Air mass trajectories and land cover map reveal cereals and oilseed rape as major local sources of *Alternaria* spores in the Midlands, UK.

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Abstract

Transport of *Alternaria* spores from both local agricultural and remote areas have been implicated as sources of the spores in urban areas. The purpose of this study was to understand the relative contribution of local sources versus long distance transport to *Alternaria* spore concentrations, with applicability to *Alternaria* and other spore sampling sites worldwide. This was achieved by comparing two spore sampling sites at Worcester and Leicester in the UK ~90 km apart over a three year period (2016-2018) and focusing on a period of time when both sites experienced high spore counts. The study found 61 and 151 days of clinical significance (>100 spores/m^3 air) at Worcester and Leicester, respectively. The spore concentrations were considerably higher in Leicester than in Worcester. Analysis of the crop map showed higher amounts of winter barley and oilseed rape near to Leicester than Worcester. HYSPLIT modelling during the episode revealed that the air masses arrived at both Leicester and Worcester from Ireland and the Atlantic Ocean. Long distance transport probably had a small but equal contribution to the observations at both sites. HYSPLIT particle dispersion simulations showed that the spores were dispersed and deposited from local sources. The

results indicate that substantially higher concentrations of *Alternaria* spores will be realised in areas with high amounts of cereals and oilseed rape, here illustrated with Leicester, compared to a region, illustrated with Worcester, with fever crop areas and higher amounts of other crops than cereals and oilseed rape.

Keywords: Harvesting, *Alternaria*, HYSPLIT, Pathogen, Allergen.

1.0 Introduction

Alternaria is a saprophytic fungus of the phylum Ascomycota (Woudenberg et al., 2015). It is a ubiquitous fungus found in water, soil, air and on decaying matter (Nowicki et al., 2012; Thomma, 2003). Airborne *Alternaria* spores are known to cause allergy and can trigger asthma, rhinitis, bronchitis, eczema, and alveolitis in susceptible individuals (Gabriel et al., 2016; Pastor & Guarro, 2008; Pulimood et al., 2007; Singh & Denning, 2012). In the UK, the prevalence of sensitization to *Alternaria* spores is 7.3% (Bousquet et al., 2007). The threshold for determining a high *Alternaria* day is most commonly taken to be 100 spores/m³ (Gravesen, 1979), although an earlier study suggested it to be as low as 50 spores/m³ (Frankland & Davies, 1965). The relative abundance of airborne spores in urban areas is strongly influenced by geographic location, climate and land use (Haberle et al., 2014). Land use patterns especially crop production in the outskirts may enhance the spore abundance in nearby urban areas, thereby increasing exposure (e.g. Skjøth et al., 2012). The geographical variation in crop production and its relationship with airborne *Alternaria* spore concentrations is therefore important in relation to understanding the exposure to spores.

Alternaria is also a common plant pathogen (Rotem, 1994). The *Alternaria* genus has more than 300 species (Seifert & Gams, 2011) and some species are pathogenic both during the growing and postharvest stages (Nowicki et al., 2012; Seifert & Gams, 2011). Agricultural yield losses caused by *Alternaria* diseases has been estimated at over 80% in several years of production (Nowicki et al., 2012; Maude and Humpherson-Jones, 1980). For instance, *A. solani* causes early blight in potatoes and tomatoes (Escuredo et al., 2011), *A. triticina* and *A. infectoria* infect wheat, *A. dauci* affects carrots, *A. brassicae* and *A. brassicicola* infect crucifers and *A. alternata* affects fruits (Lee et al., 2015; Logrieco et al., 2009). Spores from *Alternaria*-infected vegetation have often been shown to be transported across regions and continents and have the potential to cause infection in such new environments (Burshtein et al., 2011; Sadyś et al., 2015; Skjøth et al., 2012). This mechanism of cross-continental atmospheric spore transport and establishment in the new environment has previously been demonstrated for other pathogens, ultimately causing substantial economic losses for the agricultural sector (Isard et al., 2005; Isard et al., 2007).

Pathological studies are typically focused on local scales due to factors of labour intensiveness, the effects of other pathogens, and sensitivity to environmental conditions that are associated with field studies (Chaerani & Voorrips, 2006). Consequently, there is limited information on the spatial distribution of pathogens on a large biogeographical scale (Bégué et al., 2018). The fungal infection can develop rapidly due to its short reproductive cycles during the growing season (Agriculture and Horticulture Development Board, 2014). The agricultural industry considers early blight caused by *A. alternata* and *A. solani* to be the most significant foliar disease in many continents (Agriculture and Horticulture Development Board, 2014). The effect of spraying to manage the disease varies between the individual pesticides and the type of infection (e.g. *A. alternata* or *A. solani).* As a result, farmers have limited possibility to respond to any outbreaks of fungal diseases caused by the import of *Alternaria* spores from distant sources.

In the UK, about 19% of the land is utilized for arable farming including wheat and barley. Cereals, vegetables, and potatoes are the top most important crops in the UK, accounting for more than £5 billion of agricultural production in 2014 (DEFRA, 2018). Combine harvesting of cereals have previously been reported to release large amounts of *Alternaria* spores (Friesen et al., 2001; Hill et al., 1984; Skjøth et al., 2012). Understanding the spatial distribution of cereal production in relation to airborne concentrations of *Alternaria* spores provides information about both source and potential new host areas. Knowledge about source areas, atmospheric transport, and new host areas have previously been shown to be an efficient tool in the management of developing diseases (Isard et al., 2007) as it allows the agricultural industry to better prepare and manage important crop pathogens such as *Alternaria.* This aim of this study, therefore, was to understand the relative contribution of local sources verses long distance transport to *Alternaria* spore concentrations, with applicability to *Alternaria* and other spore sampling sites worldwide.

2.0 Materials and Methods

2.1 Sampling location

Alternaria spores were sampled at the University of Worcester and the University of Leicester. The trap at St Johns campus of the University of Worcester (hereafter referred to as Worcester) was located 10 m above ground level (agl) on the roof of Edward Elgar building (52.1970, -2.2421) (e.g. Sadyś et al., 2014) while that of the University of Leicester (hereafter referred to as Leicester) was located 12 m agl on the roof of the Bennett building (52.6231, -1.1227) (Pashley et al., 2009). Worcester and Leicester are ~90 km apart and are located in the West and East Midlands regions of England, the UK, respectively (Fig. 1). Both sites are located in urban areas and surrounded by agricultural areas under rotation. The nearest crop fields to Worcester trap are half a km away to the west while those for Leicester are within 5 km of the trap.

2.2 Spore sampling and analyses

Daily and bi-hourly *Alternaria* spore concentrations for the period 1st March to 31st October 2016-2018 were obtained using Hirst-type volumetric spore traps of the Burkard model (Hirst, 1952). *Alternaria* spores were sampled and slides prepared according to standard procedures used for over 50 years in England and other European countries (Skjøth et al., 2008, 2015; Adams-Groom et al., 2002; Kasprzyk, 2008; Makra et al., 2010; Sadyś et al., 2014). Optical microscopy is the traditional spore identification method although it requires considerable expertise and it is constrained by overlap of morphological features among species (Grinn-Gofroń et al., 2016; Kaczmarek et al., 2009; Lawrence et al., 2016; Pashley et al., 2012). Hence, *Alternaria* was identified to the genus level using an optical microscope based on size, shape, colour, and septa (Grant Smith, 1990; Simmons, 2007).

Slides at Worcester were counted using the 12 transverse method at X400 magnification (BAF, 1995; Käpylä & Penttinen, 1981) and those at Leicester were counted using the single longitudinal method at X630 magnification (Corden & Millington, 2001; Fairs et al., 2010; Sterling et al., 1999). Each counting approach generates daily values of atmospheric spore concentrations in agreement with minimum requirement (Galán et al., 2014) allowing for a direct comparison of daily values between sites, independent of the counting approach (Skjøth et al., 2016). Selected slides from Leicester were reanalysed using the 12 transverse method to allow a comparable daily and bi-hourly analyses using the same counting technique at both Leicester and Worcester. The 95% method was used to determine the spore seasons (Goldberg et al., 1988). Seasonal summaries of the season start and end, spore integral, duration, days above 100 spores/m^3, and peak concentrations were calculated for the years 2016-2018, according to Galán et al. (2017). Analyses were conducted using R software (R Core Team, 2018).

