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Co-exposure to highly allergenic airborne pollen and fungal spores in Europe

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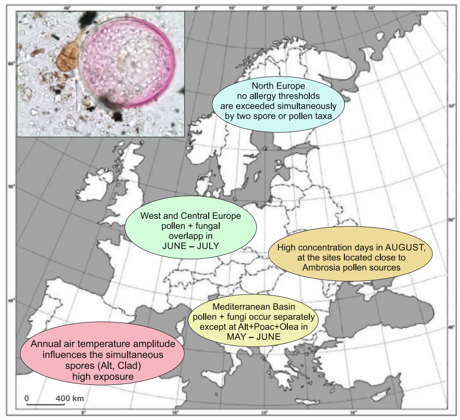
HIGHLIGHTS

- Co-occurrence of high pollen and high spore concentrations in Europe was studied.
- Five pollen and two fungal spore taxa were considered in different climatic zones.
- In central and north Europe, pollen and spores mainly overlapped in June and July.
- High pollen concentrations in the South occur usually outside of the spore season.
- The annual temperature amplitude influences the simultaneous spores high exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

The study is aimed at determining the potential spatiotemporal risk of the co-occurrence of airborne pollen and fungal spores high concentrations in different bio-climatic zones in Europe. Birch, grass, mugwort, ragweed, olive pollen and *Alternaria* and *Cladosporium* fungal spores were investigated at 16 sites in Europe, in 2005–2019. In Central and northern Europe, pollen and fungal spore seasons mainly overlap in June and July, while in South Europe, the highest pollen concentrations occur frequently outside of the spore seasons. In the coldest climate, no allergy thresholds were exceeded simultaneously by two spore or pollen taxa, while in the warmest climate most of the days with at least two pollen taxa exceeding threshold values were observed. The annual air temperature amplitude seems to be the main bioclimatic factor influencing the accumulation of days in which *Alternaria* and *Cladosporium* spores simultaneously exceed allergy thresholds. The phenomenon of co-occurrence of airborne allergen concentrations gets increasingly common in Europe and is proposed to be present on other continents, especially in temperate climate.

1. Introduction

Pollen and fungal spores are the main natural sources of inhalant allergens, responsible for respiratory allergic diseases, such as allergic rhinitis (AR) and asthma (Singh and Kumar, 2022). Allergic rhinitis affects up to 40 % of the world's population, including approximately 23–30 % of the European population (Tong and Lin, 2015). The prevalence of asthma varies depending on the region, but >5 % of any investigated population suffers from this disease (Virchow, 2021). In children, asthma may impair airway development and reduce maximally attained lung function, while in adults it can accelerate alveoli function decline and increase the risk of fixed airflow obstruction (Dharmage et al., 2019). Allergic rhinitis and asthma frequently co-exist as manifestations of the same disease expressed in either the upper or the lower airways (Damayanti et al., 2019).

The elicitation and exacerbation of AR symptoms in the sensitised individuals closely relate to exposure to airborne allergens, among which house dust mites, pollen and fungal spore allergens are the dominant ones (Bousquet et al., 2008; Smith, 2015). The risk of co-exposure to pollen and fungal spores is considered to be a greater risk for rhinitis (intermittent or persistent, depending on the clinical manifestation) in the outdoor contamination (Small et al., 2018).

In Europe, the most allergenic pollen-producing plants belong to grasses, birch family, mugworts, ragweed, olive, cypress and wall pellitory. Pollen from grasses are important all over the continent, pollen from birches prevail in western, northern and Central Europe, and from olive, cypress and wall pellitory constitute a main problem in the Mediterranean basin (Damialis et al., 2019 and citations therein). Grass-induced pollinosis is the most common pollen allergy worldwide, also in Europe affecting >95 % of sensitised-patients (D'Amato et al., 2007). In central and northern Europe, birch pollen is responsible for sensitization in 6.4 %–22.4 % of the population, while about of 3–15 % of people with inhalation allergy have a positive reaction to mugwort allergens.

Olive pollen, which is predominant in southern Europe, may differ in the way it releases allergens and contain allergens that cross-react with pollen from other species (González et al., 2000; Barderas et al., 2005).

Depending on the region under study, pollen seasons may cover more than half a year, whereas the length of the growing season varies across Europe, from 120 days in the northern part of Scandinavia, to 170–200 days in central Europe and up to >220 days in the Mediterranean region (Rötzer and Chmielewski, 2001). It must be stressed that the length of the growing seasons changes from year to year, primarily due to both high and low temperature extremes and the different precipitation patterns, adversely affecting plant development and vitality (Baumbach et al., 2017). These fluctuations can be reflected to the airborne pollen occurrence, which closely depends on the weather conditions of a given year and the climatic changes at a larger scale (Storkey et al., 2014; D'Amato et al., 2015; Ziska et al., 2019).

Compared to pollen, fungal spores have been much less studied (Beggs, 2010). The factors which influence the allergenicity of the different fungal spore taxa are little-known (Anees-Hill et al., 2022). Similarly to pollen, allergic responses to each spore type differ between individuals and fungal allergens vary in the severity of the allergic reaction they induce. For the dominant *Alternaria* and *Cladosporium* spores, the threshold concentrations for provoking allergic symptoms are very different, 100 spores/m^3 air and 3000 spores/m^3 air, respectively (Gravesen, 1979). Although *Cladosporium* spores are dominant in the air, they only induce mild allergic reactions (Katotomichelakis et al., 2016). However, *Alternaria* fungal spore taxa are responsible for hospital admissions due to severe asthma attacks in sensitised individuals, with a prevalence among children and with symptoms sometimes manifested as acute respiratory failure (Bush and Prochnau, 2004). The highest concentrations for both taxa typically occur in summer.

Until now, few examples of pollen and spore seasons co-occurrence have been published. Stępalska et al. (2017) indicated that in Poland *Ambrosia* and *Artemisia* pollen seasons can overlap and this depends on the air masses inflow and appears more often in the southeastern part of the country. It was found also in Rzeszów (Southern Poland), that the probability of becoming exposed to very high concentrations of allergenic pollen and fungal spores at the same time was, especially in July, when the highest concentrations of Poaceae, *Alternaria* and *Cladosporium* were recorded (Kasprzyk, 2008). Apart from the co-occurring pollen and fungal spores, anthropogenic pollutants, such as surface ozone, may be also found at high concentrations in the air affecting people's health synergistically (Grewling et al., 2019).

Because most patients with inhalant allergy are co-sensitised to different pollen and spore allergens, so seasons overlapping may be a cause of the intensified symptoms, particularly under the ongoing climate changes. Pollen seasons have been thoroughly documented, beginning earlier and existing longer (e.g. Damialis et al., 2019; Ziska et al., 2019). It is therefore anticipated that multiple allergen seasons are gradually overlapping, constituting increased health risks in the case of co- and multi-sensitisations which is being frequently ignored during the therapy.

