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Airborne *Cladosporium* and *Alternaria* spore concentrations through 26 years in Copenhagen, Denmark.

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Abstract

Cladosporium spp. and *Alternaria* spp. spores are dominating the airspora of Denmark. Currently, little is known about the influence of climate change on the fungal spore abundance in the air. The aim of this study was to examine temporal changes in airborne *Alternaria* and *Cladosporium* spores over 26 years. This is the first report of long-term airborne *Cladosporium* spore occurrence in Denmark. Air spore concentrations were obtained with a Burkard volumetric spore sampler placed in Copenhagen, Denmark, during June – September, 1990-2015. The highest monthly Spore Integrals (SIn) for *Alternaria* were measured in August, whereas for *Cladosporium* July SIn was nearly as high as August SIn. Average *Alternaria* Seasonal Spore Integral (SSIn) was 8615 Spores day m^{-3}, while average 3-month (July – September) *Cladosporium* SIn was 375,533 Spores day m^{-3}. Despite increasing annual temperature and decreasing relative humidity, we found a decreasing trend for *Alternaria* seasonal SIn (Slope = -277, R^2=0.38, p<0.05), *Alternaria* (Slope = -31, R^2=0.27, p<0.05) and *Cladosporium* (Slope = -440, R^2=0.23, p<0.05) annual peak concentrations. We did not find statistically significant trends for airborne *Alternaria* seasonal characteristics and duration, and neither for *Cladosporium* 3-month SIn and peak dates. Mean temperature was the main meteorological factor affecting daily spore concentrations. However, effect of meteorological parameters on daily spore concentrations was stronger for *Cladosporium* (R^2=0.41) than for *Alternaria* (R^2=0.21). Both genera had diurnal peaks during the day hours, earlier for *Cladosporium* (11:30 – 14:30) and later for *Alternaria* (15:00 – 19:00). Although *Alternaria* and *Cladosporium* daily concentrations were moderately correlated (Spearman's correlation coefficient: r_s = 0.55, p<0.05), their overall annual

indices were different, which indicates different sources and different factors determining spore release. We explain temporal decreasing trends in *Alternaria* SSIn by growing urbanisation around Copenhagen and by changes in agricultural practices.

1. Introduction

Cladosporium spp. and *Alternaria spp.* spores are omnipresent in the environment and are two dominating genera in the total airspora in Denmark (Larsen and Gravesen 1991). Fungal spores are well-known sources of airborne allergens (Twaroch et al. 2015; Levetin et al. 2016), amongst which *Alternaria* spp. and *Cladosporium* spp. are the most prevalent and well-studied with suggested health thresholds of 100 Spores m^{-3} for *Alternaria* and 3000 Spores m^{-3} for *Cladosporium* (Bagni et al. 1977; Gravesen 1979). In Denmark (Odense), 8.2% and 7.9% of patients with a suspected inhalant allergy were found to be sensitised to *Alternaria* and *Cladosporium*, respectively (Heinzerling et al. 2009). Increase in outdoor fungal spore concentrations have been associated with allergic rhinitis severity (Katotomichelakis et al. 2016); asthma hospital admissions/visits and severity (Stieb et al. 2000; Atkinson et al. 2006). However, knowledge on the determinants of spore production, seasonal dynamics, sources, and dispersion is still insufficient (Kasprzyk 2008; Crameri et al. 2014).

Influence of temperature and geographical location on seasonal indices of aerobiological particles is undeniable and was confirmed in multiple studies. Thus, the spatial pattern of *Alternaria* seasons was shown to have a gradient of earlier start and longer duration from the South to the North of Europe, with the latest and shortest season in Denmark (Skjøth et al. 2016). Seasonal length, however, does not appear to correlate with *Alternaria* spore abundance: relatively short *Alternaria* seasons in northern locations can have higher Seasonal Spore Integral (SSIn) values than those in locations with longer seasons (Skjøth et al. 2016). A similar geographical pattern seems to be true for *Cladosporium*: longer seasons in the South with bi-modal distributions and lower SSIn (Katotomichelakis et al. 2016; Sousa et al. 2016; Bardei et al. 2017; Almeida et al. 2018) than in central European locations with mono-modal *Cladosporium* distributions (Chrenová et al. 2004; Kasprzyk et al. 2016; Sindt et al. 2016), although *Cladosporium* incidence and seasonality are currently underreported.

Climate change is evident: rising temperatures are being observed and foreseen to increase worldwide and in Denmark (Olesen et al. 2014; Cappelen et al. 2017). Multiple biological responses to changes in climate have been examined over recent decades (Walther 2010; Boddy et al. 2014), and production of aeroallergens is believed to be increasing along with temperatures, aggravating the burden of allergic diseases (Cecchi et al. 2010; D'Amato et al. 2015; D'Amato et al. 2018). There have been several reports on long-term effect of climate change on pollen concentrations and seasonality (Rasmussen 2002; García-Mozo et al. 2014; Bruffaerts et al. 2018). Fungal spore patterns are less clearly manifested with weaker rates of temporal changes than those for pollen (Damialis et al. 2015b). In contrast to pollen, sources of airborne fungal spores are not straightforward. However, grain crops and the process of harvesting were related to increase in both *Alternaria* and *Cladosporium* air concentrations (Friesen et al. 2001; Skjøth et al. 2012; Olsen et al. 2019). Climate change has been reported to extend fungal fruiting seasons in Europe (Boddy et al. 2014), however, airborne *Cladosporium* and *Alternaria* conidia result from asexual reproduction and thus the relationship between length of fruiting seasons and airborne spore abundance is not direct. The results of only few studies, that have attempted to assess long-time trends in spore air concentrations, are inconsistent, reporting increasing *Alternaria* and *Cladosporium* trends at one location, and decreasing at another, despite common increase in temperature (Corden and Millington 2001; Corden et al. 2003; Damialis et al. 2015b; Sindt et al. 2016; Ščevková et al. 2016). Evaluating the impact of changes in climate that have taken place during recent decades on airborne fungal spore concentrations and seasonality aids in modelling future exposures and mitigating associated risks.

The aim of this study was to present the existing data and examine temporal changes in airborne *Alternaria* and *Cladosporium* fungal spore load over 1990-2015 in Copenhagen. We hypothesize that increasing temperatures positively influence fungal growth, followed by increase in spore production and, consequently, in air spore abundance.

