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MINI-REVIEW

Fungal secondary metabolites as harmful indoor air contaminants: 10 years on

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Abstract From the epidemiological studies conducted on the effect of mould and dampness on health a decade ago, the role of toxin-producing fungi in damp and mouldy buildings involved opinion more than evidence. Very little was known about the metabolites that were produced by the fungi that grew on damp building materials, and almost nothing had been reported on their occurrence in buildings. As a consequence, the focus was on speculations involving the fungal toxins that occur in agriculture. Over the past decade, particularly in the last 5 years, considerable progress has been made concerning the relevant toxins from fungi that grow on damp building materials. This paper summarizes the available data on the low-molecular-weight toxins reliably known from fungi common on damp building materials, the toxins that have been measured on mouldy building materials and the new understanding of the role that they play in the documented health effects of individuals living and working in damp and mouldy buildings.

Keywords Damp buildings · Fungi · Secondary metabolites · Inflammatory responses · Respiratory disease asthma

Introduction

In the early 1990s, dampness and mould became a matter for public discussion such that by August 2001, an American newspaper comic strip, Rex Morgan MD, featured a story line about toxigenic *Stachybotrys* in a home. The discussion emerged not because mould growth in homes had become suddenly dangerous but rather houses have changed; the undesirability of mouldy buildings is discussed in Leviticus. The issue of fungal toxins in the built environment in the literature emerged in the mid-1980s (Croft et al. 1986; Flannigan 1987; Samson 1985; Sorenson 1989; Tobin et al. 1987). This resulted from the fact that some of the fungi that are found on damp building materials were familiar to agricultural scientists working on mycotoxins. Inhalation exposure to mycotoxins in dusts from grain handling was known to be a serious health hazard (IARC 2012; Miller 1994a; Sorenson 1989).

Mycotoxins are low-molecular-weight secondary metabolites of fungi that have been demonstrated to be harmful to human and animal health. Large investments are made to manage the concentrations of several toxins in the food supply requiring actions from farmers, grain handlers, food producers and governments. Since mycotoxins contaminate staple crops, there is a great deal known about their impact on health and the economics of cereal farming (IARC 2012; Miller et al. 2014). Approximately 10,000 fungal metabolites have been identified and screened for biological activity (Kurtböke 2012). In many papers and presentations, it appears that virtually any fungal secondary metabolite is casually but incorrectly referred to as a mycotoxin. However, there are only five agriculturally important mycotoxins with worldwide distribution: aflatoxin, deoxynivalenol, fumonisin, zearalenone and ochratoxin. There are some less-common metabolites including T-2 and HT-2 toxins and ergot alkaloids that occur in some regions in particular years (Miller 1994b; Miller and Richardson 2013; Scott 2009). Additionally, there are a number of *Penicillium* toxins that occur in silage, mould-damaged food and sometimes corn left in the field after harvest (Nielsen et al. 2006; Samson et al. 2010; Sumarah et al. 2005). None of the fungi that produce these toxins in crops can grow on building materials indoors (AIHA 2008a; Andersen et al. 2011; Flannigan and Miller 2011) or are not known to produce toxins under these conditions (Rao et al. 1997; Ren et al. 1999).

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From the mid-1980s, many people attributed the association of nonspecific symptoms of individuals living or working in damp and mouldy buildings to toxins that occur on crops. For example, reports of aflatoxin, T-2 toxin and deoxynivalenol on building materials (e.g. Hay 1995; Smoragiewicz et al. 1993; and etcetera) are not credible (Jarvis 2003; Nielsen and Frisvad 2011).

This confusion resulted from many factors not least because there was considerable literature on mycotoxins in crops, very little information on the toxins from fungi that actually occur in buildings and even less on the occurrence of those toxins on building materials (Hendry and Cole 1993). Jarvis and Miller (2005) offered a perspective of these problems based on the literature of the day. Here, a decade later, we review the progress that has been made on the toxigenic potential of fungi common on damp building materials, low-molecular-weight compounds/toxins from fungi reliably detected on mouldy building materials and the state of the art on the health relevance of exposure. Metabolites reliably known to be produced by building-associated fungi are reviewed below and listed in Table 1. Common species on damp building materials for which little is known about the metabolites from strains that occur on damp building materials include *Aspergillus sydowii*, *Paecilomyces variotii*, *Scopulariopsis brevicaulis* and *Ulocladium* species. This review does not consider volatile compounds in part because exposure to volatiles cannot take place absent exposure to fungal particulates. Thus, there is no epidemiological evidence that these compounds affect health. Additionally, they are only produced during active fungal growth (Horner and Miller 2003; Schleibinger et al. 2005).

Housing factors leading to mould growth in buildings

With the expansion of single-family dwelling construction in the post-war period, research was conducted on sources of moisture in homes (e.g. Hite and Bray 1949) to determine the ventilation rates needed to prevent condensation which damaged paint. Ventilation rates began to decline in the 1960s and 1970s to save heating and cooling energy. By 1995, 25 % of homes in the USA were poorly ventilated (Murray and Burmaster 1995). In 2010, one third of Canadian homes had ventilation rates below the recommended standard of 0.3 air changes per hour putting them at risk for moisture problems (Johnson and Miller 2012). In 1950, air conditioning in homes was rare. In 1960, the national prevalence in the USA was 13 %; 1970, 36 %; and 1980, 59 ranging to 80 % in some areas (Biddle 2008). Large increases in the use of air conditioning during summer increased the risk for condensation in humid climates. Moreover, changes in the building materials used included a shift from plaster to paper-faced gypsum board, which is very susceptible to fungal growth. Other changes in construction details reduced resistance to water intrusion (Miller 2011; NAS 2000)

Sources of water indoors include routine activities such as showering and cooking, water leaks, flooding and ventilation failure leading to condensation (AIHA 2008b; Dales et al. 2008). A typical family generates 0.5–1 kg of water vapour per day (Tenwolde and Pilon 2007). Further, moisture can enter the building from small cracks in the slab and other sources. Since internal surfaces are almost always at a different temperature than the air, condensation takes place on walls, floors and in building envelopes (Bomberg 2013). The nature of the biodegradable compounds and the chemistry of common building materials vary considerably. Unsurprisingly, this means that different building materials are colonized by different fungal communities (AIHA 2008a; Andersen et al. 2011; Flannigan and Miller 2011).

