Critical Moisture Conditions for Mould Growth on Building Materials

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Critical Moisture Conditions for Mould Growth on Building Materials

Pernilla Johansson
Rapport TVBH-3051 Lund 2012
Avdelningen för Byggnadsfysik, LTH
Critical Moisture Conditions for Mould Growth on Building Materials

Pernilla Johansson
Licentiate thesis
Preface

This work has been made possible through funding from FORMAS – The Swedish Research Council and from WoodBuild, a research project for the Swedish forest-based industries within the Sectoral R&D programme 2006-2012. The funding is gratefully acknowledged.

I want to thank the homeowners who allowed us to use their attics and crawl spaces in the study.

I especially want to thank my colleagues at SP, particularly the following:

Annika Ekstrand-Tobin: Without your hard work in the laboratory and in the field, this study could not have been performed at all. Thomas Svensson: What would I have done without you! You have entangled my world with statistics, but you have also taught me so much and have always been there to answer my questions. Gunilla Bok: You were always there to help, both in practical matters and in discussions. Kristina Mjörnell, one of my co-supervisors: You always took the time in your busy schedule to discuss issues whether they were big or small and you gave me many valuable advices. Eva Sikander: Your encouragement has meant so much, especially during bad days. Susanne Ekendahl: Together we figured out how to perform mould resistance tests, and without your cooperation it would have been hard to even begin this study. Carina Johansson: You have edited this report with such patience.

I also want to thank Jesper Arfvidsson, LTH, my supervisor: I have appreciated your support and advice, and that you have been a calmful influence, even I stressful times. Nils Hallenberg, GU, my co-supervisor, I am grateful for your valuable comments on this work and for introducing me to the subject 16 years ago, when you laid the foundation for the knowledge I have today.

Finally, I want to thank those who matter most to me personally: my family, Johan, Filippa, Viktor and Joel, for putting up with a wife and mother who often had thoughts elsewhere. Without your support and love I don’t know how I would have managed. Thanks, Mom and Dad, Tove and Ingemar Lundin, you have through my whole life encouraged and inspired me to learn new things, not least through your own reading. And Linda Rehnman, my dearest friend, your care and your prayers mean so much to me.

Pernilla Johansson
Borås, February 2012
Abstract

Materials that are stored or used in damp climates may be subject to mould growth. However, all materials are not equally susceptible to mould growth. For each specific material, there is a critical moisture level. If this is exceeded, there is a risk that mould fungi will develop on the material. In a building, different constructions are exposed to different climatic conditions. To minimise the risk of microbial growth, building materials should be chosen that are tolerant to the expected climatic conditions.

In this study, the critical moisture levels for ten building materials were evaluated in constant climates, favourable for mould growth, in the laboratory. Samples of the building materials were inoculated with mould spores and incubated in climate chambers at different relative humidities and temperatures; growth of mould was analysed weekly for at least 12 weeks.

In order to verify that the laboratory test is relevant also for real life conditions a field study was performed where pieces of the same materials as in the laboratory test were placed in three outdoor ventilated crawl spaces and three outdoor ventilated attics, where the climate was varying, and mould growth on the test pieces was studied over 2.5 years. Material specific mould growth curves were produced based on critical moisture levels. Overall, there was agreement between the laboratory test and the field study. When the climate in a test site exceeded the mould growth limit curves, there was also mould growth on the test pieces if the time was sufficiently long.

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 that the same test method is used. It is also important to state the temperature at which the critical moisture level applies and how long the material is exposed to the climatic conditions during the test. Another conclusion from the study is that although conditions in laboratory studies are simplified and accelerated, the results serve well to indicate mould growth in within a building construction.

The methodology, as well as experiences and conclusions, from the laboratory test were used when formulating a new test method for determining the critical moisture level of a building material.

Keywords: mould; critical moisture level; building material; relative humidity; mould resistance; crawl space; attic
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List of appended papers

Paper I

Mould growth on building materials under different climatic conditions: determining the critical moisture level

Pernilla Johansson, Annika Ekstrand-Tobin, Thomas Svensson

Submitted to *International Biodeterioration and Biodegradation*

Paper II

Validation of critical moisture conditions for mould growth on building materials

Pernilla Johansson, Thomas Svensson, Annika Ekstrand-Tobin,

Submitted to *International Biodeterioration and Biodegradation*

Paper III

Test method for determining critical moisture level for mould growth on building material

Pernilla Johansson, Annika Ekstrand-Tobin, Gunilla Bok
1 Introduction

Materials that are stored or used in damp climates may be subject to mould growth. Moulds are microfungi that live on the surface of materials. They do not cause any significant degradation of the material, but use easily assimilated nutrients for their growth. The growth process may occur inside buildings, with risk that the indoor environment may be adversely affected, for example, by a mouldy odour.

The factors controlling mould growth on building materials are due to characteristics of the material, the environment to which the materials are exposed and the characteristics of the fungi itself. The susceptibility of building materials to mould growth varies. The lowest relative humidity at which mould grows on building materials is considered to be 75–80% (Grant et al., 1989; Adan, 1994; Rowan et al., 1999). On the other hand, some materials can withstand high relative humidity without mould growth, e.g. stone based materials (Ritschkoff et al., 2000).

Within a building, the climate is expected to vary from one part of the construction to another. To minimise the risk of microbial growth, materials that can tolerate the climate in question should be chosen. Swedish building regulations (BFS 2011:6, BBR) prescribed that a well-researched and documented critical moisture level is to be used to determine the maximum permitted moisture level in order to design buildings without microbial growth which can affect human health. If the critical moisture level for a material is not known, 75% RH is to be used.

At present, there is no standardised test method for determining the critical moisture level of a material. There are a number of methods for evaluating the fungal resistance of a material, and these are performed in high RH, between 90% and 95%. However, it is expected that the critical moisture level for some materials will lie somewhere in between these RH, around 75% to 95%. Although in several published studies the mould growth on building materials has been tested at various climate conditions and those results may be used to estimate the critical moisture level, the results may not be adaptable to all similar products. Treatment of and additives to the material may affect the mould growth. Also, new materials are continuously being developed which have not been tested.

Therefore, there is a need for a test method for determining a material’s critical moisture level. When the critical moisture level of a material can be
ascertained, a particular material or manufacturer can be chosen, taking the expected climate into account, to minimise the risk of mould growth.

The study presented in this report had three aims. The first was to study the mould growth development on different building materials in steady state conditions in the laboratory at different combinations of temperature and relative humidity, and to determine the critical moisture levels for mould growth for the materials tested. The second was to compare the results from this study with real-life conditions where the temperature and relative humidity were expected to fluctuate. The third purpose of the study was to formulate a test method for critical moisture levels for mould growth on the surface of building materials.
2 Background

2.1 Biology of mould fungi

Mould is an artificial grouping of a number of species of microfungi that have common life strategies. They grow on the surface of materials, use easily assimilated compounds as nutrients and energy sources, and produce spores as dispersal and survival units. Some species of mould fungi can spoil food, while others may be beneficial to humans, such as giving taste to blue cheese and producing antibiotics. In ecosystems, microfungi have a crucial role as decomposers of dead organic matter so that it can be recycled into the ecosystem. At outdoor construction, these microfungi can cause discoloration, and mould growth in buildings may affect the indoor environment negatively, for instance, by producing volatile, bad-smelling compounds.

The spores (also called conidia) of the fungi are produced in high amounts and since mould fungi are so widely spread across different environments on the Earth, there is no natural place where air and materials are free from spores. When favourable conditions are present, the spore will germinate and a small germ tube will develop; if the favourable conditions prevail, a hypha will be produced. A hypha is a tubular cell structure which extends at the tip. By continuously branching during growth, the hyphae form a mycelium. Eventually, specialized structures (conidiophores) develop from the hyphae and from them the spores are produced and dispersed. It is in the hyphae that the activity of the fungi takes place. Figure 1 presents a schematic picture of the lifecycle of a mould fungus.

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 by microbiologists as water activity, $a_w$, which is equivalent to the relative humidity of the air at equilibrium but expressed in hundredths instead of a percentage.
Each fungal species has a minimum requirement for availability of water in order to grow, although the majority of fungi will grow well at high moisture conditions, above 0.9 $a_w$, which corresponds to an equilibrium relative humidity of 90%. Fungi that grow below 0.98 $a_w$ are called xerophilic fungi (Magan and Lacey, 1984); the most extreme xerophiles may grow at $a_w$ as low as 0.62. Moisture requirements are also related to temperature; at lower temperatures, the fungus requires more available water to germinate and grow (Ayerst, 1969). Also, the growth rate of fungi is dependent on the RH and temperature. The relationship between temperature, moisture and growth rate on nutrient media in laboratory studies has been described for a number of fungal species by so-called isopleths (e.g. Ayerst, 1969; Magan and Lacey, 1984; Smith and Hill, 1982); an example is shown in Figure 2. At an optimum temperature, the required water availability for growth is at minimum, and at both sides of this temperature the moisture requirements are higher.
A large number of species of microfungi are commonly found in damp buildings. Several studies have attempted to survey which are the most common species and on which building materials they exist. Andersen et al. (2011) found at least 45 species; Hyvärinen et al. (2002) found fungi from at least 22 genera; Wessen (2006) found at least 49 species. Flannigan and Miller (2001) lists 52 species isolated from building interiors. No matter how big the exact number, the conclusions are that there are many varied species, they all represent a broad range of demand for water, and they vary in their potential for growth. Some will only grow in very specific environments, while others may be able to colonize more diverse environments (Caddick, 1993). Often, species occur together on a building material (Andersen et al., 2011; Hyvärinen et al., 2002), while at other times only one species dominates, as reported for *Penicillium corylophilum* in crawl spaces in southern Sweden (Bok et al., 2009).

Some species produce dark pigment in the cell wall of the hyphae or spores or both. When abundant growth of such fungi is present in a building, it can be seen by the naked eye as a discoloration. However, often mould growth in buildings is represented by fungi that lack such pigmentation and, although mould growth is abundant and extensive, the mould cannot be seen with the naked eye.
The various species produce different compounds, such as volatile organic compounds, of which some cause a mouldy odour, mycotoxins, etc. Even the same species might produce different substances, depending on which material it is growing on, the temperature and moisture conditions and which other species are present (Fog Nielsen et al., 2004; Sunesson et al., 1996).

