Grain harvesting as a local source of *Cladosporium* spp. in Denmark

Yulia Olsen*, Tanja Begovic, Carsten Ambelas Skjøth, Karen Rasmussen, Ulrich Gosewinkel, Ole Hertel, Torben Sigsgaard

Yulia Olsen; Torben Sigsgaard
Department of Public Health, Aarhus University, Bartholins Allé 2, 8000 Aarhus, Denmark

Tanja Begovic; Ulrich 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, UK

Karen Rasmussen
The Asthma and Allergy Association, Universitetsparken 4, 4000 Roskilde, Denmark

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

Abstract: *Cladosporium* spp. are omnipresent moulds that grow on multiple substrates. Their spores possess a high allergenic potential. Currently, little is known about the incidence and the sources of airborne *Cladosporium* spores in Denmark. Air samples were collected between 31 May and 22 September 2015 in Viborg (Jutland, western Denmark). Eighteen out of 21 days with daily average concentrations exceeding the health relevant threshold of 3,000 Spores m-3, including the day with peak daily (13,553 Spores m-3) and 3-h concentrations (35,662 Spores m-3), occurred in August. The air masses that approached Viborg during the longest episode of elevated spore concentrations originated from northern Poland, the Baltics, passing over southern Sweden and the eastern Danish island of Zealand. The *Cladosporium* spore concentrations from Viborg were compared with the *Cladosporium* spore concentrations from the operational monitoring station in Copenhagen (Zealand, eastern Denmark). During the episode concentrations in Viborg were on average 2,268 spores m-3 higher than in Copenhagen. On the peak day between 8:00-15:00 concentrations in Viborg were 4-7 times higher than in Copenhagen, which we associated with grain crop harvesting in eastern Jutland. Elevated day time concentrations in Viborg on the days with daily average concentrations exceeding the threshold also indicate the local character of the sources.

Keywords: Aerobiology; *Cladosporium* spp.; Back Trajectories; HYSPLIT; Airmass Transport; Grain harvesting.

**Corresponding author e-mail: yo@ph.au.dk*

1 Introduction

In many areas conidia of genus *Cladosporium* can account for more than 80% of all measured air spora. Spores of *Cladosporium* spp. are well known aeroallergens, that affect the severity of asthma and allergic rhinitis in sensitized persons (Denning et al. 2006). *Cladosporium* spp. grow on multiple substrates but mainly on decaying organic material, which complicates assigning them to specific sources (Awad 2005). Additionally, due to their small size, these spores can remain airborne for days while being transported from the source over great distances by wind in the absence of precipitation. Identifying the geographical source area assists in determining the possible sources of *Cladosporium* spores. The biological cycles and physical treatments, as e.g. agricultural operations, typical for the established sources, can provide an important insight on the expected time window for intensive spore release, which can aid in managing allergy outbreaks.

2 Materials and methods

Airborne spores of *Cladosporium* spp. were sampled by the Asthma and Allergy Association on the roof of Regionshospitalet, 21 m above sea level in Viborg (56° 27'N 9° 24'E) during 115 days, 31 May–22 September 2015, on the 48x14mm slides using a Hirst-type spore trap (Hirst 1952). The spores were counted at the genus level along 8 transversal transects at 6 mm distance between each under 630x magnification with further conversion to the spore air concentrations with time resolution of 3h. Spore Integrals (SIns) were calculated by summing the average daily concentrations over the specified periods (Galan et al. 2017).

The longest period of days (14–25 August) with daily average concentrations above the health relevant threshold of 3,000 Spores m^{-3} (Gravesen 1979), containing the peak daily (Fig.1) and 3-h concentrations (Fig.2a), together with the preceding day (13 August) and the following day (25 August) were selected for a detailed analysis of air mass transport by back trajectories (Fig.2b). The back trajectories were computed by the HYSPLIT model (Draxler and Hess 1998) with the Global Data Analysis System (GDAS) meteorological files in the form of a 1^{o} latitude-longitude grid with a receiving height of 500m and plotted 48h back in time with 3-h interval. The character of air mass transport on these days presented an opportunity to compare 3-h *Cladosporium* spp. concentrations at the Viborg station and at the Copenhagen station, the latter provided by the Asthma and Allergy Association. The description of the Copenhagen station can be found elsewhere (Skjøth et al. 2012). The maps of grain agricultural fields (Fig.2c, Fig.2d) were created in QGIS ver. 2.18 (https://qgis.org/en/site/).

The intra-diurnal *Cladosporium* spore cycles were plotted for every 3h of day as an average of corresponding concentrations at the Viborg station.

Daily precipitation, daily averages of temperature (T_{mean}) and relative humidity (RH) were measured at the meteorological station in Foulum (56° 29'N 9° 34'E).

3 Results

SIn over the study period totalled 218,151 Spore day m^{-3} with the average T_{mean}= 14.3 ℃ and RH = 79.5%. The largest rainfall was observed in June (109.5mm), followed by July (72.5mm), September (64.4mm) and August (55 mm). August was the warmest month (T_{mean}=16.9℃), whereas June was the coldest (T_{mean}=12℃). A positive statistically significant relationship with daily spore concentrations was found only with T_{mean} (r=0.69), whereas insignificant close to zero spearman correlation coefficients were found for daily precipitation (r=-0.03) and RH (r=-0.06). The highest spore concentrations were measured in August, which monthly SIn constituted 59.21% of the study SIn and equalled to 129,171 Spore day m^{-3}, while July and September SIns were around 40,000 Spore day m^{-3} each.

