significant

association was found between *Cladosporium* presence and air mass direction in 2008 (Table 5.3).

The analysis of spore dependence on wind direction examined using linear-circular correlation is also presented in Table 5.3. Both associations with local wind and air mass direction with *Cladosporium* spores were found to be statistically significant in each investigated year with an exception of 2007 and partly 2006 (Table 5.3). The vector of these relationships was found to be proportional, yet very weak (Table 5.3). In 2006 and 2008 a slightly larger impact on spore occurrence revealed local wind, while this has changed in favour of air mass in final two years of this investigation.

Table 5.3 Results of Spearman's rank test (r_s) and linear-circular correlation (r_c) between *Cladosporium* spore concentration and local wind (*a*) and air mass (b) directions.

Year	**2006**		**2007**		**2008**		**2009**		**2010**	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
r_s	**-0.15***	**-0.09***	**-0.27***	**-0.20***	**-0.22***	-0.08	**-0.16***	**-0.39***	**-0.09***	**-0.12***
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
r_c	**0.01***	0.00	0.03	0.03	**0.04***	**0.03***	**0.01***	**0.17***	**0.01***	**0.05***

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

5.2.2.4. An analysis of local wind direction

A detailed analysis of local wind direction is given in Table 5.4. Both the Chi-square von Mises and the Rayleigh tests revealed that the null hypothesis must be rejected, and hence local wind direction did not have a uniform circular distribution. The correlation between observed and expected from a von Mises distribution, expressed as kappa, showed a moderate (0.41-0.60) to good agreement (0.61-0.80). The results of circular statistics also indicated that most dominant winds originated from the southern directions (166°-251°).

Table 5.4 Results of descriptive circular statistics for local wind and air mass direction, when high *Cladosporium* spore count occurred (*n*=131).

Class		Year				
		2006	**2007**	**2008**	**2009**	**2010**
Air mass [a]	Mean direction	243.87°	195.58°	151.20°	208.04°	254.37°
	Circular standard deviation	99.78°	91.00°	80.04°	76.99°	81.36°
	Mean resultant length	0.22	0.28	0.38	0.40	0.36
	Skewness	0.50	0.66	-0.23	0.01	0.19
	Kappa estimate	0.45	0.59	0.81	0.89	0.78
	Prob. test of randomness	0.00	1.00	1.00	1.00	0.00
	Prob. Rayleigh test of uniformity	0.00	0.00	0.00	0.00	0.00
	Chi-square von Mises*	270.40	805.52	669.98	394.34	104.50
	Prob. Chi-square von Mises	0.00	0.00	0.00	0.00	0.00
Local wind [b]	Mean direction	251.10°	181.54°	166.43°	214.71°	243.94°
	Circular standard deviation	93.48°	93.44°	87.64°	87.71°	84.36°
	Mean resultant length	0.26	0.26	0.31	0.31	0.34
	Skewness	0.06	0.24	-0.03	-0.17	-0.14
	Kappa estimate	0.55	0.55	0.65	0.65	0.72
	Prob. test of randomness	0.00	1.00	1.00	1.00	0.00
	Prob. Rayleigh test of uniformity	0.00	0.00	0.00	0.00	0.00
	Chi-square von Mises*	65.59	787.07	680.06	435.65	53.76
	Prob. Chi-square von Mises	0.00	0.00	0.00	0.00	0.00

a Measured at 500 m above ground level.

b Measured at 10 m above ground level.

* All results with 5 degrees of freedom

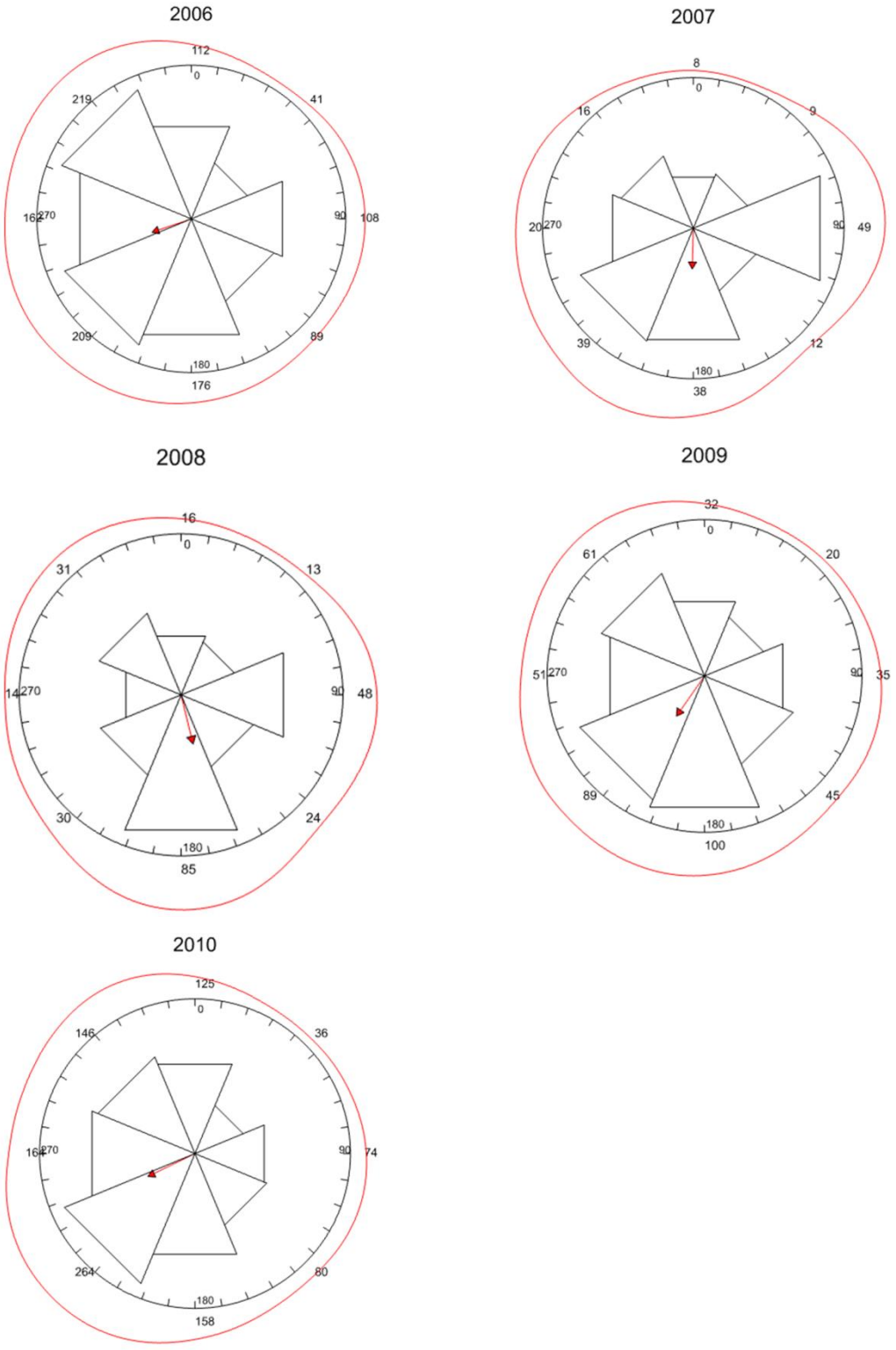


Fig. 5.14. Annual variations in local wind direction recorded in Worcester (England) with an indication of mean angle (arrow) and Kernel density (circle).

Figure 5.14 showed wind roses for each individually examined year, produced upon spore concentration threshold equal to or above 6000 s m^{-3}. Although the size of analysed samples varied between years, a characteristic pattern was noticeable that repeated annually (Fig. 5.14). High spore counts of *Cladosporium* conidia were recorded, when wind direction was observed within the span of 91°-360° (E-NW) directions (Fig. 5.14). Little contribution to the spore concentration was detected, if local wind direction changed to 46°-90° range (Fig. 5.14).

5.2.2.5. An analysis of air masses transport and its relation to local winds

The analysis of the back trajectories revealed that the time air masses spent over the non-UK areas were only a minor fraction of the time within the 24 hours before they reached Worcester (Table 5.5). In the annual summaries for high spore count days, this fraction of the time was found mainly below 10%, except for 2008. Influence of possible sources of *Cladosporium* spores from Ireland was estimated at 5% or less.

Table 5.5 Location of the air mass according to hourly trajectories on days with high *Cladosporium* spore counts from 2006 to 2010 (*n*=131).

Areas	Year, overall contribution of trajectory points (%) *				
	2006	**2007**	**2008**	**2009**	**2010**
Overall	100	100	100	100	100
Land areas	58	63	61	58	51
Sea areas	42	37	39	42	49
British Isles	53	55	48	54	49
Non-UK areas	5	8	13	4	1
UK areas	50	53	47	52	44

* Percentage of the total number of trajectories points.

Figure 5.15 presented visually the distribution of the air masses for each studied year, when daily mean concentration of *Cladosporium* spores was equal to or above 6000 s m^{-3}. Overall, the air masses were coming from the E to the W direction, while none or very little contribution was detected from N-NE bearing (Fig. 5.15). Results of the circular statistics indicated a dominance of SE-SW direction of the air masses. Throughout the period of study the mean angle remained more or less constant (151°-254°), (Table 5.4). A lack of uniformity in the sampled data was

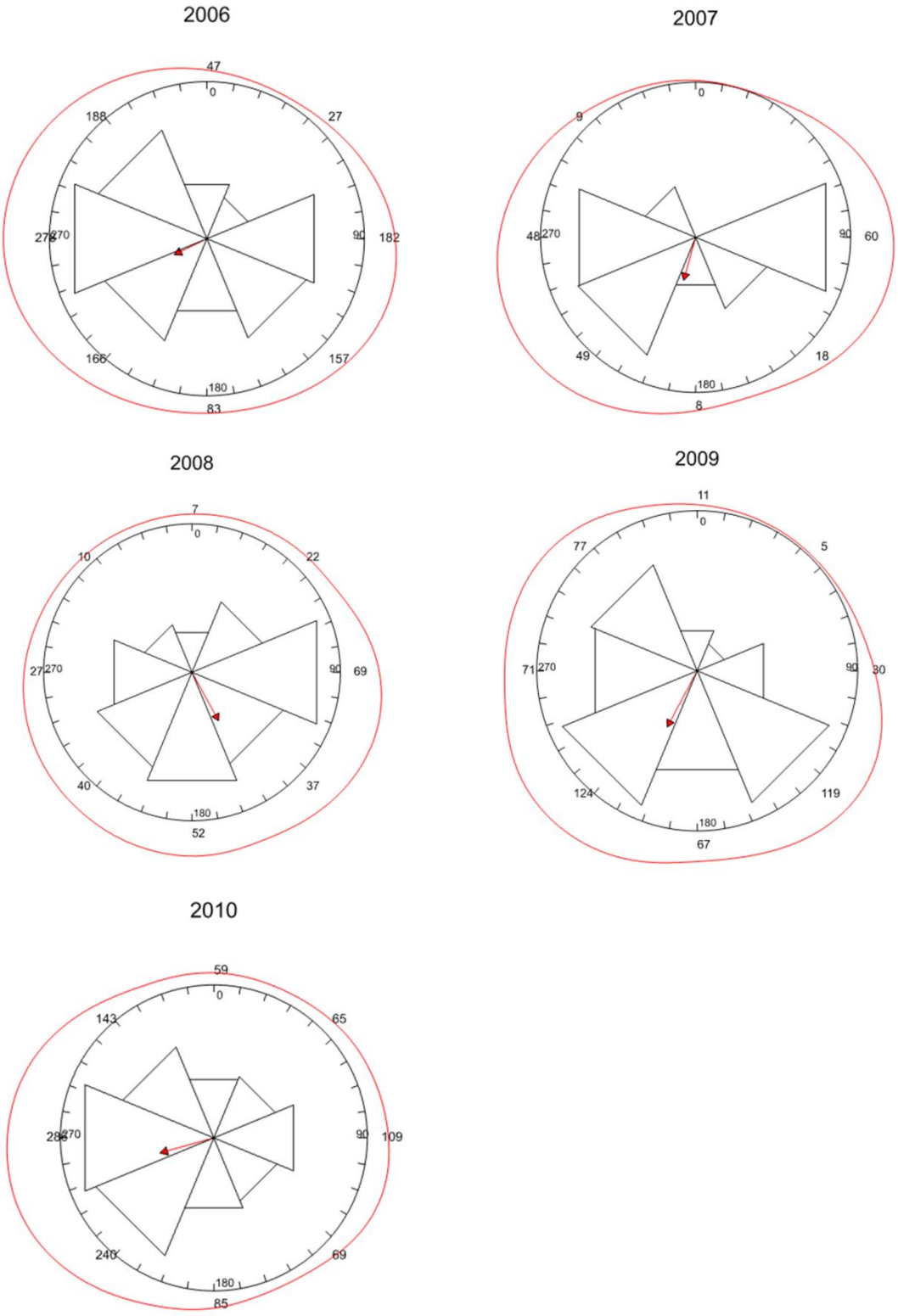


Fig. 5.15 Annual variations in air mass direction recorded in Worcester (England) with an indication of mean angle (arrow) and Kernel density (circle).

confirmed jointly by von Mises and Rayleigh tests (Table 5.4). The values of kappa also greatly varied, with an agreement from moderate (0.41-0.60) to very good (0.81-1.00). The relationship between local wind direction and air mass direction (Table 5.6) showed a moderate level of association (r_s=0.50-0.70) according to Mukaka (2012).

Table 5.6 Results of Spearman's rank test (r_s) between local wind and air mass direction.

Year	2006	2007	2008	2009	2010
r_s	0.57***	0.74***	0.34***	0.65***	0.53***

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

5.2.3. Discussion and conclusions

During five years of study the maximum daily mean concentration of *Cladosporium* conidia occurred between end of June and beginning of August with a maximum concentration reaching 47000 s m^{-3} (Table 5.2). Lacey (1981) estimated that at the climax of the spore season, daily mean concentration of *Cladosporium* spores may reach 240000 s m^{-3}. In general, very high concentrations of bioaerosols require strong local inoculum sources (Skjøth et al., 2013). Human activities such as hay making, mowing of grasslands and harvesting of cereal crops infected by *Cladosporium* species may also lead to an artificial emission of up to one billion spores per cubic meter of air (Lacey, 1981). Worcestershire is an agricultural region, where such activities often take place, while in Worcester city and at the university campus, where spore trap was operating, these agricultural activities do not occur. Hence, a lower maximum concentration of 47000 s m^{-3} would then be evidence for absence of strong local inoculum sources, and moreover a reasonable estimate for the regional background concentration for Worcestershire.

By making a detailed statistical evaluation of wind direction this study showed that the fungal spore concentrations of *Cladosporium* occurred due to strong local inoculum sources. Despite statistical significance, the obtained correlations were very weak (Table 5.3). This finding was further supported by the maximum concentration reaching 47000 s m^{-3} that was a factor of five lower than

the values suggested by Lacey (1981). Similarly, results indicated that *Cladosporium* concentrations were entirely related to regional scale of air mass transport. The statistical tests improved by using circular statistics compared to traditional Spearman's rank test (Table 5.3). Overall air mass transport yielded in higher statistical connection with fungal spore concentration, but with annual correlation coefficients ranging from r_s= -0.09 to r_s= -0.39 (Table 5.3). This suggested that if atmospheric transport was the governing process in relation to *Cladosporium* spore concentrations, then it would be important to cover both local wind and regional scale transport in order to describe atmospheric concentrations of *Cladosporium*. Most likely, the importance of either local sources or regional scale atmospheric transport will vary from location to location.

The first neural network model for *Cladosporium* spores was produced by Grinn-Gofroń and Strzelczak (2009), who applied a threshold value of 3000 s m^{-3} and examined ten variables. They found that although wind direction gained a level of statistical significance, as indicated by the sensitivity analysis of the MLP 10:10:1 neural model, its influence on the distribution of *Cladosporium* spores was not confirmed in Spearman's rank test. Likewise, O'Connor et al. (2014) did not find such a relationship using correlation analysis. However, their results could be affected by a sample size, as data were collected for a period of 3 months, and daily mean values were examined. On the other hand, Recio et al. (2012), who investigated daily and weekly means of several variables did find a statistically significant correlation between wind direction and occurrence of *Cladosporium* spores in Malaga (Spain) for the former type of data only; the correlation coefficient varied from r_s=0.07 ($p \leq 0.05$) to r_s=0.12 ($p \leq 0.001$). All of these studies confirmed the importance of local wind direction, which varied from location to location, thus supporting the findings in this study.

The maturation of spores takes place overnight and they are ready to be released early in the morning (Rich and Waggoner, 1962). Soon after spore liberation new conidia are formed within 12-24 h (Meredith, 1962; Rich and Waggoner, 1962). Hence, *Cladosporium* has a mid-day (10:00h-16:00h), occasionally double-peak (08:00h-10:00h and 14:00h-18:00h) pattern in spore

release (Lacey, 1981; Meredith, 1962). This has been assessed both by aerobiological sampling in outdoor environment and study of *Cladosporium* biology at the laboratory (Grinn-Gofroń, 2007; Harvey, 1967; O'Connor et al., 2014; Pady et al., 1969). In Worcester, *Cladosporium* spores were mainly captured from 08:00h to 18:00h, in agreement with the overall description of the fungus biology and expected time of the spore release (Fig. 5.13). A steep increase in *Cladosporium* spore concentration before 12:00h was observed for all years (Fig. 5.13). The day time concentrations for the years 2006 and 2010 were similar to the other years, but the night time concentrations were considerably larger. This could suggest that a large fraction of the observed spores in 2006 and 2010 was due to long distance transport, as previously observed for *Ganoderma* (Sadyś et al., 2014a) and *Alternaria* (Sadyś et al., 2014b; Skjøth et al., 2012). The year 2007 had a somewhat different daily pattern compared to the remaining years, e.g. with a daily peak at 18:00h. This could be explained by a low number of high spore count days in that particular year (Table 5.2). However, once the number of days with high spore count (≥ 6000 s m^{-3}) increased, *i.e.*: in 2006 and 2010, the mid-day pattern was detected. This was also true for 2008, although less apparent (Fig. 5.13). Results similar to ours were reported by Harvey (1967) for Cardiff in Wales and O'Connor et al. (2014) for Cork in Ireland, who also underlined that a sufficient large data set was needed in order to observe an actual diurnal profile.

In this study, it was assumed that local wind spent 100% of its time above the land surface, as the sampling site was 160 km (100 mi) away from the coast to the North, 160 km (100 mi) away from the coast to the East, 65 km (40 mi) away from the coast to the South, and therefore it should be considered as an inland location. Another proof for that could be a comparative study between Cork and Worcester with regard to the spore levels of selected common fungi present in the air (O'Connor et al., 2014). Results of the back trajectory analysis in Worcester showed that air mass, despite their frequent origin over the sea areas, spent vast of their time over the land surface (Table 5.5). The analysis of air mass transport indicated one major direction, where inoculum sources of *Cladosporium* spores could be located, *i.e.*: SE-SW (Table 5.5.). This result was correlating to that

obtained for the local wind direction, where majority of the local wind also originated from the same bearing (Table 5.4, Fig. 5.14). This regularity of the prevalent wind directions in Worcester must be greatly influenced by the regional topography, as previously described by Sadyś et al. (2014a), which is one of the reasons why there is only a moderate to good agreement between wind direction and over all air mass pattern (Table 5.6). The back trajectories were calculated at 500 m above ground level, while the highest point (Beacon) of the surrounding Worcester – Malvern Hills, is 425 m above sea level. As such, the back trajectories should have been influenced by the Malvern Hills, what has never been examined. The reason is the coarse spatial scale in the meteorological data set (GDAS) that is used by the HYSPLIT model. In fact Hernández-Ceballos et al. (2014b) showed that a replacement of the GDAS data by more detailed data from the WRF model improved considerably the quality of the results (Skamarock and Weisman, 2009). The same study also showed that sufficient topographic effects on the trajectories were visible in the WRF results but not in the GDAS data. It is therefore likely, that the use of higher resolution meteorological data can provide a better connection between air mass direction and observed fungal spore concentrations. Hence, it would be advisable to conduct a follow-up study, with the HYSPLIT model but using a meteorological data set, which will reveal the effects of the landscape in the Midlands of England in order to detect differences between local wind and air mass.

Obtained results, based on five years of observation, showed a weak, but yet statistically significant ($p \leq 0.05$) correlation between high *Cladosporium* spore counts (≥ 6000 s m^{-3}) and both local wind and overall air mass directions. Better results were obtained using Spearman's rank test than with linear-circular association test. The inoculum sources of the investigated fungus seemed to have a regional origin, but yet must be located within the UK territory at SE-SW direction from the monitoring station situated in Worcester.

5.3. *Didymella*

5.3.1. Introduction

Didymella spores constituted an important fraction of the total spore load observed in Worcester during the study period (Table 3.4). This has been described in more detail in Chapter 3. Methods used in this study were described in Chapter 2.

5.3.2. Results

5.3.2.1. Back trajectories and source maps

Seven individual days were selected for investigation, when daily mean *Didymella* spore concentration was equal to or above 9000 s m^{-3}. Back-trajectories were calculated for the following dates: 21st of July 2007, 24th of July 2007, 11th of July 2008, 12th of July 2008, 7th of August 2008, 13th of August 2008, and 7th of August 2009, with an hourly resolution (*n*=168).

The back trajectories drawn for six out of seven events showed that there was no contamination from the continental Europe (Table 5.7). In five cases it was observed that the air masses spent a minor fraction of time over Ireland, and this varied from 1.02% (7th of August 2008) to 9.44% (7th August 2009), (Table 5.7). On the 21st of July 2007 air masses spent 100% of time over the land areas of England. During selected period, the majority of the time that air masses spent over the sea areas varied between 50.60% and 82.82% (Table 5.7).

The source map presenting the distribution of crops under rotation expressed in percentage in 5 x 5 km grid cells has been described in detail in section 5.1.2.2. (Fig. 5.2).

Table 5.7 Location of the hourly trajectories on days with high *Didymella* spore counts during the period 2006-2010 (*n*=7).

Date	Area	Number of points	Percentage[a]
21 Jul 2007	Overall	1176	100.00
	Sea areas	923	78.49
	Land areas	253	21.51
	British Isles	253	21.51
	Non-UK areas	0	0.00
	United Kingdom	253	21.51
24 Jul 2007	Overall	1176	100.00
	Sea areas	718	61.05
	Land areas	458	38.95
	British Isles	414	35.20
	Non-UK areas	135	11.48
	United Kingdom	323	27.47
11 Jul 2008	Overall	1176	100.00
	Sea areas	934	79.42
	Land areas	242	20.58
	British Isles	242	20.58
	Non-UK areas	21	1.79
	United Kingdom	221	18.79
12 Jul 2008	Overall	1176	100.00
	Sea areas	945	80.36
	Land areas	231	19.64
	British Isles	231	19.64
	Non-UK areas	53	4.51
	United Kingdom	178	15.14
7 Aug 2008	Overall	1176	100.00
	Sea areas	887	75.43
	Land areas	289	24.57
	British Isles	289	24.57
	Non-UK areas	12	1.02
	United Kingdom	277	23.55
13 Aug 2008	Overall	1176	100.00
	Sea areas	974	82.82
	Land areas	202	17.18
	British Isles	202	17.18
	Non-UK areas	78	6.63
	United Kingdom	124	10.54
7 Aug 2009	Overall	1176	100.00
	Sea areas	595	50.60
	Land areas	581	49.40
	British Isles	581	49.40
	Non-UK areas	111	9.44
	United Kingdom	470	39.97

[a] Percentage of the total number of back trajectory points.

5.3.2.2. Day 1: 21st of July 2007

The 21st of July 2007 was the first day that was selected for a more detailed analysis as the daily mean *Didymella* spore concentration was 9961 s m^{-3}. The weather synopsis maps showed that beginning on the 20th of July 2007 a low pressure system that was located in the central part of the Ireland (1017 hPa), moved gradually towards the United Kingdom. The next day central England was fully covered by this low pressure system and the air pressure decreased to 1012 hPa. No weather clear up was found on the 22nd of July 2007, as UK was still under the influence of the same low pressure system, although the pressure system shifted slightly towards Northern Scotland and Norway. During this event, the mean temperature recorded in Worcester dropped from 14°C to 12°C and then increased to 15°C. The relative humidity varied from 91% to 79% and it corresponded to the amount of rainfall that occurred within these three days. Over 60 mm of rain was recorded on the 20th of July 2007, followed by light rain showers during the two consecutive days, when 2.80 mm and 0.20 mm were recorded respectively.

The hourly variations in *Didymella* ascospore concentration were presented visually in Fig. 5.16. The maximum spore concentration occurred on the 21st of July 2007at 23:00h with 65000 s m^{-3} of air recorded (Fig. 5.16). A smaller shadow peak was found four hours later on the following day (~41000 s m^{-3}). After that the concentration of *Didymella* spores decreased to double the amount recorded before the start of this episode.

The air masses that reached Worcester on the 21st of July 2007 originated from the North Sea as shown by the back trajectory analysis (Fig. 5.17). The most dominant height of the air masses over the sea areas was 500 m above ground level (Fig. 5.17 a), while after contact with the mainland the height increased to 1000 m above ground level. This was mainly observed for the air masses moving over Yorkshire and the West Midlands directly to Worcester. However, the air masses that passed over the East Midlands were characterized as having height of 500 m above ground level (Fig. 5.17 a). The height but also the speed of the two plumes of the air masses was different (Fig. 5.17 b).