The conclusion from these observations is that mould growth in different buildings may be very diverse; thus, it is be very hard, if not impossible, to predict the composition of a fungal population, whether the growth will be visible to the naked eye or not, or whether it will affect indoor air negatively. The latter also depends on where in the construction the growth is present, the presence of air movements, and other factors.

In the following, the term mould growth is used to describe a collection of fungi, and not individual species.

2.3 Critical moisture conditions for mould growth on building materials

Much of what we know about microfungi comes from studies on food. In the food sector, the factors controlling mould growth area are often described as intrinsic and extrinsic. Intrinsic factors are the characteristics of the food itself, while extrinsic factors are the characteristics of the environment in which the food is stored. Also, implicit factors will affect the growth and survival of microfungi, that is, the characteristics of the fungi itself and how it behaves in the presence of combinations of intrinsic and extrinsic factors. (Blackburn, 2000). These key controlling factors can be adapted to mould growth on building materials. The extrinsic factors comprise the climate that the materials in a building construction are exposed to, and the intrinsic factors the characteristics of the particular building material.

In general, if there is a high content of organic compounds in a substrate, the requirements for water for mould growth is lower, and the biodiversity of fungi may be higher than if the concentration of these compounds is low. (Hyvärinen et al., 2002) found that mould growth was highest on wooden materials and paper materials, which are rich in organic compounds, and lowest in samples of mineral insulation, ceramic products, and paints and glues were the content of organic compounds is expected to be low. (Pietarinen et al., 2008) also found the highest diversity of microbes on wooden materials.

Since the concentration of organic compounds, as well as other characteristics like pH, surface structure, etc., varies among materials, the expected critical
moisture conditions also vary. One of the earliest studies of mould growth on different types of building materials was by (Block, 1953). He concluded that the most sensitive materials will be subject to mould growth at 80% RH at room temperature. Numerous studies have since attempted to identify the climates in which different types of building materials begin to mould (e.g. Ritschkoff et al., 2000; Nielsen et al.; 2004, Hofbauer et al., 2008). Some of the published studies were used to estimate the critical moisture limit for different groups of building materials (Johansson et al., 2005). However, differences in methodology and/or variations in evaluation of the data complicated the comparison between the different studies and the estimation of critical moisture levels. Factors that vary among the experiments include the fungi used, inoculation method, climate, duration, analytical method and frequency of analyses. Studies also vary in their criteria of when growth is considered to be critical.

Although a material has a high resistance to mould growth, mould might grow if the material is soiled. (Chang et al., 1996) studied the growth of Penicillium chrysogenum in three different types of material in a duct and found that on all the materials there was mould growth when they were contaminated with organic dust, even if there was no growth on the clean material. (Grant et al., 1989) found that by adding a carbon source to wallpaper, the minimum aw level for mould growth was lower for some species compared to when they were growing on untreated references of the same material.

On the basis of the isopleths for several mould fungi common on building materials, (Sedlbauer, 2001b) defined a lowest isopleth of mould, the LIM 0 curve shown in Figure 3. It defines the limiting conditions for mould growth on an optimal media. The curves were modified to include three groups of building materials. Later, (Hofbauer et al., 2008) used laboratory data from tests of mould growth on building materials and constructed material-specific isopleths by the closest approximation to the LIM 0 curve, for an example see Figure 4. By calculating the expected climate of a construction or part of a building, these limiting growth curves may be used to assess the risk for mould growth to occur.
**Figure 3**  Isopleths for spore germination of various fungus species and the lowest isopleth for mould (LIM 0) (Sedlbauer, 2001a).

**Figure 4**  Material specific isopleth based on results from laboratory tests and approximation to the LIM 0 curve (Hofbauer et al., 2008).
2.4 Testing of the resistance for mould growth of a material

A number of standardized methods are available to test the mould resistance of a material. The principles of those methods are generally the same: spores from mould fungi are introduced onto the surface of test pieces of the material; these are then incubated in a climate favourable for mould growth and after some weeks, usually four, the surfaces of the test pieces are analysed for mould growth. The methods differ, depending on the species used, the number of spores and how these are introduced to the test pieces, the climates in which the materials are tested, the assessments of growth of the test pieces, and evaluation criteria. A selection of common methods is summarised and compared by (Adan 2011) and in table 6.

The test methods assess the resistance of a material to mould at high humidity levels (at least 90–95% RH) and are not directly applicable to lower humidity levels. At lower RH, the expected time before growth starts is longer; four weeks testing might be too short. Also, in tests where the materials are exposed at RH lower than the optimum for many species, it is important that xerophilic fungi are also used to give a relevant result. The method should also include different temperatures because the critical moisture level for mould growth is expected to be higher at lower temperatures.

2.5 Laboratory test versus real life conditions

The minimum conditions for mould growth discussed in section 2.1 are generally based on laboratory studies on media that are rich in nutrients and where water availability is optimal. Actual building materials are not as rich in nutrients, and the lowest requirement for growth is probably slightly higher (Flannigan and Miller, 2001). Although there are studies of the minimum mould growth on building materials, they are nevertheless performed in a laboratory with favourable and usually constant conditions. These conditions will differ from what the materials encounter in “real life”. Cooke and Whipps, (1993) writes,”The capability of a fungus to grow under specific laboratory conditions may explain, in part, how it can occupy a particular realized niche under competition of other fungi in similar niches. However, it must be remembered that in nature, at any one time, the mycelium may exist in several discrete microsites, each influenced by different biotic and abiotic factors. Similarly, environmental conditions, such as temperature and water availability, may vary both spatially and temporally.” This is applicable also for the in conditions encountered in buildings.
In many parts of a construction, there is a fluctuation in relative humidity and temperature, due to both seasonal and shorter-term variations. This causes stress to the fungi growing on materials in the building, which affects not only the rate of growth but also how long the fungi will survive. The tolerance for extreme periods varies from species to species and probably relates to its natural growth environment. Fungi whose natural habitat is on the surface of leaves (phylloplane fungi) cannot grow at low $a_w$ but have an excellent ability to adjust to fluctuating water and temperature conditions (Deacon, 2005). Park (1982) found that the hyphal tip of these fungi could restart growing at the tips, although exposed to a dry period of up to 21 days, in contrast to the other microfungi studied, whose natural habitat is more stable, such as soil.

On building materials, the rate and extension of mould growth have been shown to be lower when favourable conditions alternate with less favourable (Adan 1994; Viitanen and Bjurman, 1995). In addition, how long these periods last is also of importance.

The critical moisture level for a building material can be exceeded during a shorter or a longer period, while during other periods the level may be such that it does not favour mould growth. It is then expected that the risk of mould growth to occur is low if the variations are such that the critical limits are undercut by a good margin over a sufficiently long period.

Factors other than climate conditions vary among laboratory and natural environment, and some are listed in Table 1.
Table 1  
Comparison between a general laboratory test for evaluating the resistance of a material to fungal growth, as described in section 2.4, and the natural conditions that a material will encounter in a building construction

<table>
<thead>
<tr>
<th></th>
<th>Laboratory tests</th>
<th>Natural conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungal species</td>
<td>A few species (1–6) introduced to the surface, although other microorganisms may be present if test pieces not sterilized before testing</td>
<td>Potentially a large number of species</td>
</tr>
<tr>
<td>Spore exposure</td>
<td>More or less controlled; high concentration of spores</td>
<td>Unknown, continuous supply, varying among places and time of year</td>
</tr>
<tr>
<td>Climate (relative humidity and temperature)</td>
<td>Constant or varying according to a predefined scheme</td>
<td>Varying by time</td>
</tr>
<tr>
<td>Duration</td>
<td>Limited to some weeks</td>
<td>Material expected to be exposed for several years</td>
</tr>
<tr>
<td>Status of the material</td>
<td>New, clean material</td>
<td>Material possibly contaminated, either during construction phase or during use of building</td>
</tr>
<tr>
<td>Other environmental factors</td>
<td></td>
<td>Presence of other organisms, e.g. mites that eats fungi</td>
</tr>
</tbody>
</table>
The present study

The present study consists of three parts: a laboratory test, a field study and the formulation of a test method.

The mould growth on ten building materials listed in Table 2 that are commonly used in the building construction sector in Sweden was studied under laboratory conditions (Paper I), and the growth on test samples from nine of them were studied in the field study (Paper II). The purpose was to determine the critical moisture conditions of each material and then to compare the results with those from the field study (Paper II) to estimate if the results from the laboratory test could be used to predict mould growth in constructions. The methodology, results and conclusions from the laboratory test were further used to formulate a test method for determining the critical moisture conditions for mould growth and to calculate growth limit curves in order to estimate the critical moisture levels at temperatures not tested in the laboratory (Paper III).

Table 2 Building materials used in the study

<table>
<thead>
<tr>
<th>Material</th>
<th>Material description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cement-based board</td>
<td>8 mm cement-based board consisting of cement, limestone, and cellulose fibres, covered with a plastic dispersion</td>
</tr>
<tr>
<td>EPS insulation board</td>
<td>50 mm expanded polystyrene insulation board</td>
</tr>
<tr>
<td>Glass fibre board</td>
<td>15 mm rigid glass wool insulation board</td>
</tr>
<tr>
<td>Asphalt paper</td>
<td>1.5 mm windproof barrier of asphalt-impregnated cellulose paper</td>
</tr>
<tr>
<td>Wet-room gypsum plaster board</td>
<td>13 mm gypsum board with cardboard surfaces</td>
</tr>
<tr>
<td>Exterior gypsum plaster board</td>
<td>13 mm gypsum board with cardboard surfaces</td>
</tr>
<tr>
<td>Plywood</td>
<td>12 mm softwood plywood</td>
</tr>
<tr>
<td>Thin hardboard</td>
<td>3.2 mm high-density hardboard made of wood fibres and lignin</td>
</tr>
<tr>
<td>Chipboard</td>
<td>12 mm particle board</td>
</tr>
<tr>
<td>Pine sapwood</td>
<td>19 mm tongued and grooved board</td>
</tr>
</tbody>
</table>
3.1 Experimental setup of laboratory test (Paper I)

The laboratory test, as described in Paper I, was performed according to standard test methods MIL-STD 810 F and ASTM C 1338, with some modifications. The principle of the test was to inoculate specimens of the material with a suspension of spores from mould fungi, to incubate the samples in climate chambers under specified conditions of RH and temperature, and at defined intervals inspect the samples for fungal growth. The critical moisture level for mould growth was then determined by considering the lowest RH at which mould growth appeared. Six test pieces sized 50x100 mm from each material were exposed to each climate.

