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Laboratory study to determine the critical moisture level for mould growth on building materials

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ABSTRACT

The susceptibility of building materials to mould growth varies. Some are tolerant to high relative humidity in the ambient air without mould growth occurring, while others are less tolerant, and mould can grow in relative humidity as low as 75%. Within a building, constructions are exposed to different temperatures and relative humidities. To minimise the risk of microbial growth, building materials should be chosen that are tolerant to the expected conditions. In this study, the critical moisture levels for ten building materials with a range of expected critical moisture levels (wood-based materials, gypsum boards and inorganic boards) were evaluated. Samples of the building materials were inoculated with spores from six species of mould fungi (*Eurotium herbariorum*, *Aspergillus versicolor*, *Penicillium chrysogenum*, *Aureobasidium pullulans*, *Cladosporium sphaerospermum*, *Stachybotrys chartarum*) and incubated in test cabinets at specified temperature (10 °C and 22 °C) and relative humidity conditions (75–95%); growth of mould was analysed weekly for at least 12 weeks. One of the conclusions is that two similar building materials or products may have considerably different resistance to mould growth, and so the results from one type of building material cannot be applied to the other. Also, in order to compare results from different tests, it is important to use the same test method. It is also important to state the temperature at which the critical moisture level applies and how long the material is exposed to the temperature and relative humidity conditions during the test.

1. Introduction

Mould is a colloquialism for a range of micro fungi belonging to different systematic categories. However, in some aspects they share common traits. They live on the surfaces of materials, produce airborne spores and use easily assimilated nutrients for growth. Moulds act as decomposers in the natural cycle, and their spores are found everywhere in the air and on various kinds of surfaces. When the right conditions are present, the spores germinate and hyphae grow to form a mycelium. This process may occur in parts of a building construction and on interior surfaces, with risks that the indoor environment and human health may be adversely affected. The costs associated with this growth, e.g., due to renovation, are substantial. There are both economic and health arguments for reducing the risk of mould growth in buildings.

Conditions for mould growth include nutrient availability, temperature, pH, and moisture. In general, the availability of water in the material is regarded as the crucial element for growth to occur. The water available to microorganisms is often referred to as water activity, A_w. It is defined as the vapour pressure in the substrate divided by that of pure water at the same temperature.

Each fungal species has a minimum requirement for availability of water to grow, and species can be divided into groups depending on the amount of moisture needed for growth. The minimum A_w for hydrophilic fungi is 0.9, while for the most extreme xerophiles it is 0.75. Moderately xerophilic fungi begin to grow at a water activity of 0.75–0.79, and slightly xerophilic fungi at 0.80–0.89 (Lacey et al., 1980). These levels are based on growth experiments on nutrient medium, where nutrient conditions are optimal. For building materials, where nutrient availability is not as good, the requirement for available moisture is probably slightly higher (Flannigan and Miller, 2001). Moisture requirements are also related to temperature; at lower temperatures, the fungus requires more available water to germinate and grow (Ayerst, 1969).

Air always contains a certain amount of water vapour, but the maximum vapour content depends on temperature. Relative humidity (RH) is defined as the current vapour content in relation to the vapour content at saturation, expressed as a percentage. Building materials stand in relation to the ambient air, from which

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they can absorb moisture, or to which they release moisture. When equilibrium is reached between material and ambient air, water activity in the material is RH/100 (Flannigan and Miller, 2001).

The susceptibility of building materials to mould growth varies. Some materials tolerate being in air with high relative humidity without mould growth occurring, while on others mould can grow at a relative humidity as low as 75%. Numerous studies have attempted to identify the temperature and humidity conditions in which different types of building materials begin to mould (e.g. Ritschkoff et al., 2000; Nielsen et al., 2004; Hofbauer et al., 2008). However, much remains to be learned about the complex relationship between mould growth on building materials and factors such as temperature, humidity and time. In addition, new products are constantly being developed, and their resistance to mould is unknown.

Within a building, the humidity and temperature is expected to vary from one construction to another. To minimise the risk of microbial growth, materials should be chosen that can tolerate the prevailing conditions. Materials manufacturers should be able to determine and account for a material's critical moisture level with respect to mould growth; that is, the moisture level above which there is a risk of mould developing. To the best of our knowledge, there is no standardised testing method to determine critical moisture level. Test methods are available that assess the resistance of a material to mould at high humidity levels (at least 90–95%), but these methods are not directly applicable to lower humidity levels.

This study aimed to investigate mould growth on building materials in different temperature and relative humidity conditions. Samples of ten building materials commonly found on the Swedish market were inoculated with mould spores and incubated in test chambers; growth of mould was analysed weekly for at least 12 weeks. The results, together with results from field tests, will be the basis for a test method to determine the critical moisture level of a material.

2. Materials and methods

2.1. Building materials

Ten building materials commonly used in new Swedish buildings were examined in the study. They were selected in collaboration with damage investigators at SP Technical Research Institute of Sweden and experienced buyers at building construction companies. The materials were expected to vary in critical moisture level (Table 1).

Three boards of each material were bought from a local building supply store and cut into test pieces of size 50 × 100 mm. There were four replicates of each board in each humidity and temperature combination studied, and thus a total of twelve replicates of each material. The test specimens of asphalt paper all came from one roll. All materials were handled in such a way as to minimise risk of contamination that might lead to mould growth.

2.2. Fungal species

Different species of fungi often occur together on the materials used in building (Hyvärinen et al., 2002; Andersen et al., 2011). To emulate real-life situations, a mixture of spores from six fungal species was used in the study (see Table 2). These species frequently occur on different types of building materials in damp houses (Hyvärinen et al., 2002; Wessen, 2006; Nilsson et al., 2009; Andersen et al., 2011), vary in their water requirements and represent different groups in the successional colonisation order (Grant et al., 1989). Freeze-dried strains from each of the fungi were provided from Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). They were treated according to the instructions from CBS and cultivated in Petri dishes with malt agar (20 g agar and 20 g malt extract to 1000 ml water) until sporulation occurred.