2.3 Meteorological data

Meteorological data for Worcester was provided by a meteorological station co-located with the spore sampler. Leicester City Council Air Quality group whose weather station is located 5 km from Leicester spore trap provided the meteorological data for Leicester. The half-hourly weather data for Worcester and hourly data for Leicester were independently averaged to provide the daily weather data for each station in order to match the daily concentrations of *Alternaria* spores during the periods of interest.

2.4 Potential *Alternaria* spore sources

Potential source areas for *Alternaria* spores were based on crop maps produced by the Centre for Ecology and Hydrology in collaboration with the National Environment Research Council and the weekly harvest progress reports produced by Agriculture and Horticulture Development Board (Agriculture and Horticulture Development Board, 2019). The crop map is a field-based land cover map, where the crop type has been identified using Copernicus Sentinel-1 C-band synthetic aperture radar and Sentinel-2 satellite optical images. The map covers Great Britain with a minimum mapping unit of two hectares and an overall accuracy of 86% with respect to identifying the correct crop type. The crops include winter wheat intercropped with oat, winter barley, spring wheat, spring barley, maize, field beans, oilseed rape, potatoes, beet and other crops (CEH, 2018).

In this study, the crop map for 2017 (Fig. 1) was utilized to evaluate the potential source areas of *Alternaria* spores in Worcester and Leicester since crop-producing areas have already been reported as major hosts of *Alternaria* spores (O'Connor et al., 2014; Skjøth et al., 2012). The land cover data was downloaded from the EDINA digimap website (EDINA, 2018). The crop map was analysed using the Analysis tools extension of ArcGIS v.10.5.1. A radius of 30 km from the sampling trap was extracted as representative of the land cover following previous aerobiological studies that suggest that the overall spore load in a region is mainly from local sources with intermittent long-distance transport from remote sources (Avolio et al., 2008; Isard et al., 2007; Isard et al., 2005; Skjøth et al., 2009; Smith et al., 2008). Daily *Alternaria* spore concentrations during the period 27 Jul-1 Aug 2017 and 2-7 Aug 2017 were cumulated and averaged to match the weekly crop harvest reports. The relationship between the weekly crop harvest and airborne spore concentrations were examined.

2.5 Back-trajectory calculations

Back-trajectories were calculated using the HYSPLIT model and the Global Data Analysis System (GDAS) meteorological data of the Air Resources Laboratory (ARL) (Draxler et al., 2016; Stein et al., 2015). The finest resolution of 0.5° x 0.5° was used as HYSPLIT model calculations generally are sensitive to the grid resolution of the input data (Bilińska et al., 2017; Hernández-Ceballos et al., 2014). Back-trajectories of 24 hours with a 2-hour step between trajectories, corresponding to the time of *Alternaria* spore observation, were calculated for the entire spore season of 2016, 2017 and 2018 for Worcester and Leicester, as previously applied by Fernández-Rodríguez et al. (2015) and Skjøth et al. (2012). Back trajectories were calculated at a receiving height of 500 m agl, an approach commonly used in aerobiological studies (Fernández-Rodríguez et al., 2014, 2015; Sadyś et al., 2015; Skjøth et al., 2012; Stach et al., 2007).

The back-trajectories from each site were sorted into two groups: (i) days with daily mean *Alternaria* spore concentration above 100 spores/m³ of air (high days) and (ii) days with spore concentration below 100 spores/m³. This method follows that of Skjøth et al. (2009) and Sadyś et al. (2014), where trajectories for the entire observational period and those for high days alone are both analysed with a HYSPLIT model. Group (i) of high days for each site and year were further analysed considering back-trajectories of air masses that passed via Worcester to

Leicester– here choosing a 30 km distance corresponding to the typical biogeographical coverage of the pollen trap, according to a methodology described by Skjøth et al. (2008). The trajectories for each site and year were sorted using ArcGIS 10.5.1. The most severe days with back-trajectories of air masses that passed via Worcester to Leicester formed the episode. The daily spore concentrations from both sites during the episode were then analysed and compared. This comparison explored if the daily spore concentrations at Leicester during the episode were higher compared to Worcester when air masses passed via Worcester. The episode was further analysed using bi-hourly spore data from both sites and a density map based on particle dispersion modelling using the HYSPLIT model.

2.6 Backward particle dispersion modelling

Particle dispersion modelling was conducted to ascertain whether *Alternaria* spores released from their different sources were dispersed and deposited within the sampling sites of Worcester and Leicester. HYSPLIT model was used to simulate the dispersion of particles (Draxler et al., 2016; Stein et al., 2015) during the episode. The model was set to release 2500 particles of 19 µm (McCartney et al., 1993) at 100 m agl for 24 hrs with 2 hrs step between each trajectory corresponding with the time of *Alternaria* spore observation, similar to the studies by Skjøth et al. (2012) and another by de Weger et al. (2016) and taking into account both dry and wet deposition.

Settling velocity (fall speed or terminal velocity) of pollen and spores plays an important role in their dispersal and deposition. Settling velocities are therefore important in modelling particle dispersion (Di-Giovanni et al., 1995; McCartney & West, 2007). In this study, the HYSPLIT particle dispersion model was set at *Alternaria* spore settling velocity of 0.55 cm/s as determined by Gregory (1961) and McCartney et al. (1993). The HYSPLIT model setup covers simulations for both dry and wet particle deposition that takes into account both in-cloud and below cloud scavenging. The model output is the geographical location and height of each particle in the calculated particle cloud. This cloud is available for every 2 hr time step. All data were further analysed using point density method in the Spatial Analyst tool of ArcGIS v. 10.5.1 to produce density maps of the entire episode as well as the day with the largest difference in spore concentration between the two sites. The trajectories for the day with the largest difference in spore concentration between the two sites were further analysed and the percentage of particles dispersed below and above a trajectory height of 500 m was done, in a similar way as de Weger et al. (2016).

3.0 Results

3.1 Annual and seasonal *Alternaria* spore concentrations

Leicester recorded higher Annual and Seasonal Spore Integrals than Worcester throughout the three years of observation (Fig. 2). For instance, Leicester recorded seasonal spore integral five times greater than Worcester in 2017 (Table 1). Furthermore, Leicester (151 days) recorded more high days than Worcester (61 days) in the three years of observation. *Alternaria* spore season at Worcester started in early June and ended late September. Meanwhile, the season in Leicester started either in early May or June and ended late September or early October. Whereas the two sites had comparable season duration in 2017, Leicester had a longer season (by 31 days) in 2018. The maximum daily spore concentrations at Leicester were considerably higher than those at Worcester during the three years of observation. For instance, a maximum daily peak of 3700 spores/m^3 was recorded at Leicester while Worcester observed 796 spores/m^3 in the 2017 season.

3.2 Spore concentrations during the episode: 27 July-7 August 2017

During the episode, the daily mean *Alternaria* spore concentration at Worcester ranged from low to high (10-273 spores/m^3) while Leicester maintained a high spore concentration of above

100 spores/m³ (176-659 spores/m³) (Table 2**)**. Dispersal of *Alternaria* spores at both sites mainly occurred from the afternoon (12:00 hr) to late evening (22:00 hr) (Fig. 3). Leicester observed two peaks of comparable spore concentrations at 13:00 hr and 21:00 hr while Worcester recorded a minor peak at 13:00 hr and the major one at 21:00 hr.

3.3 Trajectory calculations, weather synopsis, and potential source areas

3.3.1 Trajectory calculations and weather synopsis

The meteorological observations show that there was an oscillation of a high- (1016 hPa) and low-pressure system (998 hPa) during the episode. The trajectories show that this caused the air masses to be pushed from the Atlantic Ocean and Ireland and in a SW to NE direction **(**Fig. 4) towards Wales and England (Worcester and Leicester). The winds blew at an average speed range of 1.9-4.4 m/s and in the WSW direction (average 255º) on arrival at the Worcester trap. During the same period, Worcester received a light amount of precipitation (0.05-1.6 mm/hr) for 11 days of the episode except on 06 Aug 2017. The temperature at Worcester was recorded in the range of 15-17 ºC (Supplementary Table 1a). During the same episode, Leicester experienced a similar range of daily average temperatures (13-17 ºC) as Worcester (Supplementary Table 1b) but received no precipitation. Winds blew in a SSW direction (average 186º) and at a lower speed (1.2-2.1 m/s) than for Worcester on their arrival at Leicester trap.