The fungi used in the study (Table 2 of Paper I) were chosen to emulate real-life situations. Different species of fungi often occur together on the materials used in buildings (Hyvärinen et al., 2002; Andersen et al., 2011) and interactions between the species are inevitable. Mostly, those interactions mean different ways of competition for space and nutrients (Cooke and Whipp, 1993). A mixture of spores from six fungal species was therefore used in the study. The species varied in their water requirements: among the six species were some requiring high moisture levels and others only needing lower levels for mould growth. The 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). The strains used were originally isolated from building materials in European houses and are part of a collection where they are preserved to maintain their original characteristics.

A spore suspension was prepared according to MIL-STD 810 F. This procedure is common in many standardised test methods; in short, it means that spores from each of the fungi are dissolved in a salt solution or sterile water in a known amount. The solution was sprayed onto one surface of each test specimen so the spores were more or less evenly distributed over the surface of the test pieces. Prior to inoculation, six test pieces of the material to be tested in each climate were dipped in sterile water for 20 minutes to simulate the effect of flooding or a heavy rainfall.

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. An external humidity and temperature transmitter (Vaisala HUMICAP® HMT330, Helsinki, Finland) was mounted in each of the climate chambers. The values of temperature and relative humidity were saved in a computer-based program (Exomatic) every
five minutes. 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 they showed RH values up to 11% above the target values. The calibrated values were adjusted for the drift. The sensors were later replaced by new ones, which were stable. 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. During the whole test period, the climate 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 of the sensors, and therefore the mean value from the manual readings was used to describe the climate.

The mean values and standard deviation of the ten climates that test pieces were exposed to and the incubation time are presented in Table 3. The test period of each climate was set to 12 weeks, although the tests at 75% and 80% RH and 22 °C were continued for some additional incubation time. The measurement uncertainty was calculated for each climate tested, based on calibration data, according to EA-4/02.
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.

<table>
<thead>
<tr>
<th>RH (%)</th>
<th>T (°C)</th>
<th>Maximum incubation time (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 (0.1)(1)</td>
<td>10 (0.0)(1)</td>
<td>12</td>
</tr>
<tr>
<td>85 (0.5)</td>
<td>10 (0.0)</td>
<td>12</td>
</tr>
<tr>
<td>90 (0.8)</td>
<td>10 (0.1)</td>
<td>12</td>
</tr>
<tr>
<td>95 (0.9)</td>
<td>10 (0.3)</td>
<td>12</td>
</tr>
<tr>
<td>93(1)(2)</td>
<td>10(1)(2)</td>
<td>12</td>
</tr>
<tr>
<td>75 (0.5)(1)</td>
<td>22 (0.1)(1)</td>
<td>12+20(3)</td>
</tr>
<tr>
<td>79 (1.4)</td>
<td>22 (0.3)</td>
<td>12+7</td>
</tr>
<tr>
<td>85 (1)</td>
<td>22 (0.6)</td>
<td>12</td>
</tr>
<tr>
<td>89 (0.7)</td>
<td>22 (0.2)</td>
<td>12</td>
</tr>
<tr>
<td>95 (0.3)</td>
<td>22 (0.0)</td>
<td>12</td>
</tr>
</tbody>
</table>

(1) Values are based on manual readings.
(2) Standard deviation is not available.
(3) During the additional 20 weeks, samples were incubated over saturated salt solution at about 76% RH and 23°C.
3.2 Field test setup (Paper II)

Test pieces from the same materials as in the laboratory test, except pine sapwood, were placed in three outdoor ventilated attics and crawl spaces. The houses were selected to represent both high- and low-risk construction with regard to mould growth. They were all single-family houses situated close to Borås in the southwest of Sweden. All houses were buildings with light wooden framework structure and are representative of the Swedish housing stock. They varied in construction year, heating system, construction design, building materials etc.

Outdoor ventilated crawl spaces and attics are constructions where the climate is highly governed by the outdoor climate. Therefore, there is a seasonal fluctuation in the climate in these structures. In Scandinavia, the relative humidity in the crawl spaces is highest in the summer and autumn when warm, moist outdoor air enters the cooler space. Since cold air can hold less moisture, the saturation moisture content is lower, and the relative humidity will increase. In outdoor ventilated attics the relative humidity is instead usually at its highest during the winter months. There are also short-term variations, for example, during clear nights when heat radiates from the roof to the atmosphere, which leads to lower temperature at the surface of the interior surface if the roof, which enhances the risk for condensation and mould growth. In both types of construction, there is often extensive mould growth on the building materials (e.g. Pasanen et al., 2001; Bok et al., 2009; Hagentoft and Kalagasidis, 2010).

Not all materials used in the study were intended to be used in crawl spaces or attics, but because the climate of these constructions was expected to be such that mould growth is favoured and the climate expected to fluctuate, they were suitable as test environments. In addition, the materials were easily accessible, and the test pieces could easily be evaluated for mould growth at the defined intervals.

The relative humidity and temperature at each test site was registered every fourth hour by data loggers with internal sensors (Testo 177-H1). These were placed in close proximity to the specimens to ensure that the climate logged was as close as possible to that which the specimens was exposed to. One logger was placed at each test site.

The sensors were calibrated before and after exposure at the test sites. The calibration made it possible to adjust the measured values of RH with the correction factor from calibration (reference RH minus measured RH).
However, since this factor was expected to be dependent on temperature (Fernicola et al., 2008), and the temperature and relative humidity were not constant in the field measurements, the latter calibration was performed at two temperatures and three values of RH, which made it possible to make a more accurate adjustment of data. A multiple regression was performed, and both temperature and relative humidity were included. The measured values were adjusted according to an equation, where temperature, relative humidity and drift (which for some of the loggers was considerably large) were variables.

3.3 Analysis and assessment of mould growth tests, definition of failure and critical moisture levels (Paper I and Paper II)

Mould growth on the inoculated surface of each test sample, excluding the edges, was assessed at defined intervals, once a week for the laboratory tests and every six months, in April and October for the field test. The samples were then analysed under a stereo microscope at 10–40x magnification. During this procedure, it was important to use low-angle light to detect hyaline as well as coloured hyphae. The mould growth was assessed according to the rating scale shown in Table 4. A test piece was considered to have failed when the rating of mould growth first reached rating 2 or higher.

A limitation of the method is that it is somewhat subjective, as different persons that perform the analyses will vary in their assessment of the extent of mould. To investigate the 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. The true value of mould growth on each test piece was defined as the median of each of the ratings and the relative frequencies of the judgements given for each true rating were collected in a matrix, with the number in the matrix position ij representing the probability of judging a rating j when the true rating is i. This matrix was regarded as an estimate of the probability of a judgement, given a certain true rating.

Using this matrix, it was 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 rating of ≥ 2 and non-failure as a rating of < 2.
Table 4. Rating scale for the assessment of mould by microscope at 40 x magnification. Illustration: Agneta Olsson-Jonsson

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

Two alternative definitions were used to determine the critical moisture level:

a) when the median growth of the six test pieces was equal to or exceeding rating 2

b) when the rating of at least one of the six test pieces was equal to or exceeding rating 2

Since the tests were carried out in climates of constant RH, with RH set at intervals of 5%, the precise RH that corresponds to the critical level cannot be determined. Mould will grow at one RH, and not at the next lower RH; therefore the critical moisture level will fall into this range. For example, if the criteria for critical mould levels were met at 79% RH, the critical moisture
level for this particular material was assumed to be in the range of $75\% < RH_{\text{crit}} \leq 79\%$.

For each material there were two ranges: one valid for 10°C and one for 22°C. However, we wanted to fit the data so as to also be valid for other temperatures. To create growth limit curves, we used the same technique as (Hofbauer et al., 2008), where material specific isopleths were constructed from the closest approximation to the LIM 0 curve (Sedlbauer 2001).

The equation for the LIM 0 curve was not published, so we chose to fit the data from this curve to a polynomial of second degree. By considering a minimum temperature, the equation could be formulated using only two parameters, and the critical moisture limits from the laboratory study were used to create material-specific growth limit curves, Equation 1 - Equation 3. Two growth limit curves, one upper and one lower, were produced for each material. The “true” critical moisture level may therefore lie between the two growth limit curves, or at the upper curve.

$$RH = a + c(t^2 - 54t) \quad [\%]$$  \hspace{1cm} (1)

where $t$ is the temperature in °C.

The parameters were estimated by using Equation 2 and Equation 3, where the data for $RF_{\text{crit}}$ and corresponding temperature come from the results in the laboratory tests.

$$c = \frac{RH_{\text{crit}1} - RH_{\text{crit}2}}{t_1^2 - t_2^2 - 54(t_1 - t_2)} \quad (2)$$

$$a = RH_{\text{crit}1} - c(t_1^2 - 54t_1)$$  \hspace{1cm} (3)

In order to find out if the climate in the test sites had exceeded the growth limit curves, the curves were drawn into plots of monitored relative humidity and temperature. Mould growth was then expected when the relative humidity and temperature exceeded the growth limit curves (Rowan et al., 1997). In Figure 5 an example is shown. The expected growth was then compared to the results from the assessment of mould growth on the test pieces at each test site.
Testing to find the critical moisture level must be done at several RH, and the same procedure must be followed at two temperatures in order to estimate the parameters in the equation for the growth limit curve. This entails extensive testing in practice. One way to make the test feasible is to test at one temperature and to predict the expected critical moisture level at other temperatures. For this method, one of the parameters in Equation 1 must be known. Based on the values of \( a \) that were estimated for each material in the study according to Equation 2, an average was calculated, which was then used for all materials. This was then used to calculate parameter \( c \) for each material and new limit curves could be created.

### 3.4 Results and discussion of field test and laboratory test

In this study test pieces of 9-10 building materials have been exposed to different constant climates in the laboratory and to fluctuating temperature and relative humidity in three crawlspaces and three attics.

The development of mould growth on test pieces of the materials in the laboratory test (Paper I) was plotted against time of incubation in two ways, as the median of rating in the weekly assessments and as Kaplan-Meier curves, which show the percentage survival of the samples as a function of time. Survival in this case was defined as there being no established growth on a sample; that is, the rating according to Table 4 was below 2. Once a test piece
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. Examples of the charts, for the most sensitive material, plywood, and one of the most resistant products among the materials that showed growth, asphalt paper, are presented in Figure 6 and Figure 7. In the charts, the arrow points out where the critical moisture conditions were met according to the definitions in section 3.4.