Eighteen (out of 21) days with daily average concentration above 3,000 Spores m^{-3} occurred in August, contributing up to 80% of August SIn. The peak daily average and 3-h concentrations were measured on 16 August (Fig.1; Fig.2A).

During the 11 days, 14–24 August, the air masses were arriving from the East and South East, i.e. originating in the areas in Poland, the Baltic countries, and north-west Russia, passing over the Baltic sea and southern Sweden (Fig.2b). Conversely, on 13 August and on 25 August the wind directions were distinctively different with the air masses arriving from the North- and South-West (Fig. 2b). In the course of those 11 days the corresponding daily average concentrations at the Copenhagen station were lower than at the Viborg station, with the exception of 15 August, when the daily spore concentration in Viborg dropped down to 1,979 Spores m^{-3} while precipitation increased to 12.1 mm. On 16 August the daily average concentration at the Copenhagen station was less than half (5,685 Spores m^{-3}) of the concentration at the Viborg station (13,553 Spores m^{-3}).

Maps of possible agricultural sources associated with the increase in *Cladosporium* spore air concentrations during 14-24 August are shown in Fig.2c-d. In the area of eastern Jutland agricultural fields with winter grain seeds were the main agricultural land cover (Fig.2c), with winter wheat as the dominating type of crop (Fig.2d).

The diurnal cycle of *Cladosporium* spores (Fig.3) on the days with daily average concentrations above 3,000 Spores m^{-3} had a maximum between 08:00 and 10:00. However, after excluding the high concentrations on 14 August and 16 August, the diurnal distribution reflected elevated concentrations between 06:00 and 20:00 (Fig.3). No clear diurnal pattern was found for the other 94 days with daily average concentrations below the threshold (Fig.3).

4 Discussion

Cladosporium conidia belong to the so-called dry spores, as higher temperatures and absence of precipitation have been frequently observed to facilitate the increase in their air concentrations (Kasprzyk et al. 2016; Aira et al. 2012). The value of spearman correlation coefficient for the T_{mean} found in this study is comparable to the reported previously, and despite the absence of correlation with precipitation, the highest concentrations of spores were observed on the dry days.

The monthly SIns, the number of days with average concentration above 3,000 Spores m^{-3}, and the value of the peak concentrations in Viborg were lower compared with the values reported from Poland (Kasprzyk et al. 2016; Grinn-Gofroń and Mika 2008), England (Sadyś et al. 2016), and France (Sindt et al. 2016) but higher than those found in Morocco (Bardei et al. 2017), Croatia (Peternel et al. 2003) and northern Portugal (Oliveira et al. 2009). The climatic conditions and the type of land cover determine both the availability of organic material in the form of vegetation, that is prerequisite nutrient for fungi, and the growth of fungi mycelium leading to sporulation. Therefore, *Cladosporium* air concentrations vary between different locations and between different years at the same location. We found August to be the most important month in terms of airborne *Cladosporium* spore concentrations in 2015. This mono-modal monthly distribution could reflect a typical pattern for *Cladosporium* in Viborg. However, in 2015 the T_{mean} in July was 2 degrees lower than in August. This is unusual for Denmark, as July and August are normally the warmest months of the year with ca. equal monthly T_{mean} values (www.dmi.dk). The daily pattern with higher concentrations between 08:00 and 17:00 based on the 19 days when *Cladosporium* concentrations were exceeding the health relevant threshold in Viborg (Fig.3) indicates a local character of the sources rather than a distant.

On the peak day (16 August) the air masses originated in Latvia and western Belarus, passing over Lithuania, north-western Poland, southern Sweden, and northern Zealand including Copenhagen (Fig.2b). Long distance transport of air masses originating from the sources in Poland, Germany, and southern Sweden has been shown to affect air concentrations of birch pollen and *Alternaria* spores in Copenhagen (Skjøth et al. 2012; Skjøth et al. 2007). Similarly, the influence of the distant sources in southern Sweden, the Baltics, and Poland on *Cladosporium* air concentrations in

Viborg was visible in this study during the longest period of elevated concentrations (14–24 August) (Fig.2b). The coastal position of Copenhagen is exceptional in the sense that its urbanised territory extends 20-30 km to the West and North, whereas the East and South of the city are surrounded by the sea. Thus, in case of the wind coming from the East and South, high concentrations of aerobiological particles in Copenhagen would be mainly influenced by the sources located overseas. Viborg, unlike Copenhagen, is located in the centre of the Jutland peninsula and separated from the east coast by ca. 100 km of mainly agricultural land. Therefore, the concentrations in Viborg which were 4-7 times higher than in Copenhagen on 16 August (between 08:00-15:00) may have been supplemented by the yield of the sources situated in closer proximity, i.e. in eastern Jutland. Additionally, during 14–24 August the daily *Cladosporium* concentrations in Viborg were on average 2,268 Spores m^{-3} higher than in Copenhagen, which also indicates the influence of local sources. According to the annual Danish report by the Agricultural Agency (Pedersen 2015), 14-24 August coincided with spring and winter grain crops harvesting activities.

This study provides the evidence that distant sources located in the Baltics, northern Poland, and southern Sweden can affect air concentrations of *Cladosporium* in both eastern and western Denmark. However, harvesting of grain crops, such as winter wheat, spring barley, winter rapeseed, barley, rye, winter and spring oats may have contributed to the peak concentrations of *Cladosporium* in Viborg. A longer time-series in the eastern part of Denmark will help to estimate the yield of local sources to peak *Cladosporium* concentrations in the area and to establish *Cladosporium* monthly distribution and season duration. Additionally, the routine monitoring of spore concentrations in Viborg can improve the accuracy of public information on *Cladosporium* concentrations, which is highly relevant for sensitized individuals.

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