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► To cite this version:

Denis Caillaud, Marion K. Keirsbulck, Charlotte Leger, Bénédicte Leynaert. Outdoor Mold and Respiratory Health: State of Science of Epidemiological Studies. *Journal of Allergy and Clinical Immunology: In Practice*, 2022, 10 (3), pp.768-784.e3. 10.1016/j.jaip.2021.09.042 . hal-03604071

HAL Id: hal-03604071

<https://hal.science/hal-03604071v1>

Submitted on 22 Jul 2024

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OUTDOOR MOULD AND RESPIRATORY HEALTH: State of Science of Epidemiological Studies.

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Funding: No

Word count: Abstract: 250; Text: 3667

Highlight box

1. What is already known about this topic?

There is increasing evidence that outdoor fungal spores increase the risk of severe asthma exacerbations in children. Outdoor fungal spores might also increase asthma symptoms occurrence, impact other health outcomes, and also affect adults.

2. What does this article add to our knowledge?

Increases in total outdoor fungal spores, Ascospores, Basidiospores, *Alternaria*, and *Cladosporium* are associated with increases in asthma exacerbations among children. *Coprinus*, *Ganoderma*, *Aspergillus/Penicillium*, *Botrytis* and *Epicoccum* might also be harmful. Evidence remains limited in adults.

3. How does this study impact current management guidelines?

Outdoor mould exposure should be investigated as a potential cause in patients with mild or severe asthma exacerbations. Recommendations to reduce spore exposure might be proposed using regional fungal calendars.

Key words: Asthma. Epidemiology. Mould. Outdoor. Rhinitis. Spores.

Abbreviations used

D: Day

ECRHS: European Community Respiratory Health Survey

EDV : Emergency department visit

NHANES: National Health and Nutrition Examination Survey

PF: Peak flow

ABSTRACT

BACKGROUND: Fungal spores are the predominant biological particulates in outdoor air. However, in contrast to pollens or outdoor air pollution, little is known about their respiratory health risks.

OBJECTIVES: The objectives were to conduct the first review of epidemiological studies on the short and long-term effects of outdoor mould exposure on respiratory health in children and adults.

METHODS: Health outcomes included asthma, lung function and rhinitis. Cross-sectional and longitudinal epidemiological studies using quantitative measures of outdoor mould exposure (optical microscopy, culture-based methods) were selected, providing that important confounding factors including temporal trends or meteorological factors were accounted for. A systematic literature search was performed up to June 2020, leading to the selection of 37 publications.

RESULTS: Most studies were longitudinal and investigated short-term effects. There is evidence of an association between outdoor fungal exposure and an increase in asthma exacerbation among children for total spores, two phyla (Ascomycetes, Basidiomycetes), and two taxa (*Cladosporium*, *Alternaria*). A few studies also suggested an association for *Coprinus*, *Ganoderma*, *Aspergillus-Penicillium*, *Botrytis* and *Epicoccum* in children, but this needs to be confirmed. Some studies reported mould associations with rhinitis, lung function, and among adults but these were few in number or inconsistent.

DISCUSSION: Further ecological studies in different regions that measure exposure to all taxa over several years are required to better understand their impact on rhinitis, asthma exacerbations and lung function. Larger panel studies are necessary to identify threshold effects in susceptible individuals. Finally, further research should assess the long-term effects of outdoor mould.

INTRODUCTION

Fungal spores are the predominant biological particulates in outdoor air, and frequently exceed pollen by 100 to 1000 fold (1). They are very common throughout the environment, and exposure occurs throughout most of the year. They are usually recorded as total spores, phyla, or specific taxa.

Total spore concentrations usually range from 50 spores/m³ during the cold season to more than 50,000 spores/m³ during summer. Fungi can be divided into two broad phyla, called Ascomycota and Basidiomycota, depending how their sexual spores are formed (2). In ascomycetes, the spores are produced within tubular cells, called asci. At maturity, asci usually contain eight ascospores. In basidiomycetes, the spores develop on projections formed outside microscopic cells, called basidia, commonly elongated and club-like shaped; there are generally four basidiospores produced on each basidium. Ascomycota are the most abundant microfungi in the environment and are estimated to contain 65,000 species. Basidiomycota are morphologically complex, and include mushrooms, puffballs, bracket fungi, rusts and smuts (2). In temperate climates, outdoor basidiospores comprise between 5 and 30% of total spores. *Cladosporium* is the most commonly identified outdoor fungus worldwide, and represents 50% to 90% of total spores in temperate regions (3). *Alternaria* is also frequently identified, because it occurs at almost all geographical latitude (4). Other specific taxa often identified on the basis of their morphology are *Aspergillus-Penicillium*, *Botrytis*, *Cercospora*, *Chaetomium*, *Coprinus*, *Didymella*, *Entomophthora*, *Epiccocum*, *Erysiphe*, *Fusarium*, *Fusicladium*, *Ganoderma*, *Helicomyces*, *Helminthosporium*, *Myxomyetes*, *Nigrospora*, *Peronospora*, *Pithomyces*, *Polythrincium*, *Sporobolomycetes*, *Stemphyllium*, *Tilletia*, *Torula*, *Uredospores* and *Ustilago* (5, 6).

In contrast to the large pollens mostly associated with rhinitis, most fungal spores are smaller (less than 10 µm), making them more likely to cause bronchial manifestations (1). However, compared to other outdoor exposure such as to pollens or air pollution, there is little public awareness or information (e.g. air-quality reports or alerts for high-risk days) regarding the health risks associated with outdoor mould (7, 8). As with indoor mould (9-11), outdoor mould can potentially affect respiratory health (12, 13) Some studies have reported significant correlations between the high amounts of fungal spores released during thunderstorms and asthma hospitalizations (14-17). Climate changes are likely to increase the duration and frequency of exposure to fungal spores (17).

To date, no comprehensive review has investigated the relationships between outdoor total spores, phyla, or exposure to specific taxa and asthma incidence, degrees of asthma exacerbation (whether mild, moderate or severe), lung function or rhinitis. This review systematically presents and evaluates the current literature on the relationships between outdoor fungi and respiratory health in children and adults.

METHODS

This study forms one section of a scientific assessment commissioned by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). Seven experts with complementary areas of expertise (epidemiologists, clinicians, toxicologists, microbiologists, mycologists), convened over 10 days from 2018-2020, with the report published in November 2020 (18).

A review of studies published in English up to June 2020, on the effect of outdoor mould on human health was performed (online supplement). The review was conducted based on the four steps from the PRISMA statement for systematic reviewing: identification, screening, eligibility, and inclusion (19, 20) (Figure 1). We selected those epidemiological studies that investigated the following respiratory health outcomes: respiratory symptoms, wheezing, asthma symptom score, exacerbations (EDV or hospitalizations for asthma) and development of asthma, anti-allergic or respiratory drug use or sales, lung function (especially peak flow [PF]), or rhinitis.

In contrast to studies on indoor mould which show significant variations in sampling methods (11, 13), the methods used for outdoor mould sampling varied little between studies. Many studies used an outdoor volumetric (Hirst or Burkard) sampler which is a compact unit containing a vacuum pump designed to sample airborne particles that are impacted on an adhesive coated plastic tape continuously for periods of up to seven days, or (in few studies) directly on to culture medium. Other studies, in Canada and the US, used a Rotorod sampler, a rotating-arm impactor that recovers airborne particles on two rapidly moving coated plastic collector rods. In most studies, samples were analyzed through morphological spore recognition allowing results to be expressed as concentrations (spores/m³) (21). Alternatively, a few studies combined the volumetric sampling method with the culture on media and expressed results in Colony Forming Units/m³.

