Olsen Yulia, Gosewinkel Ulrich, Skjøth Carsten, Hertel Ole, Rasmussen Karen, Sigsgaard Torben.

Regional variation in airborne *Alternaria* spore concentrations in Denmark through 2012-2015 seasons: the influence of meteorology and grain harvesting.

Yulia Olsen; Torben Sigsgaard

*Department of Public Health, Aarhus University, Bartholins Allé 2, 8000 Aarhus, Denmark*

Ulrich Bay Gosewinkel

*Department of Environmental Science – Environmental microbiology & biotechnology, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark*

Carsten Ambelas Skjøth

*School of Science and the Environment, University of Worcester, Henwick Grove, Worcester WR2 6AJ, United Kingdom*

Ole Hertel

*Department of Environmental Science – Atmospheric chemistry and physics, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark*

Karen Rasmussen

*The Asthma and Allergy Association, Universitetsparken 4, 4000 Roskilde, Denmark*

*\*Corresponding author e-mail:* [*yo@ph.au.dk*](mailto:yo@ph.au.dk)*, tlf. +45 51809660*

Abstract:

High airborne *Alternaria* spore concentrations measured in eastern Denmark have been associated with local agricultural sources. However, the density of agricultural areas is highest in western Denmark.

This is the first report of airborne *Alternaria* spore concentrations obtained with Burkard volumetric spore sampler in western Denmark, Viborg. We compared the concentrations of airborne *Alternaria* spores and the patterns of air mass transport using HYSPLIT model between Copenhagen and Viborg for the seasons 2012-2015, with the main focus on the days with daily average *Alternaria* spore concentrations ≥ 100 s m-3 (high concentration days).

Except for 2012, Annual Spore Integrals (ASIns) were on average 3,335 s day m-3 higher in Viborg than in Copenhagen. The high concentration days during 2012-2015 occurred more frequently and with higher values in Viborg (96 days; mean=381 s m-3) than in Copenhagen (79 days; mean=270 s m-3). We found increased shares of trajectories coming from South-East on the high concentration days and increased shares of trajectories coming from the West and North-West on the days with concentrations below 100 s m-3 for both stations. July and August had the highest spore integrals matching the periods of grain harvesting in Denmark. The absence of the concurrent grain harvesting in Denmark was associated with the lowest ASIns in 2012. The results of this study support the hypothesis that local sources cause the main load of airborne *Alternaria* sporeconcentrations in Denmark, however, the contribution from the remote source areas in northern Germany, Poland and southern Sweden remains unquantified.

Keywords: *Alternaria* spp., spore dispersion, HYSPLIT, back trajectories, grain harvesting

1. Introduction

*Alternaria* spp. is a cosmopolitan fungal genus which includes mainly saprophytic, but also endophytic and pathogenic species. Beingone of the most frequent endophytes found in grasses and other plants, after the host tissue death, *Alternaria* spp. can undergo a transition from endophytic to a saprobic phase, followed by mycelial growth and development of reproductive structures forming airborne conidia under appropriate environmental conditions (Vázquez de Aldana et al. 2013). As a part of soil filamentous fungi mycobiota, *Alternaria* spp., are involved in forest ecosystems nutrient/litter recycling (Song et al. 2010) and can be found prevalent as on senescent or old tissues, such as petals, old leaves, and ripe fruits (Barnes 1979) widely available during fall. Species of the genus *Alternaria* also inoculate a variety of crops in the field, negatively affecting crops quality and quantity (Ram and Chauhan 1998; Saharan et al. 2016; Andersen et al. 1996; Broggi et al. 2007; Farrar et al. 2004; Van der Waals et al. 2001; Disalov et al. 2018).

Furthermore, *Alternaria* spp. spores are well-known aeroallergens (Levetin et al. 2016; Twaroch et al. 2015) and daily average *Alternaria* spp. spore concentration of 100 m-3 has been widely applied in aerobiological research as a health relevant threshold for respiratory allergy risk (Gravesen 1979). A critical review of occupational fungal spore exposures, reported lowest-observed-adverse-effect level of around 105 spores m-3 in mixed environments after short term exposures (up to 8 hours) of non-sensitized highly exposed workers, not differentiating between species and genera (Eduard 2009). However, taking into consideration, that occupational exposure is dramatically different from environmental (Semple 2005) and that the effect in the general population is an allergic reaction in sensitized persons, working on the top of seasonal exposure protracted over a few weeks or months, the true ambient *Alternaria* spp. spore threshold for inducing allergic response lies somewhere between the two extremes. Elevated ambient *Alternaria* spore levels were associated with asthma symptom scores in sensitized asthmatics (Delfino et al. 1997), with asthma hospital visits and admissions in adults (Stieb et al. 2000) and children (Atkinson et al. 2006). Peak *Alternaria* season has been observed to coincide with asthma attacks in case-studies with *A.* *alternata* sensitized patients (Bush and Prochnau 2004; Hyde et al. 1956; O'Hollaren et al. 1991).

