



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 any statistically significant trends for airborne *Alternaria* seasonal characteristics and duration, and likewise for *Cladosporium* 3-month SIn and peak concentration dates.

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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.

Keywords *Cladosporium* · *Alternaria* · Annual trends · Climate change · Respiratory allergy · Land use

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), among 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* (Bagini et al. 1977; Gravesen 1979). In Denmark (Odense), 8.2% and 7.9% of patients with a suspected inhalant allergy were found to be sensitized 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) and 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 (Skjødth 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 (Skjødth 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, 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; Skjødth et al. 2012; Olsen et al. 2019a, b). 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) 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 under 640 × magnification at the genus level by the method of vertical transects, i.e. 12 transects along the slide for *Alternaria* spores and eight 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.

3 Meteorological data

Daily temperature averages, i.e. minimal daily temperature— $T_{\min}$, maximal daily temperature— $T_{\max}$, 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.

3.1 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 three 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 every *Alternaria* spp. season and for every 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-min 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-min 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.

4 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 given 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 (1990–2002) 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 (2003–2015) with the minimal value in 2012 (2351 Spores day m^{-3}), followed by higher SSIn in 2011 and 2003 (Table 1). *Alternaria* SSIn was strongly positively correlated with the annual number of days when daily average concentrations exceeded 100 Spores m^{-3} ($r_s = 0.93$, $p = 0.0000$) and with

Fig. 1 Airborne *Cladosporium* (blue) and *Alternaria* (red) spore annual daily average distribution (over 26 years)

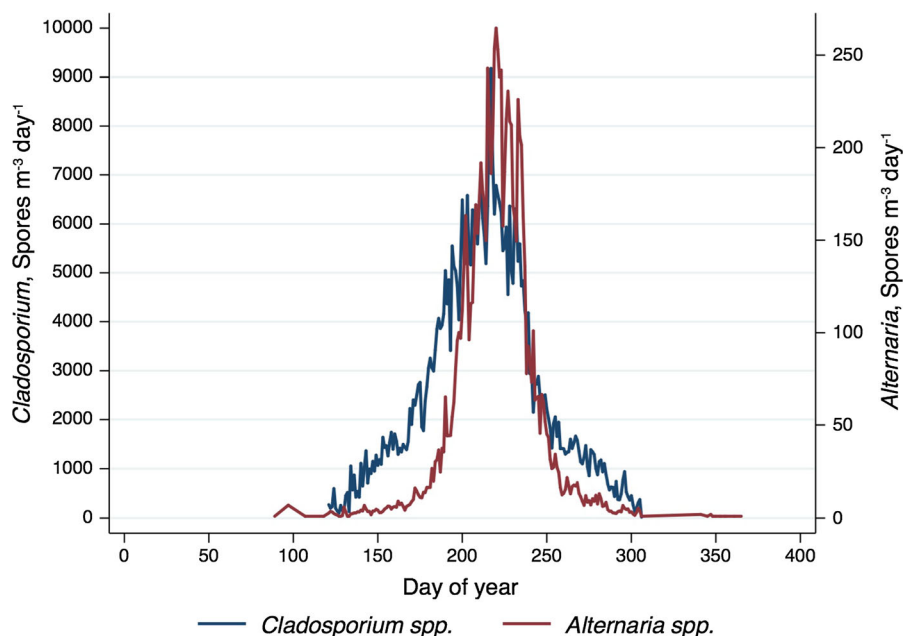


Table 1 Seasonal characteristics of airborne *Alternaria* spp. during 1990–2015 (Start and End of season—day of year and date, Duration—days, Peak date—day of year and date with peak daily average concentration, Peak value—peak daily

average concentration (Spores m^{-3}), SSIn—seasonal spore integral (Spores day m^{-3}), *N* above—number of days with daily average concentration ≥ 100 Spores m^{-3})

