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Title

A systematic review of outdoor airborne fungal spore seasonality across Europe and the implications for health.

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Abstract

Fungal spores make up a significant proportion of organic matter within the air. Allergic sensitisation to fungi is associated with conditions including allergic fungal airway disease. This systematic review

analyses outdoor fungal spore seasonality across Europe and considers the implications for health. Seventy-four studies met the inclusion criteria, the majority of which (n=64) were observational sampling studies published between 1978 and 2020. The most commonly reported genera were the known allergens *Alternaria* and *Cladosporium*, measured in 52 and 49 studies, respectively. Both displayed statistically significant increased season length in south-westerly (Mediterranean) *versus* north-easterly (Atlantic and Continental) regions. Although there was a trend for reduced peak or annual *Alternaria* and *Cladosporium* spore concentrations in more northerly locations, this was not statistically significant. Peak spore concentrations of *Alternaria* and *Cladosporium* exceeded clinical thresholds in nearly all locations, with median peak concentrations of 565 and 18,827 per m³, respectively. Meteorological variables, predominantly temperature, precipitation and relative humidity, were the main factors associated with fungal seasonality. Land-use was identified as another important factor, particularly proximity to agricultural and coastal areas. While correlations of increased season length and decreased annual spore concentrations with increasing average temperatures were reported in multi-decade sampling studies, the number of such studies was too small to make any definitive conclusions. Further, up-to-date studies covering underrepresented geographical regions and fungal taxa (including the use of modern molecular techniques), the impact of land-use and climate change will help address remaining knowledge gaps. Such knowledge will help to better understand fungal allergy, develop improved fungal spore calendars and forecasts with greater geographical coverage, and promote increased awareness and management strategies for those with allergic fungal disease.

Keywords

Aerobiology; fungi; season; outdoor air; *Alternaria*; *Cladosporium*

1. Introduction

Fungi are eukaryotic organisms defined by their heterotrophic means of acquiring nutrition from the environment. Highly diverse in morphology and nutrition, these organisms play an essential role within a healthy ecosystem due to their ability to decompose and degenerate organic matter. Fungi

have been found to thrive in a wide variety of environments, with many being colonisers and parasites of plants and animals. As a consequence, humans are frequently exposed to airborne fungal spores, driving the need to consider associated health outcomes.

1.1 Fungal contribution to allergic disease

The role of fungi in the pathogenesis and exacerbation of atopic conditions is an ongoing area of study. The airborne, microscopic nature of fungal spores results in unavoidable inhalation, with more than 80 genera of fungi associated with allergic sensitisation (Horner et al., 1995; Pashley and Wardlaw, 2021). Sensitisation varies according to the taxonomic genera and species of fungi, with *Alternaria*, *Cladosporium* and *Aspergillus* recognised as important sensitisers (Idrose et al., 2020; Levetin et al., 2016). Given the large taxonomic and proteomic diversity within the fungal kingdom, there may well be taxonomic groups with allergenic properties yet to be identified (Crameri et al., 2014; Denning et al., 2006). Onset of atopic asthma has been linked with fungal spore exposure for some time, with associated conditions ranging from sensitisation to allergy (Denning et al., 2014; Pavord et al., 2018). Increasing evidence of fungal involvement with asthma has led to recognition of allergic bronchopulmonary mycosis (ABPM) as an endotype of asthma (Lötvall et al., 2011). ABPM is a pattern of disease caused by fungal allergy to airway colonising fungi, archetypically *Aspergillus fumigatus*. However, fungal sensitisation plays a role in asthma far beyond the scope of ABPM, as evidenced by the plethora of terminology used to classify such conditions, including severe asthma with fungal sensitisation (SAFS), allergic fungal airways disease (AFAD), airway mycosis and fungal asthma (Bush, 2020; Denning et al., 2006; Li et al., 2019; Pashley and Wardlaw, 2021; Rick et al., 2016). Globally, fungal sensitisation is estimated to affect 3-10% of the general population, 7-20% of asthma sufferers, 35-75% of people with severe asthma and 54-91% of people with life-threatening asthma (Del Giacco et al., 2017; Horner et al., 1995; Pashley, 2014; Pashley and Wardlaw, 2021). Fungal exposure is also implicated in thunderstorm asthma and allergic fungal rhinosinusitis (AFRS), the latter being a distinct form of nasal-focussed allergy that frequently concurs with allergic rhinitis and asthma (Hoyt et al., 2016; Idrose et al., 2020).

1.2 Air sampling and analysis of fungal composition

Fungal spore sampling can be performed via active or passive methods, with the appropriate method directed by the study question. Active methods involve use of a pump to draw air through a filter (filter sampling), into media or buffer (impinger sampling), or onto a solid surface (impactor sampling) (Mainelis, 2019). These methods are typically utilised in long-term sampling studies, and benefit from a variable rate of flow that can be set to mimic the rate of human respiration (Nunez et al., 2021). Sampled material will also include fungal hyphae and fragments of fungal material (Green et al., 2005). Passive methods rely upon the gravitational deposition of airborne matter upon a solid surface and benefit from low running and setup costs but are unable to sample large volumes of air or impinge or impact material into or onto suitable vessels for downstream identification and quantification.

Fungal spore identification following either active or passive sampling can involve viable or non-viable approaches. The use of a culture-based analysis relies upon subsequent growth of collected viable-fungi, and is associated with selection bias due to the varying nutritional requirements of fungi in their ability to grow on the media (Denning-Gofron, 2011). Non-viable approaches do not require the growth of fungi, with microscopy-based identification and counting remaining the most common method. As such, active volumetric impactor sampling combined with microscopy-based analysis has benefited from consistent and standardised methodology for many decades, and is the 'gold standard' for outdoor fungal spore monitoring (Grewling et al., 2019). Non-viable sample analysis remains valid within allergy research as the spores need not be viable to induce an allergic response (Denning et al., 2014). However, microscopy limits analysis to a few identifiable genera or groups of fungi, with a degree of expertise required to perform this. Recent advances in high throughput sequencing (HTS) have provided the opportunity to identify a wider spectrum of airborne fungi by amplifying and sequencing species-specific variable regions of the highly conserved ribosomal RNA genes, so-called metabarcoding. These advances have been brought in part by increased affordability and the continuing development of reference fungal DNA sequence

databases.

Other sampling considerations can impact upon the quantity and composition of the fungal spores collected. For example, the height at which an air sampler operates is important due to varying fungal spore concentrations at different elevations, with greater heights thought to produce samples representative of a greater region (Khattab and Levetin, 2008).

1.3 Fungal seasonality and associated driving factors

The composition and concentration of both indoor and outdoor airborne fungal spores is strongly governed by geographical location and seasonality. This is particularly true for outdoor fungal spores, which are typically higher in concentration compared to those within dry indoor environments (Garrett et al., 1997; Jara et al., 2017; Lee et al., 2006; Shin et al., 2015; Stern et al., 1999). Seasonal variations in outdoor fungal spores have been studied in many regions of the world (Eymedio et al., 2018; Gusareva et al., 2020; Irga and Torpy, 2016; Patel et al., 2018; Priyamvada et al., 2017). This seasonality is driven by several interplaying factors, including meteorological conditions, local vegetation sources, and anthropological activities such as agriculture and large-scale composting (Gioulekas et al., 2004; Górriz-Gofroń et al., 2020; Pearson et al., 2015), all of which affect fungal growth and sporulation. The effect of each factor varies by fungal species, with the complex dynamics not fully understood, even for fungi associated with allergy. Fungal spores associated with allergenicity are typically linked with crop farming or products of agriculture. For example, *Alternaria* is associated with disease in potato and tomato plants, *Cladosporium* with cereal plants, and *Aspergillus* with decomposing vegetation and compost sites (Mousavi et al., 2016; Pearson et al., 2015; Robertson et al., 2019). Seasonality of airborne spores from other fungal genera are relatively less well documented. There is, however, an enhanced understanding of spore seasonality for a class of fungi known as basidiomycetes, where there are visible fruiting bodies to mark the sporulation of fungi (Kausrud et al., 2008; Sato et al., 2012).

1.4 Allergic disease seasonality

Asthma also displays seasonality, with higher rates of exacerbations occurring in summer and

autumn (Denning et al., 2006). Viruses and pollens have been found to be associated with these, however, there is a growing base of evidence linking fungal spore exposure with allergenic conditions including asthma (Barnes, 2019; Denning et al., 2006; Forkel et al., 2021). The relative paucity and inconsistency of studies concerning species-specific sensitisation creates difficulties in establishing the concentration of outdoor fungal spores above which allergy-related clinical symptoms are observed (Cecchi et al., 2010). Only *Alternaria* and *Cladosporium* have defined clinical thresholds at 100 and 3000 spores per cubic metre, respectively (Gravesen, 1979; Katotomichelakis et al., 2016). Unfortunately, the high diversity of fungi in air also makes defining the allergenicity of individual fungi and establishing clinical thresholds a difficult task. Improved understanding of the factors that drive fungal seasonality, as well as the use of HTS in combination with health surveillance data, will help to both identify a wider range of fungi to greater taxonomic levels and improve identification of specific associations with allergy-related symptoms.

1.5 Aim

The aim of this systematic review was to analyse Europe-wide fungal spore seasonality and associated driving factors, and consider the relevance of this information for health.

2. Methods

2.1 Search strategy and selection criteria

Methodology for this systematic review was informed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2010). These provide an evidence-based checklist to guide the reporting of review-based findings in a transparent, reproducible manner. Search strings related to the sampling of outdoor air were used to search the OVID Medline, Web of Science and OpenGrey databases between January 1960 and May 2021 (detailed in Supplementary Information 1). Studies were screened first by title and abstract and then full text (author SA). Partial checks on 10% of the search results were performed on randomised entries by PD and EM (5% each). Discrepancies were resolved by discussion.

Studies were included if they:

- Were an original study published in English.
- Described airborne sampling of the outdoor air within Europe, across multiple meteorological seasons. Different seasons could be within a year or across multiple years, as long as sampling took place across multiple seasons.
- Identified airborne fungal spores to the taxonomic level of genera for at least one fungal taxon sampled.

Studies were excluded if:

- They did not describe outdoor, airborne fungal spores.
- Sampling was conducted within the confines or close proximity of an area known to possibly cause skewed fungal spore compositions or increased quantities of spores in the air. For example, composting sites, vineyards and forest plantations, which contain high volumes of substrates leading to high airborne concentrations of fungal spores (from specific taxa).
- They only identified fungal taxa following a culture-based approach.
- They were conference proceedings, a review, or an editorial.

2.2 Data extraction

Using a pre-designed template, the following study characteristics were extracted: authors, year, sampling locations, sampling period, sampling methodology, composition analysis method and fungal taxa identified. Spore concentration metrics, relevant seasonal dates and meteorological variable correlations with concentrations were also extracted for downstream meta-analyses. Values for total fungal spores within an air sample were not extracted due to the heterogeneous approaches of presenting actual totals or totals from select groups of fungi. Details were extracted for each sampler where multiple samplers were used within a study.