Airborne dispersion is the main mechanism for *Alternaria* inoculum distribution and spatial propagation (Rotem 1994). *Alternaria* spp. is the second most dominant out of 30 genera measured at the Copenhagen station comprising 3-9.4% to the total airspora (Larsen 1981; Larsen and Gravesen 1991). Although, several mechanisms of active spore liberation have been described in the literature, there is a general consensus that passive release via wind plays the main role in the detachment of *Alternaria* spores from the inoculum (Rotem 1994; Money 2016). *Alternaria* spores belong to so called “dry dispersed” spores, because warm, dry weather facilitates their release (Smith et al. 2012; Savage et al. 2012) and distribution over long distances (Skjøth et al. 2012; Sadyś et al. 2015; Smith et al. 2012). Higher air concentrations of *Alternaria* spores in rural sites compared with urban is related to greater abundancy of organic material (Kasprzyk and Worek 2006; Oliveira et al. 2010) and have been associated with local cropping activities and crop maturation (Mitakakis et al. 2001; Corden et al. 2003; Oliveira et al. 2009; Kasprzyk et al. 2013). The importance of local landscape management, vegetation structure and local climate for *Alternaria* spore production were found to prevail over year-to-year variations in weather in a recent study with 15 annual time series from the 23 sites in Europe (Skjøth et al. 2016).

In aerobiological research the increase in the atmospheric concentrations of *Alternaria* spores has been attributed to harvest activities in grain crop fields (Corden et al. 2003; Skjøth et al. 2016). It is known, that *Alternaria* spp. frequently infect cereal grains (Logrieco et al. 2003), thus, several experimental studies have isolated *Alternaria* species and the mycotoxins specific to *Alternaria* spp. from the harvested grains (Mercado Vergnes et al. 2006; Logrieco et al. 2009; Logrieco et al. 1990; Gavrilova et al. 2016; Lôiveke et al. 2004; Müller and Korn 2013; Gannibal 2018). Moreover, already in the 1950s high *Alternaria* spore air concentrations were measured within the fields of barley and wheat (Last 1955). Combine harvesting has been assumed to passively trigger *Alternaria* spore release for a number of reasons. First of all, the maturity of the crop is defined by the low moisture level of biomass, which determines the start of harvesting. Furthermore, harvesting takes place mainly during dry, warm weather, which facilitates air dispersal of *Alternaria* spores, and at last, timing of grain harvest has been noticed to occur within airborne *Alternaria* main spore season (MSS). This assumption has been proved to be correct, when billions of spores were measured in the air during wheat combine harvesting experiment by Friesen et al. (2001). Additionally, a couple of field experiments directly measured abundant *Alternaria* spore emissions during combine operations with grains (Mitakakis et al. 2001; Skjøth et al. 2012), e.g. from the exhaust airstream of harvesting combine in Danish barley and wheat fields (Skjøth et al. 2012). According to the agricultural crop area annual statistics, winter wheat and spring barley cover the largest areas in Denmark, followed by straw and grasses (SM Tab.1). An analysis of 10 airborne *Alternaria* spore seasons in Denmark, Copenhagen, showed that the Annual Spore Integrals (ASIns) of *Alternaria* spores were dominated by the days with daily average concentrations above the health threshold of 100 s m-3 (Skjøth et al. 2012). The majority of those days were associated with local agricultural sources, and the density of these sources were found to be higher in western Denmark versus eastern (Skjøth et al. 2012).

Back trajectory analysis of air masses in combination with potential source area maps have received an increasing attention in aerobiological research, since a study by Skjøth et al. (2007) with birch pollen in Denmark. In recent source receptor studies high *Alteranaria* spore concentrations have been associated with the air masses/winds passing over/originating from the agricultural areas and arable lands (Fernández-Rodríguez et al. 2015; Skjøth et al. 2012; Sadyś et al. 2015; Reyes et al. 2009; Corden et al. 2003). The only study that modelled *Alternaria* spore transport in Denmark found agricultural areas in northern/central Poland and Germany, and southern Scania (Sweden), as possible remote sources, that affected high *Alternaria* concentrations in Copenhagen on the days, when bi-hourly concentrations diverted from the established diurnal pattern (Skjøth et al. 2012).

Currently aero-mycological analysis of *Alternaria* spore concentrations in Denmark is based on routine monitoring at a single urban station in Copenhagen, located at the eastern border of Denmark. This can compromise the quality of national daily air spore concentrations report, due to the existing spatial variation of possible local and remote sources throughout the country.

This is the first study that attempts to look into the differences in airborne *Alternaria* spore occurrence between two stations in Denmark, placed in western (Viborg) and eastern (Copenhagen) Denmark. The aim of this study was to compare the load of *Alternaria* spores at the Viborg and Copenhagen stations, with special attention to the days with daily average *Alternaria* spore concentrations ≥ 100 s m-3 (high concentration days), hypothesizing, that the ASIns and the total number of days equal or exceeding 100 s m-3 are larger in western Denmark than in eastern, due to higher density of potential *Alternaria* sources in western Denmark (Skjøth et al. 2012). Additionally, we tried to investigate, if the time of grain harvesting coincides with the main *Alternaria* spore release during the seasons. Furthermore, to better understand which source areas can be associated with the high concentrations measured at the stations, we examined the patterns of air mass transport on the high versus low concentration days (<100 s m-3) during periods with high *Alternaria* counts at both stations.

2. Materials and Methods

2.1 Sites location and spore identification

The data were obtained using 7-day volumetric spore samplers of the Hirst design (Hirst 1952; Levetin 2004) by the Asthma and Allergy Association as a part of ongoing pollen and spore monitoring programme, providing time-series of *Alternaria* spores in the ambient air. The first trap is placed in the city centre of the country capital, Copenhagen, Eastern Zealand, on the roof of the Danish Meteorological Institute (55⁰43’N 12⁰34’E) building at a height of 15 m above sea level. Danish Meteorological Institute is located next to the city highway and in approximately 2 km distance from the sea shore. The second trap is placed in the Central Jutland, in the southern part of Viborg (56⁰27’N 9⁰24’E), on the roof of Regional Hospital, 21 m above sea level. The distance between the traps is approximately 240 km. The Hirst trap is assumed to capture the spores released from the sources within a 30-50 km range (Skjøth et al. 2010). The dominating land cover types around 30 km of the Viborg trap are non-irrigated arable (56%) and agricultural lands with areas of natural vegetation (14%), whereas the dominating land cover types around the Copenhagen trap, apart from the sea (49%), are non-irrigated arable land (17%) and discontinuous urban fabric (13%) (SM Tab.2).