Studies were assessed for adequate control of potential confounding variables such as meteorological factors and/or temporal trends and seasonal variations. Studies reporting simple statistical correlations (e.g. Spearman) without adjusting for potential confounding factors were excluded, as were studies initially intended to study another risk factor, in which mould exposure was insufficiently described.

RESULTS

Of 2796 publications (2770 peer-reviewed), 37 original epidemiological studies with relevant exposures and health outcomes and adequate control for confounders were included in this review.

We identified 4 major groups of studies. Three birth cohort studies investigated the effects of early exposure to outdoor mould on asthma occurrence (Table I). Fifteen ecological studies investigated the short-term associations between mould exposure and EDV, hospitalizations or drug sales for asthma exacerbations, as did two case-crossover studies (where the exposure of a subject at the time of admission is compared to the exposure of the same subject at a control period) (Table II). Twelve panel studies focused on selected groups of individuals with repeated measures of asthma-like symptoms, treatment use or lung function, and one cross-sectional study examined respiratory function (Table III). Finally, we reported separately five studies exploring associations with rhinitis (Table IV).

When available, we first considered studies that investigated all identifiable taxa. However, most studies focused on a few *a-priori selected* specific taxa, the three major phyla (Ascomycota, Basidiomycota and Deuteromycota (until 2006) (3), or total spores. The list of exposure variables considered in each study is shown in the 4th column of tables I to IV. Exposure variables that are not described in the results showed either no association with respiratory outcomes or inconsistent results within the one study. Study findings with regard to total spores and phyla are summarized in Table V.

1. Early-life exposure to outdoor mould and asthma onset among children.

Birth-cohort studies (Table I) showed that children born during periods of high ascospore and basidiospore concentrations in California (22) were at greater risk of wheezing by age 2, and children with high culturable levels of outdoor *Cladosporium* at the age of 3 months in Boston were at increased risk of ‘any wheeze’ by age one, (23) as compared to children with low exposure. However, within the Boston cohort, the only association reported at age 13 related to rhinitis (24).

2. Asthma exacerbations: ecological and case-crossover time-series

Several time-series analyses have investigated the short-term effects of outdoor mould but only five considered more than 10 taxa (Table II) . As regards specific taxa (online supplement), significant increases of asthma exacerbation surrogates (EDV, hospitalizations,

general practitioner consultations or short-acting β 2-agonist bronchodilator sales) were reported in at least one of these studies for *Agrocybe*, *Alternaria*, *Aspergillus*–*Penicillium*, *Botrytis*, *Chaetomium*, *Cerebella*, *Cladosporium*, *Coprinus*, *Didymella*, *Drechslera*, *Epicoccum*, *Ganoderma*, *Leptosphaeria*, *Periconia*, *Stemphylium* and *Sporormiella*. Negative associations were reported in one study for *Puccinia*, *Polythrincium* and *Stemphyllium*.

Other time-series studies (with many conducted in North-America) have examined pre-selected sets of specific taxa (Table II and online supplement). In particular, Dales and co-workers have run several time-series analyses in Canada, first in Ottawa and then considering ten cities (25-29). We identified two other studies in Canada (30, 31), one in the US (32), two in Europe (33, 34), and four in tropical locations (35-38). Positive associations were reported for *Alternaria*, *Aspergillus*/*Penicillium*, *Cladosporium*, and *Ganoderma*. However, null findings or even negative associations were also reported.

At the phyla level, the Trent-region study showed that EDV increased during days of high ascospore counts, in children and adults (41). The London study showed associations with Ascomycota and Basidiomycota among children (42). The 10-city study in Canada showed fairly consistent findings across the cities (27): in the total population, a daily increase in exposure equivalent to the mean value was associated with an increase of 3.3% in asthma hospitalizations for basidiomycetes, 3.1% for ascomycetes, and 3.2% for deuteromycetes. However, these associations varied with age and gender, with basidiomycetes and deuteromycetes showing stronger associations among younger males and older females, and ascomycetes showing associations only in females (28). Other studies revealed increases in EDV associated with ascospores for children (25, 35) and adults (30), and with basidiospores in children (25, 31, 35).

Total spores were associated with a significant increase in children EDV or asthma hospitalizations in 3 studies (25, 39, 40). Four studies in children and/or adults showed no association (32, 36, 41, 42), and one study revealed an association with insurance-claimed asthma outpatient-visits among the total population, but not with EDV (38).

3 Asthma disease activity in panel studies and studies on lung function.

Some studies have investigated the variations in disease activity indicators (symptom score, PF or treatment for asthma attack), airway responsiveness or lung function according to fungal exposure, in panels of individuals followed on a daily basis over a relatively short period of time (from 1 month to 1 year), or at several time points over a longer period. Some studies were conducted among asthmatic patients, some comprised only sensitized

individuals, and some were carried-out in population-based samples such as school-children (Table III).

We identified 10 panel studies in the US (43-46), Oceania (47-49), Taiwan (50, 51) and South-Africa (48) in which fungi were quantified after microscopic examination of morphology. Only one of these studies considered the entire set of identified taxa (51). Two additional studies used culture-based quantification (52, 53).

A significant increase in disease activity indicators was reported in at least one study for *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Coprinus*, *Curvularia*, *Drechslera*, *Epicoccum*, *Ganoderma*, *Penicillium*, *Periconia*, although a few taxa also showed negative associations (online supplement). Of the three panel studies reporting on ascospores (43, 44, 47), one showed a significant increase of symptoms in children (43). Three panel studies showed some association between basidiospores and symptoms in children or adults, but there was little or no evidence regarding PF or treatment (43, 44, 47). With respect to total spores, most studies showed an increase in symptoms, and some also reported an association with asthma treatment use or PF (43-45, 52).

Four of these longitudinal studies (41-51,54) and one additional cross-sectional study (55) investigated the associations between lung function and outdoor mould, and showed decreased FEV1 or FVC associated with higher levels of *Alternaria*, *Cladosporium*, *Coprinus*, *Drechslera* and *Ustilago/smut* or total spores (Table III and online supplement).

5. Outdoor mould and rhinitis.

Five studies investigating relationships between outdoor mould and rhinitis were identified (Table IV). Within the Boston birth cohort (24), *Cladosporium* and non-sporulating moulds at a child's home at age 3 months were associated with an increased risk of rhinitis onset by the age 13.

In Ottawa (56), ascomycetes, basidiomycetes and deuteromycetes were associated with EDV for rhinitis among children (increase significant only for basidiomycetes). In tropical Australia, Johnston, (57) reported no association between total spores and drug sales for allergic rhinitis treatment (obtained from pharmacies). Two European studies used drug sale information obtained from health insurance data. In Brussels, Guilbert (58) found an unexpected negative association between *Alternaria* and *Cladosporium* spores and sales of oral antihistamines. Finally, in central France, Caillaud (5) analysed daily sales of oral antihistamine prescribed together with a topical nasal or ocular anti-allergic treatment, for associations with all identifiable taxa (i.e. none selected *a priori*). *Cladosporium* and

Aspergillus-*Penicillium* were associated with a significant increase in sales of anti-allergic treatments. The association with *Cladosporium* was stronger in boys than in girls, and was significant in males aged 0 to 49 years.

DISCUSSION

This comprehensive review concerned the effect of outdoor mould exposure on human respiratory health, reflected in the following health outcomes: asthma onset, severe (EDV or hospitalizations) or mild (symptoms, respiratory drug use or sales) asthma exacerbations, lung function and rhinitis. Most studies investigated short-term effects. The various published studies show an association between outdoor mould and asthma exacerbations in children, with evidence for an association with total spores, ascospores, basidiospores, *Alternaria*, *Cladosporium*, and a probable but yet to be confirmed association with *Coprinus*, *Ganoderma*, *Aspergillus/Penicillium*, *Botrytis* and *Epicoccum*.