2.3 Statistical analysis

The relationship between fungal spore seasonality and the geographical location of air samplers was determined by regression analysis. Modelled dependent variables of interest included dates of fungal spore seasonality comprising season start, peak, end dates, and fungal season length. Fungal

season length was calculated by subtracting season end dates from season start dates where both dates were available. For inclusion as a model parameter, geographical location was calculated by converting decimal latitudinal and longitudinal values of sampler locations into kilometres from a fixed point via the haversine formula to account for the curvature of the earth. Biogeographical regions, as defined by the European Environment Agency (EEA) were also utilised as factor variables for models and map-based visualisations (Cervellini et al., 2020).

Fungal taxa were grouped according to their correlation with maximum temperature, precipitation and relative humidity using k-means clustering of available Pearson and Spearman coefficients. Principal component analysis (PCA) was used to summarise the variance across the selected parameters to enable bivariate plotting. Elbow and gap statistic methods determined the optimal number of clusters as 4 (Supplementary Information 2).

All statistical analyses were performed using R version 4.0.2 using the code provided within Supplementary Information 3.

2.4 Quality assessment

A quality-scoring tool was adapted from a previous review on bioaerosols with explicit scoring categories for environmental exposure assessment (Douglas et al., 2018). This enabled assessment of seven domains deemed essential for the accurate measurement of fungal spore seasonality, providing an overall quality score for each study. A detailed explanation of the quality-scoring tool is available in Supplementary Information 4. Briefly, the seven domains were fungal spore diversity and relevance to allergy, temporal coverage, sampling methodology, meteorological variables, statistical methods, seasonal data availability and descriptive detail. Scores were classified as high, moderate, low, or very low.

3. Results

3.1 Study selection

There were 14,333 studies identified, of which 86 were deemed to meet the criteria for inclusion (Fig. 1). Most studies removed at the abstract screening stage were due to lack of fungal spore

relevance. Exclusions at the full text assessment stage were mostly due to insufficient characterisation of sampled fungal spores or a lack of seasonal coverage. Of the 86 studies included, 12 were found to cover the same sampling period, location, and involved all or some of the same results as another study. Where this occurred, selection of the publication most tailored towards the explanation of seasonality was chosen to prevent duplicate findings within this review. This resulted in a final count of 74 included studies.

The characteristics of these 74 studies are detailed in Table 1. They were primarily observational sampling studies ($n = 68$), but also included methodological validation studies ($n = 2$), and studies with an epidemiological element ($n = 4$). Over half ($n = 43$) were published in or after 2010, including 8 published within or after 2020. The remaining were published within the years 2000-2009 ($n = 21$), 1990-1999 ($n = 8$), and 2 prior to 1990. Most involved sampling within one location only ($n = 48$), with fewer using multiple locations within a single country ($n = 23$), or multiple countries ($n = 3$). The most represented fungal genera reported in studies were *Alternaria* and *Cladosporium*, with concentrations measured in 52 and 49 unique studies, respectively. Other genera identified in over 10 studies included *Epicoccum*, *Drechslera*, *Canoderma*, *Torula*, *Leptosphaeria*, *Stemphylium*, *Pithomyces*, *Aspergillus/Penicillium* and *Didymella* (Supplementary Information 5). A more detailed table of study characteristics can be found in Supplementary Information 6.

3.2 Sampling and analysis methodology

Air sampling within the 74 studies was performed in 124 unique locations, resulting in good geographical spread across Europe, except for Iceland, much of Southern Italy, and the Scandinavian and Balkan Peninsulas (Fig. 2). Sampling across all studies spanned the years 1969 to 2018, with most studies taking place within the years 2000-2018 ($n=50$). Exact geographic coordinates for sampler locations were provided in 36 studies. Otherwise, building location ($n = 6$) or area name ($n = 32$) were provided. Sampling was mostly performed with use of a Hirst-style active volumetric impactor sampler ($n = 65$ studies). Use of other air sampling equipment was more likely where composition analysis involved methods other than microscopy. Sampling heights above ground were

more frequently provided than total sampling height above sea-level ($n = 63$ and $n = 30$ studies, respectively), with the majority of studies ($n = 54$) sampling at a height of 10 m above ground or higher. Where included, sampling heights above mean sea level ranged between 5 m to 2367 m. Fungal composition was characterised with light microscopy in 95% ($n = 70$) of studies. Concurrent methods included fungal culture ($n = 4$), lateral flow ($n = 2$), elemental analysers and gas chromatography ($n = 1$). DNA-based identification of fungal genera was only performed in 5% ($n = 4$) of the 74 studies, and included use of both Illumina MiSeq (Nunez et al., 2021; Tignat-Perrier et al., 2020), and Ion Torrent sequencing technologies (Banchi et al., 2020). In an older study, Sanger-based sequencing was utilised (Frohlich-Nowoisky et al., 2009). All used metabarcoding of the ITS2 region of the ribosomal RNA gene, identifying the most relative abundant genera as *Cladosporium* typically followed by *Alternaria* and *Epicoccum*, with greater fungal diversity in autumn and winter (Banchi et al., 2020; Frohlich-Nowoisky et al., 2009; Nunez et al., 2021; Tignat-Perrier et al., 2020). Where taxonomic division-level compositional analysis was performed, the relative abundance of basidiomycetes was higher than that of Ascomycetes in the air (Frohlich-Nowoisky et al., 2009).

3.3.1 Season length

Counts in spores per cubic metre of air per day were the most common form of measurement due to the predominant use of microscopy. These measurements enabled the use of season start, peak and end dates to visualise the seasonality of fungal taxa, and were available in 22 studies, made up of 58 sampling locations. The 90% method was used in all studies reporting seasonal dates, with the season start date defined as the point where 5% of the annual cumulative counts of a fungal taxa have been reached, and similarly the end date at 95%.

Alternaria and *Cladosporium* genera were identified more often than other genera, with counts too infrequent for other genera/groups to enable effective trend analysis. For both *Alternaria* and *Cladosporium* genera, length of season generally increased with decreasing latitude, which in turn appeared to create higher variability in the date of peak concentration (Fig. 3). A similar pattern may be seen for other genera, including *Leptosphaeria* and *Epicoccum*, although there is insufficient

detail to enable inference (Supplementary Information 7). The group of *Aspergillus/Penicillium* was especially variable with peak dates reported within different meteorological seasons dependent on the year, even within a single location (Supplementary Information 7).

Geographical latitude and longitude were found to predict seasonal trends for *Alternaria* and *Cladosporium* in regression analysis (Table 2). In particular, strong associations between season length and geographical location were observed for both taxa. Season start date appeared to be more strongly influenced by location than season end date. While the effect of latitude was much greater than that of longitude, both were significant ($p < 0.01$) predictors of season start and end date with the exception of longitude for predicting *Alternaria* season end date. These models suggest that seasons become longer in south-westerly compared to north-easterly European regions. Regression analysis using biogeographical regions as dummy variables provided significant ($p < 0.05$) evidence that the season length of both genera was longer in the Mediterranean compared to other European regions (Supplementary Information 8).

3.3.2 Daily and annual/seasonal airborne concentrations

Airborne concentrations were typically presented with peak and mean spores per cubic metre values for an entire calendar year ($n = 25$ studies) or fungal season ($n = 14$ studies). In nearly all locations, a peak date concentration of *Cladosporium* and *Alternaria* exceeded the defined clinical thresholds (Fig. 4). High variation was also evident between locations, with an apparent trend for higher spore concentrations in northernly locations. Although geographical location was shown to significantly predict season length, using annual or peak concentrations and their associated dates as dependent variables did not result in statistically significant associations (data not shown). Across all locations, peak spore concentrations for *Alternaria* and *Cladosporium* had median values of 665 and 18,827 spores per m^3 , respectively. The highest daily concentrations for all spore taxa was *Cladosporium* with a count of 106,896 spores per $m^3 \text{ day}^{-1}$ in Szczecin (Poland) in 2008 (Grinn-Gofron et al., 2016). This was followed by *Didymella* in Worcester (England) at 19,966 spores per $m^3 \text{ day}^{-1}$ in 2008 (Sadys et al., 2016), *Venturia* in Payerne (Switzerland) at 9,657 spores per $m^3 \text{ day}^{-1}$ in 2013 (Sadys et al.,

2016), and *Aspergillus/Penicillium* in Derby (England) at 5,560 spores per m³ day⁻¹ in 1999 (Millington and Corden, 2005). The highest daily concentration for *Alternaria* was 2,295 spores per m³ in Poznan (Poland) in 2010 (Kasprzyk et al., 2015).

The taxa with the highest total annual counts, and therefore abundance, was *Cladosporium*, with counts of >750,000 spores per m³ occurring in Seville (Spain) in 1998 (Aira et al., 2012) and Worcester (England) in 2006 and 2010 (O'Connor et al., 2014). The taxa with the second highest annual count was *Didymella* with 190,186 spores per m³ in Worcester (England) in 2008 (Sadys et al., 2016), followed by *Coprinus* with 73,925 spores per m³ in Bratislava (Slovakia) in 2016 (Scevkova and Kovac, 2019). Annual spore concentrations of *Alternaria* and *Cladosporium* followed a similar pattern to that of maximum daily spore concentrations (Supplementary Information 9).

3.4 Meteorological factors driving seasonality

Meteorological measurements were included in the analysis of fungal spore concentrations within 85% of studies (n=63). Correlation statistics were most frequently used to identify/measure any associations, with Spearman's rank correlation method favoured over Pearson or other methods due to the typically non-parametric nature of the data. Meteorological parameters of interest were varied and diverse, with the most common relating to air temperature, precipitation, relative humidity, wind speed and direction (Supplementary Information 10). The strongest factor driving seasonal fungal spore concentrations was temperature. Strong positive correlations with mean, maximum and minimum temperature were observed for most taxa, including the allergenic species *Alternaria*, *Cladosporium*, *Epicoccum* and *Ganoderma*. Water-related climate variables including precipitation and relative humidity were also moderate to strong influencers of seasonal fungal spore concentrations, particularly with members of the Basidiomycota phyla. Other variables, including wind speed and direction, air pressure, and solar radiation were less commonly measured, showing weaker correlations with fungal taxa in general. Thus, the most universally relevant meteorological factors were temperature, precipitation and relative humidity.

Extracted correlation coefficients were grouped into 4 distinct clusters via k-means clustering for

maximum temperature, precipitation and relative humidity. These variables provided the best separation and explained 82% of the total variance (Fig. 5). Clusters 2 and 3 contain fungi with generally opposing dependencies on temperature and water, with moderate to strong correlations for these factors. Cluster 2 contains many genera typically classified as 'wet' fungi, while cluster 3 contains many classified as 'dry' fungi. Clusters 1 and 4 contain genera with weaker associations to precipitation and humidity. Cluster 1 contains genera with no strong patterns of correlation for any of the specified features, while cluster 4 contains genera with moderate to strong positive correlations with temperature.