**Figure 6.** Median value of mould growth on test pieces (n=6) of building materials at different RH at 22°C over 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.
The lowest RH at which mould growth established, that is the critical moisture levels, varied among materials. These are summarised for all materials in Table 5. There was correspondence between the values for critical moisture levels elicited using the two different criteria for critical moisture, with two exceptions at 10°C. However, the time before the critical moisture level was reached varied depending on which of the criteria were met; the time before mould growth established at the first test piece with was shorter when the median growth was considered, since not all test pieces failed at the same time.

In the climates where several materials did show mould growth, there was also variation in the weeks at which the mould growth was rated 2 or higher. Considering both these aspects – critical moisture levels and time before established growth – pine sapwood and plywood were the most susceptible to mould growth, followed by chipboard, thin hardboard, exterior plaster board, wet-room plaster board, and asphalt paper, in that order. No growth was detected on any of the samples of glass fibre board, cement-based board, or expanded polystyrene boards in any of the climates tested.
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 when first test piece failed, criterion (b).

<table>
<thead>
<tr>
<th>Material</th>
<th>Temperature 22°C</th>
<th>Temperature 10°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine sapwood</td>
<td>$75 &lt; \text{RH}_{\text{crit}12\text{w}} \leq 79$</td>
<td>$85 &lt; \text{RH}_{\text{crit}12\text{w}} \leq 90$</td>
</tr>
<tr>
<td>Plywood</td>
<td>$75 &lt; \text{RH}_{\text{crit}12\text{w}} \leq 79$</td>
<td>$75 &lt; \text{RH}_{\text{crit}12\text{w}} \leq 85^*(1)$</td>
</tr>
<tr>
<td>Chipboard</td>
<td>$79 &lt; \text{RH}_{\text{crit}12\text{w}} \leq 85$</td>
<td>$90 &lt; \text{RH}_{\text{crit}12\text{w}} \leq 93$</td>
</tr>
<tr>
<td>Thin hardboard</td>
<td>$85 &lt; \text{RH}_{\text{crit}12\text{w}} \leq 89$</td>
<td>$93 &lt; \text{RH}_{\text{crit}12\text{w}} \leq 95^*(2)$</td>
</tr>
<tr>
<td>Wet-room gypsum plaster board</td>
<td>$89 &lt; \text{RH}_{\text{crit}} \leq 95$</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
</tr>
<tr>
<td>Exterior gypsum plaster board</td>
<td>$89 &lt; \text{RH}_{\text{crit}} \leq 95$</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
</tr>
<tr>
<td>Asphalt paper</td>
<td>$89 &lt; \text{RH}_{\text{crit}} \leq 95$</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
</tr>
<tr>
<td>Cement-based board</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
</tr>
<tr>
<td>Glass fibre</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
</tr>
<tr>
<td>Expanded polystyrene</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
<td>$95 &lt; \text{RH}_{\text{crit}}$</td>
</tr>
</tbody>
</table>

* This is based on the criterion (b). When using the criterion (a) concerning median rating $\geq 2$, the result was $^{(1)} 85 < \text{RH}_{\text{crit}} \leq 90 \quad ^{(2)} 95 < \text{RH}_{\text{crit}}$.

The length of incubation will affect the growth of mould. Testing over a long period increases the risk of mould growth. In this study, no growth was found on any of the materials tested after 12 weeks at 75% RH, yet mould began to grow on plywood after 16 weeks and on pine sapwood after 32 weeks. The critical moisture level was thus reduced to 75%, or below, from having been between 75% and 79% RH at 22 °C. Had the test been allowed to continue for longer than 12 weeks in other climates too, it is possible that the critical moisture level would have been reduced also for some of the other materials. Had the incubation time been shorter than 12 weeks, the critical moisture level for some materials may also have been different, depending on how long the time would have been.

If a building material has high moisture content, mould may begin to grow even when the humidity in the air surrounding the material 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, this was not confirmed by the findings in the laboratory study presented here; the critical
moisture level was the same as for the non-wetted material. One reason could be that the time for moistening the sample, 15 minutes, was too short. A more likely explanation is that the high air exchange rate in the climate cabinets quickly achieved equilibrium between the surface of moistened samples and the prevailing climate in the climate cabinet. The surfaces will therefore have become comparable to the surfaces of samples that were not subjected to moistening.

In the attics and crawl spaces where test pieces were exposed (Paper II), the relative humidity and temperature fluctuated. The conditions varied among the test sites, some being more humid and favourable to mould growth than others. Mould fungi grew on several of the material samples. The limit curves for mould growth are presented in figure 6 and figure 7 of paper 2, together with the measured data of RH and temperature for each test-site. Measured relative humidity is plotted against measured temperature, where each point corresponds to one measurement. If points were above the limit lines, growth was expected on the samples.

The expected growth was compared to actual growth. In most cases, the predicted growth, or absence of growth, was consistent with the results from the analysis at the end of the test period. For some materials and test sites mould growth was predicted, but there was no mould growth at the test pieces of the materials. In those cases, the number of occasions on which the relevant growth limit curves were exceeded was counted. Each measurement point was then regarded as constant for four hours because RH and temperature were logged at this interval. The cumulative time was calculated as the sum of these values. If the time was shorter than the shortest time before the critical moisture level was achieved in the laboratory, no growth was expected, and then there were consistency between actual and expected mould growth in those cases as well.

The sequence in which the mould established on the materials was the same in the field test as in the laboratory test, with one exception; on thin hardboard in attic 3 mould started to grow earlier than on chipboard. On material that did not show any mould growth in the laboratory, there was no growth on any test piece at any of the test sites.

Overall, there was therefore agreement between the laboratory tests and the field study. However, there are limitations and unknown uncertainties in this comparison. Using the cumulative time for climate that was favourable is a simplified method of considering the conditions that affect mould growth. These favourable conditions were followed by less favourable, and how long
they last, how far they are from the limits, and how rapidly the climate changes between favourable and unfavourable conditions are significant to the risk that mould growth will occur, as well as to its scope and its growth rate (Viitanen and Bjurman, 1995; Adan, 1994; Pasanen et al., 2000). Also, the mould growth in the field study was considered after 2.5 years of exposure. In some cases, however, mould growth was established earlier. The values of critical moisture levels on the calculated growth limit curves are most valid at temperatures in the range 10°C-22°C, also, the model underlying the production of growth limit curves has its uncertainties. Also the number of test pieces in the field test is low, which leads to a higher uncertainty.

Although limitations and uncertainties exist, the results in this study indicate that the accelerated laboratory test can be used to predict mould growth in buildings. Therefore the methodology, as well as experiences and conclusions, from the laboratory test were used when formulating a new test method for determining the critical moisture level of a building material. This is presented in Paper III, and some aspects of the method are discussed in section 4.1.

3.5 The test method (Paper III)

A new test method for critical moisture conditions for mould growth on building materials, SP method 4927, is presented in Paper III. The routines in the test method follow the methodology used in the laboratory tests in Paper I and are based on standardised test methods for testing resistance of materials to mould growth. Table 6 compares some important parts of the new test method to a selection of standard methods, and some of these are discussed in this section.

3.5.1 Climate

The tests in SP method 4927 are carried out at four levels of constant RH: 80%, 85%, 90% and 95% at 22°C. To ensure that the climate will not deviate more than allowed from the prescribed, there are specifications for the homogeneity and stability of the test chambers and for the loggers used to monitor the climate in the chambers. In the majority of the other methods, no such monitoring is prescribed. Often, a high humidity level is achieved by putting the samples directly onto the surface of a humidifying medium, and it is not possible to know the exact moisture level to which the material is exposed. However, the purpose of these methods is to test resistance to mould at climates that are optimal for mould growth; it may be of minor importance to measure the exact climate at which the test pieces are exposed. When testing to find the critical moisture level of a material, however, it is essential
to know which climate the test was performed in, so as to draw an accurate conclusion about the RH at which mould starts to grow.

One way to verify that the climate in each chamber is suitable for mould growth is to use controls with high nutrient together with the samples of the material to be tested; this is used in the majority of tests.

### 3.5.2 Inoculum

A suspension of fungal spores is used, as in many of the standard test methods, and the inoculation procedure includes spraying the solution onto the surface of the test piece. The purpose of this step is to make the process repeatable because the number of spores in the solution and sprayed on the surface is known; likewise the area of the test piece and the number of spores per mm² supplied to the surface is more or less the same from time to time.

SP method 4927 uses controls for the viability of spore suspension and the purity of the solution. These are to make sure that no nutrients are available in the solution that might affect the test results. To achieve this, it is important to use distilled water with a minimum of nutrients in the solution. Also, all spores can swell in an aqueous solution, which may affect the start of growth (Dantigny et al., 2006); therefore, the suspension must be used as soon as possible. In the laboratory study, there was no growth on three of the materials tested even in the climate that was most favourable to mould growth (95%, 22°C), which indicates that the spore suspension as such has no impact on mould growth. It can be argued that the water in the solution will lower the critical moisture level. However, it is a very small amount of water that is applied to the surface, 0.4 ml. In the laboratory, wetting of test pieces did not affect the critical moisture level even though test pieces were dipped in water for 20 minutes before inoculation and incubation. Therefore it can be concluded that the water that is added to the test pieces in the spraying of spore suspension has no significant impact on the results.

### 3.5.3 Number of test specimens

Generally, the more test pieces that are used in a study the better. However, for practical reasons, the number of test pieces in a commercial test method must be limited. The number in the test methods in Table 1 varies from three to six. In SP method 4927, the number of test pieces in the study is prescribed as seven, at a minimum. This provides a 95% level of confidence for the median value of the ratings according to the results from comparison between raters, see section 3.1.
3.5.4 Analysis methodology and rating of mould growth

In SP method 4927, mould growth at the surface of the test pieces is studied under a stereo microscope. This analytical procedure is common to many test methods, but the assessment criteria for growth in this test method differ from others. Both non-visible and discolouring growth is assessed according to the same rating scale, and the growth is described in terms of both distribution over the surface as well as biomass development at different spots at the surface. Many test methods assess distribution in terms of percentage of surface and assume that a high percentage of distribution causes discolouration. However, the experience from the laboratory tests shows that, 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. This conclusion is also reached by (Shirakawa et al., 2002).