Fungi can tolerate conditions where there is sometimes much less available water. Using the example of paper-faced gypsum wallboard, some water is bound to the gypsum salts (21 % by weight), which is unavailable for mould growth. Depending on the relative humidity of the room, moisture occupies the pores in the material that is also unavailable. As water from condensation, leaks or floods wets wallboard, more water becomes available for fungal growth. This principle is the same for all materials capable of supporting mould growth. Materials with the same amount of available water may have entirely different water contents (Richards et al. 1992). For example, at a moisture content that will permit the growth of many fungi, the moisture content of softwood will be approximately 17 %, wallpaper 11.3 % and gypsum plaster 0.7 %.

The biologically available water or water activity (a_w), the quality of the nutrients, the carbon/nitrogen ratio and ambient temperature interact to determine the ultimate fungal community on a mouldy building material. Full discussions of these and other factors that affect fungal growth on damp building materials are found elsewhere (AIHA 2008a; Flannigan and Miller 2011; Miller 2011). Adan et al. (2011) demonstrated that the growth of fungi on interior surfaces depends on the amount of time that the surface has a relative humidity (RH) >80 %. This has been confirmed by elegant experiments by Li and Wadsö (2013) who measured the growth of fungi by imagining the heat of their metabolism on wood that was wet and subsequently dried. They found that *Penicillium brevicompactum* was able to recover from periods of dryness quickly whereas *Trichoderma viride* was sensitive to periods of drying and failed to regrow. This indicates that "time of wetness" in a given environment affects species distributions.

The house dust in carpets is hydroscopic and provides both nutrients and shelter for dust mites as well as some fungi such as *Aspergillus versicolor* and *Wallemia sebi* (Bloom et al. 2009; Desroches et al. 2014; Visagie et al. 2014). Active fungal growth in dust will begin when the RH is consistently above 84 % (Korpi et al. 1997). If the floor is cooler than the

Table 1 Metabolites reliably known to be produced by isolates of fungi from mouldy building materials[a]

Fungus	Metabolites
Alternaria tenuissima	Altenuene, alternariol, alternariol monomethyl ether, altertoxin, tentoxin, tenuazonic acid
Aspergillus amstelodami[b]	Neoechinulin A and B, echinulin, epiheveadride, flavoglaucin, auroglaucin, isotetrahydroauroglaucin
A. calidoustus	TMC-120 A–C, methyl isoquinoline alkaloids, drimane sesquiterpene
A. glaucus[c]	Cladosporin, neoechinulin A and B, echinulin, epiheveadride, flavoglaucin, auroglaucin, isotetrahydroauroglaucin
A. insuetus	TMC-120 A–C, methyl isoquinoline alkaloids, drimane sesquiterpene
A. ochraceus	Ochratoxin A, penicillic acid, xanthomegnin, viomellein, vioxanthin
A. ruber[d]	Neoechinulin A and B, echinulin, epiheveadride, flavoglaucin, auroglaucin, isotetrahydroauroglaucin
A. versicolor sensu lato	Sterigmatocystin, 5-methoxysterigmatocystin
Chaetomium globosum	Chaetoglobosins, chlorinated azaphilones, chetomin
Penicillium brevicompactum	Brevianamide A, mycophenolic acid, asperphenamate
P. chrysogenum	Roquefortine C, penicillin G, xanthocillin X, meleagrin, andrastins A and B, chrysogine, secalonic acid D and F
P. corylophilum	Andrastin A, citreohybridinol, phomenone, koninginins, isochromans, α-pyrones
P. crustosum	Andrastin A, roquefortine C, penitrem A, viridicatols, terrestic acid
P. rubens	Roquefortine C, penicillin G, xanthocillin X, meleagrin
Trichoderma atroviride	α-Pyrones
T. citrinoviride	Bisvertinol, trichotetronine, spirosorbicillins, vertinolide
T. harzianum	Almethicins, α-pyrones
T. koningiopsis	Koninginins
T. longibrachiatum	Trilongins
Stachybotrys chartarum sensu stricto chemotype S	Macrocyclic trichothecenes (satratoxin G and H, roridin E), trichodermin, trichodermol, spirocyclic drimanes
S. chartarum sensu stricto chemotype A	Atranones, dolabellanes, trichodermin, trichodermol, spirocyclic drimanes
S. chlorohalonata	Atranones, dolabellanes, trichodermin, trichodermol, spirocyclic drimanes
S. echinata	Dechlorogriseofulvins, griseofulvin, trichodermin, trichodermol, spirocyclic drimanes (different from *S. chartarum*), xanthone
Wallemia sebi	Walleminone, wallimidione, tryptophol, other phenolics

[a] References are provided in the text

[b] Formerly *Eurotium amstelodami*

[c] Formerly *E. herbariorum*

[d] Formerly *E. rubrum*

centre of the room, the RH will be higher near the floor and condensation will occur. Pasanen et al. (1993) showed that when the RH exceeded 84 %, active fungal growth in air ducts could be detected. As with interior surfaces, floors are typically cooler. De Boer (2000) measured temperature and RH at the floor surface in a house over a 24 period. While at night, the RH was low, during the day, the RH was well above that where fungal growth could occur.

Toxigenic potential of fungi common in the built environment

Since Jarvis and Miller (2005), considerable data have been collected on the fungi that actually occur on mouldy building materials from the USA and Canada as well as parts of Europe. A systematic survey of accredited commercial laboratories was conducted representing samples from the length and breadth of Canada and the USA including Hawaii. Data were reported from thousands of mouldy gypsum wallboard samples, wood, manufactured wood and ceiling tiles in four categories: common, frequent, infrequent and rare (AIHA 2008a). Similar comprehensive data have been reported for Scandinavia (Andersen et al. 2011). These studies are supported by many smaller studies summarized in Flannigan and Miller (2011). From this, the most common fungi can be discerned to inform studies of their toxigenic potential and analysis of damp building materials for their toxins.

