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Crop harvest in Central Europe causes episodes of high airborne *Alternaria* spore concentrations in Copenhagen

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Abstract

This study tests the hypothesis that Danish agricultural areas are the main source to airborne *Alternaria* spores in Copenhagen, Denmark. We suggest that the source to the overall load is mainly local, but with intermittent Long Distance Transport (LDT) from more remote agricultural areas. This hypothesis is supported by investigating a 10 yr bi-hourly record of *Alternaira* spores in the air from Copenhagen. This record shows 232 clinically relevant episodes with a distinct daily profile. The data analysis also revealed potential LDT episodes almost every year. A source map and analysis of atmospheric transport suggest that LDT always originates from the main agricultural areas in Central Europe. A dedicated emission study in cereal crops under harvest during 2010 also supports our hypothesis. The emission study showed that although the fields had been treated against fungal infections, harvesting still produced large amounts of airborne fungal spores. It is likely that such harvesting periods can cause clinically relevant levels of fungal spores in the atmosphere. Our findings suggest that crop harvest in Central Europe causes episodes of high airborne *Alternaria* spore concentrations in Copenhagen as well as other urban areas in this region. It is likely that such episodes could be simulated using atmospheric transport models.

1 Introduction

The importance of understanding the spatial and temporal distribution of fungal spores has recently been highlighted by Lang-Yona et al. (2012) by presenting seasonal variations of airborne fungal spore concentrations in 2009 at a site in Israel, based on qPCR analysis (Lang-Yona et al., 2012). Similarly, studies from the same group in Israel suggest that fungal spore concentrations peak during spring and autumn (Burshtein et al., 2011). The authors discuss whether these peaks could be related to spring blooms and autumn decomposition of the vegetation.

Fungal spore concentrations can also be obtained using volumetric spore traps of the Hirst design (Hirst, 1952). The advantage of the Hirst trap is that it provides a daily or bi-hourly record of fungal spore concentrations, that may be used to construct actual calendars of bioaerosols (e.g., Ceter et al., 2012; Melgar et al., 2012; Skjøth and Sommer, 2012). The disadvantage of the Hirst trap is that it typically only provides observations of fungal spores at the genus level (e.g., Ceter et al., 2012; Skjøth and Sommer, 2012), whereas qPCR can quantify fungal spores at the species level. This disadvantage is outweighed by the long time series with high temporal resolution, often covering several years with bi-hourly records (Oliveira et al., 2009; Stepalska et al., 1999; Stepalska and Wolek, 2009) or even up to 10 yr or more, (Aira et al., 2008; Hjelmroos, 1993; Skjøth and Sommer, 2012). Despite these advantages, data of fungal spores from Hirst traps are rare compared to pollen data. This is even more pronounced when the numbers of studies on data from Hirst traps are compared to studies on atmospheric trace gasses such as ozone. This lack of scientific attention has been recognized for a number of years, e.g., by an Editorial in the Lancet in (2008) and by the recommendation in Allergy by Cecchi et al. (2010) as they both suggested further studies in aerobiology. Studies on bio-aerosols such airborne fungal spores are therefore highly needed.

The first long term study on fungal spores in the air of Copenhagen by Skjøth and Sommer (2012) have shown that the genus *Cladosporium* and *Alternaria* both have their peak concentrations during summer, but that *Cladosporium* has a much longer season. This suggests that the source to these two important genera of fungal spores can be different.

Observations from Hirst traps have the last 5–10 yr improved knowledge of aeroallergens concerning the temporal distribution and possible source locations to aeroallergens. These studies include source-receptor studies on pollen from *Fagus* (Belmonte et al., 2008), *Betula* (Mahura et al., 2007; Skjøth et al., 2007; Veriankaite et al., 2010), Poaceae (Smith et al., 2005), Olea (Hernandez-Ceballos et al., 2011b), *Ambrosia artemisiifolia* (Cecchi et al., 2007; Fernández-Llamazares et al., 2012; Sikoparija et al., 2009) and Quercus (Hernandez-Ceballos et al., 2011a), respectively. This suggests

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that similar analysis on fungal spore observations (e.g., *Alternaria*) from the Hirst trap also can identify sources to the most important genera of fungal spores.

Fungal spore that are among the most often observed genera are *Cladosporium* and *Alternaria* (Larsen, 1981). The genus *Alternaria* includes numerous plant pathogens (Gravesen et al., 1994) and *Alternaria* spores are considered an important part of the total fungal spectrum in e.g., potato crops (e.g., Escuredo et al., 2011; Iglesias et al., 2007). *Alternaria* spores can also threaten human health (Damato and Spieksma, 1995) and cause allergic symptoms in sensitized individuals when the atmospheric concentrations are high (Gravesen, 1979). The sources of airborne *Alternaria* spores are considered to be mainly vegetation such as forest and agricultural land (e.g., Stepalska et al., 1999), during the drying and decomposition of above-ground plant tissues (Burshtein et al., 2011; Escuredo et al., 2011; Iglesias et al., 2007). The studies by Skjøth and Sommer (2012) showed that in Denmark the *Alternaria* season ends in the middle of September, which is about a month before leaf fall in the forests. Similar studies from Poland showed that the peak of the *Alternaria* spore season is found in July–August, while October–November has a relatively small load of *Alternaria* spores (Stepalska et al., 1999). This suggests that decomposition of tree leaves does not contribute to the overall *Alternaria* load in Northern and Central Europe. In the UK, agricultural areas near Cardiff and Derby have been suggested as a potential source to high *Alternaria* concentrations (Corden et al., 2003), and studies from Northern Portugal and Poland have shown that rural areas have a higher load of *Alternaria* than nearby urban areas (Oliveira et al., 2009). Wheat harvesting has previously been shown to release large numbers of spores into the air (Friesen et al., 2001), exposing harvesters to large amounts of viable fungi (Hill et al., 1984). Vegetation in agricultural areas is therefore a likely main source of *Alternaria* spores in many parts of Europe. Studies from the USA have shown that spores from agricultural areas that are infected with Soybean rust (*Phakopsora meibomiae* and *P. pachyrhizi*) has the potential to be transported more than 1000 km under favourable weather conditions (Isard et al., 2007; Isard et al., 2005). European studies on other aeroallergens have shown that the overall

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load in a region is typically due to local sources with intermittent long distance transport from remote regions (Skjøth et al., 2009; Smith et al., 2008). It is therefore likely, that *Alternaria* spores have a similar potential for atmospheric transport as other aeroallergens. This suggests that the temporal and spatial variation of *Alternaria* spores is mainly dependent on the proximity of local sources and only secondarily on Long Distance Transport (LDT) from areas with a high load of *Alternaria*.