Table 1
Building materials used in the study, showing expected critical moisture levels, based on a proposal in Johansson et al. (2005). The references in the right column refer to the studies which, together with experience, are the basis for the proposed critical moisture conditions.

Material	Material description	Expected critical moisture level, % RH (Johansson et al., 2005)	References
Cement-based board	8 mm cement-based board consisting of cement, limestone, and cellulose fibres, covered with a plastic dispersion	90–95	Nielsen et al., 2000 Ritschkoff et al., 2000 Nielsen et al., 2004 Viitanen, 2004
XPS insulation board	50 mm extruded polystyrene insulation board	90–95	Authors' estimation
Glass fibre board	15 mm rigid glass wool insulation board	90–95	Chang et al., 1995 Nielsen et al., 2000 Nielsen et al., 2004 Viitanen, 2004
Asphalt paper	1.5 mm windproof barrier of asphalt-impregnated cellulose paper	90–95	Authors' estimation
Wet-room gypsum plaster board	13 mm gypsum board with cardboard surfaces	80–85	Pasanen et al., 1992
Exterior gypsum plaster board	13 mm gypsum board with cardboard surfaces	80–85	Ritschkoff et al., 2000 Nielsen et al., 2000 Doll and Burge 2001 Horner et al., 2001 Nielsen et al., 2004
Plywood	12 mm softwood plywood	75–80	Wang, 1992
Thin hardboard	3.2 mm high-density hardboard made of wood fibres and lignin	75–80	Ritschkoff et al., 2000 Pasanen et al., 2000
Chipboard	12 mm particle board	75–80	Nielsen et al., 2000 Nielsen et al., 2004
Pine sapwood	19 mm tongued and grooved board	75–80	Hallenberg and Gilert, 1988 Viitanen and Ritschkoff, 1991 Pasanen et al., 1992 Nielsen et al., 2000

Table 2
Mould species used in the study.

Species	Strain used in this study		A_w minima for growth on 2% malt extract agar (Grant et al., 1989)	
	CBS number[a]	Origin	Temperature	
			12 °C	25 °C
Eurotium herbariorum	115808	Interior mortar (cement), Germany	0.82[b]	0.78[b]
Aspergillus versicolor	117286	Wall in bakery, Netherlands, 2005	0.83	0.79
Penicillium chrysogenum	401.92	Gypsum, Netherlands, 1992	0.79	0.79
Aureobasidium pullulans	101160	Window frame, Sweden, 1998	0.87	0.89
Cladosporium sphaerospermum	122.63	Betula plywood, Finland, 1997	0.83	0.84
Stachybotrys chartarum	109.292	Building material, Finland, 2000	0.91	0.93

[a] CBS numbers refer to strains maintained by Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
[b] Growth on flow wheat-sucrose agar (Abellana et al., 1999).

2.3. Inoculum preparation

In order to make each test reproducible, a suspension of spores was prepared in a standardised way, mainly according to MIL-STD-810G (Department of Defense, 2010). First, 10 ml of distilled, autoclaved water was poured onto each of the subcultures. The surface of the fungi was scraped to liberate spores into the water, and the liquid was then poured into a sterile flask containing glass beads and 45 ml of autoclaved water. One flask was used for each species. The flask was shaken to liberate the spores from the conidiophores, and the contents were then filtered through sterile glass wool, contained in a glass funnel, into a centrifuge tube. The suspension was centrifuged until a spore pellet was formed. The supernatant was poured off, and the spores were washed with distilled, autoclaved water; the solution was then centrifuged in the same manner as before. This procedure was repeated three times, the aim being to wash out any nutrients from the agar that could affect the test results and to avoid hyphae in the final solution.

The spore concentration in the final washed residue for each species was determined using a counting chamber (Bürker, Marienfeldt, Lauda-Königshofen, Germany). The residue was then diluted so it contained approximately 10^6 spores per ml. The final spore suspension was prepared by mixing equal volumes of suspension from each species.

2.4. Inoculation of test specimens

A volume of 0.4 ml of the spore suspension was sprayed onto one surface of each test specimen by using an airbrush (Claes Olson Model AB-119, Insjön, Sweden) attached to a Minicompressor (Cotech, Claes Olson, Insjön, Sweden) with a pressure regulator with water separator. The working pressure was 2 bar. During spraying, the airbrush was swept along at an even speed. The aim of spraying the suspension on the surface was to distribute the spores more or less evenly over the surface of the test pieces.

2.5. Incubation

2.5.1. Incubation chambers

Following inoculation, the test specimens were incubated horizontally in the dark in Climate test chambers (CTS C-20/350, CTS GmbH, Hechningen, Germany). Air with the desired relative humidity and temperature streamed over the test pieces at a velocity of 0.3–0.5 m/s. The chambers were calibrated regularly (and adjusted when needed) by an accredited consultant (CTS, Alingsås, Sweden) to ensure correspondence between the set point, displayed value, and actual value of relative humidity and temperature.

2.5.2. Registration of incubation conditions

An external humidity and temperature transmitter (Vaisala HUMICAP® HMT330, Helsinki, Finland) was mounted in each of the chambers. The values of temperature and relative humidity were saved in a computer-based program (Exomatic) every 5 min. The setup made it possible to monitor the stability of these values, and to calculate their means and standard deviations during the incubation time.

The transmitters were calibrated regularly at an accredited laboratory (SP Technical Research Institute, Energy Technology, Borås, Sweden). The recorded data were adjusted according to the results of the calibrations. Early in the test period, the sensors in the transmitters drifted more than expected, and after one year's use showed RH values up to 11% above the target values. The calibrated values were adjusted for the drift, which was calculated for each measuring point. The sensors were later replaced by new ones, which were stable. During the whole test period, the temperature and relative humidity in the chambers was also monitored by regular manual reading of the displays in the moisture chambers. For one of the cabinets, it was difficult to estimate the drift, and therefore the mean value from the manual readings was used to describe the incubation conditions.