3.5 Local factors driving seasonality

The only objective metric for the measurement of nearby agriculture as a factor effecting spore seasonality was Growing Degree Days (GDD) (Skjoth et al., 2016). This was used as a proxy for agricultural growth and demonstrated a significant correlation with fungal season start and peak dates. The same study also utilised Geographic Information Systems (GIS) to measure the agricultural land surrounding the sampling stations. Two other studies in Denmark and the UK determined sources of fungal spores from further afield using methods including back-trajectory analysis, where the movement of airborne *Alternaria* spores was attributed to either long-distance transport or local agricultural events (Apangu et al., 2020; Olsen et al., 2019a).

Comparison of total spore concentrations in rural *versus* urbanised areas were performed in four studies, with rural concentrations of fungal spores typically being greater. A range of allergy-associated spore concentrations were higher within a rural compared to an urban area of Poland (Kasprzyk and Worek, 2006). Greater rural-associated concentrations of *Alternaria* and *Cladosporium* were observed in Spain (Rodriguez-Rajo et al., 2005). Higher concentrations of *Alternaria*, *Aspergillus/Penicillium*, *Cladosporium* and smut spores were measured in rural areas, compared to higher concentrations of *Coprinus* and *Pleospora* spores in the more urbanised city of Porto (Oliveira et al., 2009). The effect of rurality was less pronounced in an English study analysing eight sites within a 60km area, including measurements of six fungal taxa inclusive of *Alternaria* and

Cladosporium (Brown and Jackson, 1978). The study did, however, note the presence of farmland near to the urbanised areas studied.

Proximity to the coast was associated with reduced airborne concentrations of total/peak spores in multiple studies. These included lower *Alternaria* spore concentrations in addition to a shorter season in coastal sites closer to the Atlantic Ocean in a study comparing 12 locations across north-eastern Europe (Kasprzyk et al., 2015), and reduced *Alternaria*, *Cladosporium*, *Ganoderma* and *Didymella* spore concentrations in coastal Cork compared to inland Worcester (O'Connor et al., 2014). Lower *Alternaria* spore concentrations were sampled on the Atlantic island of Tenerife compared to mainland Spain, although other factors include a large separating distance and different terrains (De Linares et al., 2010).

3.6 Long-term trends

While 51 studies sampled across multiple years, only 7 reported analysis of long-term trends, all of which were in relation to changes in average temperatures. Prolonged *Alternaria* and *Stemphylium* season lengths correlated with an increase in mean temperature in 13 years of sampling in Bratislava (Scevkova et al., 2016). Decreases in total and/or peak concentrations of *Alternaria* and/or *Cladosporium* correlated with a rise in average temperature across 26 years in Copenhagen (Denmark) (Olsen et al., 2019b), 10 years in Szczecin (Grinn-Gofron et al., 2016), 19 years in Northern Italy (Marchesi, 2019) and 8 years or more in Southern France (Sindt et al., 2016). Conversely, total and/or peak *Aspergillus*/*Penicillium* spore concentrations correlated with increasing temperature across 33 years in Derby (Millington and Corden, 2005). No significant associations between total/peak fungal spore concentrations and changes in temperature were observed across 15 years in Thessaloniki (Greece) (Gioulekas et al., 2004).

3.7 Quality assessment

Overall quality was assessed as high for over half of the studies (Fig. 6). Individual quality scores for each study are available within Supplementary Information 11. Fungal spore diversity and relevance to allergy scores were rated as high in 36% of the studies, with 41% rated as low or very low. Many

of the studies opted to focus on the allergenic genera of *Alternaria* and *Cladosporium* only, or on the seasonality of taxa relevant to fungal diseases of crops. Temporal coverage was rated as high for the majority (64%) of the studies due to the use of multiple samplers, across multiple locations and years or seasons. Sampling methodology was highly consistent in use of active volumetric impactor samplers with a high rating assigned for 85% of studies. A common but not universal analysis included the use of meteorological measurements in correlation with fungal spore concentrations, with 50% of studies scoring high to moderate (42% scoring as high). Appropriate statistical methods for the study design were used in the majority of studies, with a score of moderate to high assigned to 93% of studies. The final two quality scoring categories were based on the inclusion of detail within a study, including results relevant to fungal spore seasonality and descriptive detail of the study. Both were rated as moderate to high in 68% and 82% of studies, respectively, indicating that sufficient detail was more often included than not.

4. Discussion

Fungal spore exposure can impact on allergic conditions including asthma. A critical topic within this area of research is fungal spore seasonal patterns, which can often align with seasonal exacerbations of allergic conditions. The aim of this systematic review was therefore to analyse fungal spore seasonality and associated driving factors across Europe, and consider the relevance of this information for health.

4.1 Sampling and analysis methodology

Methodology across the studies was highly consistent, particularly in the use and operation of active volumetric impactor samplers, which are considered the 'gold standard' for fungal spore sampling. Furthermore, these methods have established guidelines to standardise the monitoring of bioaerosols (Galán et al., 2014). This helps to ensure consistent sampling methodology with homogenous measurement units and sampling parameters, which in turn provides the opportunity to compare sampling studies across Europe and has enabled the meta-analyses presented here. Identification of spores to genera-level is not possible for the majority of fungal taxa via microscopy

since many species are morphologically indistinguishable from each other (Lacey, 1996; Trejo et al., 2013). The more recent development of molecular techniques such as HTS provides the opportunity to better characterise a wider spectrum of environmental microorganisms, offering the ability to identify many hundreds of genera within multiple samples. Although only 4 of the 74 studies used metabarcoding, it is beginning to be more widely used as costs fall and it becomes accessible to more researchers. It is important to note, however, that metabarcoding has its own caveats. The main issue being the difficulty in providing an absolute quantification of spores, due to the diverse copy number variation (CNV) within the metabarcoded regions of different fungal taxa. Relative abundance metrics provide a composition-based analysis of fungal spore seasonality. This is valid for comparing the abundance of the same fungal taxa within different samples (eg. from different time points and locations), but cannot yet be used to compare the absolute abundance of different fungal taxa within the same sample (eg. a specific environment) (Gloor et al., 2017). Consequently, integrating metabarcoding results with microscopy data remains challenging. Greater use of metabarcoding alongside microscopy, together with approaches to address CNV normalisation (such as DNA spike-ins, internal controls and/or validation with quantitative PCR), will enable improved comparison and integration of data generated by molecular and traditional methods (Jian et al., 2020; Tkacz et al., 2018). Furthermore, the use of molecular techniques to improve exposure assessment may help to identify novel associations between fungal genera and allergic disease in epidemiological cohorts, especially as part of longitudinal analyses.

4.2 Fungal spore seasonality in Europe and associated driving factors

The 74 studies represented much of Europe with the exception of Iceland, Southern Italy, and the Scandinavian and Balkan Peninsulas. While summer and autumn are generally recognised as the main fungal spore seasons within local areas, we confirm it here on a larger Europe-wide scale. Allergenic taxa typically present within this period were *Alternaria*, *Cladosporium*, *Botrytis*, *Coprinus*, *Epicoccum*, *Ganoderma*, *Leptosphaeria*, *Sporobolomyces*, *Stemphylium* and basidiospores. The longer *Alternaria* and *Cladosporium* seasons in south-westerly Mediterranean compared to north-

easterly Atlantic and Continental regions were likely driven by differences in general meteorological trends and local vegetation sources. In particular, the strong dependence of many allergenic fungal taxa on temperature may explain the increased fungal season lengths in warmer Mediterranean countries. The reason why peak and annual concentrations demonstrated the opposite trend, with many of the highest concentrations occurring in the relatively cooler regions of Northern Europe, was unclear. It could be speculated that this trend is related to plant growth patterns, which in colder countries may be more concentrated within the warmer months, and consequently produce more material for fungal growth (Körner, 2016). While peak and annual concentrations as a function of geographical location were not statistically significant in this study, the analysis did not control for land-use as a variable. Inclusion of a GIS-based analysis of features surrounding a sampler may help to better account for the effects of geographical location, especially when samplers are within areas associated with skewed concentrations of spores.

Effects of other meteorological variables were generally weaker, although a clear pattern was demonstrated with respect to humidity and precipitation. This is unsurprising considering the 'wet' groups of fungi, such as *Didymella* and *Leptosphaeria*, require a specific level of humidity to sporulate (Jones and Harrison, 2004). Unfortunately, the ability to deduce the impact of combined variables was limited by the use of simple correlation statistics, rather than multivariate models, in many studies. The correlation of single variables with fungal spore concentrations does not control for the effect of multiple variables, including other meteorological factors, other particulate matter, land-use or sporadic events such as thunderstorms and droughts, all of which can influence fungal spore concentrations (Castro e Silva et al., 2020; Giner et al., 2001; Grinn-Gofroń and Strzelczak, 2013; Kasprzyk et al., 2016; Kasprzyk et al., 2015; Pakpour et al., 2015).

Composition and quantity of local vegetation sources are intrinsically linked, as they provide a resource for fungal growth and subsequent sporulation and spore release. Meteorological variables therefore govern the timing and magnitude of a season through effects on both the fruiting fungus and the host vegetation's ability to thrive. Agricultural crop farming, particularly during harvest, is

associated with the release of allergenic fungal spores (Skjøth et al., 2012). The direct influence of agriculture is difficult to ascertain due to a lack of a standardised measurement of crop growth and yield within an area. However, studies made use of alternative indirect means, such as proxies for crop growth and air mass transport models. In general, reduced fungal spore concentrations and diversity were measured in locations with a lower density of agricultural activity. These included coastal locations and highly urbanised cities, with lower concentrations explained by a relative lack of growth substrate such as that provided by nearby agricultural activities (Sindt et al., 2016). The post-harvest period was associated with decaying crops and may contribute to the high fungal spore concentrations in late summer and autumn (Grinn-Gofron, 2008).

4.3 Relevance to health and impact

The most important patterns observed in relation to health were longer season lengths and reduced peak concentrations of *Alternaria* and *Cladosporium* in warmer European countries/regions and with increasing temperature. Together such data suggests that as average global temperatures rise, northern-European *Alternaria* and *Cladosporium* fungal seasons may become closer in characteristic to those within the south. Increased fungal season lengths result in longer exposure periods, potentially resulting in more allergy symptom days. Reduced spore concentrations are only beneficial when they fall below clinical thresholds. In the vast majority of locations, peak *Alternaria* and *Cladosporium* spore concentrations were above clinical thresholds, even in the more southerly warmer European regions. Further studies are required to establish clinical thresholds and provide daily spore concentrations for a wider range of fungal taxa over long-term sampling periods (decades). This will enable a more conclusive analysis of the effect of climate on fungal spore seasonality and any associated changes in clinically relevant exposures. The former may well be taxa specific, since the only study including a long-term analysis of *Aspergillus/Penicillium* spore trends reported a positive correlation of total and/or peak spore concentrations with increasing temperature (Millington and Corden, 2005). Low numbers of studies with long-term sampling are most likely due to the costs and practical difficulties associated, which currently limits inferences on

the impact of climate change, as well as changes in land-use, plant diversity and air quality, on general and specific fungal spore seasonality and subsequent health outcomes.