	Start	End	Duration	Peak date	Peak value	SSIn	<i>N</i> above
1990	179 28 Jun	241 29 Aug	63	227 15 Aug	1713	15,695	45
1991	204 23 Jul	248 05 Sep	45	220 08 Aug	2262	15,419	38
1992	192 10 Jul	255 11 Sep	64	203 21 Jul	681	8433	31
1993	182 01 Jul	246 03 Sep	65	213 01 Aug	1039	10,730	31
1994	196 15 Jul	224 01 Sep	49	233 23 Aug	534	7220	21
1995	194 13 Jul	251 08 Sep	58	202 21 Jul	684	13,631	41
1996	192 10 Jul	251 07 Sep	60	233 20 Aug	1107	12,417	37
1997	193 12 Jul	243 31 Aug	51	226 14 Aug	977	11,822	33
1998	184 03 Jul	250 07 Sep	67	223 11 Aug	1272	9547	27
1999	186 05 Jul	262 19 Sep	77	242 30 Aug	627	7702	26
2000	175 22 Jun	262 18 Sep	88	233 20 Aug	873	9239	24
2001	191 10 Jul	242 30 Aug	52	229 17 Aug	1,016	9474	24
2002	190 09 Jul	248 05 Sep	59	210 29 Jul	567	6447	21
2003	196 15 Jul	255 12 Sep	60	200 19 Jul	279	4199	18
2004	195 13 Jul	250 06 Sep	56	219 06 Aug	607	5129	18
2005	192 11 Jul	266 23 Sep	75	222 10 Aug	468	7826	31
2006	187 06 Jul	257 14 Sep	71	223 11 Aug	682	9879	27
2007	177 26 Jun	236 24 Aug	60	198 17 Jul	588	9497	22
2008	184 02 Jul	247 03 Sep	64	244 31 Aug	313	4771	17
2009	188 07 Jul	241 29 Aug	54	221 09 Aug	595	9497	31
2010	193 12 Jul	246 03 Sep	54	215 03 Aug	689	6912	26

Table 1 continued

	Start	End	Duration	Peak date	Peak value	SSIn	N above
2011	180	246	67	215	535	3691	8
	29 Jun	03 Sep		03 Aug			
2012	186	259	74	207	359	2351	4
	04 Jul	15 Sep		25 Jul			
2013	197	250	54	217	390	5048	18
	16 Jul	07 Sep		05 Aug			
2014	185	256	72	196	665	11,156	31
	04 Jul	13 Sep		15 Jul			
2015	192	256	65	226	1326	8658	26
	11 Jul	13 Sep		14 Aug			
Mean	189	250	62	219	804	8615	26
SD	7	7	10	13	451	3450	9

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 the longest in 2000 (88 days) and shortest in 1991 (45 days) and there was no systematic temporal 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 the 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. *Cladosporium* 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-year

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}) (Table 2). 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 100 Spores m^{-3} (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 concentration exceeding 3000 *Cladosporium* 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).

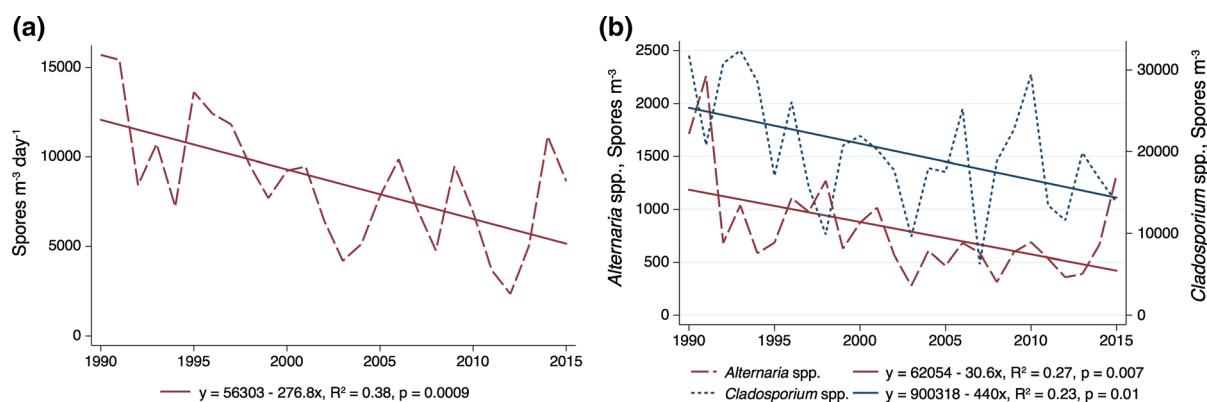
Cladosporium and *Alternaria* peak concentration dates were not synchronized ($r_s = 0.02$, $p = 0.92$), and in 15 years, except in 2004 (when the peak