2. Methods

2.1 Aerobiological data

This study was conducted using the data on airborne *Alternaria* and *Cladosporium* spore concentrations over 26 years from routine monitoring by the Danish Asthma and Allergy Association at the Copenhagen station. Daily spore samples were obtained by use of the Hirst type spore trap (Hirst 1952), mounted on the roof of the Danish Meteorological Institute (55°43'N 12°34'E) building at a height of 15 m above sea level. The description of the Copenhagen station can be found elsewhere (Skjøth et al. 2012). The spores were counted at 640x magnification at the genus level only by the method of vertical transects, i.e. 12 transects along the slide for *Alternaria* spores and 8 transects along 2 mm of the horizontal centre of the slide for *Cladosporium* spores, with subsequent conversions of the counts to daily average concentrations. *Alternaria* spore seasons were established using the 90% method (Nilsson and Persson 1981).

Due to the short observation periods, *Cladosporium* spore seasons could not be calculated by commonly used methods. Therefore, the investigations for *Cladosporium* air spore temporal patterns were restricted to the period of three months (from 01 July to 30 September) over 26 years (1990-2015). Spore integrals (SIn) were calculated as a sum of daily average spore concentrations during specified periods (Galan et al. 2017). Following the methodology chosen in previous aerobiological studies (Smith et al. 2014; Damialis et al. 2015a; Ugolotti et al. 2015; Sindt et al. 2016), we used simple linear regression analysis to examine annual spore trends. For *Alternaria* the following seasonal characteristics were included into the analysis: start, end, and duration of the season; peak date; and peak concentration value; seasonal spore integral (SSIn); and number of days above 100 Spores m^{-3}. For *Cladosporium* the analysis included 3-month SIn, number of days above 3000 Spores m^{-3}, peak date, and peak concentration value.

Diurnal patterns for *Cladosporium* spores were calculated as mean concentrations of 3-hourly averages over 12 years (2001-2010, 2014-2015), as only this fraction of the dataset has been digitized, while diurnal patterns for *Alternaria* spores were calculated as mean concentrations of 2-hourly averages over 26 seasons (1990-2015) with the entire dataset available in digitized format. Subsequently, spore diurnal concentrations were standardized in relation to cumulative spore concentrations within a day (assigned as 100%). To examine possible differences in the diurnal distributions, a set of diurnal profiles were plotted on the basis of daily average concentrations: above/below 100 Spores m^{-3} and 3000 Spores m^{-3} (for *Alternaria* and *Cladosporium* respectively), below 75%-centile, equal to/above 75%-centile below 95%-centile, and equal to/above 95%-centile.

2.2 Meteorological data

Daily temperature averages, i.e. minimal daily temperature – Tmin, maximal daily temperature – Tmax, daily average temperature – T mean were obtained from Kastrup station, located at the Copenhagen airport (55°36' N, 12° 38' E; 5 m above sea level). Daily averages of relative humidity (RH), wind speed (WS), and global radiation (GR), also 30-minute measurements of WS and wind direction were obtained as a part of Aarhus University monitoring programme from the station located on the roof of H.C. Ørsted institute (55°42' N, 12°33' E; 24 m above sea level), i.e. the HCØ station for 1993-2015 (Ellermann et al. 2016). Accumulative daily precipitation was measured at the Botanisk Have station (55°24' N, 12°20' E; 6 m above sea level) (Cappelen et al. 2017). As such, all meteorological stations are located 14 km or less from the Copenhagen station.

2.3 Statistical analysis

Daily spore data distribution deviated from normality, the relationship between daily spore concentrations and daily meteorological parameters was analysed by a stepwise regression performed on logarithmically transformed daily average spore data, i.e. logarithm of each datum plus one. Due to collinearity of daily mean temperature with maximal and minimal temperatures (SM Table 1), each of four temperature parameters were included separately for the analysis, and the model with the highest R^2 was chosen as final. Spearman correlation coefficients with daily meteorological parameters were calculated for each *Alternaria* spp. season and for each 3-month period (July – September) for *Cladosporium* spp. Meteorological annual trends are also presented to provide a broader picture of changes in the local environment. Wind roses were drawn in Grapher. 8.0 by using 30-minute time-series of wind direction data during the periods of all *Cladosporium* spore measurements and during *Alternaria* seasons from 1993 to 2015. Furthermore, to understand the relationship between spore concentrations and wind direction, 30-minute wind speed/direction measurements were divided into four groups according to the corresponding daily average spore concentrations: 1). below 25%-centile 2). equal to/above 25%-centile – below 75%-centile, 3) equal to/above 75%-centile – below 90%-centile, and 4). equal to/above 90%-centile and wind roses were drawn for each group separately. All statistical analyses were performed using STATA 14.2.

3. Results

Daily *Alternaria* and *Cladosporium* concentrations during *Alternaria* season were moderately correlated (r_s=0.55, p=0.000). However, for all 26 years *Cladosporium* spore seasons started earlier and lasted longer than *Alternaria* seasons with the concentrations outnumbering those of *Alternaria* (Fig. 1). On average *Cladosporium* daily mean exceeded 3000 Spores m^{-3} two-three weeks earlier than *Alternaria* daily mean exceeded 100 Spores m^{-3} (Fig. 1).

The main seasonal characteristics of annual spore records are presented in Table 1 for *Alternaria* and in Table 2 for *Cladosporium*.

Alternaria SSIn on average equalled 8615 Spores day m^{-3}, varying through the years with the highest values within the first 13 years of study including the maximum value in 1990 (15,695 Spores day m^{-3}), followed by the values in 1991 and 1995. The lowest *Alternaria* SSIns were observed in the second 13 years with the minimal value in 2012 (2351 Spores day m $^{-3}$), followed by higher SIn in 2011 and 2003 (Table 1). *Alternaria* SSIn was strongly positively correlated with the annual number of days when daily average concentrations exceeded the 100 Spores m^{-3} threshold (r_s=0.93, p=0.0000) and with seasonal peak concentration values (r_s=0.81, p=0.0000). Notably, *Alternaria* seasonal duration was not correlated with SSIn (r_s= –0.12, p=0.55) and was longest in 2000 (88 days) and shortest in 1991 (45 days) and there was no systematic pattern towards later/earlier start/end of the season. August on average had the highest *Alternaria* concentrations and monthly SIn (mean August SIn = 5501 Spores day m^{-3}; mean July SIn=2688 Spores day m^{-3}). In 19 out of 26 years, annual peak daily average concentrations were measured in August (the absolute peak on 08 August 1991: 2262 Spores m^{-3}; the absolute minimum on 17 July 2008: 313 Spores m^{-3}), in the other 7 years annual peak concentrations were measured in the second part of July (Table 1).

