ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2009, 16, 63–70

SEASONAL OCCURRENCE OF *ALTERNARIA* (1993–2004) AND *EPICOCCUM* (1994–2004) SPORES IN TRIESTE (NE ITALY)

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Rizzi-Longo L, Pizzulin-Sauli M, Ganis P: Seasonal occurrence of *Alternaria* (1993–2004) and *Epicoccum* (1994–2004) spores in Trieste (NE Italy). *Ann Agric Environ Med* 2009, **16**, 63–70.

Abstract: Fungal spores are known to trigger allergic respiratory diseases. Very little is known about airborne fungal spore presence in Trieste. This paper reports the seasonal (June-October) *Alternaria* (1993–2004) and *Epicoccum* (1994–2004) spore concentrations during these years, using a Hirst sampler. Daily spore counts fluctuate considerably from one day to the next. Airborne spores of *Alternaria* and *Epicoccum* tend to display a similar temporal pattern, with the peak daily count occurring in late summer – early autumn; a shifted occurrence towards the early autumn of the maximum peaking day of *Epicoccum* was observed, compared to *Alternaria*, on average. A great variability in seasonal spore concentrations was also found throughout the years. Seasonal totals of 6,076 *Alternaria* spores and 2,796 *Epicoccum* spores were found over the examined years, on average. 2003 was an exceptionally high year for *Alternaria* with a seasonal total of 15,021 spores. Statistical analyses comparing daily spore counts with meteorological parameters were carried out. Weather variables have been shown to affect the spore concentrations in the air. *Alternaria* and *Epicoccum* respond in a similar way to meteorological factors. Daily spore levels showed a significant positive correlation with temperatures; weak significant negative correlations have been found with the other parameters.

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Key words: airborne spores, *Alternaria, Epicoccum*, seasonal variation, meteorological factors, Trieste, Italy.

INTRODUCTION

Many species of phytopathogenic moulds are sources of aero-allergens which trigger respiratory diseases [5, 9]. Spores of mitosporic fungi are liberated actively or passively in the atmosphere and are transported by air currents [12]. The airspora of different sites was examined in relation to allergic symptoms and/or meteorological parameters [1, 3, 4, 10, 16, 20, 24, 26]. The incidence of fungal spores in the air of Trieste is not well known. Only a preliminary study on the occurrence of *Alternaria* airborne spores in the town and the relationship between *Alternaria* spore levels and sensitization in patients with respiratory allergies was carried out [17]. *Alternaria* airborne spores are usually sporadic in Trieste between November and April, and daily concentrations increase during May becoming higher from June [17].

The present study documents the seasonal (June–October) spore incidence of *Alternaria* and *Epicoccum* in the atmosphere of the town; the effects of weather on the concentration of these spores were also investigated. *Alternaria* and *Epicoccum* are common moulds. *Alternaria* is a plant pathogen and lives as a parasite or saprophyte on plants and fruits, and in the soil; *Epicoccum* is a saprophyte that colonizes wild plants, crops and rotting vegetation. Their spores are common aeroallergens with a world-wide distribution. Detailed studies regarding the occurrence of *Alternaria* in the atmosphere of some European sites have been carried out by Fernández *et al.* [11], Corden and Millington [6], Munuera Giner *et al.* [22], Corden *et al.* [7] and Aira

Received: 19 June 2008
Accepted: 3 March 2009

et al. [1]. The relationship between spore concentrations of *Alternaria* and meteorological parameters were also investigated [1, 2, 13, 14, 30]. The occurrence of *Epicoccum* in the air was investigated to a lesser extent.

MATERIALS AND METHODS

The aerobiological survey was carried out over a period of 12 consecutive years (1993–2004) for *Alternaria* and 11 years (1994–2004) for *Epicoccum*. Airborne spores of *Alternaria* and *Epicoccum* were collected each year by means of a Hirst type 7-day volumetric spore trap (Burkard until 1999, VPSS 2000 Lanzoni since 2000), located at about 20m above ground level on the Bastione Fiorito of San Giusto Castle, in the town centre. Sampling method, slide preparation and data interpretation were performed according to the standard method adopted by the Italian Aeroallergen Network [19]. The spore counts were expressed as the number of spores per cubic meter of air (sp/m^3).

The seasonal (June–October) spore incidence of *Alternaria* and *Epicoccum* in the atmosphere of the town was investigated, since *Alternaria* airborne spores are usually sporadic in Trieste between November–April, increase during May and becoming higher from June [17].