Our results confirm and extend the previous findings of the few reviews considering the health effects of outdoor mould. A 2014 review examining the relationships between outdoor spores and emergency department visits (EDV) or hospitalization for asthma in children (59) noted the small number of previous studies at that time, and inconsistencies among findings. However, it found increasing evidence that outdoor fungal spores may increase susceptibility to severe asthma exacerbations, but there was limited understanding of the contributions of specific taxa. In the 2015 update to the 2000 review by the Institute of Medicine (60), it was concluded that there was a sufficient evidence of a causal association between outdoor culturable fungal exposure and exacerbation in asthmatics sensitized to fungi. Another review in the 2016 Mould Theme issue of the Journal (13) further underlined the possible increase in asthma morbidity related to high concentrations of outdoor fungal spores in sensitized individuals whereas un-sensitized individuals appeared to be relatively unaffected. Fungal exposure might however be associated with less severe asthma exacerbations and other respiratory outcomes. For example, a recent study identified a cluster of patients with chronic obstructive pulmonary disease highly sensitized to outdoor air fungi, with frequent exacerbations and poor lung function (61). In addition to severe asthma exacerbations, the present review encompasses the effects of outdoor mould exposure on disease activity, development of wheeze in children, lung function and rhinitis. In contrast to indoor mould (11), the inconsistency among results and the small number of studies to date prevent the drawing of conclusions about associations between outdoor mould and asthma development, lung function or rhinitis. In addition, no threshold for triggering health effects could be determined. Noteworthy, our search identified several studies, particularly ecological studies, showing evidence for an overall detrimental effect of outdoor mould on asthma

morbidity despite the sensitization status was not considered. Our results highlight the possible effects of total spores, two main phyla, and a few individual spores on asthma exacerbation, but only in children.

Most panel studies have been conducted on children, and of the five ecological studies reporting stratified analyses of the entire population (28, 35, 41, 42, 62), all but one (28) found associations among children, whereas most found no association in adults. These results are consistent with our findings on the effect of indoor moulds on asthma exacerbations (11).

Eight studies showed associations with total spores (Table V): three concern hospitalizations (25, 39, 40) and five concern asthma symptoms, PF and/or treatment use (43, 44, 48, 52, 53). Seven studies exploring EDV, hospitalizations or asthma symptoms revealed associations with ascospores (25, 28, 30, 35, 41-43) and basidiospores (25, 28, 31, 35, 42-44). Finally, studies examining exposure to individual taxa showed associations in 9 studies for *Alternaria* (25, 30, 37, 39, 44, 49, 52, 54, 62), and 8 studies for *Cladosporium* (25, 31, 40, 44, 46, 48, 52, 54). Four studies reported associations with *Coprinus* (39, 40, 44, 46) and *Ganoderma* (25, 31, 40, 46), 3 studies with *Aspergillus-Penicillium* (25, 52, 62), *Botrytis* (41, 42, 44) and *Epicoccum* (42, 45, 46) with no studies finding opposite effects for these taxa.

An early study (63) conducted in Chicago showed asthma mortality to be higher when total spores exceeded 1000 spores/m³. Total spore were associated with increases in asthma EDV among children in approximately half the ecological studies.

Sensitization to basidiomycetes in Europe and the US varies from 4% to 25% in outpatients (64, 65), and their clinical relevance has been demonstrated through provocation tests (66, 67).

Ascomycota comprise some 65,000 species (2) including the genera *Chaetomium* and *Didymella*. *Chaetomium* sensitization rate was 7% among outpatients in West Virginia and 21% among inpatients with asthma in Poland (68, 69). Damp spores, such as *Didymella*, have been documented at unusually high levels during thunderstorm asthma (16).

Cladosporium very high species diversity helps to explain its year-long presence (70). In Europe, sensitization of 1.7% was recorded in an ECRHS population-based study (71) and 4.9% among outpatients (72). Its presence is usually considered to be associated with more severe asthma (73, 74).

Alternaria is frequently found in outdoor air but in concentrations not exceeding 10% of total spores. Sensitization of 3.3% was recorded in Europe in the ECRHS study (71), 8.6% in the USA from NHANES data (75) and 8.9% in outpatients (72). Sensitization is associated

with severe asthma across the general population (74) and seasonal asthma exacerbations (76).

Coprinus is a common basidiospore, accounting for 3% of total spores in Seville (77) and up to 14.6% in Melbourne (78), and is a known allergic trigger (79). In outpatients, sensitization to *Coprinus* in Europe and US varies between 2% and 5% (65). Positive bronchial provocation tests (67) have demonstrated potential clinical relevance to asthma.

Ganoderma is commonly known as wood decay basidiospore (65). Maximum spore release occurs during the late summer and autumn. Among outpatients, sensitization is around 10% (64), with *Ganoderma* inhalation inducing immediate bronchospasms in sensitized patients (67).

Aspergillus and *Penicillium* are closely related species. As their spores are indistinguishable under an optical microscope, they are reported as *Aspergillus-Penicillium*. Present in the outdoor air all year round with an autumnal increase (3), they represent less than 10% of total spore numbers. *Aspergillus* sensitization among the general population was found to be 6.4% using NAHNES data (75) and 4.4% among European outpatients (72). It is classically associated with severe asthma (80) and with allergic broncho-pulmonary aspergillosis (81).

Botrytis is a hydrophilic fungus found mainly in humid regions (82). Although present in the outdoor air most of the year at low concentrations, the median sensitization rate was found to be high (18.8%) in Danish patients workers (83).

Epicoccum is a dry spore, and in Italy it peaks in September (84). Despite relatively low outdoor concentrations, high sensitization rates have been observed, particularly in patients admitted in ICU for acute asthma attack (85).

It is important to identify the strengths, limitations and potential bias among the reviewed studies.

Most ecological and panel studies relied on a single spore trap, allowing daily collection over a long period. Subjects lived within close proximity of traps, which are considered to represent spore concentrations within a radius of at least 20 km (42).

Traditionally, airborne fungal studies were based on culture-dependent methods but this technique is biased towards fungi that can be cultured on generic fungal growth media (86) and continuous sampling is impossible. Most of the studies we have identified used volumetric collectors, the same as for pollen collection (Hirst-type spore traps) (87) with morphological identification of spores. The advantage of this method is that it can give

continuous results, the limitation is the time and expertise required to identify the spores. A number of taxa found under the optical microscope do not grow in culture, such as *Coprinus* and *Ganoderma*. Therefore, studies using culture-based methods may show different results. Within the past 20 years, molecular methods such as polymerase chain reaction (PCR), quantitative PCR (qPCR) and Next-generation sequencing (NGS) have greatly increased our understanding of outdoor bioaerosol diversity. Few studies have compared the level of airborne fungal diversity detectable by the molecular approach to results generated using morphological recognition (88). High-throughput DNA sequencing applied to aerobiological samples obtained with Hirst spore traps provide a more comprehensive determination of the taxa (89, 90) than morphological analyses, which recognize about 40 fungal categories, only some of which can be classified to the genus (91). However, as some taxa are identified only by one of the two techniques, both approaches are still to be implemented to capture airborne fungal diversity at the regional scale (92, 93).

Most studies have focused on a few specific taxa for ease of identification, or selected *a priori* for their previously recorded effects on asthma exacerbations. Overall, only six studies (39-42, 51, 62) have analysed the relationships to asthma exacerbations for all identifiable outdoor mould taxa (i.e. without *a priori* assumptions regarding their possible pathogenic effect). Usually between 10 and 20 taxa were found in sufficient quantity to be analysed, after measurement of sufficient duration.

In many studies performed in temperate climates, the measurement periods were limited from spring to early autumn, because the same sensor is used for both moulds and pollens (which are absent in winter). However, even if winter spore concentrations are lower, winter spores might have adverse respiratory health effects. Ideally, spore measurement should be performed over several years (at least 3 years) to overcome variations in mould concentrations related to interannual weather variations.