The measurement uncertainty was calculated for each humidity and temperature combination tested, based on calibration data, according to EA-4/02.

2.5.3. Incubation conditions

The materials were tested in ten specific temperature and humidity settings (Table 3), with the test period originally set to 12 weeks. After 12 weeks of incubation and weekly assessments of growth, there was no established growth on the test pieces of wood

Table 3
Relative humidity and temperature at which materials were tested. Maximum measurement uncertainty is 2.5% for RH and 0.2 °C for the temperature.

Mean value (standard deviation)		Maximum incubation time (weeks)
RH (%)	T (°C)	
75 (0.1)[a]	10 (0.0)[a]	12
85 (0.5)	10 (0.0)	12
90 (0.8)	10 (0.1)	12
95 (0.9)	10 (0.3)	12
93[a,b]	10[a,b]	12
75 (0.5)[a]	22 (0.1)[a]	12 + 20[c]
79 (1.4)	22 (0.3)	12 + 7
85 (1)	22 (0.6)	12
89 (0.7)	22 (0.2)	12
95 (0.3)	22 (0)	12

[a] Values are based on manual readings.
[b] Standard deviation is not available.
[c] During the additional 20 weeks, samples were incubated over saturated salt solution at about 76% RH and 23 °C.

or wood-based boards at 75% RH. As mould growth on wood is expected in this relative humidity, the tests at 75% and 80% RH and 22 °C were continued for some additional incubation time.

Prior to inoculation and incubation, six of the test specimens from each material were dipped in sterile water for 20 min; the other six were just sprayed with the solution before incubation. The purpose of the dipping was to assess the effect of a shorter period of flood or rainfall.

2.6. Assessment of mould growth

Mould growth on the inoculated surface of each test sample, excluding the edges, was assessed once a week. The samples were then analysed under a stereo microscope at 10–40× magnification. During this procedure, it was important to use low-angle light to detect hyaline as well as dematiaceous hyphae. The mould growth was assessed according to the rating scale shown in Table 4.

In order to minimise further contamination with spores and dirt, which could enhance the risk of mould growth, the analyses were performed in a laminar airflow (LAF) bench and the test pieces were handled with gloves.

2.7. Validation of ratings

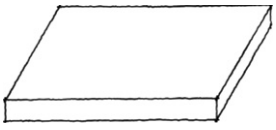
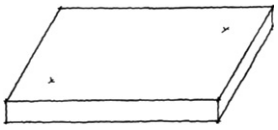
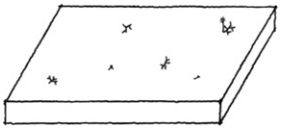
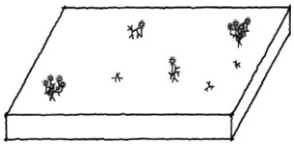
The method of analysis was non-destructive, since the studied surfaces were not touched during analysis. This made it possible to follow the mould growth on the same test piece during the entire study. A limitation of the method is that it is somewhat subjective, as different raters will vary in their assessment of the extent of mould. To investigate the amount of variation between the raters, a comparative study was performed. Four persons, trained and well experienced in analysing mould growth on materials, analysed 63 test pieces, independently of each other. Mould growth from all of the rating grades in Table 4 was expected to be represented on the test pieces.

Since the classification was based on human judgement, the obtained values cannot be regarded as numerical values, and so statistical measures such as average and standard deviation are not appropriate for analysis. In order to still control the measurement uncertainty, a new idea was implemented, based on simulations from a calibration matrix: Each judgement in the calibration procedure was compared to a "true" value, and the relative frequencies of the judgements given for each true rating were collected in a matrix, i.e. the number in the matrix position ij represents the probability of judging a rating j when the true rating is i. Simulations from this matrix then allowed estimation of the overall measurement uncertainties. The calibration indicated that the variation among operators was negligible, and therefore all observations were regarded as independent. As the "true" rating was unknown, the median of the four assessments of each test piece was defined to be "the truth". Since an even number (six) of assessments was performed, there was a problem with defining the median in cases with two non-equal middle values. The usual way of taking the average is not possible with non-numeric values. The problem was solved in this particular case by taking the average value of a large number of relative frequency matrices, each generated by taking random choices of truth in cases of ambiguity.

Simulations from the matrix cannot produce confidence intervals from the measurement uncertainty, but they make it possible to assess how many test pieces must be used to obtain a confidence of 95% for the median of ratings.

In particular, we were interested in the simplified judgement, "Has the test piece failed or not?" where "failure" is defined as a test piece having a rating of ≥ 2 and "non-failure" a rating of <2.

Table 4
Rating scale for the assessment of mould. The analysis is performed in microscope at 40× magnification. The growth may not be visible to the naked eye. The illustrations are intended to give an idea of how each rating might look like.

Rating		Description of extent of growth
0		No mould growth.
1		Initial growth, one or a few hyphae and no conidiophores.
2		Sparse but clearly established growth; often conidiophores are beginning to develop.
3		Patchy, heavy growth with many well-developed conidiophores.
4		Heavy growth over more or less the entire surface.

Another question was, "What is the confidence level for the rating of the six test pieces that were used in the study?" Again, the even number posed a problem in defining the median. In order to prioritise the discovery of failure, in cases where the two middle values were equal, we chose to define the median as the larger value.

2.8. Definition of critical mould growth and critical moisture level

The results were analysed based on the simplified judgement given in the previous section 2.7. A test piece was considered to have failed when the rating of mould growth first reached 2 or higher. Two alternative definitions were then used to determine the critical mould level: (a) when the median growth of the six test pieces was equal to or exceeded rating 2, and (b) when the rating of at least one of the six test pieces was equal to or exceeding rating 2.