3.5.5 Unique features of this method

3.5.5.1 Critical moisture level

A test piece is considered failed when the mould growth equals or exceeds rating 2. The critical moisture level is considered reached at the RH when at least two test pieces have failed and when no growth is detected on the test pieces at the next lower RH tested. The actual critical moisture level is then expected somewhere between these two values or at the RH when test pieces failed.

This criterion – the failure of at least two test pieces – results in a greater uncertainty due to the use of the rating procedure in comparison to the use of the median value. However, we believe that the uncertainty can be reduced, since at the end of the test period, at twelve weeks, the analysis must no longer be non-destructive and the mould growth can be verified by a more careful analysis. Also, in the laboratory study, we found that the values of critical moisture levels were the same at week 12 when either of the criteria, median growth or first sample with growth, was considered. Growth on just a single piece out of the seven might be due to contamination of this particular test piece, and were therefore not chosen as the criterion.
3.5.5.2 Incubation time and prediction of service life

The critical moisture level is based on the results from a 12-week test, but the analysis for mould growth can be performed at defined intervals during incubation. The time before mould growth is established in the different RH can then be given as extra information. However, this information must be handled properly. It is only an indication of how long a material can be exposed at that particular RH and temperature before mould starts to grow. If the actual climate exceeds this RH, the growth process might start earlier.

3.5.5.3 Reporting of test results

The result from a test according to SP method 4927 is critical moisture level at 22°C for the material tested. Since the critical moisture level is defined as being reached somewhere between the RH at which at least two test pieces have a rating of at least 2 and the next lower RH where no growth occurred, the value is reported as a range. The limits of the range are reported as mean value of the test climate and standard deviation, and not the reference value. From the test result, a growth limit curve can be calculated so that the critical moisture conditions for mould growth may also be assessed also for temperatures other than those tested.
### Table 6  Comparison of some variables in selected test methods for assessing mould resistance of materials

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Material</strong></td>
<td>Insulation materials and their facings</td>
<td>Panel products, in particular building materials required to present a decorative finish</td>
<td>Synthetic polymeric materials</td>
<td>Plastics</td>
<td>A variety of materials commonly used in the construction of military materiel</td>
<td>Building materials</td>
</tr>
<tr>
<td><strong>Specimen</strong></td>
<td>Size</td>
<td>Surface area not defined</td>
<td>40 x 40 mm</td>
<td>50x50mm/dia. 50 mm/length 76 cm (rods)</td>
<td>Not defined</td>
<td>Not defined</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>Minimum 3</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>Not defined</td>
</tr>
<tr>
<td><strong>Inoculum</strong></td>
<td>Number of species</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Spore solution</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Concentration of spores</td>
<td>$10^6 \pm 200\ 000$ spores/ml</td>
<td>Not specified</td>
<td>$10^6 \pm 200\ 000$ spores/ml</td>
<td>$10^6$ spores/ml</td>
<td>$10^6 \pm 2%$ spores/ml</td>
</tr>
<tr>
<td></td>
<td>Inoculation methodology</td>
<td>Spraying</td>
<td>Spraying</td>
<td>Spraying</td>
<td>Spraying or pipetting</td>
<td>Spraying</td>
</tr>
<tr>
<td></td>
<td>Amount of solution</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>Until surface is moistened</td>
<td>0.1</td>
<td>Not specified</td>
</tr>
<tr>
<td></td>
<td>Number of spores/mm$^2$</td>
<td>$(a)$</td>
<td>$(b)$</td>
<td>$(c)$</td>
<td>$(a)$</td>
<td>$(a)$ and $(c)$</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
<td>------------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Incubation Temperature</td>
<td>30 ± 2 °C</td>
<td>24 ± 1 °C</td>
<td>28-30 °C</td>
<td>24 ± 1°C or 29 ± 1°C</td>
<td>30 ± 1°C</td>
<td>22 ± 1 °C</td>
</tr>
<tr>
<td>RH</td>
<td>95 ± 4%</td>
<td>Not specified</td>
<td>&gt;95%*</td>
<td>&gt;95%*</td>
<td>At least 90% but less than 100%</td>
<td>80 ± 3 % 85 ± 3 % 90 ± 3 % 95 ± 3 %</td>
</tr>
<tr>
<td>Climate control</td>
<td>No</td>
<td>No</td>
<td>Automatic recording of wet and dry-bulb temperature recommended</td>
<td>No</td>
<td>Record chamber temperature and humidity versus time</td>
<td>Recording of temperature and relative humidity every 10 min</td>
</tr>
<tr>
<td>Incubation time</td>
<td>Min 28 days</td>
<td>4 weeks</td>
<td>28 days</td>
<td>4 weeks or longer</td>
<td>28 days or up to 84 days</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Storage of suspension</td>
<td>28 days at 6 ± 4 °C¹</td>
<td>Must be used the same day</td>
<td>Not more than 4 days at 3–10°C</td>
<td>6 h</td>
<td>6 ± 4 °C for not more than 14 days</td>
<td>Must be used the same day</td>
</tr>
<tr>
<td>Analysis Method</td>
<td>40x magnification</td>
<td>Hand lens 10x or microscope 10x–50x</td>
<td>Microscope used only to confirm growth less than 10%</td>
<td>Visible effect, microscope (x50) if necessary</td>
<td>Visible effects</td>
<td>40x magnification</td>
</tr>
<tr>
<td>Assessment</td>
<td>No rating scale</td>
<td>6-point rating scale based on percentage coverage</td>
<td>6-point rating scale based on percentage coverage</td>
<td>5-point rating scale based on % of surface with growth</td>
<td>5-point rating scale and analysis of species</td>
<td>5-point rating scale</td>
</tr>
</tbody>
</table>

¹ Or until the viability test indicates poor growth or until growth appears in the sealed storage bottle
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth compared to reference material; if bigger on test specimen, considered to have failed. If no growth is criterion, any growth on any test piece considered a failure</td>
<td>Calculate the notional mean ratings. No pass or fail criterion</td>
<td>No pass or fail criterion</td>
<td>Depending on rating of growth, material is classed as fungistatic, containing small amounts of nutrients or is not resistant to fungal attack</td>
<td>No guidance</td>
<td>Critical moisture level reached at climate and week when first test piece has established growth</td>
</tr>
</tbody>
</table>

notes:
(a) Cannot be calculated since the area is unknown
(b) Cannot be calculated since the concentration of spores is unknown
(c) Cannot be calculated since the amount of spore suspension is unknown
*Specimens are placed on solidified nutrient salt agar and incubated in an incubator that according to ISO 846 is >95% in the Petri dishes.
4 Conclusions

Mould growth on building materials in existing construction is a complicated process depending on interactions of climate conditions, material characteristics and the biology of the microfungi that constitute the artificial group called mould. Although conditions in laboratory studies are simplified and accelerated, the method presented in the study serves well to indicate mould growth in building constructions, as represented by three crawl spaces and three attics. If the expected climate in a construction is known, knowledge of the material’s critical moisture limits and the calculated growth limit curves may therefore be used as a tool when choosing the right materials for the construction, with a minimum risk for mould growth. The method may therefore be used as a helpful tool when designing buildings.

The specific results of critical moisture levels in this study can be used to roughly estimate which climate conditions a type of material can withstand without development of mould growth. However, the particular material must be tested separately to more accurately determine the critical moisture conditions for that specific product. Two similar materials may have considerably different resistance to mould growth, and so the results from one cannot be applied to the other. Treatment of a material can considerably change its susceptibility to mould growth and new products are continuously being developed. Thus, the results presented for the different materials in this study, in a strict sense, only apply to the products included in the laboratory test.

Limitations of the test method presented in this study include the time factor, the definition of critical mould growth and inter-rater variability. The latter can be minimized by training and calibration among persons that are making the analysis, so that there is a consensus of how growth is to be rated, and by using sufficient numbers of test pieces. Further, the methodology makes it possible to easily change the criteria for when the mould growth is considered to be critical and a material fails, for example, when there is more knowledge or a consensus in the field. More challenging is how the duration of laboratory tests corresponds to the actual service life of a material in a construction and how constant climates in the laboratory correspond to fluctuating climate in real constructions.
5 Further studies

Further studies are needed to understand how fluctuating climates will affect mould growth and how constant climate conditions in laboratory can be used to assess the time a material can withstand a certain climate without mould growth. This may be done by further analysis of the results from this study and by performing additional field tests.

It would also be interesting to further explore how the values of critical moisture levels could be used in existing models for calculating expected climate conditions in constructions and evaluating the risk for mould growth.

In order to validate the assessment of critical moisture levels for temperatures other than those tested, further tests at different temperatures should be performed.
6 References


Swedish National Board of Housing. Building Regulations, BFS 2011:6, BBR


Mould growth on building materials under different climatic conditions: determining the critical moisture level

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Mould growth on building materials under different climatic conditions: determining the critical moisture level

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Keywords: mold; critical moisture level; building material; relative humidity; mold resistance

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%. In a building, different constructions are exposed to different climatic conditions. To minimise the risk of microbial growth, building materials should be chosen that are tolerant to the expected climatic conditions. In this study, the critical moisture levels for ten building materials were evaluated. Samples of the building materials were inoculated with mould spores and incubated in climate chambers at different relative humidities and temperatures; 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 that the same test method is used. It is also important to state the temperature at which the critical moisture level applies and how long the material is exposed to the climatic conditions during the test.
1 Introduction

Moulds are micro fungi that live on the surfaces of materials and produce airborne spores. They do not cause any significant degradation of the material, but use easily assimilated nutrients for their 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, hyphae will grow and finally a mycelium is formed. This process may occur inside buildings, with risks that the indoor environment and human health may be adversely affected.

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, Aw. 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 Aw 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 0.8–0.98 (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 et al., 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 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 relative humidities as
low as 75%. Numerous studies have attempted to identify the climates 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 climate is expected to vary from one construction to another. To minimise the risk of microbial growth, materials should be chosen that can tolerate the climate in question. Materials manufacturers should be able to disclose a material's critical moisture level regarding 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 climatic conditions. Samples of ten building materials commonly found on the Swedish market were inoculated with mould spores and incubated in climate 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 the expected 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 50x100 mm. There were four replicates of each board in each climate 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.

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.