Table 2 Airborne *Cladosporium* spp. spore monthly characteristics in June, July, August, September and over July–September periods during 1990–2015 (SIn—spore integral, Spores day m⁻³, *N*—number of days with daily averageconcentration ≥ 3000 Spores m⁻³, Peak date—day of year and date, Peak value—the highest daily average concentration over the period, Spores m⁻³, SD—standard deviation)

	June		July		August		September		July–September			
	SIn	<i>N</i>	SIn	<i>N</i>	SIn	<i>N</i>	SIn	<i>N</i>	SIn	Peak date	Peak value	<i>N</i>
1990	180,204	22	192,209	24	204,441	21	36,944	1	433,594	224 12 Aug	31,734	46
1991	78,731	8	154,722	20	245,824	25	68,484	6	469,030	221 09 Aug	20,793	51
1992	21,322	0	282,069	22	148,569	23	47,401	3	478,039	208 26 Jul	30,776	48
1993	105,912	17	238,917	23	145,812	15	43,311	0	428,040	200 19 Jul	32,345	38
1994	38,966	2	132,108	17	349,011	27	46,162	5	527,281	216 04 Aug	28,604	49
1995	32,864	1	152,599	19	138,197	17	94,902	13	385,698	239 27 Aug	17,061	49
1996	24,398	0	97,309	13	249,174	28	72,909	7	419,392	214 01 Aug	26,094	48
1997	59,420	9	95,769	8	174,419	17	32,814	4	303,002	218 06 Aug	15,726	29
1998	70,033	6	68,545	6	78,895	11	36,032	2	183,472	204 23 Jul	9842	19
1999	49,064	3	113,798	14	157,983	19	87,364	11	359,145	221 09 Aug	20,718	44
2000	72,679	8	187,559	23	230,938	25	40,485	3	458,982	194 12 Jul	21,921	51
2001	47,082	5	206,681	24	209,611	19	27,222	0	443,514	200 19 Jul	20,257	43
2002	54,155	5	179,347	22	148,404	22	32,384	1	360,135	217 05 Aug	17,728	45
2003	—	—	68,488	8	55,572	5	27,546	4	151,606	218 06 Aug	9579	17
2004	—	—	84,790	10	141,660	16	20,708	1	247,158	219 06 Aug	17,992	27
2005	38,570	0	183,554	23	157,870	23	78,555	10	419,979	210 29 Jul	17,522	56
2006	—	—	234,623	28	222,789	26	55,396	4	507,955	217 05 Aug	25,220	58
2007	62,093	5	78,666	9	57,739	8	12,876	0	149,281	203 22 Jul	6238	17
2008	75,866	6	210,450	26	229,202	26	58,593	8	498,245	221 08 Aug	18,827	60
2009	37,572	1	258,708	26	111,299	18	53,723	3	423,820	206 25 Jul	22,702	47

Table 2 continued

	June		July		August		September		July–September			
	SIn	N	SIn	N	SIn	N	SIn	N	SIn	Peak date	Peak value	N
2010	–	–	158,389	23	265,840	22	40,982	4	465,211	228 16 Aug	29,449	49
2011	58,847	4	134,716	20	66,968	8	25,856	0	227,540	192 11 Jul	13,442	28
2012	58,716	7	117,013	16	100,073	10	40,061	2	257,147	231 18 Aug	11,594	28
2013	–	–	77,145	8	194,909	23	46,198	3	318,252	222 10 Aug	19,851	34
2014	48,970	4	235,838	25	125,281	15	126,832	22	487,951	201 20 Jul	16,695	62
2015	36,441	2	120,413	21	148,623	22	86,492	14	355,528	218 06 Aug	13,801	57
Mean	59,615	5	156,328	18	167,658	19	51,547	5	375,533	214	19,866	42
SD	34,152	5	63,338	6	70,860	6	26,494	5	113,309	11.49	7081	13

**Fig. 2** Temporal trends (1990–2015) for: **a** *Alternaria* spp. SSIn; **b** *Alternaria* (red long dash) and *Cladosporium* (blue short dash) peak daily concentrations

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, and 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-month mean meteorological parameters

including cumulative precipitation over the corresponding periods, except for moderate positive correlation between *Alternaria* 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).