In this study we hypothesize that Danish agricultural areas are the main source of airborne *Alternaria* spores in Denmark and that the source to the overall load is mainly local but with intermittent LDT from non-Danish areas with both a high density of agricultural areas and a potentially high load of *Alternaria* due to harvest. We have adapted a protocol that has been used in several similar studies in several European studies on allergenic pollen since 2007 (Hernandez-Ceballos et al., 2011b; Sikoparija et al., 2009; Stach et al., 2007). Here we investigate our hypothesis by analysing a 10 yr record of bi-hourly *Alternaria* spore observations from Copenhagen with respect to seasonality, overall daily pattern and potential source areas to LDT, combined with a dedicated field study on potential emission sources in agricultural areas.

2 Methodology

2.1 Spore trap data and analysis of episodes

Measurements from Copenhagen obtained within the Danish pollen and spore program (Skjøth and Sommer, 2012; Sommer and Rasmussen, 2009) have been analysed for 2001–2010 with respect to *Alternaria* spores. The near surroundings to the trap in Copenhagen are urban, while nearby areas within a distance of ≥ 30 km are mainly agricultural in both Southern Sweden and Denmark as described by Skjøth et al. (2008). Summaries of the data for the 10 yr are organised in two tables in a similar way as Kasperzyk et al. (2011) with respect to the annual spore index, which by convention in aerobiology is dimensionless (Mandroli et al., 1998), peak day and start of season (Table 1). The *Alternaria* seasons were defined using the 95 % method (Goldberg et al., 1988), as this is the standard analytical method in the Danish pollen and spore program (Skjøth and Sommer, 2012; Sommer and Rasmussen, 2009). The daily average concentration of 100 *Alternaria* spores m^{-3} has been reported as a clinical threshold for allergic symptoms (Gravesen, 1979; Ricci et al., 1995). Therefore, days with daily average concentration above 100 spores m^{-3} were investigated for the mean diurnal variation (Fig. 1). *Alternaria* episodes (> 100 spores m^{-3}) that showed diurnal patterns that were markedly different from the mean daily cycle (Table 2) were investigated further using back trajectory analysis, in a similar way as in related studies on *Betula* (Skjøth et al., 2009), *Quercus* (Hernandez-Ceballos et al., 2011a), *Olea* (Fernández-Rodríguez et al., 2012; Hernandez-Ceballos et al., 2011b) and *Ambrosia artemisiifolia* (Fernández-Llamazares et al., 2012; Kasprzyk et al., 2011; Sikoparija et al., 2009), respectively.

2.2 Field observations and emission flux estimates

Measurements of emission fluxes of *Alternaria* spores during harvest were taken at four locations around Tune, Roskilde, Denmark. Samples were obtained from wheat and barley fields between 18 August 2011 and 16 September 2011. The measurements were taken as grab samples from the exhaust air stream of the harvesting machine. The grab samples were allowed to immediately sediment onto glass slides, for later microscopic counting of spores. Visual inspection of the fields revealed that none of the crops displayed signs of fungal infection. The fields were treated with fungicide and the farmer informed that he had used a recommended application scheme of chemical fungicides and therefore considered the crops to be free of fungal diseases, including *Alternaria*.

The harvester was a CASE-IH Agriculture model 7120 Axialflow combine with a type 3050 cutter table (width 915 cm). During sampling of emission fluxes, the harvester advanced at ca. 4 km h^{-1}, with its air throughput being set to ca. 950 (unitless machine

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value, corresponding to ca. 570 $m^3 min^{-1}$). The straw shredder was running on two sampling dates, on the two others it was turned off.

Samples of emissions were obtained by manually directing the harvester's exhaust air stream through a 155 cm long piece of ventilation pipe (polished steel; inner diameter, 20 cm) and abruptly closing the input end with a padded nylon-covered lid. Immediately after closure, the pipe was positioned upright, with the lid on the upper end. The bottom end was maintained open for 10 s to allow coarse particles to escape. Then the bottom was sealed using a standard ventilation pipe stopper (polished steel, with a rubber seal around the edge). The stopper had a glass slide centred on the flat inner side. The effective sedimentation distance, from the surface of the padded lid to the surface of the glass slide, was 155.5 cm. Samples of emissions were produced by allowing particles to sediment onto the glass slide from the air column inside the pipe for 9 min. After sedimentation, the slide was removed and archived for later microscopic analysis. In several cases, residual control samples were taken by continuing the sedimentation for another 9 min on a fresh glass slide and maintaining the pipe firmly in an upright position. Negative control samples included environmental air from the middle and from the upwind end of each field. Prior to each sampling, the inside of the pipe was cleaned with a stream of clean air.

The glass slides for emission flux sampling were identical to those slides that are used in the Danish pollen and spore program (Skjøth and Sommer, 2012; Sommer and Rasmussen, 2009). The surfaces of the slides were inspected for *Alternaria* spores by using the spore counting method for *Alternaria* in the Danish pollen and spore program (Skjøth and Sommer, 2012). The microscopic counts were then converted to spores per volume of air in the exhaust air of the harvesting machine by using the sedimentation distance inside the pipe for the third dimension. The spore concentrations were converted to estimates of *Alternaria* spores per ha harvested field by using the width of the cutting table and driving speed of the harvesting machine and assuming that the grab sample was representative for the exhaust air stream of the harvesting machine.

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Microscopic counts and calculated fungal spore densities for each sample, along with estimated emission factors for the fields, are presented in Table S1 (Supplement).