Following Corden and Millington [6], June–October seasonal totals were calculated for each year from 1993–2004 (1994–2004 for *Epicoccum*). Monthly totals and daily spore frequencies were studied for all examined seasons; the number of days when the spore counts of *Alternaria* were above the allergenic threshold of 100 sp/m^3 proposed by Bagni *et al.* [3] were also counted.

Alternaria and *Epicoccum* spore counts and some meteorological parameters, even after logarithmic transformation, are not normally distributed. For this reason, the non-parametric Spearman correlation coefficient was used to examine, for each year, both the daily distribution similarity of the 2 taxa spores in the air and the relationship between their spore counts and some daily meteorological parameters: mean, minimum and maximum temperature (expressed in °C), relative humidity (%), rainfall (expressed in mm), maximum and mean wind speed (expressed in m/s). All calculations were made using SPSS [28] and STATISTICA [29] software packages. The meteorological data was supplied by the Department of Earth Sciences, University of Trieste.

RESULTS

Airborne fungal spore content in Trieste varies largely from month to month and also from year to year. Daily spore counts also fluctuate considerably from one day to the next. *Alternaria* and *Epicoccum* seasonal patterns are rather similar: the highest levels are usually recorded in the late summer or early autumn (Figs 1, 2). Also, the trends of their total spores from January–October along the considered years appear to correspond (Fig. 3).

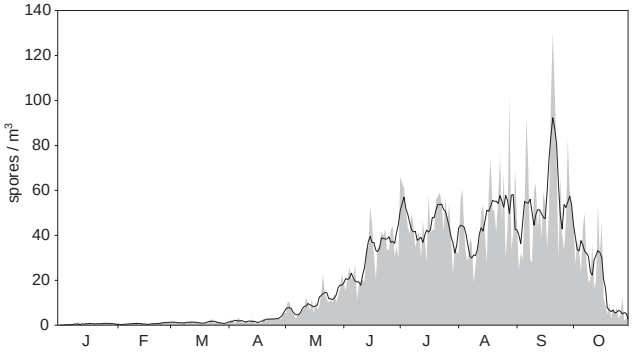

Figure 1. Daily variation (January–October) of the average of *Alternaria* daily spore counts in Trieste for the studied years (1993–2004); the line shows the average of the 5-day running means.

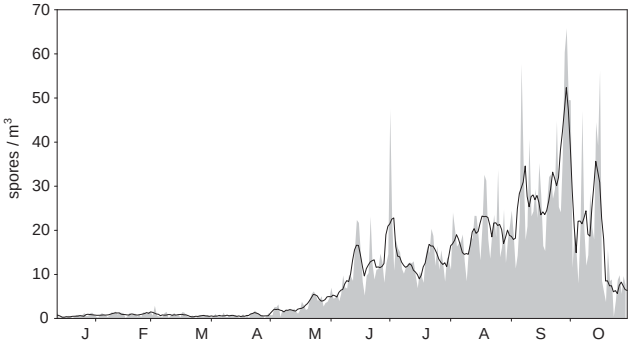

Figure 2. Daily variation (January–October) of the average of *Epicoccum* daily spore counts in Trieste for the studied years (1994–2004); the line shows the average of the 5-day running means.

The seasonal (June–October) concentrations of *Alternaria* spores between 1993–2004 display a mean total of 6,076 spores (Tab. 1). 2003 was an exceptionally high year with a seasonal total of 15,021 spores and particular climatic conditions: 87 days with mean temperatures above 23°C against an average of 64 days for the studied years; a total rain of 300 mm against an average of 463 (from June–September, only 135 mm against an average of 344).

Within the seasons, *Alternaria* daily counts in Trieste were rather low, often below the putative threshold value for provoking allergic symptoms (100 sp/m^3). On average, there are 13 days in the season when spore concentrations exceed this value. The highest number of days with over 100 spores occurred in the exceptionally high year of 2003, when daily counts over 1,000 sp/m^3 were occasionally found. The mean pattern of daily counts shows various peak periods, with the highest values occurring in September (Fig. 1). The peak day per year occurred on various dates: the earliest was in 2002 and the latest in 1998 (Tab. 1). Yearly peak values were rather different; the highest daily spore count (1,374 sp/m^3) was recorded in 2003, on 20 September, and the lowest (150 sp/m^3) was observed in 1993, on 31 August.

