Int J Biometeorol (2015) 59:1157–1167
DOI 10.1007/s00484-014-0927-0

ORIGINAL PAPER

Fungi in a changing world: growth rates will be elevated, but spore production may decrease in future climates

Athanasios Damialis · Aqilah B. Mohammad · John M. Halley · Alan C. Gange

Received: 28 July 2014 / Revised: 23 October 2014 / Accepted: 23 October 2014 / Published online: 21 November 2014

Abstract Very little is known about the impact of climate change on fungi and especially on spore production. Fungal spores can be allergenic, thus being important for human health. The aim of this study was to investigate how climate change influences the responsive ability of fungi by simulating differing environmental regimes. Fungal species with high spore allergenic potential and atmospheric abundance were grown and experimentally examined under a variety of temperatures and different nutrient availability. Each represented the average decadal air temperature of the 1980s, 1990s and 2000s in the UK, along with an Intergovernmental Panel on Climate Change (IPCC) climate change scenario for 2100. All tests were run on six fungal species: *Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *Cladosporium oxysporum* and *Epicoccum purpurascens*. Mycelium growth rate and spore production were examined on each single species and competitive capacity among species combinations in pairs. All fungal species grew faster at higher temperatures, and this was more pronounced for the temperature projection in 2100. Most species grew faster when there was lower nutrient availability. Exceptions were the species with the highest growth rate (*E. purpurascens*) and with the highest competition capacity (*A. alternata*). Most species (except for *E. purpurascens*) produced more spores in the richer nutrient medium but fewer as temperature increased. *C. cladosporioides* was an exception, exponentially increasing its spore production in the temperature of the 2100 scenario. Regarding competitive capacity, no species displayed any significant alterations within the environmental range checked. It is suggested that in future climates, fungi will display dramatic growth responses, with faster mycelium growth and lower spore production, with questions risen on relevant allergen potential.

Keywords Climate change · Experimental warming · Fungal ecology · Fungal spores · Global warming · IPCC projection

A. Damialis · A. B. Mohammad · A. C. Gange
School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK

A. Damialis (✉) · J. M. Halley
Department of Biological Applications and Technology, University of Ioannina, 451 10 Ioannina, Greece
e-mail: th_damialis@hotmail.com

A. B. Mohammad
School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, Kuala Terengganu 21030, Malaysia

Introduction

The ecology and biology of living organisms has been highly affected by climate change (Penuelas et al. 2002; Walther et al. 2002); these alterations usually refer to long-lived organisms, i.e. mammals, woody plants etc. For example, during the last decades, plants' flowering occurs earlier and lasts longer, and more pollen has been produced by numerous taxa (Ziello et al. 2012). Changes in climate (and also those expected in the coming decades) are expected to also have an impact on fungal biodiversity patterns, which, in turn, can also have implications in public health. Hence, it is critically important to evaluate ecological responses of fungi.

It is well known that fungal spores are implicated in respiratory allergy symptoms (D'Amato et al. 1997; Mari et al. 2003; Gioulekas et al. 2004). Specific taxa (e.g. *Alternaria*, *Aspergillus*, *Cladosporium*) have been found to be 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 (Dales et al. 2000; Bush and Prochnau 2004). Therefore, it is critically important to assess and evaluate the

potential risks of the atmospheric presence of fungal spores. For pollen, the frequency and the severity of allergic reactions in sensitised individuals have been already found to increase over the last decades (Bousquet et al. 2004). This is possibly the result of the increase of the airborne pollen concentration (Linneberg et al. 1999). Crameri et al. (2014) have highlighted fungi as a neglected and underestimated source of respiratory allergy, e.g. compared to pollen allergies. Beggs (2004) has reviewed the need for research on fungal spores; however, very little has been done since, and the Intergovernmental Panel on Climate Change (IPCC) (Confalonieri et al. 2007) have stated that no studies on fungal spores and on interactions with climate change exist. Cecchi et al. (2010) have concluded that there is limited and inconsistent evidence of trends in fungal spore production.

To date, only a few relevant studies have reported earlier and longer fungal fruiting seasons in the UK (Gange et al. 2007) and Norway (Kauserud et al. 2012). Boddy et al. (2014) have suggested that many European countries have experienced similar changes. Long-term alterations in spore production and atmospheric abundance have likewise received little attention so far. This is mainly due to the lack of representative fungal spore data. Only very few aerobiological stations worldwide, such as Derby (UK), Kraków (Poland), Thessaloniki (Greece) or Copenhagen (Denmark), monitor fungal spore concentration of various taxa on a regular basis for more than 15 years. In contrast, such changes have been widely studied for pollen, and bio-indicator taxa of environmental change have been sometimes suggested (Damialis et al. 2007). In Europe, Corden et al. (2003) have investigated long-term trends from a 26-year dataset of airborne fungal spores of the genus *Alternaria* in the UK and attempted to attribute any changes found to various environmental factors; however, the results were inconclusive as they were largely dependent upon regional vegetation and other factors. A most recent study by Damialis et al. (2015) has revealed a start of a long-term trend in airborne spore concentrations in Thessaloniki during 1987–2005. Berman (2011) has also expressed concerns both from the allergic sensitisation and also from the biodiversity perspective regarding the increasing or decreasing long-term trends in aeroallergen concentrations, particularly in relation to the current and the projected climatic change scenarios.

Helfer (2014) has already shown that specific fungal taxa could be vulnerable to global environmental change. As sporulation and also fruiting of fungi are influenced by several meteorological factors, such as rainfall, air temperature (Straatsma et al. 2001; Carlile et al. 2007) and relative humidity (Cecchi et al. 2010), variability at different stages of fungal reproduction could lead to modifications in fungal diversity and the dynamics of their performance. However, the exact influence of the current and projected global change on fungal ecology is still not understood. There are indications that changing climate may lead to alterations in phenology (Corden et al. 2003; Gange et al. 2007; Kauserud et al. 2010) and dynamics of fungal communities (Gange et al. 2011; Mohammad 2013).