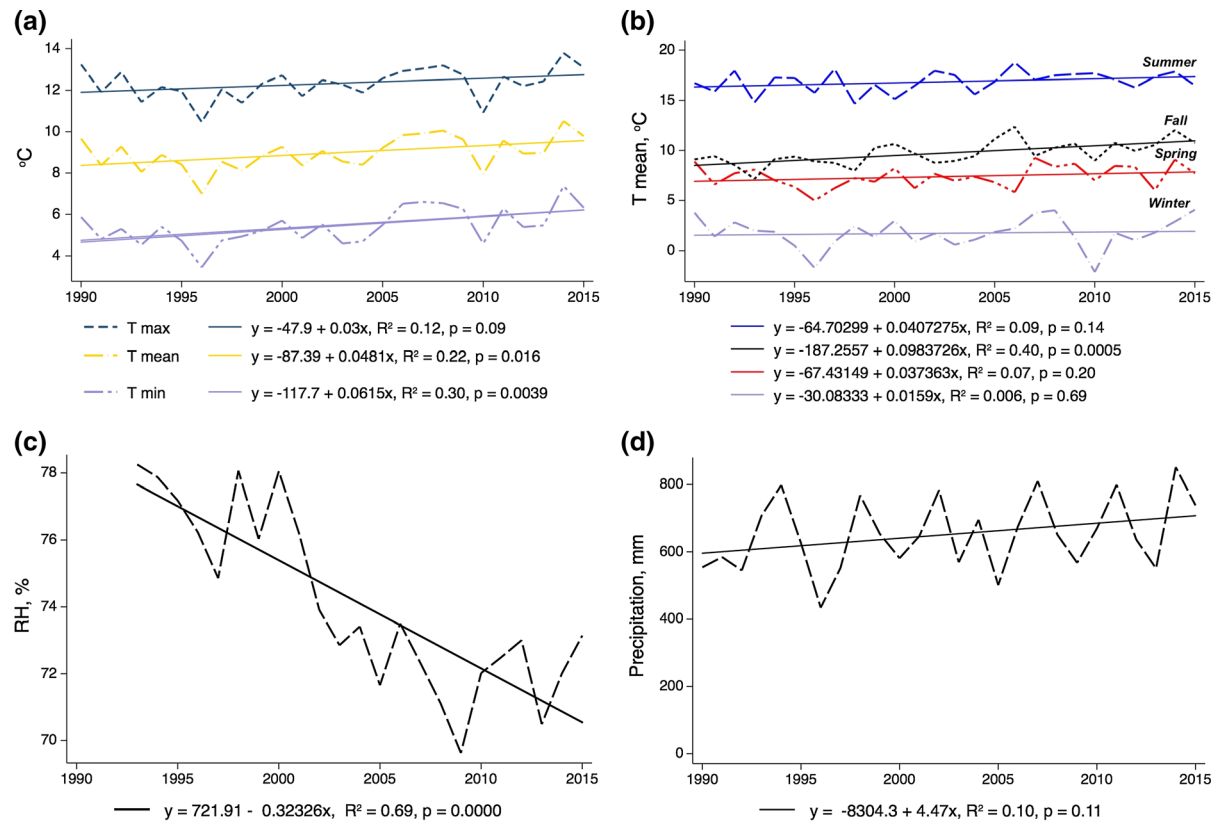


Fig. 3 Temporal trends of **a** annual: T_{max} —average maximal daily temperature, T_{mean} —average daily temperature, T_{min} —average minimal daily temperature over 1990–2015; **b** seasonal

T_{mean} —average daily temperature over 1990–2015; **c** annual average RH relative humidity over 1993–2015; **d** annual precipitation over 1990–2015

Table 3 Results of stepwise regression on logarithmically transformed daily average spore data for *Alternaria* and *Cladosporium* and daily meteorological parameters: mean temperature— T_{mean} , wind speed—WS, global radiation—GR, relative humidity—RH, cumulative precipitation

	<i>Alternaria</i> spp.		<i>Cladosporium</i> spp.	
	Slope	P	Slope	P
T_{mean}	0.0873233	0.000	0.1125479	0.000
RH	− 0.005659	0.001	0.0086351	0.000
GR	Removed	0.7375	− 0.0003561	0.017
Precipitation	− 0.0086425	0.000	Removed	0.0731
WS	0.0408656	0.000	Removed	0.1295
R^2	0.21	0.000	0.41	0.0000
Intercept	0.5431353	0.005	0.9026544	0.000

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 05:30 h for *Cladosporium* and between 05:00 h and 07:00 h 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 (11:30 h–14:30 h) was only more evident with increasing daily concentrations (Fig. 4c, d), *Alternaria* diurnal peak shifted to later hours on the days with daily average concentration above 100 Spores m^{-3} (Fig. 4a, b). Thus, on the days with the highest daily