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.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain used in this study</th>
<th>$A_w$ Minima for Growth on 2% Malt Extract Agar (Grant et al., 1989)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$CBS$ number$^{a}$</td>
<td>Origin</td>
</tr>
<tr>
<td>Eurotium herbariorum</td>
<td>115808</td>
<td>Interior mortar (cement), Germany</td>
</tr>
<tr>
<td>Aspergillus versicolor</td>
<td>117286</td>
<td>Wall in bakery, Netherlands, 2005</td>
</tr>
<tr>
<td>Penicillium chrysogenum</td>
<td>401.92</td>
<td>Gypsum, Netherlands, 1992</td>
</tr>
<tr>
<td>Aureobasidium pullulans</td>
<td>101160</td>
<td>Window frame, Sweden, 1998</td>
</tr>
<tr>
<td>Cladosporium sphaerospermum</td>
<td>122.63</td>
<td>Betula plywood, Finland, 1997</td>
</tr>
<tr>
<td>Stachybotrys chartarum</td>
<td>109.292</td>
<td>Building material, Finland, 2000</td>
</tr>
</tbody>
</table>

$^{a}$ 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. 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 cell (Bürker) in the microscope, and this 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 climate

An external humidity and temperature transmitter (Vaisala HUMICAP® HMT330, Helsinki, Finland) was mounted in each of the climate chambers. The values of temperature and relative humidity were saved in a computer-based program (Exomatic) every five minutes. 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 climate 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 climate.

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

2.5.3 Incubation climate

The materials were tested in ten climates (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 or wood-based boards at 75 % RH. As mould growth on wood is expected in this climate, the tests at 75 % and 79 % 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 minutes; 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.
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.

<table>
<thead>
<tr>
<th>RH (%)</th>
<th>Mean value (standard deviation)</th>
<th>T (°C)</th>
<th>Maximum incubation time (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 (0.1)</td>
<td>10 (0.0)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>85 (0.5)</td>
<td>10 (0.0)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>90 (0.8)</td>
<td>10 (0.1)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>95 (0.9)</td>
<td>10 (0.3)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>93 (0.1) (2)</td>
<td>10 (0.3)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>75 (0.5) (1)</td>
<td>22 (0.1)</td>
<td>12+20 (3)</td>
<td></td>
</tr>
<tr>
<td>79 (1.4)</td>
<td>22 (0.3)</td>
<td>12+7</td>
<td></td>
</tr>
<tr>
<td>85 (1)</td>
<td>22 (0.6)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>89 (0.7)</td>
<td>22 (0.2)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>95 (0.3)</td>
<td>22 (0)</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

(1) Values are based on manual readings.
(2) Standard deviation is not available.
(3) During the additional 20 weeks, samples were incubated over saturated salt solution at about 76% RH and 23°C.

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 x 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.
Table 4. Rating scale for the assessment of mould by microscope at 40 x magnification.

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

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. Instead, the calibration results were analysed in the following way. Each judgement was compared to a “true” value, and the relative frequencies of the judgements given for each true rating were collected in a matrix, with the number in the matrix position $ij$ representing the probability of judging a rating $j$ when the true rating is $i$. This matrix was regarded as an estimate of the probability of a judgement, given a certain true rating. 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 were 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.

Using this matrix, it was 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: “Is the test piece damaged or not?” where “damaged” is defined as a rating of $\geq 2$ and “non-damaged” as a rating of $< 2$. 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 damage, in cases where the two middle values were equal we chose to define the median as the largest 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 exceeding 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 in climates of 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 climate case with lowest RH where any of the above criteria were met, and the lower limit by the climate case with the next lowest RH. For example, if the criteria for critical mould levels were met at 79% RH, the critical moisture level for this particular material was assumed to be in the range of $75% < \text{RH}_{\text{crit}} \leq 79%$. 

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 expanded polystyrene boards in any of the climates tested.

Mould development according to definition (a) is shown in Figures 1 and 3 as the median of the weekly assessments at 22°C and 10°C respectively. Figures 2 and 4 present the results according to definition (b) of tests at 22°C and 10°C as Kaplan-Meier curves, which show the percentage survival of the samples as a function of time. Survival in this case is defined as there being no established growth on a sample; that is, the rating according to Table 4 was below 2. 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. 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, EPS 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.
Figure 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 $\geq 2$, represented as a horizontal dotted line. The arrow indicates the point when this is reached.
Figure 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 indicated the points when this is reached.
Figure 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.

Figure 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.
Figure 5. Median value of mould growth in 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 $\geq 2$, represented as a horizontal dotted line. The arrow indicates the point when this is reached.

Figure 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 $\geq 2$, represented as a horizontal dotted line. The arrow indicates the point when this is reached.

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 79%. 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 79% RH and 22°C (Figures 5 and 6).
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).

<table>
<thead>
<tr>
<th>Material</th>
<th>22°C</th>
<th>10°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine sapwood</td>
<td>75 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 79</td>
<td>85 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 90</td>
</tr>
<tr>
<td>Plywood</td>
<td>75 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 79</td>
<td>75 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 85&lt;sup&gt;*1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chipboard</td>
<td>79 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 85</td>
<td>90 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 93</td>
</tr>
<tr>
<td>Thin hardboard</td>
<td>85 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 89</td>
<td>93 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 95&lt;sup&gt;*2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wet-room gypsum plaster board</td>
<td>89 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 95</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
</tr>
<tr>
<td>Exterior gypsum plaster board</td>
<td>89 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 95</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
</tr>
<tr>
<td>Asphalt paper</td>
<td>89 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt; ≤ 95</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
</tr>
<tr>
<td>Cement-based board</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
</tr>
<tr>
<td>Glass fibre</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
</tr>
<tr>
<td>Expanded polystyrene</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
<td>95 &lt; RH&lt;sub&gt;cr12w&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

* This is based on the criterion (b). When using the criterion (a) concerning median rating ≥ 2, the result was <sup>1</sup> 85 < RH<sub>cr12w</sub> ≤ 90 <sup>2</sup> 95 < RH<sub>cr12w</sub>.

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

<table>
<thead>
<tr>
<th>Material</th>
<th>22 °C</th>
<th>10 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Criterion (a), median value ≥ 2</td>
<td>Criterion (b), first rating ≥ 2</td>
</tr>
<tr>
<td>Asphalt paper</td>
<td>3 &lt; w ≤ 3</td>
<td>2 &lt; w ≤ 3</td>
</tr>
<tr>
<td>Cement-based board</td>
<td>12 ≤ w</td>
<td>12 ≤ w</td>
</tr>
<tr>
<td>Chipboard</td>
<td>6 &lt; w ≤ 7</td>
<td>3 &lt; w ≤ 4</td>
</tr>
<tr>
<td>Exterior gypsum plaster board</td>
<td>0 &lt; w ≤ 1</td>
<td>0 &lt; w ≤ 1</td>
</tr>
<tr>
<td>Expanded polystyrene board</td>
<td>12 ≤ w</td>
<td>12 ≤ w</td>
</tr>
<tr>
<td>Glass fibre board</td>
<td>12 ≤ w</td>
<td>12 ≤ w</td>
</tr>
<tr>
<td>Pine sapwood</td>
<td>7 &lt; w ≤ 8</td>
<td>4 &lt; w ≤ 5</td>
</tr>
<tr>
<td>Plywood</td>
<td>5 &lt; w ≤ 6</td>
<td>4 &lt; w ≤ 5</td>
</tr>
<tr>
<td>Thin hardboard</td>
<td>11 &lt; w ≤ 12</td>
<td>3 &lt; w ≤ 4</td>
</tr>
<tr>
<td>Wet-room gypsum plaster board</td>
<td>4 &lt; w ≤ 5</td>
<td>3 &lt; w ≤ 4</td>
</tr>
</tbody>
</table>
Table 7 The week when mould growth could first be determined

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Rating ≥ 2 for at least one test piece</th>
<th>Median ≥ 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative humidity</td>
<td>85 %</td>
</tr>
<tr>
<td>Chipboard</td>
<td>10 °C</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22 °C</td>
<td>7</td>
</tr>
<tr>
<td>Pine sapwood</td>
<td>10 °C</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22 °C</td>
<td>3</td>
</tr>
<tr>
<td>Plywood</td>
<td>10 °C</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>22 °C</td>
<td>1</td>
</tr>
<tr>
<td>Thin hardboard</td>
<td>10 °C</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22 °C</td>
<td>-</td>
</tr>
</tbody>
</table>

On the basis of simulations from the estimated matrix performed according to section 2.7, we conclude that a correct judgement of mould growth with 95% confidence can be achieved by taking the median of seven judgements. However, only six test pieces were used in this study. For the simplified judgement between a damaged and undamaged piece, with a test piece considered to have failed when it has reached a rating of 2 or higher, a correct judgement of damaged pieces can be made with 97% confidence. This higher confidence compared to the case with seven pieces is obtained at the price of a higher risk of misjudgement in the other direction; namely, a correct judgement of non-damaged pieces is 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; Johansson et al., 2006). Sometimes the results presented in this article are consistent with the results from the studies that formed the basis for the proposal, 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 set-up of the experiments and/or variations in evaluation of the data. Factors that vary among the different experiments include the fungi used, inoculation method, climate,
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 79% RH. The critical moisture level is therefore between 75% and 79%. This study used RH levels differing by 5 percentage points, with two exceptions. The fewer percentage points between two tested climates, the narrower the interval for RH\textsubscript{crit}. However, measurement uncertainty limits how narrow these intervals may usefully be. In our case, uncertainty was at most 2.5 percentage points RH, so climates differing by less than 3 percentage points became irrelevant. To ensure stable climatic conditions during the tests and to minimise measurement uncertainty, it is important to use climate chambers that are stable and to continuously log the climate 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 Figure 5 and Figure 6. Viitanen tested a number of materials over a long 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 79% RH at 22°C. Had the test been allowed to continue for longer than 12 weeks in other climates too, 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 79% 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 climates 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 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 Figure 1, Figure 3 and Figure 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 (Doll and Burge, 2001). 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 damage should be allowed and still be considered acceptable. In this study, the definition of 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 median 2 for the first time. However, this method of analysing the results provides 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 reasonable conservative estimate of the material property. Method (a) is instead based on the estimated median of the material behaviour. However, in case of no material variation, method (b) underestimates the true critical level, since the worst case is solely caused by 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 minutes, was too short. A more likely explanation is that the high air exchange rate in the climate cabinets quickly achieved equilibrium between the surface of moistened samples and the prevailing climate in the climate cabinet. 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 climate into account, to minimise the risk of mould growth. The tests in this study were carried out under constant RH and temperature. In buildings, these factors fluctuate more or less, which affects mould growth (Adan, 1994; Viitanen, 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.