Based on analyses of settled dust collected in homes by pyrosequencing, there is little doubt that there remains more to learn about the fungi in settled dust (Amend et al. 2010; Visagie et al. 2014). However, the data from mycological

analysis of damp materials represent those fungi that dominate indoors. Another line of evidence of this comes from the fact that the human antigens of many fungi that have been commonly identified growing on damp building materials have been investigated. The number of sensitized, atopic patient sera that respond to a specific mould allergen typically correlates with the frequency that those species are found in damp buildings in Canada and the USA. *Penicillium rubens* is the most common species of *Penicillium* on damp building materials (AIHA 2008a). For this species, just over 50 % of the patient sera reacted to the allergen (Wilson et al. 2009). Approximately 50 % of the sera tested responded to a *Chaetomium globosum* chitosanase (Provost et al. 2013), and for *W. sebi*, 36 % responded to its antigen (Desroches et al. 2014). In the case of another very common fungus, *A. versicolor*, 20 % of sera reacted (Liang et al. 2011). In the case of *Stachybotrys chartarum*, *Stachybotrys chlorohalonata* and *Stachybotrys echinata*, 10 % of the patient sera responded to its antigen (Xu et al. 2007). Finally, a similar percentage of atopic sera, 10 %, had antibodies to the antigen common to *Aspergillus glaucus*, *Aspergillus amstelodami* and *Aspergillus ruber* (formerly *Eurotium herbariorum*, *Eurotium amstelodami* and *Eurotium rubrum* respectively; corrected after Samson et al. 2014) that grow on building materials (Levac 2011).

Common food-borne or storage fungi typically produce secondary metabolites optimally when the a_w of the substrate is near the upper end of the particular optimum (Cairns-Fuller et al. 2005; Frisvad and Thrane 1995). The majority of studies concerning fungal metabolites on damp building materials have been performed when the material a_w was between 0.95 and 0.99 at 25 °C (Nielsen 2003). The lower a_w limit for fungal growth on wood and wood-based products is 0.78 at 20–25 °C which increased to 0.90 at 5 °C (Nielsen et al. 2004). For most strains of the hydrophilic species *S. chartarum* sensu lato, growth is optimal at 0.98 at 30 °C (Ayerst 1969; Frazer et al. 2012. For one strain (IBT 7711), production of the trichothecene satratoxin G was maximal at 20 °C at a a_w of 0.98 whereas at 0.995, the toxin was maximally produced at 15 °C (Frazer et al. 2011). When *S. chartarum* sensu lato is grown on autoclaved rice, yields of satratoxin G and other trichothecenes are increased after incubation at lower temperatures (Jarvis et al. 1986). Disregarding the impact on growth at lowered temperatures, the effect on metabolite synthesis relates to higher oxygen tensions in water at colder versus warmer temperatures.

When the a_w of common building materials was lowered, fungal growth was dominated by some of the more xerophilic *Penicillium* and *Aspergillus* including the former *Eurotium* species. Their metabolite production was drastically reduced on building materials at this lower a_w in comparison to when it exceeded 0.95. The exception was a xerophilic *Aspergillus* species, identified then as a *Eurotium* species, which produced its metabolites optimally at ca. 0.80 a_w (Nielsen et al. 2004). The production of walleminone by the xerophilic fungus *W. sebi* increased as the amount of solute was added to the liquid medium lowering the a_w (Frank et al. 1999; Wood et al. 1990). *W. sebi* isolated from the built environment in Canada only produced walleminone or wallimidione on media of lower a_w (Desroches et al. 2014; Fig. 1). Similar observations were made with indoor strains of the moderate xerophile *Penicillium corylophilum* (McMullin et al. 2014b).

Penicillium

The most common species of *Penicillium* found on damp materials in the USA and Canada is the Fleming clade of *Penicillium chrysogenum*, now called *P. rubens*. This clade is habitually isolated from the built environment as it was by Sir Alexander Fleming but much less so from other sources (De La Campa et al. 2007; Houbraken et al. 2011; Scott et al. 2004). *P. rubens* makes penicillin G, roquefortine C, meleagrin and xanthocillin X (De La Campa et al. 2007). The other clades of *P. chrysogenum* produce these metabolites with the addition of secalonic acid D and F and a lumpidin-related compound (Houbraken et al. 2011). Meleagrin and roquefortine C have been reported on building materials experimentally inoculated with *P. chrysogenum* (Gutarowska et al. 2010; Nielsen et al. 1999) and from mould-damaged building materials (Täubel et al. 2011; Vishwanath et al. 2009; Fig. 2).

Metabolites from another *Penicillium* species common on damp building materials, *Penicillium brevicompactum*, include brevianamide, mycophenolic acid (Rand et al. 2005; Fig. 3), asperphenamate and a tanzawaic acid (Nielsen et al. 1999, 2004). Mycophenolic acid has been reported on mouldy building materials (Täubel et al. 2011). Another *Penicillium* species typically found on wet cellulose-based materials is *Penicillium crustosum* (AIHA 2008a; Andersen et al. 2011). A strain of this species from damp building materials in Denmark-produced roquefortine C, penitrem A, viridicatols, terrestic acid and andrastin A (Sonjak et al. 2005). *P. crustosum* from saw lumber in Canada produced roquefortine C and penitrem A (Seifert and Frisvad 2000).