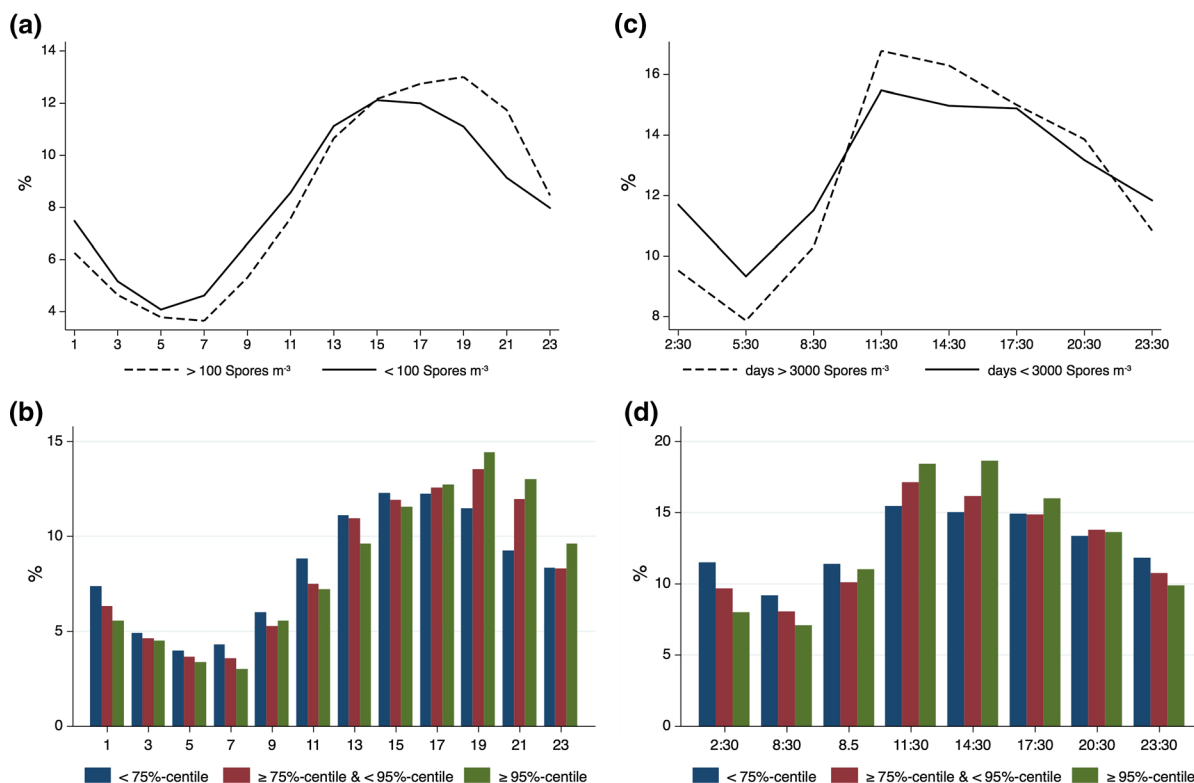


Fig. 4 Diurnal profiles: *Alternaria* spp. **a** on the days with daily average concentration above and below 100 Spores m^{-3} **b**; on the days with daily average concentration: below 184 Spores m^{-3} in blue (below 75%-centile), above/equal to 184 Spores m^{-3} and below 458 Spores m^{-3} in brown (above 75%-centile—below 95%-centile), above/equal to 458 Spores m^{-3} in green (above 95%-centile); *Cladosporium* spp. **c** on the days

with daily average concentration above and below 3000 Spores m^{-3} , **d** on the days with daily average concentration: below 4107 Spores m^{-3} in blue (below 75%-centile), above/equal to 4107 Spores m^{-3} and below 10,453 Spores m^{-3} in brown (above 75%-centile—below 95%-centile), and above/equal to 10,453 Spores m^{-3} in green (above 95%-centile)

concentrations, i.e. above 75%-centile (184 Spores m^{-3}), the diurnal maxima occurred at 19:00 h (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 of 37–39% of all 30-min 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). The 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 the increase in east-north-east to east wind directions (Fig. 5).