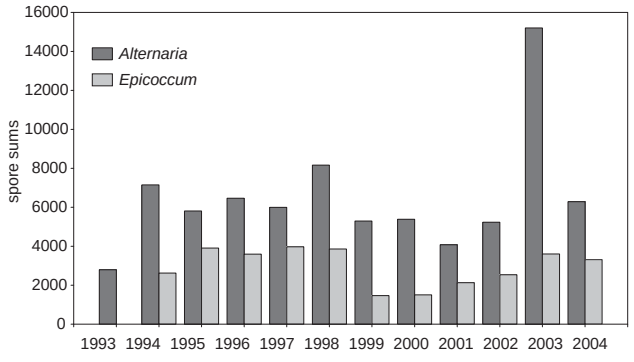

Figure 3. January–October total sums of the daily *Alternaria* and *Epicoccum* spore concentrations in Trieste in each of the studied years.

There is a great variation in monthly spore concentration of *Alternaria* per year (Fig. 4). From 1993–2000, the highest monthly sum was found in July or August, when the maximum number of days with the daily mean temperature > 23°C occurred; in some of these months, minimum rainfall was also observed. This relation is not so clear for the other years. In 2002, the spore levels for June were the highest; in 2001, 2003 and 2004, the pattern of monthly sums showed a delayed occurrence of *Alternaria* airborne spores, September had the highest monthly total.

The seasonal (June–October) spore concentrations of *Epicoccum* from 1994–2004 display a mean total of 2,796 spores, 1997 being the highest season and 1999 the lowest (Tab. 2).

The daily counts of *Epicoccum* were lower than those of *Alternaria* and the mean pattern shows increasing values from mid August, with the highest mean values occurring in the late summer–early autumn (Fig. 2). The peak day per year usually occurred in September, the earliest was in 2002 and the latest in 1998 (Tab. 2). Yearly peak values were rather different, with the highest daily spore count (433 sp/m^3) occurring in 1995, on 1 July, and the lowest (85 sp/m^3) in 2000, on 13 September.

Table 2. Aerobiological data of *Epicoccum* June–October spore seasons 1994–2004 in Trieste, Italy.

Year	Peak value	Peak date	Seasonal totals
1994	307	6–9	2,444
1995	433	1–7	3,677
1996	299	30–9	3,459
1997	166	13–9	3,800
1998	388	16–10	3,675
1999	101	25–9	1,178
2000	85	13–9	1,450
2001	292	28–9	1,989
2002	150	15–6	2,283
2003	258	20–9	3,564
2004	393	6–10	3,240
mean	261	7–9	2,796

The highest monthly spore concentrations of *Epicoccum* per year (Fig. 5) were usually found in September. In the years 1995, 1997 and 2002, the highest monthly sum occurred earlier, in those months in which the highest mean temperature in the season occurred. In 1998, a delayed occurrence of *Epicoccum* airborne spores occurred, October having the highest monthly total. The association between the monthly levels of *Epicoccum* and the meteorogical parameters are usually not evident.

The very high correlation between the spore counts of *Alternaria* and *Epicoccum* in each year (Tab. 3) suggests both taxa respond in similar way to the weather variables.

All temperature parameters are strongly correlated in all years with *Alternaria* and *Epicoccum* spore concentrations (Tab. 3), suggesting a significant and positive effect of

Table 1. Aerobiological data of *Alternaria* June–October spore seasons 1993–2004 in Trieste, Italy.

Year	Seasonal data		Number of days						Seasonal totals of spores
	Peak value	Peak date	Spores: 0–99	100–199	200–499	500–999	1000–1999	≥100	
1993	150	31–8	149	2	0	0	0	2	2,582
1994	665	28–8	133	7	1	2	0	10	6,531
1995	313	14–10	135	7	2	0	0	9	5,372
1996	313	15–7	115	18	1	0	0	19	6,014
1997	176	13–9	123	15	0	0	0	15	5,893
1998	324	16–10	111	16	5	0	0	21	7,655
1999	242	17–8	137	6	2	0	0	8	4,527
2000	190	2–7	131	11	0	0	0	11	4,994
2001	500	28–9	143	3	1	1	0	5	3,687
2002	216	15–6	141	4	2	0	0	6	4,623
2003	1,374	20–9	67	29	9	3	2	43	15,021
2004	392	6–10	133	4	6	0	0	10	6,015
mean	405	30–8	126	10	2	2	0	13	6,076