4.4 Recommendations for future work

The following recommendations are based on our review of the included literature:

1. Monitor a greater range of fungal taxa. There was insufficient evidence to effectively characterise European trends for fungal taxa other than *Alternaria* and *Cladosporium*. Increased use of modern molecular techniques such as HTS for the characterisation of fungal spores in the outdoor air will also help to identify additional taxa of interest.
2. Sample missing/underrepresented regions and/or different land-use types within Europe. More sampling is needed in regions such as Iceland, Southern Italy, and the Scandinavian and Balkan Peninsulas, and the inclusion of land-use variables provides an important opportunity to understand the wider spatial distribution and sources of fungal spores.
3. Include simultaneous measurement and appropriate analysis of meteorological and local factors in relation to spore data. Many studies relied upon correlation-based tests without controlling and accounting for other determinants. Metrics related to land-use were also underutilized. Improved use of such measurements and analyses will help to better define factors impacting upon fungal spore seasonality.
4. Combine fungal spore sampling data as exposure data with health surveillance data. The use of HTS in these studies may enhance the ability to identify novel associations between specific fungal genera and allergy outcomes.
5. Raise the awareness of patients and health practitioners to the contribution of fungal spores to allergic disease, and how knowledge of fungal spore levels can help manage allergy. Pollen calendars and forecasts are widely available. In contrast, fungal spore calendars and forecasts are only produced in limited regions and are not widely available or publicised. This is understandable, due to both the technical challenges of identifying spores in comparison to pollen, and in the relative importance of pollen as an allergen. However, for individuals with asthma, the risk of

hospitalisations, worse lung function and overall morbidity are greater for fungal sensitisation than for pollen (Pashley and Wardlaw, 2021; Woolnough et al., 2017).

6. Explore the potential impact of climate change and altered land-use on seasonal fungal trends. For example, the analysis of Europe-wide *Alternaria* and *Cladosporium* seasonal trends presented here suggests that increasing temperatures may result in prolonged fungal spore seasons. This has significant clinical implications for those sensitised to fungal spores and warrants both the collection of new data and the re-evaluation of the existing European datasets with the inclusion of appropriate climate and land-use variables.

5. Conclusions

The outcome of this systematic review is the first comprehensive meta-analysis of studies related to the seasonality of fungal spores across Europe. We have provided an overview of Europe-wide trends for the outdoor spores of the genera *Alternaria* and *Cladosporium*. However, specific geographical regions and other fungal taxa were underrepresented and remain poorly understood in terms of seasonal trends and their driving factors, as do long-term temporal trends in relation to changes in climate and land-use. The predominant method used for fungal identification was microscopy. While this is the 'gold standard' for many of the human relevant fungal taxa, it does not enable characterisation of the full spectrum of airborne fungal spores. Greater use of molecular techniques such as HTS will help to improve our characterisation of fungal seasonality, allowing the trends of under-explored or novel taxa to be explored. The importance of fungal spores as allergens is becoming increasingly recognised. More long-term sampling studies in combination with health surveillance data will help to identify the diverse risks and dynamics attributed to allergenic fungal spores and their seasonality. This will in-turn help to better understand fungal allergy, develop improved fungal spore calendars and forecasts with greater geographical coverage, and promote increased awareness and management strategies for those with allergic fungal disease.

Author contributions

Samuel Anees-Hill: Conceptualization, methodology, validation, formal analysis, investigation, data

curation, writing – original draft, writing – review and editing, visualization; **Philippa Douglas**:

Conceptualization, methodology, validation, writing – review and editing; **Catherine H Pashley**:

Writing – review and editing; **Anna Hansell**: Conceptualization, methodology, writing – review and

editing; **Emma L Marczylo**: Conceptualization, methodology, validation, writing – review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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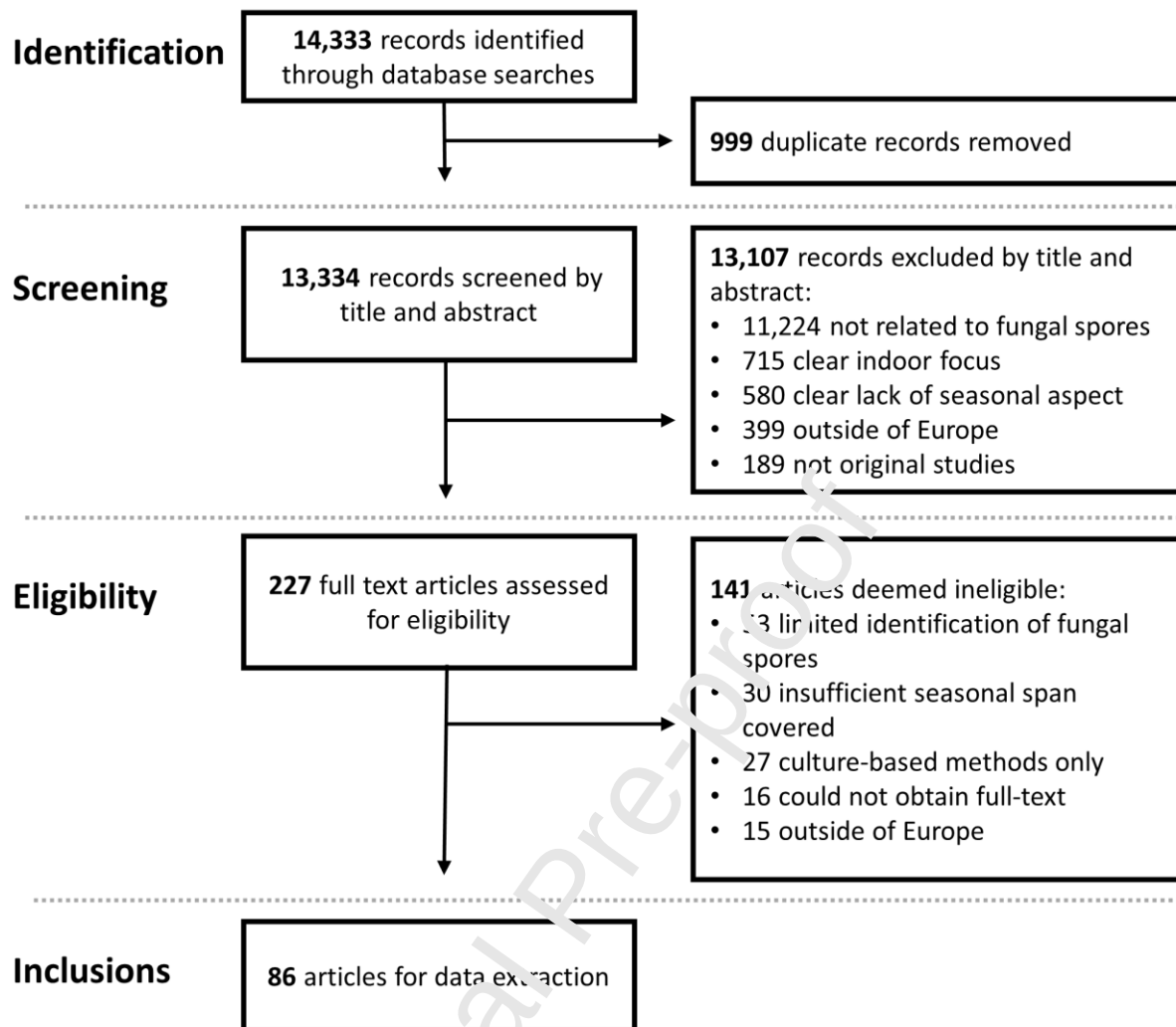


Fig. 1. Flow chart summarising the systematic review screening process.

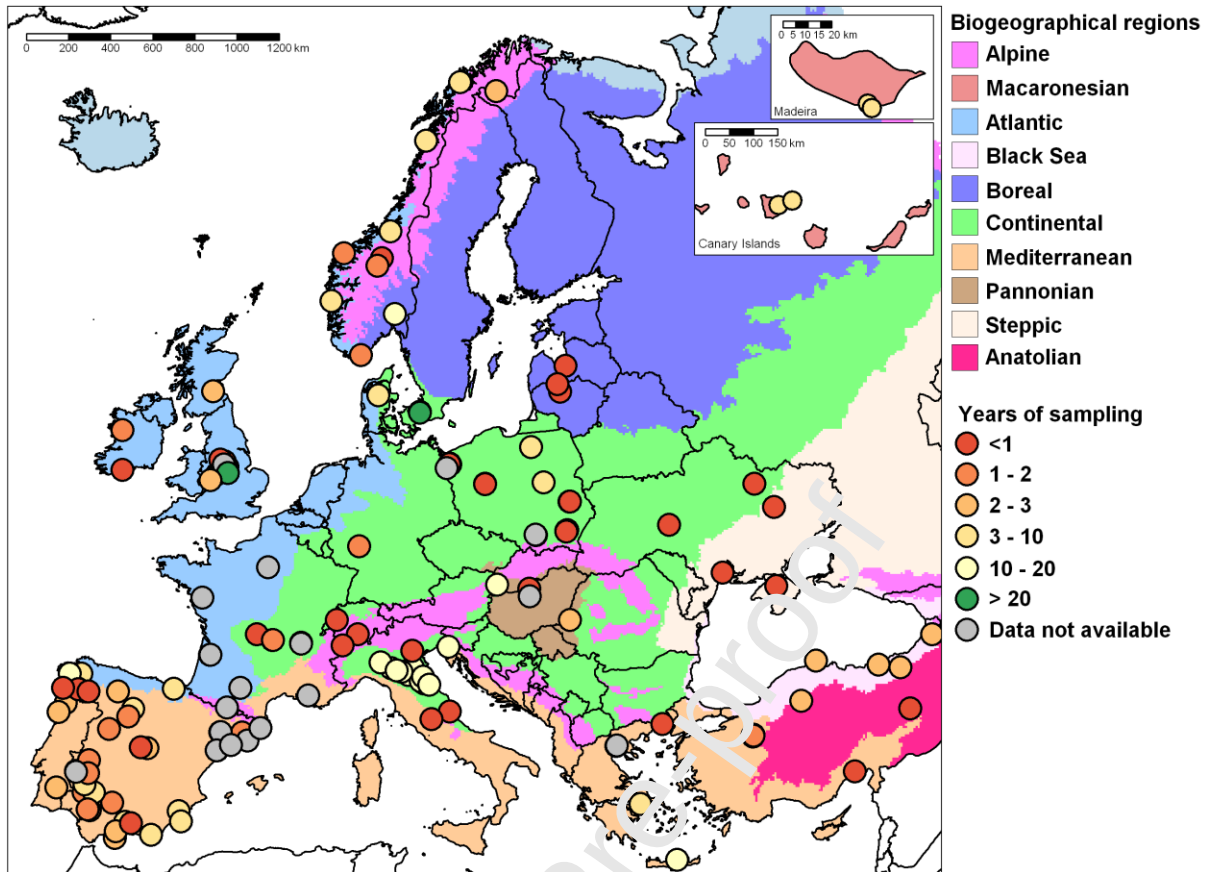


Fig. 2. Sampling locations across Europe within included studies using latitudinal/longitudinal coordinates. European Environment Agency (EEA) defined biogeographical regions are shown by coloured shading (Cervellini et al., 2020). The colour of the circles represents the number of years of sampling and was calculated by dividing total days of sampling by 365.