5 Discussion

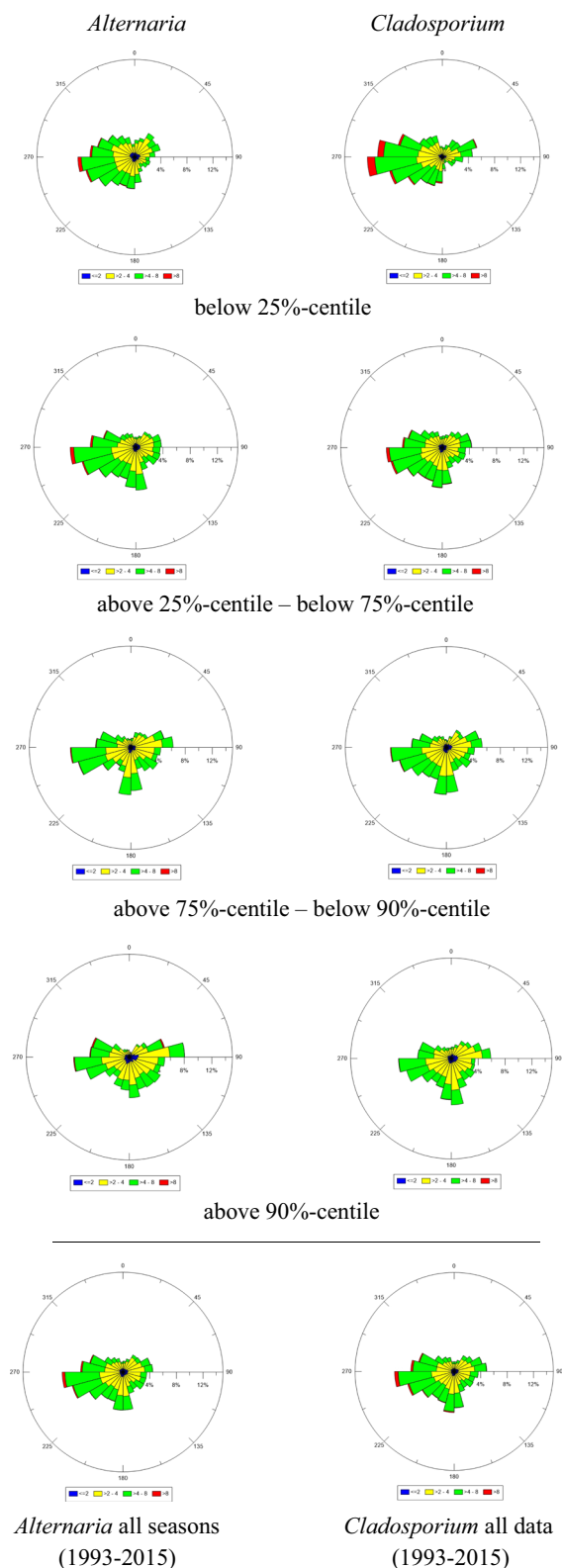
Our results confirm previous findings of peak *Alternaria* concentrations in Denmark occurring between late July and mid-August with an average season duration of 2 months, which is shorter than reported for more southern locations (Skjøth et al. 2012, 2016).

The *Cladosporium* season in Denmark starts earlier and ends later than that of *Alternaria*'s with the highest *Cladosporium* spore concentrations in July and August. Every year, *Cladosporium* daily concentrations exceeded 3000 Spores m^{-3} more frequently

Fig. 5 Rose winds drawn using 30-min wind direction measurements divided according to airborne *Alternaria* daily average spore concentrations during 90% spore seasons (left column) through 1993–2015 and *Cladosporium* daily average spore concentrations for all available data during 1993–2015 (right column), i.e. starting from top: below the 25% centile (*Alternaria*: below 26 Spores m^{-3} , *Cladosporium*: below 696 Spores m^{-3}), above 25%—below 75% centiles (*Alternaria*: above/equal to 26 Spores m^{-3} —below 169 Spores m^{-3} , *Cladosporium*: above/equal 696 Spores m^{-3} —below 3863 Spores m^{-3}), above 75%—below 90% centiles (*Alternaria*: above/equal to 169 Spores m^{-3} —below 331 Spores m^{-3} , *Cladosporium*: above/equal 3863 Spores m^{-3} —7166 below Spores m^{-3}), above 75% centile (*Alternaria*: above 331 Spores m^{-3} , *Cladosporium*: above 7166 Spores m^{-3}); the lowest two wind roses show all 30-min wind directions for all days during *Alternaria* seasons (1993–2015) and for all days when *Cladosporium* spores were measured (1993–2015). Colour of spikes indicates the wind speed: blue $\leq 2 \text{ m s}^{-1}$, yellow $> 2\text{--}4 \text{ m s}^{-1}$, green $> 4\text{--}8 \text{ m s}^{-1}$, red $> 8 \text{ m s}^{-1}$