3.3.2 Potential sources of *Alternaria* spores

Crops located along the path of the air masses are possible sources of *Alternaria* spores at Worcester and Leicester. Statistical analyses of the crop map within a radius of 30 km from Worcester and Leicester spore traps (Table 3) show that crop density at Leicester (114,844 ha) was considerably greater than that at Worcester (86,739 ha). Furthermore, Leicester (73,312 ha) had more cereals cultivated than Worcester did (54,164 ha). Cereals like winter wheat intercropped with oat and winter barley and non-cereal like oilseed rape were the most cultivated crops at both sites. The crop harvest progress report 2017 (Fig. 5) shows a gradual increase in the harvest of winter barley and winter oilseed rape which similarly results in a gradual increase in spore concentrations at Leicester and Worcester during the episode. However, the cumulative spore concentrations at Leicester were higher than those at Worcester. Meanwhile, there was a minimum harvest of the other crops like the winter wheat, spring barley, and oats during the period (Fig. 5).

3.4 Particle dispersion during the episode

The density maps (Fig. 6a and b) of the particles dispersed during the episode reveal that the particles were dispersed from similar directions (SW) before their deposition at Worcester and Leicester traps. For Worcester, the highest concentration of particles (14,134-23,554) was dispersed from the SW-W direction, particularly in the areas of Leigh, Hereford, and Wales (Fig. 6a). The lowest concentration of particles (1-14,133) was dispersed from the NW-SW direction of Worcester, particularly within the Atlantic Ocean and Ireland.

For Leicester, the highest (13,634-22,721) concentration of particles was dispersed from the SW-W direction (Fig. 6b) while the lowest concentration of particles (1-13,633) was emitted from the NW-SW direction of Leicester. The possible places of origin of the highest concentration of particles before arrival at Leicester trap are the cereal and non-cereal farms outside the cities of Nuneaton, Coventry, Birmingham, and Redditch. The lowest particle concentrations were observed to have originated from the Atlantic Ocean, Ireland and Wales.

August 1st, 2017 was the date with the highest difference in daily *Alternaria* spore concentration between the two sites during the episode. Although on this date the density map for the particle clouds (Fig. 7a and b) revealed that the air masses moved in SW direction towards the spore traps of both Worcester and Leicester, Leicester received 6 times more spore concentration (659 spores/m³) than Worcester did (115 spores/m³). Geostatistical analyses of particle clouds deposited at Worcester revealed that 30% of the particles were dispersed and deposited from England and Wales, 21% in Ireland and 49% in the Atlantic Ocean (Table 4). Thirty-five percent of the particles were dispersed at a height below 500 m (Table 5). For Leicester, 40% of the particles were dispersed and deposited within England and Wales, 22% in Ireland and 38% in the Atlantic Ocean (Table 4). Thirty-one percent of the particles were dispersed at a height below 500 m (Table 5).

4.0 Discussion

The study supports the hypothesis that cereal-producing areas within a 30 km radius were likely sources of airborne *Alternaria* spores in urban areas of Worcester and Leicester during spore season. However, Leicester recorded considerably higher annual *Alternaria* spore concentrations than Worcester did throughout the three seasons. This high annual spore concentration at Leicester could be attributed to several factors including larger geographical coverage of croplands acting as host of *Alternaria* within the local source area of Leicester's trap, human activities e.g. harvesting of crops and environmental factors (Mitakakis et al., 2001; Sabariego et al., 2012; Skjøth et al., 2012). Moreover, the two study sites show distinct differences in spore concentrations across the sampling years. This could be attributed to environmental conditions such as weather and anthropogenic activities e.g. farming (Calderón et al., 1997; Corden et al., 2003; Stennett & Beggs, 2004).

Some previous studies also reported extremely high *Alternaria* spore concentrations. For instance, Stępalska et al. (1999) reported an annual *Alternaria* load of 32,000 and Grewling et al. (2018) reported a seasonal load of over 46,000 all in Poznan, Poland. Angulo-Romero et al.

(1999) and Bartra et al. (2009) also observed annual spore loads of over 20,000 and 27,000, respectively in Spain. Corden and others observed seasonal *Alternaria* spore loads of over 35,000 in 1992 and 1996 (Corden et al., 2003) and over 50,000 in 1997 in Derby (Corden & Millington, 2001), which are comparable to the seasonal values observed in Leicester. With the right climatic and weather conditions, such high annual and seasonal loads can result in some *Alternaria* spores being dispersed to new and uninfected geographical areas thus leading to widespread infection (Savage et al., 2012), as was the case with soybean rust (*P. meibomiae* and *P. pachyrhizi*) in the United States (Isard et al., 2005, 2007).

Cereal crop harvesting has long been associated with the emission of large amounts of *Alternaria* spores (Corden et al., 2003; Friesen et al., 2001; Hill et al., 1984; Skjøth et al., 2012). Cereals e.g. wheat (Nicolaisen et al., 2014; Uddin & Chakraverty, 1996) and barley (Müller & Korn, 2013) have shown high infection rates with *Alternaria* both in-field and in post-harvest stages. Oilseed rape is a non-cereal *Alternaria* host crop (Kumar et al., 2014; Giamoustaris & Mithen, 1997). In this study, analysis of crop density revealed that cereals were the most dominant crops cultivated in both Worcester and Leicester. However, Leicester (73,312 ha) had more hectares cultivated with the cereals than Worcester did (54,164 ha). Oilseed rape was also found to be grown abundantly at Worcester (11,494 ha) and more extensively at Leicester (23,154 ha). The harvest progress report 2017 showed that the progressive harvesting of winter barley and winter oilseed rape coincided with an increase of spore concentrations at Leicester and Worcester. This suggests that winter barley and winter oilseed rape were likely an important contributing source of the high concentration of *Alternaria* spores during the spore season in Worcester and Leicester urban areas. However, Leicester recorded higher concentrations than Worcester. This could be caused by the observed higher density of crops near the Leicester trap compared to the Worcester trap. However, a contributing factor may be that the agricultural areas near to Leicester may have a higher emission potential, either caused by local environmental conditions or that some crops may possess a very high emission potential. Previously, Mitakakis et al. (2001), in an experimental study, found a correlation between *Alternaria* spore concentrations recorded in towns with those recorded in the field during harvesting of wheat and cotton crops. In Spain, several crops and orchards harvested during the summer were also found to be important sources of *Alternaria* spores during the season (Fernández-Rodríguez et al., 2015). In the UK, potatoes and field beans are harvested in autumn and early winter (Agriculture and Horticulture Development Board, 2015; Processors and Growers Research Organisation, 2016). This shows that potatoes and field beans were unlikely contributors to the high concentration of *Alternaria* spores recorded in Worcester and Leicester during the cereal harvest period (July-August) although they are known hosts of *Alternaria* (Abd-El-Kareem, 2007; Landschoot et al., 2017). Although the cereals and oil-seeds are cultivated using rotational system in the Midlands region of Leicester and Worcester (Sadyś et al., 2015; Skjøth et al., 2016), crop rotation probably had a minimum and equal effect on spore concentrations at the two cities. Crop rotation may be expected to impact *Alternaria* spore emission from a few individual fields due to a change in crop types. However, within a larger geographical area, the abundance of specific crops varies to a smaller degree. According to Eurostat (https://ec.europa.eu/eurostat/data/database), the amount of areas cultivated with cereals in East Midlands was 466, 462 and 458 thousand hectares. Furthermore, *Alternaria* is difficult to be solely controlled by rotation since it has soil, seed/plant and airborne life cycle stages (Chaerani & Voorrips, 2006; Escuredo et al., 2011). Besides, growers tend to shorten rotation periods due to production demands (Pryor et al., 1998) which can result in continued habitation and release of *Alternaria* spores in an area. Moreover, annual changes in crop rotational areas cause changes in crop maturation resulting in varying concentrations and time series of *Alternaria* spores in different areas (Skjøth et al., 2016). Consequently, some high *Alternaria* spore concentrations can be observed beyond the main harvest period. Previously, Sadyś et al. (2015) produced a map showing large areas of permanent crops e.g. orchards in Worcester while Leicester had large areas of crops under rotation. Therefore, this study suggests that harvesting of cereals and oilseed rape could be a cause of elevated concentrations of *Alternaria* spores.