5 Conclusions

Many factors affect the critical moisture level that can be assigned to a building material. 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.

Acknowledgements

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References


Validation of critical moisture conditions for mould growth on building materials

Pernilla Johansson, Thomas Svensson, Annika Ekstrand-Tobin

Submitted to *International Biodeterioration and Biodegradation*
Validation of critical moisture levels and prediction of risk for mould growth on building materials in constructions based on results from laboratory tests.

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Keywords: mold; critical moisture level; building material; relative humidity; mold resistance; crawl space; attic

Abstract

Materials that are stored or used in damp climates may be subject to mould growth. However, all materials are not equally susceptible; for each specific material, there is a critical moisture level for mould growth. If this is exceeded, there is a risk that mould fungi will develop on the material. This level can be determined in accelerated laboratory test, at constant RH and temperatures, favourable to mould growth. Within a building however, the climate is expected to vary from one part of the construction to another and it is seldom constant. This means that there is a fluctuation in relative humidity and temperature, due to seasonal or shorter-term variations. In this study, test pieces of the same materials tested in a laboratory environment were placed in three outdoor ventilated crawl spaces and three outdoor ventilated attics, where the climate was varying, and mould growth on the test pieces was studied over 2.5 years. Material specific mould growth curves were produced based on critical moisture levels, as determined in laboratory experiments under constant climatic conditions. When the climate exceeded this curves, there was mould growth on the test pieces if the time was sufficiently long. The conclusion from the study is that although conditions in laboratory studies are simplified and accelerated, the results serves well to indicate mould growth in within a building construction.
1 Introduction

Different parts of a building are exposed to different climates; for instance, as a result of construction design and moisture produced by human activity. Materials in construction are affected by the surrounding climate, and if it is favourable to mould fungi, there is a risk that mould will develop on the materials. However, all materials are not equally susceptible to mould growth. For each specific material, there is a critical moisture level for mould growth. If this is exceeded, there is a risk that mould fungi will develop on the material. Critical moisture conditions are also dependent on temperature. Mould fungi require more available water to grow at lower temperatures; consequently, critical moisture levels will be higher at lower temperatures than they will be at higher temperatures.

The relationship between temperature, moisture and rate of growth on nutrient media in the laboratory has been described for a number of fungal species by so-called isopleths (e.g. Ayerst, 1969; Smith and Hill, 1982; Magan and Lacey, 1984). Some of these isopleths have been adapted to predict the risk of mould growth on building materials (Rowan et al., 1997; Sedlbauer, 2001). By calculating the expected climate of a construction or part of a building, these limiting growth curves may be used to assess the risk for mould to occur. It is then important that the data for critical moisture conditions for the various materials are available, since the value for one material cannot be used to predict the properties of another, although they may seem similar (Johansson 2012). Also, different treatments of a material may alter resistance to mould growth (Vacher et al., 2010).

Although there are optimal and minimal growth conditions for the different microfungi that are included in mould fungi, the organisms can survive in periods of unfavourable conditions. How well they can tolerate fluctuating periods varies among species (Park, 1982). On building materials, the rate and extension of mould growth have been shown to be lower when favourable conditions alternate with less favourable (Viitanen and Bjurman, 1995). In addition, how long these periods last is also of importance. In constructions, the climate that building materials are exposed to is seldom constant. Instead, there is a variation in both relative humidity and temperature. This variation can be long-term, such as seasonal variation, or shorter-term, for example, due to human activity or local climatic conditions. Therefore, the critical moisture level for a building material may be exceeded during either a shorter or a longer period, while during other periods the level may not favour mould growth. It is thus expected that the risk of mould growth occurring is low if the variations are such that the moisture levels are substantially under the critical
limits over a sufficiently long period and that the critical limits are exceeded only for short periods.

Outdoor ventilated crawl spaces and attics are constructions where the climate is highly governed by the outdoor climate. Therefore, there is a seasonal fluctuation in the exposed climate in these structures. In Scandinavia, the relative humidity in crawl spaces is highest in the summer and autumn when warm, moist outdoor air enters the cooler space. Since cold air can hold less moisture, the saturation moisture content is lower, and the relative humidity will increase. In outdoor ventilated attics, the relative humidity is instead usually at its highest during the winter months. There are also short-term variations, for example, during clear nights when heat radiates from the roof to the sky, which leads to lower temperature at the interior surface of the roof, which in turn enhances the risk for condensation and mould growth. In both types of construction, there is often extensive mould growth on the building materials (e.g. Pasanen et al 2001; Bok et al., 2009; Hagentoft and Kalagasidis, 2010).

The purpose of this study was to investigate whether results from laboratory testing of materials for critical moisture levels, where constant climate conditions are used, can predict mould growth in constructions where the climate fluctuates. The same materials that were tested in a laboratory environment (Johansson et al., unpublished results) were placed in three outdoor ventilated crawl spaces and three outdoor ventilated attics. The development of mould on the test pieces was followed by analysing each test piece twice a year, in spring and autumn, over 2.5 years. Not all materials used in the study were intended to be used in crawl spaces and/or attics, but because the climate of these constructions was expected to be such that mould growth was favoured, and the climate expected to fluctuate, they were considered suitable as test environments. In addition, the materials were very easily accessible, and the test pieces could easily be evaluated for mould growth at the defined intervals.

2 Materials and methods

2.1 Building materials

Nine building materials (see Table 1) were bought from a local building supply store. The materials are common in the Swedish construction market and have different critical moisture limits, according to a laboratory study on the same material (Johansson et al., unpublished results). Nine of the materials
were boards, and from each brand there were three boards. The ninth material was asphalt paper.

Table 1 Building materials used in the study.

<table>
<thead>
<tr>
<th>Building material</th>
<th>Description</th>
<th>Critical moisture level (Johansson et al., unpublished results)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>22°C</td>
</tr>
<tr>
<td>Pine sapwood</td>
<td>19 mm tongued and grooved board</td>
<td>75&lt;( \text{RH}<em>{\text{crit}} )≤79 85&lt;( \text{RH}</em>{\text{crit}} )≤89</td>
</tr>
<tr>
<td>Plywood</td>
<td>12 mm softwood plywood</td>
<td>75&lt;( \text{RH}<em>{\text{crit}} )≤79 85&lt;( \text{RH}</em>{\text{crit}} )≤89*(1)</td>
</tr>
<tr>
<td>Chipboard</td>
<td>12 mm particle board</td>
<td>79&lt;( \text{RH}<em>{\text{crit}} )≤85 93&lt;( \text{RH}</em>{\text{crit}} )≤95*(2)</td>
</tr>
<tr>
<td>Thin hardboard</td>
<td>3.2 mm high density hardboard made of wood fibres and lignin</td>
<td>85&lt;( \text{RH}<em>{\text{crit}} )≤89 95&lt;( \text{RH}</em>{\text{crit}} ) *(3)</td>
</tr>
<tr>
<td>Wet-room gypsum plaster</td>
<td>13 mm gypsum board with cardboard surfaces</td>
<td>89&lt;( \text{RH}<em>{\text{crit}} )≤95 95&lt;( \text{RH}</em>{\text{crit}} )</td>
</tr>
<tr>
<td>Exterior gypsum plaster</td>
<td>13 mm gypsum board with cardboard surfaces</td>
<td>89&lt;( \text{RH}<em>{\text{crit}} )≤95 95&lt;( \text{RH}</em>{\text{crit}} )</td>
</tr>
<tr>
<td>Asphalt paper</td>
<td>1.5 mm windproof barrier of asphalt-impregnated cellulose paper</td>
<td>89&lt;( \text{RH}<em>{\text{crit}} )≤95 95&lt;( \text{RH}</em>{\text{crit}} )</td>
</tr>
<tr>
<td>Cement-based board</td>
<td>8 mm cement based board consisting of cement, limestone and cellulose fibers, covered with a plastic dispersion</td>
<td>95&lt;( \text{RH}<em>{\text{crit}} ) 95&lt;( \text{RH}</em>{\text{crit}} )</td>
</tr>
<tr>
<td>Glassfibre board</td>
<td>15 mm rigid glass wool insulation board</td>
<td>95&lt;( \text{RH}<em>{\text{crit}} ) 95&lt;( \text{RH}</em>{\text{crit}} )</td>
</tr>
<tr>
<td>Expanded polystyrene board</td>
<td>50 mm expanded polystyrene insulation board</td>
<td>95&lt;( \text{RH}<em>{\text{crit}} ) 95&lt;( \text{RH}</em>{\text{crit}} )</td>
</tr>
</tbody>
</table>

From each board, one test piece sized 50 x 100 mm was prepared for each test site, so the number of test pieces for each material was three. For plywood and chipboard, an additional test specimen was prepared from two of the boards,
and consequently there were five replicates of those materials. From the asphalt paper, all test pieces were prepared from one roll, and three samples placed on each test site.

The test pieces were placed in stainless steel spring clips mounted on aluminium strips placed in the underlay roof of the attics and blind floor of the crawl spaces. The test pieces were able to be easily dismantled from the clips when they were to be analysed for microbial growth.

All materials were handled in such a way as to minimise risk of contamination that might lead to mould growth, for example, by using plastic gloves.

### 2.2 Test-sites

The houses where the test pieces were exposed were all single-family houses situated close to Borås in the south-west of Sweden. All these houses were buildings with light wooden framework structure and were representative of Swedish housing stock. They varied in construction year, heating system, construction design, building materials, and other characteristics and were selected to represent both high- and low-risk construction with regard to mould growth.

#### 2.2.1 House A; Attic 1 and Crawlspace 1

House A was built in 1923 and was the oldest house in the study. The house was heated by a pellet boiler located in one of the outbuildings and a stove connected to the building’s central chimney. The house roof was hipped with a 45° angle in the ridge. Roof material from the outside and inwards consisted of concrete tiles, thin hardboard and wooden rafters. The foundation was of stone, the floor consisted of soil and stone, and the height of the crawl space was about 1 m. The joist was insulated with approx. 10 cm of wooden chips.