Daily *Cladosporium* concentrations exceeded 3000 Spores m^{-3} in June and September in most years, whereas highest concentrations of *Alternaria* were mainly distributed over a shorter period during the end of July and August. July and August were the months with the maximal presence of *Cladosporium* spores in the air (Table 2). Monthly June and September SIns were on average (21 years) by factor 3 less than those of July and August, and were negatively correlated between each other (r_s = –0.43, p=0.0495). This study is the first attempt to assess annual variation in airborne *Cladosporium* spore concentrations in Copenhagen. June SIn was highest in 1990 (180,204 Spores day m^{-3}) and September SIn in 2014 (126,832 Spores day m^{-3}) (Table 2). The 26 years average value of 3-month *Cladosporium* SIn was 375,533 Spores day m^{-3}, with the maximum 3-month SIn measured in 1994 (527,281 Spores day m^{-3}), and the minimal in 2007 (149,281 Spores day m^{-3}). The highest daily average for *Cladosporium* was measured on 19 July 1993 (32,345 Spores m^{-3}) (Table 2). Similarly to *Alternaria*, number of days with *Cladosporium* daily average exceeding 3000 Spores m^{-3} (r_s =0.81, p=0.0000) and peak concentrations values (r_s =0.72, p=0.0000) were strongly correlated with 3-month *Cladosporium* SIn.

We found a statistically significant negative trend over 26 years for *Alternaria* SSIn (Fig. 2a). Highly correlated with *Alternaria* SSIn, number of days exceeding the threshold (Slope = –0.7, R^2=0.36, p=0.001) and peak concentration values (Fig. 2b) also had negative statistically significant trends. No significant statistics were found for the start, end, or peak dates, nor for the seasonal duration.

Simple linear regression of 3-month *Cladosporium* SIn against 26 years had a negative but not significant direction (Slope = –3433, R^2 = 0.05, p = 0.26). The number of days with daily average concentrations exceeding 3000 *Cladosporium* spp. Spores m^{-3}, correlated with 3-month SIn, did not change with time (Slope = 0.02, R^2= 0.0001, p=0.97). However, the value of peak *Cladosporium* concentrations had a statistically significant decrease (Fig. 2b).

Cladosporium and *Alternaria* peak concentration dates were not synchronized (r_s= 0.02, p=0.92), and in 15 years, except in 2004 (when the peak concentrations were measured on the same day), *Alternaria* concentrations peaked after *Cladosporium*.

Temporal trends of selected meteorological parameters are shown in Fig. 3. Mean and minimal temperatures had positive and RH negative statistically significant annual trends (Fig. 3a, c-d). Seasonal changes in T mean were statistically increasing only in fall (Fig. 3b), minimal temperature had significant increases in summer and fall, RH had statistically significant negative slope in all seasons. Neither *Cladosporium* annual (three-months) SIn, nor *Alternaria* seasonal SSIn were correlated with annual/seasonal/3-months mean meteorological parameters including cumulative precipitation over the corresponding periods, except for moderate positive correlation between *Alternari*a SSIn and annual RH (r_s=0.46, p=0.03).

Both genera showed moderate correlations with daily temperature parameters (SM Table 2). Daily average RH had a weak negative and GR had equally weak positive correlation with both genera. Both *Cladosporium* and *Alternaria* daily spore concentrations exhibited little correlation with wind speed and daily precipitation, although the latter was stronger correlated with *Alternaria* than with *Cladosporium* (SM Table 2).

In stepwise regression model, daily average concentrations for both genera had the best fit with daily mean temperature. Second important parameter for *Alternaria* was WS, whereas it was not significant for *Cladosporium*. The final results suggest that variation in *Cladosporium* spore concentrations can be better explained by daily changes in meteorological parameters (R^2=0.41) than variation in *Alternaria* (R^2=0.21) (Table 3).

Diurnal patterns for both *Cladosporium* and *Alternaria* had minimal concentrations in the morning, i.e. at around 05:30h for Cladosporium and between 05:00h to 07:00h for Alternaria (Fig. 4a-d). However, *Cladosporium* concentrations peaked earlier (11:30 – 14:30) (Fig. 4c-d), compared with *Alternaria* (15:00 - 19:00) (Fig. 4a-b). Furthermore, on the contrary to *Cladosporium*, for which the diurnal peak (11:30h –14:30h) was only more evident with increasing daily concentrations (Fig. 4c-d), *Alternaria* diurnal peak shifted to later hours on the days with daily average above 100 Spores m^{-3} (Fig. 4a-b). Thus, on the days with the highest daily concentrations, i.e. above 75%-centile (184 Spores m^{-3}), the diurnal maxima occurred at 19:00h (Fig. 4b), compared with the peak at 15:00 –17:00 on the days with lower daily concentrations (Fig. 4a-b).

Overall through 26 years, westerly wind direction was dominating during the periods of available spore concentrations data and during *Alternaria* seasons: spikes from South-West to North-West direction comprised 37%-39% of all 30-minute wind direction data, followed by 15-18% from South-South-West to South-South-East directions, and 8-10% from East-North-East to East directions with very few winds coming from North (Fig. 5). Highest wind speeds (above 8 s m^{-1}) were associated mainly with western winds. Increases in daily average spore concentrations (both *Alternaria* and *Cladosporium*) were associated with higher shares of South to South-East directions (Fig. 5), for *Alternaria* higher concentrations were also associated with increase in East-North-East to East wind directions (Fig. 5).

4. Discussion

Our results confirm previous findings of peak *Alternaria* concentrations in Denmark occurring between late July and mid-August with an average season duration of 2 months, which is shorter than reported for more southern locations (Skjøth et al. 2012; Skjøth et al. 2016).

The *Cladosporium* season in Denmark starts earlier and ends later than that of *Alternaria*'s with highest *Cladosporium* spore concentrations in July and August. Every year *Cladosporium* daily concentrations exceeded 3000 Spores m^{-3} more frequently than *Alternaria* daily concentrations exceeded 100 Spores m^{-3}. While in majority of European countries sensitisation rates to *Alternaria* are much higher than those to *Cladosporium* among individuals with a suspected inhalant allergy, in Denmark the rates are nearly equal (Heinzerling et al. 2009). This indicates that in Denmark airborne *Cladosporium* may be a more important aeroallergen than *Alternaria*.