From a socio-economic perspective, the health-related implications due to airborne fungal spores are also very important: asthma is a common respiratory disease, observed in up to 30 % of the adult European population (Burney et al. 1994; Beasley et al. 1998; Annesi-Maesano 1999). It is estimated that 300 million people suffer from asthma symptoms worldwide. Annually, 13.2 million emergency visits to health practitioners (in the public or private sector) and approximately 4000 deaths related to asthma incidents are recorded (Selgrade et al. 2006). Asthma frequency has risen significantly in many countries where it has doubled or tripled during the last decades (Strachan and Ross Anderson 1992; Bousquet et al. 2004). If we also consider the very high economic cost for the medical and pharmaceutical manifestation of respiratory diseases (Bousquet and Daures 2005; Selgrade et al. 2006), knowledge on airborne concentrations of allergenic fungal types is very important. It is documented that the annual cost for visits to allergy specialists and for drug therapy, only for the less severe form of allergy, viz. allergic rhinitis, can reach up to 3.5 billion dollars in the USA (Storms et al. 1997). If other forms of respiratory diseases are included (i.e. asthma) and indirect effects are co-assessed, e.g. lost working hours, reduced working efficiency etc., the potentially additional cost rises to 16.1 billion dollars per annum (Selgrade et al. 2006).

The aim of this study was to investigate fungal responses under differing environmental regimes. To achieve this, the responsive ability of different fungal species was examined under a variety of temperature and nutrient availability levels. The latter factors simulated temperature increases during past and current climate change conditions over the last three decades (1980–2010). To assess fungal adaptability to the parameters examined, an IPCC projected global change scenario for 2100 was also checked and the responsive behaviour of fungal species was observed. This experimental evidence will be the first to examine as to whether and to what extent environmental changes may affect fungal species' growth and sporulation patterns. We hypothesised that if fungal growth rates are elevated at higher temperatures, then spore production would also increase. However, given the known individualistic responses of fungi to climate (Gange et al. 2007), we also hypothesised that the extent of any changes would vary between species.

Materials and methods

Fungal species

Six different fungal species were selected, namely *Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *Cladosporium oxysporum* and *Epicoccum*

purpurascens. The selection was based on the following criteria: (i) on fungal spore atmospheric abundance: particularly *Alternaria* and *Cladosporium* species have been frequently reported as the most abundantly represented worldwide and specifically in the Mediterranean climate study area (Damialis and Gioulekas 2006); (ii) on fungal spore allergenic properties: spores from all studied species are well documented as the most allergenic worldwide and particularly from *Alternaria*, *Aspergillus* and *Cladosporium* (e.g. Gioulekas et al. 2004), being responsible for the most difficult and severe cases of asthma which sometimes lead to mortality (Zukiewicz-Sobczak 2013); (iii) on fungal phytopathogenic properties: all selected species, i.e. *Alternaria*, *Botrytis* and *Epicoccum* (Carlile et al. 2007) and *C. cladosporioides* (Jacyno et al. 1993) are well-known phytopathogens of commercial crops; (iv) on produced mycotoxins: mainly *Aspergillus* species are well known for being implicated in mycotoxicoses which can have dire effects on human health (Kourousekos 2008); and (v) on usefulness in industry and biotechnology: specific species, viz. *A. niger* and *B. cinerea*, are widely used for beverage and wine production, respectively (Carlile et al. 2007).

Sample and substrate preparation

All species were isolated as endophytes from plant tissue, as described in Wearn et al. (2012). Cultures from that study were maintained on potato-carrot agar (PCA) until required for this research.

Isolation and culturing of fungi and a variety of microbiological plate assays were undertaken to determine growth rates and interactions between fungi. Species were sub-cultured (so as to obtain equally fresh samples) in an ESCO class II cabinet in sterile conditions. Two different substrates were used: a nutrient-rich one, potato-dextrose agar (PDA; Oxoid, CM0139) and a nutrient-poor one, potato-carrot agar (PCA).

Experimental simulations

Fungal species were investigated for their responsive ability under differing environmental regimes and over time. All fungal species (cultures) were tested in an incubator for a combination of different temperatures and nutrient availability. In total, five elevated temperature experiments were run. Each represented an average decadal air temperature of the 1980s, 1990s and 2000s in the UK, from where the samples were taken, along with control temperature 22 °C, stable throughout the experiment. As most fungal species sporulate on senescent leaf tissue from spring to autumn (Hodgson 2010), the averages were estimated after excluding winter periods. In all experiments and so as to resemble the natural conditions as closely as possibly, temperature was fluctuated daily every 12 h together with light so as to simulate day and night. The respective averaged decadal temperatures are given in Table 1. Another experiment simulated the projected temperature under the 'pessimistic' IPCC scenario, *A1Fl*. This scenario assumes a world of very rapid economic growth, a global population that peaks in mid-century and rapid introduction of new and more efficient fossil-intensive technologies (Bernstein et al. 2007). So as to check for a comparatively much higher temperature and since the six selected species are quite common in a wide range of ecosystems and at various latitudes, the IPCC projection was conducted for a warmer Mediterranean-type climate, that of Greece. This selection was based on the already intense regional effects of climate change on plants (Damialis et al. 2007) and fungi (Damialis et al. 2015), compared to the rest of the European regions (Ziello et al. 2012). Thus, the responsive ability of all six species would be tested against temperatures of two different geoclimatic regions, temperate-oceanic UK and Mediterranean Greece. The above simulation corresponded to a +6.4 °C increase in temperature (upper limit forecast; Bernstein et al. 2007) compared to that in Greece during 1980–1999 (Universite de Thessaloniki 1981–2000). In particular, the above equated to a minimum temperature of 17.7 °C and a maximum of 27.1 °C (Table 1).

Fungi were cultivated and grown in two different nutrient-level agars, so as to check for the effect of nutrient availability. All tests were run on each fungal species alone to check for its growth rate and spore production and in pairs to examine competitive capacity among all species combinations. For fungal growth experiments, a total of 240 Petri dishes were prepared and observed (6 species × 2 agars × 5 temperatures × 4 replicates each). From the above samples and for the assessment of fungal spore production, a total of 720 microscope slides were prepared and used (240 Petri dishes × 3 replicates). For fungal competition experiments, a total of 600 Petri dishes were prepared and observed (15 species combinations for the six species × 2 agars × 5 temperatures × 4 replicates each).