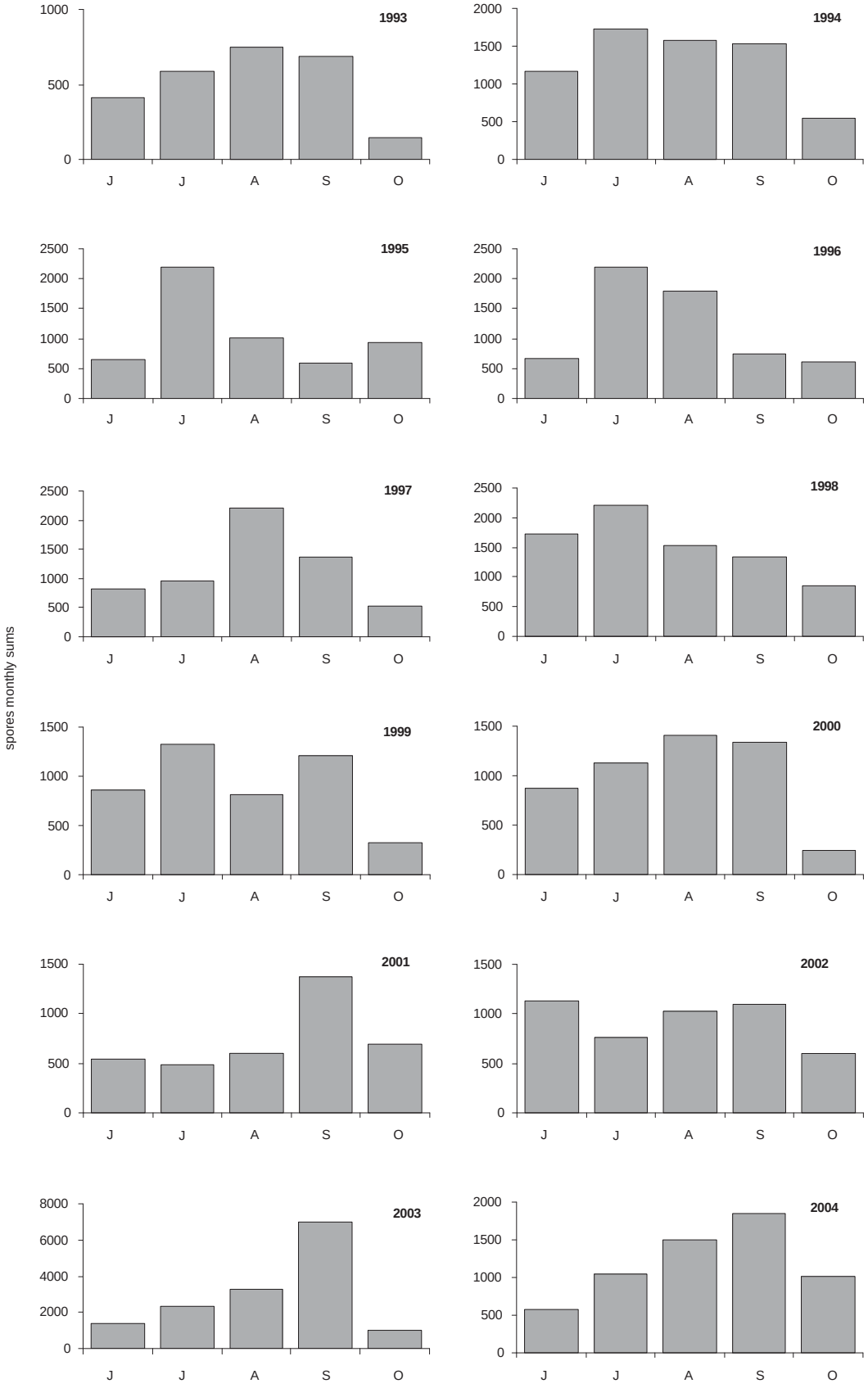

Figure 4. Monthly *Alternaria* spore concentrations in Trieste in each of the studied seasons (June–October). Note the different scales.