Mono-modal *Cladosporium* annual distributions, similar to those found in our study, were reported mostly in central and northern European locations, e.g. France (Sindt et al. 2016), Poland (Grinn-Gofroń and Mika 2008), the UK (Hollins et al. 2004; Sadyś 2017), Slovakia (Chrenová et al. 2004; Ščevková and Kováč 2019). The average, 3-month *Cladosporium* SIn in Copenhagen was higher than long-term SSIn averages in the southernmost areas of France Bordeaux and Toulouse, but lower than in three other French cities (Sindt et al. 2016). However, annual *Cladosporium* SIn averages over 1993-2009 at 10 out of 12 stations in the Iberian Peninsula had lower values: 54,459-238,214 Spores day m^{-3}, with higher values in Merida (579,953 Spores day m^{-3}) and Sevilla (933,485 Spores day m^{-3}) (Aira et al. 2012). Annual SIn, lower than 3-month SIn averages in Denmark, were reported for Southern Portugal (Almeida et al. 2018) and Madeira Island (Sousa et al. 2016) at 92,351 – 123,863 Spores day m^{-3}, and for the Western Thrace region in Greece at 120,464 Spores day m^{-3} (Katotomichelakis et al. 2016). *Cladosporium* SIn values close to or lower than the 3-month Copenhagen *Cladosporium* SIn were reported for northern Poland (annual SIn = 392,670 Spores day m^{-3}) (Bednarz and Pawlowska 2016) and North-West Morocco (SSIn 296,707 – 417,929 Spores day m^{-3}) (Bardei et al. 2017), whereas annual SSIn reported for western and eastern Poland were at least twice higher than the 3-month Copenhagen *Cladosporium* SIn

(Kasprzyk et al. 2016; Weryszko-Chmielewska et al. 2018). Peak daily values followed the same geographical trend as SIn, i.e. with lower values generally reported in North West Morocco 6242 – 13,270 Spores m^{-3} (Bardei et al. 2017), Southern Portugal 1273 – 1966 Spores m^{-3} (Almeida et al. 2018), at 11 stations in Iberian Peninsula 4920 – 16,182 Spores m^{-3} (Aira et al. 2012), in Slovakia 13,812 Spores m^{-3} (Ščevková and Kováč 2019) and in Ireland 11,397 Spores m^{-3} (O'Connor et al. 2014). Higher peak values were reported for France 20,212 – 67,392 Spores m^{-3} (Sindt et al. 2016), the UK 46,831 Spores m^{-3} (O'Connor et al. 2014), and for eastern and western Poland 27,005 – 63,983 Spores m^{-3} (Kasprzyk et al. 2016).

Only few studies have been conducted on long-term trends of airborne fungal spore concentrations. Upward trends of long-time series of *Alternaria* through 1970-1996 and 1970-1998 were reported for Derby, UK (Corden and Millington 2001; Corden et al. 2003) and were associated with increased cereal production and rising temperatures, while the trend was downward at the coastal station in Cardiff which is remote from arable lands (Corden et al. 2003). Our results provide an additional evidence of a downward trend for *Alternaria*, measured at the station that bears some similarity to the Cardiff station, due to its coastal position and urbanised land cover. Damialis et al. (2015), on the contrary, found a positive, though not significant, trend for *Alternaria* concentrations in Thessaloniki, Greece during 1987-2005. However, out of 14 analysed fungal taxa, 12 had negative trends, including statistically significant decrease in *Cladosporium* concentrations (Damialis et al. 2015b). Rizzi-Longo et al. (2009) published a detailed paper on seasonal occurrence of *Alternaria* during 12 years (1993 – 2004) in Trieste. Although the authors have not reported temporal trends, the data presented on *Alternaria* SSIn were clearly not decreasing with a more than twice higher annual SIn than the average SIn observed in 2003 (Rizzi-Longo et al. 2009). Absence of a temporal trend over 12 years in airborne *Alternaria* spore data in Bratislava (Slovakia), was associated with reduced agricultural land use around the city (Ščevková et al. 2016).

The negative trend in peak *Cladosporium* spore concentrations, found in this study, indirectly implicates a possible decreasing tendency for average *Cladosporium* spore concentrations rather than increasing, which would be in agreement with the negative trend in Thessaloniki. Sindt et al. (2016) found decreasing *Cladosporium* concentrations at two southernmost stations out of five stations in France, where three remaining stations showed an upward tendency in parallel with an increase in annual temperature, giving a statistically significant trend in Bordeaux (Sindt et al. 2016).

Similar to several of the previous studies, day-to-day *Alternaria* and *Cladosporium* concentration variations were best explained by temperature parameters, with daily average showing the best fit in the analysis. Sadyś (2017) found that the highest *Cladosporium* concentrations were associated with WS below 2.5 m s^{-1}. In our results, daily *Cladosporium* concentrations were not correlated with WS, and the highest centile (above 90%-centile) of daily average concentrations were associated with WS in the range above 2 m s^{-1} and below 8 m s^{-1}. The same was observed for *Alternaria*. However, as discussed earlier (Grinn-Gofroń et al. 2019), long-term precipitation data that are often available only at daily temporal resolution and, therefore, cannot be used to relate spore concentrations to rain fall. Thus, in this study precipitation and RH had very weak negative correlation with *Alternaria*, and even weaker correlations with *Cladosporium*, such that precipitation was removed from the final stepwise regression model for *Cladosporium*. Likewise, daily cumulative precipitation was found to be the least important factor for *Alternaria* and *Cladosporium* spore concentrations in a recent study on long-term monitoring data (1987-2015) from 18 sites across Europe (Grinn-Gofroń et al. 2019).

The positive trend for the annual temperatures that we found for Copenhagen in our research is in agreement with reported climate changes during the last decades in Denmark (Cappelen et al. 2017). Moreover, the decreasing trends in SIn and peak concentrations were accompanied by statistically significant positive trends in annual T mean, T min and a negative trend in RH. Notably, positive trend in T min was statistically significant during the period when highest concentrations for both *Alternaria* and *Cladosporium* are normally measured, i.e. in summer and fall, whereas the annual trend for T mean was mainly driven by statistical increase in fall temperatures. It is known that *Cladosporium* and *Alternaria* spores belong to so-called dry spores, whose concentration in the air is increased during hours with lower RH and higher temperatures. Average daily temperature is the main meteorological factor affecting day to day variation in both genera (Aira et al. 2012). However, observed annual changes in climate contradict the observed decline in *Alternaria* spore concentrations. Interestingly, in an experimental study with various temperatures, spores of *A. alternata* were found to be decreasing along with increasing temperatures, while mycelium growth was increasing, and the authors concluded that spore production in the future will be decreased (Damialis et al. 2015a).