Table 1 Experimental design: averaged decadal temperatures* for the last three decades and IPCC projection for 2100

Simulated period	Air temperature (°C)	
	Minimum (night)	Maximum (day)
1980s	7.2	14.5
1990s	7.8	15.2
2000s	8.8	15.4
2100	17.7	27.1
Control	22.0	22.0

*Excluding winter period

Estimation of fungal characters

Estimation of mycelium growth

The mycelial growth of all fungal species was measured daily for a total of 6 months. This was performed for all experimental simulations (different nutrient availability and temperatures) in two perpendicular to each other's diameters and was expressed as average radial growth of mycelium expressed in millimeters per day.

Estimation of spore production

The number of spores produced by each species was counted through destructive sampling and for a total period of 4 months. This was performed for all combinations of parameters, and samples were taken at least three times for each species' life cycle, per factor and replicate. Fungal spore production was expressed as number of spores expressed in per cubic centimeter of agar for the given sampling day. To assess spore production, random circular surfaces (10-mm diameter) were sampled from each replicate (sub-culture) for all manipulations and species and were placed into Eppendorf tubes, which were filled with 80 % glycerol (so as to avoid spore clumps) up to a solution of total volume of 1.5 ml. The solutions were stained with saffranine and stirred vigorously using Vortex so that all spores were released. A small suspension was taken with a micropipette and was placed on a microscope slide under a cover slip. All spores were then counted using an optical microscope under a magnification of ×400. The suspensions taken were 2 μl for *Alternaria* and *Cladosporium* species, 1 μl for *Aspergillus* and *Botrytis* and 20 μl for *Epicoccum*. The above different dilution rates were adjusted according to the total spore production of each species examined. Three sub-samples (replicates) were taken for each manipulation and species.

Estimation of competition capacity

The competition capacity of all fungal species was tested by observing the outcome of the interaction of all species pairings. This was observed daily for a total period of 6 months and was performed for all manipulations. From the day when the two species started touching each other and interaction initiated, each species' growth was measured daily so as to identify the type of interaction between them. The interaction types could be: (a) overgrowth, where the mycelium of the most vigorous species encroaches upon the weaker one's colony, partially or totally taking over its domain or (b) deadlock, where an inhibition at a distance takes place, with a mycelium barrage or clear zone getting formed and separating the two colonies, thus resulting in a final 'equilibrium' (Mohammad 2013).

Meteorological data

So as to simulate the average decadal air temperatures of the UK, we obtained daily air temperature time series for the area of Southampton for the last three decades (MET Office, UK), from which the fungal samples were taken. For the IPCC projection, we obtained air temperature data from the Mediterranean climate area of Thessaloniki, Greece (Université de Thessaloniki 1981–2000).

Statistical analysis

We checked for differences (using one-way ANOVA) in mycelial growth and sporulation (dependent variables) between: (i) species, (ii) temperatures, and (iii) nutrient availability. We checked for covariance (ANCOVA) and interaction effects (factorial ANOVA) in growth and sporulation (dependent variables) for each species against nutrient availability and temperatures (categorical predictors) and sampling date (continuous variable). Post hoc tests (Bonferroni) provided significant categories of variables. For all analyses, we checked both raw values and the natural logarithms of the response variables. The normality of data was estimated for each taxon using the Kolmogorov-Smirnov test, which finally determined the best scale (log-transformed or not) for the estimation of regressions (Chatfield 1989). All data analyses were carried out in Statistica 7.

Results

Mycelium growth

All fungal species grew faster at higher temperatures ($p<0.001$, $R^2=0.51$). This was even more pronounced for the projected IPCC temperature and particularly for *Epicoccum* ($p<0.001$, $R^2=0.88$) and *Alternaria* ($p<0.001$, $R^2=0.84$), followed by *Botrytis* ($p<0.001$, $R^2=0.76$), *Aspergillus* ($p<0.001$, $R^2=0.74$), *C. oxysporum* ($p<0.001$, $R^2=0.72$) and *C. cladosporioides* ($p<0.001$, $R^2=0.71$) (Fig. 1). Mycelium growth was slightly faster when there were fewer nutrients, which was particularly apparent for the fastest growing species of all examined, viz. *Alternaria* ($p<0.001$, $R^2=0.81$) and *Epicoccum* ($p<0.001$, $R^2=0.65$). After applying Bonferroni post hoc test, it was also found that, on average, mycelium growth increased more intensely in the 1990s and 2000s. This rise seems to peak in 2100, when the highest fungal growth for all species examined is recorded, regardless of nutrient availability ($p<0.05$) (Fig. 1). This peak is particularly intense (exponential increase) for *B. cinerea* and *C. oxysporum* (Bonferroni post hoc test, $p<0.05$). Overall, nutrient availability seems to play an important role for the commencement of mycelium growth, whereas temperature for