The tests were carried out at constant RH, with RH set at intervals of 5%, with two exceptions. The critical moisture level therefore fell into a range, with the upper limit determined by the case with lowest RH where any of the above criteria were met, and the lower limit by the case with the next-lowest RH. For example, if the criteria for critical mould levels were met at 80% RH, the critical moisture level for this particular material was assumed to be in the range of $75\% < RH_{crit} \leq 80\%$.

2.9. Description of mould development by time

Mould development by time is described in two ways: the median of the weekly assessments and Kaplan–Meier curves, which show the percentage survival of the samples as a function of time. The former is a traditional way of describing data while the latter is a more modern approach that has many advantages (Singer and Willett, 2003).

Survival in this particular case is defined as there being no established growth on a sample; that is, a rating below 2 according to Table 4. Once a sample had received a rating of 2 or higher for the first time, it was considered to be "dead"; that is, it had reached critical mould growth. On each occasion that a test piece failed, the percentage of surviving specimens decreased. Samples that did not fail during the test period were censored in the plots.

3. Results

The materials most susceptible to mould growth were pine sapwood and plywood, followed by chipboard, thin hardboard, plaster boards and asphalt paper. No growth was detected on any samples of glass fibre board, cement-based board, or extruded polystyrene boards in any of the conditions tested.

Mould development according to definition (a) is shown in Figs. 1 and 3 as the median of the weekly assessments at 22 °C and 10 °C respectively. Figs. 2 and 4 present the results according to definition (b) of tests at 22 °C and 10 °C as Kaplan–Meier curves. No plots are shown for materials where there was no growth in any of the RHs tested. At 22 °C these materials comprised cement-based board, XPS insulation board, and glass fibre board. At 10 °C there was also no growth on asphalt paper, wet-room gypsum board, or exterior plaster board.

Table 5 presents the estimated critical moisture levels, based on twelve weeks incubation, for the materials tested. The maximum relative humidity in the test was 95%, and so the critical level for materials that did not show any mould growth during the test period was above this value. The lowest RH at which mould growth appeared was 80%. Some test pieces that showed no growth during the twelve weeks of incubation did show mould growth when incubated for additional time at 75% or 80% RH and 22 °C (Figs. 5 and 6).

Mould growth was not affected by wetting the test pieces prior to incubation; the critical moisture level was the same as for the non-wetted material.

There was correspondence between the values for critical moisture levels elicited using the two different criteria for critical moisture, with three exceptions at 10 °C. However, the time before the critical moisture level was reached varied depending on which of the criteria were met, as can be seen in Table 6. Exactly when this level was reached is not known, since the analysis was performed only once a week; the time is therefore presented as a range. Table 7 presents an analysis of the week when growth was first seen at each RH.

On the basis of simulations from the estimated matrix performed according to section 2.7, we concluded that a correct judgement of mould growth with 95% confidence could be achieved by taking the median of seven judgements. However, only six test pieces were used in this study. A test piece was considered to have failed when it had reached a rating of 2 or higher. For this simplified judgement between a failed and non-failed test piece, a correct judgement of failed pieces was made with 97% confidence. This higher confidence, compared to the case with seven pieces, was obtained at the price of a higher risk of misjudgement in the other direction: namely, a correct judgement of non-failed pieces was made with only 90% confidence.

4. Discussion

Based on a literature review, critical moisture levels for different groups of building materials have previously been proposed (Johansson et al., 2005). Sometimes the results presented in this article are consistent with the results from the studies that formed the basis for the proposal, as presented in Table 1, but sometimes they are not. Where differences exist, they may be due to variations in the sensitivity of the individual materials to mould, despite belonging to the same group of materials (e.g. wood-based panels). Other reasons for these differences include variations in the setup of the experiments and/or variations in evaluation of the data. Factors that vary among the different experiments include the fungi used, inoculation method, temperature, relative humidity, duration, analytical method and frequency of analyses. Studies also vary in their assessments of when growth is considered to be critical. Following is a discussion of how a number of these factors can influence the critical moisture level attributed to a material, in light of the results and experiences from the present study.

To determine the critical moisture level of a material, it is necessary to test it at different humidity levels. The critical moisture level will then lie somewhere between the two closest humidity levels tested. For example, with 12 weeks of testing at 22 °C, no mould growth was established on plywood at 75% RH, but mould did appear at 80% RH. The critical moisture level is therefore between 75% and 80%. This study used RH levels differing by 5 percentage points, with two exceptions. The fewer percentage points between two tested humidity levels, the narrower the interval for RH_{crit}. However, measurement uncertainty limits how narrow these intervals may usefully be. In our case, the uncertainty was at most 2.5 percentage points RH, so settings of RH in ranges smaller than 3 percentage points became irrelevant. To ensure stable conditions during the tests and to minimise measurement uncertainty, it is important to use test chambers that are stable and to continuously log the temperature and relative humidity with calibrated sensors.

The duration of an experiment is important, since the period needs to be long enough for mould to have time to germinate and grow. Testing over a long period increases the risk of mould growth (see Figs. 5 and 6). Viitanen tested a number of materials over a long