A number of variables including gravitational settling, eddy-diffusion, temperature, precipitation, wind speed, wind direction, and atmospheric pressure system influence the dispersal of fungal spores from their sources to distant places (Hirst et al., 1967). Notwithstanding, atmospheric processes such as wet deposition and cloud processing in low-level clouds limit travel distances in the boundary layer thus reducing concentrations of bioaerosols such as *Alternaria* spores in the air mass (Sesartic et al., 2012; Huffman et al., 2013). This study found that during the episode the air masses that arrived at Worcester and Leicester originated from the Atlantic Ocean and Ireland. Long distance transport probably had a small but equal contribution to spore observations at both Worcester and Leicester. Besides, the marine environment is known to contain low concentrations of fungal spores (Sadyś et al., 2014; Urbano et al., 2011; Elbert et al., 2007). The daily spore concentration during the episode shows that Leicester recorded 5 times more spores than Worcester did. This suggests that the high spore concentrations recorded at Leicester were probably contributed by local sources with strong emission potential. The low concentrations in Worcester were also likely from its local sources that had a weaker emission potential. Fernández-Rodríguez et al. (2015) investigated sources of *Alternaria* spores in Badajoz (Spain) and found that local sources of crops and grasslands were the greatest contributors of spores in the area with supplements from distant sources. Sadyś et al. (2015) also examined *Alternaria* spore sources in Worcester (UK) and found that local sources within West Midlands were the main contributors to total spore load in Worcester. Both studies agree with ours, that local sources are a major contributing source of *Alternaria* spores, while this study extends previous findings by identifying both specific crop types and harvesting as likely causes to high spore concentrations.

The analysis during the episode shows that the local sources at Worcester and Leicester mostly released spores in the afternoon and late evening. Similar diurnal patterns of *Alternaria* spores were observed in Worcester (O'Connor et al., 2014; Sadyś et al., 2015), Derby (Corden & Millington, 2001), Copenhagen (Skjøth et al., 2012), Krakow (Stępalska & Wołek, 2009), Northern Portugal (Oliveira et al., 2009; Rodríguez-Rajo et al., 2005), Northern Spain (Aira et al., 2008), Southern Spain (Angulo-Romero et al., 1999) and Italy (Ricci et al., 1995). In the UK, the cereal harvesting activities peak in the afternoon or early evening (Agriculture and Horticulture Development Board, 2018). This pattern matches well with the diurnal patterns in *Alternaria* concentrations. It is therefore likely that harvesting of cereals and oilseeds coupled with optimum weather conditions could have increased *Alternaria* spore concentrations in the urban areas of Leicester and Worcester.

Precipitation is one known weather parameter that can affect airborne *Alternaria* spore concentrations (Rotem, 1994). In this study, Worcester received light precipitation of 0.05-1.6 mm/hr for 11 days of the episode. During the same period, daily spore concentrations remained low at Worcester except on 31 Jul, 04 Aug, and 07 Aug 2017 when they surpassed 100 spores/m^3. The low spore concentration could be due to the washing down of *Alternaria* spores from the atmosphere during the rainy days (O'Connor et al., 2014; Peternel et al., 2004; Sakiyan, 2003). It also suggests that the precipitation wetted the soils hence hindering harvesting of the crops. However, there was no remarkable increase in spore emissions observed after the precipitation, although moisture is known to encourage *Alternaria* spore growth and emission (Kumar et al., 2014; Green & Bailey, 2000). Skjøth et al. (2012) and Friesen et al. (2001) also had similar observations as ours for *Alternaria* and other fungal spores. The lack of harvesting during the precipitation period coupled with other factors e.g. unfavourable weather conditions after the precipitation probably resulted in low spore emissions at Worcester. Meanwhile, there was no precipitation recorded at Leicester throughout the episode. The consistent lack of precipitation (dry weather) allows easy and fast harvesting of cereals during the summer and hence eventual release of large amounts of *Alternaria* spores to the atmosphere (Skjøth et al., 2012). However, this phenomenon of high *Alternaria* spore concentrations during cereal harvesting coupled with good weather (e.g. no precipitation) may apply to mainly non-irrigated arable crops. In another study, Maya-Manzano et al. (2013) found that there was no relationship between the harvesting of irrigated maize and tomatoes and airborne *Alternaria* spore concentrations. This suggests that local emission of fungal spores strongly depends on the climate, weather and agricultural activities (Gyldenkærne et al., 2005; Sommer et al., 2006).

Studies on the effect of weather and agricultural production on *Alternaria* spore emission in inland and coastal areas have been documented and it is known that inland areas emit more spores than coastal areas (Corden et al., 2003; Rodríguez-Rajo et al., 2005). Leicester and Worcester are located in the East Midlands and West Midlands counties of the UK, respectively and both are inland urban/rural areas in the UK. However, Leicester recorded comparatively higher spore concentrations than Worcester during the episode. The difference in daily spore concentration between the two inland observation sites is possibly attributed to a strong local source of *Alternaria* spores near Leicester trap with high emission potential. Previously, Friesen et al. (2001) experimentally studied source strength in fungal spores in a field and found high fungal spore concentrations including *Alternaria* spores near the main source (combine harvesting) than far from the source. Quantifying the biological source strength of spores dispersed from a local or regional source remains a challenge since it is highly uncertain (Aylor, 1999).

In this study, the dispersal of *Alternaria* spores from their sources was simulated to estimate the spatial scale from the observation sites. The HYSPLIT particle dispersal simulation revealed that the highest concentration of particles was dispersed in the areas SW of Worcester and Leicester. It further showed that 35% and 31% of the particles were dispersed at a trajectory height below 500 m on their arrival at Worcester and Leicester traps, respectively. A previous study found a high concentration and diversity of pollen grains and fungal spores including *Alternaria* spores in the airstream sampled at an altitudinal range of 200-2000 m above sea level in Thessaloniki, Greece (Damialis et al., 2017). Furthermore, de Weger et al. (2016) found that *Ambrosia* pollen grains were dispersed from distant sources located at the Pannonian plain and in the Rhone valley. They were carried by the air masses to higher altitudes (above 1500 m) and were eventually deposited at Leicester and Leiden. Therefore, it was likely that the low altitude air masses passing over the local source areas of Worcester and Leicester could have mixed with the airborne *Alternaria* spores emitted from the cereal farms and eventually depositing them at the traps. However, there is a potential for high altitude air masses to contribute to the daily spores recorded Leicester and Worcester since *Alternaria* spores are constantly present in small amounts in the atmosphere (Bashan et al., 1991). Wind speed is a known weather parameter that can affect *Alternaria* spore dispersal and concentrations (Rotem, 1994). The lower wind speeds recorded at Leicester compared to Worcester could have contributed to the higher spore concentrations observed at Leicester than at Worcester, as was the case in previous studies (Sabariego et al., 2012; Stennett & Beggs, 2004). However, with very low wind speeds, spores need days to travel over large distances. Such spores may arrive at any time of the day. In our study, the typical harvesting time matches the typical peak in spore concentrations and the increase in spore concentration coincided with actual harvesting in the region. This suggests that most *Alternaria* spores that were detected at Worcester and

Leicester were dispersed from local sources with a potential minor contribution from remote sources and that wind speed is an important parameter in the spore dispersal process.

5.0 Conclusion

Alternaria spore concentrations of clinical importance were observed at Leicester and Worcester throughout the study period. However, Leicester recorded a higher spore concentration than Worcester did. The study found that the local sources (oilseed rape and winter barley) located outside the urban boundaries of Leicester and Worcester likely contributed to the high daily *Alternaria* spore concentration in the urban areas. Back-trajectory calculations during the episode showed that the air masses originated from the Atlantic Ocean and Ireland and arrived at Leicester and Worcester. Long distance transport probably had a small but equal contribution to the observations at both sites. The conclusion is therefore that the substantially higher concentrations of *Alternaria* spores observed at Leicester are caused by specific local sources with high emission potential: winter barley and oilseed rape. Such local sources of spores are important aspects to consider in understanding aeroallergens and dispersal of plant pathogens.