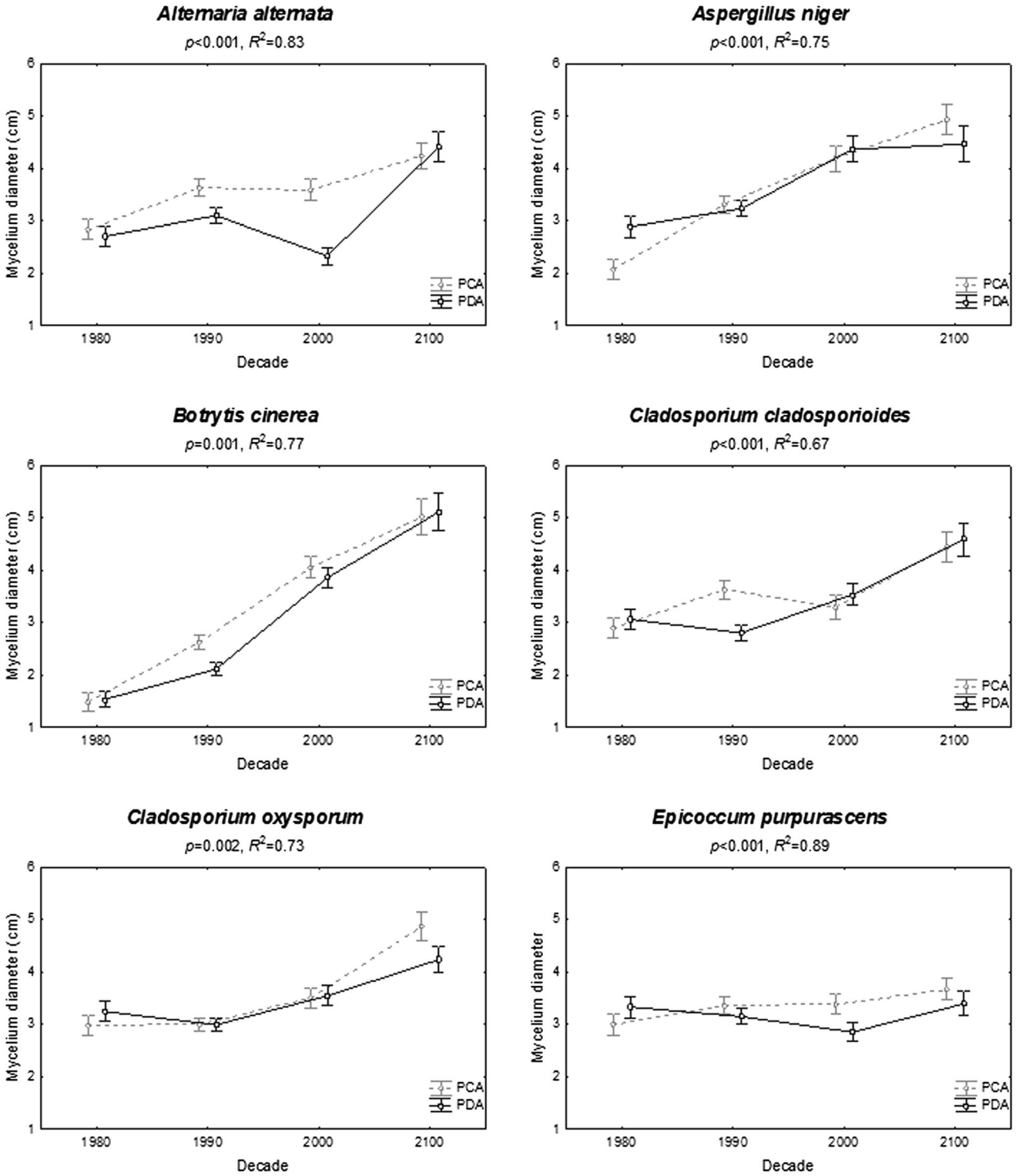

Fig. 1 Differences (full factorial ANOVA) in fungal growth (*dependent variable*) between different nutrient levels, PCA and PDA, and temperature decadal averages during 1980–2010 and 2100 projection (categorical predictors; *X axis*) and over sampling time (covariate). Fungal growth (*Y axis*) is expressed as mycelial diameter in centimeter. Standard deviation bars are also given. The respective averaged decadal temperatures are (i) for the 1980s: minimum (night) temperature =7.2 °C and maximum (day) temperature =14.5 °C, (ii) for the 1990s: 7.8 and 15.2 °C, (iii) for the 2000s: 8.8 and 15.4 °C, and (iv) for the 2100 IPCC projection: 17.7 and 27.1 °C (see also Table 1)

the end of growth. Particularly, minimum temperature (in all species: $p<0.001$, $R^2=0.41$–0.74) displayed a negative exponential effect on mycelium growth, particularly for the fast-growing *Alternaria* and *Epicoccum*.

Spore production

Spore production differed greatly among species (Fig. 2) with the highest producer being *A. niger* (producing 5.1×10^7 spores per cm^3 of agar), followed by *B. cinerea* (2.2×10^7 spores per cm^3 of agar) and *C. oxysporum* (3.5×10^6 spores per cm^3 of agar) and the lowest being *E. purpurascens* (1.1×10^4 spores per cm^3 of agar) (Bonferroni posthoc test, $p<0.05$). All species produced more spores when there were more nutrients available ($p<0.001$), and this was particularly true for *Alternaria* ($p<0.001$), *Aspergillus* ($p<0.001$), *Botrytis* ($p<0.001$) and *Epicoccum* ($p=0.001$) at temperatures seen over the last three decades. *A. niger* tripled its spore production at richer nutrient medium (7.8×10^7 vs. 2.5×10^7 spores cm^{-3}), while *B. cinerea* (2.8×10^7 vs. 1.3×10^7 spores cm^{-3}) and *C. cladosporioides* (9.1×10^5 vs. 4.0×10^5 spores cm^{-3}) doubled it; *A. alternata* showed a ninefold increase in its spore production (9.1×10^5 vs. 1.0×10^5 spores cm^{-3}).

Considering temperature effects over time and as temperature increased during 1980–2010, spore production decreased exponentially ($p<0.001$). Regardless of the nutrient availability and with increasing temperatures, only *C. cladosporioides* increased its spore production over time ($p<0.001$). At the IPCC projected temperature in 2100, all other species either decreased or did not alter the amount of spores they produced. The direct and interaction effects of all species at different nutrient levels and at increasing temperatures are given in Fig. 3. Bonferroni post hoc tests revealed a greater decrease for *A. alternata* and *B. cinerea* ($p<0.05$), which lowered the spores produced with increased temperature regardless of the available nutrients. Particularly minimum temperature (in all species: $p<0.001$) displayed a negative relationship with spore production, particularly for *A. alternata*, *B. cinerea* and *C. oxysporum*. This effect was even more pronounced after the 1990s and for the 2100 projection.

Competition capacity

A. alternata overgrew all other species, comprising the most competitive among the examined species. The encroachment was total on *A. niger* and *B. cinerea*, whereas it was only partial upon *Epicoccum* and *Cladosporium* genera. *E. purpurascens* was the second most competitive, growing over four out of five species. Encroachment of this species' mycelium was always partial on all other interacting colonies. For all other species combinations, deadlocks were observed, for all the manipulations applied; that is, as soon as mycelia from different species touched each other and interaction initiated, they were soon proven to be equally vigorous and no overgrowth was observed. This equilibrium was established via a clear zone that was present in all cases.