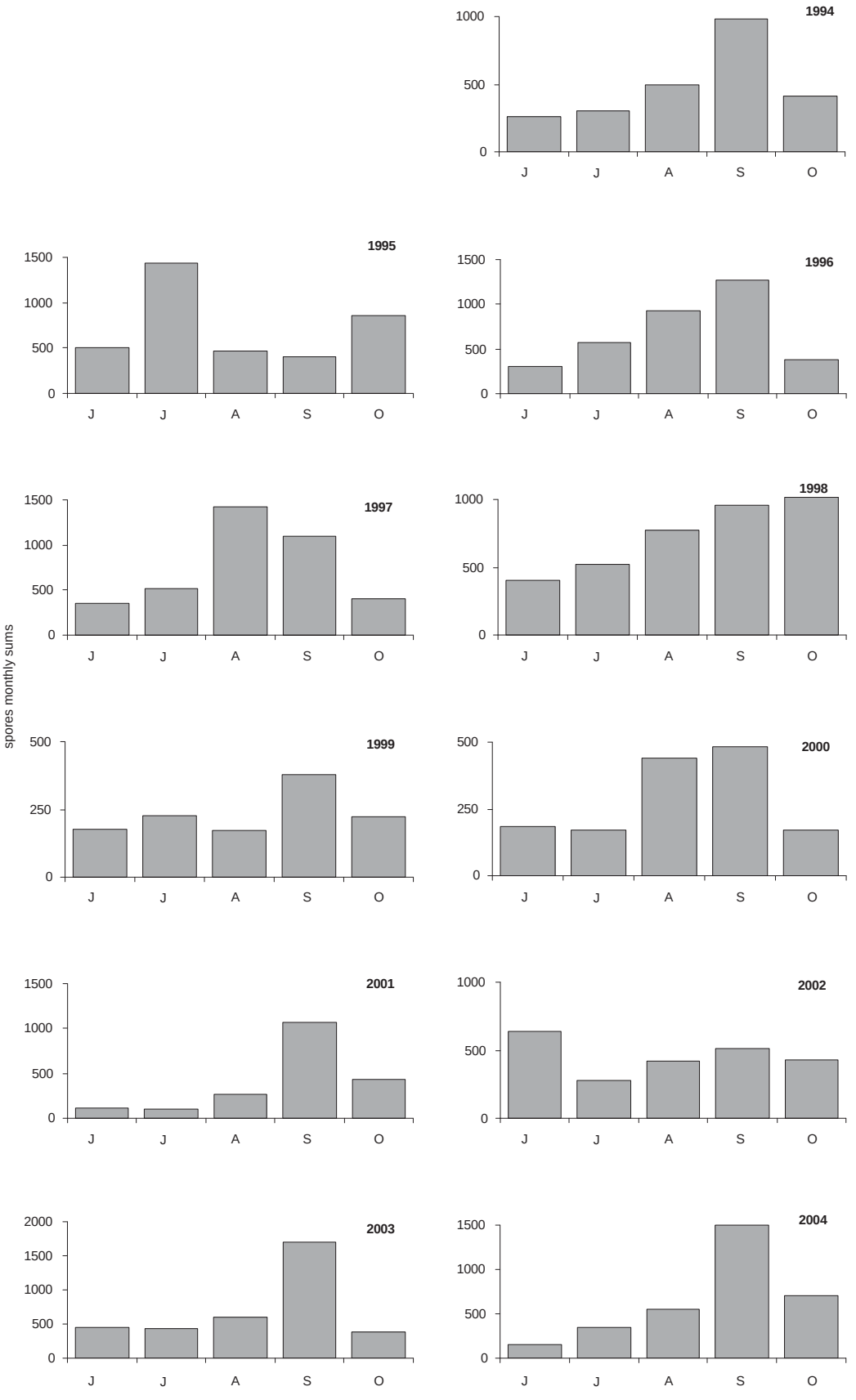

Figure 5. Monthly *Epicoccum* spore concentrations in Trieste in each of the studied seasons (June–October). Note the different scales.

Table 3. Spearman's correlation coefficients for daily spore counts of *Alternaria* (1993–2004) and *Epicoccum* (1994–2004) according to meteorological parameters.