Climate change can affect the availability of nutrients for fungal growth in terms of amount and timing and seasonality. It can be speculated that warmer temperatures, especially in fall, may prolong the green season thereby delaying both the seasonal peak and the end of season. Thus, in an *Alternaria* trend study in Bratislava, increasing winter temperatures were correlated with earlier start, later end and peak concentration dates, resulting in a longer *Alternaria* season (Ščevková at al. 2016). We did not find systematic temporal changes in those *Alternaria* seasonal characteristics. Our results neither support the hypothesis of tendency to shorter duration and earlier onset of main spore seasons reported by Damialis et al. (2015). The influence of temperature on airborne *Cladosporium* seasonal duration was recently reported (Kasprzyk et al. 2016). However, we could not analyse trends for seasonal characteristics of *Cladosporium* spore concentrations due to the limitations in our data. However, interestingly, however, peak dates did not have a tendency for delay.

Changes in local climate, vegetation patterns, and management of landscape were found to be the governing parameters for airborne *Alternaria* spore concentrations (Skjøth et al. 2016). The increase in annual average temperatures and the decrease in relative humidity contradict the negative direction of *Alternaria* seasonal load observed in our study. Nonetheless, there is a growing burden of evidence, that remoteness from agricultural fields plays a significant role in fungal spore abundance in the air, with most reports concerning *Alternaria* (Simeray et al. 1993; Mitakakis et al. 2001; Şen and Asan 2001; Corden et al. 2003; Awad 2005; Rodríguez-Rajo et al. 2005; Rizzi-Longo et al. 2009; Skjøth et al. 2012; Aira et al. 2013; Kasprzyk et al. 2013; Fernández-Rodríguez et al. 2015; Sadyś et al. 2015; Olsen et al. 2019b). Thus, synchronized *Alternaria* peak concentrations for central-northern Europe indicate that despite the existing differences in climate and seasonal durations, agricultural processes such as grain harvesting can stimulate peak *Alternaria* concentrations on a large scale if they happen simultaneously (Skjøth et al. 2016). In particular in Denmark, high *Alternaria* concentrations have been shown to be associated with local wheat harvesting (Skjøth et al. 2012), and the periods of highest concentrations of *Alternaria* spores at two stations (Viborg and Copenhagen) coincided with the periods of grain harvesting (Olsen et al. 2019b). Since 1990, the major trend in the regional land use around Copenhagen has been an increase (by 7.2%, data for 1990-2006) in artificial surface covers, including settlements, industry and infrastructure at the expense of arable land, especially of land utilized for grain production, with around 25% of farms situated around the city shifting from agricultural activities towards agricultural services (e.g. use of equipment and machinery) (Busck et al. 2006; Zasada et al. 2011; Fertner 2012). On the assumption, that agricultural crops are the main source for *Alternaria* spores in the air, increased urbanisation in the suburban areas around Copenhagen during last decades may have negatively influenced the number of *Alternaria* spores, due to reduction of agricultural sources and change in agricultural practices.

We propose that the longer *Cladosporium* season, the earlier diurnal maximum and the stronger relationship between meteorological parameters and daily concentration are related to the different nature and location of *Cladosporium* and *Alternaria* sources as well to different spore physical properties, in particular their gravitational settling. In this context, agricultural sources have a stronger influence on *Alternaria* spore release compared with *Cladosporium*, while the much smaller *Cladosporium* spores settle more slowly and transported over longer distances. Data on possible *Cladosporium* sources are scarce in the literature. There is a commonly accepted link between agriculture and higher *Cladosporium* spore concentrations (Weryszko-Chmielewska et al. 2018; Olsen et al. 2019a). However, the ever-presence of *Cladosporium* in the air with spore concentrations dominating over other fungal air spora, suggests, that its sources can exist also within urban areas, such as parks or other green zones, typical for the city. Thus, in south-eastern Poland, *Cladosporium* spores seasonal sum was higher at the urban cite, than in the rural, although it occurred equally frequent at both sites (Kasprzyk and Worek 2006). The decrease in the peak *Cladosporium* values within our study years was most probably a response to changes in the availability of sources than to increase in the temperatures. Thus, *C. cladosporioides* was the only species that exponentially increased its spore production in higher temperatures in the study by Damialis et al. (2015a). Sindt et al. (2016) found increasing trends for *Cladosporium* in three stations in France, located in more continental and Mediterranean climates, and downward trends for the southernmost stations in Aquitanian climate. Kasprzyk et al. (2016) demonstrated that rapid increase in temperatures and decrease in rainfall at three monitoring stations in Poland were associated with increased *Cladosporium* concentrations and longer season duration (Kasprzyk et al. 2016). We did not find that *Cladosporium* abundance was changing within 1990-2015. However, data availability for *Cladosporium*, confined within June-September months, was the main limitation of our research.

Most reports on diurnal distribution of fungal spores are based on one to five years of observations. Several studies showed that *Alternaria* and *Cladosporium* peak at the same time: at noon in Poland (Stępalska and Wołek 2009), later in the evening (16:00 – 20:00) in Portugal (Oliveira et al. 2009), in the late evening (Rodríguez-Rajo et al. 2005) or at the central hour of the day and at night (Reyes et al. 2009) in Spain, before noon in Croatia (Peternel et al. 2003), or without peak in Morocco (Bardei et al. 2017). We found an earlier peak for *Cladosporium* (11:30 – 14:30) than for *Alternaria* concentrations (17:00 – 19:00). Our results confirm and add to recently reported diurnal patterns for *Alternaria*, based on data for ten seasons (Skjøth et al. 2012), and for *Cladosporium* based on one season in Denmark (Olsen et al. 2019a). Comparable diurnal profiles were observed in Ireland and UK (Corden and Millington 2001; O'Connor et al. 2014). As it was discussed earlier, the late afternoon *Alternaria* maximum may be related to conidia release from local sources or from sources, situated 15-30 km away (Giner et al. 2001). Our results show that time of diurnal peak for *Cladosporium* occurred independently of corresponding daily concentrations, which advocates close proximity of the sources. The later diurnal maximum for *Alternaria* suggests a different nature of *Alternaria* sources or their longer distance from the sampling station or both, however, on the majority of days (75%) within *Alternaria* season, *Alternaria* diurnal concentration peaked between 15:00h and 17:00h. Furthermore, despite a moderate correlation between *Alternaria* and *Cladosporium* daily concentrations, probably due to daily temperature changes, we did not observe a correlation between *Alternaria* and *Cladosporium* overall annual indices. Thus, the peak SIn for *Cladosporium* was observed in 1994, while *Alternaria* SIn was highest in 1990, the lowest 3-month SIn for *Cladosporium* was observed in

2007, when *Alternaria* SSIn had a value above the 26 years average. This supports the hypothesis that the sources for *Alternaria* and *Cladosporium* spores have different origins and dynamics.