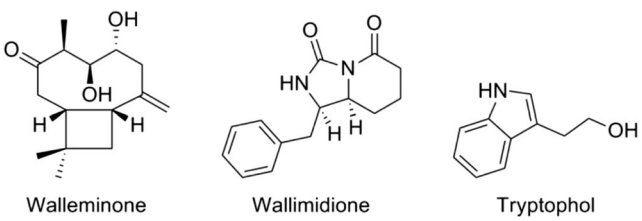

Fig. 1 Metabolites of *Wallemia sebi*

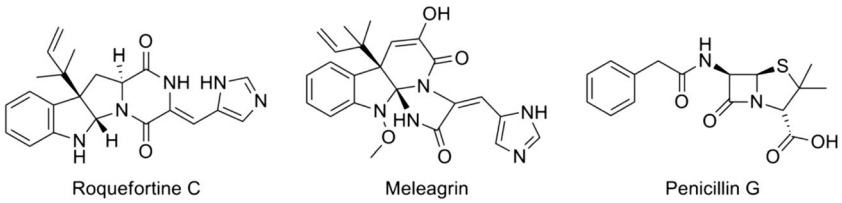

Fig. 2 Secondary metabolites of *Penicillium rubens*

P. corylophilum is found on paper-faced gypsum wallboard and was also reported from fibrous insulation, wood and manufactured wood in the USA and Canada (AIHA 2008a). This species comprised approximately 5 % of the Penicillia isolated from damp buildings in Scandinavia and various countries in Western Europe (Andersen et al. 2011; Samson et al. 2010) and is common in damp buildings in Japan (Ohnishi et al. 2002). Strains collected across Canada produced phomenone, the meroterpenoids citreohybridonol and andrastin A as well as koninginin A, E and G. In addition, a number of new α-pyrones and isochromans were produced by all strains studied (McMullin et al. 2014a, b; Fig. 4).

Aspergillus

One of the most common species found growing on water-damaged building materials, *A. versicolor*, (AIHA 2008a; Andersen et al. 2011) produces the aflatoxin precursor sterigmatocystin and 5-methoxy-sterigmatocystin. These two compounds have been directly detected on water-damaged building materials (Bloom et al. 2007, 2009; Nielsen et al. 1999, 2004; Täubel et al. 2011; Vishwanath et al. 2009) and dust collected from carpets and floors (Bloom et al. 2007, 2009; Engelhart et al. 2002).

The isoquinoline alkaloids TMC-120 A–C, their derivatives and a drimane sesquiterpene were isolated from *Aspergillus insuetus* and *Aspergillus calidoustus* obtained from mouldy building materials (formerly *Aspergillus ustus*; Slack et al. 2009). TMC-120 A has been detected on building materials contaminated with this species (Nielsen 2003). *Aspergillus ochraceus* is also common indoors, however, is more often associated with concrete and flooring (Andersen et al. 2011). This species produces a variety of metabolites including the nephrotoxic ochratoxin A, penicillic acid, xanthomegnin, viomellein and vioxanthin (Frisvad and Thrane 2002). Ochratoxin A was reported indoors from mouldy pet food debris in house dust (Richard et al. 1999), although the fungal source was not identified. Under laboratory conditions, pure cultures of *A. ochraceus* produced ochratoxin A on water-saturated ceiling tiles and carpet (Salazar 1997). Ochratoxin A has been detected from mouldy building materials (Täubel et al. 2011) with penicillic acid and viomellein on experimentally inoculated gypsum wallboard (Gutarowska et al. 2010; Fig. 5).

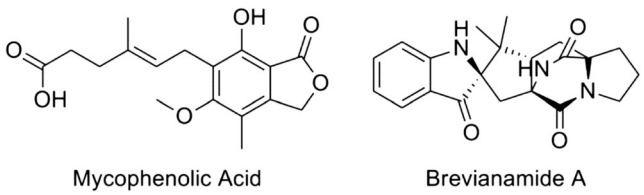

Fig. 3 Secondary metabolites of *Penicillium brevicompactum*

In North American buildings, the dominant species of the more xerophilic Aspergilli are *A. glaucus* and *A. amstelodami*; *A. ruber* is less common (formerly *Eurotium* species; AIHA 2008a; Samson et al. 2014). Neoechinulin A and B, epiheveadride, flavoglaucin, auroglaucin and isotetrahydroauroglaucin were produced by these three species. A metabolite of *A. glaucus*, cladosporin, was not isolated from *A. amstelodami* or *A. ruber*. All three species produced questin (Slack et al. 2009). Echinulin and questin have been detected on experimentally inoculated building materials (Nielsen et al. 2004; Fig. 6).

Stachybotrys

In North America, two species of *Stachybotrys*, *S. chartarum* and *S. chlorohalonata*, are common on wet gypsum wallboard, wood, manufactured wood and ceiling tiles and occur frequently on wet insulation. A third species, *S. echinata* (formerly *Memnoniella echinata*; Samson et al. 2010) is less common being more associated with warmer regions (AIHA 2008a; Flannigan and Miller 2011). Likely due to important differences in interior finishing materials, *Stachybotrys* species are much less common in Europe (Andersen et al. 2011; Flannigan and Miller 2011). These species produce many biologically active compounds (Miller et al. 2003). *S. chartarum* sensu lato was separated into two distinct chemotypes and two species, *S. chlorohalonata* and *S. chartarum* (Andersen et al. 2002, 2003). *S. chartarum* chemotype S produces the macrocyclic trichothecene satratoxins G and H as well as roridin E. Chemotype A produces atranones A, B, C, F and E; their precursors; and dolabellanes (Andersen et al. 2002; Hinkley et al. 1999, 2000). Semeiks et al. (2014) demonstrated that chemotype-specific gene clusters are the genetic basis for these mutually exclusive toxin chemotypes. Regardless of chemotype, toxigenic strains produce the simple trichothecenes trichodermin and trichodermol. The dominant metabolites produced by

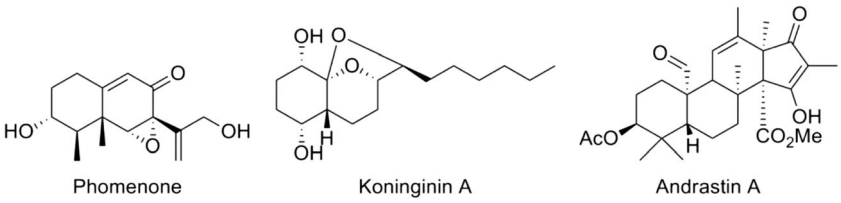

Fig. 4 Secondary metabolites of *Penicillium corylophilum*

both *S. chartarum* chemotypes and *S. chlorohalonata* are spirocyclic drimanes and their precursors. However, strains of *S. chlorohalonata* generally produce lower amounts of these compounds (Andersen et al. 2002). Of these, the spirocyclic drimanes, satratoxins G and H have been found on damp building materials (Nielsen et al. 2003; Täubel et al. 2011; Vishwanath et al. 2009.