Alternaria	d.f.	*Epicoccum*	T_{mean}	T_{min}	T_{max}	H_{rel}	R	W	W_{max}
1993	263	.	0.813 ***	0.814 ***	0.797 ***	-0.019	-0.021	0.003	-0.101
1994	249	0.803 ***	0.814 ***	0.815 ***	0.807 ***	-0.155 *	-0.109	-0.192 **	-0.223 ***
1995	279	0.816 ***	0.801 ***	0.800 ***	0.794 ***	-0.079	-0.126 *	-0.045	-0.132 *
1996	266	0.814 ***	0.813 ***	0.793 ***	0.805 ***	-0.003	-0.080	-0.161 **	-0.192 **
1997	211	0.855 ***	0.771 ***	0.770 ***	0.754 ***	0.122	0.030	-0.103	-0.175 *
1998	257	0.830 ***	0.778 ***	0.772 ***	0.754 ***	-0.026	-0.079	-0.041	-0.108
1999	287	0.749 ***	0.789 ***	0.787 ***	0.765 ***	-0.165 **	-0.215 **	-0.021	-0.108
2000	266	0.743 ***	0.805 ***	0.796 ***	0.791 ***	-0.213 ***	-0.051	0.087	0.007
2001	277	0.790 ***	0.697 ***	0.697 ***	0.670 ***	-0.063	-0.160 **	-0.095	-0.152 *
2002	229	0.715 ***	0.564 ***	0.566 ***	0.541 ***	-0.084	-0.104	0.016	-0.017
2003	229	0.857 ***	0.595 ***	0.586 ***	0.581 ***	0.122	0.069	0.005	-0.067
2004	238	0.771 ***	0.657 ***	0.661 ***	0.644 ***	-0.077	-0.196 **	-0.170 **	-0.193 **
Epicoccum	d.f.	*Alternaria*	T_{mean}	T_{min}	T_{max}	H_{rel}	R	W	W_{max}
1993	263	.							
1994	249	0.803 ***	0.714 ***	0.717 ***	0.708 ***	-0.170 **	-0.152 *	-0.180 **	-0.237 ***
1995	279	0.816 ***	0.685 ***	0.680 ***	0.685 ***	-0.090	-0.211 **	-0.059	-0.143 *
1996	266	0.814 ***	0.676 ***	0.663 ***	0.667 ***	-0.036	-0.150 *	-0.138 *	-0.182 **
1997	211	0.855 ***	0.667 ***	0.668 ***	0.653 ***	0.175 *	-0.021	-0.170 *	-0.225 **
1998	257	0.830 ***	0.669 ***	0.651 ***	0.657 ***	-0.145 *	-0.155 *	0.002	-0.062
1999	287	0.749 ***	0.530 ***	0.528 ***	0.519 ***	-0.178 **	-0.261 **	-0.064	-0.143 *
2000	266	0.743 ***	0.667 ***	0.683 ***	0.653 ***	-0.185 **	-0.084	0.065	-0.012
2001	277	0.790 ***	0.565 ***	0.578 ***	0.536 ***	-0.010	-0.077	-0.064	-0.110
2002	229	0.715 ***	0.434 ***	0.418 ***	0.431 ***	-0.156 *	-0.115	0.049	0.020
2003	229	0.857 ***	0.545 ***	0.531 ***	0.537 ***	0.017	-0.017	0.026	-0.053
2004	238	0.771 ***	0.551 ***	0.555 ***	0.541 ***	-0.142 *	-0.188 **	-0.050	-0.086

T_{mean} – mean daily temperature, T_{min} – minimum daily temperature, T_{max} – maximum daily temperature, H_{rel} – relative humidity, R – daily rainfall, W_{mean} – wind speed, W_{max} – maximum wind speed, d.f. – degrees of freedom. Level of significance: * $p<0.05$; ** $p<0.01$, ***$p<0.001$

temperature on these spore concentrations. Not all correlation values with the other meteorological parameters are significant, but if they are, they are always negative, except the correlation between *Epicoccum* and relative humidity in 1997. Significant correlations of the spores with all temperature parameters computed also on 5-day running mean values are all confirmed with higher values, whereas correlations of the spore concentrations with the other weather parameters, especially with rainfall, are often contradictory, changing the sign.

DISCUSSION

A great variability in daily and seasonal total concentrations of *Alternaria* and *Epicoccum* spores was found over the years, as observed in other sampling sites [10, 26, 27]. Highest daily values were usually recorded in late summer.

The seasonal pattern of *Alternaria* and *Epicoccum* airborne spores is similar. The similarity of the temporal pattern of different dry spores have already been observed in other studies [13, 15, 31]. Differences are based only on concentrations and temporal occurrence of their maximum peaks. *Epicoccum* concentrations are almost always less than *Alternaria*.