A consistent change towards a certain wind direction on the high concentration days might be related to a systematic contribution from distant sources. The episodes with unusual *Alternaria* diurnal distributions have been associated with atmospheric transport form the main agricultural areas in central Europe and southern Sweden (Skjøth et al. 2012), whereas high *Cladosporium* concentrations coincided with a period of air masses transport from northern Poland, the Baltics, and southern Sweden (Olsen et al. 2019a). We found increasing incidence of South and South-East wind directions in parallel to increasing spore concentrations, and this was more evident for *Alternaria*, which may be related to patterns in long distant transport. However, the time of diurnal maxima for *Cladosporium* and *Alternaria* on the days with highest concentrations, indicates, that events with unusually high concentrations during night hours, which can be associated with long distance transport (Skjøth et al. 2007; Skjøth et al. 2012) are rare. Continental agricultural sources situated approx. 100 km away from the Copenhagen station and therefore, shares in spore concentrations from distant sources may be mainly related to atmospheric transport from a neighbouring southern Sweden (Skjøth et al. 2012).

5. Conclusion

- We found a statistically significant negative trend for *Alternaria* SSIn, as well as for the number of days exceeding a commonly assumed health threshold, as well as for the peak day values. We did not find a trend in July-September SIn for *Cladosporium*. However, *Cladosporium* peak concentrations had a statistically significant annual decrease towards lower values. Although we were able to study the most intensive period of *Cladosporium* spore concentrations, assuming mono-modal annual distribution, longer data-series are necessary to determine season duration and its characteristics.
- We explain an observed decrease in *Alternaria* SSIn through 1990-2015 by concurrent temporal changes in land use, i.e. by urbanisation around the greater Copenhagen area and by changes in agricultural practices. Therefore, the influence of increasing temperatures on *Alternaria* and *Cladosporium* spore temporal abundance and seasonality awaits to be established and examined in the areas with minor temporal changes in the surrounding land cover.
- The shape of diurnal distributions confirms that both *Cladosporium* and *Alternaria* spores are released locally. However, the differences in the diurnal pattern dynamics between two genera, and earlier seasonal peak for *Cladosporium*, as well as year-to-year differences between *Cladosporium* and *Alternaria* suggest that sources of *Cladosporium* are located closer to the station, and are less dependent on agricultural practices. However, this hypothesis needs further investigation.

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References

Aira, M.-J., Rodríguez-Rajo, F.-J., Fernández-González, M., Seijo, C., Elvira-Rendueles, B., Abreu, I., et al. (2013). Spatial and temporal distribution of *Alternaria* spores in the Iberian Peninsula atmosphere, and meteorological relationships: 1993–2009. *International Journal of Biometeorology,* 57(2), 265-274.

Aira, M.-J., Rodríguez-Rajo, F.-J., Fernández-González, M., Seijo, C., Elvira-Rendueles, B., Gutiérrez-Bustillo, M., et al. (2012). *Cladosporium* airborne spore incidence in the environmental quality of the Iberian Peninsula. *Grana,* 51(4), 293-304.

Almeida, E., Caeiro, E., Todo-Bom, A., Ferro, R., Dionísio, A., Duarte, A., et al. (2018). The influence of meteorological parameters on *Alternaria* and *Cladosporium* fungal spore concentrations in Beja (Southern Portugal): preliminary results. *Aerobiologia,* 34(2), 219-226.

Atkinson, R. W., Strachan, D. P., Anderson, H. R., Hajat, S., & Emberlin, J. (2006). Temporal associations between daily counts of fungal spores and asthma exacerbations. *Occupational and Environmental Medicine,* 63(9), 580-590.

Awad, A. H. A. (2005). Vegetation: A source of air fungal bio-contaminant. *Aerobiologia,* 21(1), 53-61.

Bardei, F., Bouziane, H., Trigo, M. d. M., Ajouray, N., El Haskouri, F., & Kadiri, M. (2017). Atmospheric concentrations and intradiurnal pattern of *Alternaria* and *Cladosporium* conidia in Tétouan (NW of Morocco). *Aerobiologia,* 33(2), 221-228.

Bednarz, A., & Pawlowska, S. (2016). A fungal spore calendar for the atmosphere of Szczecin, Poland. *Acta Agrobotanica,* 69(3), 1-9.

Boddy, L., Büntgen, U., Egli, S., Gange, A. C., Heegaard, E., Kirk, P. M., et al. (2014). Climate variation effects on fungal fruiting. *Fungal Ecology,* 10, 20-33.

Bruffaerts, N., De Smedt, T., Delcloo, A., Simons, K., Hoebeke, L., Verstraeten, C., et al. (2018). Comparative long-term trend analysis of daily weather conditions with daily pollen concentrations in Brussels, Belgium. *International Journal of Biometeorology,* 62(3), 483-491.

Busck, A. G., Kristensen, S. P., Præstholm, S., Reenberg, A., & Primdahl, J. (2006). Land system changes in the context of urbanisation: Examples from the peri-urban area of Greater Copenhagen. *Geografisk Tidsskrift-Danish Journal of Geography,* 106(2), 21-34.

Cappelen, J., Kern-Hansen, C., Laursen, E. V., Jørgensen, P. V., Jørgensen, P. V., & Jørgensen, B. V. (2017). DMI Report 17-02. In C. John (Ed.), *Denmark - DMI Historical Climate Data Collection 1768-2016*. Copenhagen, Denmark: Danish Meteorological Institute.

Cecchi, L., D'Amato, G., Ayres, J., Galan, C., Forastiere, F., Forsberg, B., et al. (2010). Projections of the effects of climate change on allergic asthma: the contribution of aerobiology. *Allergy,* 65(9), 1073-1081.

Chrenová, J., Mišík, M., Ščevková, J., Mičieta, K., & Mlynarčík, D. (2004). Monitoring of microscopic airborne fungi in Bratislava. *Acta Facult. Pharm. Comenianae,* 51, 68-72.

Corden, J. M., & Millington, W. M. (2001). The long-term trends and seasonal variation of the aeroallergen *Alternaria* in Derby, UK. *Aerobiologia,* 17(2), 127-136.

Corden, J. M., Millington, W. M., & Mullins, J. (2003). Long-term trends and regional variation in the aeroallergen *Alternaria* in Cardiff and Derby UK – are differences in climate and cereal production having an effect? *Aerobiologia,* 19(3/4), 191-199.

Crameri, R., Garbani, M., Rhyner, C., & Huitema, C. (2014). Fungi: the neglected allergenic sources. *Allergy,* 69(2), 176-185.

D' Amato, M., Cecchi, L., Annesi-Maesani, I., & D'Amato, G. (2018). News on Climate Change, Air Pollution, and Allergic Triggers of Asthma. *Journal of Investigational Allergology and Clinical Immunology,* 28(2), 91-97.