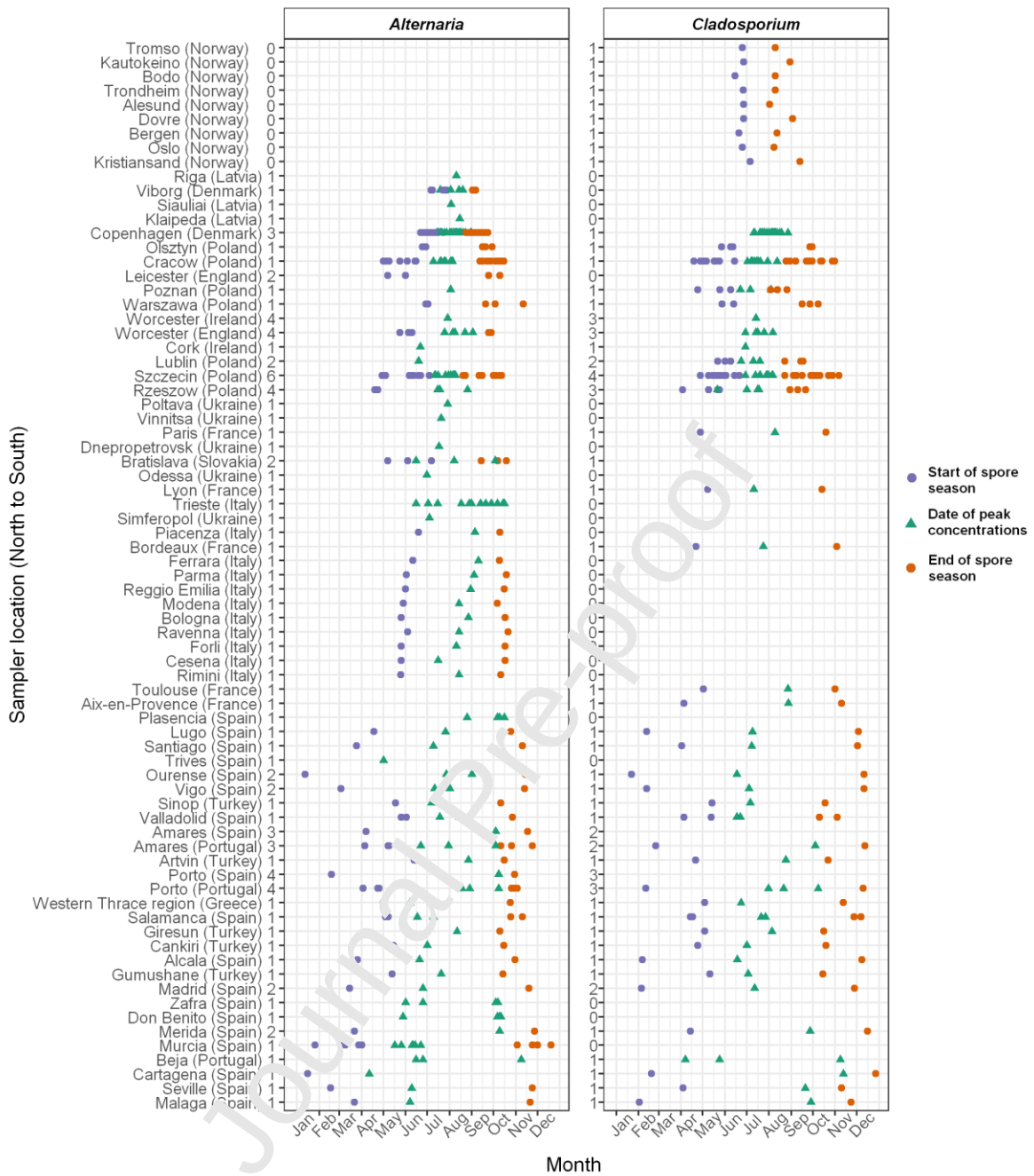


Fig. 3. Seasonal fungal index (SFI) start, end, and peak concentration dates by sampling location. All seasons are defined using the 90% method. Multiple data points are plotted where multiple years of data were available. Numbered Y-axis displays the number of studies associated with adjacent data points.

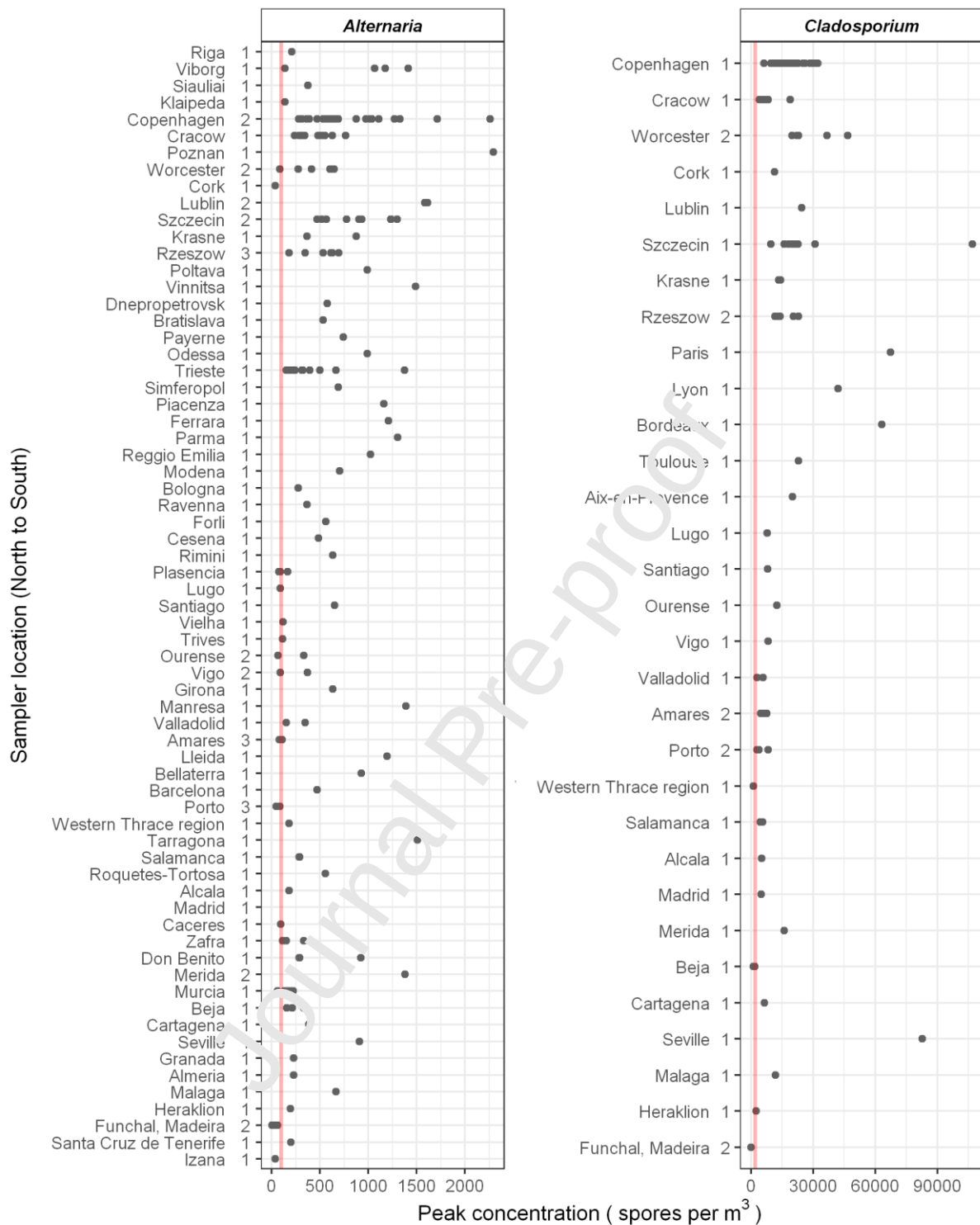


Fig. 4. Peak concentrations for *Alternaria* or *Cladosporium* across different locations and seasons.

Each point represents a peak date concentration for a given fungal season. The red vertical line indicates the defined daily clinical threshold of 100 or 3,000 spores per m³ for *Alternaria* and *Cladosporium*, respectively. Y-axis displays the number of studies associated with adjacent data

points.

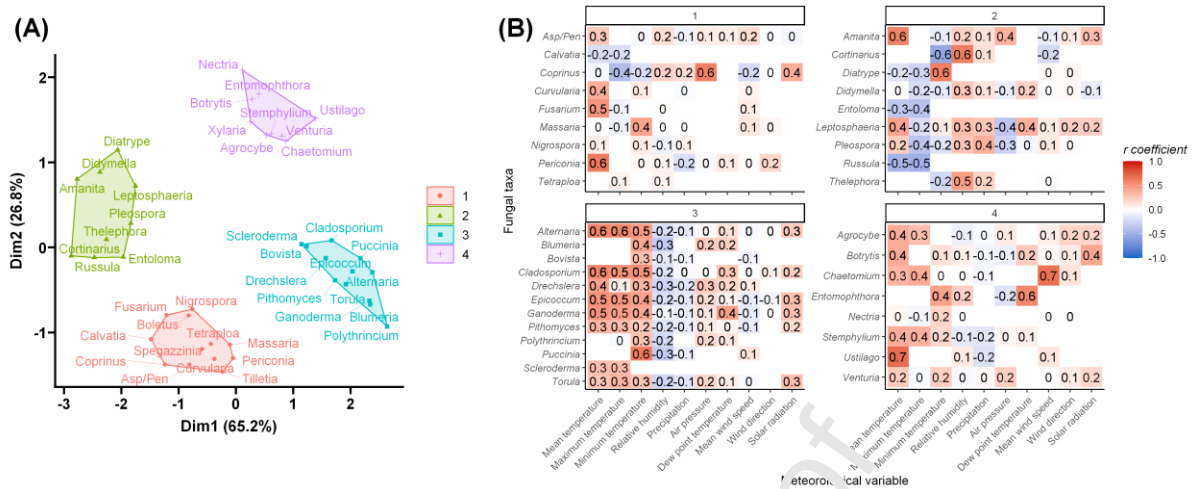


Fig. 5. Clustering of coefficients related to the correlation of various meteorological variables against fungal spore concentrations. (A) Principal components plot showing k-means clustering of fungal taxa according to their correlation with maximum temperature, precipitation and relative humidity using available Pearson and Spearman coefficients. Figure legend displays clustered groups 1-4. (B) Spearman and Pearson correlation coefficients for meteorological variables of interest, grouped by clusters. Coefficient values were grouped and averaged by sampler location across one or more studies for each fungal taxon. Blank cells indicate a lack of data for the fungal taxon.