2.3 Model calculations and potential source map

Agricultural areas under rotation and with mechanical harvesting methods have been identified in the CLC2000 dataset to consist of following three land cover types: non-irrigated arable land (code 211), permanently irrigated land (code 212) and pastures (code 231), respectively. The land cover data have been extracted for Central and Northern Europe (Fig. 2) and gridded to a tenth of the EMEP50 grid (http://www.emep.int/grid/griddescr.html) using a similar methodology as Skjøth et al. (2010) and Fernández-Rodríguez (2012). The EMEP grid is commonly used for inventories in European air quality studies including the use of the chemistry transport models EMEP (Fagerli and Aas, 2008; Simpson et al., 2012), the EMEP4UK (Vieno et al., 2010) and DEHM (Brandt et al., 2012; Skjøth et al., 2011). This procedure allows for easy comparison of density of relevant emission areas throughout the region and analysis in relation to atmospheric transport (e.g., Fernández-Rodríguez et al., 2012).

Back trajectories were computed using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model (Draxler et al., 2007). Trajectories were calculated using the GDAS (Global Data Analysis System) meteorological files maintained by ARL, with a temporal resolution of 3 h and a spatial resolution of $1^{\circ} \times 1^{\circ}$. Air mass trajectories were calculated at Copenhagen during the identified episodes with a receiving height of 500 m. Air mass trajectories were plotted 48 h back in time with 2 h steps, corresponding to the time step of the fungal spore observations, following the method described by Stach et al. (2007) and later used by Skjøth et al. (2008, 2009), Smith et al. (2008), Sikoparija et al. (2009), Hernandez et al. (2011a, b), by using either the ACDEP (Skjøth et al., 2002) in the matrix style or the HYSPLIT (Draxler et al., 2007) models. Measured precipitation from weather and climate stations have been used as an estimate for potential *Alternaria* spore release due to harvest in the potential source

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regions (Fig. 2 and Table S2, Supplement) by assuming that dry weather and dry fields are required for intense harvesting.

3 Results

3.1 Seasonal and daily variations of *Alternaria* in Copenhagen

The annual spore index of *Alternaria* in Copenhagen varied with more than a factor of two in the spore index from 4488 in 2003 to 10781 in 2006 (Table 1). The mean of the season start was day number 182, while the mean peak day during the season was number was 218, with a standard deviation of 7 and 13 days, respectively. The highest daily concentration was 1016 spores m^{-3} on the 17 August 2001 (the second largest was 853 spores m^{-3} on the following day, not shown). The highest observed bihourly concentration was 2727 spores m^{-3} (not shown). 232 days during the 10 yr period had peak concentrations above the clinical threshold of 100 spores m^{-3}. The contribution from individual years ranged from 17 days in 2003 and 2008 to 31 days in 2009. The analysis of bi-hourly *Alternaria* spore concentrations at peak days shows a typical daily pattern (Fig. 1) with peak concentrations in the late afternoon reaching 378 spores m^{-3} and a minimum at 112 spores m^{-3} early in the morning. 16 of the 232 peak days had a very different pattern compared to the typical daily pattern (Table 2). Except for the year 2002, each year had one or more of these 16 non-typical peak days. Trajectory calculations show, that all of the 16 non-typical peak days had air masses arriving from main agricultural areas in Southern Scania (Sweden), Denmark, Poland or Germany. The 3 most outstanding episodes with respect to both load and pattern are discussed in detail in Sect. 3.3 using trajectories and the source map (Figs. 3–5).

3.2 Alternaria emission sources in local agricultural fields

Analysis of the field data revealed between 10^6 and 10^7 *Alternaria* spores m^{-3} in the exhaust air of the harvesting combine (Table S1). These have been converted into an emission of between 1.2×10^{10} and 6.7×10^{10} *Alternaria* spores ha^{-1} during harvest. Residual control slides from a second sedimentation period gave 10–15 % of the initial slide. This suggests, that the sedimentation efficiency in the pipe was 85–90 % for *Alternaria* spores when using 9 min sedimentation time. Negative controls always counted at zero (data not shown).

3.3 Trajectory calculations, potential source map and long distance transport

The inventory of potential sources to *Alternaria* spores in Central and Northern Europe reflects the density of managed agricultural areas that are under rotation. The inventory shows that the potential sources to *Alternaria* spores are found in many parts of the studied area. The highest densities (70–100 %) are found in Western Denmark, Central and Northern Germany, Southern Scania (Sweden) and Central Poland. Much lower densities (0–20 %) are found in most of Southern Sweden, Southern Germany, along the border between Germany and Poland, the southern parts of Poland and the Baltic countries.

In Sects. 3.3.1–3, the 3 most outstanding episodes with respect to both load and pattern are discussed in detail, using trajectories and the source maps and the overall weather pattern including measured accumulated precipitation in the potential source region.

3.3.1 Episode 1: 30–31 August 2008

Daily average *Alternaria* spore concentrations observed on the 30 and 31 August 2008 in Copenhagen were 161 and 313 spores m^{-3}, respectively. Hourly *Alternaria* spore concentrations was low in the beginning of the period and increased quickly to above 700 spores m^{-3} late in the evening of the 30 and remained at a level of around 600 spores m^{-3} until mid-day the 31 (Fig. 3a). From mid-day the 31 and until late in the evening, the concentrations gradually decreased to below 100 spores m^{-3}. The weather in the study region had during the study period a high pressure ridge extending

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from Iceland (1029 hPa) over Scandinavia (∼ 1020 hPa) to Northern Germany and Central Poland (1022–1023 hPa), which in the beginning of the period pushed air masses from the North towards Copenhagen. Around mid-day of the 30 wind speeds decreased and the air masses remained for a number of hours over Denmark and Scania before arriving in Copenhagen. Similar situation with low wind speeds were also present the 31 (Fig. 3b and c). A few mm of precipitation were recorded on the 29 in the entire region, and the most eastern parts of Denmark and Sweden also recorded precipitation on the 28 and 27 (Table S2). This suggests harvesting possibilities in Denmark and Sweden starting the 30 and good harvesting possibilities on the 31 August.