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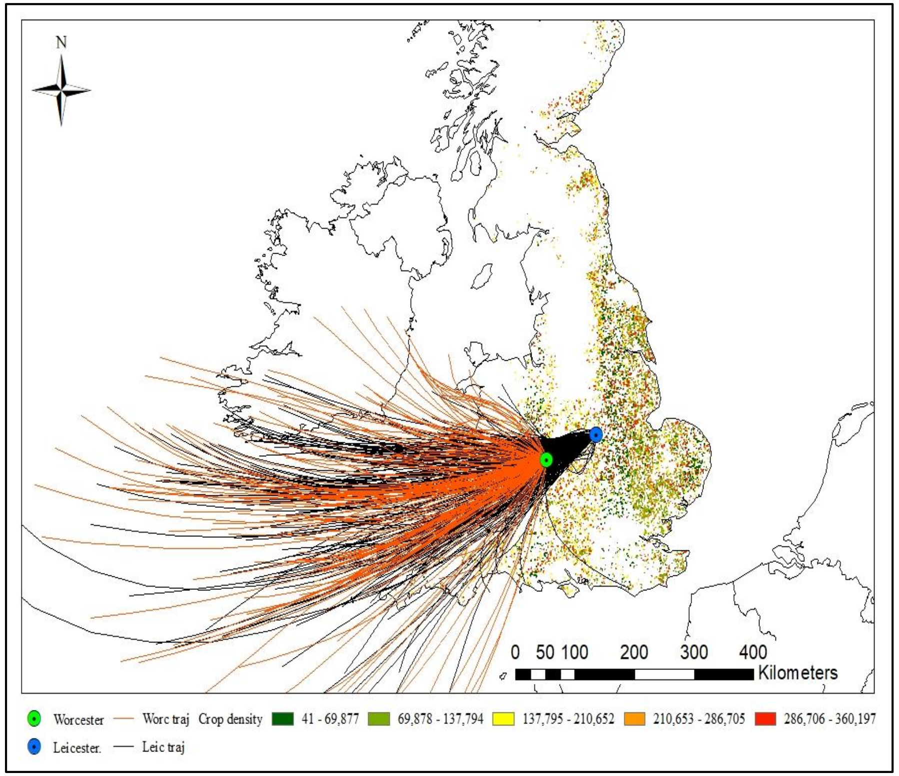
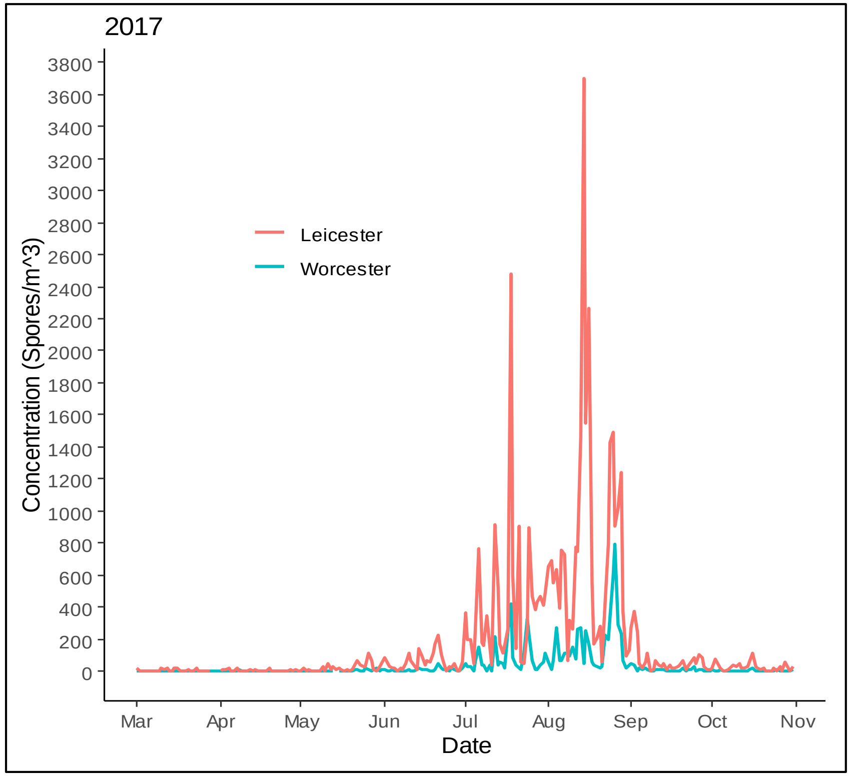
Highlights

- Cereals and oilseed rape are potential hosts of *Alternaria* fungus.
- Leicester had a higher density of the crops grown in its local area than Worcester.
- Harvesting of the crops coincided with higher *Alternaria* spore counts at Leicester.


N
0 50 100 200 300 400
Kilometers
Worcester
Worc traj
Crop density
41 - 69,877
69,878 - 137,794
137,795 - 210,652
210,653 - 286,705
286,706 - 360,197
Leicester.
Leic traj


2017
Leicester
Worcester
Concentration (Spores/m^3)
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Date

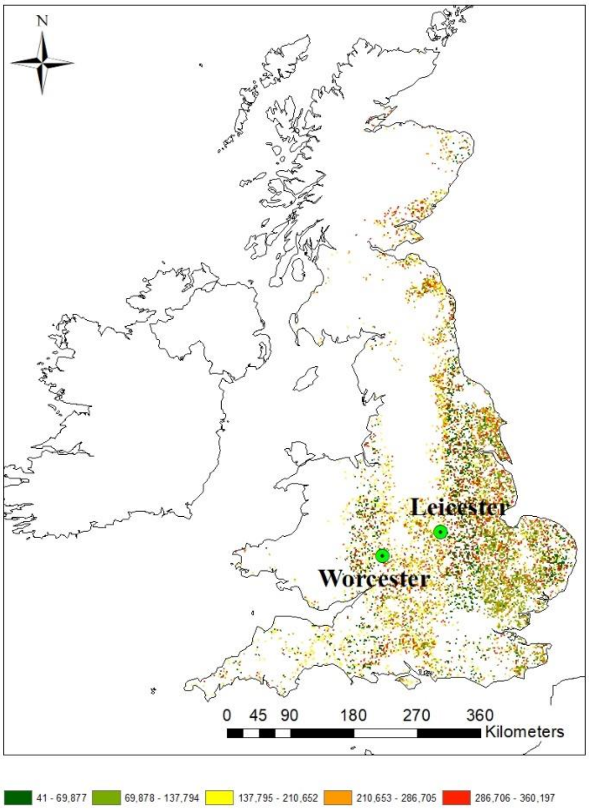


Fig. 1 Sampling locations and crop density map for Great Britain 2017. Range of figures indicate the amount of crops (Ha).