Given the above, the main aim of this study was to provide evidence for the first time regarding the phenomenon of co-occurrence of high fungal spore and pollen concentrations of major allergenic taxa in Europe, which may cause elevated sensitization and symptomatology

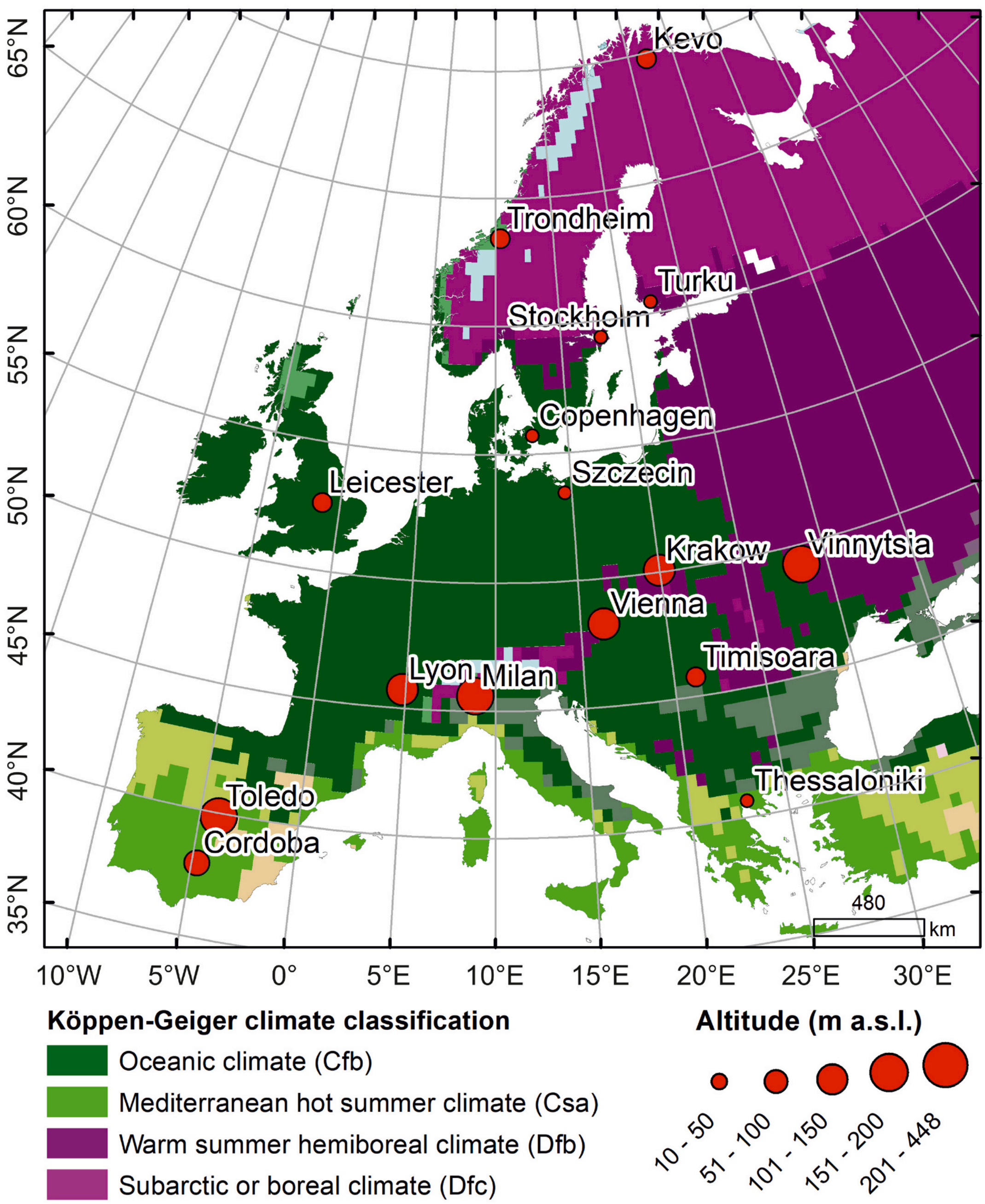

Fig. 1. Köppen-Geiger climate zones covering the study area. Apart from the location, altitude above sea level was shown (size of red points) Source: based on http://koeppen-geiger.vu-wien.ac.at/.

patterns beyond the identified thresholds. We specifically aimed to identify the number and timing of co-occurrence days, their long-term trends and general dependency on bioclimatic variables across a variety of European climates. Results of this study could also explain possible increase in the symptoms intensity beyond those expected above the identified thresholds.

2. Materials and methods

2.1. Study sites

Pollen and fungal spore data were obtained from 16 European aerobiological stations, selected to cover the four dominant climate zones in Europe: Mediterranean hot summer climate (Csa), Oceanic climate (Cfb), Warm summer hemiboreal climate (Dfb) and Subarctic or boreal climate (Dfc), according to Köppen-Geiger classification (Rubel and Kottek, 2010) (Fig. 1). These climates are delimited based on seasonal temperature and precipitation pattern and the delimitation criteria are listed in Kottek et al. (2006). Our dataset practically covers almost the whole European continent, from high-north Finland (Kevo), to southwest Spain (Cordoba), and east and southeast Ukraine (Vinnytsia) and Greece (Thessaloniki) (Fig. 1; Table S1; Supplementary materials).

2.2. Aerobiological and meteorological data

Pollen and fungal spore concentration were collected in 2005–2019, but in some stations the observation period was a bit shorter (Table S1). All the sites met the minimum requirements for aerobiological monitoring (Galán et al., 2014). Hirst-type volumetric traps were used to collect airborne pollen and fungal spores. Then samples were treated in the lab onto glass slides and under cover clips, and their content was identified and counted under an optical microscope according to the procedures proposed by the European Aerobiology Society (more details in Galán et al., 2014) and the European Standard EN 16868 (European Standard, 2019). The following highly allergenic pollen taxa were taken into account: birch (*Betula* sp.), grasses (Poaceae), mugwort (*Artemisia* sp.), ragweed (*Ambrosia* sp.), olive (*Olea europaea*), and two spore genera, *Alternaria* and *Cladosporium*, as the most allergenic and the most abundant in Europe (D'Amato et al., 2007).

Meteorological data in daily resolution were obtained from the OGIMET database by a climate R package (Czernecki et al., 2020). For simplicity, only thermal and precipitation variables were used to characterize climatic conditions of a particular site to meet Köppen-Geiger classification that is based on temperature and precipitation.

2.3. Data processing

First, we cleaned the daily aerobiological datasets from all sampling sites by carefully unifying different file formats, double-checking the timing and magnitude of spore and pollen time series, identifying gaps and removing years in which gaps covered main pollination/sporulation period of specific taxa/site, so that in the end all data were comparable. During the study period, general land use changes in the surrounding of measurement sites were minimal (Fig. S1; Supplementary materials). Here, we focused on fungal spore and pollen concentrations exceeding clinically significant thresholds, which are expected in real-life to potentially be responsible for allergic responses in sensitised individuals. Days with high pollen concentrations were selected according to what Pfaar et al. (2017) suggest for birch, grasses, ragweed, mugwort and olive as high pollen days. As the term "High pollen days" introduced by Pfaar et al. (2017) refers only to pollen, we propose a new general term for pollen and fungal spores: High concentration days (hereafter HC days). HC day corresponds to a day when daily spore or pollen concentrations exceed specific threshold values. Specifically, HC days referred to days that pollen concentration exceeded 100 pollen/m^3 for birch and olive, but 50 pollen/m^3 for grasses and ragweed according to the European Academy of Allergy and Clinical Immunology (Galán et al., 2017; Bastl et al., 2018). Additionally for mugwort the threshold for high concentration – was considered as 50 pollen/m^3 as reported by de Weger et al. (2013). In the case of fungal spores, the threshold values of 100 spores/m^3 for *Alternaria* and 3000 spores/m^3 for *Cladosporium* were considered, the most frequently indicated as having the minimum impact for causing manifestation of allergy symptoms (Grinn-Gofroń et al., 2019; Olsen et al., 2020). As the selection of thresholds is crucial for this study, we also applied the alternative approach to show how the variation in pollen or fungal spore thresholds may affect the results – mainly the density or number of days with high concentrations recorded for at least two taxa. We considered thresholds available in the literature recommended by de Weger et al. (2013). The allergy threshold studies were conducted in France (Déchamp et al., 1997; Thibaudon, 2003), Canada (Comtois and Gagnon, 1988; Banken and Comtois, 1990), Russia (Ostroumov, 1989), Austria (Jäger, 1998), Hungary (Juhász, 1998), Finland (Viander and Koivikko, 1978; Rantio-Lehtimäki et al., 1991), Poland (Rapiejko et al., 2007), Israel (Waisel et al., 2004), Spain (Antepara et al., 1995; Florido et al., 1999) and Great Britain (Davies and Smith, 1973) (Fig. S2; Supplementary materials). The threshold values were aggregated to low and high severity classes (Fig. S3; Supplementary materials). High severity class contained all thresholds marked at least as causing moderate symptoms or affecting majority of patients. Then we calculated the median threshold value for high severity class in all the pollen or spore types (Table S2; Supplementary materials).