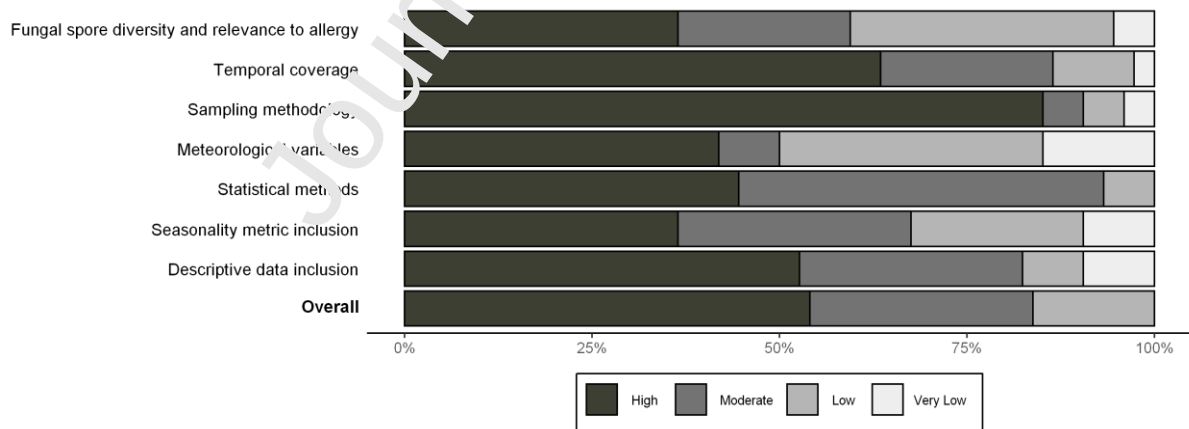


Fig. 6. Overall composition of quality scores assigned to studies based upon the seven quality-scoring domains and an overall score. A detailed explanation of the quality scoring tool is available in Supplementary Information 4, and the individual quality scores of each study is shown in Supplementary Information 11.

Table 1. Study characteristics from included articles. A more detailed version of this table is available in Supplementary Information 5.

Author(s), Year	Sampling location(s))	Sampling period	Sampling methodol ogy	Composit ion analysis method	Fungal taxa identified	Overlap ping studies*
(Brown and Jackson, 1978)	England: 8 locations.	All locations: 01/06/1969 - 30/09/1969 (continuous sampling).	All locations: - Morrow Brown 7- day volumetric trap. Operated between 0.5 and 10 m above ground.	All locations : Microscop y.	<i>Cladosporium</i> , <i>Sporobolomyces</i>	
(Corden and Millington, 1994)	England: Derby.	01/06/1986 - 28/09/1988 (continuous sampling).	- Morrow Brown 7- day volumetric trap. Operated at 10 m	Microscop y.	<i>Didymella</i>	

			above ground.			
(Millington and Corden, 2005)	England: Derby.	21/04/1970 - 31/12/2003 (mixture of partial and continuous sampling)	- Hirst- style 7- day volumetric trap.	Microscop py.	<i>Aspergillus/Penic illium</i>	
(Sadys et al., 2016)	England: Worcester.	01/01/2006 - 31/12/2010 (continuous sampling)	- Burkard 7-day volumetric trap. Operated 10 m above ground.	Microscop py.	20 groups: <i>Alternaria</i> , <i>Aspergillus/Penic illium</i> , <i>Blumeria</i> , <i>Botrytis</i> , <i>Cladosporium</i> , Basidiospores, <i>Didymella</i> , <i>Drechslera</i> , <i>Entomophthora</i> , <i>Epicoccum</i> , <i>Ganoderma</i> , <i>Leptosphaeria</i> , <i>Periconia</i> , <i>Pithomyces</i> , <i>Pleospora</i> , <i>Polythrincium</i> ,	(Sadys et al., 2014)

					rusts, smuts, <i>Stemphylium</i> , <i>Torula</i> .	
(Sadys, 2017)	England: Worcester.	01/01/2006 - 31/12/2010 (continuous sampling)	- Burkard 7-day volumetric trap. Operated 10 m above ground.	Microscopy.	<i>Cladosporium</i>	
(Richardson, 1996)	Scotland: Edinburgh	01/07/1992- 31/10/1994 (continuous sampling).	- Burkard 7 day volumetric trap. Operated 20 m above ground, 120 m above mean sea level (a.m.s.l.).	Microscopy.	<i>Didymella</i>	
(Skjoth et al.,	England:	Range from	All	All	<i>Alternaria</i>	(Velez-

2016)	3 locations. Denmark : 1 location. France: 6 locations. Greece: 1 location. Hungary: 1 location. Poland: 4 locations. Spain 7 locations.	2000 – 2014. Continuous sampling.	locations: - Hirst- style 7- day volumetric trap.	locations : Microsco py.		<i>Pereira et al., 2021)</i>
(Apangu et al., 2020)	England: Leicester and Worceste r.	Both locations: 01/03/2016 - 31/10/2018 (continuous sampling)	- Burkard 7-day volumetric trap. Operated 10-12 m above ground.	Both locations : Microsco py.	<i>Alternaria</i>	
(Olsen et al.,	Denmark	- 01/07/1990	- Hirst-	Microsco	<i>Alternaria,</i>	

2019b)	:	- 30/09/2015	style 7-	py.	<i>Cladosporium</i>	
	Copenha gen.	(continuous)	day volumetric trap. Operated 15 m above ground.			
(Olsen et al., 2019a)	Denmark : Copenha gen and Viborg	Range: 01/06/2012 - 01/10/2015. (continuous sampling)	Copenhag en: Hirst- style 7- day volumetric trap. Operated 15-21 m above ground.	All locations Microsco py.	<i>Alternaria</i>	
(Sindt et al., 2016)	France: 5 locations	Range: 01/01/1999 - 31/12/2014. (continuous sampling).	All locations: - Lanzoni 7-day volumetric trap. - Sampler	All locations : Microsco py.	<i>Cladosporium</i>	

			location heights (m a.m.s.l): Aix-en- Provence (173), Bordeaux (6), Lyon (198), Paris (60), Toulouse (145).			
(Caillaud et al., 2018)	France: Clermont -Ferrand	01/01/2010 - 31/12/2015 (continuous sampling).	- Burkard 7-day volumetric trap.	Microscop py.	Aspergillaceae, <i>Cladosporium</i>	
(Tignat-Perrier et al., 2020)	France: Puy de Dôme	01/01/2016 - 30/08/2017 (w eekly sampling)	- Size selective high- volume air sampler.	Illumina MiSeq sequenci ng of ITS2 region.	Not applicable – de-novo sequencing.	
(Frohlich- Nowoisky et al., 2009)	Germany : Mainz	01/03/2006- 30/04/2007 (sampling	- Self- built high- volume	Cloning, RFLP, and DNA	8 taxa: <i>Alternaria</i> , <i>Blumeria</i> ,	

		period varied)	dichotomous sampler. Sampler location 130 m a.m.s.l.	sequencing of ITS1, ITS2, 5.8S, and 18S regions.	<i>Cladosporium</i> , <i>Penicillium</i> , <i>Itersonilia</i> <i>perplexans</i> , <i>Stereum</i> , <i>Heterobasidion</i> <i>annosum</i> , <i>Bjerkandera</i> .	
(Gioulekas et al., 2004)	Greece: Thessaloniki	01/01/1987 - 31/12/2001 (continuous sampling).	- Burkard 7-day volumetric trap.	Microscopy.	14 taxa: <i>Cladosporium</i> , <i>Alternaria</i> , <i>Ustilago</i> , <i>Drechslera</i> , <i>Leptosphaeria</i> , <i>Agrocybe</i> , <i>Botrytis</i> , <i>Stemphylium</i> , <i>Pleospora</i> , <i>Nigrospora</i> , <i>Epicoccum</i> , <i>Fusarium</i> , <i>Torula</i> , <i>Phoma</i> . Also ascospores.	(Damiali s et al., 2015)

(Gonianakis et al., 2006)	Greece: Heraklion	01/01/1994 - 31/12/2003 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 18 m above ground (30 m a.m.s.l.).	Microscopy.	17 taxa: <i>Cladosporium</i> , <i>Alternaria</i> , <i>Drechslera</i> , <i>Stemphylium</i> , <i>Arthrinium</i> , <i>Aspergillus niger</i> , <i>Torula</i> , <i>Epicoccum</i> , <i>Asperisporium</i> , <i>Curvularia</i> , <i>Agrocybe</i> , <i>Coprinus</i> , <i>Ustilago</i> , <i>Puccinia</i> , <i>Leptosphaeria</i> , <i>Chaetomium</i> , <i>Venturia</i> . Also ascospores.	(Goniana kis et al., 2005)
(Pyrri and Kapsanaki-Gotsi, 2015)	Greece: Athens	01/01/1998 - 31/12/2001 (half-an-hour runs)	- Burkard portable air sampler. Operated 31 m	Microscopy.	33 groups: <i>Alternaria</i> , <i>Arthrinium</i> , <i>Cladosporium</i> , <i>Coprinus</i> , <i>Curvularia</i> ,	(Pyrri and Kapsana ki-Gotsi, 2007)

			above ground.		<i>Drechslera</i> , <i>Emericella</i> , <i>Epicoccum</i> , <i>Ganoderma</i> , <i>Helminthosporiu</i> <i>m</i> , <i>Humicola</i> , <i>Nigrospora</i> , <i>Periconia</i> , <i>Pestalotiopsis</i> , <i>Pithomyces</i> , <i>Pleospora</i> , <i>Polythrincium</i> , <i>Puccinia</i> , <i>Scopulariopsis</i> , <i>Stachybotrys</i> , <i>Stemphylium</i> , <i>Torula</i> , <i>Trichothecium</i> , <i>Ulocladium</i> , <i>Aspergillus/Penic</i> <i>illium</i> , Erysiphales, Ustilaginales. Also Ascospores, Basidiospores,	
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					Uredospores, Unknown, Hyphal fragments.	
(Katotomichelakis et al., 2016)	Greece: Western Thrace region	01/01/2013 - 31/12/2013 (continuous sampling).	- Burkard 7-day volumetric trap. Oper- ated 20 m above ground.	Microscopy.	<i>Alternaria</i> , <i>Cladosporium</i>	
(Jarajkomlodi, 1991)	Hungary: Budapest	01/02/1989 - 31/10/1989 (continuous sampling)	- Burkard 7-day volumetric trap.	Microscopy.	10 taxa: <i>Cladosporium</i> , <i>Alternaria</i> , <i>Leptosphaeria</i> , <i>Epicoccum</i> , <i>Ustilago</i> , <i>Stemphylium</i> , <i>Torula</i> , Uredinales, <i>Phaeosphaeria</i> , <i>Helminthosporium</i> .	
(McDonald and O'Driscoll,	Ireland: Galway	01/05/1977 - 30/09/1978	- 7-day volumetric	Microscopy.	<i>Cladosporium</i> and	