Microscope slides from the Hirst traps were stained with fuchsin and examined by use of light microscopy at the genus level only on the basis of conidial shape, septation and pigmentation under 640x magnification on 12 latitudinal transects, with a time resolution of 2 hours (Stepalska and Wolek 2009). The total examined surface corresponded to the 9.75% of entire impaction area. *Alternaria* spore concentrations for 2012-2015 from the Copenhagen trap and for 2012 from the Viborg trap were counted within the Asthma and Allergy Association monitoring program, whereas *Alternaria* spore concentrations for 2013-2015 were counted at the laboratory of Aarhus University under the same protocol as used at the Asthma and Allergy Association.

2.2. Meteorological data

Daily meteorological parameters, i.e. accumulative precipitation, average temperature and average relative humidity (RH), were provided by the Danish Meteorological Institute from the nearby stations at Copenhagen Airport (Lat: 55.6140 Lon: 12.6454, height 5 m above sea level), and at Foulum (Lat: 56.4931 Lon: 9.5709, height 53 m above sea level).

2.3. Season definitions and statistical analysis

Relationships between spore concentrations and meteorological parameters were assessed applying the Spearman’s rank correlation, due to non-normality of the analyzed variables. Correlation between the daily average concentrations at the stations was studied monthly for each year.

In Denmark, aerobiological monitoring of *Alternaria* spores is limited to the time period of June – middle October and geographically to the trap at the Copenhagen station. The trap at the Viborg station is used for pollen monitoring and normally stopped one month prior to the Copenhagen trap, making the period of available data for Viborg one month shorter than for Copenhagen (SM Tab.3). *Alternaria* main spore seasons, were established using the 90% method, commonly used in aerobiological studies with fungal spores (i.e. the onset of the season was defined as the first day with the daily averaged concentration above 5% of the total annual spore catch, and the last day of the season – as the last day below 95% of the total annual spore catch) (Nilsson and Persson 1981).

Following the recommended terminology for the aerobiological studies (Galan et al. 2017), the Annual Spore Integral (ASIn) was calculated as the sum of the daily average concentrations over the whole period of available measurements.

In order to investigate the difference in the daily and bihourly load of *Alternaria* spores on the high concentration days, daily averages and bihourly concentrations on these days were compared between the stations using Wilcoxon rank-sum test with significance level set below 5%. Statistical analysis was performed using STATA Version 14.

2.4. Back-trajectory analysis

To avoid the days with very low concentrations for a given station, but to include all the days with the daily average concentration ≥ 100 s m-3, a period between the first and the last date in a year with daily average concentration ≥ 100 s m-3 at a station was chosen for the back-trajectory analysis. The origin of air masses and potential source regions for each station were estimated by computing 48h back-trajectories with 2h temporal resolution in PC Windows-based Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model (Stein et al. 2015; Draxler et al. 2018) using the Global Analysis System (GDAS) meteorological files (spatial resolution of 1 degree) with receptor height of 500 m above ground level, which is typically used in aerobiological studies (Sadyś et al. 2014; Sadyś et al. 2015; Stach et al. 2007) . The trajectories were calculated for all days within the chosen periods for the years 2012-2015. Subsequently, those trajectories were sorted into two groups according to the corresponding daily average concentrations: trajectories for the high concentration days (≥100 s m-3) and for the low concentration days (<100 s m-3). The trajectories from the same group were merged by means of cluster analysis, which has been previously applied (Maya-Manzano et al. 2016; Sadyś et al. 2014), where each day with spore concentrations is represented by 12 trajectories. This approach reduces the uncertainties associated with the limited quality of the meteorological data and with the calculation of the individual trajectories (Stach et al. 2007). A similar procedure with forming groups of trajectories according to the threshold concentration was applied in the recent footprint studies on ragweed, alder, and birch pollen (Skjøth et al. 2015; Bilińska et al. 2017) and in the study on *Ganoderma* sp. fungal spores transport (Sadyś et al. 2014).

3. Results

Table 1 presents annual *Alternaria* spp. and meteorological indices. The ASIns varied substantially between the four studied years at both stations with the highest ASIn in 2014 and the lowest in 2012 (Tab. 1). Except for 2012, ASIn was on average 3,335 s day m-3 higher in Viborg than in Copenhagen. Over the whole period 96 days in Viborg and 79 days in Copenhagen had high daily average concentrations above or equal to the clinical threshold of 100 s m-3 (high days). The majority of those days (62) concurred at the two stations. A total of 27 and 26 high concentration days were measured in July, 49 and 61 in August, and 3 and 9 in September in Copenhagen and in Viborg, respectively. In all four years the first day in the season with daily average concentrations above the threshold happened earlier in Copenhagen: almost four weeks earlier in 2012, a day earlier in 2013 and 2014, and 11 days earlier in 2015 (Tab.2). High concentration days constituted on average around 73% of ASIn in Copenhagen and around 88% in Viborg, with the exception of 2012 when high concentration days contributed to the ASIn noticeably less, i.e. 26.5% in Copenhagen and 15.2% in Viborg.