High concentration days referred to days of HC simultaneously for at least two taxa, and in three combinations: (1) at least two pollen types, (2) two spore types, (3) at least one spore and one pollen type. Then, the frequency of these overlapping HC days was calculated, separately for each site showing the dominant period when these days occurred. This was presented by density plots, where density refers to the distribution of a numeric variable, in this case the number of overlapping HC days. For the most frequent combinations of specific fungal spore and pollen types, violin plots were generated. The width of a plot reflected the number of overlapping HC days with particular combinations. Also, the number of overlapping HC days was calculated per year and site for each of the four considered Köppen-Geiger climate types. All these plots were created using R software (R Core Team, 2022) and R packages ggplot2 (Wickham, 2016) and ggridges (Wilke, 2022).

2.4. Statistical analyses

Long-term trends of the timing and number of overlapping HC days for the most frequent combinations were tested using simple linear regression and *t*-test. The timing of overlapping HC days was quantified by indicating the first and last days (referred as the start and the end, respectively) when two taxa exceeded the appropriate thresholds in each study year. Moreover, trend parameters of duration of overlapping period (time between the start and the end day) were calculated. Finally, simple linear regression was used to show the relationship between the number and timing of overlapping HC days and bioclimatic variables calculated using dismo R package (Hijmans et al., 2017).

3. Results

3.1. Pollen and fungal spore concentrations across Europe

The studied pollen taxa were found to vary by region. During the study years, while olive pollen reached the highest concentrations in Southern Europe, grass pollen was abundant in West (Leicester, Lyon) and Central Europe (Vienna, Kraków, Szczecin). The highest airborne birch and mugwort pollen concentrations were recorded in hemiboreal climate: birch in Turku and Stockholm (North-East Europe), while mugwort in Vinnytsia (Eastern Europe) (Fig. 2). High ragweed pollen levels were primarily recorded in sites close to the Pannonian Plain, Po

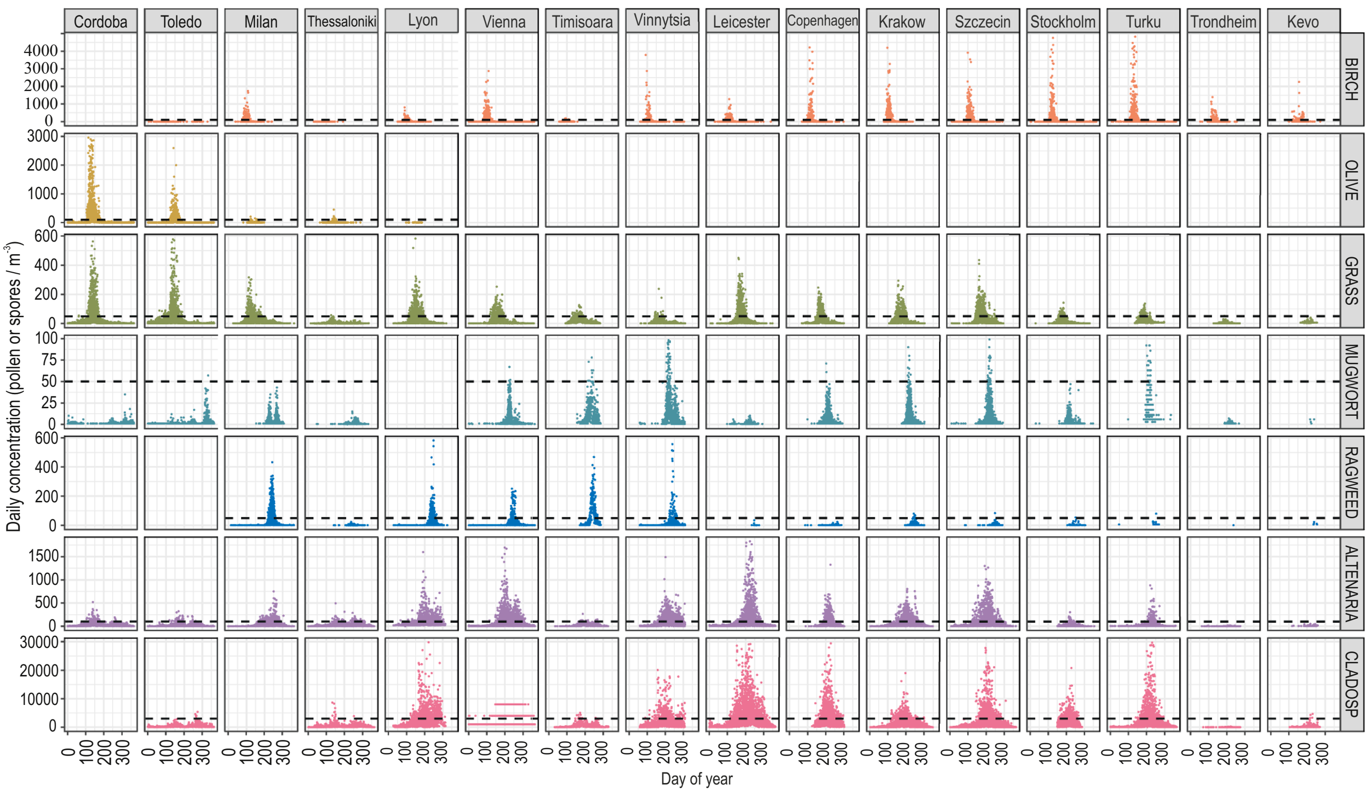

Fig. 2. Seasonal variation of clinically relevant airborne pollen and spore concentrations in Europe in 2005–2019. Note: for some stations the period with available data was shorter (see Table S1). For each taxon, the threshold values are marked as dashed lines.

Valley and Rhone Valley, known as centres of ragweed occurrence in Europe. In turn, fungal spores in humid conditions (western Europe, also coastal locations) outnumbered concentrations recorded in southern Europe and inland sites (Fig. 2).

