

Wall relative humidity: a simple and reliable index for predicting *Stachybotrys chartarum* infestation in dwellings

Abstract Because the indoor mold *Stachybotrys chartarum* has been considered as potentially responsible for serious health effects, its identification in dwellings with water damages is of utmost importance. As such dwellings are many, it would be of great value to have a simple and reliable index for predicting its presence. The aim of the study was to compare measurements of wall relative humidity (RH) to mold identification in 458 samples from 100 dwellings. Mold identification was performed by direct microscopic examination of a sample collected on the wall by the gummed paper technique. Mean ($\pm$ s.d.) wall RH (%) was much higher (97.0 ± 6.1) on the 30 samples where *S. chartarum* was identified compared with the 291 samples where other molds were identified (41.8 ± 36.9) and to the 137 samples where no molds were identified (38.9 ± 34.8). There was no straightforward relationship between wall and room RH. In conclusion, this study clearly demonstrate that the simple measurement of wall RH can be used as a reliable index for discarding and suspecting *S. chartarum* infestation in dwellings.

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Practical Implications

This paper suggests that very high relative humidity (RH) within walls is a strong risk factor for their infestation with the 'toxic mold' *Stachybotrys chartarum*. Besides, data from the literature demonstrate that other molds are able to produce mycotoxins when RH is very high. Thus, measurement of wall RH, which is easy to perform and very cheap, could be used as a screening tool to select those dwellings where mold identification should be performed and remediation should be promptly carried out.

Introduction

Among indoor molds, *Stachybotrys chartarum* deserves a special consideration because it has been considered as potentially responsible for serious deleterious health effects, including death in newborn babies (Kuhn and Ghannoun, 2003; Nielsen, 2003). Thus, its identification may have very important implications for the family, including a quick evacuation out of the damaged house. As molds and water damage are found in many buildings, i.e., a prevalence of typically 23–79% in tropically monsoon climate and 4–25% in cold areas such as Nordic countries (Bornehag et al., 2001), it would be of great practical interest to discard or suspect *S. chartarum* infestation on the basis of a simple indice and to turn to mycologic identification in

a limited number of houses. In this paper, we measured relative humidity (RH) inside the wall and correlated it with the identification of molds collected on the same wall.

Material and methods

Houses selected

Houses were selected in this study when a general practitioner or chest physician asked our institution to visit and study them because their inhabitants were suffering from a respiratory disease, which could be influenced by an unhealthy dwelling. From March to October 2002, our team visited 100 such dwellings located in or around Marseille, France.

The visit included: (i) a questionnaire on building and housing characteristics (results not reported here); (ii) a visual examination of the whole house, with a special attention to visible molds; (iii) measurement of temperature and RH in the room (Digital Hygrometer; Protimeter plc, Marlow, UK) and inside the wall (Protimeter Surveymaster; Protimeter plc) of a moldy room. In each step, three different measurements were performed and the mean value retained for analysis; (iv) identification of mural molds by sampling on the outer part of the moldy area using the gummed paper technique (Porto, 1953; Vanbreuseghem, 1954), which has proved much more reliable for identifying *S. chartarum* than sampling room air. The sampling material includes a cylindrical plastic book containing four glass slides covered by a transparent gummed paper. The latter is removed from the slide, gently applied on moldy areas, then transferred back to the slide for light microscopic examination. *Stachybotrys chartarum* identification relies upon characteristics structures such as phialides and conidiophores. Thus, identification of this genus species can be performed by microscopic examination (Haugland et al., 2001). All analysis were performed in the Mycologic Department of Louis Pasteur Scientific Institute, Brussels, Belgium. If in a given house, several rooms showed moldy areas, we took off a sample per room.

Statistical analysis

Because the issue was to identify a dwelling contaminated by *S. chartarum* in any of its room, we divided the houses into three subgroups: houses with at least one sample including *S. chartarum*, houses with other molds, houses without any identified molds. Mean ($\pm$ s.d.) RH was computed in each subgroup and compared using an analysis of variance technique. Two by two comparisons were performed using the Bonferroni–Dunn statistics. The level of significance was considered as $\alpha = 5\%$. A sensitivity analysis was carried out to identify the threshold level under which *S. chartarum* presence could be dismissed.

Results

In the 100 dwellings studied, 458 samples were performed. Overall 13 dwellings (13%) and 30 samples (6.5%) demonstrated *S. chartarum* molds on at least one of the samples collected. In all instances, *S. chartarum* was associated with other mold species. Table 1 shows the distribution of molds, which have been identified.