Regardless of the outcome recorded, overgrowth or deadlock, there was no change at all in the competition pattern observed between species because of varying temperature or nutrient availability: the competitive capacity of all the species examined was not significantly affected within the environmental range tested. In Table 2, the matrix for the outcomes of the competition experiments is given.

Discussion

The present study revealed that fungi seem to grow more vigorously under elevated temperature and seem to be either unaffected or to produce lower spore amounts as temperature increases over time. In fact, they are expected to do so even

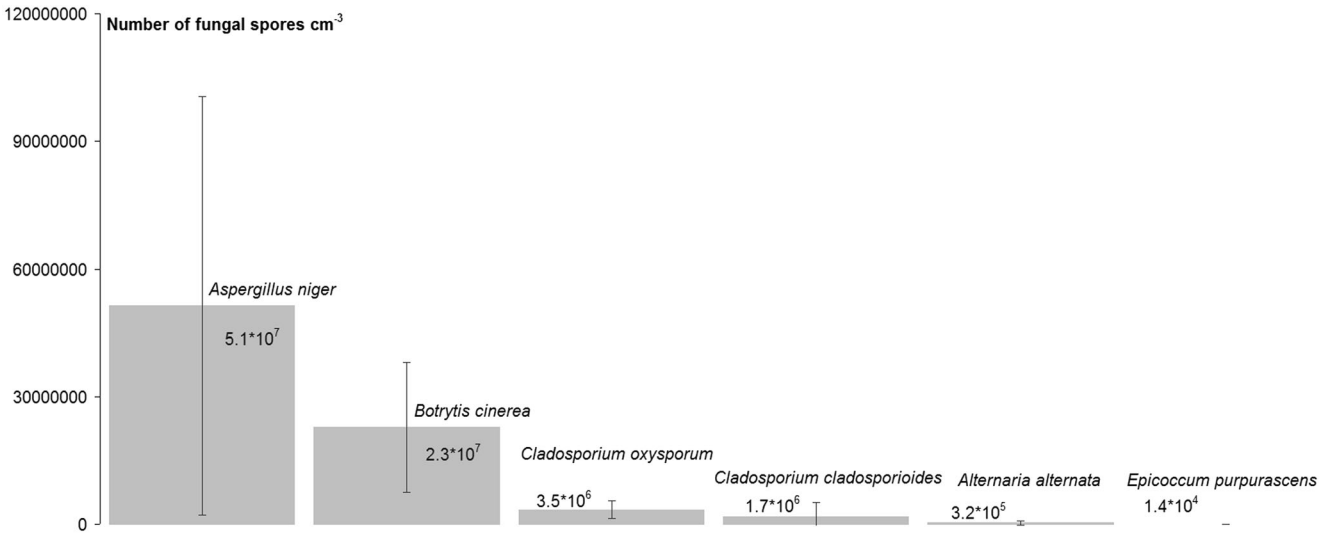

Fig. 2 Spore production averages (number of spores per cm^3 of agar; *Y axis*) for six species and for all manipulations conducted (temperature and nutrient availability range). Standard deviation bars are also given.