3.2. Density of overlapping high concentration (HC) days

At first, we estimated general severity and timing of HC (high concentration) days of at least two taxa, be it pollen, spores or their combination. We detected 14 possible combinations (4723 days for all stations, 3640 using alternative approach) of simultaneously recorded HC days for two taxa and 7 possible combinations (390 days, 349 using alternative approach) for three taxa. HC of airborne spores and pollen occurred simultaneously in June and at the beginning of July regardless of the climate zone and the site location (Fig. 3). In addition, periods of co-occurrence of spores and pollen HC days was noted at the beginning of August in Eastern Europe or and late August to September in sites located close to *Ambrosia* pollen sources, near Lyon, Vienna, Timisoara and Milan. At least two pollen types simultaneously exceeded thresholds primarily in southern Europe (April–May) and exceptionally in Pannonian Plain (August). Interestingly, in southern Europe (Cordoba, Milan, Toledo, Thessaloniki) no co-occurrence of pollen HC days was recorded in July. *Alternaria* and *Cladosporium* spores generally showed low levels in Southern and Northern Europe (Trondheim, Kevo), but in the remaining sites they simultaneously exceed thresholds from late May (Thessaloniki, Kraków) to the beginning of October (Vienna, Lyon). The highest number of overlapping HC days for *Alternaria* and *Cladosporium* was recorded from the beginning of July to the middle of August, being delayed along the increasing latitudes. When using alternative thresholds, the pattern described above is similar except for one major difference – *Ambrosia* and *Artemisia* exceed thresholds simultaneously in greater number of cities (Milan, Timisoara, Vienna, Vinnytsia). Moreover, Poaceae and *Artemisia* pollen sometimes simultaneously exceed the alternative thresholds from late July to September in cities located close to Baltic coast (Turku, Szczecin) (Figs. S4, S5; Supplementary materials).

Considering two-taxa combinations, it is clear that the most frequent combinations were found for *Alternaria* and *Cladosporium* spores (in 68.8 % of all sites, located in Mediterranean, oceanic and hemiboreal climate), *Alternaria* spores and Poaceae pollen (62.5 % of sites, Mediterranean, oceanic and hemiboreal climate) and *Cladosporium* spores and Poaceae pollen (56.2 % of sites, oceanic and hemiboreal climate) (Fig. 4). HC days of *Alternaria* spores also coexisted with *Artemisia* or *Ambrosia* HC days (37.5 % of sites for each combination). HC days of *Cladosporium* spores similarly co-occurred with *Artemisia* (31.3 % of sites) and *Ambrosia* (18.8 % of sites) HC days. The co-occurrence of only pollen HC days is rarely noted in stations, apart from Poaceae-*Olea* pollen, which was recorded in southern Europe (Fig. 4). In two northern sites (subarctic or boreal climate) we did not detect any overlapping of HC days. Taking into account three-taxa combinations, only four possible combinations were recorded at more than one site. *Alternaria*, *Cladosporium* spores and Poaceae pollen HC days occurred simultaneously at 43.8 % of sites (mainly in oceanic climate), whereas both spore types and *Artemisia* pollen HC days were observed simultaneously at 31.3 % of sites (oceanic and hemiboreal climates) (Fig. 5). It is interesting that only *Alternaria* spores coincided with two pollen types, as in the case of Cordoba and Toledo (Mediterranean hot summer climate). The number of overlapping HC days per year and taxa both for two- and three-taxa combinations is shown in Fig. S6 (Supplementary materials). Using alternative thresholds (median approach) the pattern is very similar but with one additional combination: *Alternaria Artemisia Ambrosia*, which occurred together in Vienna (second half of August) and Vinnytsia (August to September) (Fig. S7).

It is clearly visible that in the coldest climate (Dfc – Northern Europe) no allergy thresholds are exceeded simultaneously by two spore or pollen taxa (Fig. 6). In the warmest climate (Csa – Mediterranean), the

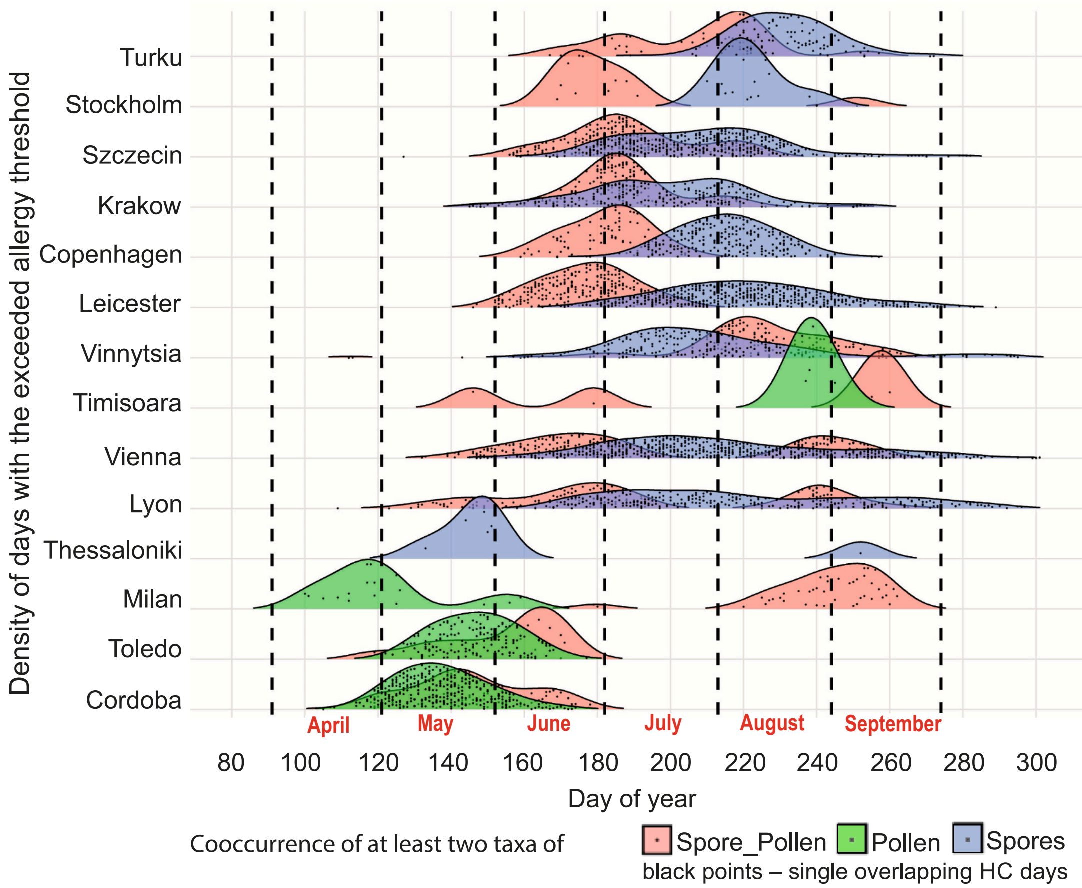

Fig. 3. Overall density of high concentration (HC) days co-occurrence for at least two taxa. Curve height indicates the frequency of co-occurrence. Only sites with at least one overlapping day are shown.

highest number of days was recorded when at least two major allergenic pollen taxa exceed high allergy thresholds, as evident in the case of *Olea* and Poaceae pollen. On the other hand, in oceanic climate (Cfb), with higher precipitation in summer, spore types play more important role and the number of days with *Alternaria* and *Cladosporium* spores exceeding allergy thresholds simultaneously was higher there. A similar situation was observed in hemiboreal climate (Dfb), but the number of overlapping days is slightly lower. Interestingly, the phenomenon of co-exposure to spores and pollen varies across the South to the North of Europe, showing that *Alternaria* spores are exceeding high thresholds more frequently in the areas of Mediterranean climate, while *Cladosporium* spores in areas with hemiboreal climate.