S. echinata, is closely related to *S. chartarum*, produces many of the same toxins and is found on similar building materials (Jarvis et al. 1998). It produces the simple trichothecenes trichodermin and trichodermol, xanthone and some spirocyclic drimanes not reported from *S. chartarum*. However, *S. echinata* indoor strains primarily produce several griseofulvins including dechlorogriseofulvin and epidechlorogriseofulvin (Jarvis et al. 1996; 1998; Nielsen et al. 1998a, b; Fig. 7).

C. globosum

In the USA and Canada, *C. globosum* is common on wet gypsum wallboard and wood while it frequently occurs on wet insulation, manufactured wood and ceiling tiles. In contrast to other hydrophilic *Stachybotrys* species, this fungus is also very common on damp building materials in Europe (Andersen et al. 2011). Strains isolated from damp buildings across Canada produce chaetoglobosins A, C and F; chaetomugilin D; and chaetoviridin A. Other chaetoglobosins and azaphilones are also produced in minor amounts (McMullin et al. 2013a, b). Chaetoglobosins A and C (Fogle et al. 2007; Nielsen et al. 1999; Täubel et al. 2011) and chetomin (Vishwanath et al. 2009) have all been reported from mouldy building materials (Fig. 8.)

Trichoderma

A number *Trichoderma* species have been reported from damp building materials in Europe including *Trichoderma longibrachiatum Trichoderma citrinoviride*, *Trichoderma harzianum*, *Trichoderma atroviride*, *Trichoderma viride*, *Trichoderma hamatum* and *Trichoderma koningii* (Andersen et al. 2011; Lubeck et al. 2000). In the USA and Canada, species of *Trichoderma* are common on insulation, wood and manufactured wood (AIHA 2008a). The simple trichothecenes trichodermin and harzianum A were originally reported from *T. viride* and *T. harzianum*; however, these species identifications proved to be incorrect. Isolates of *T. viride*, *T. harzianum*, *T. atroviride*, *T. longibrachiatum* and *T. citrinoviride* from European buildings did not produce either trichothecene (Nielsen et al. 2005). Canadian strains of *Trichoderma koningiopsis* produced koninginins A, B, D and F where *T. harzianum* and *T. atroviride* strains produced the simple α-pyrone, 6-pentyl-2H-pyran-2-one (McMullin 2014). The production of low-molecular-weight pyrones and lactones is characteristic of this genus (Reino et al. 2008). An isolate of *T. citrinoviride* produced various sorbicillin-derived compounds including the (*R*) isomer of vertinolide, spirosorbicillinol A–C, bisvertinol and trichotetronine. From the related species, *T. longibrachiatum*, one 11- and eight 20-

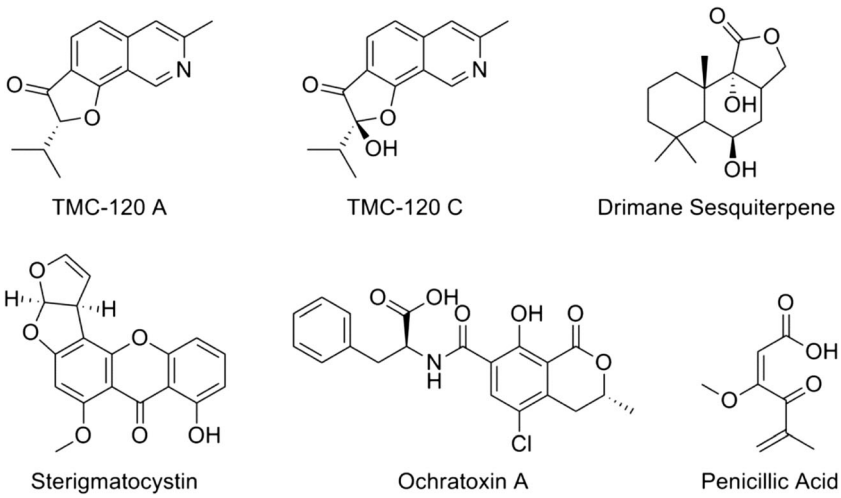

Fig. 5 Secondary metabolites of *Aspergillus insuetus*, *A. calidoustus*, *A. versicolor* and *A. ochraceus*

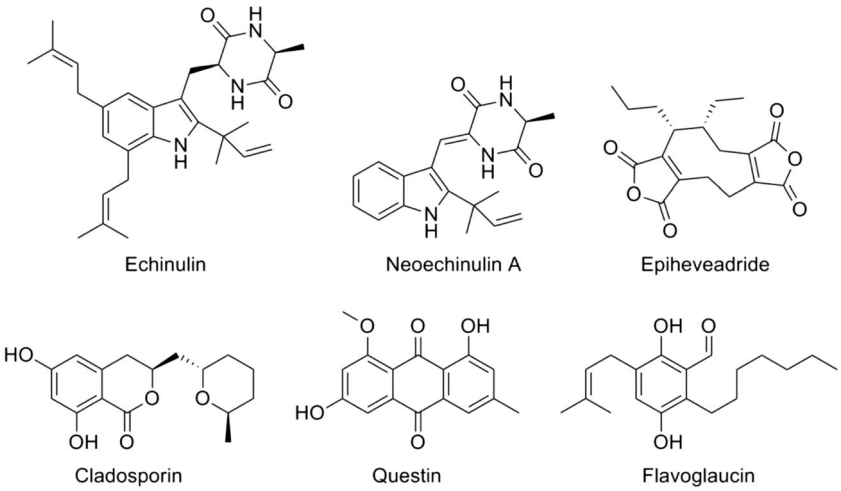

Fig. 6 Secondary metabolites of *Aspergillus glaucus*, *A. amstelodami* and *A. ruber*

residue cytotoxic peptaibols named trilongins were identified from a Finish indoor strain (Mikkola et al. 2012).