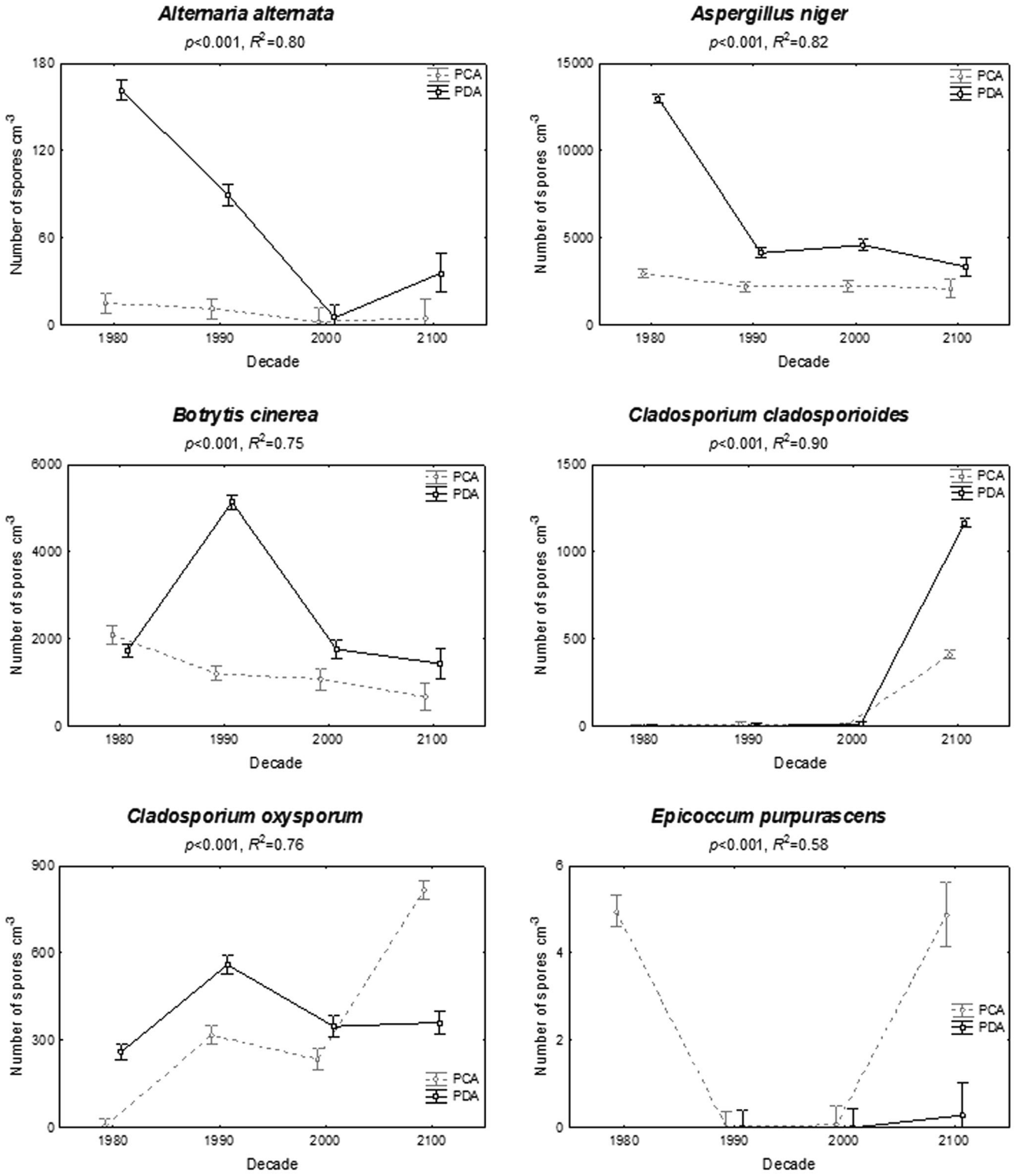

Fig. 3 Differences (full factorial weighted ANOVA) in spore production (dependent variable) between different nutrient levels, PCA and PDA, and temperature decadal averages during 1980–2010 and 2100 projection (independent variables; *X axis*). Spore production (*Y axis*) is expressed as number of spores per cm^3 of agar ($\times 10^4$). Standard deviation bars are also given. The respective averaged decadal temperatures are (i) for the 1980s: minimum (night) temperature =7.2 °C and maximum (day) temperature =14.5 °C, (ii) for the 1990s: 7.8 and 15.2 °C, (iii) for the 2000s: 8.8 and 15.4 °C, and (iv) for the 2100 IPCC projection: 17.7 and 27.1 °C (see also Table 1)

more intensely in climate projected for 2100. Thus, our first hypothesis of increased future spore production was not upheld, while our second of individualistic responses was shown to be true.

Table 2 Matrix for the outcomes (species of first column per row against the others) of competition experiments between combinations among six fungal species

	A. alternata	*A. niger*	*B. cinerea*	*C. cladosporioides*	*C. oxysporum*	*E. purpurascens*
Alternaria alternata		(+)	(+)	(+)	(+)	(+)
Aspergillus niger			(=)	(=)	(=)	(-)
Botrytis cinerea				(=)	(=)	(-)
Cladosporium cladosporioides					(=)	(-)
Cladosporium oxysporum						(-)
Epicoccum purpurascens						

Dark grey cells: deadlock [(=) denotes no overgrowth]. Light grey cells: overgrowth. Per row, (+) denotes the winner of the competition and (−) the loser. Competition outcomes for all species are similar for all handlings (temperature and nutrient availability)

Simulations like those performed in the current study have not been systematically explored for fungi and neither any assessment for IPCC temperature projections have been made so far. Only Wolf et al. (2010) have investigated the effect of varying carbon dioxide levels on a single species (*A. alternata*). In that study, elevated CO_2 and temperature increased spore production and antigenic protein content, thus suggesting that climate change will increase the responsive ability of fungi via increased sporulation rates. Although this is particularly important in terms of higher chances for more frequent and severe respiratory incidents because of potentially more *Alternaria* spores in the air, it is not in total agreement with the results from our study. Here, we found that *A. alternata*—like almost all other species examined—has likely decreased its spore production rates during the last three decades. Possibly, the decisive factor for the difference between the two studies could be temperature, since Wolf et al. (2010) tested a range of 20 and 30 °C night and day temperatures, respectively. This temperature range is even higher than the worst IPCC climate change scenario that we applied for our experiment (17.7 and 27.1 °C, respectively) for the Mediterranean climate of Greece. To a certain extent, it was observed that temperature played the leading role, since changes in both fungal growth and sporulation rates were more intense in the temperature projection for 2100, regardless of nutrient availability. Hence, we strongly believe that the interaction effects of temperature and nutrient levels (and consequently of CO_2) are probably responsible for the decrease or increase of the reproductive output. Because of the resource allocation limitations for each fungal species, it is evident that it is not feasible—at least for this smaller scale, decadal, variability—for a species to increase both the length and intensity of its vegetative stage (mycelium growth) *and* sporulation stage (spore production) at the same time. Cecchi et al. (2010) have strongly recommended that the effects of interaction between fungi and their hosts need to be elaborated, in contrast to solely isolated environmental factors, i.e. drought, temperature and carbon dioxide increase. This, in combination with more long-term relevant aerobiological datasets, can potentially clarify the general picture of fungal responsiveness to global change.

Conclusive results for more fungal species and for a variety of environmental factors have only rarely been reported by other researchers. Gange et al. (2007) found a consistent shift towards earlier and extended fungal fruiting seasons, using 58-year, rigorous observations for more than 300 species. Similar analyses and results were reported for the UK and Norway by Kauserud et al. (2012). On the other hand, Corden et al. (2003) studied airborne spore concentrations of *Alternaria* spp. and found region-specific trends of this fungal genus with no further evidence of any consistent, global trends thereafter. A very recent study by Damialis et al. (2015) has revealed a consistent downward trend in airborne fungal spore concentrations in Thessaloniki for a wide spectrum of taxa and during the period 1987–2005. Therefore, the results of the current study are among the first to reveal long-term changes, in parallel with and attributed to climate change (as expressed via experimental warming), for a variety of fungal species.

What has not been emphasised adequately is the role of nutrient availability in fungal fruiting. Gange et al. (2013) have recently emphasised the potential role for resource availability in the phenology of fungal fruiting. Even if the factors leading to current climate change cease at once, we expect that the delayed responsive ability of organisms, i.e. fungi, will not simultaneously react dramatically in the same direction. Thus, climate change effects will probably be visible in their ecology for many years or decades after the supposed (and hoped for) policy change. In particular, fungi seem to display a long memory for environmental alterations (Damialis et al., unpublished data) and hence, our experimental results can be considered as the genuine fingerprint of current and projected global change.