Outdoor mould concentrations vary by country (e.g. temperate vs tropical) (94), seasons and regions (1). In some tropical countries, Basidiomycetes are the predominant phyla in the atmosphere (95) with spore assemblages varying between dry and wet seasons (96). In temperate countries, *Cladosporium* predominates, with spore concentrations peaking during summer. Even within the same country, concentrations vary across regions. In the UK, *Ganoderma* spore concentrations are much lower in dense cities such as London (50/m³) (97) than in less dense towns, as in Cardiff (around 400/m³), correlating with the number of trees in the vicinity of the spore trap (98). *Alternaria* concentrations are higher in Derby, an area of high cereal production, than in Cardiff, (99). It is therefore important that future studies

encompass geographical areas with varied climates and among different regions with diverse surrounding vegetation.

The number of events should also be large enough to ensure sufficient statistical power. Four ecological studies showing no association between total spores and health effects involved study periods of less than three years (32, 36, 38, 42). The average number of daily admissions for respiratory disorders also appears somewhat low (2.2/day) in the negative study by Hanigan (36). By comparison, in two positive studies, Dales recorded 7.5 EDV/day (25) while Caillaud recorded 20 asthma treatment sales/day (62).

Ecological studies reporting simple statistical correlations (e.g. Spearman) were excluded. Among the studies included in this review, some failed to consider all of the known potential confounding factors such as air pollutants, meteorological factors or viral infections. Few studies have adjusted for pollens. Simultaneous pollen and mould exposure may lead to false positive associations with moulds, when pollen is the actual driver. Pollen exposure preceding exposure to moulds may mask or even reverse the association between mould and health indicators. For example, in the study by Guilbert (58), daily sales of rhinitis treatments were strongly associated with *Poaceae*, which pollinates just before the fungi period. Therefore, patients sensitive to fungi might already have been under treatment at the time of the fungal spore peak, thus explaining the unexpected findings in this study. Exposure assessment may also be impacted by the absence of data on indoor mould exposure (11) although, in contrast to outdoor mould, it is unlikely to change on a daily basis. Very few studies have simultaneously measured indoor and outdoor fungal exposure. In the Inner-City Asthma Study in the US, both indoor and outdoor exposure were assessed. Indoor fungal exposure more strongly increased severe exacerbations (unscheduled emergency department visits) whereas outdoor fungal exposure was more strongly related to symptom impairment. Noteworthy, all significant associations between indoor fungi and asthma symptoms became not significant after adjustment for outdoor fungal exposure, except for indoor *Penicillium* (52). In the prospective Boston birth cohort study, outdoor *Cladosporium* was associated with an increased risk of wheeze by age 1 after adjustment for indoor dust and air exposure (23).

Testing for different lag responses is important as the effect may be delayed. In the case of asthma EDV after exposure to Deuteromycetes and *Cladosporium*, significant increased risk has been observed starting at Day 3 (D) and diminishing by D6 (31). In a panel study on respiratory function, a significant deficit was reported for *Cladosporium* at D0 and D5, suggesting the possibility of a dual reaction to this mould (54). For drug sales, the lag might extend up to 10 days due to the need to obtain a prescription (5).

Temporal studies have further inherent limitations. Ecological studies estimate the association between short-term changes in mould levels and health events at the population level, such as hospitalization or drug sales for severe or mild asthma. In contrast, panel studies relate mould concentrations to symptom severity at an individual level, e.g. individuals with mild asthma or sensitized to fungi monitored over time. For practical reasons, panel studies are usually performed on a limited number of individuals for a shorter period. Further studies including a sufficient number of patients are required to establish possible threshold effects, as in the case of pollen (100). Further birth-cohort studies could highlight the long-term effect of outdoor mould on asthma, as their design allows the examination of temporality by assessing exposure before the onset of the disease (11).

The lack of knowledge about mould sensitization (particularly in the ecological studies) should be acknowledged. However, mould sensitization in the general population is low across Europe (71, 101) and the USA (75). In addition, both allergic and irritative mechanisms are likely to be involved in the relationships between mould exposure and respiratory health (102, 103). A few studies have compared the effects of outdoor mould exposure according to the sensitization status and generally showed stronger effects in participants sensitized to fungi (39, 48, 49, 52, 55). However, for outdoor *Alternaria* and a few other taxa, similarly adverse respiratory effects were observed among participants sensitized to other allergens but not to the specific taxa and among those sensitized (39, 49, 52, 55). Although cross-reactivity might partly explain these findings (13, 52), it has been suggested that both fungal exposure and fungal sensitization might have independent effects (52).

We cannot discount the possibility that some studies showing no association have not been published (publication bias). However, several studies included in this review that considered multiple mould-exposure variables reported no association in many cases (see the number of exposures considered vs. those showing significant results respectively in Tables column 4 and 6). Furthermore, observing no association does not mean that no association exists. If some specific taxa do have a causal effect, one must still measure the right taxa at the right time to catch this effect. Due to differences in study designs, age distributions, and types and levels of exposure, the findings of the various studies could not be meta-analysed, and we did not derive any combined estimates.

Strong recommendation is made by GINA to remediate indoor dampness or mold in order to decrease asthma symptoms and medication use (104). Besides indoor home exposure, school-based environmental interventions have been developed to reduce exposure

of children when they are outside the home, as shown for example in Online supplementary Table (105-109). In contrast, in the current state of knowledge, only panel consensus judgment recommend to reduce exposure to outdoor mold, when outdoor spore are highest, by closing windows and doors, remaining indoors, and using air conditioning (104).

The findings of increased asthma exacerbation risk associated with outdoor fungal exposure in a range of different large representative datasets from around the world supports the existence of an association. Thus, practitioners should consider outdoor mould exposure as a potential cause of asthma exacerbations. Developing regional fungal calendar and providing recommendations to reduce spore exposure as it is currently done for pollens, should benefit patients with fungi-related asthma exacerbations (87).

Further work should be done to make solid, evidence based recommendations. Temporal ecological epidemiological studies should be conducted in large cities, year-round, for at least 3 years to account for possible annual variations in mold sporulation. It is necessary to measure total spores, main phyla, and any individual spores present over a sufficient period (at least 2 months/year) to have sufficient statistical power (between 10 and 20 individual spores in temperate countries), and to take into account confounding factors (air pollution, meteorology, vacations, weekends, influenza episodes). In case of discovery of molds not yet known to be pathogenic, mold allergens should be searched, immediate reactions sought (skin test, specific IgE) and provocation tests could be proposed (110). Panel studies with a sufficient number of subjects (about 100), followed daily for a year, including questionnaires and digital validated health tools (111), may highlight the dose-response relationship (linear or not). Further birth cohort studies should assess the long-term effects of outdoor mould. However, even these types of studies remain observational, as it is not possible to randomize children at birth to varying mold exposures and monitor their health throughout life (13). Molecular analysis in conjunction with morphological analysis should ideally be performed in all cases. Finally, high quality study, such as randomized control trials should be encouraged to appraise the effect of reducing exposure to outdoor mold on respiratory health in susceptible populations.

In conclusion, relatively few studies exploring associations between ambient air mould and health effects exist in the literature. The majority of the studies identified here document short-term effects on respiratory health. Firm conclusions can only be made regarding asthma exacerbation among children, with evidence for an association with total spores, ascospores, basidiospores, *Alternaria*, *Cladosporium*, and a probable but to be confirmed association with *Coprinus*, *Ganoderma*, *Aspergillus/Penicillium*, *Botrytis* and

Epicoccum. Further ecological studies should assess the effects of all taxa measured throughout the year, over several years and in different countries, on the respiratory health of large populations. Similarly, larger panel studies performed throughout the entire year are necessary to identify the threshold effects of specific taxa. Finally, further birth cohort studies should assess the long-term effects of outdoor mould.