3.3. Trends

It was possible to assess trends in timing and severity of periods with overlapping HC days for following most frequent combinations (in 56.3 % of all sites): *Alternaria* and *Cladosporium* spores, *Alternaria* spores and Poaceae pollen, *Cladosporium* spores and Poaceae pollen (Table S1). Generally, insignificant trends were observed, although there is a general tendency to earlier start and end of overlapping HC day periods. In the case of *Alternaria*-Poaceae especially noticeable trend was found in Copenhagen and Szczecin, while in the case of *Cladosporium*-Poaceae only season end was getting earlier in both of these localizations (Table 1). In turn, the end of *Alternaria* and *Cladosporium* spores co-occurrence period was significantly delayed in Western Europe (Lyon, Leicester), while *Alternaria* and *Cladosporium* spores co-existing period ends in central Europe tended to be earlier although the only significant negative trend was recorded in Szczecin (northwestern Poland) (Table 1).

3.4. Overlapping HC days and bioclimate

We checked the possible influence of thermal- and precipitation–based bioclimatic variables (listed in https://www.worldclim.org/data/bioclim.html) on spatial and temporal pattern of HC days co-occurrence in Europe. It is evident that the number of overlapping HC days was significantly dependent on the annual temperature range (difference between mean temperature of warmest and coldest month) ($R^2 = 0.3$, $p = 0.0318$) (Fig. 7). The regression coefficient is negative which means that the larger the annual temperature range, the lower number of days when *Alternaria* and *Cladosporium* spores simultaneously exceed allergy thresholds. The relationships among the rest of the combinations are given in Fig. S8 (Supplementary materials).

4. Discussion

Pollen and fungal spore season dynamics reports deliver necessary information on when to expect airborne allergens (Myszkowska et al., 2011; Skjøth et al., 2016; Sikoparija et al., 2017; Kubik-Komar et al., 2019). These studies aimed at the evaluation of the specific features of the pollen/spore seasons of different taxa and many of them were focused on assessing the exposure of allergic individuals (Myszkowska et al., 2002; Pfaar et al., 2017; Biedermann et al., 2019; Steckling-Muschack et al., 2021). At least 10 % of the general population and between 50 % and 80 % of patients consulting allergists are poly-sensitised (Migueres et al., 2014), manifesting (i) cross-reactivity/cross-sensitization (the same IgE binds to several different allergens with common structural features) or (ii) co-sensitization (the simultaneous presence of different specific immunoglobulins E (IgEs) that bind to allergens that may not necessarily have common structural features).

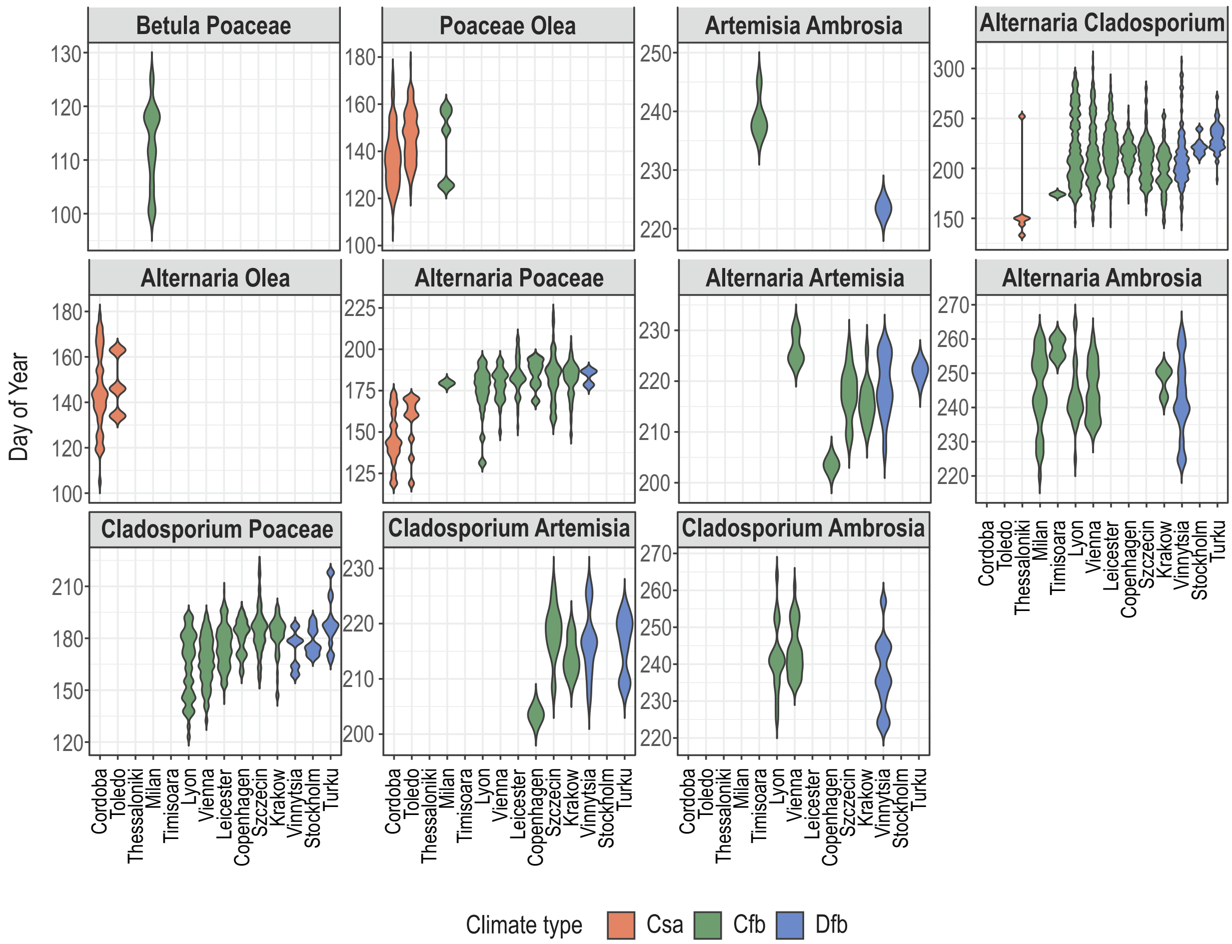

Fig. 4. Timing of two taxa co-occurrence of high concentration (HC) days per climate type. The wider the violin plot, the more frequent co-occurrence of HC days. Comparisons were conducted for the cases that at least two taxa exceeded allergy threshold at each site. Only sites with at least one overlapping day are shown. Csa – Mediterranean hot summer climate; Cfb – Oceanic climate; Dfb – Warm summer hemiboreal climate.

According to Burbach et al. (2009), who examined data from patients in a multicentre, pan-European GA2LEN study, the risk of allergic rhinitis increased with the number of sensitizations. Because polysensitization related to the new sources of allergens develops over time, it may be associated with disease severity and allergen immunotherapy (Pfaar et al., 2017). For the reasons mentioned above, the insight into the phenomenon of the pollen and fungal spore seasons overlapping can be crucial for confirming and predicting the clinical symptoms occurrence in patients with polyvalent pollen allergy. Moreover, because of the changes in the pollen seasons' dynamics caused by a variety of environmental factors (e.g. temperature increase, thunderstorms, lack of water availability), frequently documented not only in Europe (Ziello et al., 2012; Kurganskiy et al., 2021), but in the whole northern hemisphere (Ziska et al., 2019), the problem of airborne allergens co-exposure is dynamic and worthy of the permanent monitoring.