3.3.2 Episode 2: 10–11 August 2010

Daily average *Alternaria* spore concentrations observed on the 10 and 11 August 2010 in Copenhagen were 153 and 130 spores m^{-3}, respectively. Hourly *Alternaria* spore concentrations had a number of peaks (200–400 spores m^{-3}) from the beginning of the period until mid day the 11 August (Fig. 4a). After that the concentrations remained low. The weather in the study region during the study period was dominated by a high pressure system (1018–1019 hPa) over Central Germany and Poland, which during most of the period pushed air masses from the South and South-West towards Copenhagen, passing either Danish or German land areas including water areas (Fig. 4b and c). Heavy precipitation was recorded over eastern parts of Denmark and Scania on the 9 August (Table S2). Eastern, westerns and southern parts of Denmark recorded almost no precipitation from the 5 to the 11 August 2010 suggesting generally good harvesting possibilities in most of Denmark and Northern Germany until at least the 10 and partly the 11 August.

3.3.3 Episode 3: 15–16 August 2010

Daily average *Alternaria* spore concentrations observed on the 15 and 16 August 2010 in Copenhagen were 262 and 424 spores m^{-3}, respectively. Hourly *Alternaria* spore

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concentrations were low until around mid day of the 15 and then had two distinct peaks exceeding 1000 and 1400 spores m^{-3} late in the evening the 15 and during early morning the 16 (Fig. 5a). Hereafter concentrations remained high at between 200 and 400 spores m^{-3} until late in the evening the 16, when concentrations dropped to near zero. The weather in the study region had during the study period a high pressure area (~1010–1021 hPa) covering most of Poland, the Baltic countries, Russia and reaching down to the Balkan region. At the same time minor low pressure centres (1006–1013 hPa) were located over Southern Sweden and Germany. This caused in the beginning of the period that air masses from the East and the Baltic states were pushed towards Denmark, arriving at Copenhagen from the North West passing northern over parts of Scania. Around midday of the 15, winds veered to the South so that the air masses originated from either Germany or Poland. These air masses had passed the Baltic Sea and arrived directly to Copenhagen from the Sea or by passing the southern parts of Scania in Sweden. Heavy precipitation was recorded over Denmark and Scania the 13 and 15 (Table S2, Supplement). Medium precipitation was recorded at Wielkopolski the 13–15 August, while the remaining 6 Polish stations recorded limited or no precipitation. This suggests that during the episode there were limited harvesting possibilities in Denmark/Sweden and good harvesting possibilities in Poland.

4 Discussion

The measured airborne concentrations of *Alternaria* spores in Copenhagen show that the majority of the 232 peak days have a strong diurnal pattern with a maximum in the late afternoon and a minimum during night or early morning (Fig. 1). If the main source to *Alternaria* spores were remote sources, then this daily pattern could have been either non-existent or peaking at any time of the day, as the big plumes of LDT of aeroallergens can arrive in Copenhagen at any time of the day or night (Mahura et al., 2007; Skjøth et al., 2007, 2008). In fact only a small fraction of the peak days has a diurnal pattern that deviates from the overall pattern (Table 2). The potential

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source map (Fig. 2) shows that Denmark is dominated by land cover types that can be a strong source to *Alternaria* spores. Additionally, the emission flux study from a typical land cover with agricultural production in rotation show that emission during harvest releases a large amount of *Alternaria* spores, even though the fields have been treated with fungicides. Finally, then the small fraction of peak days with a different diurnal pattern than the overall pattern have been analysed with respect to air mass transport. In all cases, the air masses came from more remote areas that are also dominated by land cover types containing potential sources to *Alternaria* spores. Such episodes were identified almost every year during the study period (Table 2). Additionally, it was shown that even if a region such as Eastern Denmark and Southern Sweden had obtained very large amounts of rain making harvest very difficult, then more remote regions in e.g., Poland could have contributed with large amounts of *Alternaria* spores (Fig. 5). Overall these studies suggest that the daily load of *Alternaria* spores is dominated by local or near local sources with intermittent LDT from more remote sources in e.g., Germany and Poland and that these LDT episodes can happen almost every year.

A number of source receptor studies on aeroallergens have recently linked measured concentrations from the Hirst traps with both local sources and intermittent long distance transport from regions with high source densities. Common for all of these studies are is that they are concerned with pollen such as *Betula* (Mahura et al., 2007; Skjøth et al., 2007, 2008; Veriankaite et al., 2010), *Quercus* (Hernandez-Ceballos et al., 2011a) *Olea* (Fernández-Rodríguez et al., 2012; Hernandez-Ceballos et al., 2011b) and *Ambrosia artemisiifolia* (Fernández-Llamazares et al., 2012; Kasprzyk et al., 2011; Sikoparija et al., 2009). Our study suggests that the methodology used for allergenic pollen can be extended to fungal spores and that agricultural fields are a potential source to elevated *Alternaria* spore concentrations.

The *Alternaria* spore emissions we have measured during harvest may be considered average for state-of-the-art agricultural practice. Fungal disease was not observed in either wheat or barly fields that were harvested. Despite this, fungal spore emissions were recorded during harvest using grab samples from the exhaust of the harvesting

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machine. The grab samples were taken on four different days, from different fields containing barley and wheat. These emission flux measurements in the Danish fields gave surprisingly uniform results, about 5×10^{10} spores ha^{-1} during harvest. With this emission factor, a simple Eulerian box model calculation suggests that if 2 % of the entire surface area in a region is harvested, then the threshold of 100 spores m^{-3} would be exceeded in the harvested region. Here it is assumed that spores are kept airborne the entire day, that spores are well mixed in the atmosphere up to 1000 m, that all fungal spores are kept in the local region and that all harvested areas have an emission factor similar to the Danish areas that were treated with fungicides. Thus our emission factor could be related to the geographical location or could be a function of agricultural management, such as the use of machinery or application of fungicides. How the real distribution in the atmosphere will be in case the emission factor varies between fields, and when atmospheric transport and deposition is taken into account, is not known. However it is known that the concentrations in rural areas typically are larger than in nearby urban areas (Kasprzyk and Worek, 2006). This suggests the importance of atmospheric transport on local scale. Furthermore, the low variation in the flux samples suggests that the experimental method is robust for estimating emission fluxes; despite the crude sampling technique and that it does not require a large sample set. Surprisingly, fungal spore emissions were not increased after the rain period between 21 August and 10 September 2011, which one could have expected, as wet periods are known as periods of fungal growth. To the contrary, harvesting over moist soil after the rain period appeared to result in lower spore emissions. Similar observations as ours have been made for other agricultural fungal spores (Friesen et al., 2001) and for pollutants, such as ammonia. Here the local emission depends strongly on both climate as well as agricultural production methods (Gyldenkærne et al., 2005; Sommer et al., 2003, 2006). Our estimated emission of spores during harvest was of the same order of magnitude as in the study by Friesen et al. (2001), but using a much simpler approach. The simplicity of our method may therefore make it applicable to different areas.