Acknowledgments: The members of the ANSES expert assessment “État des connaissances relatif à l’impact sanitaire de l’exposition aux moisissures présentes dans l’air ambiant sur la population générale française et recommandations en matière de surveillance nationale” are as follows: S Achard (Paris University, France), V Bex (SPSE, Paris, France), D Caillaud (CHU Clermont-Ferrand, France), J Dupont (Museum National d’Histoire Naturelle, Sorbonne Universités, Paris, France), E Frealle (CHU Lille, France), B Leynaert (INSERM, Paris, France), I Thaon (CHU Nancy, France), M Keirsbulck (ANSES) and C Leger (ANSES)

Authors’ contribution: D Caillaud and B Leynaert were members, and M Keirsbulck and C Leger coordinated the ANSES expert assessment. CL and MK conducted the literature search and article extraction from SCOPUS and Pubmed and screening based on titles. All authors were involved in the selection based on abstract screening, and BL and DC read the full texts, examining the selection and description of the study sample, exposure and outcome assessment, appropriateness of statistical methods and adjustment for confounders. Any disagreements were discussed, together with CL and MK, until a consensus was found. All authors wrote the paper, participated in the revision of the manuscript and gave approval of the final version

Conflict of interest: None declared.

Table I. Exposure to outdoor mould and asthma occurrence: birth cohorts.

Author, Location	Time period Sample size	Tr	Fungal Exposure	Outcome definition	Results	Statistical method Confounding factors
Harley, 09 USA, California (22)	n=514 Oct 99-July 03	B	TS Asc, Bas <i>Cl</i> , <i>Asp-Pen</i> , , in the first 3 months of life	Wheeze by age 2	aOR/IQR Asc: 2.8 (1.3- 5.9) Bas: 2.1 (1-4.4)	Logistic regression adjusted for AP (PM _{2.5} , PM 10), respiratory infections, indoor moulds, humidity, cockroaches, rodents. No adjustment for pollens, but for different seasons
Behbod, 13 Boston At risk cohort (23)	n=499	B	Alt, <i>Cl</i> , <i>Asp</i> , <i>Pen</i> CFU/m ³ at 3 months	Any wheeze by age 1	aOR/IQR <i>Cl</i> : 1.68 (1.04– 2.72)	Cox model adjusted for 4 taxa (Alt, <i>Cl</i> , <i>Asp</i> , <i>Pen</i>), yeasts, respiratory infections <1 yr, birth weight, maternal smoking, asthma and atopy, gender, ethnicity, income, childcare arrangements, type of building, indoor endotoxin or allergen levels (cockroach and dust mite).
Behbod, 15 Boston At risk cohort (24)	n=408	B	Alt, <i>Cl</i> , <i>Asp</i> , <i>Pen</i> CFU/m ³ at 3 months	Asthma onset by age 13	NS	Cox model (as above)

Alt: *Alternaria*; *Asc*: Ascomycetes; *Asp*: *Aspergillus*; *Asp-Pen*: *Aspergillus-Penicillium*; B: Burkard; Bas: Basidiomycetes; *Cl*: *Cladosporium*; *Pen*: *Penicillium*; Tr: Trap; TS: Total Spores.

aOR: adjusted Odds-Ratio; IQR: Interquartile Range, NS: Not significant

Table II. Outdoor mould and exacerbation of asthma: ecological and case-crossover time-series analyses.

Author Location*	Time period Sample size Age group	Tr	Fungal Exposure	Outcome Daily attendances	Results	Statistical methods Confounding factors
Newson, 00 Trent, UK (41)	July 87-Feb 94 Whole pop 0-14; 15-64 yrs	MB 87/ 91 B 91/ 94	TS Asc, Bas 25 Taxa	H Daily mean 10.7/d 0-14 yrs 10.6/d > 14 yrs	TS: NS 0-14 yrs: some associations Asc. Some bas hyaline <i>Botrytis</i> <i>Didymella</i> <i>Leptosphaeria</i> 15-64 yrs: Asc	Ecological study Linear autoregressive model taking into account weekly and long-term trends. Not adjusted for AP and pollens
Atkinson, 06 London, UK (42)	June-Oct 92-93 Whole pop 0-14; 15-64 yrs	B	TS Asc, Bas,Deu 30 Taxa	(0-14 yrs] GP visit:median13/d EDV : Median 14/d H Median 18/d	RR vs. 1st quartile (Q1) 0-14 yrs : Asc: EDV:Q4: 1.15 (1.02-1.29) Bas: EDV: Q3 1.14 (1.02-1.27) H: Q3 1.16 (1.04-1.28) <i>Agroclybe, Botrytis, Epi</i> : some associations Adults : NS	Ecological study GAM adjusted for AP (PM10, NO2, SO2, O3), weather (T, RH), pollens, day of week, respiratory epidemics.
Caillaud, 19 Central France (62)	3 yrs (Apr-Oct] Whole pop 6-12; 13-39 yrs	B	25 taxa	SABA sales Mean: 20/d	RR [IQR] Whole pop: Alt: 1.06 [1.00- 1.12] Boys 6-12 yrs Alt:1.21 1.08 [1.04-1.12] Asp-Pen: 1.08 [1.04-1.41]	Ecological study GAM adjusted for AP (PM10, NO2, O3), weather (RH, Tmax, wind speed), total pollens, temporal trends, influenza, thunderstorm.
Tham, 17 Melbourne (Case Cross-over) (39)	Sept 09-Dec 11 Children 2-17 years n=644	B	TS 12 taxa	H	aOR per unit increase from 75- 90 Pct; [95%CI] TS: 1.05 [1.01-1.09] Alt:1.07 [1.03-1.11] <i>Coprinus</i> 1.04 [1.01-1.02] <i>Drechslera</i> 1.03 [1.00-1.05] <i>Leptosphaeria</i> 1.05 [1.02-1.07] In children Alt or Cla sensitized, associations with <i>Stemphylium and Sporormiella</i>	Case-crossover study Conditional Logistic regression adjusted for AP (PM _{2.5} , PM10, NO2, O3), weather (RH, Tmax), tree, grass and weed pollens, rhinovirus, Alt and Cla sensitization.
Tham, 17 Sydney (Case Cross-over) (40)	May 08-Dec 13 Children: 2-13; 14-18 yrs n=2098	B	TS 21 taxa	H	aOR per unit increase from 75- 90 Pct; [95%CI] TS: 1.02 [1.00-1.03] <i>Cerebella</i> : 1.01 [1.00-1.01] <i>Chaetomium</i> :1.08[1.00-1.16] <i>Coprinus</i> 1.01 [1.01-1.06] <i>Ganoderma</i> : 1.02 [1.02-1.04] <i>Periconia</i> 1.03 [1.001-1.07] Girls: -Positive associations for TS, Cla, <i>Coprinus</i> , <i>Chaetomium</i> , <i>Ustil</i> -Negative associations for <i>Puccinia</i> , <i>Polythrincium</i> , <i>Stemphylium</i>	Case-crossover study Conditional Logistic regression adjusted for AP (PM _{2.5} , PM10, NO2, O3), weather (RH, Tmax), grass pollens.
Dales, 2000 Ottawa (25)	Mar-Oct 93-97 Children 0-14 yr	R	TS Asc, Bas,Deu Alt, Asp- Pen, Cla, Epi, Gan	EDV Mean: 7.5/d	% increase : mean (SE) TS: 1,8 (0,8) Asc: 2,9% (1) Bas 3,8 (1,7) Deu: 1,8 (0,8) Alt: 1,7 (1); Cla: 1,6 (0,7) Asp/Pen: 1,8 (0,9) <i>Ganoderma</i> : 3,5 (1,5)	Ecological study GAM, adjusted for AP (O3, NO2, SO2), weather (T, RH, bar pressure), temporal trends, days of the week, season.
Dales, 04 10 Cities Canada, (27)	Mar-Oct 93-00 Whole pop	R	TS Asc, Bas,Deu	H n=60.066 in 108 hospitals in 10 cities	% increase : mean (95%CI) Asc: 3.1 (2.8-5.7) Bas: 3.3% (2.3-4.1) Deu: 3.2 (1.6-4.8)	Ecological study GAM, adjusted for AP (O3, NO2, SO2), weather (T, RH, bar pressure), temporal trends, days of the week, season.
Cakmak, 05 10 Cities, Canada (28)	Mar-Oct 93-00 Whole pop Stratified by age and gender	R	Asc, Bas,Deu	H n=356,605	% increase : mean (95%CI) Males <13 Bas: 9,3 (4,8-13,8) Deutero: 5,2 (0,2-10,2) Female <13 : Asc: 4,3 (0,3-	Ecological study GAM, adjusted for AP (O3, NO2, SO2, PM10), weather (T, RH, bar pressure), temporal