Our results show (Fig. 4) that on the South of Europe, particularly in Spain, olive (*Olea europea* L.) pollen coincide with the grass pollen seasons from April to June raising a problem of exposure to two allergenic pollen types simultaneously. When using alternative thresholds (Table S2; Supplementary materials) olive and grass pollen coincide with *Alternaria* spores, too (Fig. S7; Supplementary materials). The period of greatest risk to human health due to olive pollen in the south of Italy is limited primarily to the last 10 days of May (Bonofiglio et al., 2013). However, depending on the climate scenario considered by the authors (temperature increase from 2.2 °C to 6.0 °C in southern Europe and in the Mediterranean countries until 2100) (IPCC, 2023), the olive pollen allergy season might be significantly precocious in future decades (20–30 days earlier in the year), creating a risk of overlapping with the other pollen seasons, especially because olive pollinosis is quite rare in the form of monosensitization (Gioulekas et al., 2004).

In Milan (Italy), where the oceanic climate influence is marked, grass pollen does not coincide clearly with olive pollen, but with birch pollen, which is specific for this region only, and may occur between the second half of April and first half of May (Fig. 4, Fig. S5; Supplementary materials). Birches grow in the northern part of Italy, on the southern slopes of the Alps (Beck et al., 2016) and high concentrations could be recorded in northern Italy. *Betula pendula* Roth pollen can reach southern regions of Europe under long distance transport, such as Iberian Peninsula, South Italy and Greece (Alarcón et al., 2022) and join the grass pollen season mainly at the end of April, when it is starting. In other European sites, birch pollen seasons are ahead of the grass pollen seasons (Fig. 2), so high concentrations of these pollen types do not overlap at all (Khwarahm et al., 2017).

A major problem is observed in Romania (Timisoara) and in Ukraine (Vinnytsia), where *Artemisia* (mugwort) and *Ambrosia* (ragweed) pollen occur simultaneously, but at a different time and high concentrations overlap very rarely (1 day/year, Fig. S6; Supplementary materials). Nevertheless, ragweed and mugwort pollen may be airborne at the same time, what is also true for the area between the Pannonian Plain (Timisoara) and Vinnytsia (Ukraine) (Stępalska et al., 2017), where sensitization to pollen of both taxa has been confirmed (Ianovici and Sirbu, 2007; Afonin et al., 2018; Rodinkova et al., 2018). In many European regions, the problem of co-sensitization or cross-sensitization to mugwort and ragweed pollen exists, making the diagnostics of the

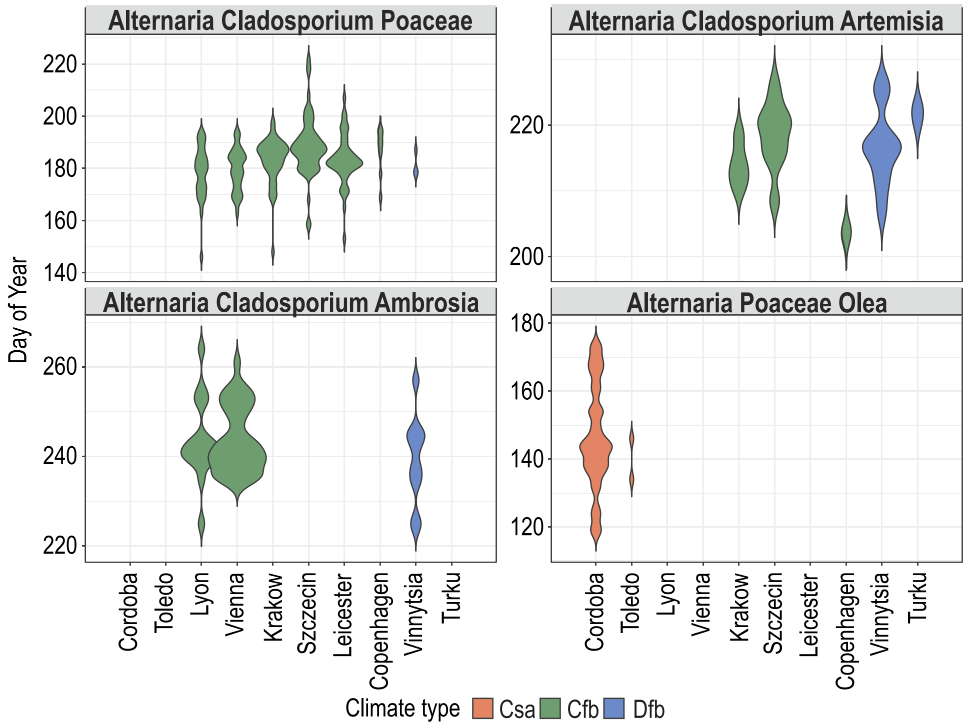

Fig. 5. Timing of three taxa co-occurrence of high concentration (HC) days per climate type. The wider the violin plot, the more frequent co-occurrence of HC days. This comparison was limited only to sites that allergy thresholds were exceed for at least three taxa, either pollen or spores or a combination of these. Csa – Mediterranean hot summer climate; Cfb – Oceanic climate; Dfb – Warm summer hemiboreal climate.

Fig. 6. Number of overlapping days with the high concentration (HC) of airborne pollen/spore types in relation to climate type, per year and site. Points show mean temperature (°C) calculated for study sites located in particular climate type. Csa – Mediterranean hot summer climate; Cfb – Oceanic climate; Dfb – Warm summer hemiboreal climate; Dfc – subarctic or boreal climate.

primary sensitizing allergen a quite difficult task and consequently the implementation of the proper immunotherapy (Asero et al., 2014).

Common occurrence of fungal spores, including the most allergenic *Alternaria* and *Cladosporium* genera (D'Amato et al., 2007), makes the individual fungal allergen exposure hard to estimate. In Europe, only the northern part of Fennoscandia is almost free of *Alternaria* spores (Fig. 2),

Table 1
Trends in the timing and numbers of overlapping high concentration (HC) days for the most frequent two-taxa combinations. Results are shown for each season's feature (start, end, duration, number of co-occurrence days) examined after performing linear regression. Significant trends ($p < 0.05$) are marked in bold. Csa – Mediterranean hot summer climate; Cfb – Oceanic climate; Dfb – Warm summer hemiboreal climate; Dfc – subarctic or boreal clime.