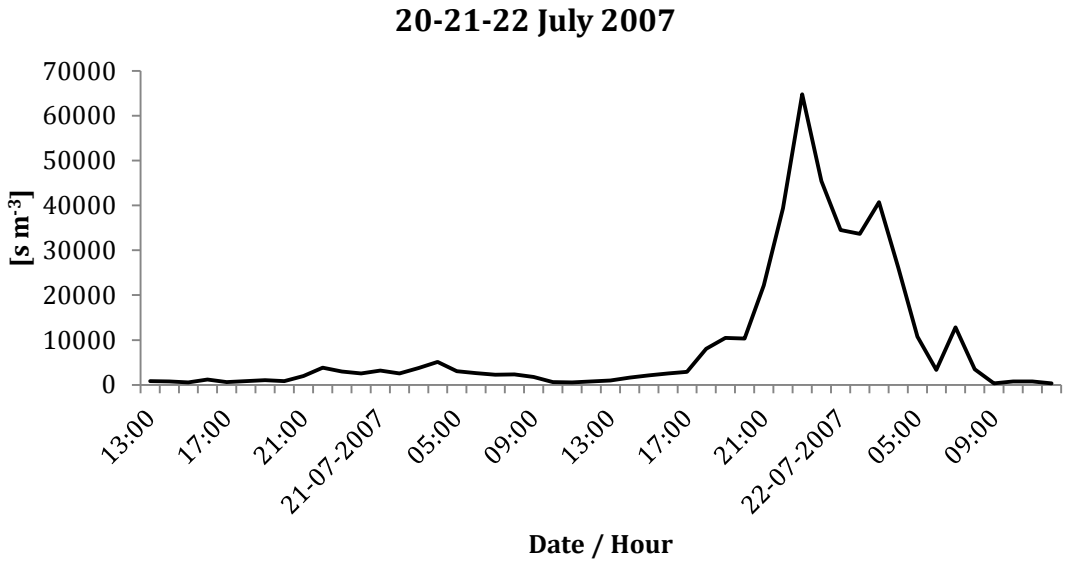


Fig. 5.16 Hourly variations in *Didymella* ascospore distribution recorded between 20th of July (13:00h) and 22nd of July (12:00h) 2007 in Worcester, UK.

The higher air masses that passed over Yorkshire were moving slower (from 7 to 18 hours), whereas the air masses that travelled above the East Midlands were moving much faster and covered a similar distance within 6 hours. The overall mean speed of the air masses recorded during the last 6 hours before they reached Worcester was 7 m s^{-1}, and within 12 hours and 18 hours 9 m s^{-1}, within 24 hours 8 m s^{-1}.

Location of potential arable areas that could be a source of *Didymella* ascospores was found to be in an arc from 360° to 45° (from North to North-East) from Worcester (Fig. 5.17), within a distance of 152 km (94 mi) and a time equal to 6 hours. Transport of the air masses that took from 12 hours to 18 hours must cross a distance of 583 km (362 mi).

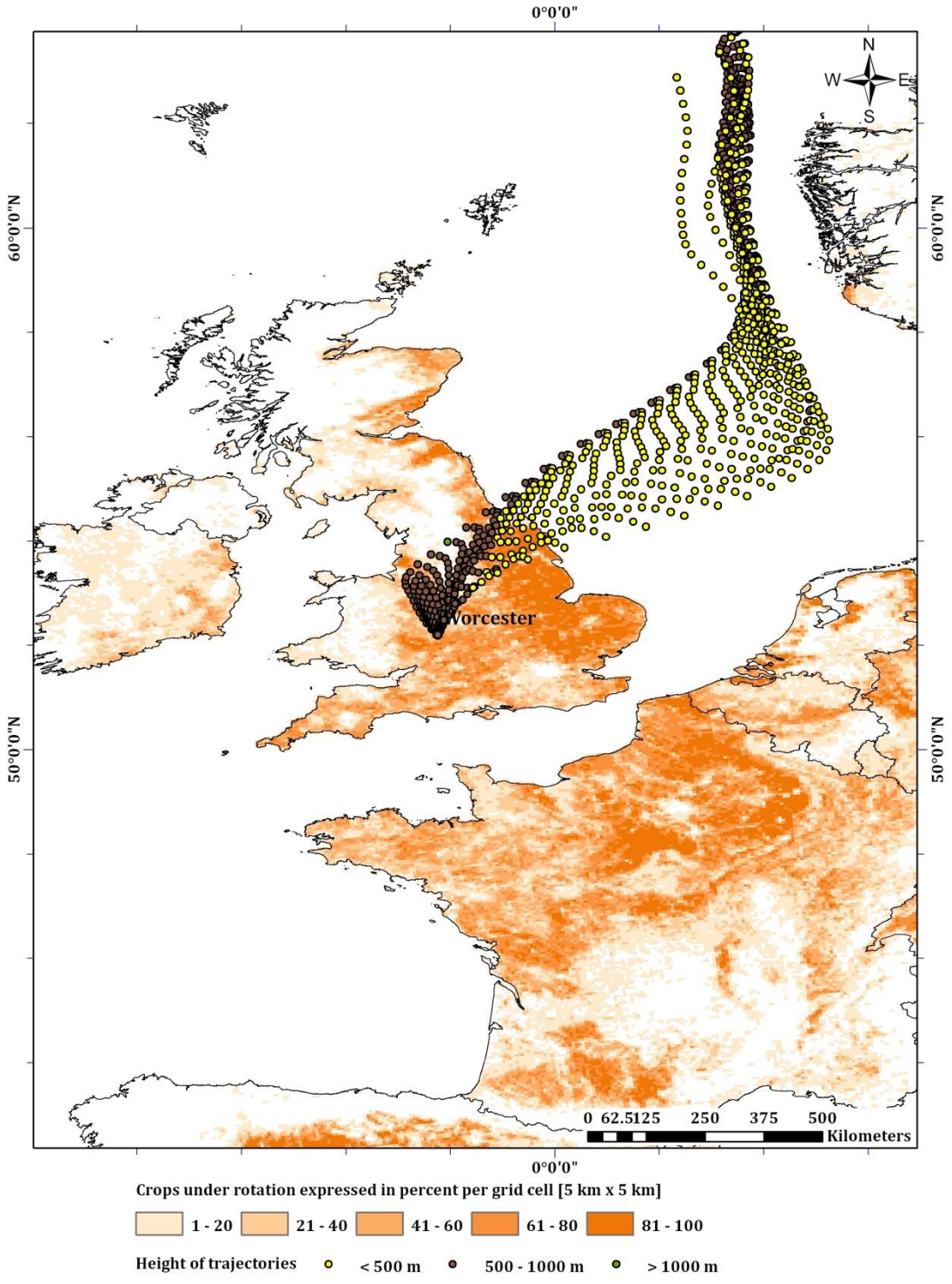


Fig. 5.17a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 21st of July 2007 (*n*=24).

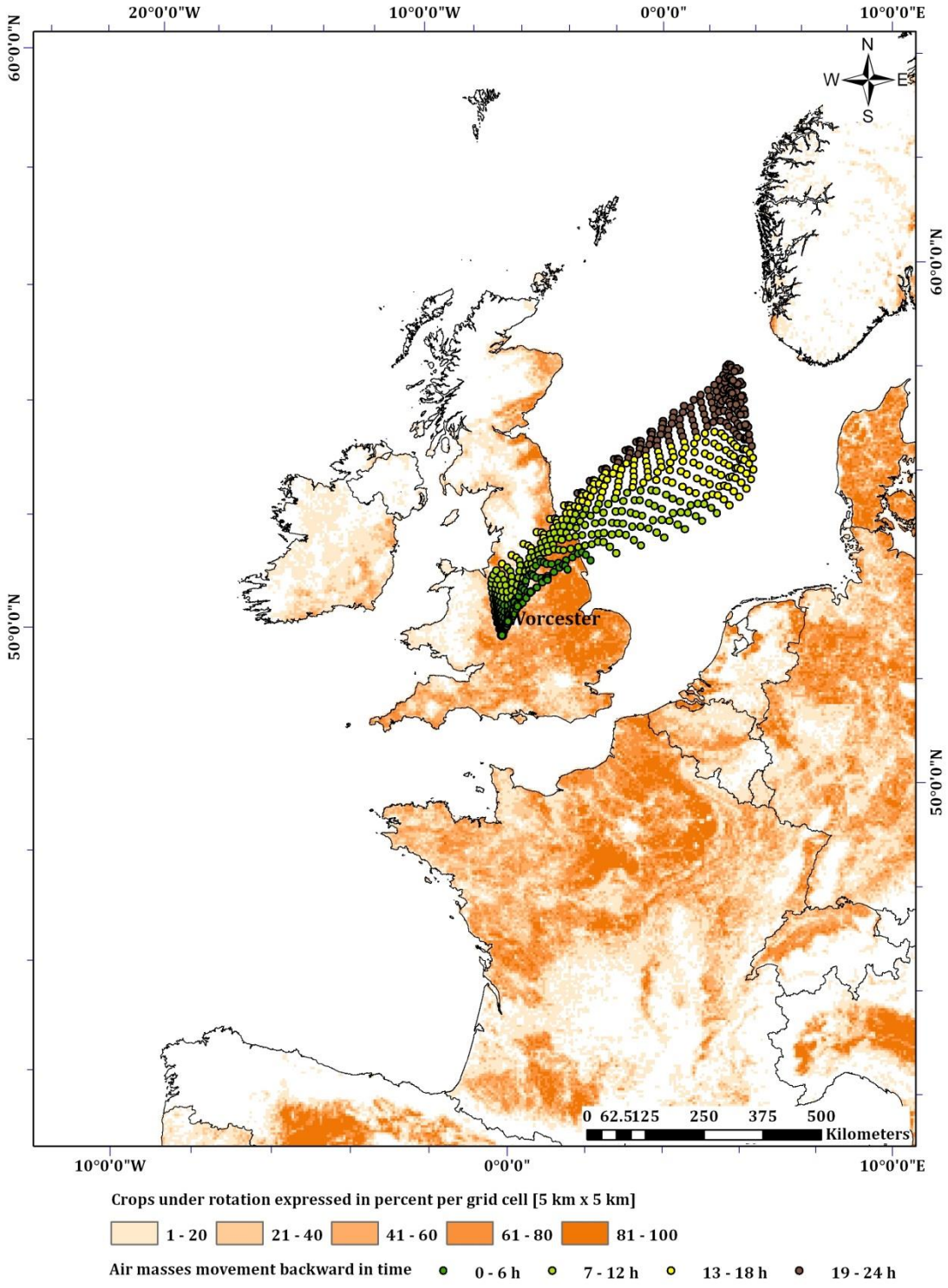


Fig. 5.17b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 21st of July 2007 (*n*=24).

5.3.2.3. Day 2: 24th of July 2007

A peak of *Didymella* spores was observed on the 24th of July 2007 (9801 s m^{-3}). The British Isles were dominated by two low pressure systems in the preceeding day. One system originated from the Atlantic Ocean with a centre situated close to the Brittany Peninslula (992 hPa), while the latter was situated above the Baltic Sea and mainly affected North England and Scotland (1004 hPa). The next 24 hours showed the movement of these low pressure systems. The first system shifted to the North towards the Svalbard Archipelago, the second passed over the south coast of England towards the Netherlands. A slight improvement in weather was seen on the 25th of July 2007, when the southern and central part of England came under the sphere of the influence of a high pressure system (1008 hPa) emanating from the south of France. The mean temparature that was recorded in Worcester within this event varied from 15°C to 16°C. Light rain showers were present on the 23rd of July and 25th of July 2007 with amounts of rain equal to 3.20 mm and 0.80 mm, respectively. The relative humidity varied between 83% and 67% with the lowest value occuring on the peak day.

The maximum *Didymella* ascospore concentration was found at 02:00h with a spore count equal to 44171 s m^{-3} (Fig. 5.18). A smaller "shadow" peak was found three hours later (38552 s m^{-3}), (Fig. 5.18). Afterwards the concentration of *Didymella* spores dropped to zero at 14:00h (Fig. 5.18).

The back trajectory analysis showed that the air masses began in the North Sea off the coast of the Shetland Islands (Fig. 5.19). They then passed over Northern Ireland, Ireland, Wales and approached Worcester from an arc between 226° and 315° (Fig. 5.19). The height of the majority of the air masses varied between 500 m and 1000 m above ground level (Fig. 5.19 a). The minor fraction of the air masses that crossed over the South West and South East of England, above the coast of France, Belgium and Netherlands were found at the height of 500 m above the ground level (Fig. 5.19 a). The air masses were present above Wales and England up to 6 hours before arrival in Worcester (Fig. 5.19 b). Within 7 to 12 hours previously the air masses were overpassing the Irish Sea, while a further 6 hours earlier they occupied the southern coast of Ireland, eastern Ireland

(Leinster) and Northern Ireland (Fig. 5.19 b). The overall mean speed of the air masses recorded during 6 hours, before they reached Worcester, was 8 m s^{-1}, and within 12 hours, 18 hours and 24 hours 7 m s^{-1}.

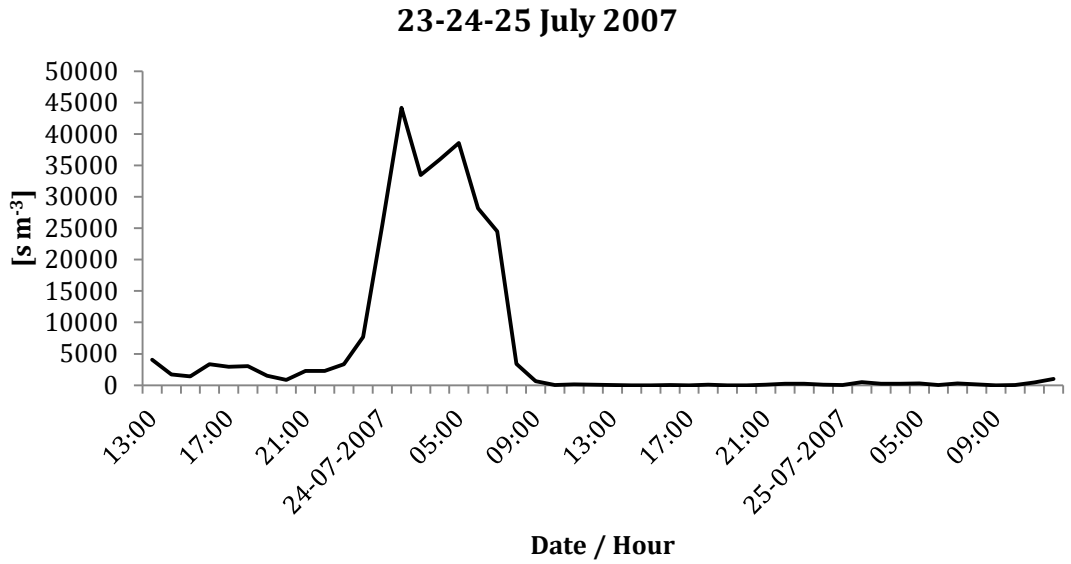


Fig. 5.18 Hourly variations in *Didymella* ascospore distribution recorded between 23rd of July (13:00h) and 25th of July (12:00h) 2007 in Worcester, UK.

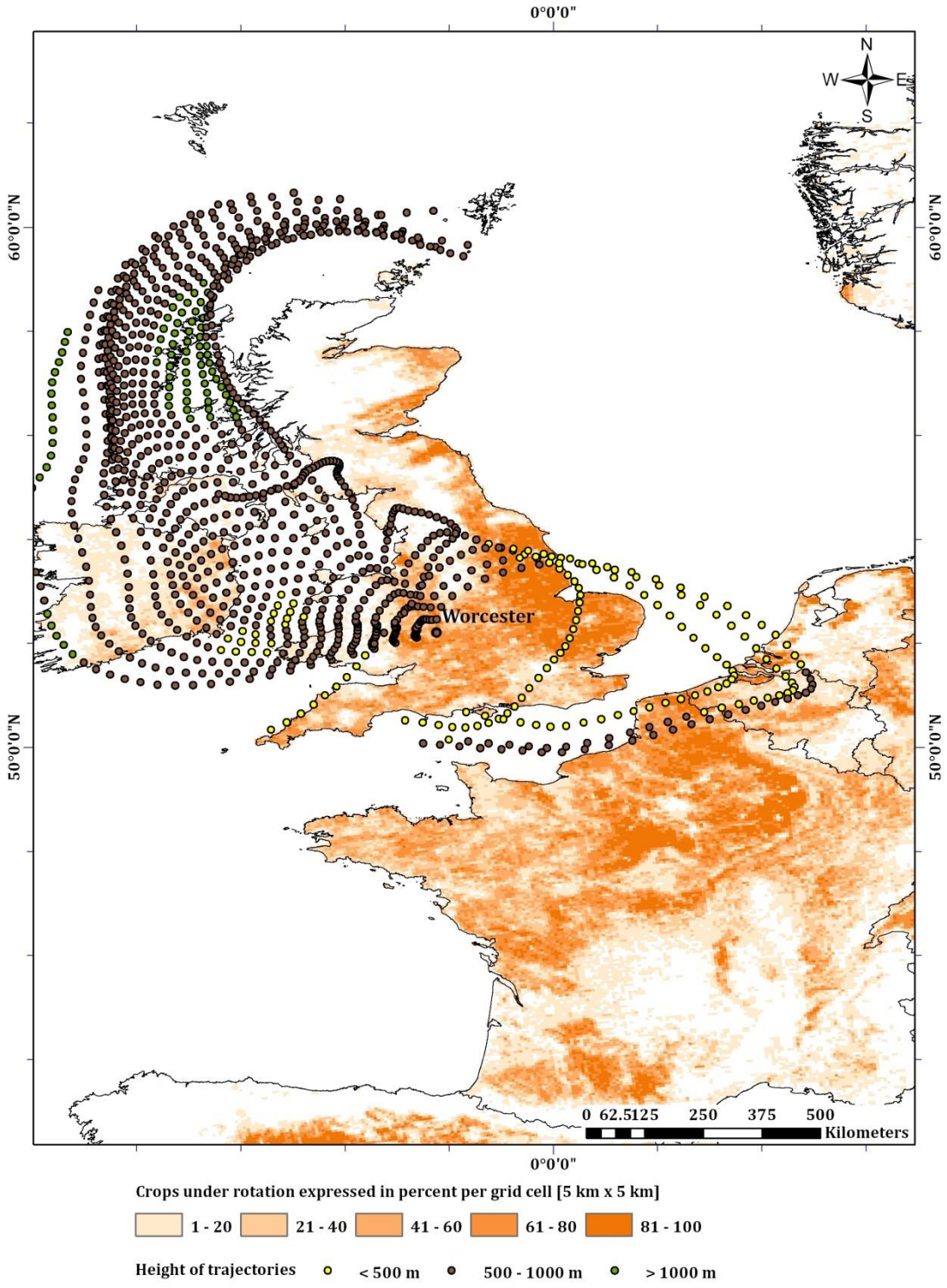


Fig. 5.19a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 24th of July 2007 (*n*=24).

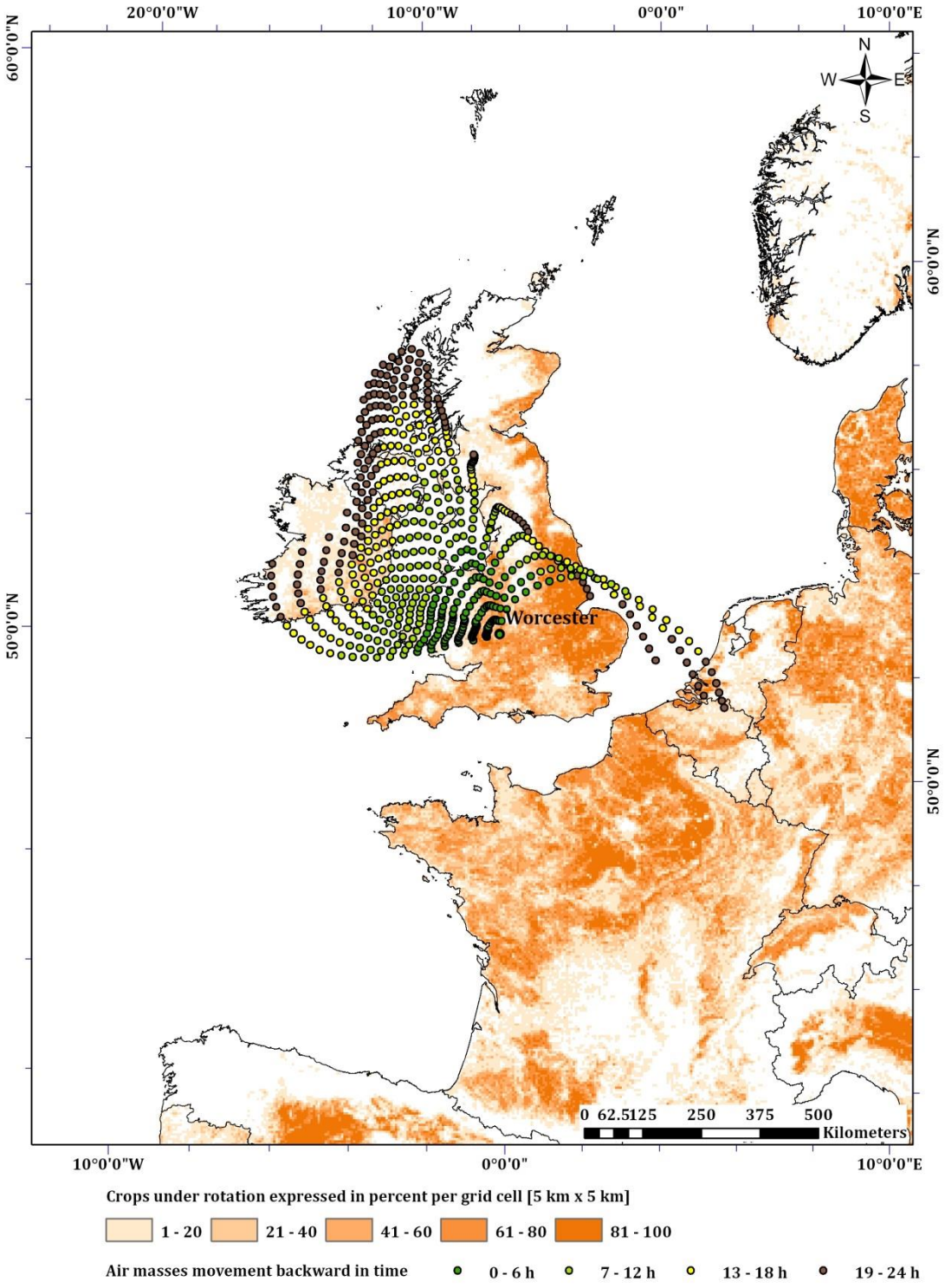


Fig. 5.19b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 24th of July 2007 (n=24).

5.3.2.4. Day 3-4: 11th and 12th of July 2008

On the 11th and 12th of July 2008 uniform concentrations of *Didymella* spores were recorded in Worcester, which were equal to 9940 s m^{-3}. Patterns produced by the back-trajectories were very similar, therefore these two days could be jointly analysed.

Beginning on the 10th of July 2008 the British Isles were fully covered by a single low pressure system, which was centred gradually moved from the Northern Ireland (997 hPa), through Isle of Man (1000 hPa) to the North Sea (996 hPa) during 72 hours. On the fourth day (13th of July 2008) most of the United Kingdom came under influence of a high pressure system (1012 hPa) originating from the Atlantic Ocean. The weather station located at the University of Worcester registered a significant decrease of the mean temperature from 16°C on the 10th of July 2008, to 15°C on the 11th of July 2008 and 13°C on the 12th of July 2008. The next day the temperature increased to 15°C. Light rain showers occurred on the 10th and 13th of July 2008, whereas 11th and 12th of July 2008 had 4.20 mm and 2.20 mm of rain, respectively. The relative humidity varied accordingly, and the highest value was found on the 11th of July 2008 (80%) with the lowest on the 13th of July 2008 (68%).

The maximum concentrations of *Didymella* spores were detected on the 11th and 12th of July 2008 at 22:00h with spore counts equal to 45770 s m^{-3} and 51907 s m^{-3}, respectively (Fig. 5.20). After the occurrence of the peak, the spore concentrations gradually decreased and between 11:00h and 12:00h it was reduced to either 173 s m^{-3} or zero (Fig. 5.20).

The overall direction of the back-trajectories, which were computed by the HYSPLIT model for 11th and 12th of July 2008, was found between 226° and 315° (Fig. 5.21). As in the previous event that took a place on the 24th of July 2007, the air masses started in the North Sea 48 hours before they reached Worcester. On the 11th of July 2008 the air masses were moving above Northern Scotland, via Northern Ireland and Wales until they finally arrived in the West Midlands (Fig. 5.21). The next day a change was observed in the angle that the air masses followed, as they crossed only over Northern Ireland, North Wales and directly

reached Worcester (Fig. 5.22). The majority of the air masses on the 11th of July 2008 passed several countries and were characterised by a low height above ground level (Fig. 5.21 a). Once they were moving above the eastern part of Wales and England their height increased up to 1000 m above ground level (Fig. 5.21 a). On the following day the height of the air masses was between 500 m and 1000 m which were dominant (Fig. 5.22 a). The speed of the air masses on the 11th of July 2008 was fairly constant at 9 m s^{-1} within 24 hours. A small increase was then found on the following day, and within the first 6 hours the speed was equal to 10 m s^{-1}, and later increased to 11 m s^{-1}.