D'Amato, G., Holgate, S. T., Pawankar, R., Ledford, D. K., Cecchi, L., Al-Ahmad, M., et al. (2015). Meteorological conditions, climate change, new emerging factors, and asthma and related allergic disorders. A statement of the World Allergy Organization. *World Allergy Organization Journal,* 8(1), 1.

Damialis, A., Mohammad, A. B., Halley, J. M., & Gange, A. C. (2015a). Fungi in a changing world: growth rates will be elevated, but spore production may decrease in future climates. *International Journal of Biometeorology,* 59(9), 1157-1167.

Damialis, A., Vokou, D., Gioulekas, D., & Halley, J. M. (2015b). Long-term trends in airborne fungal-spore concentrations: a comparison with pollen. *Fungal Ecology,* 13, 150-156.

Ellermann, T., Nygaard, J., Nøjgaard, J. K., Nordstrøm, C., Brandt, J., Christensen, J., et al. (2016). The Danish Air Quality Monitoring Programme. Annual Summary for 2015. *Scientific Report from DCE - Danish Centre for Environment and Energy No. 201* (pp. 65): Aarhus University, DCE - Danish Centre for Environment and Energy.

Fernández-Rodríguez, S., Sadyś, M., Smith, M., Tormo-Molina, R., Skjøth, C. A., Maya-Manzano, J. M., et al. (2015). Potential sources of airborne *Alternaria* spp. spores in South-west Spain. *Science of The Total Environment,* 533, 165-176.

Fertner, C. (2012). Urbanisation, urban growth and planning in the Copenhagen Metropolitan Region with reference studies from Europe and the USA. Forest & Landscape, University of Copenhagen. Forest and landscape research, No. 54/2012.

Friesen, T. L., De Wolf, E. D., & Francl, L. J. (2001). Source strength of wheat pathogens during combine harvest. *Aerobiologia,* 17(4), 293-299.

Galan, C., Ariatti, A., Bonini, M., Clot, B., Crouzy, B., Dahl, A., et al. (2017). Recommended terminology for aerobiological studies. *Aerbiologia,* 33, 293-295.

García-Mozo, H., Yaezel, L., Oteros, J., & Galán, C. (2014). Statistical approach to the analysis of olive long-term pollen season trends in southern Spain. *Science of The Total Environment,* 473, 103-109.

Giner, M. M., García, J. C., & Camacho, C. N. (2001). Airborne *Alternaria* spores in SE Spain (1993-98). Occurrence patterns, relationship with weather variables and prediction models. *Grana,* 40(3), 111-118.

Gravesen, S. (1979). Fungi as a cause of allergic disease. *Allergy,* 34(3), 135-154.

Grinn-Gofroń, A., & Mika, A. (2008). Selected airborne allergenic fungal spores and meteorological factors in Szczecin, Poland, 2004–2006. *Aerobiologia,* 24(2), 89.

Grinn-Gofroń, A., Nowosad, J., Bosiacka, B., Camacho, I., Pashley, C., Belmonte, J., et al. (2019). Airborne *Alternaria* and *Cladosporium* fungal spores in Europe: Forecasting possibilities and relationships with meteorological parameters. *Science of The Total Environment*, 653, 938-946.

Heinzerling, L. M., Burbach, G. J., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., et al. (2009). GA2LEN skin test study I: GA²LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy,* 64(10), 1498-1506.

Hirst, J.M. (1952). An automatic volumetric spore trap. *Annals of Applied Biology*, 39(2), 257-265.

Hollins, P., Kettlewell, P., Atkinson, M., Stephenson, D., Corden, J., Millington, W., et al. (2004). Relationships between airborne fungal spore concentration of *Cladosporium* and the summer climate at two sites in Britain. *International Journal of Biometeorology*, 48(3), 137-141.

Kasprzyk, I. (2008). Aeromycology–main research fields of interest during the last 25 years. *Ann Agric Environ Med,* 15(1), 1-7.

Kasprzyk, I., Kaszewski, B. M., Weryszko-Chmielewska, E., Nowak, M., Sulborska, A., Kaczmarek, J., et al. (2016). Warm and dry weather accelerates and elongates *Cladosporium* spore seasons in Poland. *Aerobiologia,* 32(1), 109-126.

Kasprzyk, I., Sulborska, A., Nowak, M., Szymanska, A., Kaczmarek, J., Haratym, W., et al. (2013). Fluctuation range of the concentration of airborne *Alternaria* condiospores sampled at different geographical locations in Poland (2010-2011). *Acta Agrobotanica,* 66(1), 65-76.

Kasprzyk, I., & Worek, M. (2006). Airborne fungal spores in urban and rural environments in Poland. *Aerobiologia,* 22, 169–176.

Katotomichelakis, M., Nikolaidis, C., Makris, M., Proimos, E., Aggelides, X., Constantinidis, T. C., et al. (2016). *Alternaria* and *Cladosporium* calendar of Western Thrace: Relationship with allergic rhinitis symptoms. *The Laryngoscope,* 126(2), E51-E56.

Larsen, L., & Gravesen, S. (1991). Seasonal variation of outdoor airborne viable microfungi in Copenhagen, Denmark. *Grana,* 30(2), 467-471.

Levetin, E., Horner, W. E., Scott, J. A., Barnes, C., Baxi, S., Chew, G. L., et al. (2016). Taxonomy of Allergenic Fungi. *The Journal of Allergy and Clinical Immunology: In Practice,* 4(3), 375-385.e371.

Mitakakis, T. Z., Clift, A., & McGee, P. A. (2001). The effect of local cropping activities and weather on the airborne concentration of allergenic *Alternaria* spores in rural Australia. *Grana,* 40(4-5), 230-239.

Nilsson, S., & Persson, S. (1981). Tree pollen spectra in the Stockholm region (Sweden), 1973–1980. *Grana,* 20(3), 179-182.

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

Olesen, M., Madsen, K. C., Ludwigsen, C. A., Boberg, F., Christensen, T., Cappelen, J., et al. (2014). Fremtidige klimaforandringer i Danmark. Damarks Klimacenter rapport nr. 6 Copenhagen: Danmarks Meteorologiske Institut.

Oliveira, M., Ribeiro, H., Delgado, J. L., & Abreu, I. (2009). Seasonal and intradiurnal variation of allergenic fungal spores in urban and rural areas of the North of Portugal. *Aerobiologia,* 25(2), 85-98.

Olsen, Y., Begovic, T., Skjøth, C. A., Rasmussen, K., Gosewinkel, U., Hertel, O., et al. (2019a). Grain harvesting as a local source of *Cladosporium* spp. in Denmark. *Aerobiologia*, 35(2), 373-378.