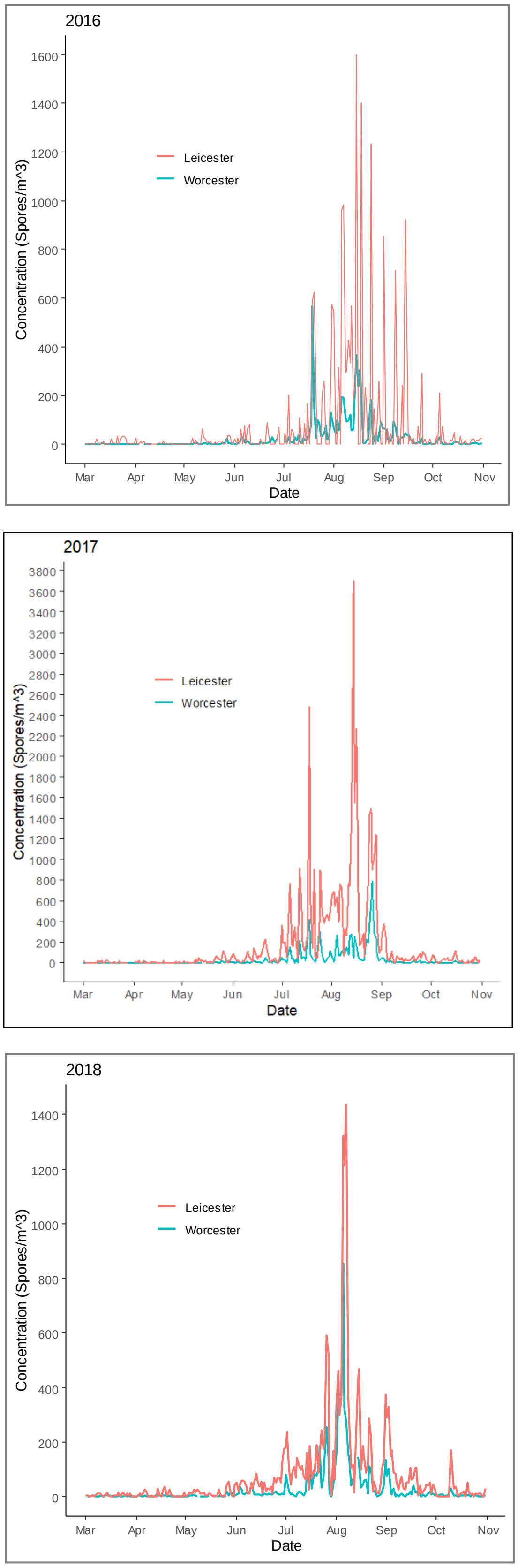


Fig. 2 Daily *Alternaria* spore concentration during the three years of observation at Worcester and Leicester.

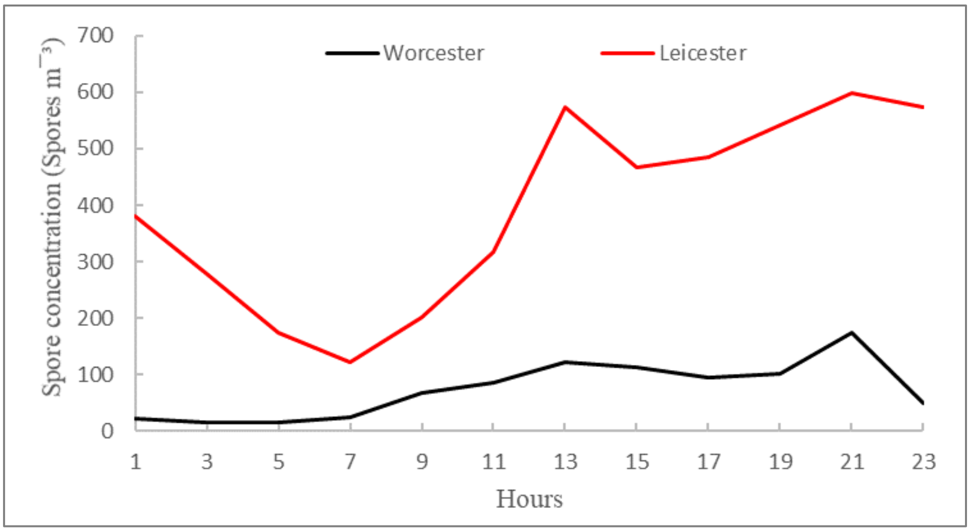


Fig. 3 Mean bi-hourly *Alternaria* spore distribution at Worcester and Leicester during the episode: 27 July-7 Aug 2017.

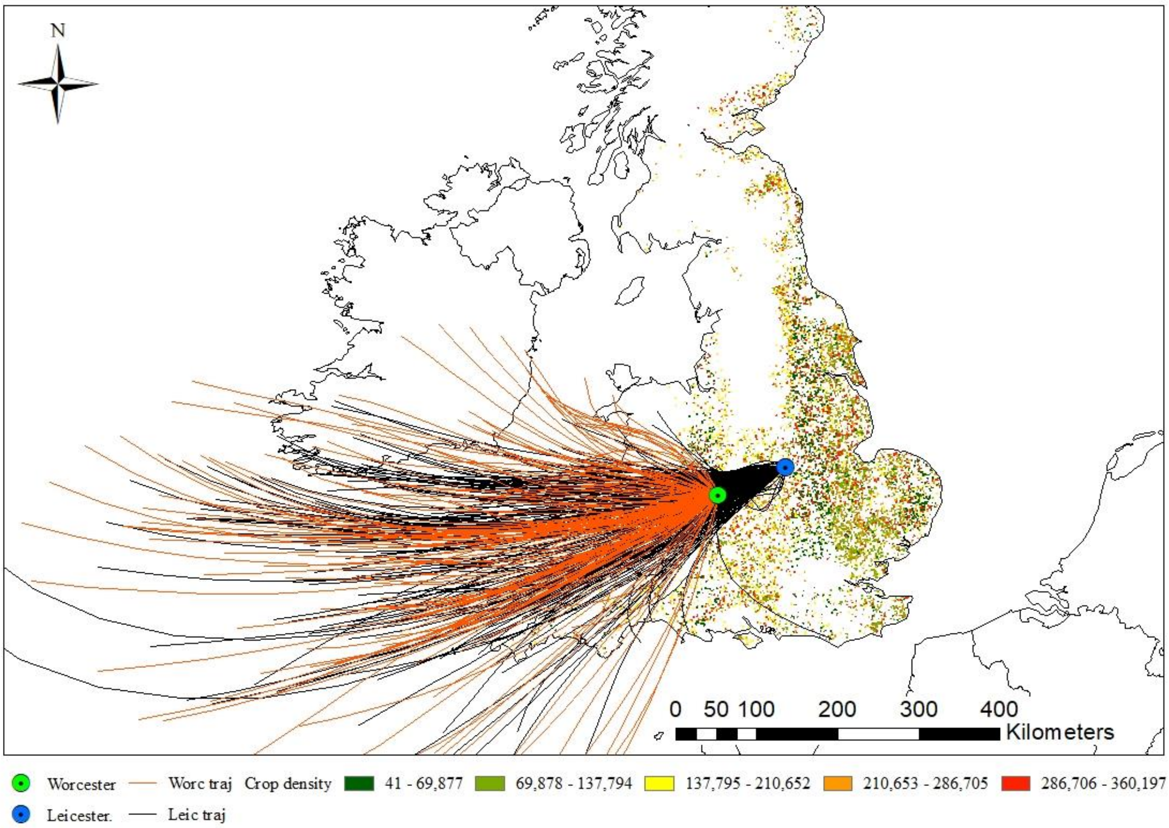


Fig. 4 Back-trajectories of air masses during the episode: 27 July-7 August 2017 that passed within 30 km radius of cropland in Worcester and Leicester. Red and black back-trajectories indicate air masses arriving at Worcester and Leicester, respectively. Range of figures indicate amount of crops (Ha)