					8.3) Female > 13 Asc: 3.0 (2.0-5.8) Bas: 7.1 (4.1-10.1)	trends, days of the week, season.
Stieb, 2000 Saint-John, Canada (30)	1992-96 Adults	R	Asc, Bas,Deu <i>Alt, Cla, Gan, Epi</i>	Asthma: 3.5 EDV/d COPD: 1.3/d	% increase : mean (95%CI) Asc: +4, 5 (1.8-7.4) <i>Alt</i> : +4.7 (1.0-8.6) <i>Gan</i> -4.7 (-7.6 to -1.8) <i>Epi</i> -5.2 (-10.1 to -0.1)	Ecological study GLM adjusted for AP (O3, NO2, SO2, PM10, PM2.5), weather (T, RH, precipitation, wind), days of the week.
Raphoz, 10 Montreal (31)	94-04: Apr-Oct Children 0-9yrs	R	Bas, Deu <i>Cla, Gan</i>	EDV 1 st visit: 9.67/d Readmission: 8.93/d	Associations 1 st admission: Bas, Deu, <i>Cla</i> . <i>Gan</i> 2d admission: Bas	Ecological study GLM adjusted for AP (O3, NO2), weather (T, P) and temporal trends
Lierl, 03 Cincinnati,US A (32)	96-97; Apr-Oct Children	R	TS	9<EDV<17/d	NS	Ecological study Generalized autoregressive model adjusted for AP (O3, PM10) and total pollens.
Lewis, 2000 Derbyshire, UK (33)	93-96; Jan-Dec >14 yrs	B	Bas <i>Alt, Cla, Didymella</i>	EDV + H: Mean: Derby Royal: 1.24/d Derby City: 0.7/d	RR :Top tertile vs bottom tertile H for asthma <i>Cla</i> : 2.1 (1.4-2.3)	Ecological study Linear autoregressive model, taking into account AP (NO2, O3, BS), weather (T, RH, rainfall, wind), temporal trend, influenza. Identical results after pollen adjustment.
Guilbert, 18 Brussels(34)	08-13; Jan-Nov Whole pop	B	<i>Alt, Cla</i>	H 2,3 H/d	<i>Alt, Cla</i> NS	Ecological study GLM adjusted for AP (PM10, NO2, O3, SO2), weather (T, RH), holidays, day of the week, influenza, 10 pollens.
Rosas, 98 Mexico (subtropical) (35)	1991 Whole pop <15,15-59,59 yrs	B	Asc, Bas, Deu	HEDV < 15 n=903 15- 59 n=1098 >59 n=238	Associations: Fungal spores associated with child admissions for both wet (Asc) and dry season (Bas, Deu)	Ecological study GLM adjusted for AP (NO2, O3, SO2; PM10), weather (T, RH), seasonal variations, tree and grass pollens.
Hanigan, 07 Darwin (tropical) Australia (36)	04-05: Apr-Nov Adults	B	TS <i>Alt</i>	H for asthma, COPD, resp infections Mean 2,2/d	TS: NS <i>Alt</i> NS	Ecological study GLM adjusted for AP (PM10), weather (T, RH, rainfall), temporal trends, respiratory viral infections, holidays. Pollens measured.
Chakraborty, 14 Kolkata (subtropical) (37)	2010 Children	B	<i>Alt</i>	H Mean: 7.42/d	<i>Alt</i> : significant increase	Ecological study GAM (non-parametric) adjusted for AP (O3, PM10), weather (T, RH, rainfall, wind speed). Pollens not measured.
Lewis, 20 Metro region Puerto Rico (tropical) (38)	2013 Whole pop	B	TS	Outpatient visit (n= 66,671) EDV (n= 2,963)	RR per 1,000 unit increase in TS Outpatient visits : RR= 1.0028 (1.0002–1.0053) EDV RR=1.0012 (0.9991–1.0034)	Ecological study Poisson regression models with cubic splines for time, adjusted for T, RH,, calendar quarters, and holidays

AH: AntiHistaminics; *Alt*: *Alternaria*; Asc: Ascospores; Av: Average; B: Burkard; Bar: barometric; Bas: Basidiospores; BS: Black Smoke; *Cla*: *Cladosporium*; COPD: Chronic Obstructive Pulmonary Disease; d: day; Deu: Deuteromycetes; EDV: Emergency Department visits. *Epi*: *Epicoccum*; GAM: Generalized Additive Model; *Gan*: *Ganoderma*; GLM: Generalized linear models; GP: General Practitioner; H: Hospitalizations; MB: Morrow-Brown; P: Pressure; pop: population; R: Rotorod; Resp: Respiratory; RH: Relative Humidity; SE: Standard Error; T: Temperature; Tr: Trap; TS: Total Spores; *Ustil*: *Ustilago*; Yrs: years.

* The table first presents studies that have investigated associations with all identified taxa (>10 taxa), ie. with no a priori focus on specific taxa, then studies are presented according to geographical area/climate, (North-America, then Europe, then other climate).

Table III. Outdoor mould and respiratory outcomes: panel and cross-sectional studies