Alternaria

Alternaria tenuissima is fairly common on some damp building materials (AIHA 2008a; Andersen et al. 2011). Strains isolated from various plants produce altenuene, alternariol, alternariol monomethyl ether, altertoxin, tentoxin and tenuazonic acid (Andersen et al. 2001). Of these, alternariol and alternariol monomethyl ether were detected on experimentally inoculated gypsum wallboard (Nielsen et al. 1999) and ceiling tiles (Ren et al. 1998). These compounds have also been reported from mouldy building materials (Täubel et al. 2011; Vishwanath et al. 2009; Fig. 9).

β-1,3-D-Glucan

Although not low-molecular-weight compounds as discussed above, some fungal glucans are potently inflammatory and contribute to adverse health effects (Akpinar-Elci et al. 2013; Neveu et al. 2011; Rand et al. 2010, 2013). β-1,3-D-Glucan polysaccharides are found in the cell membrane of fungi, higher plants and some bacteria. For "mould" fungi as defined above, glucan is predominantly present in a triple-helical conformation (Williams 1997; Williams et al. 2005). Some Basidiomycetes produce both β-1,3-D-glucan and β-1,6-D-glucan that have anti-inflammatory properties in vitro and in vivo (Jedinak et al. 2011; Pacheco-Sanchez et al. 2006). Fungi from different taxonomic groups produce varying relative amounts of β-1,3-D-glucan compared to other glucans (Odabasi et al. 2006).

The anamorphic Trichocomaceae (i.e. *Penicillium*, *Aspergillus* and related hyphomycetes), often referred to as moulds associated with damp building materials, apparently predominantly contain β-1,3-D-glucan in a triple-helical form. In the fungal genera that grow on building materials, the percentage of glucan in a cell wall is species-specific (Foto et al. 2004, 2005) and a stable property under growth conditions suitable for the normal growth of the fungi concerned. In these fungi, as with the fungal membrane sterol ergosterol (Miller and Young 1997), the amount of glucan per spore is

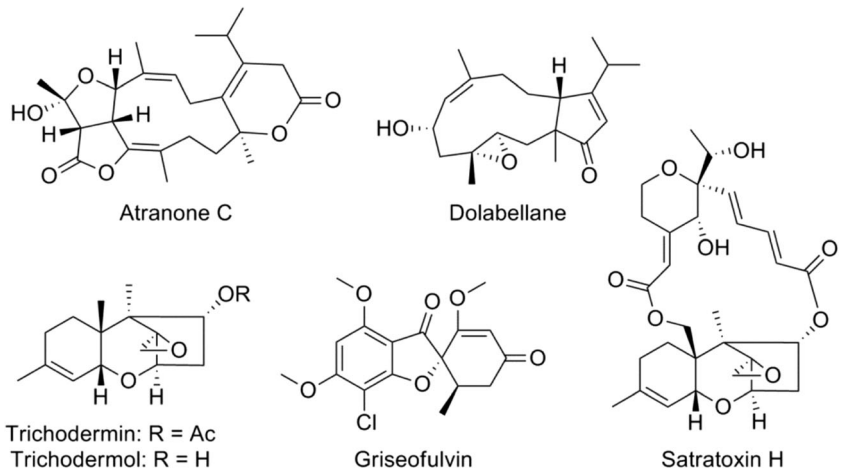

Fig. 7 Selected secondary metabolites of *Stachybotrys chartarum*, *S. chlorohalonata* and *S. echinata*

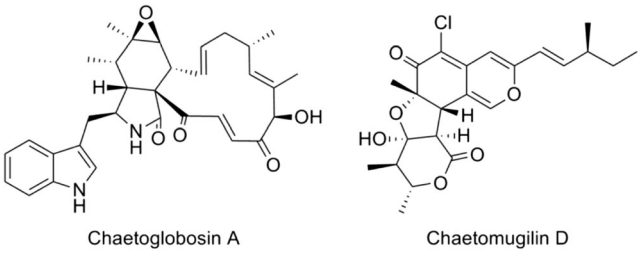

Fig. 8 Secondary metabolites of *Chaetomium globosum*

determined primarily by the size of the spore (Foto et al. 2004). The forms and relative amounts of glucan in the common damp building fungi outside the *Eurotiales* and allied genera are more diverse (Odabasi et al. 2006). *W. sebi* is a Basidiomycete (*Ustilaginomycetes*, *Heterobasidiomycetes*). Basidiomycetes of this type are known to produce β-1,6-D-glucan with sparse β-1,3 branching. The glucan of two common species in the *Hypocreales*, *C. globosum* and *Trichoderma viride*, is comprised primarily of both β-1,3 and β-1,6 glucans (Benítez et al. 1975; Maret 1972).

Health impact

Since 2005, a broad consensus has been reached on the impact of mould and dampness in buildings (Health Canada 2007; NAS 2004; NIOSH 2012; WHO 2009). Well-conducted epidemiology studies in Europe, Canada and the USA have consistently demonstrated that exposures from building/house dampness and mould have been associated with increased risks for respiratory symptoms, asthma, hypersensitivity pneumonitis, rhinosinusitis, bronchitis and respiratory infections (Jaakkola et al. 2013; Mendell et al. 2011; Park and Cox-Ganser 2011; Quansah et al. 2012). The conclusions of these observational studies are supported by intervention studies (Krieger et al. 2010; NIOSH 2012). If there are material amounts of mould and dust mites, typically, endotoxin will also be present. Regardless, the relative risk for increased asthma and other conditions remains after controlling for these exposures (e.g. Dales and Miller 1999). The question that emerged from the earliest epidemiology studies in the 1980s and early 1990s was that increased relative risks for respiratory disease did not stratify according to atopic status (Miller 2011). Indeed, recent studies have shown that exposure to mould and dampness increases the risk of allergy to other allergens such as house dust mites and pollen, not just fungi (Cox-Ganser et al. 2005)

Inhaled fungal spores and mycelial fragments contain allergens, fungal glucan and low-molecular-weight toxins. Damp buildings have a higher percentage of spore and mycelial fragments (Cho et al. 2005; Foto et al. 2005; Green et al. 2011; Reponen et al. 2007). The majority of fungal biomass is present as fine fragments (Salares et al. 2009). Although the largest of these particles do not reach the lungs in high efficiency nor penetrate deeply, the smallest do so efficiently (Phalen et al. 2008). The spores and sclerotia of toxigenic fungi typically contain very high concentrations of some or all of the toxins of that species (Miller 1992; Miller 1994a).