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The map produced in this study (Fig. 2) suggests that most of Denmark, Southern Scania (Sweden), northern and central parts of Poland and Germany, in contrast to Southern Poland, have a high density of potential *Alternaria* source areas. Previously, southern parts of Poland have been identified to have a lower *Alternaria* load compared to Central Poland, especially Poznan (Stepalska et al., 1999). This lower load stood in contrast to the longer vegetation period in Southern Poland compared to Central Poland (Stepalska et al., 1999). The study by Stepalska et al. (1999) therefore indicates that there must be a higher density of sources in Central Poland, thus supporting our map. If agricultural areas are the main source to *Alternaria* spores in Denmark, then it is likely that the fungal spore concentration is higher in Western Denmark than Eastern Denmark (Copenhagen). In our map of potential source areas, Western Denmark has a considerably higher proportion of potential source areas than Eastern Denmark. Similar relations have previously been suggested by Corden et al. (2003), as Corden et al. (2003) found a high *Alternaria* spore load in Derby with high agricultural production and a low annual spore load at the coastal site in Cardiff, which had very limited cereal production. Also in Southern Poland results from the operational trap in Rzeszow were compared with results from a rural trap 10 km away (Kasprzyk and Worek, 2006). In the year 2001 the load was about the same at the two Polish sites, while the *Alternaria* load in the rural area was more than double of the urban load in 2002 and with a markedly different seasonal pattern. For Denmark such relations remain to be investigated using more than one spore trap. Such studies would provide valuable information about Western Denmark and can also be used to test the hypothesis in this paper as well as investigating the robustness of the proposed source map.

In a Spanish potato crop treated with fungicides, *Alternaria* spores were recorded during the entire growth season, but with a peak in *Alternaria* concentration during leaf senescence (Escuredo et al., 2011; Iglesias et al., 2007). This suggests that although fields are treated against fungal disease (and visual inspection does not reveal *Alternaria* attack), spores are still present in the field and released in varying quantities throughout the entire season. The Spanish studies also showed that

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the fungal spore load in the region of Ourense is higher in the field area (Escuredo et al., 2011) compared to the load that is observed in the nearby city area (Aira et al., 2008). This again stresses the importance of atmospheric transport on the local scale. More importantly, other studies by Hill et al., (1984) and (Friesen et al., 2001) have observed very high amounts of spore release during harvesting. Similarly, Mitakakis et al. (2001) found periods of *Alternaria* burst during mowing and harvesting of grass in Australia. *Alternaria* spp. have been named among the agents of fungal diseases in wheat (alternaria leaf blight *A. triticina*; black head molds, *Alternaria* spp.; http://en.wikipedia.org/wiki/List_of_wheat_diseases) and barley (kernel blight, *Alternaria* spp.; http://en.wikipedia.org/wiki/List_of_barley_diseases). Our study did only concern a specific harvest situation, and samples were taken from a few wheat and barley fields that had been treated with fungicides. Sampling in infested crops, or crops that have not been treated with fungicides, or harvesting using different methods might therefore yield significantly different emission factors. If emission factors can be obtained from both growing crops growth and crop harvest (e.g., by using the simple methodology that we employed), then this will provide the much needed emission factors that can be used by atmospheric modellers in order to increase understanding of how *Alternaria* spores are released and distributed in the atmosphere.

The main characteristics of the spore season show that the annual variations in the spore index from Copenhagen varies with more than a factor of two from less than 5000 to more than 10000. The annual index of *Alternaria* spores and the number of days with clinical relevant levels are highly correlated ($r^2 = 0.67$, not shown), which is not surprising as these numbers are highly dependent. This annual level is generally higher than the levels that are observed on the Iberian Peninsula Spain (Aira et al., 2008; Rodriguez-Rajo et al., 2005) or in Sweden (Hjelmroos, 1993), where the annual spore index of *Alternaria* has been reported to be in the range of 1000–4000. Similar levels as in this study were also reported for Warszawa in a Polish study by Stepalska et al. (1999). The same Polish study also showed that the annual loads in Poznan can have an index that exceeds 30 000. Such large loads were also observed by

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Angulo-Romero et al. (1999) and Maya-Manzano et al. (2012) in Merida in 1997, where the spore index for *Alternaria* was about 20 000, 25 000 and 50 000, respectively. The studies from Spain also pointed out that the geographical region had large variations in the annual spore index, as another site, Caceres, only had a a spore index of about 2000. Here it is worth to note that Caceres is a region with limited crop production, while Merida is a region with large amounts of irrigated crops such as maize, tomato and fruit trees (Maya-Manzano et al., 2012). The study by Mayo-Manzano et al. (2012) as well as Stepalska et al. (1999) show, that the variation in the load in the same biogeographical region can vary with more than a factor of 10 in between years and between sites. Such large variations can be difficult to explain and map using volumetric spore traps alone. The seasonal variation found in this study with only one single peak has been found in most European studies such as Poland, Sweden, England and Spain. Bimodal peaks are only found in the Mediterranean region (Angulo-Romero et al., 1999; Cosentino et al., 1995; De Linares et al., 2010; Giner et al., 2001; Lang-Yona et al., 2012; Maya-Manzano et al., 2012). All these studies on annual loads, on seasonal variations as well as our study highlight the interrelated connection between overall weather in the geographical regions as well as the abundance of local sources. Studies that focus on various aspects of source mapping (e.g., observations of load and comparisons between sites, source-receptor studies such as using trajectories or actual mapping of potential sources) are therefore all highly needed for fungal spores. Such studies provide a much needed insight into an area that according to an editorial in the Lancet (2008) has to some degree been forgotten and therefore needs much more scientific attention (Cecchi et al., 2010).