1980)		(continuous sampling)	trap. Operated 20 m above ground.		Basidiospores	
(O'Connor et al., 2014)	Ireland: Cork. England: Worcester	Both locations: 01/05/2010 - 31/07/2010 (continuous sampling).	Both locations: - 7-day volumetric trap. Operated 20 m above ground.	Both locations : Microscopy.	Four taxa: <i>Alternaria</i> , <i>Cladosporium</i> , <i>Didymella</i> , <i>Ganoderma</i> .	
(Rizzi-Longo et al., 2009)	Italy: Trieste	01/01/1997 - 31/10/2004 (continuous sampling)	- 7-day volumetric traps. Operated 20 m above ground.	Microscopy.	<i>Alternaria</i> and <i>Epicoccum</i>	
(Favero-Longo et al., 2014)	Italy: Aosta valley region	01/06/2011 - 30/05/2012 (continuous sampling)	- Lanzoni 7-day volumetric trap.	Microscopy.	<i>Cladosporium</i> , <i>Alternaria</i> , Teloschistaceae (Lichen)	

			Operated 1.5 m above ground (1367 m a.m.s.l.).			
(Marchesi, 2019)	Italy: 11 locations.	All locations: 01/01/1999 - 31/12/2017 (continuous sampling).	All locations: - Volumetric trap.	All locations: : Microscopy.	<i>Alternaria</i>	
(Banchi et al., 2020)	Italy: 5 locations	All locations: 01/03/2017 - 30/11/2017 (18 total samples)	All locations: - Lanzoni 7-day volumetric trap.	Ion Torrent sequencing of ITS2 region.	Not applicable – de-novo sequencing.	(Tordoni et al., 2021)
(Kasprzyk et al., 2015)	Poland: 4 locations. Latvia: 3 locations. Ukraine: 5 locations.	All locations: 01/04/2010 - 30/09/2010 (continuous sampling).	All locations: - Lanzoni 7-day volumetric trap. - Sampler heights	Microscopy.	<i>Alternaria</i>	

			between (12-25 m, 322-741),			
(Ramfjord, 1991)	Norway: 9 locations	All locations: 25/03/1982 - 14/09/1984. (continuous sampling).	All locations: - 7-day volumetric trap.	All locations : Microsco py.	<i>Cladosporium</i>	
(Johansen, 1992)	Norway: Kongsvol d	Range: 22/03/1982 - 30/09/1984 (continuous sampling within sampling period)	- Burkard 7-day volumetric trap. Operated 1.5 m above ground (900 m a.m.s.l.).	Microsco py.	<i>Cladosporium</i>	
(Konopinska, 2004)	Poland: Lublin	01/01/2002 - 31/12/2002 (continuous sampling).	- Lanzoni 7-day volumetric trap. Operated 18 m above	Microsco py.	<i>Alternaria</i> , <i>Cladosporium</i>	

			ground.			
(Kasprzyk and Worek, 2006)	Poland: Krasne and Rzeszow	Range: 01/01/2001 - 31/12/2002. (continuous sampling).	Both locations: - Lanzoni 7-day volumetric trap. Operated 12 m above ground.	Both locations : Microscopical	10 taxa: <i>Alternaria</i> , <i>Botrytis</i> , <i>Cladosporium</i> , <i>Drechslera</i> , <i>Epicoccum</i> , <i>Ganoderma</i> , <i>Pithomyces</i> , <i>Polythrincium</i> , <i>Stemphylium</i> , <i>Torula</i> .	
(Grinn-Gofron and Mika, 2008)	Poland: Szczecin	01/01/2004 - 31/12/2005 (continuous sampling).	- Lanzoni 7-day volumetric trap. Operated 21 m above ground.	Microscopical	Five taxa: <i>Alternaria</i> , <i>Cladosporium</i> , <i>Didymella</i> , <i>Ganoderma</i> , <i>Leptosphaeria</i> .	
(Grinn-Gofron, 2008)	Poland: Szczecin	01/01/2004 - 31/12/2006 (continuous sampling).	- Lanzoni 7-day volumetric trap. Operated	Microscopical	Ten taxa: <i>Alternaria</i> , <i>Cladosporium</i> , <i>Didymella</i> , <i>Drechslera</i> ,	

			21 m above ground.		<i>Epicoccum</i> , <i>Ganoderma</i> , <i>Pithomyces</i> , <i>Polythrincium</i> , <i>Stemphylium</i> , <i>Torula</i> .	
(Grinn-Gofron and Rapiejko, 2009)	Poland: 3 locations	All locations: 01/01/2004 - 31/12/2006 (continuous sampling).	All locations: - Lanzoni 7-day volumetric trap. Sampler heights: 20-28m.	All locations : Microscopical	<i>Alternaria</i> , <i>Cladosporium</i>	
(Grinn-Gofron, 2011)	Poland: Szczecin	01/01/2004 - 31/12/2009 (continuous sampling).	- Lanzoni 7-day volumetric trap. Operated 21 m above ground.	Microscopy.	<i>Aspergillus/Penicillium</i>	
(Grinn-Gofron and Strzelczak,	Poland: Szczecin	01/01/2004 - 31/12/2008	- Lanzoni 7-day	Microscopy.	<i>Ganoderma</i>	

2011)		(continuous sampling).	volumetric trap. Operated 21 m above ground. (52 m a.m.s.l.).			
(Grinn-Gofron et al., 2016)	Poland: Cracow and Szczecin	Both locations: 01/01/2004 - 31/12/2013 (continuous sampling).	Both locations: - Lanzoni 7-day (volumetric trap. Operated 20 m above ground.	Both locations: Microscopy.	<i>Alternaria,</i> <i>Cladosporium</i>	
(Kasprzyk et al., 2016)	Poland: 2 locations.	All locations: 01/04/2010 - 30/09/2012 (continuous sampling).	All locations: - Lanzoni 7-day volumetric trap. - Sampler	All locations: : Microscopy.	<i>Cladosporium</i>	

			heights 12-18m.			
(Kasprzyk et al., 2021)	Poland: Rzeszow	01/04/2016 - 30/09/2016 (50 samples total).	- Burkard personal sampler (10L/min, 30 minute runs)	Microscopy.	12 taxa: <i>Cladosporium</i> , <i>Alternaria</i> , <i>Ganoderma</i> , <i>Leptosphaeria</i> , <i>Epicoccum</i> , <i>Torula</i> , <i>Periconia</i> , <i>Pithomyces</i> , <i>Polythrincium</i> , <i>Drechslera</i> , <i>Paraphaeosphaeria</i> , <i>Didymella</i> .	
(Marynowski et al., 2020)	Poland: Sosnowiec	01/11/2017 - 31/12/2017. (Weekly samples)	- Atmoservice LVS-3D sampler	Microscopy.	9 taxa: <i>Alternaria</i> , <i>Botrytis</i> , <i>Cladosporium</i> , <i>Drechslera</i> , <i>Epicoccum</i> , <i>Leptosphaeria</i> , <i>Periconia</i> , <i>Peronospora</i> , <i>Torula</i> .	
(Oliveira et al.,	Portugal:	01/01/2003 -	- Burkard	Microscopy.	22 groups:	

2005)	Porto	31/12/2003 (continuous sampling).	7-day volumetric trap. Operated 20 m above ground.	py.	<i>Alternaria</i> , <i>Aspergillaceae</i> , <i>Botrytis</i> , <i>Cladosporium</i> , <i>Coprinus</i> , <i>Corynespora</i> , <i>Didymella</i> , <i>Drechslera</i> , <i>Epicoccum</i> , <i>Fusarium</i> , <i>Ganoderma</i> , <i>Leptosphaeria</i> , <i>Oidium</i> , <i>Periconia</i> , <i>Pithomyces</i> , <i>Pleospora</i> , <i>Polythrincium</i> , <i>Rhizopus</i> , rusts, smuts, <i>Torula</i> , <i>Ustilago</i> .	
(Oliveira et al., 2009a)	Portugal: Amares	01/03/2005 - 31/10/2007 (continuous sampling).	- Burkard 7-day volumetric trap. Operated	Microsco py.	<i>Botrytis</i> , <i>Oidium</i> .	

			5 m above ground.			
(Oliveira et al., 2009b)	Portugal: Amares and Porto.	Both locations: 01/01/2005 - 31/12/2007 (continuous sampling).	- Burkard 7-day volumetric trap. - Sampler heights: 5- 20m.	Both locations : Microscopy.	14 groups: <i>Alternaria</i> , <i>Aspergillus/Penicillium</i> , <i>Cladosporium</i> , <i>Coprinus</i> , <i>Didymella</i> , <i>Drechslera</i> , <i>Epicoccum</i> , <i>Ganoderma</i> , <i>Leptosphaeria</i> , <i>Pleospora</i> , rusts, smuts, <i>Stemphylium</i> , <i>Ustilago</i> .	(Oliveira et al., 2009c; Oliveira et al., 2010b)
(Oliveira et al., 2010a)	Portugal. Amares and Porto.	Both locations: 01/01/2005 - 31/12/2007 (continuous sampling).	- Burkard 7-day volumetric trap. - Sampler heights: 5- 20m.	Both locations : Microscopy.	9 taxa: <i>Alternaria</i> , <i>Drechslera</i> , <i>Epicoccum</i> <i>Leptosphaeria</i> , <i>Paraphaeosphaeria</i> , <i>Pithomyces</i> , <i>Pleospora</i> ,	

					<i>Stemphylium,</i> <i>Venturia.</i>	
(Camacho et al., 2016)	Portugal: Funchal, Madeira	01/01/2003 - 31/12/2009 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 10 m above ground.	Microscopy.	<i>Alternaria,</i> <i>Cladosporium</i>	
(Sousa et al., 2016)	Portugal: Funchal, Madeira	01/01/2003 - 31/12/2008 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 10 m above ground.	Microscopy.	14 taxa: <i>Alternaria,</i> <i>Arthrinium,</i> <i>Botrytis,</i> <i>Cladosporium,</i> <i>Curvularia,</i> <i>Drechslera,</i> <i>Epicoccum,</i> <i>Fusarium,</i> <i>Gliomastix,</i> <i>Nigrospora,</i> <i>Polythrincium,</i> <i>Spegazzinia,</i> <i>Tetraploa,</i> <i>Torula.</i>	