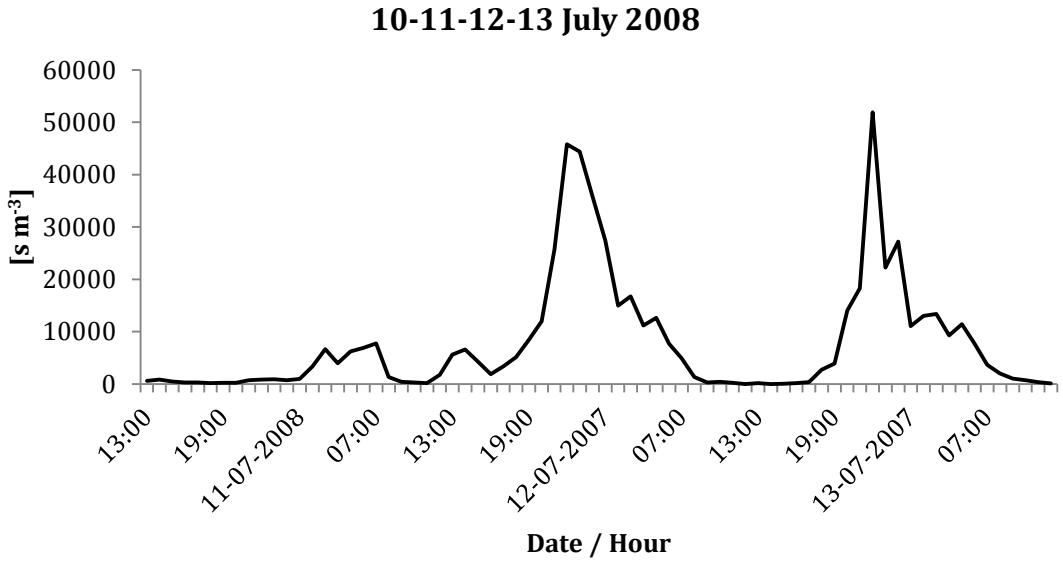


Fig. 5.20 Hourly variations in *Didymella* ascospore distribution recorded between 10th of July (13:00h) and 13th of July (12:00h) 2008 in Worcester, UK.

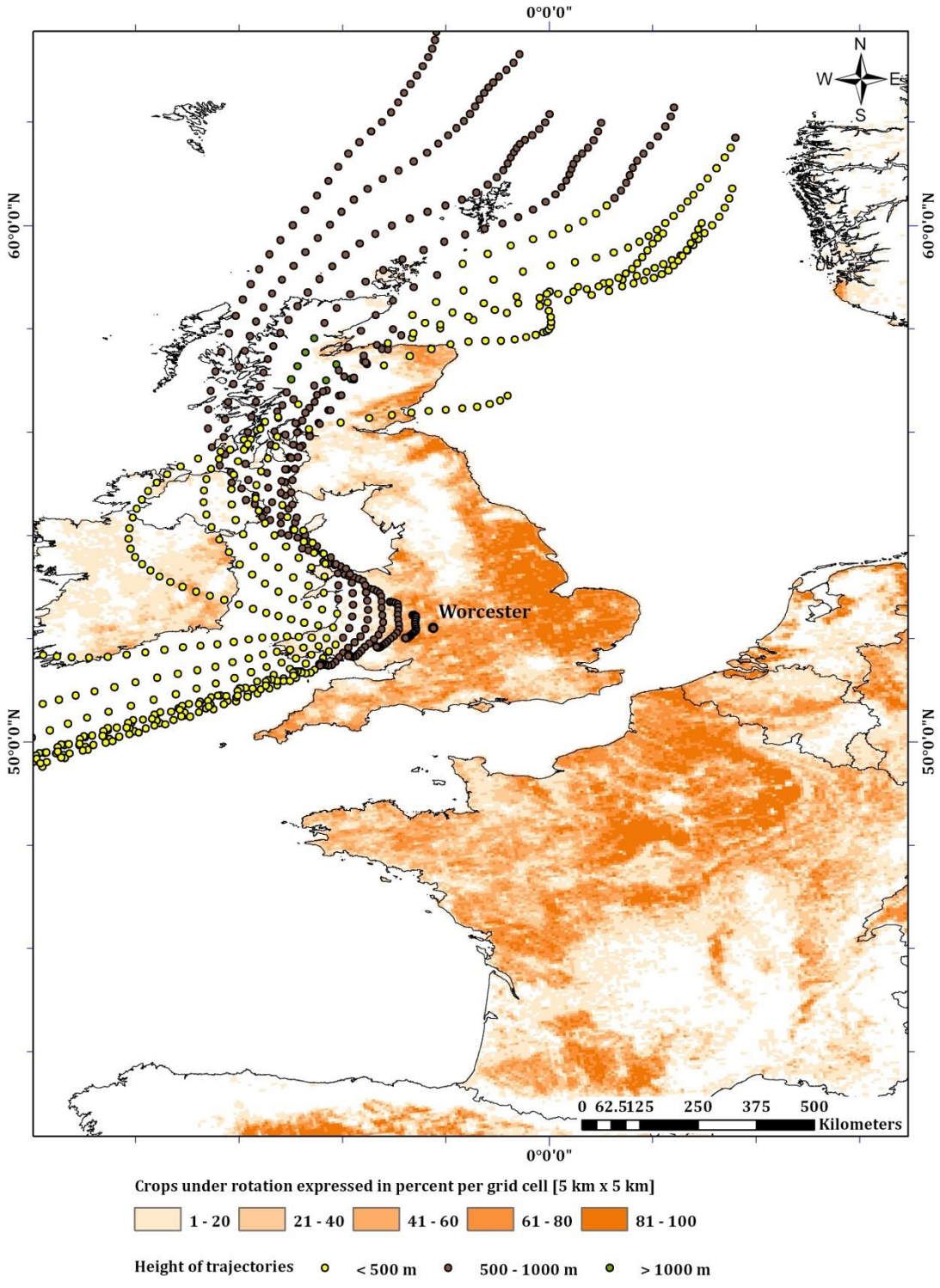


Fig. 5.21a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 11th of July 2008 (*n*=24).

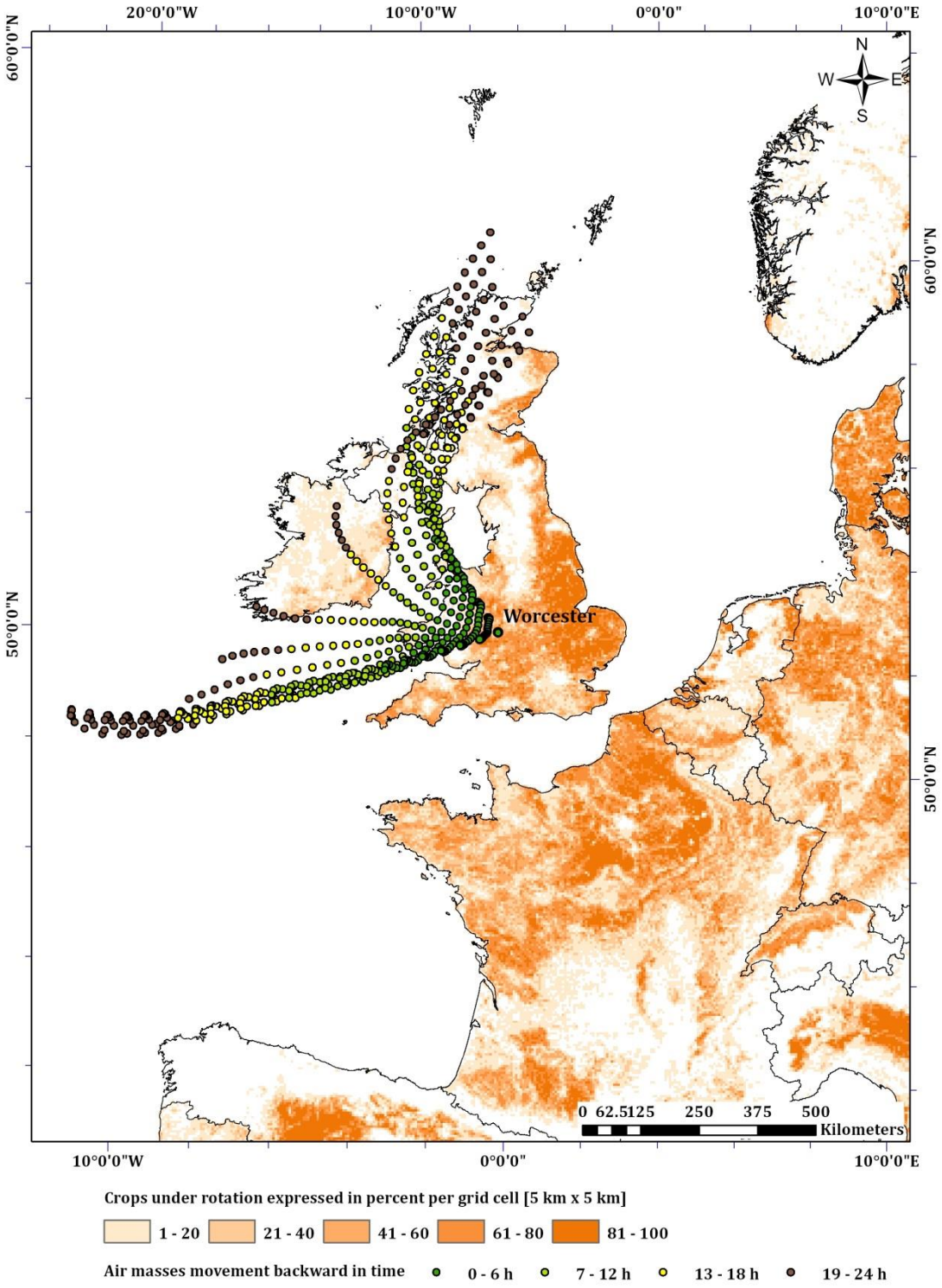


Fig. 5.21b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 11th of July 2008 (*n*=24).

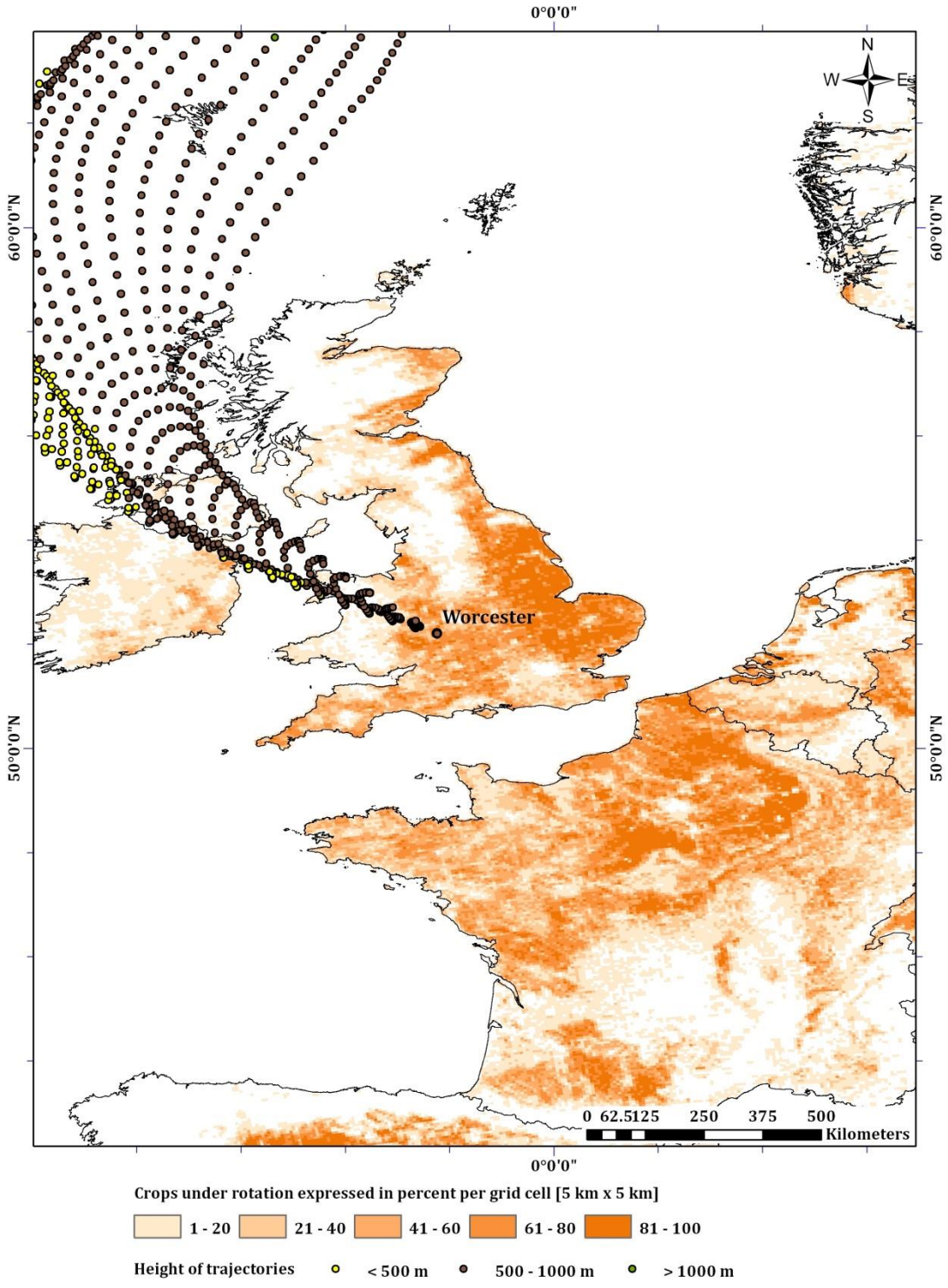


Fig. 5.22a The back trajectories showing the origin and the of the air masses 48 hours before they reached Worcester, UK on the 12th of July 2008 (*n*=24).

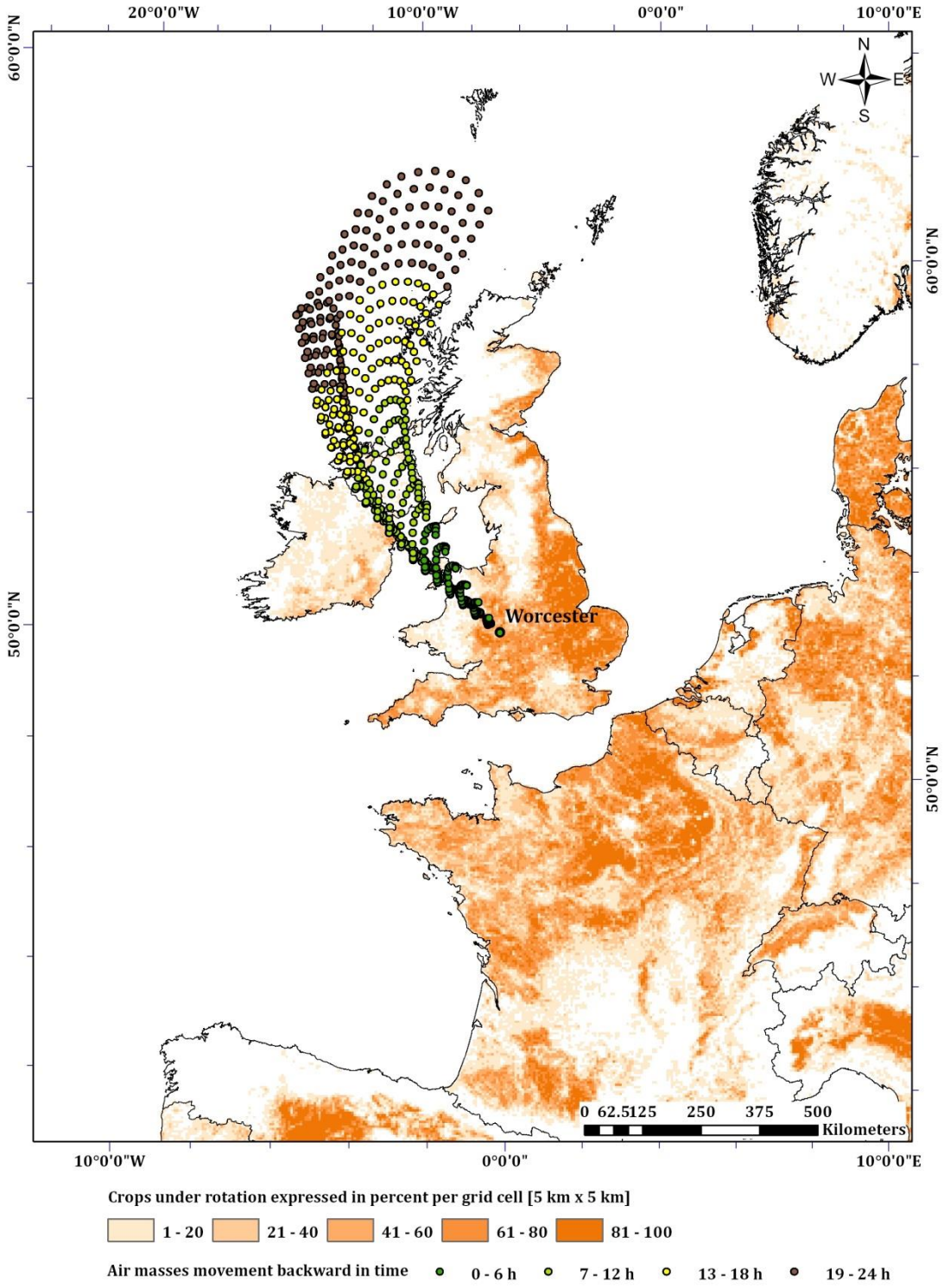


Fig. 5.22b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 12th of July 2008 (*n*=24).

5.3.2.5. Day 5: 7th of August 2008

Detailed analysis was conducted on the 7th of August 2008. This was a very unsual day, as the highest daily mean concentration of *Didymella* spores (19966 s m^{-3}) was recorded within 5 years of observations in Worcester.

On the 6th of August 2008 Great Britain was under the influence of a single low pressure system (997 hPa) that originated from the Atlantic Ocean close by the southern coast of Ireland. This low pressure system was seen to move its center closer towards Ireland (1002 hPa) on the following day. On the 8th of August 2008 the low pressure system subsequently moved into the North Sea as a new high pressure system from the Atlantic Ocean (1012 hPa) approached. Within this three day period the mean temperature dropped from 19°C to 17°C. Simultaneously relative humidity decreased from 80% to 73%. No rainfall was observed on the 6th of August 2008, in contrast to the following 48 hours, where 1.20 mm and 3.80 mm of rainfall were recorded respectively.

Two peaks were recorded on the 7th of August 2008, at 05:00h and 22:00h with spore counts equal to 66170 s m^{-3} and 72048 s m^{-3}, respectively (Fig. 5.23). The lowest concentration was then found between 17:00h and 18:00h, when spore counts of 432 s m^{-3} were recorded (Fig. 5.23).

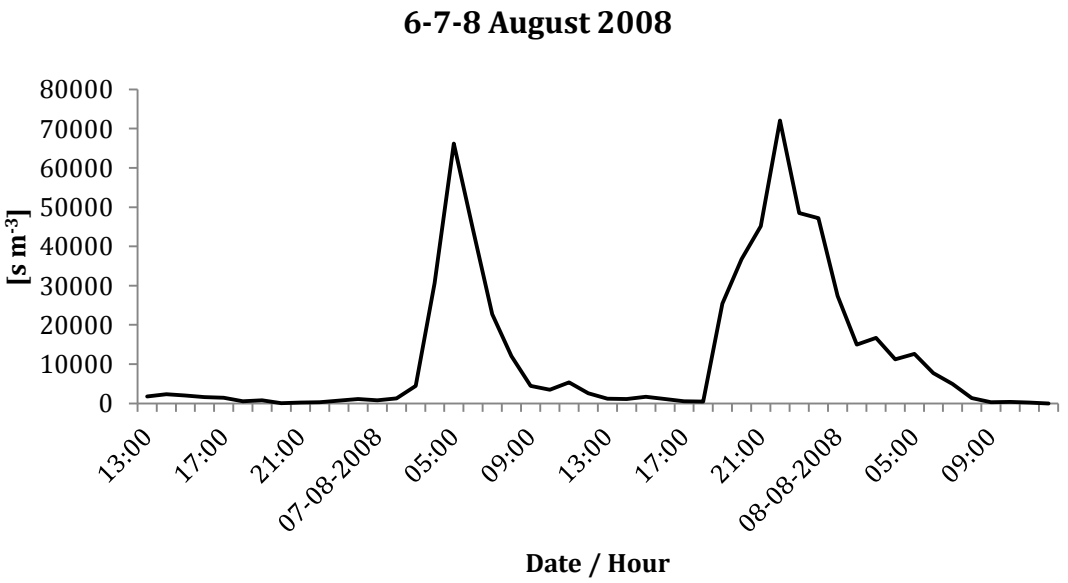


Fig. 5.23 Hourly variations in *Didymella* ascospore distribution recorded between 6th of August (13:00h) and 8th of August (12:00h) 2008 in Worcester, UK.

Figure 5.24 presented the air masses movement on the 7th of August 2008. It showed that the air masses originated from the Atlantic Ocean, and did not have contact with the land surface of the European continent. A minor fraction of the air masses was observed passing over the southern part of Ireland (Fig. 5.24). Air masses arriving in Worcester on the examined day were coming from an arc between South-West and Westerly directions (226°-270°), (Fig. 5.24). Furthermore, most air masses at this time were located below 500 m above ground level while passing over the water areas (Atlantic Ocean, Celtic Sea and Irish Sea), (Fig. 5.24 a). The height then increased up to 1000 m above ground level once they entered of England (Fig. 5.24 a). The analysis of the speed of the air masses revealed that the mean wind within the past 6 hours before arrival in Worcester was 5 m s^{-1}, between 12 to 18 hours it has 6 m s^{-1} and during the last 24 hours it was 7 m s^{-1}.

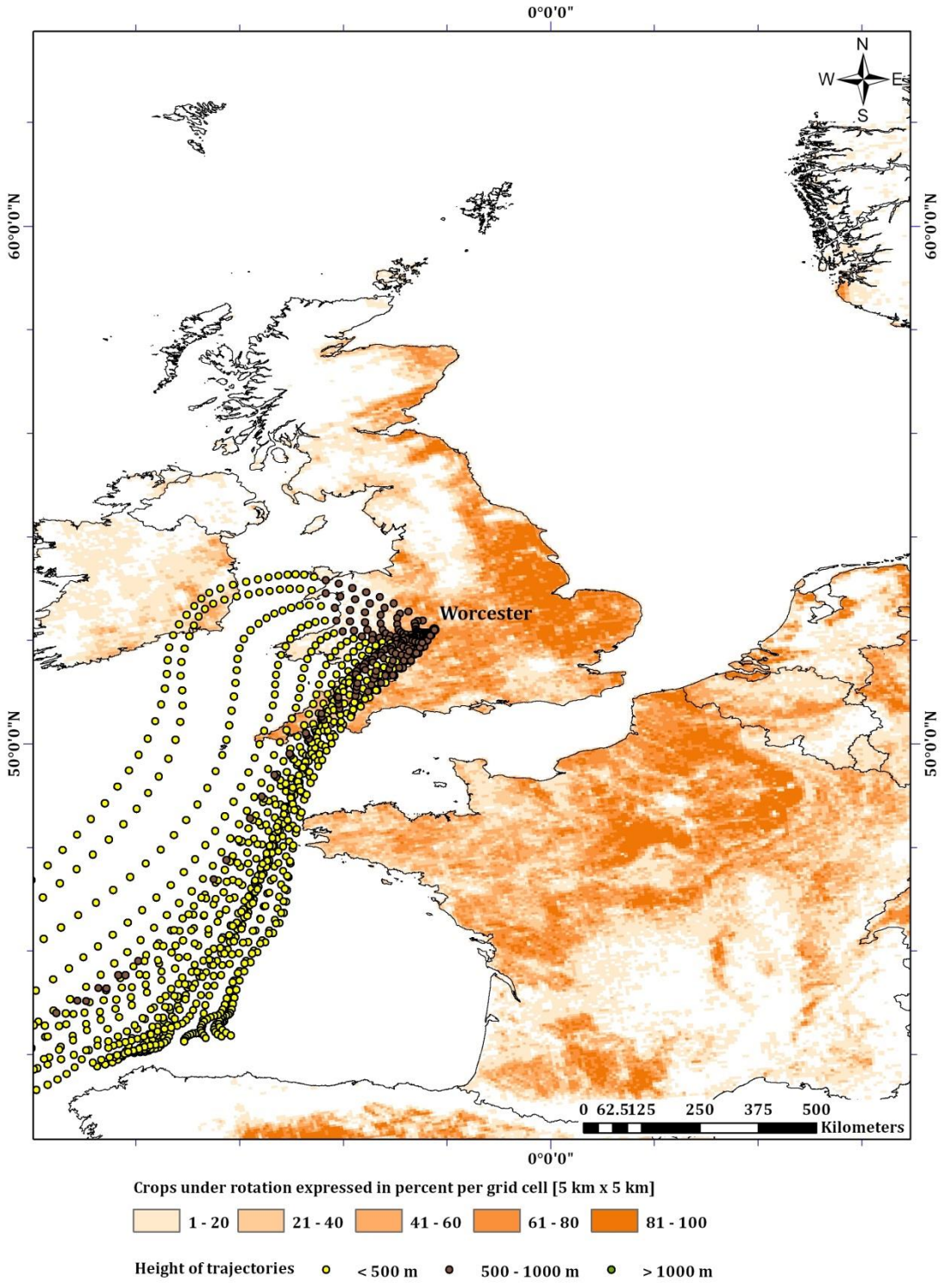


Fig. 5.24a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 7th of August 2008 (*n*=24).