A number of studies have shown similar daily patterns as our study of *Alternaria* spore concentrations. Stepalska and Wolek (2009) showed that in Krakow the distribution of peak concentrations had a similar pattern as the peak concentrations as in this study. In Krakow peak concentrations are most often observed in the late afternoon and with about a factor of three more often than during night and early morning (Stepalska and Wolek, 2009). Similar observations with a peak in the late afternoon and

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a minimum in the night or early morning were found in the north of Portugal (Oliveira et al., 2009; Rodriguez-Rajo et al., 2005), north of Spain (Aira et al., 2008), south of Spain (Angulo-Romero et al., 1999; Giner et al., 2001) and Italy (Ricci et al., 1995). This suggests that at all these sites including Denmark, the overall load of *Alternaria* is due to local sources and local dispersion. It is not known if LDT is a contributing factor at other locations than Copenhagen. This again calls for dedicated source-receptor studies on fungal spores from other sites than Copenhagen. Such studies can also be considered an answer to both the editorial in the Lancet (2008) as well as the recommendation in Allergy by Cecchi et al. (2010) such as collection and analysis of aerobiological data on large spatial scales.

In Denmark *Cladosporium* and *Alternaria* dominate the atmospheric fungal spore flora by 68.9 % and 9.4 % of the total fungal spore catch, respectively (Larsen, 1981). High season for fungal spores is June until October, but external meteorological factors affect the fluctuation from day to day and year to year (Larsen, 1981). Our studies suggest that the *Alternaria* concentrations can be explained by combining source maps with atmospheric transport. Such information can be relevant for both agriculture as well as patients that are sensitized to fungal spores. The number of patients that are sensitive to fungal spores is usually much lower than to pollen (Damato and Spieksma, 1995). A recent study estimates that 2.4 % of the entire population is sensitized to fungal spores (Elholm et al., 2010). However broken down by asthma, the same data (Elholm et al., 2010) showed that asthmatics had a significantly higher prevalence of fungal spore sensitisation compared to non-asthmatics: 6.6 % vs. 2.0 % in the two genera, respectively. For sensitisation to *Alternaria* the corresponding figures were 6.1 % vs. 1.7 %, and it has also been observed, that the clinical reaction towards fungal spores is often larger compared to the reaction towards pollen (Sigsgaard personal communication). This calls for additional efforts in research, diagnosis and treatment of allergy, which would be a direct response to the editorial in the Lancet (2008) as well as the overall recommendations on aerobiological research as given by Cecchi et al. (2010), such as collection and analysis of aerobiological data on large spatial scales.

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5 Conclusions

The present study supports the hypothesis that Danish agricultural areas are the main source to airborne *Alternaria* spores in Denmark, that the source to the overall load is mainly local, but with intermittent LDT from more remote agricultural areas. This hypothesis is supported by the analysed data of the 10 yr bi-hourly record of *Alternaira* in Copenhagen that show a distinct daily profile of 232 clinical relevant episodes (Fig. 1) and the identification of potential Long Distance Episodes (Table 2) from areas that could be a potential source region (Figs. 3–5, respectively). The emission studies in cereal crops under harvest also support our hypothesis. The results showed that although the fields had been treated against fungal infections, harvesting still produced large amounts of airborne fungal spores. The findings agree well with related studies that shows high *Alternaria* spore load in agricultural areas in Central Europe. This supports the hypothesis that crop harvest in Central Europe causes episodes of high airborne *Alternaria* spore concentrations in Copenhagen as well as other urban areas in this region.

Our findings have several implications. Firstly, forecasting of fungal spore quantities relevant to allergy patients in Denmark must take into account long-range transport, and cannot be based on measured concentrations in Denmark alone. Secondly, allergy patients need a warning several days ahead to plan their medical intake. This information is not available for fungal spores, as the Danish information system on fungal spores is very simplistic and is based on information from Copenhagen alone (Skjøth and Sommer, 2012). An extension of the spore monitoring programme, using several spore traps would most likely be very useful, as our study suggests that the fungal spore load might be higher in other parts of the country. An alternative is to supplement the current information system with the mathematical model systems from chemical weather forecasting (e.g., Kukkonen et al., 2012) and extend these to include the spore production and emission from countries such as Germany and Poland, as well as the local agricultural production in Denmark. This approach might however be

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very difficult as all relevant *Alternaria* sources remain to be identified and also because this and other studies suggest, that the emission pattern is related to both biology and agricultural production methods. In our study we have identified possible LDT episodes, suggested a gridded inventory of potential source areas, verified potential sources to local emission peaks from harvesting and found the typical daily pattern in the observed load of *Alternaria* spores. Each of these pieces of information will be very useful in the daily information to the public as well as in forecasting. Furthermore such studies allow for additional studies using source based models such as DEHM (Brandt et al., 2012), SILAM (Sofiev et al., 2006) and COSMO-ART (Zink et al., 2012) for improved understanding of aeroallergens and ultimately better information to the public.

Supplementary material related to this article is available online at: http://www.atmos-chem-phys-discuss.net/12/14329/2012/acpd-12-14329-2012-supplement.pdf.

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Table 1. Maximum daily *Alternaria* spore concentrations (spores m^{-3}), day of season start and day of maximum spore concentration (days from 1 January) and number of days with concentrations above 100 pollen spores m^{-3} recorded in Copenhagen during 2001–2010.

Year	Seasonal spore index	Day of season start	Day of peak concentration	Peak value (spores m^{-3})	Days above 100 spores m^{-3}
2001	9431	187	229	1016	23
2002	7046	186	210	567	21
2003	4488	191	200	279	17
2004	5651	184	219	607	18
2005	8141	172	222	468	30
2006	10 781	182	221	682	27
2007	7813	171	198	588	22
2008	5276	178	244	313	17
2009	10 511	182	221	595	31
2010	7519	189	215	689	27
Mean	7946	182	218	580	23
SD	2239	7	13	208	5

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Table 2. Days with episodes (above 100 spores m^{-3}) of fungal spores with a markedly different daily pattern compared to the overall daily pattern of the 232 episodes recorded in Copenhagen during 2001–2010.