Conidia of one strain of *S. chartarum* sensu lato contained 15 μg/g of three toxins from that species (Sorenson et al. 1987). Spores of a single strain of both *Fusarium graminearum* and *Fusarium sporotrichioides* contained 30 μg/g deoxynivalenol and 50 μg/g T-2 toxin, respectively, potent toxins produced by these species (Miller 1992; Sorenson 1989; 1999). From agricultural studies, toxins present in dusts are mainly contained in the fractions containing fungal spores (Sorenson 1990). One of the early findings was the role of these low exposures of many fungal toxins on alveolar macrophage function (Sorensen 1999). It was therefore postulated that a mechanism for the health effects associated with fungal exposures in damp buildings was due to the impact of fungal metabolites on lung biology (Miller 1992). In *S. chartarum*, the allergen and toxins are in high concentrations on the fragments that are engulfed by alveolar macrophages (Gregory et al. 2004; Rand and Miller 2008). The presence of the toxins in the spores apparently affected particle clearance (Rand and Miller 2008; see also Lichtenstein et al. 2010).

In mice, a dose of 1 nM/ mouse of the *S. chartarum* sensu lato trichothecene isosatratoxin F in the lung resulted in ultrastructural changes within the lung cells that produce lung surfactants. This resulted in the accumulation of newly secreted pulmonary surfactant in the alveolar space of isosatratoxin F-treated animals (Rand et al. 2002). Since 2005, studies have been conducted on the effects of 11 metabolites from common damp building fungi on lung biology in mice and for 7 of those compounds also with murine primary lung cells. In addition, a further seven compounds have been studied in the mouse alveolar macrophage cell line, RAW 264.7. This

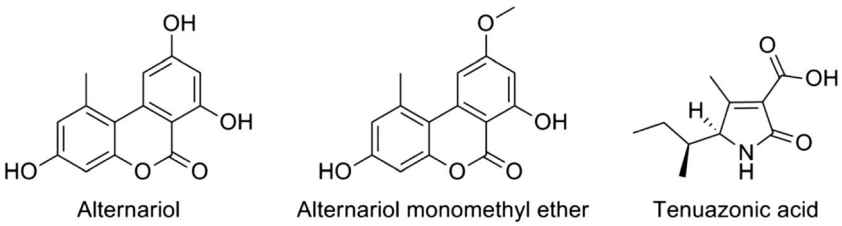

Fig. 9 Selected secondary metabolites produced by *Alternaria tenuissima*

includes compounds from fungi that grow on building materials with (1) high a_w, *S. chartarum* sensu lato (atranones A, C), *C. globosum* (chaetoglobosin A, chaetomugulin D) and *Trichoderma* species (koninginin A, bisorbibutenolide); (2) low a_w, *A. glaucus* (formerly *E. herbariorum*; neoechinulin A and B) and *W. sebi* (walleminone); and (3) intermediate a_w, *A. versicolor* (sterigmatocystin), *A. insuetus* and *A. calidoustus* (formerly *A. ustus*; TMC-120 A), *P. brevicompactum* (brevianamide A, mycophenolic acid), *P. rubens* (roquefortine C) and *P. corylophilum* (andrastin A, phomenone). The metabolites investigated for effects on lung biology in in vivo, murine primary alveolar macrophages and the cell line RAW 264.7 are listed in Table 2.

For the in vivo studies, toxin dose was based on the extensive data for $PM_{2.5}$ dosimetry (Brown et al. 2005) using measurements of β-1,3-D-glucan in triple-helical form in 3–5-day air samples conducted in 136 homes from Prince Edward Island and Ottawa (Foto et al. 2005; Miller et al. 2007; Salares et al. 2009). The glucan in $PM_{2.5}$ comprises 70–90 % of the total airborne glucan. On a 24-h basis, lung exposure to glucan in spore and hyphal fragments <2.5 μm for resting and moderate nasal breathing, and moderate oral breathing, would be in the order of 10^{-7}–10^{-9} M, focused on a number of 'hot spots' in the lungs (Phalen et al. 2008). The ratio of toxin to glucan in spores is approximately 1:1, calculated from data in Foto et al. (2004) and Miller (1992).

An in vivo study of an intratracheal exposure of the *Penicillium* toxins brevianamide, mycophenolic acid and roquefortine C was conducted. A dose range between 0.5 and 12.5 nM/g body weight was administered over a 2-day period. The higher doses of 5.0 and/or 12.5 nM/g body weight induced elevated macrophage, neutrophil, macrophage inflammatory protein (MIP-2), tumour necrosis factor (TNF) and concentrations of the interleukin (IL-6) in bronchioalveolar lavage fluid of intratracheally exposed mice. Albumin concentrations, a nonspecific marker of vascular leakage, were significantly elevated in the bronchioalveolar lavage at 12.5 nM/g body weight brevianamide (Rand et al. 2006).

A number of studies have been conducted with atranones (*S. chartarum* sensu lato) in murine models. A dose response over a 100-fold range and tissues was obtained at 3, 6, 24 and 48 h post-dosing in mice. The high doses tested, ~0.4 to 4×10^{-5} mole toxin/animal, of atranones A and C induced differentially elevated macrophage and neutrophil counts as well as MIP-2, TNF and IL-6 concentrations in the bronchioalveolar lavage fluid. Macrophage and neutrophil counts in bronchioloalveolar fluid were increased over the time course in a generally dose-dependent manner. The chemokine/cytokine marker responses were similarly increased in dose-dependent fashion and returned to control values 2 days after exposure. Atranones A and C exhibited different inflammatory potencies with different toxicokinetics where atranone C was more potent (Rand et al. 2006).