Monthly contributions of high days to the monthly (June-September) Spore Integrals (SIns) are presented in Figure 1. The highest monthly SIns were recorded in July and August, peaking in August in 2013 and 2015, in July in 2014, whereas in 2012 August SIn was larger than July in Viborg but nearly equal to July SIn in Copenhagen (Fig. 1). The overall maximum concentrations at both stations were observed on the same date, i.e. on August 14, 2015 with daily averages of 1,326 s m-3 in Copenhagen and 1,414 s m-3 in Viborg with bi-hourly maximums of 3,204 s m-3 at 9:00 in Copenhagen and 4,656 s m-3 at 13:00 in Viborg (Fig.2). During the study period the bi-hourly concentrations exceeded 1,000 s m-3 on 121 occasions in Viborg and on 27 occasions in Copenhagen. The mean MSS length was shorter in Viborg (51 days) than in Copenhagen (66 days).

During the period of measurements, the mean temperature in Viborg (14.7oC) was on average one degree lower than in Copenhagen (15.9oC), while average relative humidity (Viborg 81.69%, Copenhagen 75.08%) and total accumulative precipitation (Viborg 1134.2mm, Copenhagen 1032.2mm) were slightly higher. The maximal amount of precipitation and the number of rainy days were recorded in the season of 2012 at the Foulum station and in the season 2014 at Copenhagen station (Tab.1). However, the distributions of the precipitation amount as well as the number of rainy days were different between the stations. Thus, in 2012 the monthly sums of daily accumulative precipitation at the Foulum weather station, 11 km East-Northeast of the Viborg spore trap, were not very different between July (93.8 mm) and August (80.2 mm), whereas in 2014 the monthly sum of daily accumulative precipitation at Copenhagen station in July was more than twice lower (48.8 mm) than in August (133.8 mm). Out of those 133.8 mm, 81.3 mm were recorded in the second half of the month. In the same year (2014) a similar pattern of precipitation was observed at the Foulum station: 65.7 mm monthly sum in July, and 116.6 mm in August with 76 mm measured in the second part of the month.

The strongest statistically significant correlations between daily spore concentrations and meteorological parameters were found with daily average temperature, weaker negative correlations were found with daily average relative humidity and cumulative precipitation (Tab. 3). Atmospheric daily average spore concentrations were moderately correlated between the Viborg and Copenhagen stations in the most important months of July and August ranging from 0.42 to 0.66 (Tab. 4). The daily averages on the high concentration days (n=62), however, were poorly correlated (rs=0.24; pvalue=0.06) between the stations while the concentrations on these days were higher in Viborg (mean=381 s m-3) than in Copenhagen (mean=270 s m-3), with statistically significant result of Wilcoxon rank-sum test (pvalue=0.01).

The mean diurnal pattern of airborne *Alternaria* spore concentrations on the high concentration days showed a peak from 13:00 to 21:00 at both stations, decreasing after 21:00 down to the minimal concentrations during early morning (03:00 to 07:00). Mean bi-hourly concentrations were similar at both stations during 01:00-07:00, while after 07:00 the curve was sharper for Viborg with on average 177 s m-3 higher mean bi-hourly concentrations from 09:00 to 21:00 (Fig. 3). The results of Wilcoxon rank-sum test on high days’ bi-hourly concentrations revealed that in the late afternoon (15:00-19:00) the concentrations were also significantly higher in Viborg (Fig. 3).

Table 2 presents the amount of precipitation on the high and low concentration days within the periods chosen for the back-trajectory analysis. The amount of precipitation and the share of the rainy days (with daily precipitation above 0 mm) were larger on the low concentration days, than on the high concentration days. Figure 4 and Figure 5 show the clusters and the individual trajectories calculated for the 79 high (Fig. 4a) and 92 low concentration days at the Copenhagen station (Fig. 4b), and for the 96 high (Fig. 5a) and 61 low (Fig. 5b) concentration days at the Viborg station. For the Copenhagen station a total of 948 individual trajectories were grouped into 6 clusters for the high concentration days, and 1,104 trajectories were grouped into 6 clusters for the low concentration days. For the Viborg station a total of 1,152 individual trajectories for the high concentration days were grouped into 7 clusters (Fig.5a), and a total of 732 trajectories for the low days were grouped into 5 clusters (Fig.5b). Copenhagen high concentration days were characterized by higher shares of the air masses coming from the South and South-East (Fig. 4a), whereas the low concentration days by higher shares of the air masses coming from the North, North-West and South-West (Fig. 4b). In Viborg, this pattern was more evident, with 45% of air masses coming the South-East (26%) and East (19%) and around 23% of air masses coming from the North-West on the high concentration days (Fig. 5a), whereas on the low concentration days, the shares of air masses coming from the North-West increased to 55% while the shares of air masses coming from the South-East were close to zero and the shares of air masses coming from the East decreased to 12% (Fig. 5b).

4. Discussion

4.1 Differences between the stations

The larger annual SIns in 2013-2015 with statistically higher concentrations on the high concentration days, and larger number of days with daily average *Alternaria* spore concentration equal or exceeding the threshold value of 100 s m-3 in Viborg positively support the initial hypothesis, tested in this study, that higher density of agricultural sources in western Denmark must result in higher *Alternaria* spore air concentrations compared to the concentrations in eastern Denmark.