Hour Date	1	3	5	7	9	11	13	15	17	19	21	23
22 Jul 2001	264	276	384	216	300	216	36	132	84	156	192	180
21 Jul 2003	84	156	204	252	336	384	192	24	48	24	84	12
26 Jul 2003	168	564	432	156	24	0	0	36	0	12	0	60
5 Sep 2004	408	384	204	228	132	228	180	120	144	84	36	0
25 Aug 2005	348	324	300	492	216	84	0	48	0	24	0	36
10 Aug 2006	1452	876	840	600	504	36	96	420	228	420	384	192
25 Aug 2006	384	456	168	288	312	180	168	216	120	180	108	120
26 Aug 2006	168	144	192	84	132	180	108	180	60	12	0	12
5 Aug 2007	708	540	540	732	444	492	480	288	420	444	96	36
11 Aug 2007	216	324	264	252	252	252	444	60	0	48	48	12
31 Aug 2008	660	420	540	480	228	600	336	180	84	144	72	12
22 Jul 2009	312	264	444	264	168	216	360	192	24	36	12	48
27 Jul 2009	48	48	156	348	276	36	96	12	12	132	48	84
6 Aug 2010	468	420	420	276	324	168	396	228	312	204	72	108
11 Aug 2010	216	360	336	336	228	24	12	0	0	24	12	12
16 Aug 2010	1344	1428	300	312	204	420	204	504	312	12	0	48

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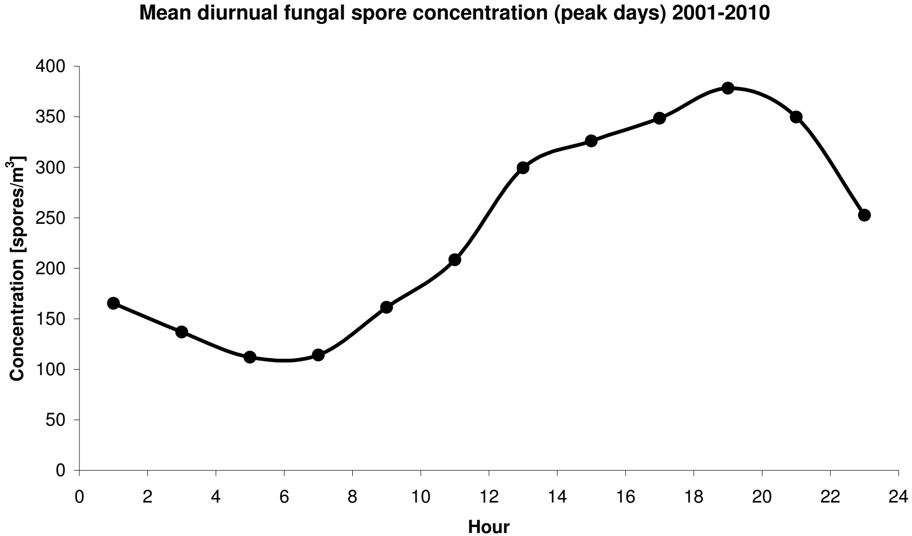

Fig. 1. Mean diurnal *Alternaria* spore concentration for days above 100 spores m^{-3}, $n = 232$.

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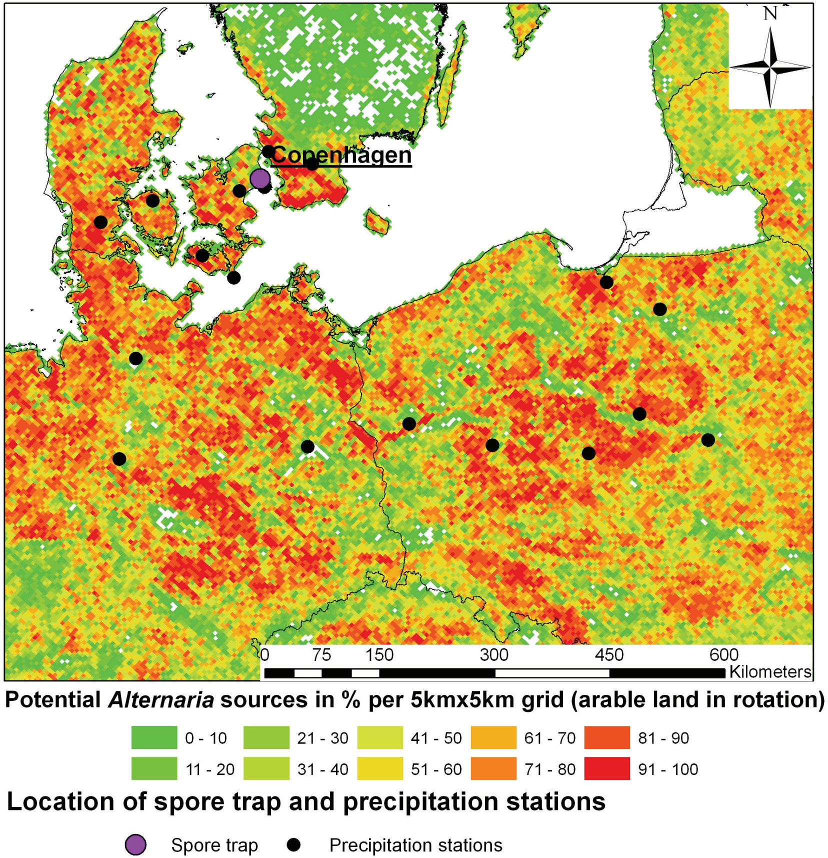

Fig. 2. Site map including the location of the spore trap in Copenhagen, the used precipitation stations (Table S2) and the density of agricultural areas under rotation – the potential source to *Alternaria* spores during harvest.