Figure 1 provides individual measurements of RH inside the wall in the three groups and Table 2 the analysis of between-subgroup differences. From Figure 1, it can be inferred that the negative predictive value for *S. chartarum* occurrence was 100% if RH was

Table 1 Distribution of mold species identified

Molds identified	Number in this category
<i>Acremonium</i> sp.	14
<i>Aerobasidium pullulans</i>	1
<i>Alternaria</i> sp.	10
<i>Alternaria alternata</i> gr.	1
<i>Apiospora</i> like	2
<i>Aspergillus</i> sp.	2
<i>Aspergillus fumigatus</i>	2
<i>Aspergillus glaucus</i>	9
<i>Aspergillus nidulans</i>	1
<i>Aspergillus niger</i>	3
<i>Aspergillus ochraceus</i>	1
<i>Aspergillus ustus</i>	2
<i>Aspergillus versicolor</i>	23
<i>Aureobasidium</i> sp.	2
<i>Chaetomium</i> sp.	15
<i>Chaetomium globosum</i>	4
<i>Cheimomyces</i> sp.	1
<i>Cladosporium</i> sp.	1
<i>Cladosporium sphaerospermum</i>	111
<i>Doratomyces</i> sp.	2
<i>Fusarium</i> sp.	2
<i>Fusarium solani</i>	1
<i>Mucor</i> sp.	1
<i>Penicillium</i> sp.	32
<i>Penicillium chrysogenum</i>	1
<i>Phoma</i> sp.	2
<i>Scopulariopsis</i> sp.	3
<i>Scopulariopsis cf. brevicaulis</i>	2
<i>Stachybotrys chartarum</i>	30
<i>Ulocladium</i> sp.	19
<i>Ulocladium botrytis</i>	17
Yeasts	3

<75%, while the positive predictive value, using the same threshold, was 67%. Table 3 demonstrates the mean ($\pm$ s.d.) RH in the main other groups of molds identified. All mean values were much lower than the one encountered in the *Stachybotrys* group but the scatter of the distribution was much larger.

Figure 2 provides the pictorial relationship between room and wall RH. Although the correlation between both variables was statistically highly significant ($P = 0.001$), the scatter of values was such that it precluded any use of the former parameter for predicting *S. chartarum* infestation.

Discussion

This paper demonstrates that measurement of wall RH is a very useful step in excluding or demonstrating *S. chartarum* infestation. The growth and metabolism of microorganisms demands the presence of water in an available form. The most useful measurement of the availability of water is water activity (a_w), which is the ratio of the water vapor pressure of a material or food to that of pure water at the same temperature. RH, like a_w , is the ratio of vapor pressures of solution and pure water, but is expressed as a percentage. To our know-

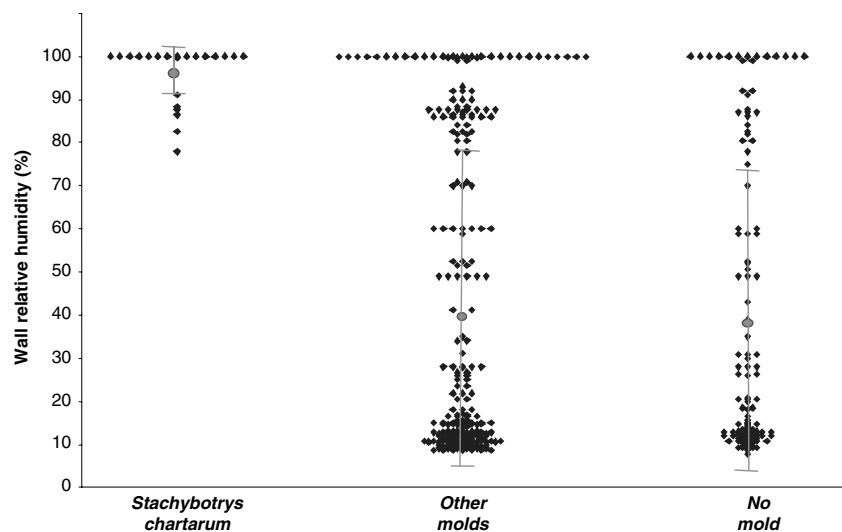


Fig. 1 Distribution of wall relative humidity measurements in the three subgroups

Table 2 Between-subgroup comparisons of mean (±s.d.) relative humidity

Subgroup	Mean	s.d.	Between-groups difference
<i>Stachybotrys chartarum</i> (n = 30)	97.0	6.1	$P < 0.001$ NS
Other molds (n = 291)	41.8	36.9	
No mold (n = 137)	38.9	34.8	

Table 3 Mean (±s.d.) relative humidity on walls where the main mold species (other than *Stachybotrys chartarum*) have been identified