The sites in the rural locations or in the locations surrounded by the concentrated agricultural fields have been previously shown to measure higher *Alternaria* spore air concentrations (Kasprzyk et al. 2013; Corden et al. 2003; RodrÍGuez-Rajo et al. 2005; Kasprzyk and Worek 2006). The importance of the surrounding site landscape was highlighted by Kazprzyk et al. (2013), as they counted the highest number of *Alternaria* spores in the capital of the region with large scale farming, despite that fact, that the city (Poznan) itself was characterized by the highest urbanization factor compared with others. Reduced agricultural land use during the last decade around Bratislava was suggested to be the reason for *Alternaria* spp. seasonal SIn decrease (Ščevková et al. 2016). The dominating land cover within 30 km around Viborg is non-irrigated arable land, whereas around Copenhagen the share of the non-irrigated arable land is 3 times less, with dominating shares of the sea, and 4.6 times larger shares of discontinuous urban fabric (SM Tab.2). The relationship between the proximity to the sea and *Alternaria* spore air concentrations was observed in the study by Corden et al. (2003), where they found the lowest numbers of spores at the coastal site of Cardiff, UK. Similarly, *Alternaria* spore concentrations decreased in more coastal areas in north-west Spain (RodrÍGuez-Rajo et al. 2005). Our findings are consistent with the previous: lower concentrations of *Alternaria* were measured in the air of a coastal city, i.e. in Copenhagen. Most notably, in Viborg we measured 96 days with *Alternaria* concentrations above 100 s m-3 versus 79 days in Copenhagen, and 34 of those days in Viborg occurred on the different dates than in Copenhagen, which questions the representativity of *Alternaria* monitoring performed in Copenhagen solely. Moreover, the concentrations on these days in Viborg had higher values than in Copenhagen.

Ambient spore concentrations, measured by microscopic counting of spores at genus level, are usually considered to be proxy for *Alternaria* spp. allergen ambient exposure and were linked to asthma severity and morbidity (Denning et al. 2006). However, the results of a recent study in Poland demonstrated that allergenic content in *Alternaria* spores might vary, possibly due to species diversity and to differences in the source area (Grewling et al. 2018). The information on the species-specific differences in spore allergenic content is not well-documented ([www.allergen.org](http://www.allergen.org/)), as most research has been directed towards the most prevalent species, i.e. *A. alternata* (Gabriel et al. 2016), although, the major allergen Alt a 1 gene homologs were also found in other *Alternaria* spp. (Hong et al. 2005). Furthermore, Alt a 1 has been expressed in other members of the *Pleosporaceae* family (Sáenz-de-Santamaría et al. 2006; Gutiérrez-Rodríguez et al. 2011), which spore air concentrations can be higher in the rural locations (Oliveira et al. 2010). Additionally, microscopic analysis neither included counting of one- /two- celled *Alternaria* spores separated from conidial chain nor parts of fungal hyphae, spore fragments or spore micro-particles, whose contribution to overall exposure can be very high (Reponen et al. 2007; Górny et al. 2002; Green et al. 2006).

Thus, our results demonstrate higher ambient *Alternaria* spp. spore concentrations in the area with lower urbanization, i.e. in Viborg, while the levels of Alt a 1 allergen exposure remains to be explored.

4.2 Inter-annual differences

In this study, the *Alternaria* season of 2012 stands out as the year with the lowest observed spore concentrations at both stations, and the only year when *Alternaria* spore concentrations were higher at the Copenhagen station. Neither harvested crop quantities nor areas, used as proxy for reasoning the increase of *Alternaria* levels by Corden et al. (2003), were significantly decreased during 2012 (SM Tab.1). The intensity of wheat and barley grain infection by *Alternaria* spp., that affects the amount of released spores, has been observed to differ annually depending on the meteorological conditions (Gannibal 2018). The great majority of aerobiological research found that *Alternaria* spore air concentrations are negatively correlated with the daily amount of precipitation and average RH, and positively correlated with average temperature. In this study, daily average temperature had the strongest correlation with daily average spore concentrations and much weaker correlation with RH and daily precipitation. We indeed measured the highest RH mean values and the lowest (out of the four years) average temperature in season 2012 at the Foulum station. However, the temperature values in season 2012 were similar to the ones measured in the season of 2015, when the ASIns for both stations were much higher (Tab.1). The total accumulative precipitation at the Foulum station during *Alternaria* spore season 2012 was nearly equal to the amount of precipitation measured in the season 2014, which was characterized by the most abundant spore release with 10 and around 5 times higher ASIns for Viborg and Copenhagen respectively. However, the number of individual days with accumulative precipitation above 0 mm was the highest in 2012 and nearly twice higher at Foulum in the season 2012 compared to the season 2014.

Airborne *Alternaria* spores originating from local sources at a distance of 65-130 km from the site can still be measured by the Hirst trap (Sadyś et al. 2015). But a meteorological station measures the precipitation in its immediate vicinity. The process of harvesting, however, depends on the very local weather conditions, such as that while it is raining in Foulum, the meteorological conditions can be favorable enough to continue harvesting at the neighboring farm.

In summer 2012 during 3 months of June, July and August, the precipitation on average for the country was 257 mm higher than typically observed in Denmark, with the highest amounts registered in Jutland and the lowest in Zealand (Pedersen 2012). Additionally, the summer in Denmark that year was on average 1.3 oC colder than the previous 10 years, and harvesting conditions were reported to be poor but in general better in West- and South- Zealand than in Jutland. In the latter the lower temperatures and the absence of sun led to the delayed start of grain harvesting, which had to be distributed over a long period, that continued into October (Pedersen 2012). When inevitable, the process of harvesting can be performed on wet crops, followed by usage of grain drying machines (Lyngvig 2011). Low humidity, however, is a prerequisite for *Alternaria* spores dispersion. Therefore, harvesting of wet grains in the conditions of increased RH cannot be as an effective mechanism of spore dispersion as the harvesting of dry grains. Accordingly, the high number of rainy days observed at the Foulum station in 2012 (63 days in 2012, compared to 22, 34, and 21 days in 2013-2015 correspondingly) must have negatively affected the dispersal of the spores into the atmosphere. This suggests that both the amount of precipitation and the frequency of precipitation events are important in relation to the abundance of *Alternaria* spores in the air. The assumption, that the harvesting process affects the airborne *Alternaria* spore concentrations, is supported by the distribution of airborne *Alternaria* monthly SIns at the sites. Thus, August SIn in Viborg (1,027 s day m-3) was two orders larger than in July (445 s day m-3), whereas SIn stayed the same in July (986 s day m-3) and August (987 s day m-3) in Copenhagen (Fig.1). Additionally, the annual peak daily average that year in Viborg was the latest out of all observed (day 232) with the modest value of 135 s m-3 (Tab. 1), which can indicate a delayed *Alternaria* release.