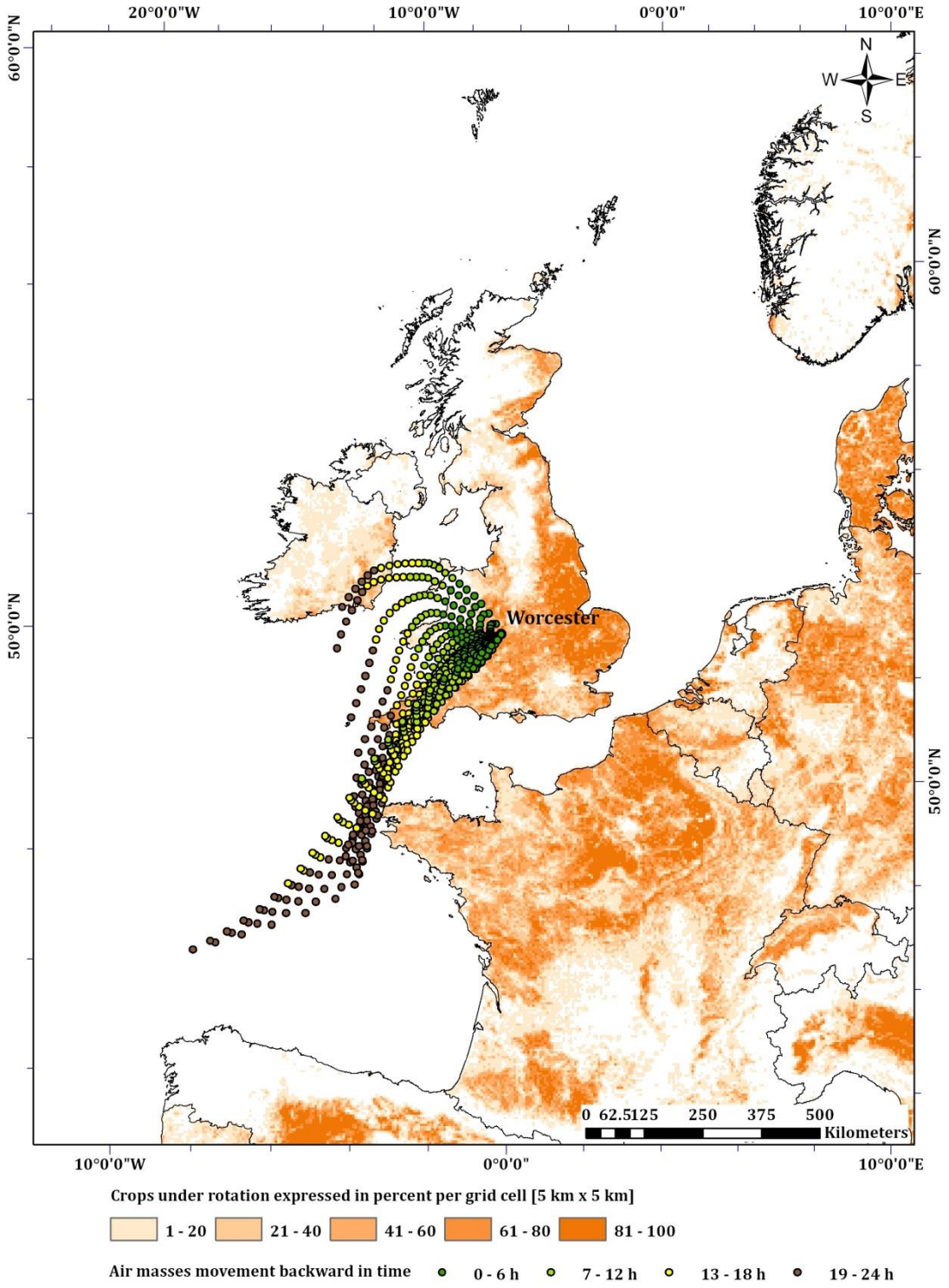


Fig. 5.24b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 7th of August 2008 (*n*=24).

5.3.2.6. Day 6: 13th of August 2008

The daily mean concentration of *Didymella* spores recorded on the 13th of August 2008 was 9351 s m^{-3}. The event commenced on the 12th of August 2008, when Great Britain was under the influence of three low pressure systems. The first was centred on the Atlantic Ocean located close to the southern coast of Ireland (987 hPa), the second was directly covering the middle part of Ireland (986 hPa) and the third occupied Lesser Britain (989 hPa). Within the following 24 hours all three low pressure systems moved towards the North and North-East directions as they moved in response to a high pressure system, which started forming in the Atlantic Ocean. On the 14th of August 2008 the development of the high pressure system and its movement towards the British Isles was observed. Within the same period of time, in Worcester a small change was seen in the mean temperature from 15°C to 14°C. Rainfall was present each day, although various amounts of rain were recorded, *i.e.*: 5.40 mm, 2.40 mm and 0.60 mm, respectively. The highest relative humidity within these three days occurred on the 13th of August 2008 (81%).

Didymella ascospores peaked on the 13th of August 2008 with a concentration equal to 9351 s m^{-3} (Fig. 5.25). Two maxima were found, one at 05:00h (31464 s m^{-3}) and a second at 21:00h (25586 s m^{-3}). The lowest spore concentration occurred at 11:00h (389 s m^{-3}).

The back trajectory analysis showed that the air masses that arrived in Worcester on the 13th of August 2008 were coming from the South West and Westerly directions (226°-270°), (Fig. 5.26). These air masses began over the waters of the North Atlantic Ocean and passed above Southern Ireland and Wales. The height of the air masses was below 500 m while travelling across the Irish land and the Irish Sea (Fig. 5.26 a). Then the air masses were found at a greater height above ground level, once they entered the United Kingdom. The air masses spent less than 6 hours over Wales and England (Fig. 5.26 b). Overall the mean speed of the air masses was 12 m s^{-1} within last 24 hours. A fraction of the air masses, which was seen above Ireland, spent 12 to 18 hours there. The possible sources of *Didymella* spores, which travelled to Worcester following the direction indicated

by the back trajectories, would be located in the Munster region (Ireland), Herefordshire and Gloucestershire (England), (Fig. 5.26).

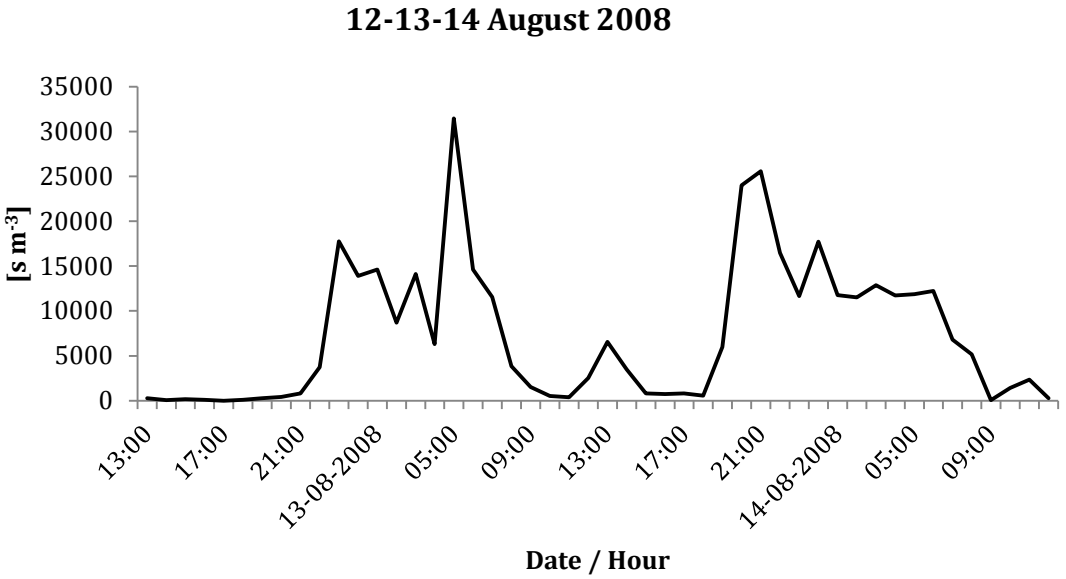


Fig. 5.25 Hourly variations in *Didymella* ascospore distribution recorded between 12th of August (13:00h) and 14th of August (12:00h) 2008 in Worcester, UK.

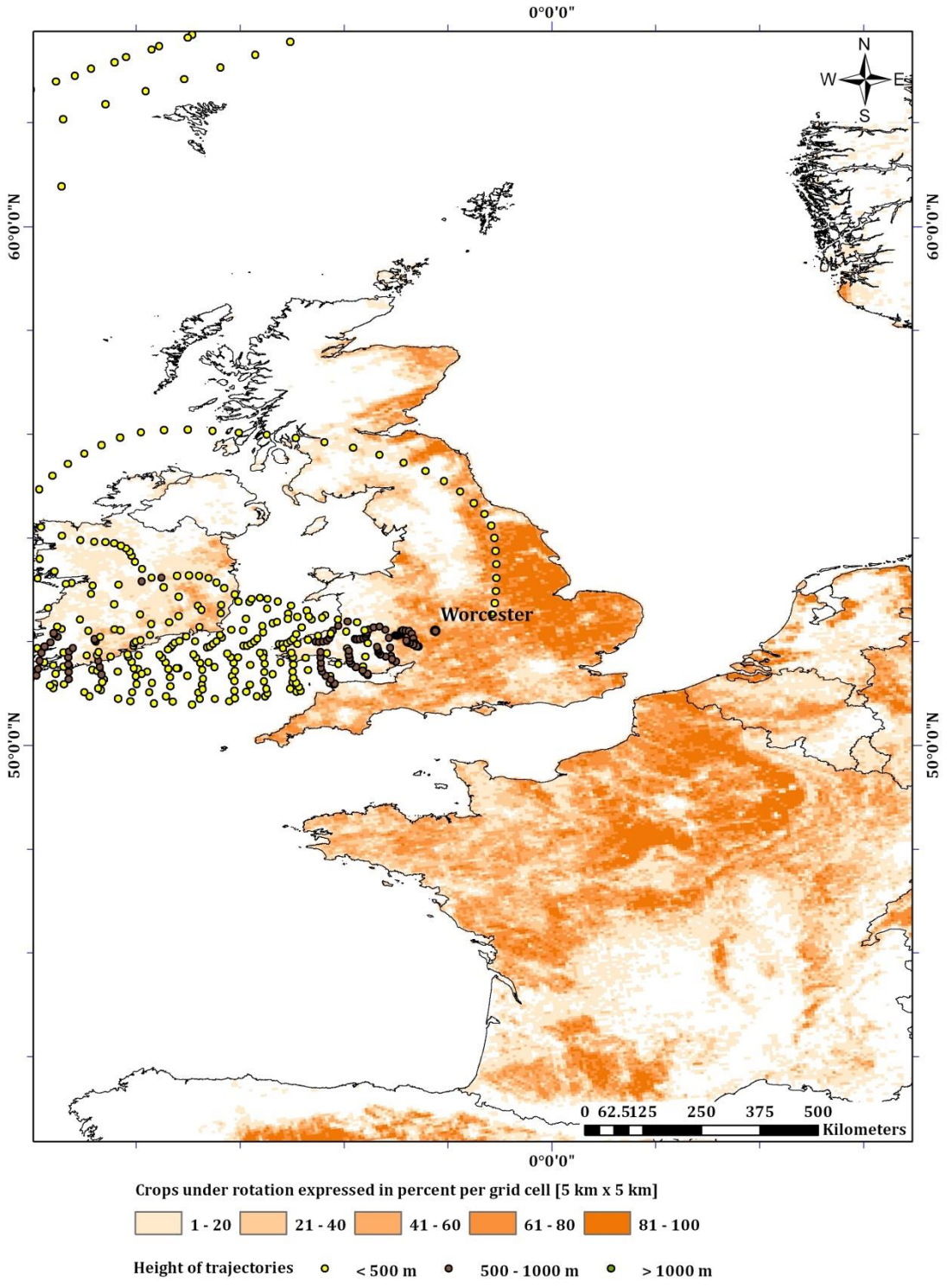


Fig. 5.26a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 13th of August 2008 (*n*=24).

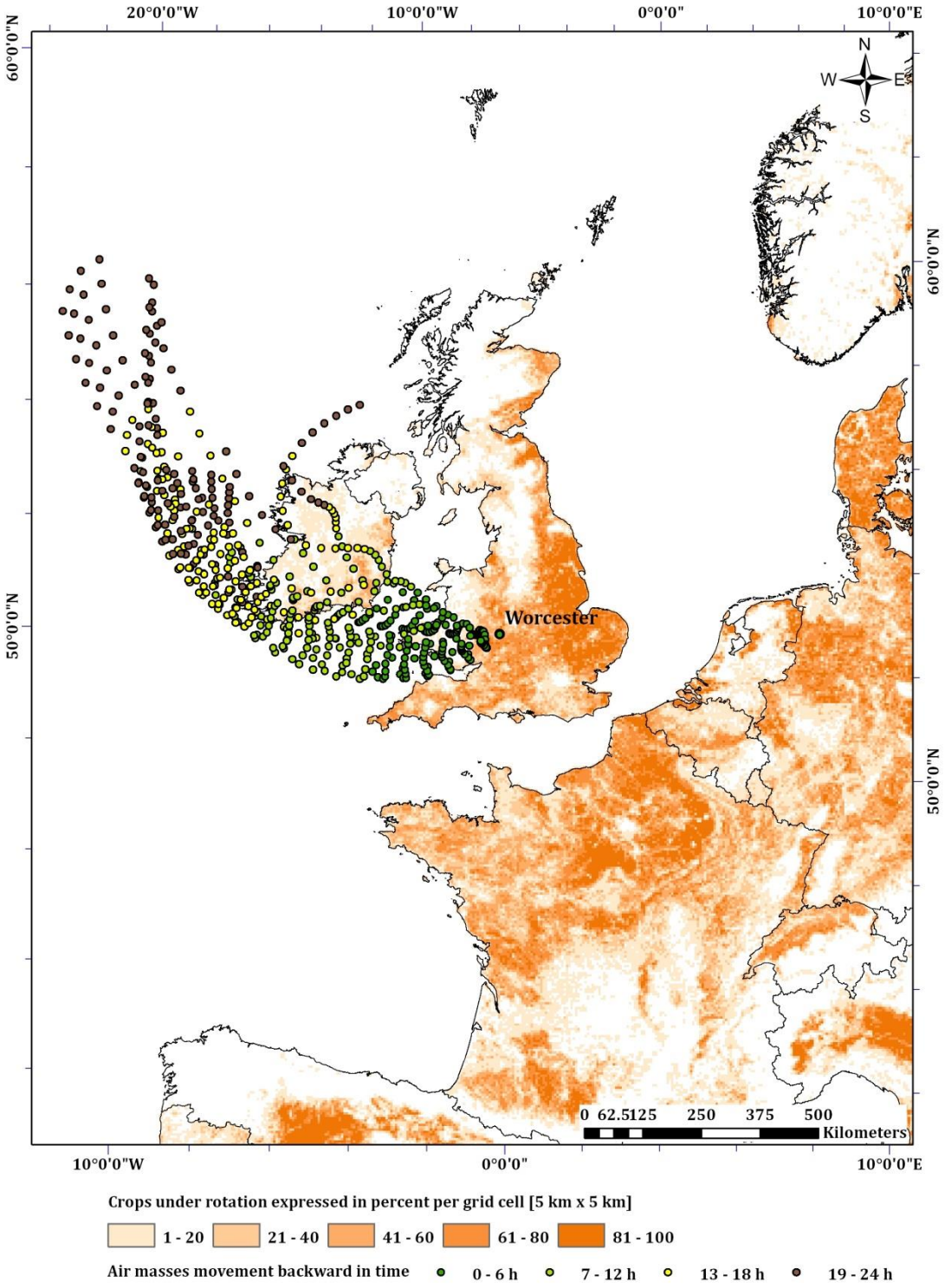


Fig. 5.26b The back trajectories showing the speed of the air masses within 24 hours before they reached Worcester, UK on the 13th of August 2008 (*n*=24).

5.3.2.7. Day 7: 7th of August 2009

The 7th of August was selected for further investigation, when the daily mean *Didymella* spore concentration was observed to be 11623 s m^{-3}. Initially, on the 6th of August 2009 the UK was under a mixed influence of a low pressure system located between Iceland and Ireland (987 hPa) and a high pressure system emerging from Scandinavia (1026 hPa). The following day did not bring much clarification, as an additional high pressure system moved towards the Atlantic Ocean (1020 hPa). The weather remained almost unchanged on the 8th of August 2009. During this period the mean temperature varied between 17°C and 18°C. Rainfall was present only on the 7th of August 2009, when 1 mm of rain fell. The relative humidity decreased from 73% to 63% within examined 72 hours.

Single peak of *Didymella* ascospores was detected in Worcester on the 7th of August 2009 (Fig. 5.27). The maximum concentration occurred at 03:00h with a spore count equal to 78574 m s^{-3} (Fig. 5.27). The concentration decreased to almost zero between 10:00h and 19:00h (Fig. 5.27). The post-dawn peak resulted in the highest hourly concentration of *Didymella* spores that has been recorded in Worcester.

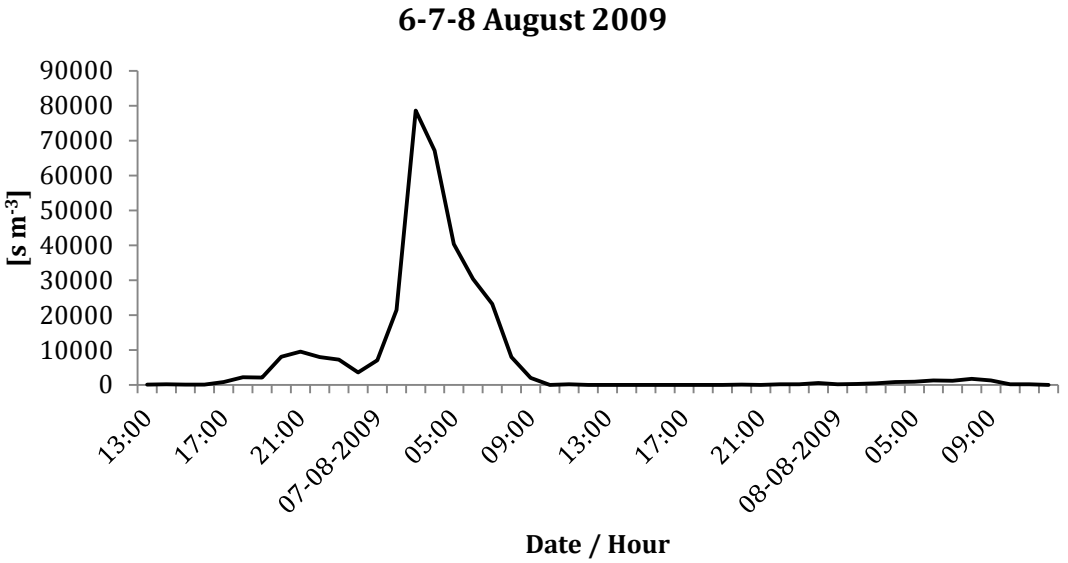


Fig. 5.27 Hourly variations in *Didymella* ascospore distribution recorded between 6th of August (13:00h) and 8th of August (12:00h) 2009 in Worcester, UK.

According to the back-trajectory analysis, the air masses arrived in Worcester from the North West and Northerly directions (316°-360°), (Fig. 5.28). The most dominant height of the air masses varied from 500 m to 1000 m above ground level, although a fraction of the air masses was also seen at greater heights (Fig. 5.28 a). The overall mean speed of the air masses was 4 m s^{-1} during the last 6 to 12 hour period and 3 m s^{-1} during the 18 to 24 hour period. The possible location of the crops, which would constitute a source of *Didymella* spores, was found between 173 and 194 km away from Worcester in the North West England (107-120 mi).

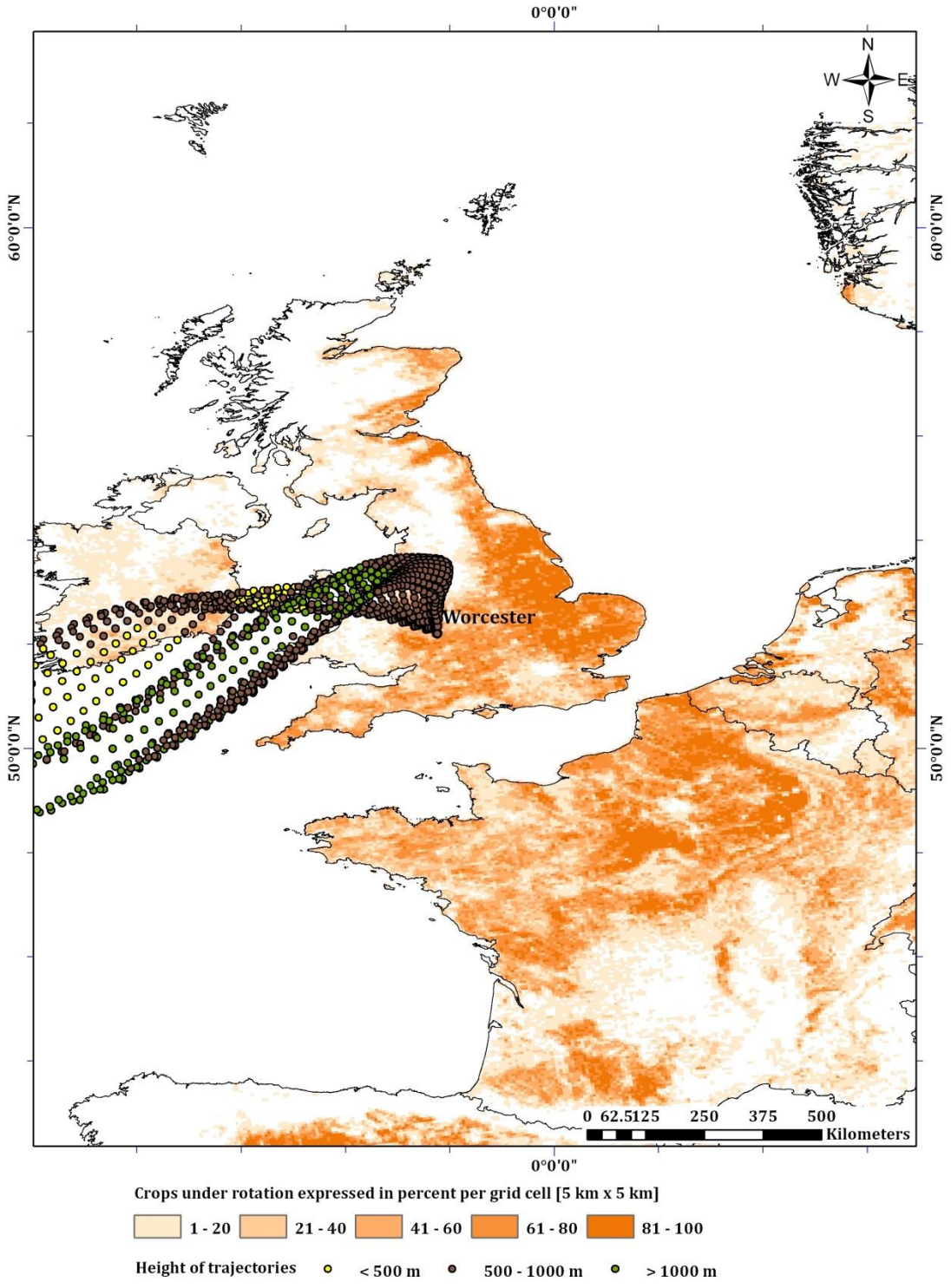


Fig. 5.28a The back trajectories showing the origin and the height of the air masses 48 hours before they reached Worcester, UK on the 7th of August 2009 (*n*=24).