Climate type and study sites												
Most frequent combinations		Csa		Cfb						Dfb		
		Cordoba	Toledo	Copenhagen	Krakow	Leicester	Lyon	Vienna	Szczecin	Stockholm	Turku	Vinnytsia
Alternaria/ Cladosporium	Start	–	–	a = −0.561 R^2 = 0.05 *p* = 0.411	a = 1.14 R^2 = 0.08 *p* = 0.336	a = 0.319 R^2 = 0.005 *p* = 0.812	a = 0.807 R^2 = 0.016 *p* = 0.746	a = −0.311 R^2 = 0.018 *p* = 0.633	**a = −0.907 R^2 = 0.26 *p* = 0.05**	a = 0.877 R^2 = 0.11 *p* = 0.473	a = 1.14 R^2 = 0.099 *p* = 0.273	a = −1.35 R^2 = 0.096 *p* = 0.384
	End	–	–	a = 0.471 R^2 = 0.05 *p* = 0.429	a = −1.02 R^2 = 0.086 *p* = 0.332	**a = 2.73 R^2 = 0.6 *p* = 0.002**	**a = 5.25 R^2 = 0.68 *p* = 0.006**	a = −1.15 R^2 = 0.034 *p* = 0.51	**a = −2.7 R^2 = 0.28, *p* = 0.044**	a = 3.43 R^2 = 0.25 *p* = 0.737	a = −0.507 R^2 = 0.026 *p* = 0.579	a = −0.692 R^2 = 0.004 *p* = 0.855
	Duration	–	–	a = 1.03 R^2 = 0.09 *p* = 0.289	a = −2.16 R^2 = 0.1 *p* = 0.285	a = 2.41 R^2 = 0.19 *p* = 0.141	a = 4.45 R^2 = 0.2 *p* = 0.231	a = −0.839 R^2 = 0.013 *p* = 0.68	a = −1.79 R^2 = 0.12 *p* = 0.204	a = −0.534 R^2 = 0.43 *p* = 0.108	a = −1.65 R^2 = 0.1 *p* = 0.266	a = 0.655 R^2 = 0.003 *p* = 0.889
	Number of days	–	–	a = −0.068 R^2 = 0.01 *p* = 0.906	**a = −1.42 R^2 = 0.27 *p* = 0.045**	a = 0.571 R^2 = 0.045 *p* = 0.485	a = 1.44 R^2 = 0.1 p = 0.411	a = 1.4 R^2 = 0.2 *p* = 0.094	a = −0.85 R^2 = 0.079 *p* = 0.31	a = 0.129 R^2 = 0.1 *p* = 0.247	a = −0.182 R^2 = 0.019 *p* = 0.628	a = 1.79 R^2 = 0.13 *p* = 0.268
Alternaria/ Poaceae	Start	a = −0.7 R^2 = 0.1 *p* = 0.597	a = −0.17 R^2 = 0.0 *p* = 0.97	**a = −1.63 R^2 = 0.89 *p* = 0.005**	a = −0.78 R^2 = 0.09 *p* = 0.302	a = −0.04 R^2 = 0.000 *p* = 0.967	a = −0.78 R^2 = 0.023 p = 0.746	a = 0.015 R^2 = 0.00 *p* = 0.98	**a = −1.69 R^2 = 0.3 *p* = 0.035**	–	–	a = −0.879 R^2 = 0.96 *p* = 0.123
	End	a = 2.6 R^2 = 0.04 *p* = 0.75	**a = −4.44 R^2 = 0.9 *p* = 0.03**	**a = −1.04 R^2 = 0.78 p = 0.02**	a = −0.55 R^2 = 0.12 p = 0.23	a = 2.0 R^2 = 0.01 *p* = 0.76	a = −0.514 R^2 = 0.1 *p* = 0.493	a = −0.954 R^2 = 0.28 *p* = 0.062	**a = −2.04 R^2 = 0.35 *p* = 0.021**	–	–	a = −0.835 R^2 = 0.99 *p* = 0.058
	Duration	a = 3.3 R^2 = 0.051 *p* = 0.715	a = −4.28 R^2 = 0.45 *p* = 0.326	a = 0.586 R^2 = 0.52 *p* = 0.106	a = 1.48 R^2 = 0.005 *p* = 0.811	a = 0.232 R^2 = 0.005 *p* = 0.818	a = 0.263 R^2 = 0.003 *p* = 0.903	a = −0.969 R^2 = 0.15 *p* = 0.188	a = −0.35 R^2 = 0.014 *p* = 0.67	–	–	a = 0.044 R^2 = 0.18 *p* = 0.725
	Number of days	–	–	a = 0.106 R^2 = 0.56 *p* = 0.087	a = 0.00 R^2 = 0.00 *p* = 1	a = 0.21 R^2 = 0.082 *p* = 0.342	a = −0.17 R^2 = 0.023 *p* = 0.695	a = −0.21 R^2 = 0.2 *p* = 0.099	a = −0.179 R^2 = 0.02 *p* = 0.612	–	–	a = −0.036 R^2 = 0.02 *p* = 0.667
Cladosporium/ Poaceae	Start	–	–	a = −0.989 R^2 = 0.21 *p* = 0.139	a = 0.632 R^2 = 0.143 *p* = 0.519	a = −0.088 R^2 = 0.003 *p* = 0.867	a = −1.13 R^2 = 0.037 p = 0.68	a = −0.428 R^2 = 0.02 *p* = 0.613	a = −0.308 R^2 = 0.02 *p* = 0.636	a = −0.401 R^2 = 0.032 *p* = 0.733	a = −0.155 R^2 = 0.003 *p* = 0.886	a = −1.46 R^2 = 0.27 *p* = 0.653
	End	–	–	**a = −1.53 R^2 = 0.46, *p* = 0.015**	a = −0.533 R^2 = 0.086 *p* = 0.355	a = −0.355 R^2 = 0.038 *p* = 0.524	a = −0.767 R^2 = 0.14 *p* = 0.406	a = −0.5 R^2 = 0.044 *p* = 0.455	**a = −1.64 R^2 = 0.38 p = 0.02**	a = −1.13 R^2 = 0.41 *p* = 0.171	a = −1.12 R^2 = 0.11 *p* = 0.309	a = 0.143 R^2 = 0.003 p = 0.967
	Duration	–	–	a = −0.543 R^2 = 0.07 *p* = 0.413	a = −1.17 R^2 = 0.21 *p* = 0.133	a = −0.247 R^2 = 0.013 *p* = 0.707	a = 0.361 R^2 = 0.006 *p* = 0.872	a = −0.071 R^2 = 0.001 *p* = 0.921	a = −1.33 R^2 = 0.17 *p* = 0.137	a = −0.727 R^2 = 0.37 p = 0.204	a = −0.964 R^2 = 0.11 *p* = 0.319	a = 1.61 R^2 = 0.96 *p* = 0.121
	Number of days	–	–	a = −0.064 R^2 = 0.01 *p* = 0.749	a = −0.404 R^2 = 0.31 *p* = 0.033	a = −0.099 R^2 = 0.003 *p* = 0.856	a = −0.368 R^2 = 0.078 *p* = 0.468	a = 0.03 R^2 = 0.001 *p* = 0.914	a = −0.214 R^2 = 0.031 *p* = 0.531	a = 0.004 R^2 = 0.00 *p* = 0.96	a = −0.089 R^2 = 0.14 *p* = 0.166	a = 0.064 R^2 = 0.051 *p* = 0.504

a – slope, R^2 – coefficient of determination, *p* – *p*-value (probability), Duration – time between the first and last day with overlapping HC, Number of days – high concentration days (HC days).

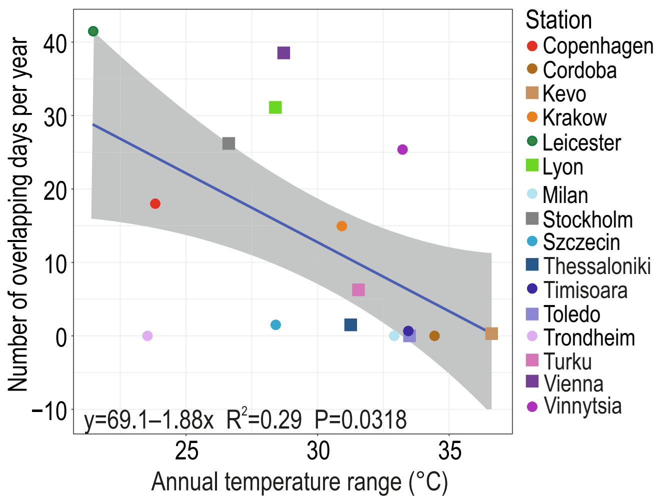

Fig. 7. Relationship between annual temperature range and *Alternaria*-*Cladosporium* co-occurrence of high concentration (HC) days. Data from the selected sites were equally considered in the linear regression analysis in order to estimate the relationship among the studied factors.

while *Cladosporium* spores' occurrence is limited by the temperature below 10 °C and not recorded during the snow cover deposition (Inglod, 1971). Consequently, as we showed in this study, there is no possibility of simultaneous co-occurrence of high concentration of *Alternaria* and *Cladosporium* spores in the boreal or subarctic climate (Dfc) (Fig. 6). In the systematic review by Anees-Hill et al. (2022), it was reported that the main fungal spore seasons occur in summer-autumn, with *Alternaria* spores' high concentrations ahead the *Cladosporium* peak days. We have also observed, that *Alternaria* and *Cladosporium* seasons are longer in southwestern Mediterranean compared to northeastern Atlantic and Continental regions (Fig. 3), which can suggest that as average global temperature rise, northern-European *Alternaria* and *Cladosporium* fungal seasons may become closer in characteristic to those within the south.