The *Alternaria* seasons of 2013 were the shortest: 53 days for Copenhagen and 40 days for Viborg, with the majority of spores registered in August, including the annual daily maximum (Fig.2, Tab. 1). The delayed (around 2 weeks) winter cereals growing period due to the late cold spring that year with unusually dry and sunny July-August, resulted in a later start of grain harvesting and a shorter period, finished by the end of August (Pedersen 2013). Grain harvesting of summer 2015 was also two-three weeks delayed due to the rainy and cold June (Pedersen 2015), and monthly SIns distributions of 2015 season mimic the ones of 2013, however, with higher values. The reason for the higher *Alternaria* release in 2015 is not clear, possibly this was related to the unusually warm and rainy spring that year on the contrary to the cold spring of 2013 (Pedersen 2013, 2015).

In the year with the maximum ASIns (2014), July SIns were higher than August SIns, and the first daily average ≥ 100 s m-3 occurred at both stations in the first week of July, which is the earliest out of the four years (Table 1). That year the *Alternaria* spore season had the highest daily average temperatures, especially noticeable in Viborg. Overall in Denmark, the spring months and the month of July 2014 were the second warmest registered since 1874 with July temperatures over the country 3.9 oC warmer than the average (Pedersen 2014). The positive influence of temperature on *Alternaria* spore air concentrations has been highlighted in several aerobiological studies (Kasprzyk et al. 2015; Maya-Manzano et al. 2016; Aira et al. 2013; Corden et al. 2003; Stennett and Beggs 2004; De Linares et al. 2010). The importance of the meteorological conditions as the dominating factor for the grain infection intensity was visible in the level of infection in grains by species of *Alternaria* between 7 different locations in the European part of Russia during three seasons (Gannibal 2018). Danish grain crops that year were reported to be mature already in July, which led to an around two weeks earlier start of grain harvesting, with most of the harvesting finished by the end of July in many places in the country (Pedersen 2014). The largest monthly SIns measured in July may be related to the early and intensive grain harvesting (Fig. 1), and the lower SIns in August may be caused by more abundant precipitation in the second part of August measured at both stations.

We observed that *Alternaria* monthly SIns coincided with the periods of grain harvesting in Denmark. However, the ASIns varied greatly at both stations, and the differences were twice larger for the Viborg station. The meteorological conditions influence several major factors that are related to *Alternaria* spore air concentrations at a certain location: the availability of the substrate that affects the start of *Alternaria* season, the scale of infection (or amount of the inoculum) and its relation to the number of mature spores, the time of sporulation, and the number of the spores, that can stay airborne and be the subject to the micro- and macro- scale dispersal.

Our findings support the hypothesis, that the process of grain harvesting principally defines the time window of *Alternaria* spore release in Denmark. However, harvesting of grains (mainly winter wheat and spring barley in Denmark), straw, and grasses takes place within the same time as grain/cereal harvesting. Straw (Rotem 1994; Al-Nadabi et al. 2018) and grasses (Rotem 1994; Gannibal and Yli-Mattila 2005) can also function as substrates for saprophytic and pathogenic *Alternaria* species. The machinery used for harvesting grasses is similar to the machinery used for grains, while the operation of presses, used for straw baling, can also be expected to facilitate massive release of spores. Spatially, the straw is harvested on the same fields as grains, whereas the locations of the grass lands are different (Sadyś et al. 2015). It should be noticed that the majority of Danish grasses are harvested for cattle feed and, therefore, still have high green biomass/moisture content during harvesting, while fungi normally form spores when the availability of nutrient is reduced (Money 2016).

It is important to remember, that the period of harvesting also depends on the local meteorology. As highlighted previously, the harvesting of cereals most probably takes place simultaneously at multiple locations in central-northern Europe, due to a synoptic scale meteorological phenomenon, settled over larger areas including neighboring lands (Skjøth et al. 2016). Therefore, the number of *Alternaria* spores in the air measured at a station consists of *Alternaria* spores from local and distant sources. In the season 2012 the timing of harvesting was delayed and was not sharply confined within a usual time period. Therefore, we could not observe the effect of synchronized harvesting (Skjøth et al. 2016) at several local and possibly distant sources that year, which was reflected in the smooth *Alternaria* seasonal distributions. Additionally, the higher than normal amount of precipitation distributed locally in Denmark may have washed out a large portion of spores from the air.