Author Location	Time period Age group Sample size	Tr	Fungal Exposure	Outcome definition	Results	Statistical method Confounding factors
Panel studies using morphological method to assess outdoor fungi						
Delfino 96 San Diego (43)	Sept-Oct 93 Children 9-16 yrs n=12 asthmatic	B	TS Asc, Bas	Daily symptoms (0-5) Inhaler use (puffs)	TS: 1000/m ³ associated with -Symptoms +0.1-0.3 -Inhaler use.+1-0.4 puff Asc, Bas associated with symptoms	GLMM adjusted for outdoor and personal O ₃ , PM ₁₀ , total pollens, day of week, respiratory infections. Confounding by Max T, H tested.
Delfino 97 Semi-rural South Ca (44)	9 May-3 Jul 94 9-46 yrs n=22 asthmatic	B	TS Asc, Bas <i>Asp-Pen, Alt, Cla Botrytis, Coprinus Drechslera, Periconia</i>	Daily symptoms (0-5) PF Mo, Ev (L/min) Inhaler use (puffs)	TS Per min-90 th percentiles (4000 spores/m ³) associated with -Symptoms +0.36 (0.16-0.56) -Ev PF: - 12.1 (-1.8-22.3) -Inhaler use+0.33 (0.02-0.69) Bas with symptoms <i>Alt, Cla, Botrytis, Coprinus, Drechslera, Periconia</i> with symptoms. <i>Cla, Dreschslera</i> with inhaler use	GLMM adjusted for outdoor and personal O ₃ , PM ₁₀ , total pollens, day of week, respiratory infections. Confounding by Max T, H tested.
Ross 02 East Moline Ill, USA (35)	24 May-25 Oct 94 5-49 yrs n=40 asthmatic	R	TS <i>Alt, Cla, Curvularia, Drechslera, Epicoccum</i>	Daily symptoms (0-3) PF Mo, Ev (L/min) Inhaler use (puffs)	<i>Curvularia</i> associated with -Mo PF: -2.23 (-0.26/-4.21) - Symptoms - Inhaler use <i>Epicoccum</i> with inhaler use	Multivariate linear regression model Sensitivity analyses adjusted for weather (max T, RH), O ₃ , but not for tobacco use or ETS
Neas 96 State College Pennsylvania, USA (46)	29 Jun-20 Aug 91 108 non-asthmatic schoolchildren Symptomatic: n=62 Asymptomatic: n=46	B	<i>Alt, Cla, Coprinus Drechslera, Epicoccum, Ganoderma, Pithomyces, Stemphylium, Xylaria</i> Other colored Bas Other colored Asc	Daily symptoms PF Mo, Ev (L/min)	- <i>Cla, Coprinus</i> and <i>Epicoccum</i> associated with Mo PF decrease - <i>Epicoccum</i> associated with morning cough incidence OR (95% CI) 1.8 (1-3.2)	Autoregressive linear regression model for PF or logistic regression model for symptoms, including linear trend, morning and evening period, weather (T, rain fall) and AP (S ₀₂ , O ₃ , PM ₁₀). Child-specific estimates using the same regression models.
Epton 97 Epstein, rural N Zealand (47)	Jul 92-Jun 93 adults n=139 asthmatic	B	Asc, Bas	Daily symptoms Nocturnal awakening Inhaler use PF Mo and Ev (L/min)	Bas associated with -Nocturnal awakening -Inhaler use	Random coefficients linear regression models adjusting for auto-correlation for PF. Random effect model for symptoms score, nocturnal awakening, inhaler use accounting to age, atopy, smoking, weather, temporal trends. AP not taken into account, but rural region.
Rutherford, 2000 Brisbane, Ipswich Australia Tropical (48)	Jun 94-Aug 95 Children and adults asthmatics. Brisbane: n=25 Ipswich n=28 Sensitized or not to pollens and molds,	B	TS <i>Cla, Alt, Epicoccum, Ustilago</i> , Other spores	PF: Mo (L/min)	-In not sensitized: NS - In Sensitized: TS, <i>Cla</i> and <i>Alt</i> and other spores associated with PF decrease in Brisbane, in wet summer in adults, especially > 54 yrs	Mixed model with auto-correlated errors taking into account AP, weather, total pollens, temporal trends, sex, age, inhaler use, and preventive medications
Downs 01 South-East Australia Hot dry inland (49)	Aug 97-Jun 99 (5 occasions) n=399 aeroallergens sensitized schoolchildren	B	<i>Alt</i>	Last month -Symptoms -wheeze -Lung function -AHR to histamine -Inhaler use	<i>Alt</i> associated with -Wheeze -AHR (stronger association in Alt-sensitized children) - Inhaler use	Generalized Estimating Equation (GEE). Association tested by stratifying according to <i>Alternaria</i> sensitization. Weather tested as a confounder
Chen 11 Panel Taiwan (50)	Oct 07-June 08 3 elementary, 2 middle schools n=33 current asthma n=30 current AR n=37 Controls	B	TS	Lung function : 9 times	TS associated negatively with FEV ₁ and FVC	Mixed effect model with repeated measurements adjusted for AP (PM ₁₀ , PM _{2.5} , S ₀₂ , NO ₂ , O ₃), weather (T, RH at school), indoor moulds or water damage.

Chen 14 Panel Taiwan subtropical (51)	Oct 07-Nov 09 3 elementary, 2 middle schools n=100 (33 current asthma, 33 current AR, 34 Controls)	B	TS, Asc, Bas, Deu 22 taxa	Lung function 5-10 times	<i>Cl</i> a associated negatively with FEV ₁ and FVC (threshold 1500 spores/m ³) Effect observed independently of other spores	Mixed effect model with repeated measurements GAM adjusted for AP (PM ₁₀ , PM _{2.5} , S0 ₂ , NO ₂ , O ₃), weather (T, RH at school), indoor moulds or water damage.
Olaniyan 20 Panel South Africa (54)	n= 313 schoolchildren in informal settlements of the Western Cape of South Africa 2 consecutive school- weeks in summer and winter 2016	B	<i>Alt</i> <i>Clado</i>	FEV1 and PF x 3/day per increase of 10 spores/m ³ of <i>Alt</i> and 1-log spores/m ³ of <i>Cl</i> a.	Effect at lag showing maximum deficit PF (L/min) <i>Alt</i> - summer Lag 5 -1.96 <i>Alt</i> - winter Lag 2 -13.78 <i>Cl</i> a - summer lag 5 -4.51 <i>Cl</i> a - winter lag 5 -8.74 FEV1 (ml) <i>Alt</i> : no consistent <i>Cl</i> a: inverted U-shape Summer: lag: 1 - 62; Lag 5 - 47 Winter: lag 1: -28; Lag 5: -2	Mixed effect model, with child as random intercept, adjusted for several factors including meteorology (T, H and wind speed), pollens and PM ₁₀ , and adjusting for co-exposure to other fungal taxa

Panel studies using culture-based methods to assess outdoor fungi (CFU/m³)

Author, Location	time period AAge group Sample size	Trap	Fungal Exposure	Outcome definition	Results	Statistical method Confounding factors
Pongracic, 10 USA Inner-City Asthma Study (52)	5 measures over 2yrs Follow-up: 2 yrs n=469 children (5-11 yrs) with mild to severe asthma monosensitized to mould	B	Total moulds <i>Asp</i> , <i>Pen</i> , <i>Alt</i> , <i>Cl</i> a every 6 months for 2 yrs	-Symptoms: Max number of days with symptoms in the past 2 weeks. - CS to Hospital or EDV Number of unplanned CS or EDV in the past 2 months	In sensitized children -Symptoms: associations with outdoor total moulds <i>Asp</i> , <i>Pen</i> , <i>Cl</i> a, <i>Alt</i> -Unscheduled CS or EDV for <i>Aspergillus</i>	GLMM with indoor and/or outdoor fungal concentration as a predictor adjusted for month of the year, ag levels (mites, cat, cockroaches). AP, weather, pollens ND
Watanabe 16 Southwest Japon (Matsue) (53)	2-27 Feb 15 n=339 schoolchildren (36 asthmatics) aged 10-12	HVS	Total moulds	PF Mo (L/min)	IQR (95 % CI) PF decrease associated with +42.6 total moulds increase All: -1.18 (-2.27-0.08) Asthmatics: -1.44 (-2.57-0.32)	Linear mixed models adjusted for AP (PM _{2.5} , S0 ₂ , NO ₂ , O ₃), weather and individual characteristics.