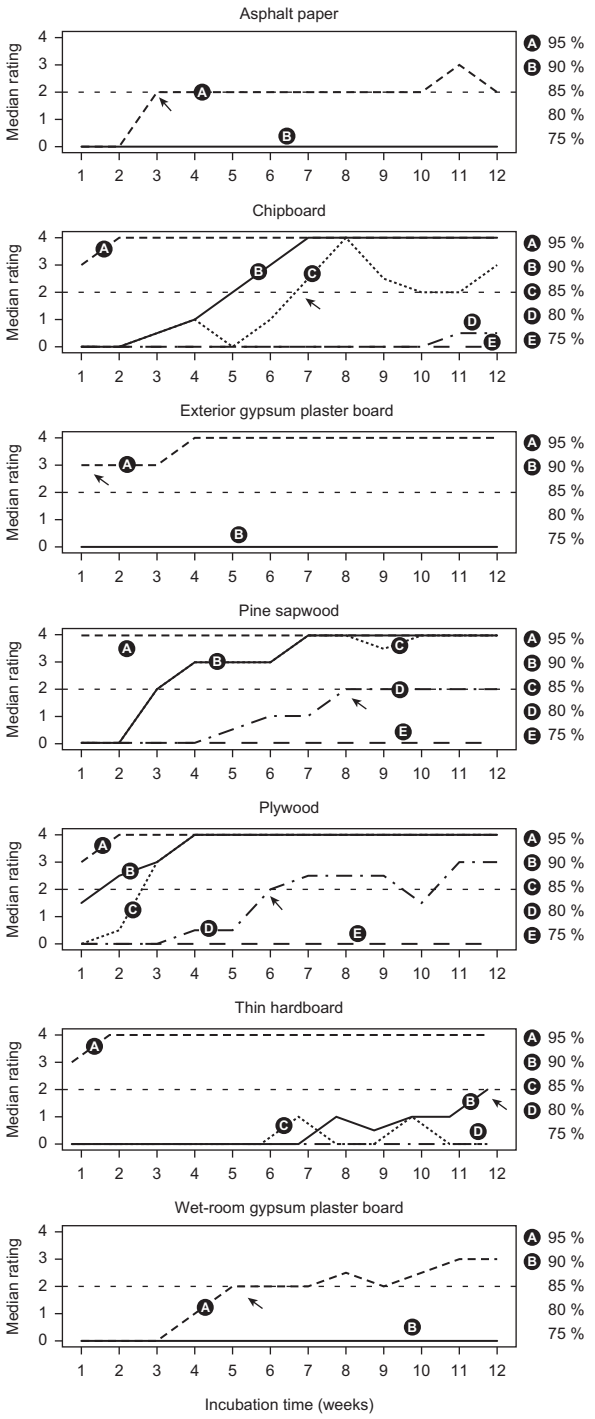


Fig. 1. Median value of mould growth on test pieces ($n = 6$) of building materials at different RH at 22 °C during 12 weeks. The critical moisture limit is reached when the median ≥2, represented as a horizontal dotted line. The arrow indicates the point when this is reached.

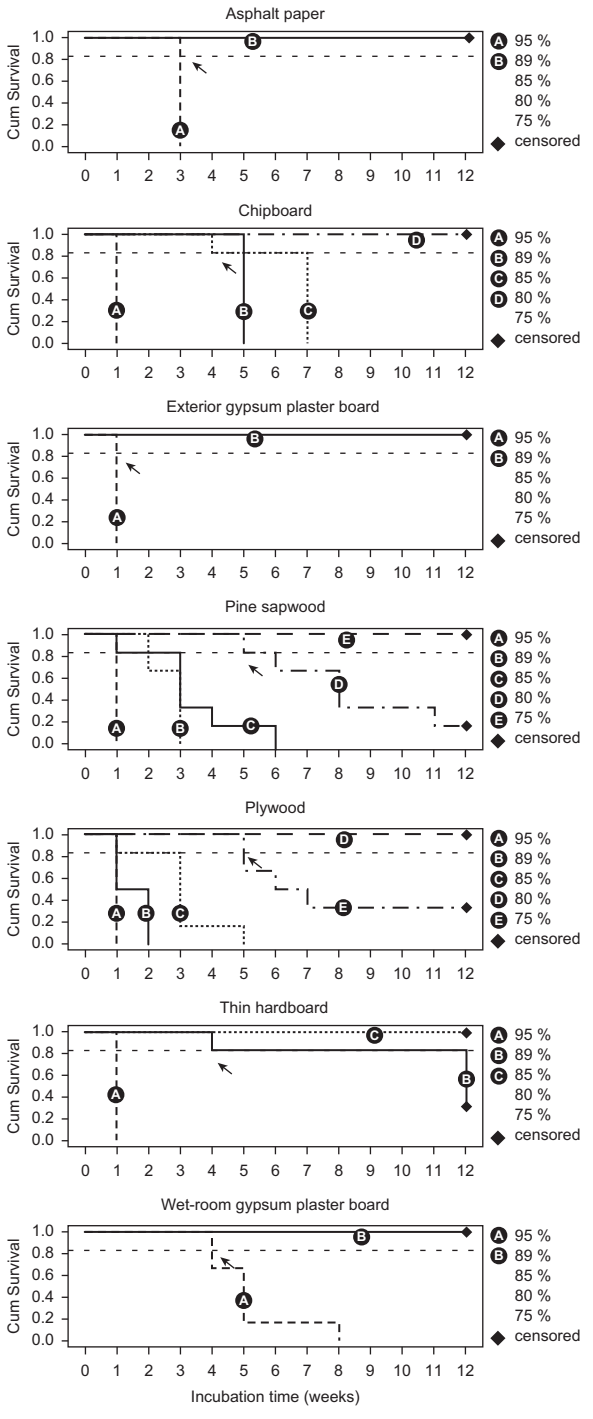


Fig. 2. Survival functions of mould growth on test pieces ($n = 6$) of building materials at different RH at 22 °C during 12 weeks. The critical moisture limit is reached when at least one of the test pieces reaches mould growth ≥2, represented as a horizontal dotted line. The arrow indicates the points when this is reached.

period, and growth did not occur on some of the materials until several months had passed (Viitanen et al., 2010) However, one can reasonably assume an upper limit for when the inoculated spores cease to be viable. Also, for practical reasons it is not possible to test over too long a period, because the results should be provided within a reasonable time. According to Hofbauer et al. (2008), 100 days is a reasonable upper level for a single test.