Airborne *Alternaria* spores were measured in higher numbers in Viborg for three out of four years, however, counting *Alternaria* spp. at genus level does not provide the information on dominant species of airborne *Alternaria* spores within a region and it does not reflect *Alternaria* species’ seasonal variation. In Denmark, there are four dominating classes of crops: grasses, grain, root vegetables, and straw (SM Tab.1). Fungal infestation of agricultural crops has been studied mainly for the purpose of disease prevention. Therefore, it is known, that *A.solani* can cause diseases in potatoes as in Denmark (Abuley et al. 2018; Nielsen 2015) as in other countries (Escuredo et al. 2011; Leiminger and Hausladen 2012). However, the period of root vegetable’s harvesting normally takes place outside the observed airborne *Alternaria* spore seasons. The species of the sections *Alternaria* and *Infectoriae* (Lawrence et al. 2015; Lawrence et al. 2013) have been often found on grain crops: *A. infectoria* in Norwegian barley, wheat, and oat grains (Kosiak et al. 2004) and harvested ripe kernels of malt barley in Denmark (Andersen et al. 1996), in wheat and barley grains in European Russia (Gannibal 2018) and in oat grains in north-western Russia (Gavrilova et al. 2016). *A. alternata* and *A. tenuissima* were also identified on grasses (Gannibal and Yli-Mattila 2005). A recent research with barcoding on fungal communities in near surface air of oilseed rape fields across north-western Europe demonstrated similar patterns of pathogen abundance for distant locations with similar climates (Denmark, Netherlands, and UK) (Nicolaisen et al. 2017). These findings suggest, that crop fields at several locations within the same climatic zone can be affected by the same species of *Alternaria*. Furthermore, we can hypothesise that as the time of grain, grasses, and straw harvesting coincides with the highest monthly SIn in Denmark, the species *A. infectoria*, *A. alternata*, and *A. tenuissima* may be dominating in the spore counts of the months with highest SIn, after being released during combine harvesting at local and distant fields. Moreover, these species’ contribution to the total number of spores may be higher for the station in Viborg than in Copenhagen, as the rooftop samples reflect a mix over a range of environments (Nicolaisen et al. 2017), different for Viborg and Copenhagen (SM\_Tab. 2).

The morphological differentiation of the dominating species in the air of Denmark as well as at other locations, can provide a missing link for defining the main sources of airborne *Alternaria*.

4.3 Back-trajectories analysis

The differences in the trajectories direction between the low and high concentration days had a similar pattern for both stations, i.e. an increase in the number of air masses coming from the South-East direction on the high days and an increase in the number of air masses coming from the North and North-West directions during the low concentration days. In case of the Viborg station this pattern was more distinctive, as very few trajectories were distributed over Germany and Poland on the low days at the station, while on the low days at the Copenhagen station, 14.5% of trajectories were coming from the South, passing over northern/north-eastern Germany.

Agricultural source-areas in northern Germany and northern/central Poland have previously been associated with high *Alternaria* spore concentrations measured at Copenhagen station (Skjøth et al. 2012). However, the summer winds coming from the South-East are also associated with dry, warm, and stable weather in Denmark (Theilgaard 2007), i.e. the weather suitable for harvesting in Denmark. We found higher precipitation on the low concentration days along with increased number of trajectories coming from the West, North-West and North (Tab.2, Fig. 4, Fig.5). Similar large-scale weather patterns (dry, warm weather with eastern and south-eastern winds and rainy, unstable weather with western, north and north-western winds) were reported in recently published research with birch pollen air concentrations in two Swedish cities, Gothenburg and, located in ≈30 km South-West from the Copenhagen station, Malmo (Grundström et al. 2017). Higher precipitation on the low concentration days may have led to the reduced harvesting conditions in Denmark, and, therefore, to the decreased *Alternaria* spore dispersion and increased spore deposition. Furthermore, on the high concentrations days diurnal bi-hourly concentrations followed the local diurnal temperature and relative humidity fluctuations (Rotem 1994), which indicates the local nature of the sources (Skjøth et al. 2012). The fact, that during the night and early morning the average bi-hourly concentrations were close in values for both stations and during the day the bi-hourly concentrations were higher at the Viborg station may also be related to the more intensive local harvesting on the larger number of agricultural sources rather, than to the contribution of spores from the remote sources.

On the high concentration days in Viborg 17% of the trajectories were originating in Scotland, 12% - in central/southern England and 20% in the North Sea (Fig. 5a). The areas in the central England (Derby and Leicester) have been shown to measure high *Alternaria* annual SIns (Skjøth et al. 2016). Considering the possibility of *Alternaria* spores long distant transport from the UK, one has to bear in mind the more than 1000 km between Denmark and the UK. Although it has been shown that *Alternaria* spores can traverse large distances, the number of spores able to undergo a long distant transport is negatively correlated with the distance. If the contribution from the long distant transport of spores released in the UK can be considered negligible, more than a half of air masses arriving in Viborg on the high concentration days were passing over western Denmark, i.e. over the areas with high number of local *Alternaria* sources (Skjøth et al. 2012). The rest of the trajectories (45%) were coming from the East and South East, originating in the areas of continental Europe (Poland, Germany) and southern Sweden, which are in closer proximity to Viborg than the UK. However, the same air masses have passed over the agricultural sources located in the central and southern Denmark, which in case of lower precipitation values on the high days, were, at least partially, undergoing harvesting.