Daily counts of *Alternaria* spores considerably increase from June, with the peak daily count usually occurring in late August or September (occasionally in July or early October), and fall in late October. A similar seasonal pattern was observed in other European sites where the highest spore concentrations were usually found during August [6, 31]. A different pattern was found in the Mediterranean region, where two main seasons occur, one in spring and the other in the late summer and early autumn, with a mid-summer decrease in spore concentrations [2, 8, 22]. Daily values of *Epicoccum* spores increase during summer and peak in September (occasionally in July or October).

On considering seasonal total counts, there are differences among years and other sampling sites. A mean seasonal total of 6,076 spores of *Alternaria* and 2,796 spores

of *Epicoccum* were found in Trieste. For *Alternaria*, similar values were recorded in Cardiff, UK, [7], whereas lower values were found in other north-European and Italian towns, higher ones in Derby, UK, [6] and in some southern Spanish towns. In Trieste, 2003 appears as an exceptionally high year for *Alternaria*, perhaps as a consequence of the exceptionally hot, dry summer, as suggested for August in Zagreb by Peternel *et al.* [23]. The seasonal totals of *Epicoccum*, although lower than those of *Alternaria*, were in most cases higher in Trieste than in other sampling sites.

When considering airborne spore concentrations in different environments, Simeray *et al.* [27] and Kasprzyk and Worek [15] observed that higher values were found in rural environments than in urban areas. The highest *Alternaria* counts were especially found during periods of grass mowing, and at harvest time [6, 21]. In the UK [7] and in the NW Iberian Peninsula [1], higher *Alternaria* spore counts were found inland rather than in coastal areas because the proximity to the sea reduces spore levels. Given the coastal position of the town and the area lacking extensive arable crops, daily *Alternaria* counts were rather low in Trieste, often below the putative threshold value for provoking allergic symptoms; the incidence of allergic sensitisation to *Alternaria* in the town, in fact, is moderate [17].

The strong association between *Alternaria* and *Epicoccum* spore concentrations is the consequence of their very similar response to meteorological parameters and the life cycle of local vegetation, as underlined by Corden and Millington [6] and Stępalska and Wołek [31]. In our study, relationships between airspora and weather are in general agreement with other studies [1, 11, 21, 22]. *Alternaria* and *Epicoccum* spores tend to be found in higher concentrations during warm, dry weather conditions, usually preceded by rain in the preceding days, as found also by Rodríguez-Rajo *et al.* [25]. Given that there is a complex relationship among the airborne spore levels, the meteorological parameters and the available substrata, the differences between years in time and intensity of peak concentration are not easily explained. Also, the association between the monthly levels of *Alternaria* and *Epicoccum* and the meteorogical parameters are usually not evident, perhaps because they are masked by the relationship with substrata availability, grass mowing and cropping activities.

Significant and positive correlations of daily *Alternaria* spore concentrations with daily mean, minimum and maximum temperatures and negative correlation with daily rainfall are consistent with the results given by some authors, such as Mitakakis *et al.* [20], Angulo-Romero *et al.* [2], Corden and Millington [6] and Aira *et al.* [1]. The negative significant correlations with the maximum wind parameter found in our study support the findings of Lyon *et al.* [18] and Munuera Giner *et al.* [22], but do not agree with the findings of Hjelmroos [14]. Correlations between *Epicoccum* and weather in Trieste are very similar to those found for *Alternaria*. They are consistent with the results of Stępalska and Wołek [31].

CONCLUSIONS

Daily spore levels in Trieste showed great variability over the years. Airborne spores of *Alternaria* and *Epicoccum* tended to display similar temporal pattern. Peaking counts were usually found in late summer/early autumn. Compared to *Alternaria*, a shifted occurrence of the maximum peak of *Epicoccum* towards early autumn was observed. Differences in seasonal (June–October) total counts between years were recorded, with 2003 as an exceptionally high year for *Alternaria*. 6,076 spores of *Alternaria* and 2,796 spores of *Epicoccum* were found in the examined seasons, on average.

Associations between daily spore of *Alternaria* and *Epicoccum* levels and daily different meteorological parameters indicate significant positive relationships with mean, minimum, maximum temperatures in all years, and negative ones with humidity, rainfall and wind in some years.

Acknowledgements

The authors are grateful to Prof. Franco Stravisi from the Department of Earth Sciences, University of Trieste, who supplied the meteorological data. Thanks to MIUR for financial aid and the Civic Museum of Trieste for the use of S. Giusto Castle as the sampling site.

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