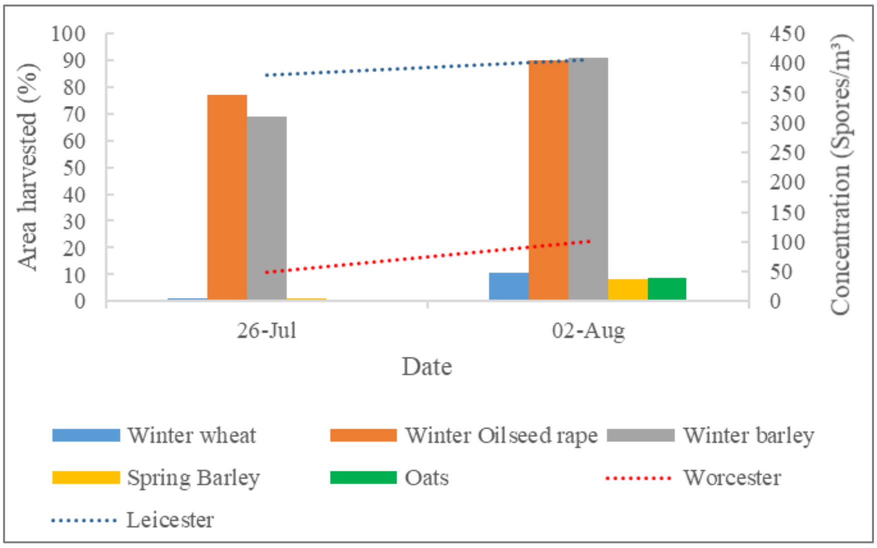
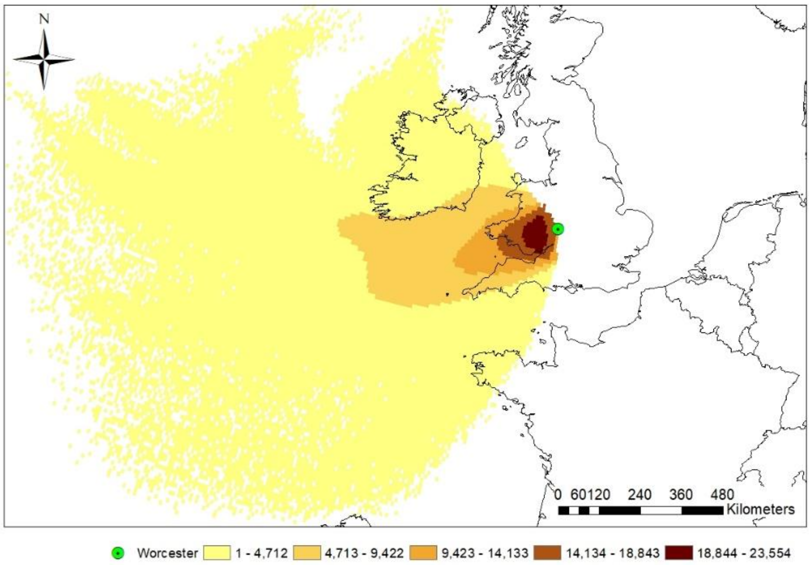
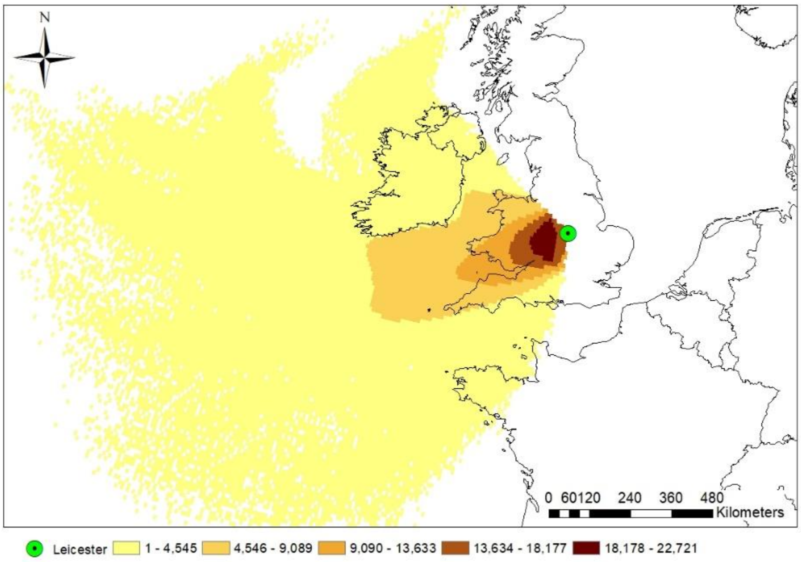


Fig. 5 Effect of harvesting of crops on airborne *Alternaria* spore concentrations. Cumulative percentage of crop harvest for the period starting 26 Jul and 2 Aug 2017 and corresponding mean *Alternaria* spore concentrations for the period 27 Jul-01 Aug and 02-07 Aug 2017 observed at Worcester (red dots) and Leicester (blue dots).

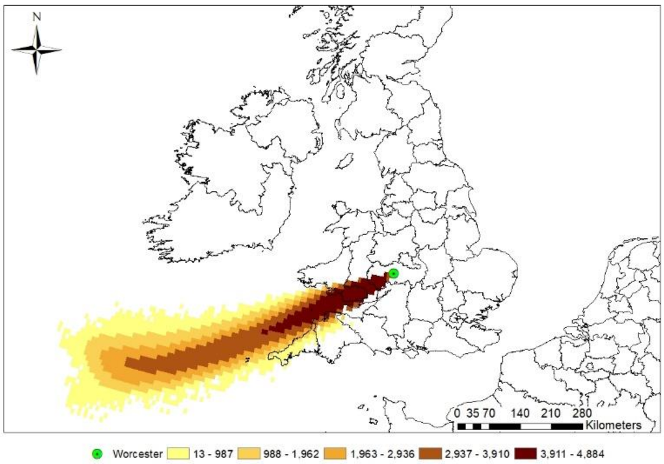


(6a)

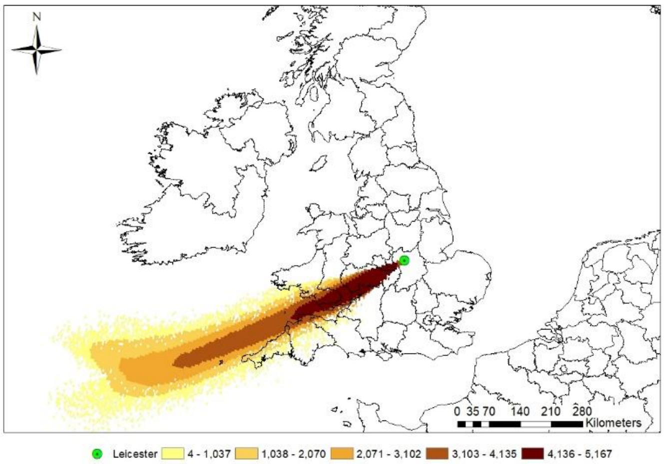


(6b)

Fig. 6 Density map for particle dispersion and deposition during the episode: 27 Jul-07 Aug 2017 at (a) Worcester and (b) Leicester at altitude 500 m agl.



(7a)



(7b)

Fig. 7 Density map of particle dispersion and deposition at (a) Worcester and (b) Leicester on 1st August 2017.

Table 1. Annual, seasonal and daily summary of *Alternaria* spore data recorded at Worcester and Leicester for the period 2016-2018.

Sampling year	**2016**		**2017**		**2018**	
Sampling location	**Worcester**	**Leicester**	**Worcester**	**Leicester**	**Worcester**	**Leicester**
Season start	5th June	NA	10th June	1st June	24th May	7th May
Season end	24th Sep	NA	24th Sep	24th Sep	28th Sep	10th Oct
Season duration (days)	110	NA	107	116	126	157
Maximum daily spore[a]	568	1,599	796	3,700	854	1,438
No. of high days[b]	17	37	23	67	21	47
Seasonal Spore Integral[c]	6,048	NA	8,194	40,986	6,803	18,126
Annual Spore Integral[c]	6,338	NA	8,588	43,038	7,157	18,919

NA-No Adequate data. [a](Spores/m^3). [b]days >100 spores m^{-3}. [c](Spore * day m^{-3}).

Table 2. Daily mean *Alternaria* spore concentration during the Episode (27 Jul-7 Aug 2017) at both Worcester and Leicester

Date	**Worcester**	**Leicester**
27/07/2017	12	185
28/07/2017	10	176
29/07/2017	38	413
30/07/2017	58	338
31/07/2017	114	504
01/08/2017	59	659
02/08/2017	14	542
03/08/2017	68	474
04/08/2017	273	343
05/08/2017	63	254
06/08/2017	66	374
07/08/2017	114	451
Sum	**889**	**4713**
Average	**74**	**393**

Table 3. Crop density (ha) within 30 km radius in 2017

Crop	**Worcester**	**Leicester**
Beet	-	65
Field beans	4,512	6,692
Oilseed rape	11,494	23,514
Other crops	14,523	10,133
Potatoes	2,046	1,127
Total-non cereals	**32,575**	**41,532**
Maize	6,261	5,659
Spring barley	5,962	4,806
Spring Wheat	3,208	6,942
Winter barley	9,330	12,955
Winter wheat and Oat	29,403	42,949
Total-Cereals	**54,164**	**73,312**
Grand total	**86,739**	**114,844**

"-" No crop

Table 4. Particles dispersed and deposited at Worcester and Leicester during the episode: 27 Jul-7 Aug 2017 when air masses passed through a 30 km radius from Worcester to Leicester

Worcester	**Particles**	**Per cent**
England and Wales	8,577	30
Ireland	6,127	21
Atlantic Ocean	14,245	49
Total particle	**28,949**	
Leicester		
England and Wales	11,972	40
Ireland	6,536	22
Atlantic Ocean	11,242	38
Total particle	**29,750**	

Table 5. Height of air masses arriving at Worcester and Leicester on 1 Aug 2017 after passing through local source areas where particle clouds were dispersed and percentage of particles at each trajectory height.