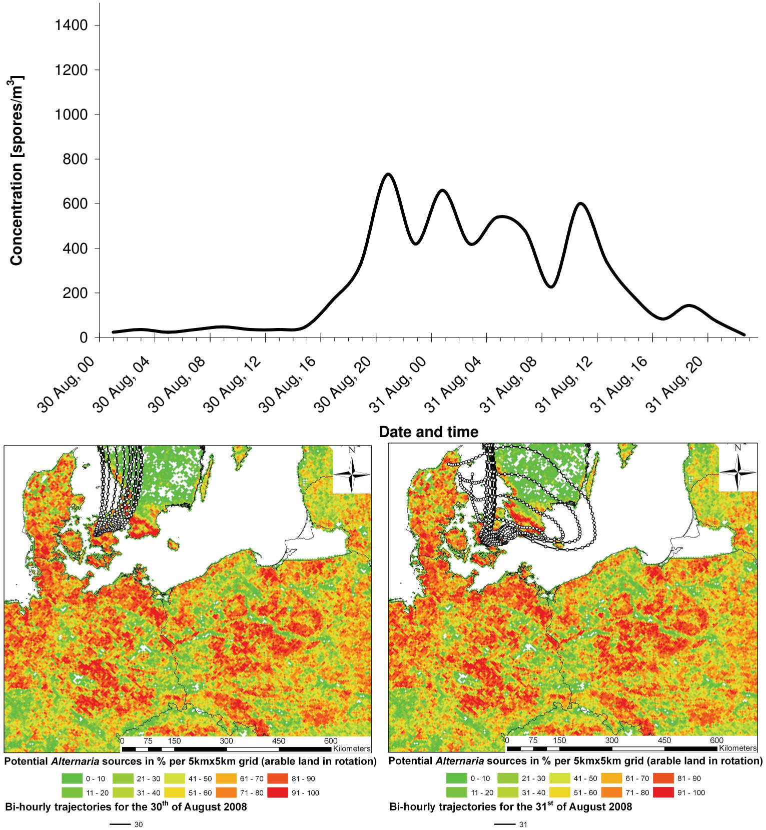

Fig. 3. (a) Hourly variation in *Alternaria* spore concentrations (spores m^{-3}) obtained in Copenhagen 30 and 31 August 2008. Back-trajectories arriving at the spore trap in Copenhagen: **(b)** 30 August; **(c)** 31 August.

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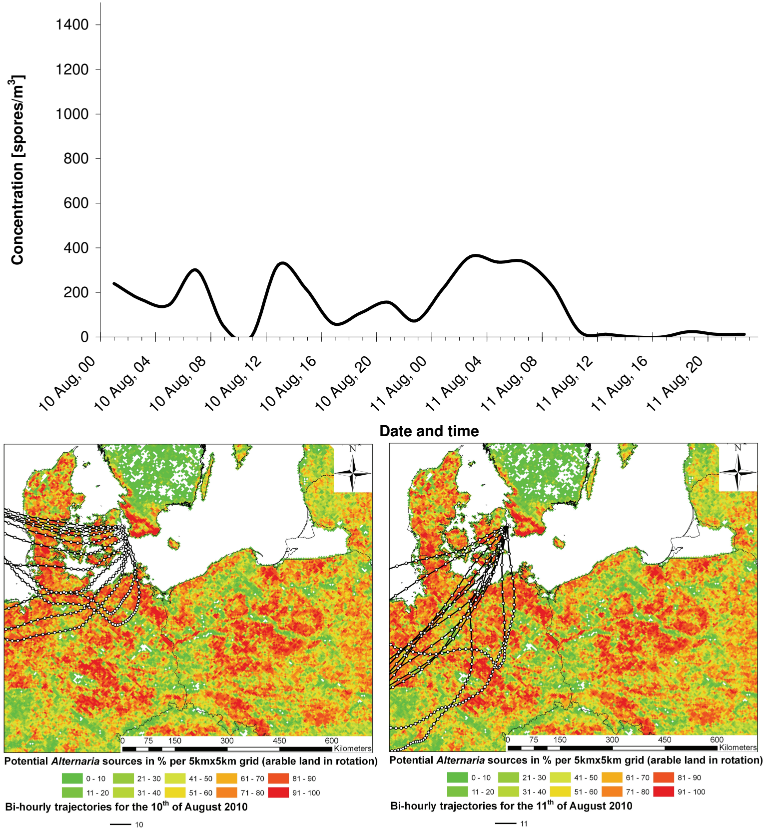

Fig. 4. (a) Hourly variation in *Alternaria* spore concentrations (spores m^{-3}) obtained in Copenhagen 10 and 11 August 2010. Back-trajectories arriving at the spore trap in Copenhagen: **(b)** 10 August; **(c)** 11 August.

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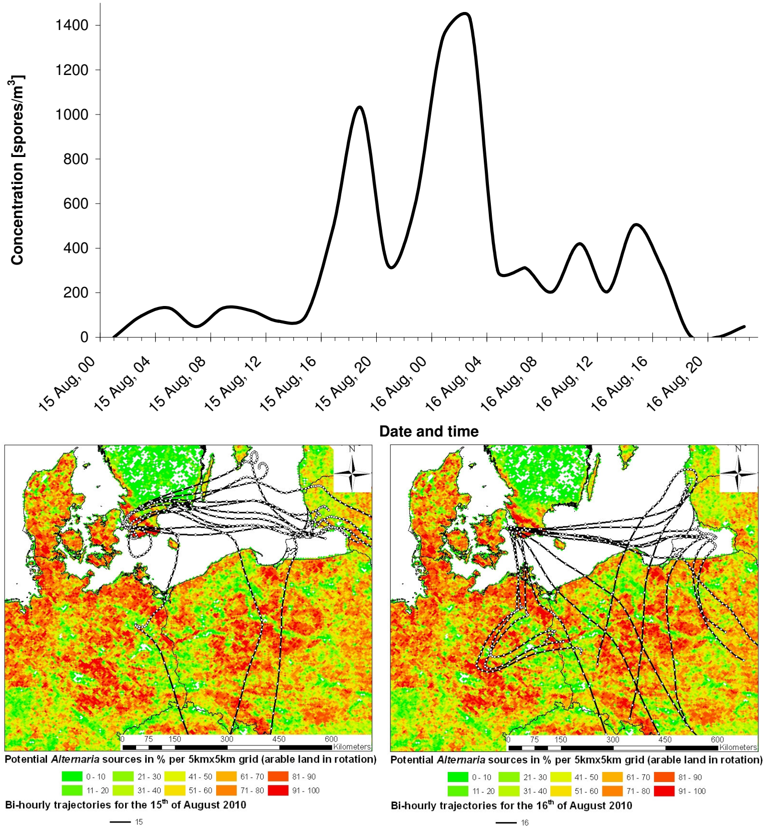

Fig. 5. (a) Hourly variation in *Alternaria* spore concentrations (spores m^{-3}) obtained in Copenhagen 15 and 16 August 2010. Back-trajectories arriving at the spore trap in Copenhagen: **(b)** 15 August; **(c)** 16 August.

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