Cross-sectional study using morphological method to assess outdoor fungi

Tham 19 Melbourne Transversal (55)	2009-11 n=936 (178 mould sensitized) from families of MACS cohort mean age 31.8±16.9	B	12 taxa	One single measure : Current asthma Lung function (LF) FEV ₁ , FVC, DEM25- 75 BDR Lung inflammation: FeNO EBC-NOx	In Total sample <i>Ustil</i> with lower LF <i>Alt</i> , <i>Drechslera</i> , <i>Ustil</i> with BDR <i>Alt</i> , <i>Cl</i> a with EBC-NOx In asthmatics <i>Alt</i> , <i>Drechslera</i> , <i>Ustil</i> with lower LF, higher EBC-pH In Mould sensitized <i>Ustil</i> /smuts, <i>Drechslera</i> associated with lower LF <i>Cl</i> a associated with higher LF and inflammation	Generalized linear models generalized estimating equations; and ordinal logistic regression. Models were adjusted for T, H and grass pollen, and further adjustment for air pollutants was tested.
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AA: Atopic Asthmatic; *Alt*: *Alternaria*; Asc: Ascospores; AP: Atmospheric Pollution; AR: Allergic Rhinitis; Asco: Ascospores; *Asp*: *Aspergillus*; Av: average; B: Burkard; Bar: Barometric; Bas: Basidiospores; BDR: BronchoDilator Response; BPT: Bronchial Provocation Test; *Cl*a: *Cladosporium*; CS: Consultation; EDV: Emergency Department Visit; *Epi*: *Epicocum*; ETS: Environmental Tobacco Smoke; Ev: Evening; GAM: Generalized Additive Model; *Gan*: *Ganoderma*; GLMM: Generalized Linear Mixed Model; HVS: High Volume air Sampler; Max: Maximum; Mo: Morning; NAA: Non Atopic Asthmatic; ND: Not Done; *Pen*: *Penicillium*; PF: Peak Flow (L/mn); R: Rotorod; SOB: shortness of breath; PF: Peak flow; RH: Relative Humidity; T: Temperature; Tr: Trap; TSP: Total Suspended Particulate.

Table IV: Outdoor moulds and rhinitis: ecological and birth cohort studies.

Author, Location, Study type	Time period Age group Sample size	Tr	Fungal Exposure	Outcome definition daily mean attendances	Results	Statistical method Confounding factors
Behbod, 15 Boston Birth cohort (24)	n=315	B	Alt, Cla, Asp, Pen CFU/m3 at 3 months	Rhinitis incidence through age 13	<i>Cla</i> : HR=2.12 [1.14-1.92] non-sporulating moulds; HR=1.41 (0.97 to 2.06),	Cox model adjusted for 4 taxa (Alt, Cla, Asp, Pen), yeasts, respiratory infections <1 yr, birth weight, maternal smoking, asthma and atopy, gender, ethnicity, income, childcare arrangements, type of building, indoor endotoxin or allergen levels (cockroach and dust mite).
Cakmak, 02 Ottawa Ecological (56)	May-Sep 93-97 Children n=502	R	Asc, Bas, Deu	EDV for rhinitis	Asc +7.4 p=0.054 Bas +14.0 p=0.0003 Deu +1.6 p=0.06	GAM adjusted for AP (S02, NO2, O3), weather, temporal trends. Pollens measured.
Guibert, 16 Brussel Ecological (58)	2005-11 January-Nov Whole pop	B	<i>Alt, Cla</i>	Anti-allergic prescriptions Oral AH ₁	NS	GLM adjusted for AP (PM10, NO2, S02, O3), weather, temporal trends, influenza, holidays. Pollens measured.
Caillaud, 18 Central France Ecological (5)	4 years April-Oct Whole pop	B	TS Asc, Bas 25 taxa	Anti-allergic prescriptions Oral AH ₁ + topical (nasal or ocular) drugs Around 50 sales/day	RR (IQR) Asp-Pen:1.05 (1.02-1.08) <i>Cla</i> : RR 1.08 (1.02-1.14)	GAM adjusted for AP (PM10, NO2, O3), weather, total pollens, temporal trends, influenza.
Johnston, 09 Darwin, Australia Tropical Ecological (57)	Apr 04-Oct 05 Whole pop	B	TS	Anti-allergic prescriptions Oral AH ₁ and topical (nasal or ocular) drugs	NS	GLM adjusted for AP (PM10), weather, temporal trends, respiratory viral infections, holidays. Pollens measured.

Asp-Pen: Aspergillus-Penicillium; Asc: Ascomycetes; AP: Air Pollution; Bas: Basidiomycetes; CFU: Colony Forming Unit; Deut: Deuteromycetes; EDV: Emergency Department Visit; GAM: generalized additive model; GLM: Generalized linear models; HR: Hazard-Ratio from Cox-Model; Weather: Meteorology; TS: Total Spores.

Table V: Summary Table for the association between total spores, ascospores, basidiospores, and deuterospores with respiratory outcomes.

Mould	Outcomes	Findings in Children	Finding in Adults or studies in children+adults that were not stratified by age groups
TS	Wheeze onset	No association with wheeze onset in birth cohorts: Harley; Behbod_2013	
	EDV or H for Asthma	Significant increase: Dales 00; Tham JACI 07; Tham ER 07 No association: Newson; ∇ but NS Atkinson; Lierl.	Significant increase in asthma outpatient-visits: children+adults, but no association with EDV Lewis 20 No association: Newson; Atkinson; in children+adults Hanigan
	Symptoms	Significant increase: Delfino 96; Delfino 97; Pongracic No/unexpected associations: Ross	Significant increase: Delfino 97
	PF	PF decrease: Delfino 97; (in asthmatic, but not in non-asthmatics): Watanabe No/unexpected associations: Ross	PF decrease: in sensitized patients, in children+adults not stratified by age and in +54yr. No association in non-sensitized patients Rutherford No association: Delfino 97
	Tt use for Asthma	Significant increase: Delfino 96; Delfino 97 No association: Ross	Significant increase: in fungi positive SPT Delfino 97 No association: Ross
	Lung function	Decrease in FEV1, FVC: Chen 2011	
	Rhinitis	No association: with rhinitis onset in birth cohort: Behbod 15	No association: with allergic rhinitis treatment sales: Johnston
Asc	Wheeze onset	Higher risk of wheeze onset in birth cohort: Harley	
	EDV or H for Asthma	Significant increase: Newson; Atkinson; Dales 00; in girls Cakmark 05; during the wet season in Mexico Rosas	Significant increase: Newson; in children+adults Dales 04; in women Cakmark 05; Stieb No or inconsistent association: ∇ in Q2 but not in upper quartiles of fungal concentration Atkinson; Rosas
	Symptoms	Significant increase: Delfino 96	No association: in children+adults Delfino 97; Epton
	PF		No association: in children+adults Delfino 97; Epton
	Tt use	No association: Delfino 96	No association: in children+adults Delfino 97, Epton
	Rhinitis	Significant increase EDV conjunctivitis: Cakmak 02	No association: with allergic rhinitis treatment sales: Caillaud
Bas	Wheeze onset	Higher risk of wheeze onset in birth cohort: Harley	
	EDV or H for Asthma	Significant increase: Atkinson; Dales 00; in boys Cakmark 05; at lag \geq 4 Raphoz; during the dry season in Mexico Rosas No association: Newson	Significant increase : in children+adults Dales 04; in men and in women Cakmark 05 No association: Newson; Atkinson; Stieb; Lewis 00; Rosas
	Symptoms	Significant increase: Delfino 96	Significant increase: in children+adults Delfino 97; Epton
	PF		No association: in children+adults Delfino 97; Epton
	Tt use	No association: Delfino 96	Significant increase: Epton No association: in children+adults Delfino 97
	Rhinitis	Significant increase EDV rhinitis conjunctivitis: Cakmak 02	No association: with allergic rhinitis treatment sales Caillaud
	Deu	EDV or H for asthma	Significant increase: Dales 00; in boys Cakmark 05; during the dry season in Mexico Rosas No or inconsistent association: Atkinson; EDV increase at lag5 but decrease at lag 2 Raphoz
Rhinitis		Significant increase EDV rhinitis conjunctivitis: Cakmak 02	

Asco: Ascospores; Bas: Basidiospores; Deu: Deuterospores; EDV: Emergency Department Visits; H: Hospitalization; LF: Lung Function; PF: Peak Expiratory Flow; Q: Quartile; NS: Non Significant; Spts: Symptoms; TS: Total Spores; Tt: Treatment;

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LEGEND FOR FIGURE 1

The Figure shows the flow of information and number of studies selected through the different phases of this review, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (www.prisma-statement.org).

Figure 1: Identification of studies and inclusion criteria.