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*, in Denmark the rates are nearly equal among individuals with a suspected inhalant allergy (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-Gofron and Mika 2008), the UK (Hollins et al. 2004; Sadyś 2017), Slovakia (Chrenová et al. 2004; Ščevková and Kováč 2019). The 26-years 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 ten 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 Seville (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: 92,351–123,863 Spores day m^{-3} (Almeida et al. 2018), for Madeira Island (Sousa et al. 2016), and for the western Thrace region in Greece: 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 for 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 concentration values followed the same geographical trend as SIn, i.e. with lower values generally reported in north-west Morocco as 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), for 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 in 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 urbanized land cover. Damialis et al. (2015a, b), 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 more than twice higher annual SIn in 2003 (15,021 Spores day m^{-3}) than the average SIn (6076 Spores

day m^{-3}) (Rizzi-Longo et al. 2009). The 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 *Cladosporium* spore concentrations, which would be in agreement with the negative trend in Thessaloniki (Damialis et al. 2015b). 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 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*. As discussed earlier by Grinn-Gofroń et al. (2019), long-term precipitation data are often available only at daily temporal resolution, that does not reflect high precipitation variability, and, therefore, cannot be used to relate spore concentrations to rainfall. 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} and T_{min} and a negative trend in RH. Notably, positive trend in T_{min} was statistically significant during the period when the 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 the increase in 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, 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 et al. 2016). We did not find systematic temporal changes in those *Alternaria* seasonal characteristics. Our results do not support the hypothesis of tendency to shorter duration or to earlier onset of main spore seasons reported by Damialis et al. (2015a, b). The influence of temperature on airborne *Cladosporium* seasonal duration was recently reported (Kasprzyk et al. 2016). We could not analyse trends for seasonal characteristics of *Cladosporium* spore concentrations due to the limited period of observations. Interestingly, however, peak concentration 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 the highest *Alternaria* spore concentrations 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 in 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 *Cladosporium* 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 airspora, 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 at 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 (15:00–19:00). 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 15:00 h and 17:00 h. 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 maximum 3-month SIn for *Cladosporium* was observed in 1994, while *Alternaria* SSIn 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, although the wind rose method normally tracks wind influences for 2–3 h back in time, loosing precision in the longer term (Fleming et al. 2012). The episodes with unusual *Alternaria* diurnal distributions have been associated with atmospheric transport from 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 the highest concentrations indicates that events with unusually high concentrations during night hours, which can be associated with long-distance transport (Skjøth et al. 2007, 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 (Skj  th et al. 2012).

6 Conclusion

- We found a statistically significant negative trends for *Alternaria* SSIn, number of days exceeding 100 Spores m⁻³, and peak day concentration values. We did not find a trend in July–September SIn for *Cladosporium*. However, *Cladosporium* peak daily 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, a longer monitoring period is necessary to determine the duration of *Cladosporium* season and its other characteristics.
- We explain the 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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Supplementary Material

SM Table 1 Spearman correlation matrix for daily meteorological parameters during spore measurements periods over 1990-2015: daily average temperature – T mean, daily maximum temperature – T max, daily precipitation – Precipitation, wind speed – WS, daily average global radiation – GR, daily relative humidity – RH; $p < 0.05$ for all data

	T mean	T max	T min	Precipitation	WS	GR
T max	0.95					
T min	0.88	0.76				
Precipitation	-0.21	-0.28	0.05			
WS	-0.23	-0.28	-0.07	0.24		
GR	0.48	0.60	0.20	-0.34	-0.20	
RH	-0.30	-0.39	-0.05	0.49	0.11	-0.76

SM Table 2 Spearman's correlation coefficients (r_s) for *Alternaria* spp. and *Cladosporium* spp. daily average concentration and daily meteorological parameters, i.e. T mean – mean temperature, T max – maximal temperature, T min – minimal temperature, Prec. – precipitation, WS – wind speed, GR – global radiation, RH – relative humidity (in bold – $p < 0.05$)