It is well documented (Carlile et al. 2007; De Aldana et al. 2013) that many of the fungal species examined here are either saprotrophs or endophytes or plant parasites. This means that they are closely related with (symbiotic relationship) or dependent on (parasitic relationship) their host organisms, that is plants, living or dead. As simulated long-term experimental patterns reveal a simultaneous increase in fungal growth rate but a decrease in sporulation rate, this may be an indication of a long-term life strategy change. It seems quite probable that various fungal species prefer, under current global change, to elongate their growth phase and delay their sporulation stage, until they exhaust the available nutrients. This, in turn, mirrors to some extent, the global increasing long-term trends in airborne pollen produced by anemophilous plants (Ziello et al. 2012). The plants respond to this prolonged symbiosis by changing their strategy themselves into increased reproductive effort (increased pollen production), which allows for both types of organisms successful survival. Gundel et al. (2013) have documented that such changes in life strategies influence the interactions between plants and fungi in a complex and species-specific manner. However, this complexity, particularly when other organisms such as bacteria participate in these processes, allows for no clear conclusions to be drawn at present. Ziska et al. (2008) have reviewed the interactions of plants and fungi and suggest that additional experiments should be conducted to elucidate the exact inter-relationship.

We originally hypothesised that fungi would show species-specific responses to changes in temperature and this was certainly upheld with spore production. In some cases, spore production decreased with increasing temperature, while in a single species, the reverse trend was seen. Thus, fungal responses to changes in climate are always going to be difficult to predict at anything above the species level. Even if the overall abundance of spores in an atmosphere does not change, the diversity is likely to be different in a projected future climate. How this altered biodiversity will affect allergenic response in humans is currently unknown but would be worthy of further attention.

For example, a prerequisite for sporulation of *Botrytis* species is the alternation of hot days during summer with cold nights (Carlile et al. 2007). If we take into account that particularly night (averaged minimum daily) temperature has greatly increased over time (e.g. Université de Thessaloniki 1981–2000), this may be a decisive factor for the decrease in sporulation rate for *B. cinerea*. Likewise, it is well known that *Botrytis* species are hydrophilic, requiring high relative humidity for growth, sporulation and spore germination. Hence, their spores can become airborne even during rainfall (Jürgensen and Madsen 2009). There are reports worldwide suggesting that there is a decrease in total annual relative humidity (Giorgi et al. 2004), which may be another reason for this species showing a decrease in spore production over elevated temperatures and over time. According to the results obtained from the present study, *E. purpurascens* may favour a life strategy of investment of resources in fast mycelial growth to compensate for its quite low spore production. In contrast, *A. alternata* probably invests in more effective chemical production and thus increased competitiveness. This is also justified by the lower competitive capacity and mycelium growth rates of *A. niger* and *B. cinera*, which nonetheless are compensated for by high spore production. All the above show that individualistic responses are common and explain a proportion of the variability found.

Therefore, if global warming continues, we suggest that fungi will display in the future a dramatic response with an earlier start of mycelium growth but with generally lower and belated fungal spore production. Such phenological changes may have important implications on fungal communities from the biodiversity perspective. Meanwhile, if allergenic spore amounts decrease, then from the public health perspective, this will probably lead to reduced risk of patient sensitisation. Given that spores from species like *Alternaria* and *Aspergillus* are well-known aeroallergens (Gioulekas et al. 2004), decreasing or unaltered levels of spore production are potential good news. However, some fungal species which are expected to

display increased spore production, like *C. cladosporioides*, may provoke more frequent or more severe respiratory allergy symptoms. In this respect, fungi have been already reported as a neglected and underestimated source of respiratory allergy (Crameri et al. 2014). Likewise, Heinzerling et al. (2009) have shown that many allergens previously regarded as untypical for some regions in Europe have been underestimated, among which several fungal taxa. Further research on long-term patterns of airborne fungal spore concentrations and additional experimental simulations of interactions among elevated temperatures, CO_2 and reduced water availability may more thoroughly clarify the impacts of climate change.

Acknowledgments The research project was implemented within the framework of the Action "Supporting Postdoctoral Researchers" of the Operational Program "Education and Lifelong Learning" and was co-financed by the European Social Fund (ESF) and the Greek State. The authors would like to thank the MET Office for providing them with meteorological data for the UK.

Conflict of interest There are no competing commercial interests in relation to the submitted work.

References

Annesi-Maesano I (1999) Epidemiological evidence of the occurrence of rhinitis and sinusitis in asthmatics. Allergy 54:7–13

Beasley R, Keil U, von Mutius E, Pearce N, Ait-Khaled N, Anabwani G et al (1998) Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 351:1225–1232

Beggs PJ (2004) Impacts of climate change on aeroallergens: past and future. Clin Exp Allergy 34:1507–1513

Berman D (2011) Climate change and aeroallergens in South Africa. Curr Allergy Clin Immunol 24:65–71

Bernstein L, Bosch P, Canziani O, Chen Z, Christ R, Davidson O et al (2007) Climate change and its impacts in the near and long term under different scenarios. In: Allali A, Bojariu R, Diaz S, Elgizouli I, Griggs D, Hawkins D et al (eds) Climate change 2007: synthesis report. An Assessment of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp 24–73

Boddy L, Büntgen U, Egli S, Gange AC, Heegaard E, Kirk PM et al (2014) Climate variation effects on fungal fruiting. Fungal Ecol 10:20–33

Bousquet J, Daures JP (2005) Modelization of cost assessment in childhood asthma is essential for policy makers. Allergy 60:135–137

Bousquet J, Ansotegui IJ, van Ree R, Burney PGJ, Zuberbier T, van Cauwenberge P (2004) European Union meets the challenge of the growing importance of allergy and asthma in Europe. Allergy 59:1–4

Burney PG, Luczynska C, Chinn S, Jarvis D (1994) The European community respiratory health survey. Eur Respir J 7:954–960

Bush RK, Prochnau JJ (2004) *Alternaria*-induced asthma. J Allergy Clin Immunol 113:227–234

Carlile MJ, Watkinson SC, Gooday GW (2007) The fungi, 2nd edn. Elsevier, UK, The Netherlands, USA

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

Chatfield C (1989) The analysis of time series. An introduction. Chapman & Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras

Confalonieri U, Menne B, Akhtar R, Ebi KL, Hauengue M, Kovats RS et al (2007) Human health. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (eds) Climate Change 2007: impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp 391–431

Corden JM, Millington WM, Mulllins J (2003) Long-term trends and regional variation in the aeroallergen *Alternaria* in Cardiff and Derby UK—are differences in climate and cereal production having an effect? Aerobiologia 19:191–199