Duration of incubation will influence the critical moisture level of the material being tested. In our study, no growth was found on any of the materials tested after 12 weeks at 75% RH, but mould began to grow on plywood after 16 weeks and on pine sapwood after 32 weeks. The critical moisture level was thus reduced to below 75%, from having been between 75% and 80% RH at 22 °C. Had the test been allowed to continue for longer than 12 weeks in

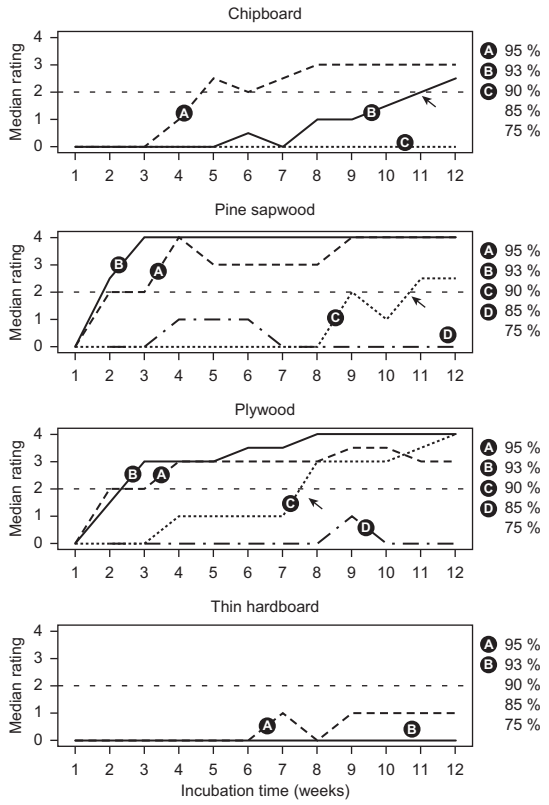


Fig. 3. Median value of mould growth on test pieces ($n = 6$) of building materials at different RH at 10 °C during 12 weeks. The critical moisture limit is reached when the median ≥2, represented as a horizontal dotted line. The arrow indicates the point when this is reached.

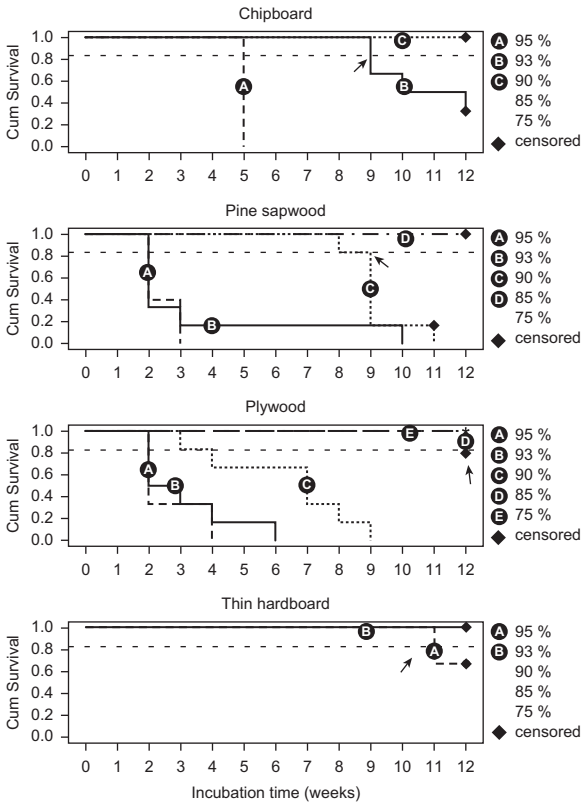


Fig. 4. Survival functions of mould growth on test pieces ($n = 6$) of building materials at different RH at 10 °C during 12 weeks. The critical moisture limit is reached when at least one of the test pieces reaches mould growth ≥2, represented as a horizontal dotted line. The arrow indicates the point when this is reached.

all the settings, it is possible that the critical moisture level would have been reduced also for some of the other materials. However, had incubation time been shorter than 12 weeks, the critical moisture level for some materials would also have been different.

As shown in Table 7, it took longer to achieve critical mould growth at a lower RH than at a higher RH. For chipboard, the time to critical moisture level at 22 °C was about 5 times longer at 90% than at 95%. The corresponding figure for pine sapwood was 3 times, while plywood showed no difference between 90% and 95%. It is therefore impossible to make a general prediction of how much longer a test needs to continue at a lower moisture level compared with one at a higher level to achieve the same results; this is material-specific.

When describing the critical moisture level of a material, temperature is also an important factor. At lower temperatures, the minimum RH level at which mould grows is expected to be higher than at higher temperatures (Flannigan and Miller, 2001); this was confirmed in the present study. For example, the critical moisture level of chipboard was between 80% and 85% at 22 °C, whereas at 10 °C it was between 90% and 93%. However, this does not mean that mould cannot grow at lower moisture levels, but again the incubation time may affect the critical moisture limit since growth is slower at lower temperatures. The results show no clear patterns for how much longer it takes for mould to become established at 10 °C than at 22 °C. Differences were found among different materials and different relative humidity levels. One explanation for the lack of pattern is that the analysis sessions were separated by one week, which may have been too long, especially in conditions that are favourable for the growth of mould fungi and where mould can become established within a few days. Another possible explanation is that the individual fungal species in the spore suspension differ in their ability to germinate and grow at different temperatures, and that these species differ regarding growth rate.

Mould grows on a surface in part through hyphal extension over the entire surface, and in part because the biomass increases at various places on the surface. We have followed mould growth both in terms of distribution over the surface and as biomass with a method that made it possible to study each sample on each occasion without affecting mould growth. Growth that can only be seen under the microscope and growth that is visible to the naked eye were assessed in the same way. This analytical method is common to many test methods and prior studies, but the

Table 5
Range in which critical moisture level is expected, based on results from 12 weeks incubation. Results are based on both median growth, criterion (a) and Kaplan–Meier estimation, criterion (b).