On high concentration days in Copenhagen 42% of the trajectories were passing over Jutland, with other 58% passing over northern Germany, central/northern Poland and southern Sweden (Fig. 4a). Although the distance between Viborg and Copenhagen is below 250 km, the location of Copenhagen in terms of distant and local (Danish) sources availability is rather different from Viborg. Copenhagen is mainly surrounded by the Baltic Sea, which divides it from continental Europe with less than 200 km distance and with around 20 km from southern Sweden. According to the inventory map of *Alternaria* potential sources in central and northern Europe, the highest density of the sources is located in Western Denmark, Southern Scania (Sweden), central/northern Germany and Poland (Skjøth et al. 2012). *Alternaria* seasons were on average two weeks shorter in Viborg, which might be attributed to the shorter period of observations at this station. However, the annual daily average time-series curve for Viborg did not reach the basal levels only in the end of 2014 season (Fig.2). The peak concentrations dates happened around the same dates at both stations (with a maximum variation of 4 days), except for 2012, when *Alternaria* annual indices and annual peak had exceptionally low values, at the Viborg station particularly (Tab.1). This complies with the previous findings of a rather synchronized peak concentrations in the middle of summer in central-northern Europe in general, presumed to be caused by accumulative effect of simultaneous crop harvesting in several European regions (Skjøth et al. 2016). Therefore, the season of *Alternaria* spores in the air of Viborg might be considered to start some days later compared to Copenhagen with the major numbers of spores distributed closely around the peak day. Assuming, that higher *Alternaria* spore air concentrations are registered earlier in more southern locations, the longer *Alternaria* season in Copenhagen may have been caused by the intermittent transport of spores from the continent, where the season had already begun. Furthermore, the accumulation of heat above the threshold, necessary for *Alternaria* sporulation during the growing season (growing degree days) and closely related to the season start (Skjøth et al. 2016), could have reached the critical values earlier in Copenhagen due to slightly warmer temperatures. The end of the season (90% method) was reported to happen later in the south-eastern direction in the central and eastern Europe (Kasprzyk et al. 2015). The same may be true for the northern Europe as the Copenhagen station, apart from being located somewhat to the South-East from the Viborg station, may be affected by *Alternaria* continental sources in Poland, which are also located to the South-East from Copenhagen, and possibly characterized by a later end of *Alternaria* season. Thus, *Alternaria* September monthly SIn can reach 2,000 day s m-3 in Poznan (Kasprzyk et al. 2013; Kasprzyk et al. 2015) or 4,000 day s m-3 in Szczecin (Grinn-Gofroń and Mika 2008) whereas in our study the highest September SIn in Copenhagen was equal to 854 day s m-3.

The areas of Southern Sweden, previously associated with distant transport episodes of high *Alternaria* concentrations in Copenhagen, may also have contributed to the high concentrations at both stations, with the maximal back trajectories shares of 17% and 19% for Copenhagen and Viborg accordingly.

5. Conclusions

Our results show that airborne *Alternaria* spore concentrations daily averages above 100 s m-3 occur more often and reach larger values in western Denmark than in eastern. Additionally, monitoring at the Copenhagen station was not able to register 34 days out of 96 with daily average above 100 s m-3 measured in Viborg. This implicates that the quality of the daily national report on *Alternaria* spores air concentrations in Denmark may benefit from additional routine monitoring in Viborg. The higher *Alternaria* spore concentrations observed in Viborg suggest higher allergen exposure in the area. However, this would have to be supported by measuring of Alt a 1 allergen air content at the stations. Alternatively, air spore monitoring at both stations can be expanded by counting spores of other genera in the order *Pleosporales*, whereas counting *Alternaria* airborne spores at the species level will allow to determine the most dominant ambient species in Denmark and provide the necessary link to understanding the nature of their sources. Additionally, data on species level can contribute to evaluating *Alternaria* species-specific differences in spore allergenicity.

The days of high *Alternaria* spore air concentrations can be expected during good summer weather conditions, defining the start of grain harvesting. Sunny, warm weather established for several consecutive days most probably means that *Alternaria* spore air concentrations have risen and will remain high (especially in the western part of Denmark) within the next days if the precipitation is minimal.

The differences between the years in the values of ASIns at a station did not correlate with the amount of harvested grain or with the amount of precipitation measured at the closest meteorological station. Instead, these variations seemed to be better explained by the overall weather conditions on a larger scale, which orchestrated the time of harvesting and probably also the *Alternaria* inoculum propagation. Colder temperatures, higher precipitation, increased number of rainy days and harvesting period distributed over longer time, with the absence of the concurrent grain harvesting in Denmark were found to be the main reasons for the lowest ASIns at the stations in 2012 (whether this was a local phenomenon needs further elucidation), whereas the warmest spring and summer along with intensive and compressed periods of harvesting were accompanied with the largest ASIns of 2014.

The monthly SIns had similar annual distributions at the stations and matched the time of grain harvesting activities in Denmark. However, measurements of *Alternaria* spore emissions at the species level during straw and grass harvesting in Danish fields may clarify the importance of these crops as possible airborne *Alternaria* spore sources, and not solely for Denmark.

We found similar patterns of air mass transport for both stations: the increased shares of trajectories coming from the South-East on the high concentration days and increased shares of air masses coming from the North-West on the low concentration days. On the high concentration days the air masses originating in the areas of northern Germany, Poland and southern Sweden comprised 58% of trajectories for Copenhagen and ≅45% for Viborg. Their shares decreased to 12% for Viborg and to 30% for Copenhagen on the low concentration days. Therefore, the sources located in these areas could influence *Alternaria* spore concentrations in the air of both eastern and western Denmark. However, more abundant precipitation on the low concentration days at both stations may have been the reason for the decreased harvesting possibilities in Denmark or for harvesting of wet crops, leading to the decreased *Alternaria* spore dispersion and to the increased wet deposition of airborne spores. The higher *Alternaria* spore concentrations in western Denmark during the days with average *Alternaria* concentrations ≥ 100 s m-3 support the hypothesis, that local sources cause the main load of airborne *Alternaria* spores in Denmark. However, the contribution from the remote sources remains unquantified.

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