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

D'Amato G, Chatzigeorgiou G, Corsico R, Gioulekas D, Jaeger L, Jaeger S (1997) Evaluation of the prevalence of skin prick test positivity to *Alternaria* and *Cladosporium* in patients with suspected respiratory allergy—a European multicenter study promoted by the Subcommittee on Aerobiology and Environmental Aspects of Inhalant Allergens of the European Academy of Allergology and Clinical Immunology. Allergy 52:711–716

Dales RE, Cakmak S, Burnett RT, Judek S, Coates F, Brook JR (2000) Influence of ambient fungal spores on emergency visits for asthma to a regional children's hospital. Am J Respir Crit Care Med 162: 2087–2090

Damialis A, Gioulekas D (2006) Airborne allergenic fungal spores and meteorological factors in Greece: forecasting possibilities. Grana 45: 122–129

Damialis A, Halley JM, Gioulekas D, Vokou D (2007) Long-term trends in atmospheric pollen levels in the city of Thessaloniki, Greece. Atmos Environ 41:7011–7021

Damialis A, Vokou D, Gioulekas D, Halley JM (2015) Long-term trends in airborne fungal-spore concentrations: a comparison with pollen. Fungal Ecol 13:150–156

Vázquez de Aldana BR, Bills G, Zabalgogeazcoa I (2013) Are endophytes an important link between airborne spores and allergen exposure? Fungal Divers 60:33–42

Université de Thessaloniki (1981–2000) Observations météorologiques de Thessaloniki 1980–1999. Annuaire de l' Institut Météorologique et Climatologique 49–68 [in French]

Gange AC, Gange EG, Sparks TH, Boddy L (2007) Rapid and recent changes in fungal fruiting patterns. Science 316:71

Gange AC, Gange EG, Mohammad AB, Boddy L (2011) Host shifts in fungi caused by climate change? Fungal Ecol 4:184–190

Gange AC, Mohammad AB, Damialis A, Gange EG (2013) Mushroom phenological changes: a role for resource availability? Proc Natl Acad Sci USA 110:E333–E334

Giorgi F, Bi X, Pal J (2004) Mean, interannual variability and trends in a regional climate change experiment over Europe. II: climate change scenarios (2071–2100). Clim Dyn 23:839–858

Gioulekas D, Damialis A, Papakosta D, Spieksma F, Giouleka P, Patakas D (2004) Allergenic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece. J Investig Allergol Clin Immunol 14:225–231

Gundel PE, Garibaldi LA, Helander M, Saikkonen K (2013) Symbiotic interactions as drivers of trade-offs in plants: effects of fungal endophytes on tall fescue. Fungal Divers 60:5–14

Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S et al (2009) GA^{2}LEN skin test study I: GA^{2}LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. Allergy 64:1498–1506

Helfer S (2014) Rust fungi and global change. New Phytol 201:770–780

Hodgson S (2010) Foliar fungal endophyte dynamics in herbaceous hosts. PhD Thesis. Royal Holloway University of London, UK.

Jacyno JM, Harwood JS, Cutler HG, Lee M-K (1993) Isocladosporin, a biologically active isomer of cladosporin from *Cladosporium cladosporioides*. J Nat Prod 56:1397–1401

Jürgensen CW, Madsen AM (2009) Exposure to the airborne mould *Botrytis* and its health effects. Ann Agr Env Med 16: 183–196

Kauserud H, Heegaard E, Semenov MA, Boddy L, Halvorsen R, Tige LCS et al (2010) Climate change and spring-fruiting fungi. P Roy Soc A – Biol Sci 277:1169–1177

Kauserud H, Heegaard E, Büntgen U, Halvorsen R, Egli S, Senn-Irlet B et al (2012) Warming-induced shift in European mushroom fruiting phenology. Proc Natl Acad Sci USA 109:14488–14493

Kourousekos GD (2008) Investigation of the possible effects of aflatoxin B1 administration on milk composition and reproductive parameters of the Greek indigenous goat. PhD Thesis. Aristotle University of Thessaloniki, Greece [in Greek with English abstract]

Linneberg A, Nielsen NH, Madsen F, Frølund L, Dirksen A, Jørgensen T (1999) Increasing prevalence of allergic rhinitis symptoms in an adult Danish population. Allergy 54:1194–1198

Mari A, Schneider P, Wally V, Breitenbach M, Simon-Nobbe B (2003) Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts. Clin Exp Allergy 33:1429–1438

Mohammad AB (2013) Spatial and temporal aspects of macrofungal community structure. PhD Thesis. Royal Holloway University of London, UK.

Penuelas J, Filella I, Comas P (2002) Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. Glob Chang Biol 8:531–544

Selgrade MJK, Lemanske JRF, Gilmour MI, Neas LM, Ward MDW, Henneberger PK et al (2006) Induction of asthma and the environment: what we know and need to know. Environ Health Perspect 114:615–619

Storms W, Meltzer EO, Nathan RA, Selner JC (1997) The economic impact of allergic rhinitis. J Allergy Clin Immunol 99:S820–S824

Straatsma G, Ayer F, Egli S (2001) Species richness, abundance, and phenology of fungal fruit bodies over 21 years in a Swiss forest plot. Mycol Res 105:515–523

Strachan DP, Ross Anderson H (1992) Trends in hospital admission rates for asthma in children. Brit Med J 304:819–820

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC et al (2002) Ecological responses to recent climate change. Nature 416: 389–395

Wearn JA, Sutton BC, Morley NJ, Gange AC (2012) Species and organ specificity of fungal endophytes in herbaceous grassland plants. J Ecol 100:1085–1092

Wolf J, O'Neill NR, Rogers CA, Muilenberg ML, Ziska LH (2010) Elevated atmospheric carbon dioxide concentrations amplify *Alternaria alternata* sporulation and total antigen production. Environ Health Perspect 118:1223–1228

Ziello C, Sparks TH, Estrella N, Belmonte J, Bergmann KC, Bucher E et al (2012) Changes to airborne pollen counts across Europe. PLoS One 7:e34076

Ziska LH, Epstein PR, Rogers CA (2008) Climate change, aerobiology, and public health in the Northeast United States. Mitig Adapt Strateg Glob Chang 13:607–613

Zukiewicz-Sobczak WA (2013) The role of fungi in allergic diseases. Adv Dermatol Allergol 30:42–45