On the other hand, in the warmer areas of southern Europe the peak and annual concentrations became lower than in oceanic climate and in most locations, especially in western and central Europe, peak *Alternaria* and *Cladosporium* spore concentrations were above clinical thresholds (Anees-Hill et al., 2022). Our results show that the high concentrations of the spores of both taxa may co-occur primarily in oceanic (Cfb) and hemiboreal (Dfb) climate zones between June and September (Fig. 4).

As the *Alternaria* spore seasons are longer in the South according to Anees-Hill et al. (2022), the problem of overlapping with *Olea* and Poaceae pollen seasons should be considered. Generally, the co-occurrence of fungal spores and two pollen taxa is rather rare and is observed in the Mediterranean region only, where *Olea* pollen seasons dominate (Negral et al., 2021). People sensitive to *Alternaria* spore allergens and *Olea* pollen allergens, who manifested severe asthma symptoms from the middle of April to the end of June (Zureik et al., 2002) should be informed about the potential double treat in this period, because this problem seems to be more unexpected than the co-occurrence of *Alternaria* spores and Poaceae pollen (May–June).

In central and northern parts of Europe, fungal spores of the studied genera overlap mostly with pollen from herbaceous plant, especially with Poaceae pollen, roughly a month later (in the case of *Alternaria* and Poaceae) than in the southern part (Fig. 3).

The co-occurrence with airborne ragweed pollen is clearly seen in the regions of high airborne *Ambrosia* concentrations, in Vinnytsia, Lyon and Vienna (Sikoparija et al., 2017; Rodinkova et al., 2018), but only in Vinnytsia this phenomenon is preceding by the overlapping by spores and *Artemisia* pollen (Fig. 5). Considering the fact that in Vinnytsia on average 76 days exceed the *Alternaria* threshold value of 100 spores/m^3 (Kasprzyk et al., 2015), patients sensitive also to ragweed pollen should expect an added problem with clinical symptoms.

The problem of triple exposure of fungal spores and Poaceae pollen is characteristic for central and western Europe, during harvest time (Skjøth et al., 2012). In the case of *Artemisia*, this phenomenon of co-occurrence with high *Alternaria* and *Cladosporium* concentrations was observed in three locations of the oceanic climate (Cfb) and in two places of the hemiboreal climate (Dfb) (Fig. 5, Fig. S7; Supplementary materials). *Artemisia* pollen season is relatively short and the threshold of 50 pollen/m^3 is rarely exceeded apart from Poland and Ukraine (Rodinkova et al., 2018 and also Fig. 3 in this study). Along with ongoing climate warming, the *Artemisia* pollen season could be delayed particularly in the regions where two peaks of mugwort concentration, related to the other species, like *A. campestris* are recorded (Grewling et al., 2019). This delay may shorten the period of co-occurrence with *Alternaria* and *Cladosporium* high concentrations.

Although there is evidence that airborne *Alternaria* and *Cladosporium* spore increase along with the increasing temperature (Almaguer et al., 2014; Grinn-Gofroń et al., 2020), higher temperature differences limit the number of days with airborne spores as we found in this study (Fig. 7). Rainfall typically decreases daily spore concentrations (e.g. Angulo-Romero et al., 1999), but in our study we tested only monthly precipitation variables included in bioclimatic dataset Worldclim designed for broad scale studies (Fick and Hijmans, 2017), which could have limited the scope of obtained dependencies. On the other hand, by using this dataset, we intended to find some continental patterns in connection with the overlapping of high airborne fungal spore and pollen concentrations (Fig. S8; Supplementary materials).

It is also worth adding, that in this study two methods for determining thresholds, obtained by proposed by Pfaar et al. (2017) and "median approach" were used. In the "median approach", the threshold values were lower for *Betula*, *Artemisia*, *Ambrosia* and Poaceae, roughly by 10–20 pollen/m^3, whereas higher for *Olea* (by 127 pollen/m^3) and *Cladosporium* (by 2000 spore/m^3). It must be emphasized that these differences in thresholds between two methods were large; for example threshold for *Ambrosia* pollen decreased from 50 pollen/m^3 to 35 pollen/m^3 what means 30 % of the initial threshold value. Despite these changes in thresholds, the general pattern of high concentrations overlapping of: at least two pollen types, two spore types or pollen and spores is similar for two methods. The only major difference is that there occur another period of at least two pollen taxa overlapping in summer in Vienna, Vinnitsa, Szczecin and Turku (when thresholds by Pfaar et al. (2017) were used, this pattern was observed only in Pannonian Plain – Timisoara). This overlapping period is mainly connected with co-occurrence of *Artemisia* and *Ambrosia* pollen in the air. It should be also remembered that in central and partially northern Europe, in early spring, the pollen seasons of two taxa – hazel and alder – overlap. This phenomenon has been described many times in the literature (Dąbrowska-Zapart, 2010; Myszkowska et al., 2010; Nowosad et al., 2015). Due to the very low probability of hazel and alder pollen exceeding thresholds together with *Alternaria* and *Cladosporium* spore concentrations, they were not analysed in this study.

5. Conclusions

Summarizing, we have concluded that the phenomenon of co-occurrence of airborne allergen concentrations is relatively common in Europe, especially in temperate climate. Days with the high fungal spores and pollen concentration occur simultaneously in June and at the beginning of July regardless the climate zone and the site location. At the beginning of August, High Concentration days overlapping prevails in Eastern Europe, while in late August to September the simultaneous HC days are noted frequently at the sites located close to *Ambrosia* pollen sources, near Lyon, Vienna, Timisoara and Milan. In the coldest climate (Dfc – Northern Europe) no allergy thresholds are exceeded simultaneously by two spore or pollen taxa, in comparison to the other climatic zones, especially the warmest climate (Csa – Mediterranean), in which there is the most days when at least two major allergenic pollen taxa exceed high allergy thresholds, as evident in the case of *Olea* and Poaceae pollen. In our analyses we have considered the verified data from 16 stations, representative for the European climate zones, although we are aware that the data pool could have been larger. However, the problem in accessing the expected data was that in many stations only pollen was counted. As, the findings of co-occurrence appear to be related to specific climatic regions in Europe, then it is likely that this phenomena is also present on other continents with similar climate. In the future studies it would be worthy to verify if the co-occurrence is present on a global scale and what extent different threshold values, than those adopted by the authors in this study, affect the overlap of pollen and fungal seasons. Finally it is relevant to compare the obtained results with the clinical symptoms severity to define the clinical significance of the overlapping exposure in a given region.

It must be emphasized that not only overlapping periods when at least two pollen or spore types exceed allergy thresholds are important for sensitised persons. Also non-overlapping periods of high pollen or spore concentration that consecutively follow each other with only short gaps in between might markedly affect the health of sensitised people, prolonging their exposure to allergens. So, future studies should not be only focused on the frequency of overlapping high-concentration periods, but also on the non-overlapping periods that closely follow each other in time.

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Declaration of competing interest

All authors declare that they have no competing interests.

Data availability

The authors do not have permission to share data.

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