Mice were intratracheally instilled with a single dose, 4×10^{-5} mole toxin/kg lung weight, comprised of either atranone C, brevianamide, cladosporin, mycophenolic acid, neoechinulin A and B, sterigmatocystin or TMC-120 A. They were followed in a time course manner with pathology and molecular studies. Twelve hours after exposure, the histopathology of lung sections revealed that bronchioli were lined with irregularly thickened and sometimes sloughing

Table 2 Metabolites of isolates of fungi from mouldy building materials tested for effects on lung biology[a]

Metabolite	In vivo	Murine primary alveolar macrophages	RAW 264.7 cell line
Brevianamide A	x	x	
Atranone C	x	x	
Cladosporin	x	x	
Sterigmatocystin	x	x	
Neoechinulin A	x	x	
Neoechinulin B	x	x	
TMC-120 A	x	x	
Mycophenolic acid	x		
Roquefortine C	x		
Atranone A	x		
Isosatratoxin F	x		
Chaetoglobosin A			x
Chaetomugilin D			x
Andrastin A			x
Walleminone			x
Phomenone			x
Koninginin A			x
Bisorbibutenolide			x

[a] References are provided in the text.

epithelium. Bronchiolar spaces showed infiltration of leukocytes and cellular and mucus-like debris while swollen macrophages and modest amorphous debris accumulations were observed in alveolar spaces. All toxin-instilled lungs exhibited copious mucus production on bronchiolar surfaces. Reverse transcription (RT)-PCR-based analysis of 83 inflammation-associated genes extracted from lung tissue demonstrated a number of patterns compared to controls. Twelve hours post-exposure, 75 genes were modulated in the different animal treatment groups. Expression of some transcriptionally modulated genes was confirmed using immunohistochemistry that demonstrated MIP-2 and TNF-α staining in bronchiolar epithelia, alveolar macrophages and alveolar type II cells. Hierarchical cluster analysis revealed significant patterns of gene transcription linking the response of the toxins at equimolar doses into three distinct groups: (1) brevianamide, mycophenolic acid and neoechinulin B; (2) neoechinulin A and sterigmatocystin; and (3) cladosporin, atranone C and TMC-120 A. Each one of the clusters is anchored by a compound known to be immunomodulatory in humans: mycophenolic acid, sterigmatocystin and TMC-120 A (Miller et al. 2010).

A follow-up study in primary mouse alveolar macrophages tested the hypothesis that this model mimicked what was observed in the whole lung. Transcriptional responses of 13 inflammation-associated genes in mouse alveolar macrophages exposed to a 10^{-8} mole dose of either atranone C, brevianimide, cladosporin, neoechinulin A and B, sterigmatocystin or TMC-120 A over a time course were monitored. Curdlan (β-1,3-D-glucan in triple-helical form) and lipopolysaccharide (LPS) endotoxin were added as reference compounds. Multianalyte ELISA was used to measure the expression of six pro-inflammatory cytokines common to the genes included in the transcriptional assays (*Cxcl1*, *Cxcl10*, *Ccl3*, *IL1β*, *Ifn-λ* and *Tnf-α*) to determine whether gene expression corresponded to the transcription data. Compared to controls, all of these compounds induced significant (≥2.5-fold or ≤−2.5-fold change at $P \leq 0.05$) time- and compound-specific transcriptional gene alterations in treatment alveolar macrophages. Among other findings, neoechinulin B had the same impact on the genes examined as LPS endotoxin albeit the time course was different. All compounds resulted in significant ($P \leq 0.05$) time- and compound-specific pro-inflammatory responses as differentially elevated concentrations of *Cxcl1*, *Cxcl10*, *Ccl3*, *Ifn-λ* and *Tnf-α* in culture supernatant of treatment alveolar macrophages. While some differences in transcriptional responses between the isolated primary alveolar macrophages and the murine model described above were observed, the results indicate that low-molecular-weight compounds from fungi that grow in damp built environments are potently pro-inflammatory (Rand et al. 2011).

Preliminary data have been obtained using the validated mouse alveolar macrophage cell line, RAW 264.7 (Rand et al. 2013) for some other toxins. For the chemokine TNF-α, up-regulation was observed at 10^{-8} M for both chaetoglobosin A and chaetomugilin D (*C. globosum*), and 10^{-7} M with andrastin A (*Penicillium* species) and walleminone (*W. sebi*) (Rand, personal communication). Fungal glucans are also potently inflammatory, but the genes that are up-regulated and the mechanisms that may apply in murine models are different than those observed with the various low-molecular-weight compounds (Miller et al. 2010; Rand et al. 2010, 2011, 2013).

Conclusions

A decade ago, there was little known concerning the repertoire of species that occur in the built environment and the toxigenic potential of these fungi growing on damp building materials. Over the intervening years, much information has been collected showing particular associations of species with different building materials (AIHA 2008a; Andersen et al. 2011). The impression that agriculturally important toxins such as T-2 toxin would occur on damp building materials has been demonstrated to be incorrect. There was a common view that fungi growing on building materials did not always make mycotoxins (e.g. Rao et al. 1996; Nikulin et al. 1997) that remained in the secondary literature until very recently. As discussed throughout this review, assumptions were made about the toxigenic potential of the fungi that were flawed. For example, 'nontoxic strains' of *S. chartarum* sensu lato were producing atranones which, although are not cytotoxic, are potently inflammatory (Rand et al. 2006). Similarly, it was generally assumed that *P. corylophilum* was not notably toxigenic despite a lack of studies until recently (McMullin et al. 2014a, b). As illustrated by Figs. 1, 2, 3, 4, 5, 6, 7, 8 and 9, the metabolites from fungi isolated from building materials represent a breadth of structural diversity with subsequent biological activities. The development of high-resolution LC-MS/MS methods has allowed the determination of fungal toxins on building materials (Nielsen and Frisvad 2011) and in settled dusts (e.g. Täubel et al. 2011). Finally, a diverse array of metabolites from fungi common on building materials and triple-helical glucan have been tested for their effects on lung biology in relevant animal models. These have revealed that toxin doses that could be achieved in damp residential housing modulate genes that are in asthma pathways. The next decade of research will illuminate the significance of this information.

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