Olsen, Y., Gosewinkel, U., Skjøth, C.A., Hertel, O., Rasmissen, K., & Sigsgaard, T. (2019b). Regional variation in airborne *Alternaria* spore concentrations in Denmark through 2012-2015 seasons: the influence of meteorology and grain harvesting. *Aerobiologia*, 35(3), 533-551.

Peternel, R., Culig, J., & Hrga, I. (2003). Atmospheric concentrations of *Cladosporium* spp. and *Alternaria* spp. spores in Zagreb (Croatia) and effects of some meteorological factors. *Annals of agricultural and environmental medicine: AAEM,* 11(2), 303-307.

Rasmussen, A. (2002). The effects of climate change on the birch pollen season in Denmark. *Aerobiologia,* 18(3), 253-265.

Reyes, E. S., de la Cruz, D. R., Merino, E., & Sánchez, J. S. (2009). Meteorological and agricultural effects on airborne *Alternaria* and *Cladosporium* spores and clinical aspects in Valladolid [Spain]. *Annals of Agricultural and Environmental Medicine,* 16(1), 53-61.

Rizzi-Longo, L., Pizzulin-Sauli, M., & Ganis, P. (2009). Seasonal occurrence of *Alternaria* [1993-2004] and *Epicoccum* [1994-2004] spores in Trieste [NE Italy]. *Annals of Agricultural and Environmental Medicine,* 16(1), 63-70.

Rodríguez-Rajo, F. J., Iglesias, I., & Jato, V. (2005). Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions. *Mycological Research,* 109(4), 497-507.

Sadyś, M. (2017). Effects of wind speed and direction on monthly fluctuations of *Cladosporium* conidia concentration in the air. *Aerobiologia,* 33(3), 445-456, doi:10.1007/s10453-017-9482-6.

Sadyś, M., Skjøth, C. A., & Kennedy, R. (2015). Determination of *Alternaria* spp. habitats using 7-day volumetric spore trap, Hybrid Single Particle Lagrangian Integrated Trajectory model and geographic information system. *Urban Climate,* 14, 429-440.

Ščevková, J., Dušička, J., Mičieta, K., & Somorčík, J. (2016). The effects of recent changes in air temperature on trends in airborne *Alternaria*, *Epicoccum* and *Stemphylium* spore seasons in Bratislava (Slovakia). *Aerobiologia,* 32(1), 69-81.

Ščevková, J., & Kováč, J. (2019). First fungal spore calendar for the atmosphere of Bratislava, Slovakia. *Aerobiologia*, 35(2), 343-356.

Şen, B., & Asan, A. (2001). Airborne fungi in vegetable growing areas of Edirne, Turkey. *Aerobiologia, 17*(1), 69-75.

Simeray, J., Chaumont, J.-P., & Léger, D. (1993). Seasonal variations in the airborne fungal spore population of the East of France (Franche-Comte). Comparison between urban and rural environment during two years. *Aerobiologia,* 9(2-3), 201-206.

Sindt, C., Besancenot, J.-P., & Thibaudon, M. (2016). Airborne *Cladosporium* fungal spores and climate change in France. *Aerobiologia,* 32(1), 53-68.

Skjøth, C. A., Damialis, A., Belmonte, J., De Linares, C., Fernández-Rodríguez, S., Grinn-Gofroń, A., et al. (2016). *Alternaria* spores in the air across Europe: abundance, seasonality and relationships with climate, meteorology and local environment. *Aerobiologia,* 32(1), 3-22.

Skjøth, C. A., Sommer, J., Brandt, J., Hvidberg, M., Geels, C., Hansen, K. M., et al. (2007). Copenhagen – a significant source of birch (Betula) pollen? *International Journal of Biometeorology,* 52(6), 453.

Skjøth, C. A., Sommer, J., Frederiksen, L., & Gosewinkel Karlson, U. (2012). Crop harvest in Denmark and Central Europe contributes to the local load of airborne *Alternaria* spore concentrations in Copenhagen. *Atmospheric Chemistry and Physics,* 12(22), 11107-11123.

Smith, M., Jäger, S., Berger, U., Sikoparija, B., Hallsdottir, M., Sauliene, I., et al. (2014). Geographic and temporal variations in pollen exposure across Europe. *Allergy,* 69, 913-923.

Sousa, L., Camacho, I. C., Grinn-Gofroń, A., & Camacho, R. (2016). Monitoring of anamorphic fungal spores in Madeira region (Portugal), 2003–2008. *Aerobiologia,* 32(2), 303-315.

Stępalska, D., & Wołek, J. (2009). Intradiurnal periodicity of fungal spore concentrations (*Alternaria*, *Botrytis*, *Cladosporium*, *Didymella*, *Ganoderma*) in Cracow, Poland. *Aerobiologia,* 25(4), 333.

Stieb, D. M., Beveridge, R. C., Brook, J. R., Smith-Doiron, M., Burnett, R. T., Dales, R. E., et al. (2000). Air pollution, aeroallergens and cardiorespiratory emergency department visits in Saint John, Canada. *Journal of Exposure Science and Environmental Epidemiology,* 10(5), 461-477.

Targonski, P. V., Persky, V. W., & Ramekrishnan, V. (1995). Effect of environmental molds on risk of death from asthma during the pollen season. *Journal of Allergy and Clinical Immunology,* 95(5), 955-961.

Twaroch, T. E., Curin, M., Valenta, R., & Swoboda, I. (2015). Mold Allergens in Respiratory Allergy: From Structure to Therapy. *Allergy Asthma Immunol Res,* 7(3), 205-220.

Ugolotti, M., Pasquarella, C., Vitali, P., Smith, M., & Albertini, R. (2015). Characteristics and trends of selected pollen seasons recorded in Parma (Northern Italy) from 1994 to 2011. *Aerobiologia,* 31(3), 341-352.

Walther, G.-R. (2010). Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences.,* 365, 2019-2024.

Weryszko-Chmielewska, E., Kasprzyk, I., Nowak, M., Sulborska, A., Kaczmarek, J., Szymanska, A., et al. (2018). Health hazards related to conidia of *Cladosporium*—biological air pollutants in Poland, central Europe. *Journal of Environmental Sciences,* 65, 271-281.

Zasada, I., Fertner, C., Piorr, A., & Nielsen, T. S. (2011). Peri-urbanisation and multifunctional adaptation of agriculture around Copenhagen AU - Zasada, Ingo. *Geografisk Tidsskrift-Danish Journal of Geography,* 111(1), 59-72.