<i>Alternaria</i>							
	T mean	T max	T min	Prec.	WS	GR	RH
1990	0.49	0.52	0.29	-0.23	-	-	-
1991	0.37	0.3	0.16	-0.31	-	-	-
1992	0.69	0.74	0.45	-0.33	-	-	-
1993	0.52	0.3	0.39	0	-0.02	0.12	0.03
1994	-0.01	-0.06	-0.01	-0.17	0.26	0.18	0.01
1995	0.42	0.44	0.18	-0.15	0.23	0.46	-0.30
1996	0.51	0.35	0.39	0.11	-0.10	-0.18	-0.02
1997	0.60	0.63	0.41	-0.27	-0.20	0.02	-0.12
1998	0.70	0.62	0.47	-0.24	0.06	0.30	-0.37
1999	0.10	0.17	-0.01	-0.19	-0.14	0.04	-0.15
2000	0.25	0.34	-0.03	-0.28	0.12	0.13	-0.28
2001	0.54	0.54	0.44	-0.42	-0.18	0.14	-0.10
2002	0.52	0.47	0.43	-0.24	0.10	0.26	-0.20
2003	0.74	0.69	0.58	-0.19	-0.23	0.53	-0.27
2004	0.60	0.59	0.4	-0.41	0.04	0.35	-0.43
2005	0.41	0.33	0.3	-0.02	0.04	0.19	-0.06
2006	0.62	0.64	0.46	-0.26	-0.21	0.40	-0.47
2007	0.69	0.76	0.34	-0.48	-0.29	0.56	-0.49
2008	0.40	0.43	0.18	-0.21	-0.25	0.58	-0.62
2009	0.46	0.49	0.25	-0.01	-0.10	0.29	-0.36
2010	0.20	0.23	0.03	0.04	-0.03	0.16	-0.06

2011	0.39	0.43	0.15	-0.31	-0.08	0.34	-0.38
2012	0.61	0.63	0.38	-0.28	-0.03	0.30	-0.37
2013	0.04	-0.05	0.1	-0.01	0.09	-0.02	-0.11
2014	0.74	0.75	0.69	-0.38	-0.19	0.39	-0.50
2015	0.66	0.7	0.38	-0.41	-0.06	0.41	-0.37
Total	0.43	0.43	0.30	-0.22	-0.04	0.27	-0.26
<i>Cladosporium</i>							
1990	0.70	0.63	0.61	-0.20	-	-	-
1991	0.50	0.43	0.57	-0.05	-	-	-
1992	0.50	0.49	0.44	0.08	-	-	-
1993	0.69	0.74	0.58	0.03	-0.02	0.44	-0.20
1994	0.59	0.51	0.54	-0.23	-0.07	0.30	-0.36
1995	0.42	0.39	0.48	-0.12	-0.16	0.11	-0.13
1996	0.58	0.49	0.58	0.01	-0.18	0.10	0.06
1997	0.65	0.62	0.56	0.05	-0.33	0.31	-0.02
1998	0.63	0.50	0.49	-0.04	0.14	0.29	-0.15
1999	0.19	0.21	0.15	0.03	0.05	-0.0008	0.13
2000	0.58	0.56	0.18	-0.01	-0.16	0.23	-0.08
2001	0.76	0.72	0.71	-0.40	-0.04	0.52	-0.46
2002	0.55	0.51	0.59	0.01	0.06	0.41	-0.11
2003	0.46	0.46	0.4	-0.03	0.01	0.31	-0.06
2004	0.60	0.65	0.53	-0.02	-0.20	0.34	-0.02
2005	0.36	0.31	0.35	0.01	0.06	0.13	0.07
2006	0.64	0.65	0.51	0.004	-0.29	0.37	-0.33
2007	0.71	0.59	0.67	-0.16	-0.20	0.37	-0.06
2008	0.51	0.57	0.42	0.13	0.10	0.22	-0.11
2009	0.57	0.62	0.40	0.08	-0.21	0.42	-0.19
2010	0.72	0.69	0.68	0.12	-0.15	0.25	-0.01
2011	0.57	0.50	0.53	-0.01	-0.28	0.33	-0.17
2012	0.59	0.56	0.57	-0.13	-0.24	0.39	-0.19
2013	0.33	0.31	0.37	0.09	0.10	0.07	0.10
2014	0.66	0.64	0.62	-0.32	-0.28	0.28	-0.27
2015	0.45	0.38	0.39	0.09	0.16	0.11	0.06
Total	0.52	0.50	0.47	-0.06	-0.11	0.27	-0.11