Worcester		**Leicester**	
Trajectory height	% in trajectory height	Trajectory height	% in trajectory height
>500	65	>500	69
<500	35	<500	31

Supplementary Table 1a. Summary of weather parameters at Worcester during the episode: 27 Jul-07 Aug 2017

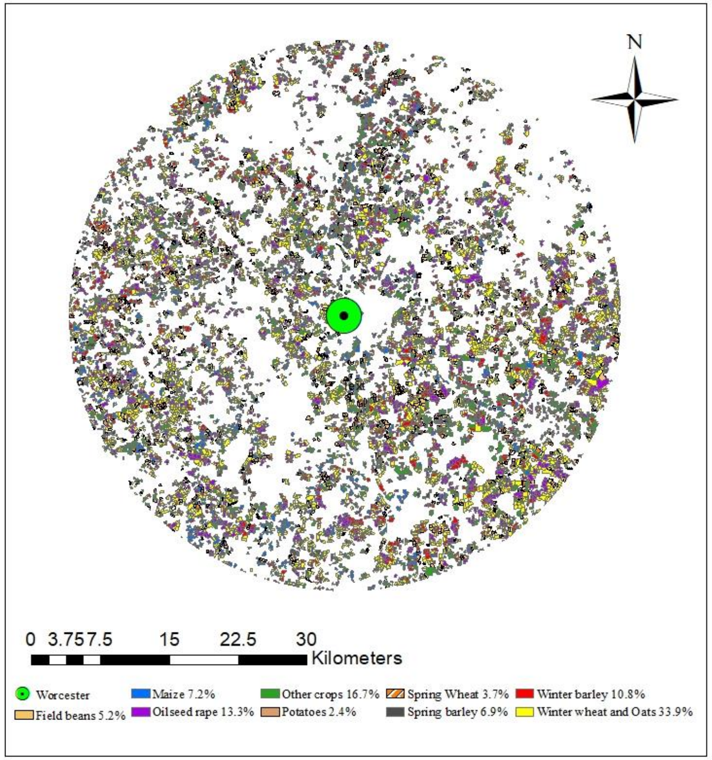
Date	Temp	Rain	WS	WD	Pressure
27/07/2017	16	0.05	3.0	170	1,001
28/07/2017	16	0.65	3.9	263	1,001
29/07/2017	16	1.00	2.6	304	1,002
30/07/2017	16	0.75	3.4	289	1,000
31/07/2017	16	0.20	3.2	251	1,005
01/08/2017	16	0.20	2.5	243	1,009
02/08/2017	17	1.60	3.4	143	1,004
03/08/2017	17	0.30	4.4	303	1,000
04/08/2017	17	0.05	3.3	271	1,006
05/08/2017	15	0.85	1.9	273	1,013
06/08/2017	15	0.00	3.5	266	1,016
07/08/2017	17	0.05	2.5	284	1,012

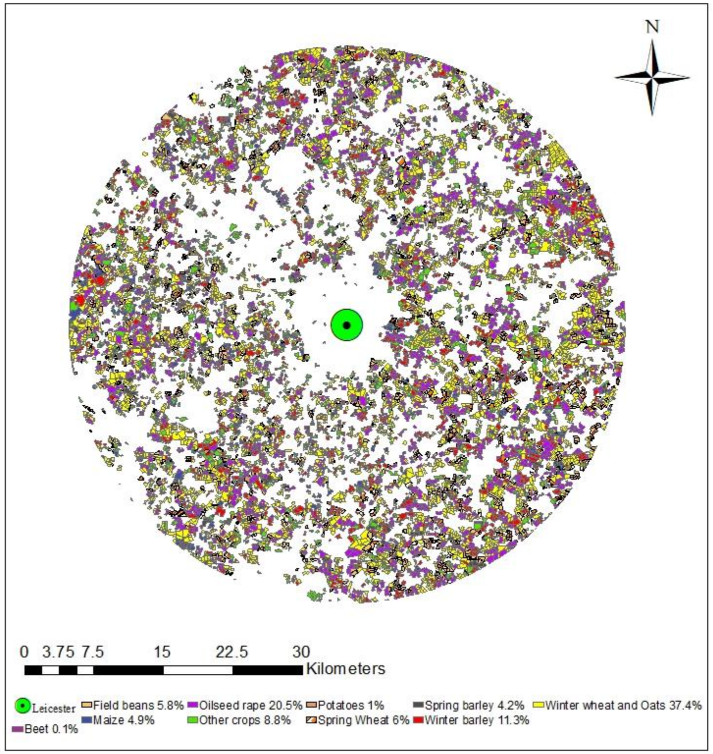
Supplementary Table 1b. Summary of weather parameters at Leicester during the episode: 27 Jul-07 Aug 2017

Date	Temp	Rain	WS	WD
27/07/2017	15	0.00	1.3	241
28/07/2017	15	0.00	1.8	81
29/07/2017	16	0.00	1.4	196
30/07/2017	15	0.00	1.4	196
31/07/2017	16	0.00	1.6	115
01/08/2017	16	0.00	1.5	150
02/08/2017	16	0.00	1.9	84
03/08/2017	17	0.00	2.1	193
04/08/2017	16	0.00	1.8	274
05/08/2017	13	0.00	1.1	298
06/08/2017	14	0.00	1.2	188
07/08/2017	16	0.00	1.1	211

Supplementary Table 2: Mean bi-hourly *Alternaria* spore concentration during the Episode (27 Jul-7 Aug 2017)

					Worcester							
Date/Time	**01:00**	**03:00**	**05:00**	**07:00**	**09:00**	**11:00**	**13:00**	**15:00**	**17:00**	**19:00**	**21:00**	**23:00**
27-Jul	18	6	31	6	122	0	0	6	0	0	0	6
28-Jul	0	0	6	0	24	6	31	0	43	0	0	0
29-Jul	0	0	6	12	55	104	147	73	18	24	0	0
30-Jul	6	0	0	24	55	61	177	184	80	73	49	0
31-Jul	0	0	6	12	141	208	122	294	110	86	220	67
01-Aug	31	24	0	67	37	86	122	122	67	18	92	18
02-Aug	61	18	37	24	80	12	12	31	12	6	0	0
03-Aug	0	0	0	18	24	147	269	184	86	61	0	12
04-Aug	18	6	0	12	116	122	233	263	288	269	1,340	288
05-Aug	49	104	92	110	80	165	147	55	92	43	43	80
06-Aug	31	6	12	6	37	24	135	61	159	171	110	37
07-Aug	43	6	0	6	31	86	73	86	190	459	251	73
Mean	**21**	**14**	**16**	**25**	**67**	**85**	**122**	**113**	**95**	**101**	**175**	**48**
					Leicester							
27-Jul	130	65	65	22	54	151	281	281	356	119	497	205
28-Jul	119	76	32	22	11	184	227	367	464	464	140	0
29-Jul	28	124	359	621	1,283	897	1,435	207	0	0	0	0
30-Jul	14	14	0	0	0	0	179	262	1,035	994	1,187	373
31-Jul	290	28	55	55	28	414	662	317	511	718	1,256	1,711
01-Aug	1,090	580	276	83	69	345	359	524	386	607	1,311	2,277
02-Aug	1,766	759	193	193	83	469	1,835	800	166	207	14	14
03-Aug	0	97	55	138	690	787	800	925	897	759	428	110
04-Aug	69	28	28	28	41	124	497	800	607	925	580	386
05-Aug	331	759	856	235	97	41	207	124	55	110	69	166
06-Aug	54	108	86	11	43	324	248	616	637	853	659	842
07-Aug	676	718	69	41	28	69	152	373	704	745	1,049	787
Mean	**381**	**279**	**173**	**121**	**202**	**317**	**574**	**466**	**485**	**542**	**599**	**573**





Supplementary Fig. 1 Worcester crop map 2017 within 30 km radius. Supplementary Fig 2. Leicester crop map 2017 within 30 km radius.