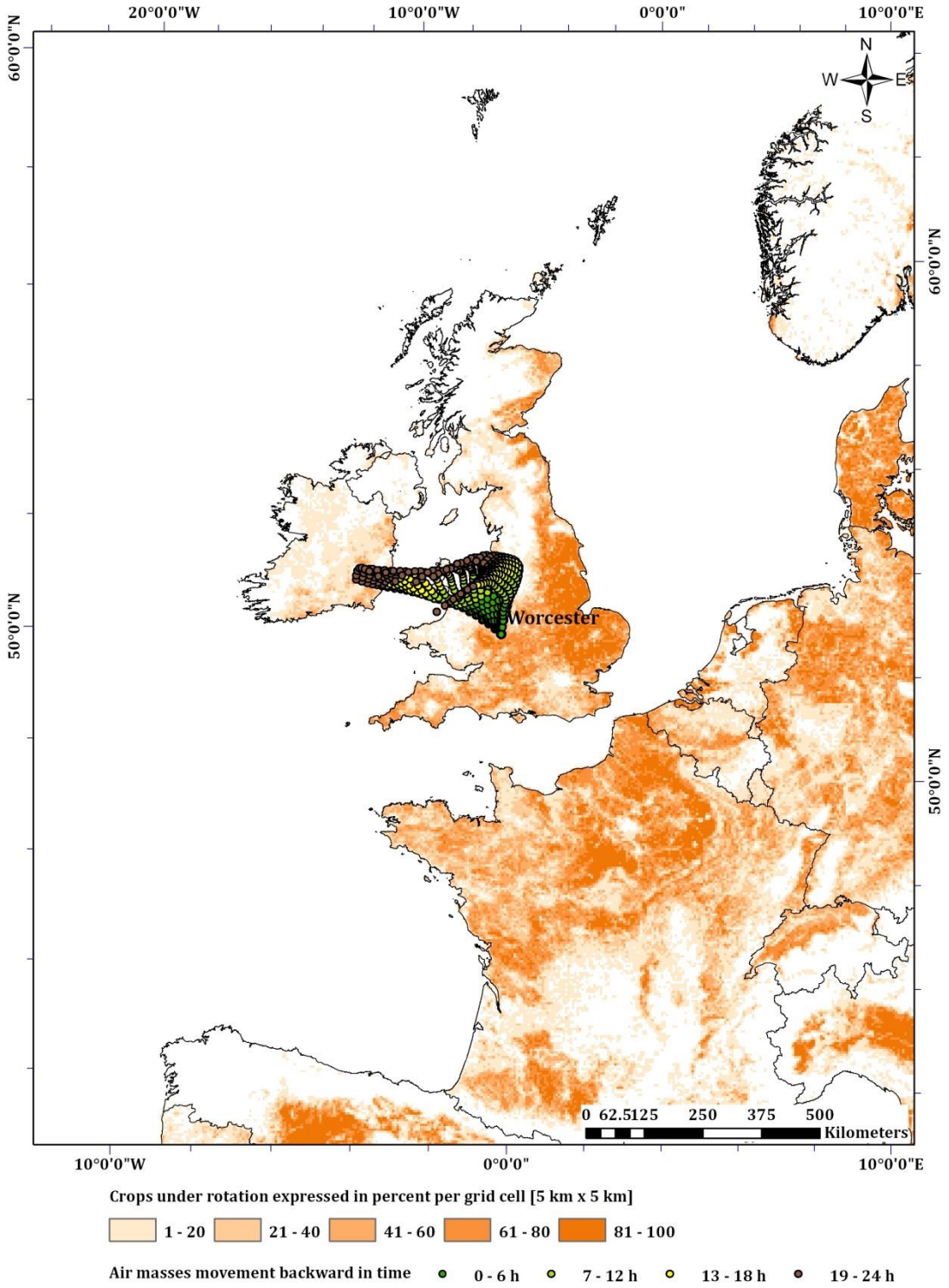


Fig. 5.28b The back trajectories showing the speed of the air masses within 24 hou$_{rs}$ before they reached Worcester, UK on the 7th of August 2009 (n=24).

5.3.3. Discussion and conclusions

The best *Didymella* spore event was seen on the 21st of July 2007 in Worcester, when according to the back trajectories, the air masses that originated from the North Sea moved directly to England (Fig. 5.17). It can be expected that the air masses were free of any biological particles as most of the time was spent over the areas of sea (Table 5.7), (Elbert et al., 2007; Urbano et al., 2011). Therefore there was no contamination from continental Europe and *Didymella* ascospores registered in Worcester must come from the sources located in England. The second plume of air masses, which passed over the East Midlands, would likely contribute to the spore load recorded in Worcester taking into consideration the distance of 151 km (93 mi) and the time of the air masses transport (6 hours). Trapero-Casas et al. (1996) observed that spore release of *Didymella rabiei* was at its greatest between 14:00h and 16:00h. Thus the time of the peaks found in Worcester reflected precisely the distance between the location of crops and the air sampler. The second plume could be an explanation for the second smaller peak, which was detected.

The second *Didymella* ascospore peak that took a place on the 24th of July 2007 presented a different scenario than the previous event (Fig. 5.19). Although the air masses originated similarly from the North Sea they overpassed Ireland and Wales entirely before they reached Worcester. Only a small fraction of the air masses was seen moving above the crops in the East Midlands of England (Fig. 5.19). Crops located in Leinster (Ireland) could be considered as a source of *Didymella* ascospores. Taking into consideration the time of the air masses transport (13-18 hours), the release of spores would have occurred between 08:00h and 16:00h. Hence, the air masses crossed over 450 km (280 mi) of distance.

The third event that was jointly presented for two consecutive days, 11th and 12th of July 2008 showed two remarkably high and uniform peaks of *Didymella* spores (Fig. 5.21-5.25). The 12th of July 2008 exhibited a very similar pattern of the air masses transport to the previous day, however the range of the air masses direction was narrower and it was oscillating around 315° (Fig. 5.22). The common

sources that the air masses passed within the investigated 48 hours were the crops located in the Ulster region (Ireland) and crops situated within the West Midlands of England. *Didymella* ascospores, which were trapped by the air sampler, were possibly originating from the local arable lands, within a distance of 194-216 km (120-134 mi) with 6 hours of transport. This hypothesis would be in agreement with the time of maximum concentration of *Didymella* ascospores recorded at 22:00h (the shorter air masses transport would cause the earlier peak).

Fourth example in this study (on the 7th of August 2008) demonstrated a bimodal distribution of *Didymella* ascospores was found (Fig. 5.24). The occurrence of the double peak pattern with peaks 17 hours apart could be explained by the presence of two plumes of the air masses that followed the same direction (Fig. 5.23). The first plume would pick up the spores from the crops located within a maximum of 6 hours of air masses transport distance equal to 108 km (67 mi). The second plume collected the spores from the source located at a maximum of 130 km (80 mi) distance from Worcester. Tracing back the movement of the air masses to Worcester indicated that possible sources of *Didymella* would be located in South West England.

The analysis of the back-trajectories on the 13th of August 2008 showed that there is a possibility that a big fraction of *Didymella* spores recorded in Worcester that day could have originated from the crops located in Ireland (Fig. 5.26). This hypothesis would be supported by the fact that during that event the air masses were moving at a fairly constant speed of 12 m s^{-1} during the last 24 hours. As the back trajectory analysis indicated the location of the crops within a range of 12 hours, then the distance between spore sources and Worcester would be equal to 518 km (321 mi). Similarly to the previously described event on the 21st July 2007, a double peak pattern in *Didymella* ascospore distribution was detected (Fig. 5.25). Once more, it is likely that the air masses could be divided into two overlapping air plumes aiming within a few hours of each other.

The last event, which occurred on the 7th of August 2009, suggested locations of additional possible source of *Didymella* ascospores (Fig. 5.28). Crops were situated within the range of 316° and 360°. Assuming that the transport of

the air masses would take up to 12 hours then the distance between crops and the spore trap was equal to 173 km (107 mi). This hypothesis is more likely to be true, as the *Didymella* spore release time was reported to occur between 14:00h and 16:00h (Trapero-Casas, 1996).

All presented days with a daily mean spore count $n \geq 9000$ s m^{-3} were mainly originating from the sources located within the British Isles. No contamination from continental Europe was detected at 6 out of 7 described events. The back trajectories indicated that potential sources of *Didymella* spores trapped in Worcester could be found within the distance of 108-216 km (67-134 mi). The majority of the spores must have originated from the crops cultivated at the East Midlands, South West England, West Midlands and North West England.

5.4. *Ganoderma*

5.4.1. Introduction

Ganoderma spores constituted an important fraction of the total spore load recorded in Worcester during the study period (Table 3.4). This has been described in more detail in Chapter 3. Methods used in this study were described in Chapter 2.

Results presented below constitute a major part of the article titled "Back-trajectories show export of airborne fungal spores (*Ganoderma* sp.) from forests to agricultural and urban areas in England", which has been accepted for publication in *Atmospheric Environment* on the 7th of November 2013.

5.4.2. Results

5.4.2.1. Spore concentrations and local meteorology

The SFI for *Ganoderma* parameters (Table 5.8) and monthly spore indices (sum of the mean daily concentrations for each month, Table 5.9) varied between 2006 and 2010. The highest SFI of *Ganoderma* basidiospores was recorded in 2009 (20612) and the lowest index was observed in 2007 (14947). The start of the season varied from end of April in 2007 to the beginning of June in 2006 and all *Ganoderma* basidiospores seasons ended in October of each year. The highest daily

mean spore concentration was usually found between mid-August and mid-October.

The total number of days, when the mean daily *Ganoderma* spore concentration was ≥ 200 s m^{-3}, differentiated from 5 in 2007 to 18 in 2009 totalling 58 days with high spore counts. The maximum mean spore concentration was observed on the 11th of September 2006 (76 s m^{-3}, Table 5.8) and the maximum hourly *Ganoderma* spore concentration (1210 s m^{-3}, Table 5.8) was found at 04:00h on the 14th of September 2008 and at 02:00h on the 21st of September 2010.

Table 5.8 Characteristics of *Ganoderma* basidiospore seasons in the air in Worcester, UK.

Year	2006	2007	2008	2009	2010
Start of season	2 Jun	23 Apr	10 May	22 May	25 May
End of season	29 Oct	29 Oct	17 Oct	29 Oct	14 Oct
Duration	150 days	190 days	161 days	161 days	143 days
Daily peak value	376 s m^{-3}	225 s m^{-3}	281 s m^{-3}	310 s m^{-3}	254 s m^{-3}
Date of daily peak	11 Sep	14 Oct	18 Sep	14 Aug	22 Sep
SFI[a]	19.103	14.947	15.793	20.612	16.946
Mean	115 s m^{-3}	71 s m^{-3}	89 s m^{-3}	115 s m^{-3}	107 s m^{-3}
Median	106 s m^{-3}	58 s m^{-3}	75 s m^{-3}	106 s m^{-3}	99 s m^{-3}
SD[b]	62 s m^{-3}	46 s m^{-3}	58 s m^{-3}	64 s m^{-3}	57 s m^{-3}
Duration ≥200 s m^{-3}	16 days	5 days	10 days	18 days	9 days
Contribution	20%	7%	15%	21%	12%
Hourly peak value	864 s m^{-3}	778 s m^{-3}	1210 s m^{-3}	1124 s m^{-3}	1210 s m^{-3}
Time of the hourly peak value	11 Sep 07:00	25 Aug 04:00	14 Sep 04:00	29 May 01:00	21 Sep 02:00

[a] SFI - Seasonal Fungal Index

[b] SD - Standard Deviation

Table 5.9 Monthly Index of *Ganoderma* basidiospores (sum of mean daily concentration) expressed in s m^{-3}.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2006	60	20	6	237	588	2105	3640	3077	5320	3284	531	235
2007	99	59	94	748	791	2059	1676	3152	2971	2711	521	66
2008	71	47	43	198	1294	1965	2806	2873	4591	1584	276	45
2009	6	21	99	465	1038	2336	3505	5551	3931	3016	569	75
2010	0	6	29	243	717	2420	3929	3496	3578	2172	349	6

The diurnal pattern in *Ganoderma* spore concentration was calculated for all days included in the study (n=805) and for all days with high counts (n=58), (Fig. 5.29). The daily profile for the concentration of *Ganoderma* spores peaked between 04:00h and 06:00h (all days) and at 03:00h (on high count days). Both profiles revealed similar trends. The peaks in concentration appeared before the dawn, and then the concentration dropped sharply within two hours (08:00h-10:00h), to gradually increase again beginning from 14:00h.

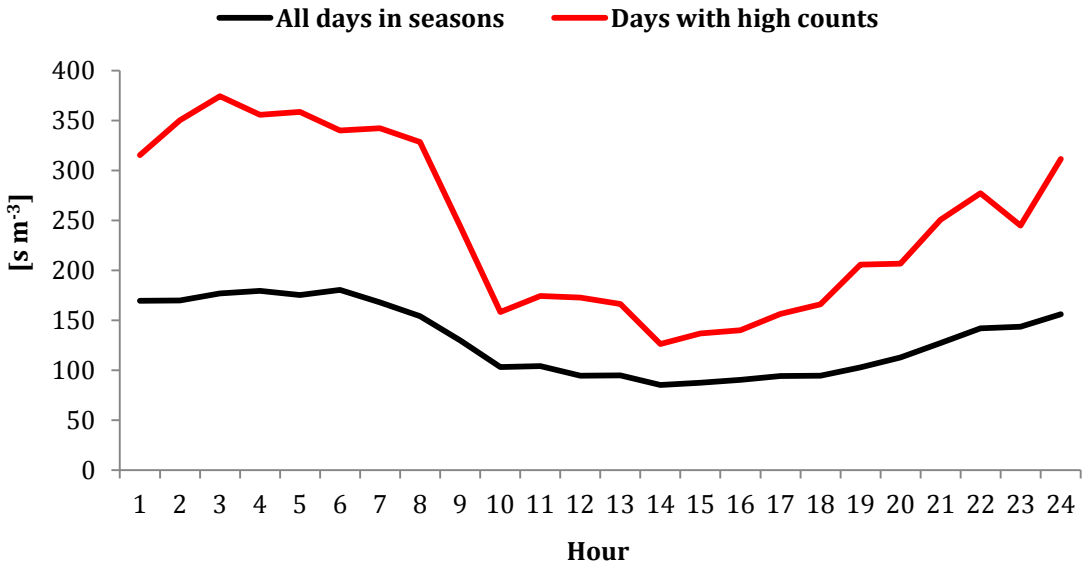


Fig. 5.29 Diurnal profile of *Ganoderma* spore concentration for all days (black) and days with counts above or equal to 200 s m^{-3} (red). Hours were expressed according to UTC (Sadyś et al., 2014).

Statistically significant relationships with the *Ganoderma* spore concentration were found with following meteorological variables: maximum, minimum, mean and dew point temperatures for all years with correlations in the range from r_S=0.22 to r_S=0.54 (Table 5.10). All other meteorological variables had correlations that vary from r_S=0.07 to r_S=0.37, where the correlations were statistically significant between one and three out of all five years.

For all days (n=805), a negative statistically significant relationship was observed with rainfall and wind direction, and after five year period also with solar radiation (Table 5.11). All of these relationships were varying from very weak to

weak strength according to Choudhury (2009). For high count days (*n*=58) moderate negative statistically significant relationships with wind direction were observed on five years of data (Table 5.11).

Table 5.10 The results of the Spearman's rank test between *Ganoderma* basidiospore concentration in the air in Worcester, UK and meteorological parameters for all days (*n*=805).

Parameter	2006	2007	2008	2009	2010	2006-10
TMA	**0.24***	**0.41***	**0.35***	**0.44***	**0.53***	**0.42***
TMI	**0.22***	**0.41***	**0.35***	**0.45***	**0.54***	**0.35***
TME	**0.22***	**0.41***	**0.35***	**0.44***	**0.53***	**0.42***
DPT	**0.34***	**0.39***	**0.40***	**0.52***	**0.54***	**0.45***
RAIN	*ns*	**-0.27***	*ns*	*ns*	*ns*	**-0.07***
RH	*ns*	*ns*	*ns*	*ns*	*ns*	**0.07***
WD	**-0.24***	*ns*	*ns*	*ns*	*ns*	**-0.12***
AP	*ns*	**0.24***	**0.37***	*ns*	*ns*	**0.14***

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

Table 5.11 The results of the Spearman's rank test between *Ganoderma* basidiospore concentration in the air in Worcester and meteorological parameters for high spore count (≥200 s m^{-3}) days (*n*=58).

Parameter	2006	2007	2008	2009	2010	2006-10
TMA	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
TMI	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
TME	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
DPT	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
RAIN	*ns*	-------	-------	*ns*	*ns*	*ns*
RH	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
WD	*ns*	*ns*	*ns*	*ns*	*ns*	**-0.31***
AP	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

Level of statistical significance: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, *ns* – not significant.

5.4.2.2. Back trajectories and source location

Back trajectories (*n*=3220) calculated for every 6 hour period during the entire *Ganoderma* spore season (2006-10) revealed, that 55% of air masses reached Worcester from a direction in a 180° arc from the East to the West (Fig. 5.30 a , 91°-270°). Within the same period 58 days were selected, when the *Ganoderma* spore concentration was equal to or above 200 s m^{-3}. For these days with high spore concentrations one back-trajectory was selected for that particular hour, when *Ganoderma* spore concentration reached its maximum (Fig. 5.30 a).

The source map (Fig. 5.30 a) shows that wooded areas are found all over the country. Usually the woodland density is below 20% in each grid cell and the largest densities are associated with the major national parks such as the South Downs and New Forest in South England and the national parks in Wales. The nearest large woodland resource was the Forest of Dean which is located about 50 km to the South-West of Worcester. The trajectories display that, during the peak hour, 78% of air masses reached the spore-monitoring site from a 180° arc direction from the East 91° to the West 270° (Fig. 5.30 a and Fig. 5.30 c) passing areas such as the Forest of Dean in Wales or South Downs in England before their arrival in Worcester.

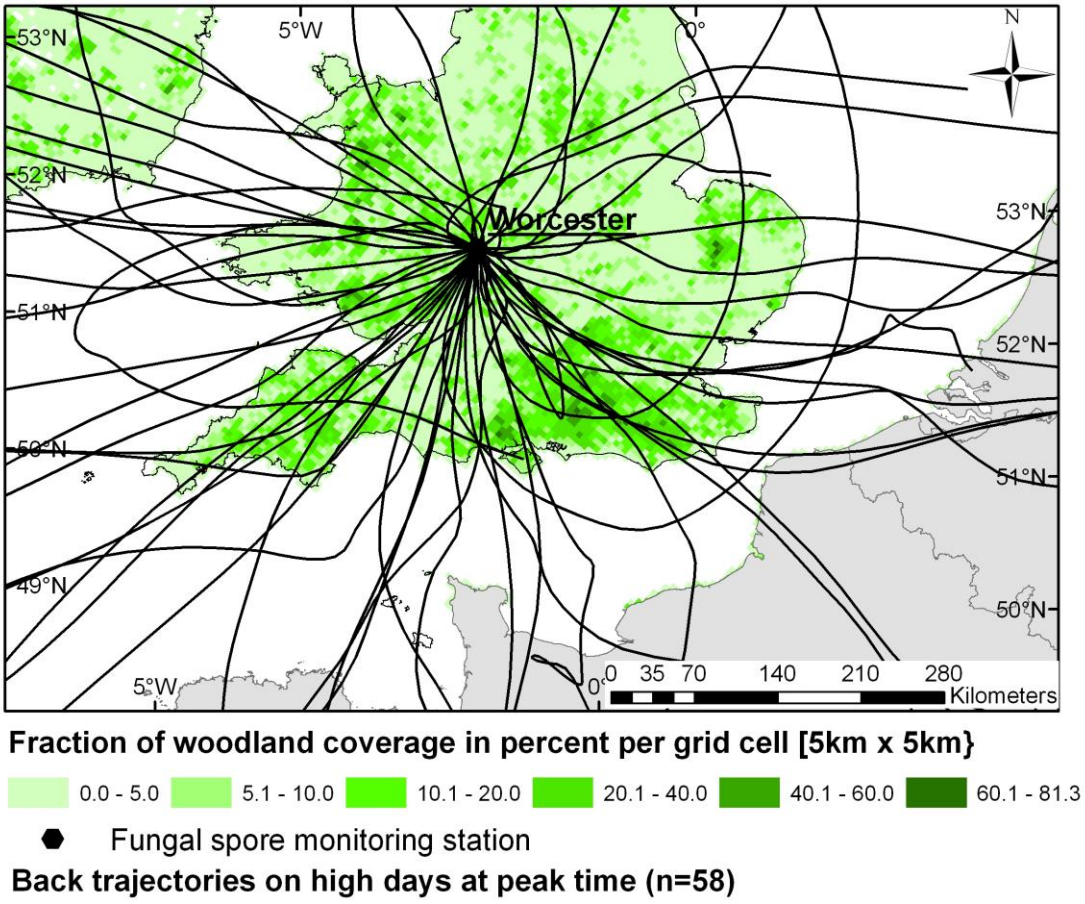


Fig. 5.30a Back trajectories on peak hour on all days with counts above or equal to 200 s m^{-3} (2006-2010), (Sadyś et al., 2014).

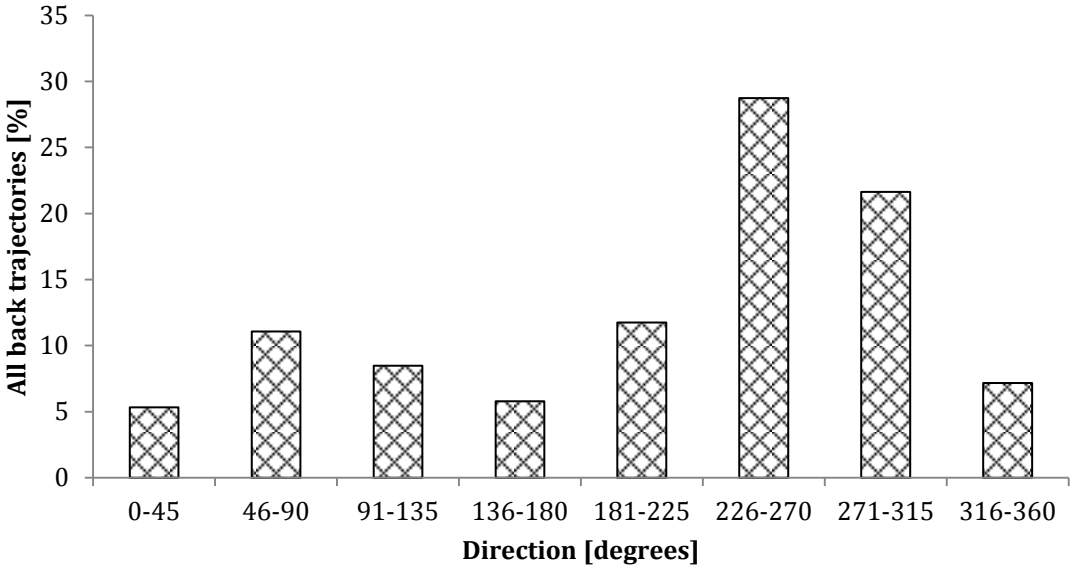


Fig. 5.30b Histograms showing the direction of the air masses before arrival in Worcester, UK for the entire period (Sadyś et al., 2014).

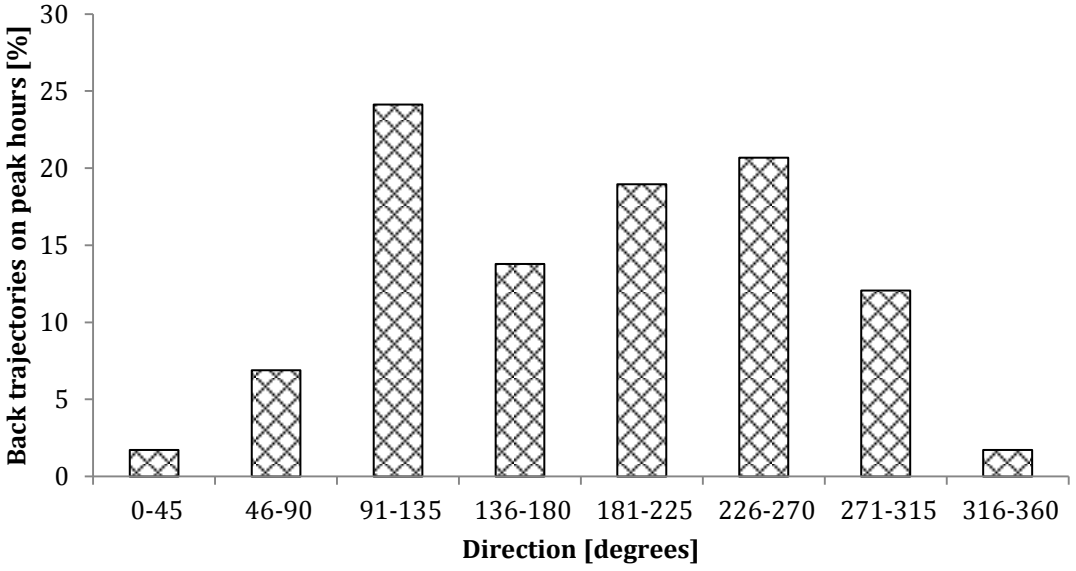


Fig. 5.30c Histograms showing the direction of the air masses before arrival in Worcester, UK on the peak hours (Sadyś et al., 2014).

5.4.2.3. Episode 1: 10th -13th of September 2006

This episode represents the first typical episode out of three, with air masses arriving mainly from areas to the South of Worcester. Daily mean *Ganoderma* spore concentration for the period 10th-13th of September 2006 in Worcester were: 236, 376, 221 and 279 s m^{-3}, respectively. Hourly spore concentration in Worcester peaked on the 11th of September 2006 at 07:00h exceeding 800 s m^{-3}. Within two hours spore concentration was reduced to zero. This pattern remained throughout the episode with a minimum during daytime (0-80 s m^{-3}) and maximum concentration approximately 400-800 s m^{-3} in the late evening or early in the morning (Fig. 5.31 a).