	22 °C	10 °C
Pine sapwood	$75 < RH_{cr12w} \leq 80$	$85 < RH_{cr12w} \leq 90$
Plywood	$75 < RH_{cr12w} \leq 80$	$75 < RH_{cr12w} \leq 85$[a,b]
Chipboard	$80 < RH_{cr12w} \leq 85$	$90 < RH_{cr12w} \leq 93$
Thin hardboard	$85 < RH_{cr12w} \leq 89$	$93 < RH_{cr12w} \leq 95$[a,c]
Wet-room gypsum plaster board	$89 < RH_{cr12w} \leq 95$	$95 < RH_{cr12w}$
Exterior gypsum plaster board	$89 < RH_{cr12w} \leq 95$	$95 < RH_{cr12w}$
Asphalt paper	$89 < RH_{cr12w} \leq 95$	$95 < RH_{cr12w}$
Cement-based board	$95 < RH_{cr12w}$	$95 < RH_{cr12w}$
Glass fibre	$95 < RH_{cr12w}$	$95 < RH_{cr12w}$
Extruded polystyrene	$95 < RH_{cr12w}$	$95 < RH_{cr12w}$

[a] This is based on the criterion (b).
[b] When using the criterion (a) concerning median rating ≥2, the result was $85 < RH_{cr12w} \leq 90$.
[c] When using the criterion (a) concerning median rating ≥2, the result was $95 < RH_{cr12w}$.

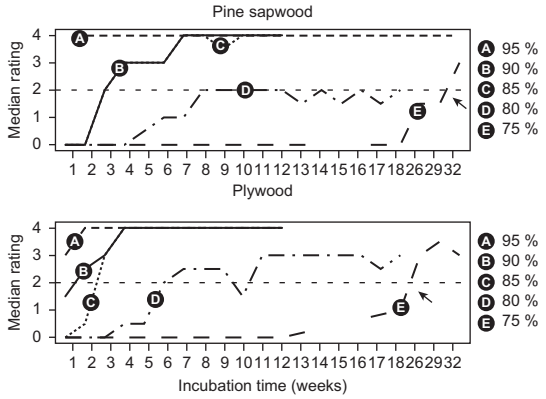


Fig. 5. Median value of mould growth on test pieces ($n = 6$) of building materials at different RH at 22 °C when incubation was extended to more than 12 weeks. The critical moisture limit is reached when the median ≥2, represented as a horizontal dotted line. The arrow indicates the point when this is reached.

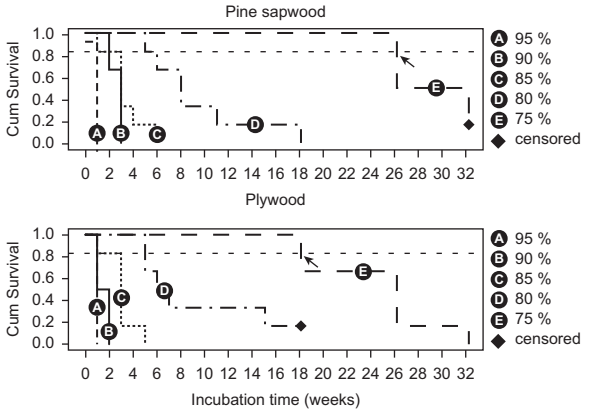


Fig. 6. Survival functions of mould growth on test pieces ($n = 6$) of building materials at different RH at 22 °C when incubation was extended to more than 12 weeks. The critical moisture limit is reached when at least one of the test pieces reaches mould growth ≥2, represented as a horizontal dotted line. The arrow indicates the point when this is reached.

assessment criteria for growth are somewhat different. Researchers often assess distribution in terms of percentage of surface and assume that a high percentage of distribution causes discolouration. However, even when nothing can be seen with the naked eye, the entire sample may be completely overgrown with mould. Furthermore, percentage of spread says little about development of biomass. Consequently, weak growth over the entire sample yields a higher percentage, even though growth is only in the initial stages. Strong, but patchy, well-established growth would yield a low percentage.

One limitation of the method we chose is that to some extent it is subjective, so different observers may sometimes assess the extent of growth on the same sample differently. The assessment can also vary for each individual analyst, as shown by the fluctuating median levels in Figs. 1, 3 and 5. When using subjective assessment, it is important to train and calibrate the people who will be performing the assessments, in order to achieve assessments that are as uniform as possible. A sufficient number of samples are expected to have a larger confidence interval for the assessments, and we have determined that a minimum of seven samples provides a 95% level of confidence. If a larger number of samples are used, the number should be odd in order to obtain unambiguous median values.

A non-destructive analytical method in which the assessment is objective would obviously be preferable. One conceivable method of this kind would be photography and digital image analysis. Frühwald et al. (2008) concluded that good correlation exists between assessments made through visually visible growth (i.e. fungi causing discolouration) and image analysis of wood samples. However, Van den Bulcke et al. (2006) argue that it is difficult to form groups based on computer analysis that are comparable to human visual assessment. It is also difficult to use this method to assess the extent of hyaline fungi (i.e. fungi without pigment), since their growth causes no visible discolouration.

Different species of fungi will grow on various building materials although the climate conditions are the same (Nielsen et al., 2004). Also, different species have different moisture requirements (Block, 1953). A test method that can be considered applicable to all types of building materials and under different climatic conditions should therefore include a mixture of fungi. The composition of the spore solution in this study represents species that commonly occur in moisture-damaged building materials and that have both high and low moisture requirements.

Mould should be acceptable in a building to a limited extent, provided conditions do not allow further growth. However, there is a theoretical limit for how much growth is acceptable. This threshold is influenced by where in the building growth can be found, which reflects the risk of affecting the indoor environment. No consensus currently exists on how much mould growth should be allowed and still considered acceptable. In this study, the definition of failure of a test piece was when the mould growth was class 2 or higher, representing the critical level for unacceptable growth. We observe that it is not until then that it is possible to show an established growth with the method of analysis that we have used. The level of judgement uncertainty concerning the class 1 assessment was excessively high in this study.

The study involved two methods to describe the development of growth and the point at which the critical moisture level was reached. Method (a) describes growth by considering medians of assessments for each sample in relation to time. This description provides an opportunity to see how development of mould occurs and describes the extent of growth. It is also analogous to other studies that describe mould growth over time. The critical moisture level was achieved once the median of the assessments reached at least 2 for the first time. However, this method of analysing results provided no information about spread in the assessments for each material.