Mold species	n	Mean	s.d.
<i>Aspergillus versicolor</i>	23	54.8	39.9
<i>Cladosporium sphaerospermum</i>	109	31.7	31.9
<i>Penicillium</i> sp.	32	44.9	38.2
<i>Ulocladium</i> sp.	19	39.9	31.0
<i>Ulocladium botrytis</i>	17	35.4	32.9

ledge, the association between mural RH and presence of *S. chartarum* has never been demonstrated in the field. However, the peculiar avidity of *S. chartarum* to water has already been pointed out. Northolt and Bullerman (Northolt and Bullerman, 1982) summarized from the literature the values of minimum a_w for growth of different fungal species under experimental conditions. The higher value, far above those of other molds, was equal to 0.94 (i.e. 94% RH) for *S. chartarum*. Other laboratory studies demonstrated that the minimum a_w for *S. chartarum* varies from 0.85 to 0.95 for germination and from 0.91 to 0.96 for growth and sporulation, depending on the temperature and medium (Ayerst, 1969; Domsch and Gams, 1972; Grant et al., 1989). Rowan et al. (1999), using previously published data and laboratory experiments, could draw curves relating temperature and RH requirements for the growth of different mold species on building materials. From their data, *S. chartarum* requires a 95% or 96% RH at temperatures varying from 19 to 24°C. Analysis of water-damaged building materials (Andersson et al., 1997) demonstrated that the dominant colonizers were *S. chartarum*, *Penicillium* and *Aspergillus* species. Increasing temperature and nutritional status of the substrate can lead to lower moisture requirements.

The demonstration of a close relationship between mural RH and growth of *S. chartarum* can have a wide practical usefulness because sanitary inspectors are used to perform this easy, reliable and very cheap measurement. If RH is higher than 75%, then a mycologic investigation is warranted because several different molds, among which *S. chartarum*, can be identified. From a practical standpoint, only 153 of 458 samples, i.e., 33% demonstrated wall RH higher than 75%. Thus, such a mycologic investigation would be performed in a limited number of houses, which is cost-effective. One could argue that other mold species,

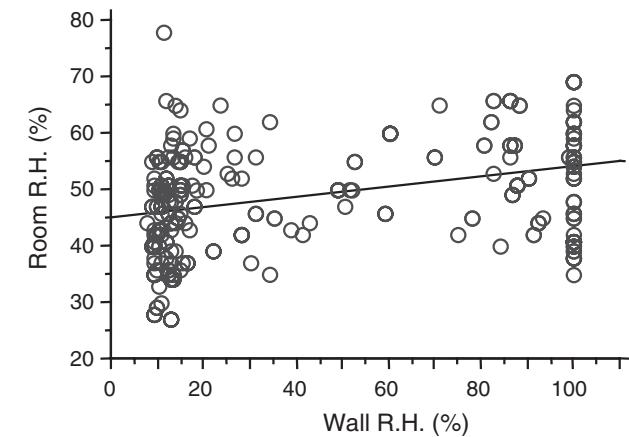


Fig. 2 Correlation between room and wall relative humidity

especially *Aspergillus* and *Penicillium*, which need less moisture to grow, may also release deleterious mycotoxins. However mycotoxin production occurs at higher RH levels than mold growth (Northolt, 1982). For example, ochratoxin A from *Aspergillus ochraceus* or *Penicillium verrucosum*, is released from an RH equal to 85–90% while growth of these molds is demonstrated from an RH equal to 80%. Similarly, release of patulin is demonstrated from an RH equal to 94% while growth of the molds involved in their production occurs from an RH equal to 82–85% (Northolt and Bullerman, 1982). Thus very high wall RH levels, such as those measured in our study, could allow both for mold growth and mycotoxin production from these molds. *Stachybotrys chartarum* obviously lies and proliferates in an extremely humid environment and is most sensitive to desiccation. Figure 1 and Table 3 clearly show that other molds can be identified on walls with very different RH levels, but mean mural RH in these instances was much lower than in walls with *S. chartarum* infestation.

The methods used in the study, both mycologic identification (Northolt and Bullerman, 1982) and RH measurements (Ayerst, 1969) are robust. The proportion of houses infested with *S. chartarum* is in keeping with other reports dealing with groups of moldy houses. Some authors (Andersson et al., 1997; Grant et al., 1989; Hunter et al., 1988; Rowan et al., 1999)

found *Stachybotrys* in 12.8% of dwellings and 4.5% of samples. Kuhn and Ghannoun (2003) report a similar frequency, while in other studies, *Stachybotrys* was present in < 3% of samples (Kozak et al., 1979; Kozak et al., 1980).

The lack of correlation between room RH and mold growth has already been pointed out (Adan, 1994; Becker, 1984; Grant et al., 1989; Gravensen et al., 1999; Hukka and Vittanen, 1999). This is so because local differences in ventilation and surface temperature can generate micro-climates with very high wall RH with an otherwise low room RH.

Conclusion

The proportion of moldy houses infested with *S. chartarum* in our area, as well as in other locations in Western countries, was equal to 13%. Because it is so high, the protocol used to investigate such cases should be rapid and cheap. From our results, we suggest that measurement of wall RH could be used to discard *S. chartarum* infestation when it is low or as a screening tool when it is high.

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