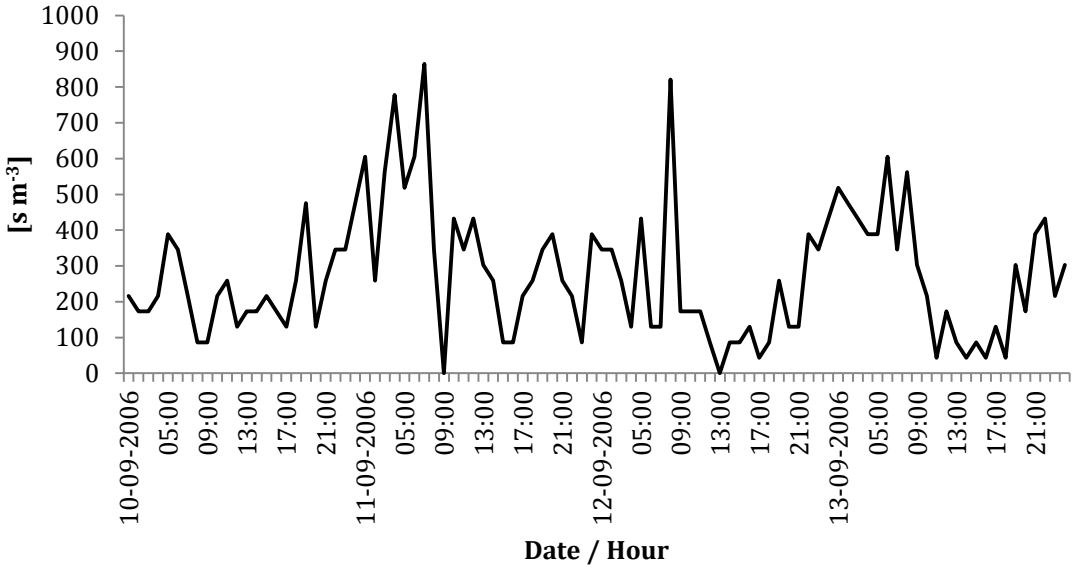


Fig. 5.31a Hourly variations in *Ganoderma* spore distribution recorded between 10th and 13th of September 2006 in Worcester, UK (Sadyś et al., 2014).

During the period 10th -13th of September 2006, a high pressure system (1031 hPa) covered the majority of the British Isles. This system was centrally located in Germany, Czech and Slovakia. Within the next three days, it gradually moved to the East towards Poland, Belarus, Ukraine and Russia simultaneously giving a way to two low pressure systems coming from Iceland and the North Atlantic Ocean. These low pressure systems joined together on the 12th of September 2006 and continued movement towards England. Within following 24 hours the air pressure dropped down from 984 hPa to 977 hPa.

Back-trajectory analysis showed that these changes in pressure systems caused air masses to approach Worcester from a south-westerly direction. Air masses passed either only water areas or limited areas with forest in France before they entered southern England. According to the trajectories, the air masses spent a fraction of 19% of their time over England during the last 48 hours before they arrived in Worcester and 28% over France. The rest of the time was spent over sea areas. Typically the last 8 hours were spent over wooded areas in Wales, and Southern England such as the Forest of Dean (Fig. 5.31 b).

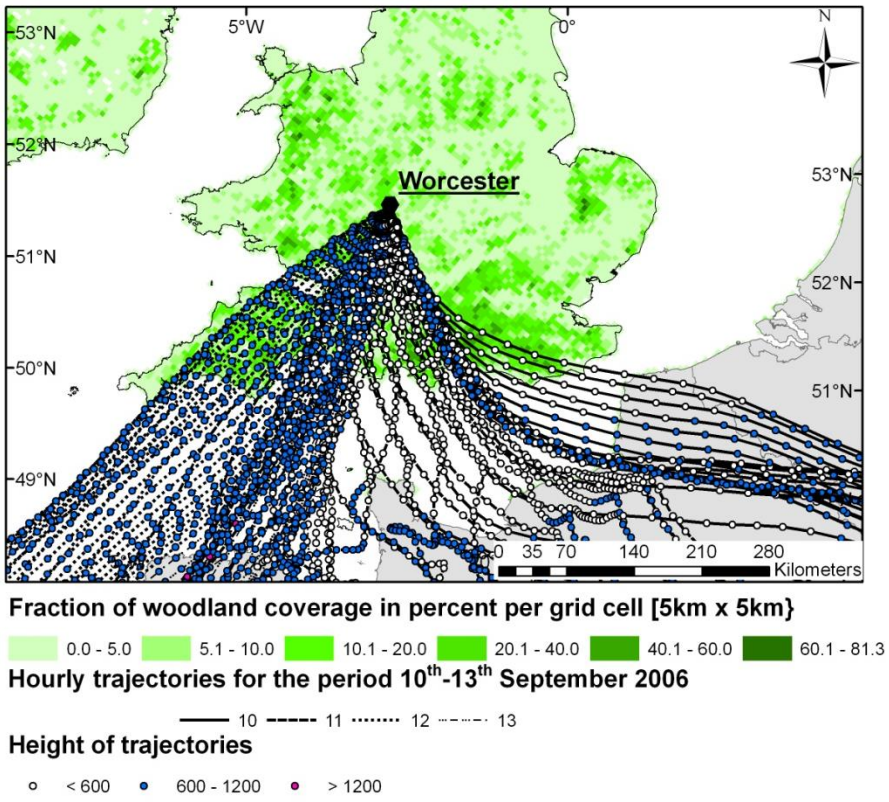


Fig. 5.31b The back trajectories showing the potential source areas (Sadyś et al., 2014).

Mean wind speed of the air masses was 6.4 m s^{-1} during the last 10 hours before arriving in Worcester. Daily mean temperatures, which were recorded in Worcester, varied between 17.6°C and 19.3°C, an increased relative humidity from 64.4% to 77.6%. 0.2 mm of rainfall was recorded during the last 24 hours of the episode.

5.4.2.4. Episode 2: 15th -18th of September 2008

This episode represents an episode, where air masses arrived mainly from areas to the East and South-East of Worcester. Daily mean *Ganoderma* spore concentrations for the period 15th-18th of September 2008 in Worcester were: 216, 211, 272 and 281 s m^{-3}, respectively. During the first 36 hours the concentrations were constant while during the rest of the period high night-time and morning values and low daytime spore concentrations were observed. Hourly spore concentrations in Worcester peaked on the 18th of September 2008 from 07:00h to 08:00h when concentrations exceeded 600 s m^{-3}. The *Ganoderma* spore concentration decreased to 40 s m^{-3} during mid-afternoon hours. This pattern remained throughout the episode with minimum concentrations during daytime (0-40 s m^{-3}) and maximum concentrations approximately 400-1200 s m^{-3} in the late evening or early in the morning (Fig. 5.32 a).

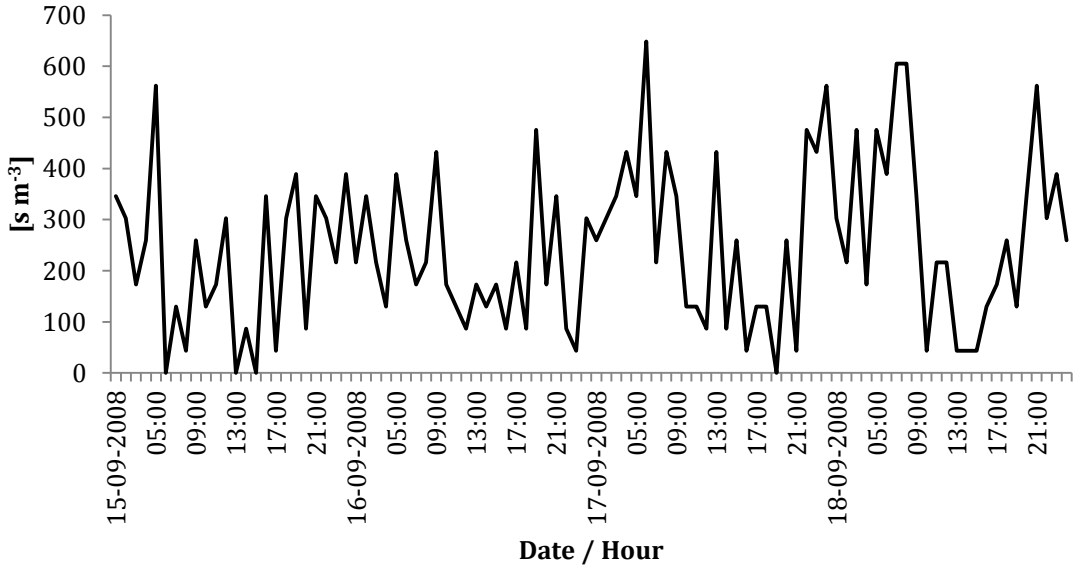


Fig. 5.32a Hourly variations in *Ganoderma* spore distribution recorded between 15th and 18th of September 2008 in Worcester, UK (Sadyś et al., 2014).

A high pressure system was dominating over the investigated period of time, with the air pressure varying between 1031 hPa and 1037 hPa. The area covered Scandinavia and most of Central and Northern Europe. Warm air masses were moving from Scandinavia to Northern Europe, overlapping the United Kingdom.

Back-trajectory analysis shows that these changes caused air masses to approach Worcester from South-Easterly and Easterly direction after passing either only the North Sea or limited areas with forests in Germany and North France before they entered Eastern England. According to the trajectories, the air masses spent a fraction of 36% of their time over England during the last 48 hours before they arrived in Worcester and 43% over France, Belgium, the Netherlands and Germany. The rest of the time was spent over areas of sea. Typically the last 12 hours were spent over wooded areas in Southern England such as the South Down National Park or woodlands to the South of London (Fig. 5.32 b).

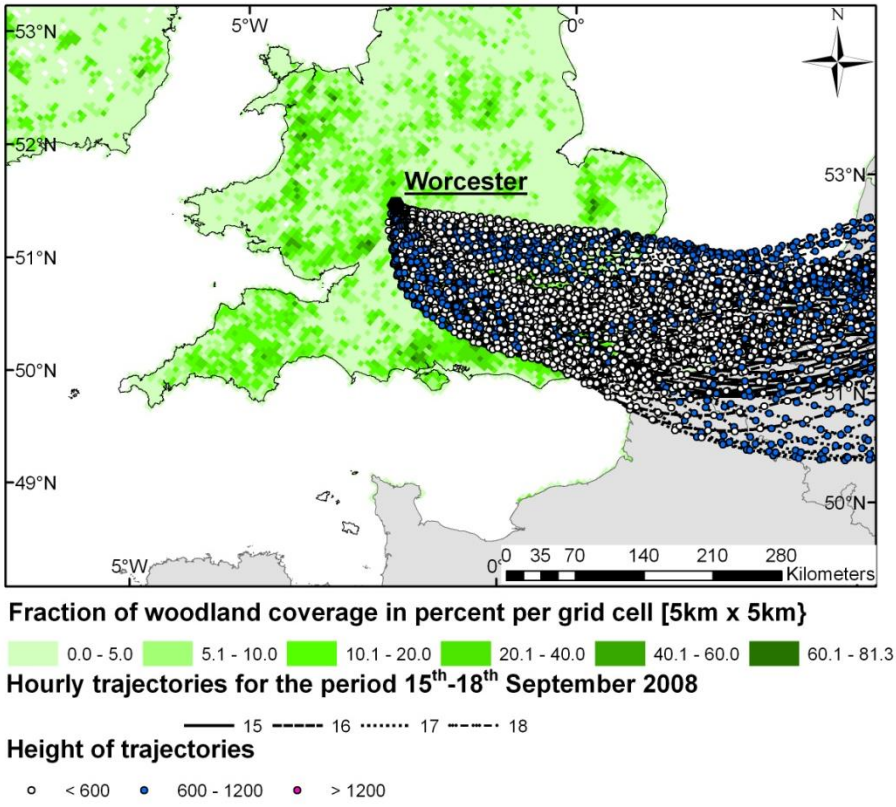


Fig. 5.32b The back trajectories showing the potential source areas (Sadyś et al., 2014).

Mean wind speed of the air masses was 3.8 m s^{-1} during the 10 hours before arriving in Worcester. Daily mean temperatures, which were recorded in Worcester, were varying from 13.6 °C to 14.6 °C, and with relative humidity from 68.3% to 73.3%. There was no rainfall within the examined four days.

5.4.2.5. Episode 3: 21st -25th of August 2009

This episode represents an episode, where air masses were arriving mainly from areas to the West of Worcester. Daily mean *Ganoderma* spore concentrations during the 21st -25th of August 2009 in Worcester were: 121, 277, 209, 230 and 142 s m^{-3}, respectively. Hourly spore concentrations in Worcester peaked on the 22nd of August 2009 at 05:00h with more than 900 s m^{-3}. Spore concentrations dropped to zero within two hours after the peak. This pattern remained throughout the episode with minimum concentrations during daytime (0-40 s m^{-3}) and maximum concentration approximately 300-500 s m^{-3} in the late evening or early in the morning (Fig. 5.33 a).

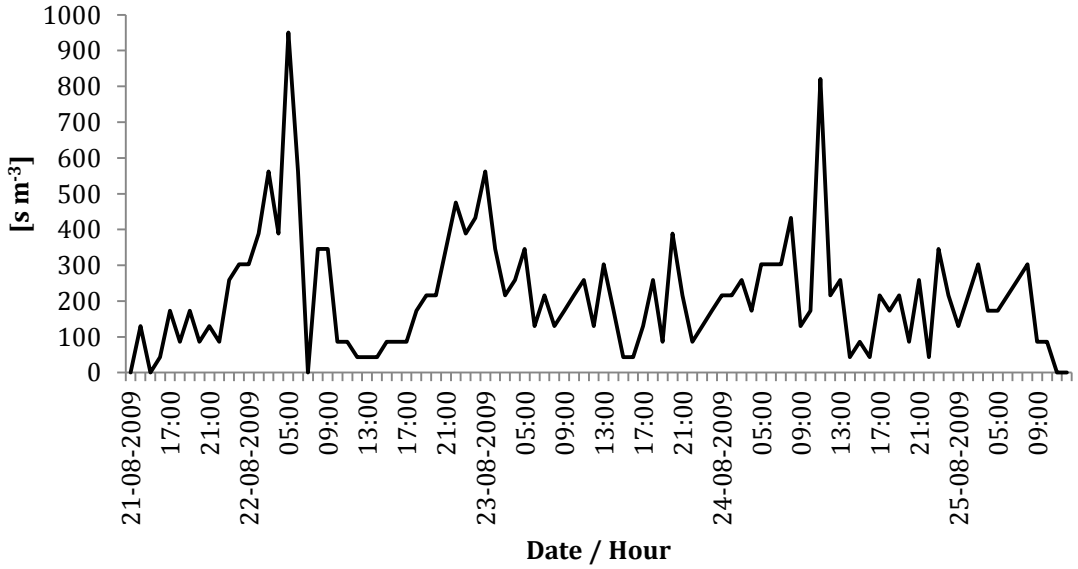


Fig. 5.33a Hourly variations in *Ganoderma* spore distribution recorded between 21st and 25th of August 2009 in Worcester, UK (Sadyś et al., 2014).

Initially Britain was under the influence of the low pressure system (985 hPa) from over Iceland. During the following 24 hours there was a movement of the high pressure system (1024 hPa) coming from France. The mixed influence of two opposite air pressure systems remained on the 23rd of August 2009, resulting

finally in the domination of a new low pressure system to the west of UK that developed during the period 23rd -25th of August 2009 (976-989 hPa).

The back-trajectory analysis shows that this caused air masses to approach Worcester from a South Westerly direction after passing either only the North Atlantic waters or limited areas with forest in Wales before arriving at the West Midlands. According to the trajectories, the air masses spent only 15% of their time over England during previous 48 hours before they arrived in Worcester and only 3% over parts of France. The rest of the time was spent over ocean areas. Typically the last 8 hours were spent over wooded areas in Wales, and Southern England such as the Forest of Dean (Fig. 5.33 b).

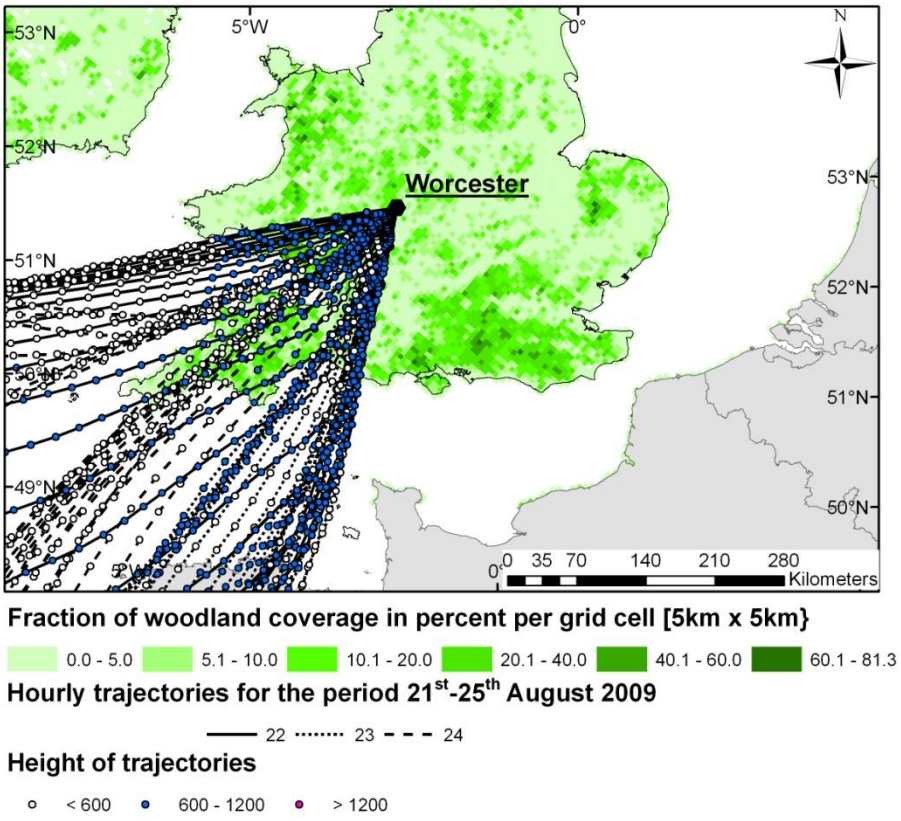


Fig. 5.33b The back trajectories showing the potential source areas (Sadyś et al., 2014).

Mean wind speed of the air masses was 7.7 m s^{-1} during the last 10 hours before arriving in Worcester. Daily mean temperatures, which were recorded in Worcester, were varying between 16.0 °C and 17.9 °C, and with relative humidity

from 74.3% to 72.0%. There was only one light rain shower recorded on the 24th of August 2009 (1.6 mm).

5.4.3. Discussion and conclusions

This investigation showed that UK woodlands were the main source of *Ganoderma* spores in all UK regions including non-wooded areas. The overall variation of the hourly spore count (Fig. 5.29) in the agricultural region of Worcestershire closely matches typical emission patterns with a peak either early in the morning or late in the evening. This suggested that in most of the cases, the source of *Ganoderma* was located a few hours transport away (less than 200 km). Within that distance there were only a few forested areas in France that could affect the UK – and only the most southern parts. This has the potential for significant effects on tree health in forests in the UK.

Furthermore, all hourly peak concentrations have been combined with air masses transport patterns (Fig. 5.30 a). The analysis of back-trajectories demonstrated that 78% of the air masses reached Worcester from a Southern to Easterly direction in a 180° arc from the East to West, while wind directions during the entire season have a different profile.

The three episodes studied showed that the air masses passed the main UK woodlands before their arrival in Worcester. The episode of August 2006 (Fig. 5.33), where the air masses mainly arrived in the UK from the Atlantic, must contain low concentration of fungal spores as marine air in general contains only low levels of fungi (Elbert et al., 2007; Urbano et al., 2011). The only source areas that could contribute were therefore woodlands in Cornwall, South Wales and West Midlands, where the confluence with mountains in Wales (Snowdonia, Cambrian, Brecon Beacon and Black Mountains) was likely to have affected a change of air masses movements. In contrary then this episode in Fig. 5.32 was likely to contain *Ganoderma* spores from the continent. As such this could be evidence of long distance transport of *Ganoderma* spores. However, the fraction of time spent over the England was 36% before arrival at the trap, which corresponded to about 18 hours. During these last 18 hours air masses also passed

the large wooded areas in South Downs and New Forest National Parks as well as the woodlands found in the South of London. During this passage the air masses spent a considerable amount of time over UK woodlands during the main emission time (late evening and early in the morning). Also, the observed time series suggested that it was mainly the first 36 hours that had a contribution from long distance transport as the rest of the time series closely followed the typical daily release pattern with low values during daytime. Fig. 5.31 could be considered as a combination between Fig. 5.33 with air masses arriving from sea and Fig. 5.32 with air masses arriving from the continent. Again the air masses passed UK woodlands during the 8 hours before they arrived in Worcester. Overall the observations of the spore counts, the diurnal pattern of the distribution and the air mass transport over identified UK woodland in combination showed that UK woodlands were the main source for *Ganoderma* spores in all UK regions including non-wooded areas.

It was interesting to observe a high trapped spore concentration in Worcester despite Worcestershire having limited woodlands. In Worcestershire the woodland area covers merely 7.6% (13445 ha) of the shire, what is below the national average of 12% (Worcestershire County Council and Forestry Commission, 2010). Furthermore, the woodlands were not found in compact large areas, but rather in small patches especially to the West of Worcester. The only exception is the Wyre Forest, situated 30 km on the North from the city, which possibly could be a source of *Ganoderma* spores in the city of Worcester. However, Fig. 5.30 suggested limited impact from that region, which highlighted the importance of including atmospheric transport in the studies of *Ganoderma*. This suggested that both climatic variables and atmospheric transport were relevant for research on the dispersal of *Ganoderma* spores.

This study agreed with earlier studies on the diurnal pattern as peaks early in the morning and late in the evening were observed (Hasnain et al., 1984). The lowest spore count occurred always at 14:00h regardless daily mean value.

Sreeramulu (1963) and Lacey (1962) found in Silwood Park, London a single peak of *Ganoderma* spores a few hours earlier than in Worcester, between 00:00h and 01:00h, and as the latter study confirmed, there was a basidiocarp in a

close neighbourhood of the spore trap (65 m away). In Canada, where *Ganoderma* concentrations were more abundant than *Cladosporium* spores (Tarlo et al., 1979), maxima were noted at 04:00h (Li and Kendrick, 1995) and 05:00h (Tarlo et al., 1979), whereas the minimum spore counts were recorded surprisingly in the evenings, at 20:00h (Li and Kendrick, 1995) and 19:00h (Tarlo et al., 1979). The double peak-pattern was observed in New Zealand, where maximum *Ganoderma* spore concentrations were observed at 00:00h and 04:00h. The concentration of spores decreased significantly at 19:00h, which has previously been reported in Canada (Hasnain et al., 1984; Tarlo et al., 1979). Haard and Kramer (1970) also reported occasionally double peaks for *Ganoderma*, with a night peak as the primary peak. Therefore, there is a possibility that observations in Worcester are a combination of one plume that was released at sources several hours away with respect to air masses transport and another plume of spores only a few hours away. The level of the first and the second peak will then be determined by the strength of these sources as the concentration of the *Ganoderma* basidiospores in general will be reduced with increased transport time. The minimum distance of local sources of *Ganoderma* would be according to the back-trajectory analysis approximately 60 km away from Worcester. Furthermore, the mixing layer in the atmosphere is in general small during night and large during day (Smith et al., 2008), which also affects the concentrations of *Ganoderma* spores, especially when there is a tendency that the main bulk of the emissions of *Ganoderma* occurs during night and is further dispersed and advected during the day. This showed the importance of including atmospheric transport in the studies of *Ganoderma*.

Summarizing, the correlations between *Ganoderma* spore presence in the atmosphere of Worcester and meteorological parameters, were found to vary from weak to moderate and occasionally statistically significant. The list of investigated meteorological factors was extended in comparison to other studies, and similar results were obtained. There was a lack of a solid link between *Ganoderma* spores caught in the spore trap with local weather conditions. Hence, spores were coming from a distant source, where different microclimates favour *Ganoderma* spore production and release.

In conclusions, this study showed that UK woodlands were the main source for *Ganoderma* spores in all UK regions including non-wooded areas. This was clearly seen by the strong daily variations in spore concentrations with high concentrations during night, which it was argued that must originate from sources within UK. This study also demonstrated that *Ganoderma* has a similar potential to pollen of *Betula* spp. and *Alternaria* spores for long distance transport. Only a small fraction of the high days (e.g. the first 36 hours of the time series in Fig. 5.32 a) had a pattern in the concentration profile that suggests long distance transport. The air masses transport analysis has been applied for the first time to *Ganoderma* spores in combination with standard techniques commonly applied in aerobiological monitoring. The potential of back-trajectory use has not been fully explored. Further investigation will be needed, in collaboration with other spore monitoring sites located in a neighbourhood to woodlands.

The pollen and spore traps are considered to cover up to 30 km of distance (Belmonte et al., 2008; Skjøth et al., 2010) and the trajectory model cannot by definition identify where the spores were released but just suggests possible locations along the route of the air masses. Plumes of aeroallergens have previously been identified with the use of trajectories and several monitoring sites (Smith et al., 2008). Such studies have been used to identify major source regions and potential conditions for long distance transport. It is likely that plumes of spores, recorded in Worcester in pre-dawn hours, can be identified in a similar manner. It is therefore important to include several monitoring sites in combination with source maps and air masses transport. Such studies can give information to the forestry industry about possible spread the pathogens and localise places where it would be necessary to reduce *Ganoderma*. These actions are likely to benefit from knowledge about spore season and duration which for the UK in general starts around the 1st of June and ends in the middle of October.