Method (b) considers a sample to have failed when it is first given a rating of 2 or more, in which case it is not further analysed. The critical moisture level for the material is considered to be reached when at least 10% of samples show at least class 2 growth. In this experiment, we used six samples, which meant that growth in one sample (17%) was enough to fail a material. This method of assessing how well a material resists growth provides an opportunity to set requirements for what is acceptable in practice. When the tolerance level is higher; that is, if a higher percentage of samples in a material package can be accepted, the limit can be changed. The threshold for acceptable growth involvement of the sample can be changed; for example, it can be raised to 3 or lowered to 1.

One way to understand the difference between methods (a) and (b) is to identify two sources for the variation between observations of the same material: one is judgement uncertainty, the other is material variation. In case of no judgement uncertainty, method (b) is based on the worst case of six and may be a reasonably conservative estimate of the material property. Method (a) is instead based on the estimated median of the material's behaviour. However, in case of no material variation, method (b) underestimates the true critical level, since the worst case is solely caused by

Table 6
Time, expressed as weeks, when critical moisture level was reached.

Material	Temperature			
	22 °C		10 °C	
	Criterion (a), median value ≥ 2	Criterion (b), first rating ≥2	Criterion (a), median value ≥ 2	Criterion (b), first rating ≥2
Asphalt paper	$3 < w \leq 3$	$2 < w \leq 3$	$12 \leq w$	$12 \leq w$
Cement-based board	$12 \leq w$	$12 \leq w$	$12 \leq w$	$12 \leq w$
Chipboard	$6 < w \leq 7$	$3 < w \leq 4$	$10 \leq w \leq 11$	$8 < w \leq 9$
Exterior gypsum plaster board	$0 < w \leq 1$	$0 < w \leq 1$	$12 \leq w$	$12 \leq w$
Extruded polystyrene board	$12 \leq w$	$12 \leq w$	$12 \leq w$	$12 \leq w$
Glass fibre board	$12 \leq w$	$12 \leq w$	$12 \leq w$	$12 \leq w$
Pine sapwood	$7 < w \leq 8$	$4 < w \leq 5$	$10 \leq w \leq 11$	$7 < w \leq 8$
Plywood	$5 < w \leq 6$	$4 < w \leq 5$	$7 \leq w \leq 8$	$11 < w \leq 12$
Thin hardboard	$11 < w \leq 12$	$3 < w \leq 4$	$12 \leq w$	$10 < w \leq 11$
Wet-room gypsum plaster board	$4 < w \leq 5$	$3 < w \leq 4$	$12 \leq w$	$12 \leq w$

judgement error, while method (a) still is based on the median material behaviour. Therefore, the method could be chosen according to a judgement of the ratio between judgement error and material variation.

If a building material has high moisture content, mould may begin to grow even when the humidity is relatively low (Horner et al., 2001; Menetrez et al., 2004). The critical moisture level is therefore expected to be lower for wetted materials. However, we were not able to confirm this finding in our study. One reason could be that the time for moistening the sample, 15 min, was too short. A more likely explanation is that the high air exchange rate in the climate test chambers quickly achieved equilibrium between the surface of moistened samples and the prevailing conditions in the chambers. The surfaces will therefore have become comparable to the surfaces of samples that were not subjected to moistening.

The design of this study can be used to assess the sensitivity to mould at different moisture levels in new materials, especially when comparing the properties of different materials. When the critical moisture level of a material can be ascertained, a particular material or manufacturer can be chosen, taking the expected temperature and relative humidity conditions into account, to minimise the risk of mould growth. The tests in this study were carried out under constant temperature and RH. In buildings, these factors fluctuate more or less, which affects mould growth (Adan, 1994; Viitanen and Bjurman, 1995). In addition, there is a risk of various kinds of contamination, which may affect mould growth (Grant et al., 1989; Chang et al., 1996). Therefore, a test with the same design as this study cannot be used to predict how long a material may be exposed, beyond the time tested in the laboratory, under real conditions with no risk of mould growth. Further research is required to make such predictions.

Table 7
The week when mould growth could first be determined.

	Temperature	Rating ≥2 for at least one test piece			Median ≥2		
		Relative humidity					
		85%	90%	95%	85%	90%	95%
Chipboard	10 °C	–	–	5	–	–	5
	22 °C	7	5	1	7	5	1
Pine sapwood	10 °C	–	8	2	–	9	1
	22 °C	3	2	1	3	3	1
Plywood	10 °C	12	3	2	–	4	2
	22 °C	1	1	1	3	1	1
Thin hardboard	10 °C	–	–	11	–	–	–
	22 °C	–	4	1	–	12	1

5. Conclusions

Many factors affect the critical moisture level that can be assigned to a building material. In this article we have identified temperature, relative humidity, incubation time and assessment criteria for mould growth. In order to compare results from different tests, it is important that such factors are controlled and the same test method used. It is also important to state the temperature at which the critical moisture level applies and how long the material is tested. We have stated this as $RH_{crit\ (temp,\ time)}$. Moreover, each individual material must be tested separately. Two similar materials may have considerably different resistance to mould growth, and so the results from one cannot be applied to the other. Thus the results of this study apply only to the materials tested here.

Two methods of describing mould growth over time and two definitions of critical moisture levels were used in this study. These methods complement each other in that one contains more information about the distribution of growth of mould on the samples, while the other makes it possible to set pass or fail criteria. Both definitions provided the same results regarding critical moisture levels, though they differed in terms of the time before such levels were achieved. In this regard, it must be noted that measurement uncertainty when assessing very low incidence of growth is greater than with more extensive growth. When evaluating growth, it is important to assess inter-rater reliability. We have provided a suggestion for how this can be done.

Further studies are needed to verify whether the laboratory tests correspond to actual conditions, and how duration affects the outcome.

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