(Almeida et al., 2018)	Portugal: Beja	12/04/2012 - 30/07/2014. (continuous sampling).	- Burkard 7-day volumetric trap. Operated 30 m above ground (256 m a.m.s.l.).	Microscopy.	<i>Alternaria</i> , <i>Cladosporium</i> .	
(Ilanovici, 2016)	Romania: Timisoara	01/02/2008 - 30/11/2010 (continuous sampling).	- Lanzoni 7-day volumetric trap. Operated 20 m above ground.	Microscopy.	Five taxa: <i>Alternaria</i> , <i>Cladosporium</i> , <i>Epicoccum</i> , <i>Pithomyces</i> , <i>Torula</i> .	
(Scevkova et al., 2016)	Slovakia: Bratislava	01/02/2002 - 30/11/2014 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 10 m above	Microscopy.	<i>Alternaria</i> , <i>Epicoccum</i> , <i>Stemphylium</i>	

			ground.			
(Scevkova and Kovac, 2019)	Slovakia: Bratislava	01/01/2016 - 31/12/2016 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 10 m above ground.	Microscopy.	54 fungal groups.	
(Fernandezgonzalez et al., 1993)	Spain: Leon	01/09/1987 - 31/08/1989 (Mon-Fri).	- Active filtration device (CAP2). Operated 12 m above ground (860 m a.m.s.l.).	Microscopy.	Four taxa: <i>Alternaria</i> , <i>Cladosporium</i> , <i>Phaeosphaeria</i> , <i>Puccinia</i> .	
(Herrero and Zaldivar, 1997)	Spain: Palencia	01/01/1990 - 31/12/1992.	- Active filtration device (CAP2). Operated 12 m	Microscopy.	<i>Alternaria</i> , <i>Cladosporium</i> .	(Herrero et al., 1996)

			above ground (743 m a.m.s.l.).			
(Angulo- Romero et al., 1999)	Spain: Cordoba	01/01/1992 - 31/12/1992 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 15 m above ground (10 m a.m.s.l.).	Microscop py.	<i>Alternaria.</i>	
(Sabariego et al., 2000)	Spain: Granada	01/01/1994 - 31/12/1994 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 23 m above ground.	Microscop py.	<i>Alternaria,</i> <i>Cladosporium,</i> <i>Ustilago.</i>	
(Giner et al., 2001)	Spain: Murcia	01/03/1993 - 31/12/1998 (continuous	- Burkard 7-day volumetric	Microscop py.	<i>Alternaria</i>	(Giner et al., 1998)

		sampling).	trap. Operated 19 m above ground (110 m a.m.s.l.).			
(Rodríguez-Rajo et al., 2005)	Spain: 3 locations.	All locations: 01/01/2002 - 31/12/2002 (continuous sampling).	All locations: - Lanzoni 7-day volumetric trap.	All locations: Microscopy.	<i>Alternaria</i> , <i>Cladosporium</i>	
(Herrero et al., 2006)	Spain: Madrid	01/01/2002 - 31/12/2002 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 24 m above ground.	Microscopy.	71 fungal groups.	
(Morales et al., 2006)	Spain: Seville	01/01/1997 - 31/12/1998 (continuous sampling).	- Hirst-style 7-day volumetric	Microscopy.	18 taxa: <i>Agaricus</i> , <i>Agrocybe</i> , Bankeraceae,	

			trap. Operated 15 m above ground.		Boletaceae, <i>Bovista</i> , <i>Calvatia</i> , <i>Coprinus</i> , <i>Cortinarius</i> , <i>Ganoderma</i> , <i>Inocybe</i> , <i>Phylacteria</i> , <i>Puccinia</i> , <i>Rhodophyllus</i> , <i>Russula</i> , <i>Tilletia</i> , <i>Tomentella</i> , <i>Uredospora</i> , <i>Ustilago</i> .	
(Reyes et al., 2009)	Spain: Valladolid	01/02/2005 - 31/01/2007 (continuous sampling).	- Burkard 7-day volumetric trap.	Microscop py.	<i>Alternaria</i> , <i>Cladosporium</i>	
(De Linares et al., 2010)	Spain: 12 locations	Range: 01/01/1995 - 31/12/2008 (continuous sampling).	All locations: - Hirst- style 7- day volumetric trap. - Sampler	All locations : Microscop py.	<i>Alternaria</i>	

			height range: 14- 2367 m a.m.s.l.			
(Aira et al., 2012)	Spain: 10 locations. Portugal: 2 locations.	Range: 01/01/1993 - 31/12/2009 (continuous sampling).	All locations: - Either Burkard or Lanzoni 7- day volumetric trap. - Sampler heights 5- 600 m a.m.s.l.	All locations : Microsco py.	<i>Cladosporium</i>	
(Maya- Manzano et al., 2012)	Spain: 3 locations.	Range: 01/01/1995 - 31/12/2010 (continuous sampling).	All locations: - Burkard 7-day volumetric trap. - Sampler heights range: 5-	All locations : Microsco py.	<i>Alternaria</i>	

			25m (184-459 m a.m.s.l.)			
(Trejo et al., 2012)	Spain: Merida	01/01/1997 - 31/12/1998 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 15 m above ground.	Microscopy.	15 taxa: <i>Chaetomium</i> , <i>Diaporthe</i> , <i>Diatrype</i> , <i>Helvella</i> , <i>Leptosphaeria</i> , <i>Massaria</i> , <i>Melanomma</i> , <i>Mycosphaerella</i> , <i>Nectria</i> , <i>Paraphaeosphaeria</i> , <i>Pleospora</i> , <i>Sordaria</i> , <i>Sporormiella</i> , <i>Venturia</i> , <i>Xylaria</i> .	
(Aira et al., 2013)	Spain: 10 locations Portugal: 2 locations	Range: 01/01/1993 - 31/12/2009 (continuous sampling).	All locations: - Either Burkard or Lanzoni 7-day volumetric	All locations: : Microscopy.	<i>Alternaria</i>	(Astray et al., 2010; Recio et al., 2012)

			trap. - Sampler location height range 5- 600 m a.m.s.l.			
(Trejo et al., 2013)	Spain: Merida	01/01/1997 - 31/12/1998 (continuous sampling).	- Burkard 7-day volumetric trap. Operated 1 m above ground.	Microscopy.	26 fungal groups.	
(Fernandez- Rodriguez et al., 2014)	Spain: Badajoz	25/03/2009 - 31/07/2011. Sampler 1: (continuous sampling).	- Burkard 7-day volumetric trap and portable Burkard spore traps. Operated 16 m	Microscopy.	<i>Alternaria</i> , <i>Aspergillus/Penicillium</i> , <i>Cladosporium</i> .	

			above ground (184 m a.m.s.l.).			
(Maya- Manzano et al., 2016)	Spain: 3 locations.	All locations: 01/02/2011 - 31/12/2014. (continuous sampling during sampling periods).	All locations: - Hirst- style 7- day volumetric trap. - Sarnier height range: 6- 17m (253- 508 m a.m.s.l.)	All locations : Microsco py.	<i>Alternaria</i>	
(Anton et al., 2019)	Spain: Salamanca a	17/02/2014 - 16/02/2016 (continuous sampling).	- Hirst- style 7- day volumetric trap. Operated 25 m above	Microsco py.	7 groups: <i>Agaricus</i> , <i>Alternaria</i> , <i>Aspergillus/Penic illium</i> , <i>Cladosporium</i> , <i>Coprinus</i> , <i>Leptosphaeria</i> ,	

			ground (802 m a.m.s.l.).		<i>Periconia.</i>	
(Nunez et al., 2021)	Spain: 11 locations.	All samplers: Summer 2015 - Spring 2017.	All samplers: - Burkard 7-day volumetric trap.	Illumina MiSeq sequenci ng of 5.8S - ITS2 region.	Not applicable – de-novo sequencing.	
(Fernandez- Rodriguez et al., 2018)	Switzerla nd: Payerne	Both samplers: 03/03/2013 - 08/08/2013 (continuous sampling).	- Sampler 1: Hi 'st- style 7- day volumetric trap, and WIBS-4 technique (fluoresce nt counting) - Height of both samplers: 490 m	Microscop y.	82 groups	

			a.m.s.l.			
(Erkara et al., 2008)	Turkey: Eskisehir	All samplers: 01/01/2000 - 31/12/2001 (continuous sampling).	All samplers: - Durham gravimetric sampler. Operated 1.75 m above ground (845, 819, and 789 m a.m.s.l.).	Microscopy.	<i>Alternaria</i> , <i>Cladosporium</i>	
(Erkara et al., 2009)	Turkey: Eskisehir	01/01/2005 - 30/11/2006 (continuous sampling).	All samplers: - Durham gravimetric sampler. Operated 1.75 m above ground.	Microscopy.	Two taxa: <i>Alternaria</i> , <i>Cladosporium</i>	
(Kilic et al., 2010)	Turkey: Adana	01/11/2006 - 31/10/2007 (continuous	- Burkard 7-day volumetric	Microscopy.	<i>Alternaria</i>	

		sampling).	trap. Operated 15 m above ground.			
(Grinn-Gofroń et al., 2020)	Turkey: 5 locations	All samplers: 01/07/2010 - 30/06/2012	All samplers: - Hirst- style 7- day volumetric trap. Sampler height range: 6- 10-15m.	Microsco py.	30 fungal groups.	
(Kilic et al., 2020)	Turkey: Elazığ	01/01/2018 - 31/12/2018	- Lanzoni 7-day volumetric trap.	Microsco py.	20 fungal groups.	

* Studies covering the same sampling period, location and fungal taxa, with no distinct results related to fungal spore seasonality.

Table 2. Multiple linear regression output for various seasonality metrics against latitude and longitude values.

Taxon	Variable	Observations (location-years)	Intercept	Latitude (km north)	Longitude (km east)	Explained variation (R-square)	Residual standard error	F statistic
<i>Alternaria</i> spp.	Season length (days)	247	258** *	- 0.066***	- 0.015***	0.68	30.3 (df = 244)	257.4*** (df = 2; 244)
	Season start date (day of the year)	247	69***	0.038***	0.014***	0.63	22.1 (df = 244)	203.3*** (df = 2; 244)
	Season end date (day of the year)	247	327** *	- 0.028***	-0.001	0.51	15.6 (df = 244)	125.3*** (df = 2; 244)

	year)							
	Peak	101	198**	0.012**	-0.002	0.07	30.1 (df =	3.86
	date		*				98)	6** (
	(day							df =
	of							2;
	the							98)
	year)							
Cladosp	Seas	203	292**	-	-	0.73	25.9 (df =	268.
orium	on		*	0.056***	0.027***		200)	7*** (
spp.	lengt							df =
	h							2;
	(days							200)
	)							
	Seas	203	57***	0.032***	0.015***	0.71	15.4 (df =	240.
	on						200)	2*** (
	start							df =
	date							2;
	(day							200)
	of							
	the							
	year)							
	Seas	203	349**	-	-	0.51	18.4 (df =	102*
	on		*	0.024***	0.012***		200)	** (df
	end							= 2;
	date(							200)

day							
of							
the							
year)							
Peak	56	231**	-0.016*	-0.004	0.16	30.3 (df =	5** (
date		*				53)	df =
(day							2;
of							53)
the							
year)							

*p<0.1; **p<0.05; ***p<0.01.

Graphical abstract



14,333 records identified



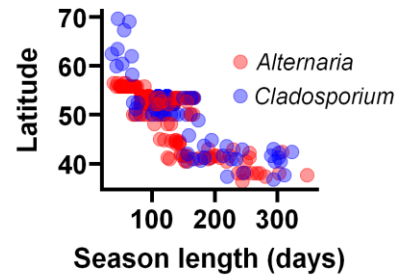
227 full-text studies assessed



Results related to seasonality extracted from 74 articles



Meta-analysis of seasonal trends and meteorological influences

Longer *Alternaria* and *Cladosporium* season length in south-westerly regions

Most important factors identified as temperature, precipitation and relative humidity



This information may benefit research in the care of those with fungal allergy

Highlights

- Outdoor airborne fungal spores are associated with allergy.
- A systematic review of outdoor fungal seasonality in Europe was conducted.
- Allergenic spores of *Alternaria* and *Cladosporium* were the focus of most studies.
- *Alternaria* and *Cladosporium* seasons were longer in south-westerly regions.
- Temperature, precipitation, and relative humidity identified as driving factors.