The mechanism of spore discharge in *Ganoderma* has not been fully understood, despite several attempts (Haard and Kramer, 1970; Ho and Nawawi, 1986; Kadowaki et al., 2010; Kramer and Long, 1970). In controlled environmental conditions, a double peak pattern was sometimes observed and sometimes it was

not. No explanation has been found for this phenomenon (Haard and Kramer, 1970). However it is certain that the fruiting body needs to be soaked with water and requires at least 24 hours before spores are released (Kramer and Long, 1970). This could be an answer, why the correlation between spore counts and meteorological parameters found in Worcester was barely found at the significance level of $p<0.05$. Presumably higher correlation coefficients would be found, if spore counts were juxtaposed together with selected meteorological parameters with one, two or three days lag. This assumption will be included in a further study.

Chapter 6

Forecasting models for investigated fungi

6.1. Introduction

Artificial Neural Networks is not a commonly used tool in the aerobiology. Their application has resulted in promising outcomes for aerobiological data (Kasprzyk et al., 2011). To date no numerical forecasting model has been produced for any of the examined fungal spore types, *i.e.*: *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* in the United Kingdom using this approach.

The methods used in this study were described in Chapter 2. Statistical modelling of all examined fungal spore types was performed in a similar manner therefore results were presented in a repetitive format. Overall 40 models were produced to detect the most optimum conditions under which the forecasting of *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores would be the most precise. Results of the best performing forecasting models produced for all examined spore types were presented in this Chapter, while all the prediction models are in Appendix VI.

6.2. Results

6.2.1. *Alternaria*

The best regression ANN model for *Alternaria* spores, where weather data without a time lag was implemented but auto-correlation between meteorological parameters could occur was MLP 8-24-1 with eight input neurons, twenty four hidden neurons and one output neuron (Table 6.1). This model was trained with 63 epochs of back propagation.

Table 6.1 Neural network model MLP 8-24-1.

	Training	Test	Validation	Algorithm	Error	Activation Function	
						Training	Validation
Value	0.54	0.36	0.66	BFGS[a] 63	SOS[b]	Tanh	Exponential

[a] BFGS Broyden-Fletcher-Goldfarb-Shanno
[b] SOS Sum of Squares

Table 6.2 shows the sensitivity analysis results, where dew point temperature, followed by relative humidity and minimum temperature were the most important parameters affecting increased concentration of *Alternaria* spores in the air in Worcester. All investigated meteorological parameters obtained ratio above 1, therefore all of them contributed significantly to the model (Table 6.2).

Table 6.2 Sensitivity analysis for neural network model MLP 8-24-1.

	TMA	TMI	TME	DPT	WD	RH	RAIN	AP
Ratio	1.09	2.15	1.37	64.60	1.22	39.49	1.03	1.26
Rank	7	3	4	1	6	2	8	5

TMA (maximum temperature); TMI (minimum temperature); TME (mean temperature); DPT (dew point temperature); WD (wind direction); RH (relative humidity); RAIN (rainfall); AP (air pressure);

Correlation between observed and predicted spore counts was r_s=0.52, and this was the supreme criterion for choosing this neural model above the others. Performance of this model has been visually presented in Fig. 6.1 and Fig. 6.2.

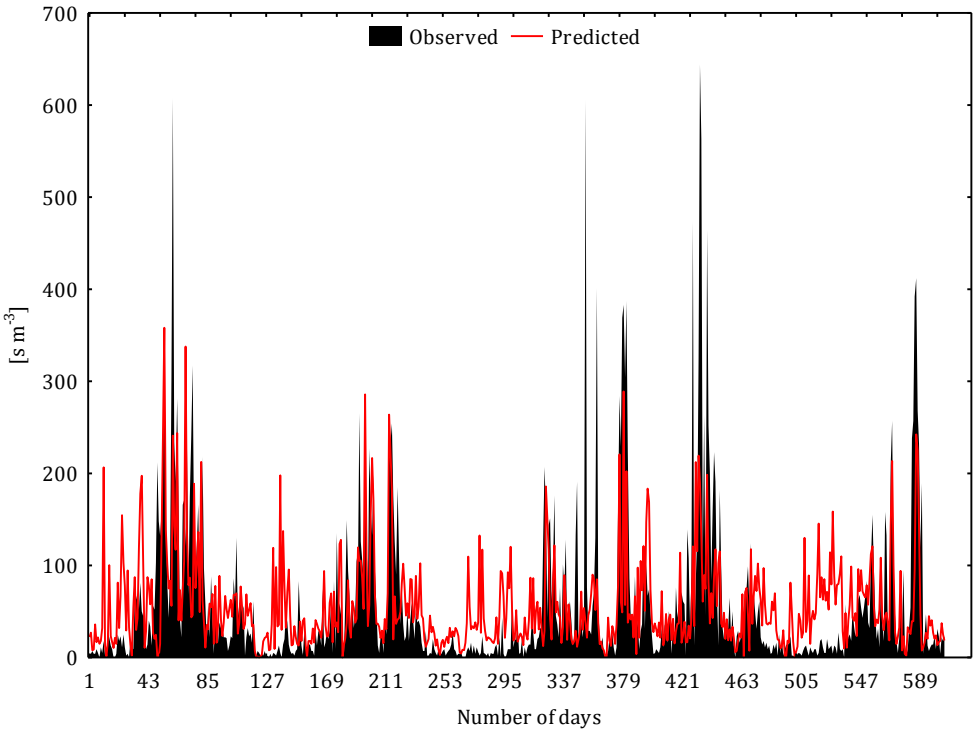


Fig. 6.1 Observed and predicted *Alternaria* spore concentrations using the MLP 8-24-1 neural network model.

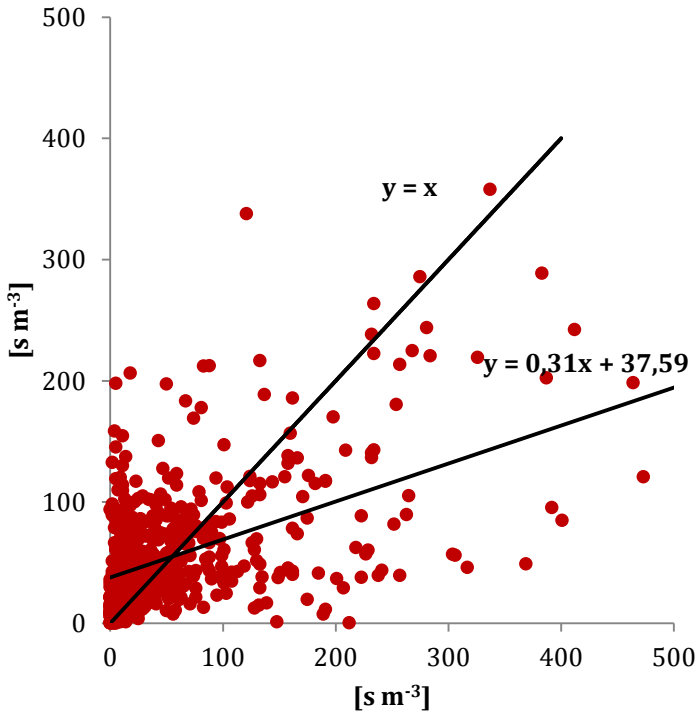


Fig. 6.2 The correlation between observed and predicted *Alternaria* spore concentrations using the MLP 8-24-1 neural network model.

6.2.2. *Cladosporium*

The best regression ANN model for *Cladosporium* spores, where weather data without a time lag was implemented but auto-correlation between meteorological parameters could occur was MLP 8-22-1 with eight input neurons, twenty two hidden neurons and one output neuron (Table 6.3). This model was trained with 110 epochs of back propagation.

Table 6.3 Neural network model MLP 8-22-1.

	Training	Test	Validation	Algorithm	Error	Activation Function	
						Training	Validation
Value	0.63	0.66	0.70	BFGS[a] 110	SOS[b]	Tanh	Identity

[a] BFGS Broyden-Fletcher-Goldfarb-Shanno
[b] SOS Sum of Squares

Table 6.4 shows the sensitivity analysis results, where maximum temperature, followed by minimum temperature and mean temperature were the most important parameters affecting increased concentration of *Cladosporium* spores in the air in Worcester. All investigated meteorological parameters obtained ratio above 1, therefore all of them contributed significantly to the model (Table 6.4).

Table 6.4 Sensitivity analysis for neural network model MLP 8-22-1.

	TMA	TMI	TME	DPT	WD	RH	RAIN	AP
Ratio	305.20	249.44	16.20	8.61	2.40	5.04	1.56	1.18
Rank	1	2	3	4	6	5	7	8

TMA (maximum temperature); TMI (minimum temperature); TME (mean temperature); DPT (dew point temperature); WD (wind direction); RH (relative humidity); RAIN (rainfall); AP (air pressure);

Correlation between observed and predicted spore counts was r_s=0.66, and this was the supreme criterion for choosing this neural model above the others. Performance of this model has been visually presented in Fig. 6.3 and Fig. 6.4.

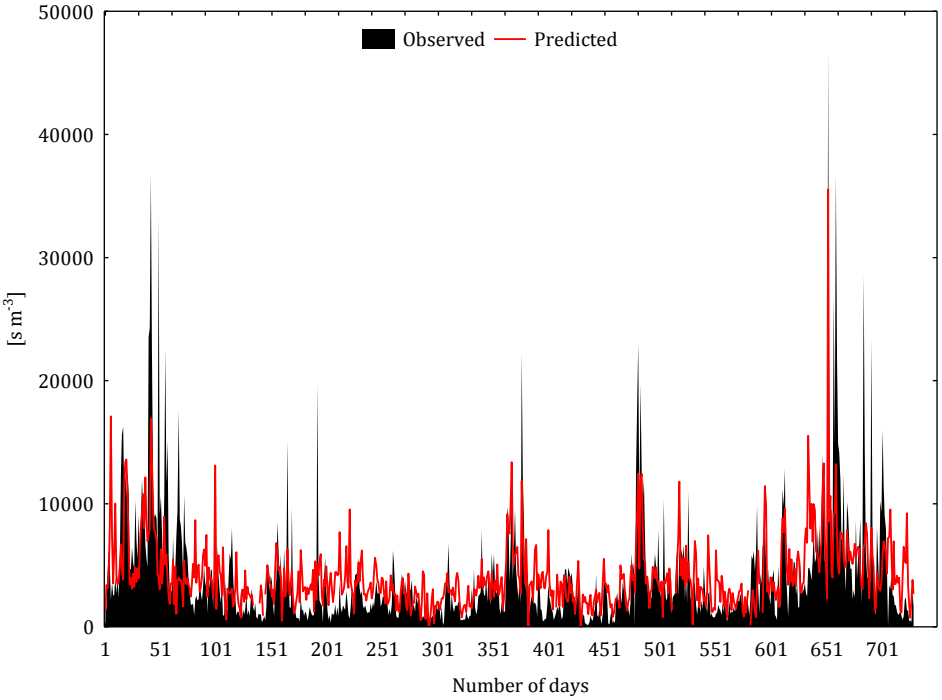


Fig. 6.3 Observed and predicted *Cladosporium* spore concentrations using the MLP 8-22-1 neural network model.

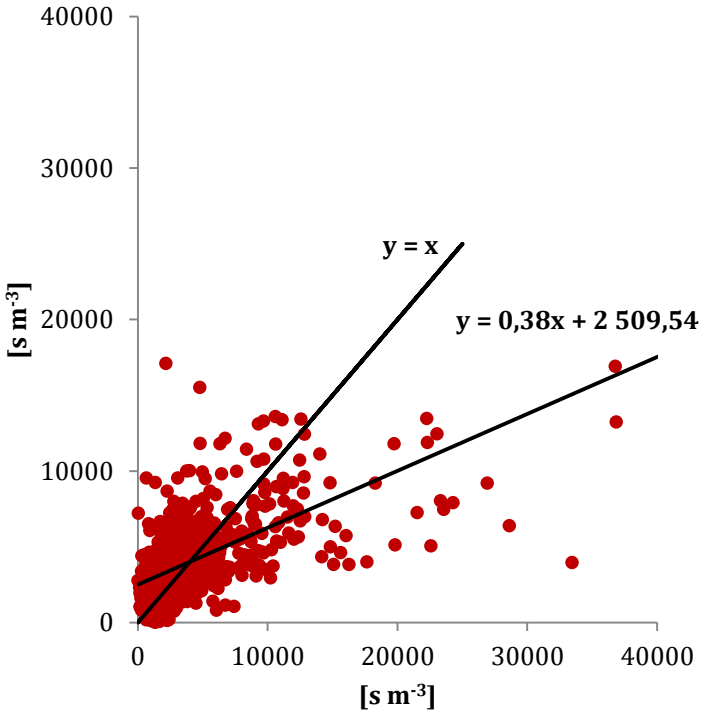


Fig. 6.4 The correlation between observed and predicted *Cladosporium* spore concentrations using the MLP 8-22-1 neural network model.

6.2.3. *Didymella*

The best regression ANN model for *Didymella* ascospores, where weather data without a time lag was implemented but auto-correlation between meteorological parameters could occur was MLP 8-6-1 with eight input neurons, six hidden neurons and one output neuron (Table 6.5). This model was trained with 44 epochs of back propagation.

Table 6.5 Neural network model MLP 8-6-1.

	Training	Test	Validation	Algorithm	Error	Activation Function	
						Training	Validation
Value	0.54	0.36	0.30	BFGS[a] 44	SOS[b]	Tanh	Sine

[a] BFGS Broyden-Fletcher-Goldfarb-Shanno
[b] SOS Sum of Squares

Table 6.6 shows the sensitivity analysis results, where minimum temperature, followed by maximum temperature and relative humidity were the most important parameters affecting increased concentration of *Didymella* spores in the air in Worcester. All investigated meteorological parameters obtained ratio above 1, therefore all of them contributed significantly to the model (Table 6.6).

Table 6.6 Sensitivity analysis for neural network model MLP 8-6-1.

	TMA	TMI	TME	DPT	WD	RH	RAIN	AP
Ratio	12.33	14.76	3.02	7.10	1.07	7.13	1.03	1.11
Rank	2	1	5	4	7	3	8	6

TMA (maximum temperature); TMI (minimum temperature); TME (mean temperature); DPT (dew point temperature); WD (wind direction); RH (relative humidity); RAIN (rainfall); AP (air pressure);

Correlation between observed and predicted spore counts was r_s=0.40, and this was the supreme criterion for choosing this neural model above the others. Performance of this model has been visually presented in Fig. 6.5 and Fig. 6.6.

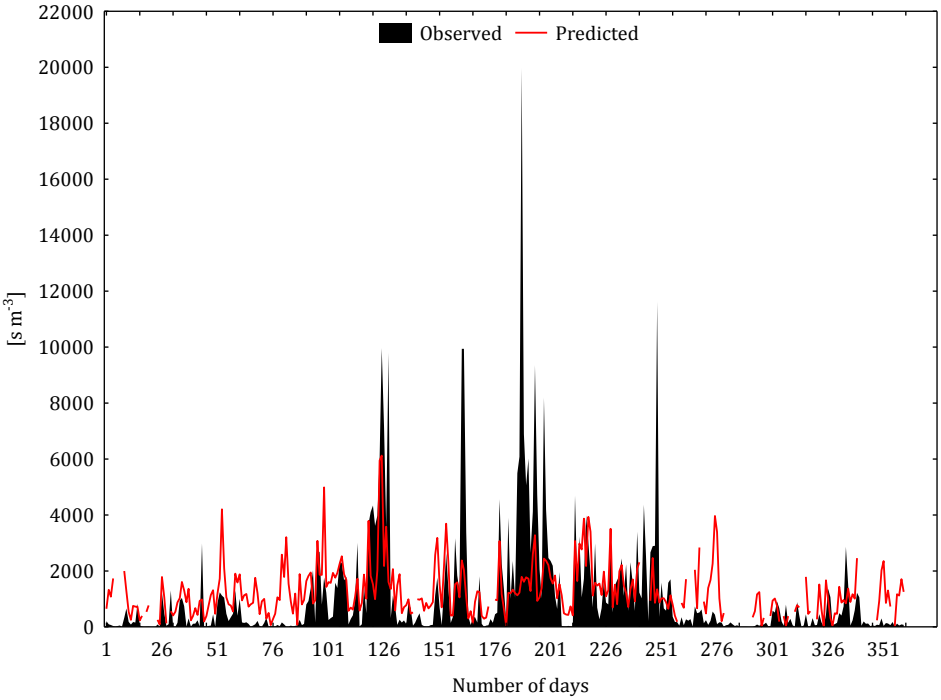


Fig. 6.5 Observed and predicted *Didymella* spore concentrations using the MLP 8-6-1 neural network model.

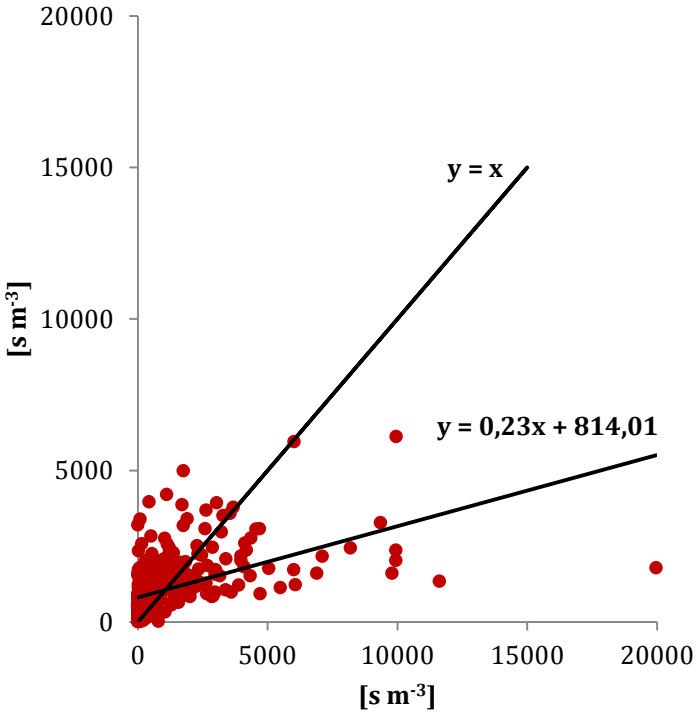


Fig. 6.6 The correlation between observed and predicted *Didymella* spore concentrations using the MLP 8-6-1 neural network model.

6.2.4. *Ganoderma*

The best regression ANN model for *Ganoderma* basidiospores, where weather data without a time lag was implemented but auto-correlation between meteorological parameters could occur was MLP 8-15-1 with eight input neurons, fifteen hidden neurons and one output neuron (Table 6.7). This model was trained with 179 epochs of back propagation.

Table 6.7 Neural network model MLP 8-15-1.

	Training	Test	Validation	Algorithm	Error	Activation Function	
						Training	Validation
Value	0.63	0.57	0.58	BFGS[a] 179	SOS[b]	Exponential	Sine

[a] BFGS Broyden-Fletcher-Goldfarb-Shanno
[b] SOS Sum of Squares

Table 6.8 shows the sensitivity analysis results, where maximum temperature, followed by minimum temperature and mean temperature were the most important parameters affecting increased concentration of *Ganoderma* spores in the air in Worcester. All investigated meteorological parameters obtained ratio above 1, therefore all of them contributed significantly to the model (Table 6.8).

Table 6.8 Sensitivity analysis for neural network model MLP 8-15-1.

	TMA	TMI	TME	DPT	WD	RH	RAIN	AP
Ratio	13.84	15.95	15.95	5.90	1.17	4.60	1.28	1.17
Rank	3	1	2	4	8	5	6	7

TMA (maximum temperature); TMI (minimum temperature); TME (mean temperature); DPT (dew point temperature); WD (wind direction); RH (relative humidity); RAIN (rainfall); AP (air pressure);

Correlation between observed and predicted spore counts was r_s=0.59, and this was the supreme criterion for choosing this neural model above the others. Performance of this model has been visually presented in Fig. 6.7 and Fig. 6.8.

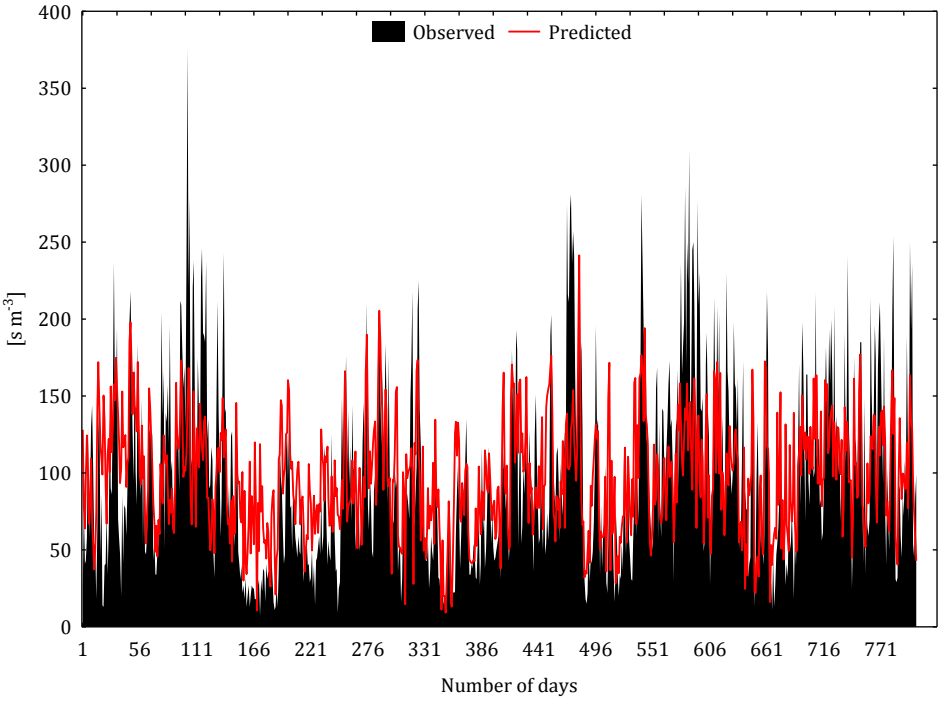


Fig. 6.7 Observed and predicted *Ganoderma* spore concentrations using the MLP 8-15-1 neural network model.

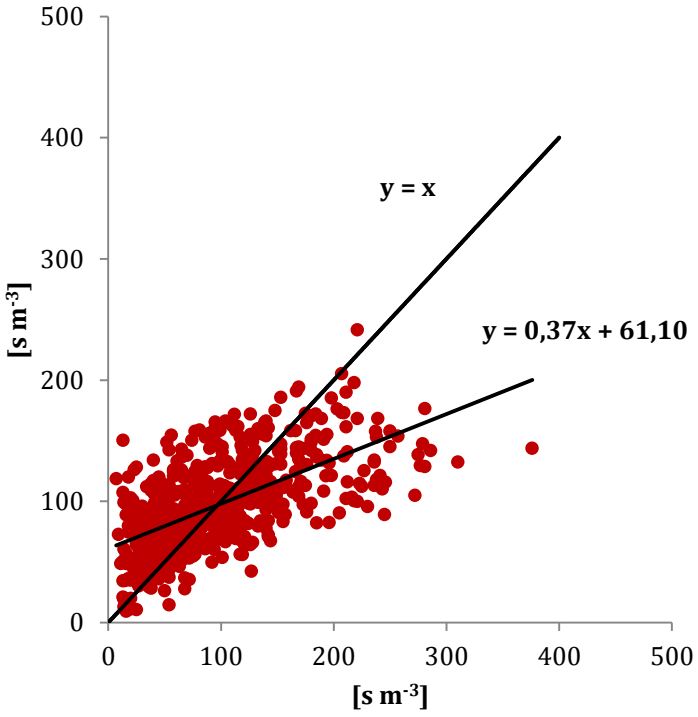


Fig. 6.8 The correlation between observed and predicted *Ganoderma* spore concentrations using the MLP 8-15-1 neural network model.

6.3. Discussion

6.3.1. *Alternaria*

Out of eight examined weather parameters, the most significant association between presence of *Alternaria* spores and minimum and mean temperature was indicated by the Spearman's rank test (Table VIII-1.6, Appendix VI). No statistically significant correlation was found between the increased concentration of fungal spores and wind direction.

Overall, the strength of the associations between *Alternaria* spore concentrations and meteorological factors decreased with time, with the exception of dew point temperature and air pressure, where slightly higher correlation coefficients were obtained when one day lag weather data were juxtaposed with the spore counts (Table VIII-1.6, Appendix VI). Similarly to the results of the Spearman's rank test, performance of the ANNs produced for *Alternaria* spores was decreasing along with an increase in the discrepancy between concentration of fungal spores and values of the meteorological parameters.

Out of ten obtained models for forecasting purposes MLP 8:24:1 was the most accurate. This choice was dictated by the coefficient of the correlation between recorded and predicted values (Table 6.1), and data points aggregation presented as the scatter plot (Fig. 6.2). The overall correlation coefficient for all three subsets (training, testing and validating) was the highest for the MLP 8:24:1 in contrast to the remaining models (r_s=0.52).

The sensitivity analysis of MLP 8:24:1 showed that the overall pattern of *Alternaria* spore distribution could be explained by changes in dew point temperature and relative humidity, as these two parameters contributed most significantly to the model (Table 6.2). These results were not confirmed by the Spearman's rank test outputs (Table VIII-1.6, Appendix VI).

Performance of this model could not be explained by the remarkably high number of hidden neurons (n=24), as three other models exhibited similar complexity of structure (*i.e.*: MLP 5:25:1, MLP 5:24:1, MLP 7:24:1), while their performance was much lower (Fig. VIII-1 D, Fig. VIII-1 H and Fig. VIII-1 I, Appendix VI). However, the greater number of epochs of the back propagation, which were

used for training the models, could possibly have a positive impact at some degree, as certain models were performing better than the others. As an example may be MLP 5:24:1 with 20 epochs, which showed correlation between observed and predicted *Alternaria* spore counts equal to r_s=0.26, while MLP 8:21:1 with 1 epoch only resulted in coefficient equal to r_s=0.20.

Application of the meteorological parameters that revealed various associations in time with concentration of *Alternaria* spores did not have an influence on precision of the prediction of the spore concentration, because similar results were obtained for the models with 2 days difference between spore and weather data. This result was in contrast to the performance of the neural model produced for Szczecin (Grinn-Gofroń and Strzelczak, 2008). Furthermore, Szczecin model revealed a slightly higher correlation value between observed and predicted *Alternaria* spore counts (r_s>0.6), although it was produced based on one year shorter data set than Worcester model (Grinn-Gofroń and Strzelczak, 2008). The reason for this may be a different selection of examined parameters, where maximum and mean wind speed were used, while in Worcester model they were replaced by wind direction and air pressure.

First attempt of deletion of auto-correlation between meteorological parameters was taken by the author, as this problem has not been yet examined by others (Grinn-Gofroń and Strzelczak, 2008). Out of five cases, no difference was detected in evaluation of the "mixed" models (MLP 7:24:1 and MLP 4:8:1), where correlation between observed and predicted spore counts was found to be r_s=0.39. Models that were produced with a time lag equal to 2 and 3 days showed that reduced number of meteorological parameters positively influenced quality of the models. However, the best performing neural network MLP 8:24:1 consisted of auto-correlated weather factors. Although there is no definite explanation, in author's opinion different weather conditions may play a key role at the beginning of the fungal spore season, in the middle of the season oscillating around the major peak, and after the post peak period. This hypothesis would be in agreement with previous results obtained by Stępalska and Wołek (2005), who examined the relationship between *Alternaria* spore concentration and weather factors in the

pre-peak and post-peak periods using multiple regression analysis. They found that each year within a three year period different meteorological parameters were indicated by the model as explanatory factors. In the pre-peak period, the key role played most frequently minimum temperature, relative humidity and rainfall, while in the post-peak period maximum temperature either solely or along with relative humidity (Stępalska and Wołek, 2005). Hence, auto-correlated meteorological parameters were used to produce the model, having a long time series of data. Possible errors were reduced to the minimum as each of them explained certain period of time with increased spore concentration.

The overall efficiency of the MLP 8:24:1 was difficult to call as satisfactory (r_s=0.52), as there was no much difference from chance whether the *Alternaria* spore concentration would be high or low. Productivity of the model, as well as weak correlation between measured concentration of *Alternaria* spores and meteorological parameters could be explained by the fact that the sources of the spores must be located at the distance greater than 30 km from the air sampler operated from the Worcester city.

6.3.2. *Cladosporium*

Out of eight examined weather parameters, the most significant association between the presence of *Cladosporium* spores and maximum and mean temperature was indicated by the Spearman's rank test (Table VIII-2.6, Appendix VI). No statistically significant correlation was found between increased concentration of fungal spores and air pressure.

Overall, the strength of the associations between *Cladosporium* spore concentrations and meteorological factors decreased in time, with the exception of dew point temperature, where slightly higher correlation coefficient was obtained when one day lag weather data were juxtaposed with the spore counts (Table VIII-2.6, Appendix VI). Similarly to the results of the Spearman's rank test, performance of the artificial neural networks produced for *Cladosporium* spores was decreasing along with an increase of the discrepancy between the concentration of fungal spores and values of the meteorological parameters.

Out of ten obtained models, for forecasting purposes MLP 8:22:1 was the most accurate. This choice was dictated by the coefficient of the correlation between recorded and predicted values (Table 6.3), and data points aggregation presented as the scatter plot (Fig. 6.4). The overall correlation coefficient for all three subsets (training, testing and validating) was the highest for the MLP 8:22:1 in contrast to the remaining models (r_s=0.66).

The sensitivity analysis of MLP 8:22:1 revealed that the overall pattern of *Cladosporium* spore distribution could be explained by fluctuations of maximum and minimum temperature. These two parameters contributed most significantly to the model obtaining ratio equal to 305.20 and 249.44, respectively (Table 6.4). The Spearman's rank test confirmed a remarkable impact of the maximum temperature on the *Cladosporium* spore concentration recorded in Worcester (Table VIII-2.6, Appendix VI).

Performance of this model cannot be explained by the remarkably high number of hidden neurons (n=22), as three other models exhibited similar or even higher complexity of structure (*i.e.*: MLP 8:25:1, MLP 5:23:1, MLP 4:24:1), while their performance was much lower (Fig. VIII-2 E, Fig. VIII-2 F and Fig. VIII-2 J, Appendix VI). However, the greater number of epochs of the back propagation, which were used for training the models, could possibly have a positive impact at some degree, although complete rely on this factor should be avoided, as for example MLP 4:24:1 trained with only 8 epochs of back propagation showed surprisingly high correlation between observed and predicted fungal spore counts (r_s=0.52), while MLP 7:10:1 trained with 75 epochs resulted in correlation coefficient equal to r_s=0.60.

Application of the meteorological parameters that revealed various associations in time with concentration of *Cladosporium* spores did not have a remarkable influence on the precision of the prediction of spore concentration, although performance of the "mixed" model MLP 7:10:1 (Table VIII-2.6, Appendix VI) was comparable to MLP 8:22:1 (Table 6.3).

Performance of the MLP 8:22:1 model obtained for Worcester could not be directly compared with any other, already described neural model produced by

Grinn-Gofroń and Strzelczak for Szczecin (2008; 2009; 2011; 2013) as different criteria were selected for their production (e.g. hourly spore counts instead of daily mean spore counts, data transformation or implementation of additional explanatory factors, such as ozone).

Performance of all the models, where possible auto-correlation between meteorological parameters could possibly occur, was much higher comparing to the models where number of examined weather factors was reduced. As previously indicated in discussion about possible contribution of each meteorological parameter to the overall model, this has been once more in agreement with obtained results. According to Stępalska and Wołek (2005), in the pre-peak period in seasonal fluctuation of *Cladosporium* spores, indicated primary, jointly impact of minimum temperature together with either solar radiation or rainfall. While, in the post-peak period, major influence on *Cladosporium* spore concentration was found to have maximum temperature, either solely or along with solar radiation or relative humidity (Stępalska and Wołek, 2005).

The overall efficiency of the MLP 8:22:1 could be considered as satisfactory (r_s=0.66), as this model showed a higher prediction than MLP 8:24:1 obtained for *Alternaria* spores. Productivity of the model, as well as slightly greater correlation between the measured concentration of *Cladosporium* spores and meteorological parameters could be partly explained by the fact that the sources of the spores must be located a little closer to the sampling station than those of *Alternaria*.

6.3.3. *Didymella*

Out of eight examined weather parameters, the most significant association between occurrence of *Didymella* ascospores and rainfall, and relative humidity was indicated by the Spearman's rank test (Table VIII-3.6, Appendix VI). Statistically significant correlations were found with all examined weather factors, although the strength of associations varied between them, as well as with regard to the relation in time (Table VIII-3.6, Appendix VI). For example the highest correlation for dew point temperature and wind direction occurred with one day lag discrepancy between weather and fungal spore data.

Similarly to the results of the Spearman's rank test, performance of the artificial neural networks produced for *Didymella* spores was decreasing along with an increase of the difference between concentration of fungal spores and values of the meteorological parameters.

Out of ten obtained models, for forecasting purposes MLP 8:6:1 was the most accurate. This choice was dictated by the coefficient of the correlation between recorded and predicted values (Table 6.5), and data points aggregation presented as the scatter plot (Fig. 6.6). The overall correlation coefficient for all three subsets (training, testing and validating) was the highest for the MLP 8:6:1 in contrast to the remaining models (r_s=0.40).

The sensitivity analysis of MLP 8:6:1 showed that the overall pattern of *Didymella* spore distribution could be explained by the changes in minimum temperature and maximum temperature, as these two parameters contributed most significantly to the model (Table 6.6). These results were not confirmed by the Spearman's rank test outputs (Table VIII-3.6, Appendix VI), while Stępalska and Wołek (2005) reported that equally in the pre-peak and post-peak periods the key role played mostly minimum temperature solely or jointly with relative humidity in distribution pattern of *Didymella* spores recorded in Kraków, Poland.

Performance of MLP 8:6:1 model could be explained by a surprisingly low number of hidden neurons (n=6), as this model presented the highest simplicity in its structure, and simultaneously the greatest efficiency (Fig. 6.6).

Application of the meteorological parameters that revealed various associations in time with concentration of *Didymella* spores did not have an influence on precision of the prediction of the spore concentration (Table VIII-3.4 and Table VIII-3.6, Appendix VI), because similar results were obtained for the models with 1 day difference between spore and weather data (Table VIII-3.1, Appendix VI).

Models that were produced with a time lag equal to 1 day and 3 days showed that reduced number of meteorological parameters positively influenced quality of the models (Table VIII-3.1, Appendix VI). However, the best performing

neural network MLP 8:6:1 consisted of auto-correlated weather factors without a time lag.

The overall efficiency of the MLP 8:6:1 was far below satisfactory (r_s=0.40). Efficiency of the model, as well as very weak correlation between measured concentration of *Didymella* spores and meteorological parameters (especially temperature) could support the hypothesis that the sources of the spores must be located at the distance far greater than 30 km from the air sampler located in Worcester.

6.3.4. *Ganoderma*

Out of eight examined weather parameters, the most significant association between presence of *Ganoderma* basidiospores and dew point temperature and maximum temperature was indicated by the Spearman's rank test (Table VIII-4.6, Appendix VI). No statistically significant correlation was found between the increased concentration of fungal spores and relative humidity.

Overall, the strength of the associations between *Ganoderma* spore concentrations and meteorological factors decreased in time, with the exception for rainfall and air pressure, where slightly higher correlation coefficient was obtained when a one day lag weather data were juxtaposed with the spore counts (Table VIII-4.6, Appendix VI). Similarly to the results of the Spearman's rank test, performance of the artificial neural networks produced for *Ganoderma* spores was decreasing along with an increase of the discrepancy between concentration of fungal spores and values of the meteorological parameters.

Out of ten obtained models, for forecasting purposes MLP 8:15:1 model appeared to be the best. This choice was dictated by the coefficient of the correlation between recorded and predicted values (Table 6.7), and data points aggregation presented as the scatter plot (Fig. 6.8). The overall correlation coefficient for all three subsets (training, testing and validating) was the highest for the MLP 8:15:1 in contrast to the remaining models (r_s=0.59).

The sensitivity analysis of MLP 8:15:1 revealed that the overall pattern of *Ganoderma* spore distribution could be explained by fluctuations of minimum and

mean temperature. These two parameters contributed most significantly to the model obtaining ratio equal to 15.95 (Table 6.8). The Spearman's rank test did not confirm an importance of the above mentioned temperature parameters on the *Ganoderma* spore concentration recorded in Worcester (Table VIII-4.6, Appendix VI). While Stępalska and Wołek (2005), in the pre-peak period in seasonal fluctuation of *Ganoderma* spores, indicated primary, the joint impact of minimum temperature together with solar radiation, rainfall or relative humidity. While, in the post-peak period, the major influence on *Ganoderma* concentration was found to have maximum temperature, either solely or along with solar radiation or relative humidity (Stępalska and Wołek, 2005).

Application of the meteorological parameters that revealed various associations in time with concentration of *Ganoderma* spores did not have a remarkable influence on precision of the prediction of the spore concentration, although performance of the "mixed" model MLP 7:9:1 (Table VIII-4.1) was comparable to MLP 8:15:1 (Table 6.7).

The regression models produced by Grinn-Gofroń and Strzelczak (2011) for *Ganoderma* spores, where both original and transformed data were examined, resulted in coefficients below 0.40 of the correlation between observed and predicted data points. Hence, the performance of the MLP 8:15:1 neural model obtained for Worcester may be considered as acceptable, although the overall efficiency was low (r_S=0.59). However, this model still showed a higher prediction than MLP 8:24:1 obtained for *Alternaria* and MLP 8:6:1 for *Didymella* spores.

6.4. Conclusions

Overall, 40 forecasting neural networks have been produced for all four examined fungal spore types (*Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* genus). Several different criteria were taken into consideration, while establishing settings of the models. The best performance showed models with a greater number of analysed weather factors, although a possible auto-correlation between them could take a place, and without a time lag in relation between fungal spore counts and meteorological data points. The prediction power, that was mainly

decided upon the correlation value calculated between observed and predicted spore counts, varied from 40% for *Didymella* to 66% for *Cladosporium* spores. The contribution of each examined weather factor varied between obtained models (sensitivity analyses) as well as between outputs of the Spearman's rank test (Table 6.9). This could be explained by the fact, that development of the fungi, along with spore production and release processes cannot be described by a single factor with a constant influence.

Table 6.9 The summary of the most important meteorological parameters.

Type of analysis	***Alternaria***	***Cladosporium***	***Didymella***	***Ganoderma***
Spearman's rank test	TMI, TME	TMA, TME	RAIN, RH	DPT, TMA
Sensitivity analysis of the best performing model	DPT, RH	TMA, TMI	TMI, TMA	TMI, TME

TMA (maximum temperature); TMI (minimum temperature); TME (mean temperature); DPT (dew point temperature); RH (relative humidity); RAIN (rainfall);

Summary and final conclusions

The sampling of airborne fungal spores was carried out continuously at the University of Worcester, Worcester, UK from 2006 to 2010. A 7-day volumetric spore sampler was used to collect air samples, which were then processed and analysed at the laboratory. Selected allergenic fungal spores were identified and counted with an hourly division under x400 magnification along one central longitudinal transect of microscope slides. Meteorological data used in this project were collected during the same period of time by the weather station, co-located with an air sampler. Out of twenty genera and groups of fungal spores for a detailed investigation four fungal genera were chosen. The overall aims of this study were to investigate atmospheric concentrations of *Alternaria*, *Cladosporium*, *Didymella*, *Ganoderma* spores and to produce forecast models for predicting their further concentration in the air in Worcester, UK. This was accomplished by examining annual, diurnal and spatial fluctuations in fungal spore concentration in relation to the changeable weather conditions within a five year period of time.

Year to year daily mean concentration of fungal spores was a subject of constant changes, which were confirmed by results of the Kruskal-Wallis test ($p \leq 0.05$). However, in these variations certain patterns were detected. *Alternaria* spores were mainly present from mid-May – mid-June until the end of September – beginning of October in the air in Worcester, with peak of the spore season occurring in August. *Cladosporium* spores were frequently recorded from mid-May until the end of October, with peak of the spore season occurring in July. *Didymella* spores were mainly found from the end of June or beginning of July, until the end of September, with peak of the spore season occurring in August. *Ganoderma* spores were mainly occurring from the beginning of May to the end of October with peak of the spore season falling towards September.

Similarly, annual variations in diurnal fluctuations in examined fungal spore concentration were observed, although of lesser importance. This mainly applied to *Cladosporium* and *Didymella*, while *Alternaria* and *Ganoderma* were

characterised with greater stability what was confirmed by results of the Kruskal-Wallis test ($p \leq 0.05$). Overall, the highest concentration of *Alternaria* spores was recorded in the evening, between 10 pm and 11pm, while the lowest between 7am and 8am. In contrast, the highest concentration of *Cladosporium* spores was observed during mid-afternoon hours (1-3pm), and the lowest in the morning hours (7-9am). *Didymella* spores as the only fungal genus revealed a double peak pattern. Maxima occurred before the dawn (4-5am) and after the dusk (10-11pm), while minima between 10am and 4pm. Similarly, greatest concentrations of *Ganoderma* spores were also found before the dawn (3-6am), while the lowest in the afternoon hours (2-5pm).

Relationship between *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spore concentration and selected meteorological parameters (maximum temperature, minimum temperature, mean temperature, dew point temperature, rainfall, relative humidity, air pressure, wind direction) was examined with regard to the annual and diurnal variations, as well as the time lagging up to three days backward using wide range of statistical tests (*i.e.*: Spearman's rank test, redundancy analysis, multivariate regression tree models and artificial neural network models).

Throughout all performed statistical tests presence and concentration of *Alternaria* spores was found to be mostly dependent on the maximum temperature, followed by minimum and mean temperature. MRT analysis indicated the most suitable range of maximum temperature varying between 13°C and 25°C. A sign of good weather and increased maximum temperature could be also explained indirectly by the higher air pressure (1022 hPa) computed by the MRT as a second important factor influencing *Alternaria* spore concentration. Some tests showed that impact of dew point temperature should be also considered as significant, what was confirmed by the sensitivity analysis of the best performing forecasting model MLP 8-24-1, while the maximum temperature was rated at the seventh position in the overall ranking of examined meteorological parameters. With regard to the hourly fluctuation in *Alternaria* spore concentration, some contrary results were obtained. While low relative

humidity (40-55%) would be fitting to the overall picture, the optimum maximum temperature was found to be from 0°C to 10°C. An increased concentration of *Alternaria* spores during light rain showers (10-15 mm) could be explained by dispersal of fungal spores through the rain splash mechanism that enabled conidia to become airborne.

The strong relationship between concentration of *Cladosporium* spores and maximum temperature was indicated by all applied examination tools. The highest correlation with this parameter was found to be equal to r_s=0.70 ($p \leq 0.05$) by Spearman's rank test and the best performing forecasting model MLP 8-22-1. Less important, although worth noting was a relationship found with dew point temperature using Spearman's rank test, while RDA indicated also minimum temperature. An increased concentration in *Cladosporium* spores in the air in Worcester was observed when the maximum temperature varied between 13°C and 21°C and air pressure was within a range from 1005 hPa and 1017 hPa according to the MRT analysis. Obtained results for daily mean *Cladosporium* spore concentration were corresponding with values of maximum temperature (20-25°C) and relative humidity (40-55%) calculated for hourly mean spore concentration. In addition, diurnal analysis of *Cladosporium* spores revealed that light rain showers (up to 5 mm within 24 hours) could help the spores to spread via the rain splash, similarly to *Alternaria* spores.

In contrast to *Alternaria* and *Cladosporium* spores other meteorological parameters favoured the occurrence and concentration levels of *Didymella* ascospores. The Spearman's rank test although showed importance of dew point temperature and air pressure, but the priority gave to the relative humidity (r_s=0.59). MRT analysis not only confirmed this dependence but also computed a threshold equal to 89% above which *Didymella* ascospores were found at significantly greater concentration (~8000 s m^{-3}). This value fell within a category range of relative humidity calculated for an hourly mean spore concentration (85-100%). Similarly, MRT indicated that maximum temperature must be oscillating around 15°C to influence positively *Didymella* spores levels in the air. An analysis of the diurnal profile once more confirmed this finding (10°C-15°C). In addition,

the best performing forecasting model MLP 8-6-1 rated relative humidity as a third factor of importance, preceded by minimum and maximum temperature.

Dew point temperature was found to play an important role in biology of *Ganoderma* fungus, what was indicated simultaneously by the Spearman's rank test (r_S=0.69) and MRT analysis ($n \geq 12$°C). These tests suggested that the maximum temperature ($n \geq 19$°C) was almost equally important to dew point temperature and played a key role in distribution of *Ganoderma* spores. An analysis of weather impact on *Ganoderma* spore levels within 24 hours confirmed previous results, where the optimal maximum temperature must varied between 20°C and 25°C simultaneously with decrease of relative humidity to 40-55%. A significantly high *Ganoderma* spore concentration recorded during heavy rain showers (15-20 mm) could be explained by the fact, that *Ganoderma* basidiocarps must soak water up to 24 hours to enable production and release of spores. In addition, the best performing forecasting model MLP 8-15-1 above all examined meteorological parameters indicated minimum temperature to be the most important factor, what was occasionally seen in one MRT model ($n \geq 14$°C).

The spatial analysis of fungal spore distribution was performed using jointly the HYSPLIT model and GIS techniques. Although different days were selected for a detailed investigation for each of examined fungal genus, all analyses showed no evidence for a long distance transport of spores from the continental Europe. A minor contribution of the fungal spores derived from the sources located in Ireland was detected in the analysis of *Alternaria* and *Cladosporium* spores.

The back trajectories showed that potential sources of *Alternaria* spores (crops under rotation) were located from the South-West to the North direction, 65-130 km (40-80 mi) away from Worcester and 6-12 hours of the air masses transport. These findings were closely matching the overall mean daily and hourly spore concentration for examined days. Mean speed of the air masses favouring the greatest concentration of *Alternaria* spores was found to be between 3 m s^{-1} and 4 m s^{-1}.

With regard to the spatial distribution of *Cladosporium* spores, obtained results showed a weak, but yet statistically significant ($p \leq 0.05$) correlation

between high *Cladosporium* spore counts (≥ 6000 s m^{-3}) and both local wind and overall air mass directions. However, better results were obtained using Spearman's rank test than linear-circular association test. The inoculum sources of the investigated fungus seemed to have a regional origin, but yet must be located within the UK territory at SE-SW direction from the monitoring station situated in Worcester.

The back trajectories showed that potential sources of *Didymella* spores collected in Worcester could be found within the distance of 108-216 km (67-134 mi). The majority of the spores must have originated from the crops (e.g. barley, wheat) cultivated at the North West England, East Midlands, West Midlands and South West England. These findings were in agreement with the results obtained using the MRT analysis, which indicated location of the potential sources within an arc from the West to the North direction. The double peak pattern characteristic for *Didymella* spores could be explained by a transport of the air masses from two sources, one closer and one farther located from Worcester, but yet lined up in the same direction. A further dispersion analysis using HYSPLIT model could definitively answer this question.

The potential sources of *Ganoderma* spores collected in Worcester could be found within the distance of less than 200 km (n < 120 mi) according to the back trajectories. The majority of the spores must have originated from the woodlands situated within an arc from the East to the West direction from Worcester. These findings were in agreement with the results obtained using the MRT analysis, which indicated location of the potential sources within an arc from the East-South East to the South-West direction. The overall transport of *Ganoderma* spores could take up to several hours of the air masses transport.

The best forecasting models constructed using artificial neural network modelling for *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores were characterized with prediction power equal to 52%, 66%, 40% and 59% respectively. Summing up the efficiency of the models must dependent therefore on several factors 1) distance between air sampler and sources of the fungal spores, 2) time of the fungal spore transport, 3) length of the fungal spore seasons,

and 4) changeable importance of the meteorological parameters on the fungi biology during a single vegetation season.

Future work

At the beginning of this thesis, in Chapter 1 Introduction two major aims were indicated as (1) investigation of atmospheric concentrations of the allergenic fungal spores of *Alternaria*, *Cladosporium*, *Didymella*, *Ganoderma* and (2) production of forecasting models for prediction of their future concentrations in the air in Worcester, UK. Both aims drawn in this project were completed using specific objectives, described in detail in section 1.7 of that chapter.

This research study is one of the first of its type conducted in the UK and many more are needed to understand spatial and temporal distribution of bioaerosols, including those consisted of fungal spores. Results of aerobiological survey have much wider application than just in allergology, as they may be also successfully applied in public health, agriculture, epidemiology, forestry.

In this study spores produced by four fungal genera were examined, although 20 spore types were sampled in total. The summary of the overall collected data should be published soon in an arrangement of fungal spore calendar for Worcester. This format is an easy way of communication with public for whose welfare it is produced for. Although Bustos-Delgado et al. (2006) performed a one year survey on fungal spores in Worcester their calendar has never been officially published. Up to date only a single fungal spore calendar was created in the UK (Derby), and hence more similar reports are needed (http://www.maara.org/2013-08-19-06-21-06/spore-calendar).

Further temporal and spatial analysis could yield in aeroallergen map production for specific regions, if not for the entire country. This would be a one step forward in contrast to daily statements released by few monitoring stations scattered across the country. More data is needed from Ireland and Northern Ireland, as only one monitoring station in Cork is currently operating (O'Connor et al., 2014). Action has been already taken in Italy (*Alternaria* spp., *Pleospora* spp.), Poland (*Leptosphaeria* spp.) and United States (*Phakopsora pachyrhizi*), while in

the UK nothing has been done yet towards data generalization (Isard et al., 2005; 2007; Jędryczka et al., 2012; Tomassetti et al., 2013).

I would therefore postulate and encourage both myself and others to continue work at the aerobiological station in Worcester and promote conduction of similar work in other locations in the UK, including both economically important rural areas as well as other densely populated urban areas. It would be advisable to perform long term studies in order to examine impact of climate change on pollen grains and fungal spore production, pollen and spore allergen production, interaction between aeroallergens and air pollution, not just weather variables. Long-term sampling could be then analysed using other time series analysis methods, such as Autoregressive Integrated Moving Average (ARIMA) models, not only Artificial Neural Network models. Also application of circular statistics, which was used here in Chapter 5 requires more attention as it could be explored to a greater extent in aerobiological studies. For further reading see Kasprzyk (2006b; 2008) as well as Borycka and Kasprzyk (2014).

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