#### 2.2.2 House B; Attic 2 and Crawlspace 2

House B was built in 1913 with a 40° pitched roof. The materials in the roof were ceiling tiles, roofing felt, wooden rafters and secondary spaced boarding. The house was in a half plane with a crawl space under two-thirds of the house. The remainder consisted of laundry room and boiler room. The heating was provided by a pellet boiler. The foundation was of stone, the floor consisted of soil and stone and the height of the crawl space was about 0.5 m. The joist was insulated with approx. 10 cm of wooden chips.
2.2.3  House C; Crawlspace 3
House C was built around 1980. The foundation of the house consisted of a crawl space under two-thirds of the house. One third consisted of a cellar including laundry and boiler room. The house was heated from a wood furnace in the basement. The crawl space section, where the test was carried out, was ventilated with outdoor air and unheated. The foundation was of concrete blocks, and the surface of the crawl space was crushed rock/gravel. The joist was insulated mineral wool insulation.

2.2.4  House D; Attic 3
House D, built in 1982, comprised 1½ storeys with a 45° pitched roof. At the beginning of the project, heating was provided by direct electricity, but this was replaced after a year or so by an air-water heat pump, though distribution via water was the same throughout the test period. Throughout the period fires were also frequently built in a centrally located wood-burning stove. The roof was built, from the outside inwards, of concrete tile, roofing felt, roof trusses and hard fibre board.

2.3  Measurement of the climates at the test-sites
The relative humidity and temperature at each test-site was registered every fourth hour by data loggers with internal sensors (Testo 177-H1). These were placed in close proximity to the specimens to ensure that the climate logged was as close as possible to that which the specimens were exposed to. One logger was placed at each test-site.

The sensors were initially calibrated at 30.1°C over three aqueous saturated salt solutions, with reference values of 83.6%, 92.3% and 75.1% respectively. After exposure in the field, a new calibration was performed, this time in moisture chambers with a calibrated reference moisture and humidity sensor, at two temperatures (22°C and 15°C) and three values of RH (90%, 85% and 60%). The calibration made it possible to adjust the measured values of RH with the correction factor from calibration (reference RH minus measured RH). However, since this factor was expected to be dependent on temperature (Fernicola et al., 2008), and the temperature and relative humidity were not constant in the field measurements, the latter calibration procedure made it possible to make a more accurate adjustment of data. A multiple regression was performed, and both temperature and relative humidity were included. Each sensor was then given an equation, with which the measured values were adjusted.
Comparing the results of RH from the initial and final calibrations, it was concluded that all of the sensors had drifted, in various extents, so that they showed higher values after, compared to before, exposure in the field. The measured values were therefore further adjusted. For each logger, the drift, which was assumed to be constant during the exposure period, was estimated by calculating the difference in the calibration error after and calibration error prior to measurement in the field. By dividing this difference by the total number of measurement occasions, which were about 5500 (6 times a day for 2.5 years), a value for the drift at each time that was logged was obtained. We call this the \textit{drift factor}.

The actual value of the RH at each time point was calculated for each sensor using the equation obtained from the multiple regression based on the final calibration, complemented by an adjustment with the service drift factor multiplied by the successive number of the time point.

\[ RH_{\text{adjusted}} = a_i + b_i \times RH_{\text{instrument}} + c_i \times t_{\text{instrument}} - \gamma \times T \quad [\%] \quad (1) \]

Where
\begin{itemize}
  \item $a_i$, $b_i$, $c_i$ Parameters from the multiple regression for each logger $i$
  \item $RH_{\text{instrument}}$ measured RH (\%)
  \item $t_{\text{instrument}}$ measured temperature (°C)
  \item $\gamma$ drift factor - error due to the drift at each measuring point
  \item $T$ The number of the measuring point
\end{itemize}

The expanded uncertainty for each sensor was calculated by considering the variance of the multiple regression together with the uncertainty of the reference sensor and the measurement uncertainty for the calibration.

\subsection*{2.4 Analysis of mould growth on test pieces}

Every six months, in April and October for a total of five occasions, the test pieces were removed from the racks where they were exposed, and they were analysed for mould growth. The surface that had been exposed to the open air in the attics and crawl spaces was studied under the microscope at 10–40 x magnification. Both mould growth visible to the naked eye and fungi only visible at this magnification were rated according to a five-point rating scale where 0=no growth; 1=sparse, initial growth with only one or a few hyphae present; 2=sparse but clearly established growth; 3= patchy, heavy growth; and 4=growth over more or less the entire surface. A test piece was considered to have failed when the rating of mould growth first reached 2 or higher, that is when mould growth was clearly established.
2.5 Comparison of results from laboratory and field studies

To create growth limit curves for each material, we used the same technique as (Hofbauer et al., 2008), where material specific isopleths were constructed from the closest approximation to the LIM 0 curve (Sedlbauer 2001).

The equation for the LIM 0 curve was not published, so we chose to fit the data from this curve to a polynomial of second degree. This contains three parameters, but in order to simplify the model we wanted only two parameters. Therefore, we fixed a function minimum at a defined temperature, namely 27°C. This value was based on the observation that, for many species of mould fungi, the temperature at which the required water availability is at minimum, i.e. the optimum temperature, is between 25°C and 30°C (Magan and Lacey, 1984). At both sides of this temperature the moisture requirements are higher, as described by the isopleths for each species. For the species used in the laboratory tests that formed the basis for the critical moisture levels for the materials in this study, the optimum temperature lies between 22°C and 30°C (according to Sedlbauer 2001), with a mean value of 27°C. The resulting growth limit curve model can then be described by Equation 2.

\[ RH = a + c(t^2 - 54t) \quad [%] \] (2)

where \( t \) is the temperature in °C.

The parameters \( c \) and \( a \) were estimated by using Equation 3 and Equation 4, where the data for \( RF_{\text{crit}} \) and corresponding temperature come from the results in the laboratory tests, Table 1.

\[ c = \frac{RH_{\text{crit1}} - RH_{\text{crit2}}}{t_1^2 - t_2^2 - 54(t_1 - t_2)} \] (3)

\[ a = RH_{\text{crit1}} - c(t_1^2 - 54t_1) \] (4)

Two growth limit curves, one upper and one lower, were produced for each material. The laboratory tests were carried out in climates of constant RH, with RH set at intervals of 5%; therefore the critical moisture level fell into a range. The upper limit was determined by the climate case with lowest RH where any of the above criteria were met, and the lower limit by the climate case with the next-lowest RH. The actual critical moisture level may therefore lie between the two growth limit curves, or above the upper curve (Hofbauer et al., 2008).
In order to find out if the climate in the test-sites had exceeded the growth limit curves, the curves were drawn into plots of monitored relative humidity and temperature. Mould growth was then expected when the relative humidity and temperature exceeded the growth limit curves (Rowan et al., 1997). This was then compared to the results from the assessment of mould growth on the test pieces at each test site.

In those cases where expected and actual mould growth were not consistent, the number of occasions on which the relevant growth limit curves were exceeded was counted. Each measurement point was then regarded as constant for four hours because RH and temperature were logged at this interval. The cumulative time was calculated as the sum of these hourly values. If the time was shorter than the shortest time before the critical moisture level was achieved in the laboratory no growth was expected.

2.6 Prediction of critical moisture levels for different temperatures

Testing to find the critical moisture level must be done at several RH, and to predict the risk at different temperatures according to section 2.5 above, using at least two temperatures. This entails extensive testing in practice. One way to make the test feasible is to test at one temperature and to predict the expected critical moisture level at other temperatures. In such case, parameter \( c \) or \( a \) needs to be known. Based on the values of \( a \) that were estimated for each material in the study, an average was therefore calculated, which was then used for all materials. This was then used to calculate parameter \( c \) for each material and then new limit curves could be created in accordance with Equation 2. The difference between the two curves obtained in this way for each material was then assessed.
3 Results

The results from measurements of RH and temperature are presented for the crawl spaces in Figure 1 and for the attics in Figure 3. Relative humidity in the attics was highest during the cold months of the year, when the temperature was also lowest. In the crawl spaces, however, RH was highest in the summer. Variation during the day was greatest in the attics. Measurement uncertainty for RH was between 1% and 1.5% for each location.

Mould grew on several of the material test pieces. However, which materials moulded, how quickly growth arose, and what was the extent of growth differed among sites. A test piece was considered to have failed (reached the critical level of mould), when growth for the first time was categorized as at least Class 2. Figure 2 and Figure 4 present the percentage of test pieces that were not damaged at each analysis time, expressed as Kaplan-Meier curves. On each occasion that a test piece failed, the percentage of surviving test pieces decreased. Pieces that did not fail during the test period were censored in the plots. Table 2 presents the number of test pieces with established growth at the end of the test. No growth was observed on any of the test pieces of cement-based board, glass fibre board or expanded polystyrene board. No growth was observed on any of the test pieces in Attic 2.

Table 2 Number of test pieces with established mould growth (rating ≥2) at the end of exposure time. The total number of test pieces for each material at each test site is shown between the parentheses in the heading.

<table>
<thead>
<tr>
<th></th>
<th>Chipboard (N=5)</th>
<th>Plywood (N=5)</th>
<th>Thin hardboard (N=3)</th>
<th>Wet room plaster board (N=3)</th>
<th>Exterior plaster board (N=3)</th>
<th>Expanded polystyrene (EPS) board (N=3)</th>
<th>Asphalt paper (N=3)</th>
<th>Glass fibre board (N=3)</th>
<th>Cement based board (N=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crawlspace 1</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Crawlspace 2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Crawlspace 3</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Attic 1</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Attic 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Attic 3</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 1  Monitored relative humidity and temperature in three crawl spaces in Sweden. Values marked with “RH$_{min}$” refers to data where the drift of the loggers entailed that the correct value could not be estimated. The specified dates refer to when microbiological analysis were performed.

Figure 2  Proportion of test pieces of six building materials, exposed for the climate in three crawlsaces, with no established growth at different times of analysis.
Figure 3  Monitored relative humidity and temperature in three crawl spaces in Sweden. Values marked with “RH_{min}” refer to data where the drift of the loggers entailed that the correct value could not be estimated. The specified dates refer to when microbiological analysis were performed.

Figure 4  Proportion of test pieces of six building materials, exposed for the climate in three attics, with no established growth at different times of analysis.

The limit curves for mould growth are presented for each material, together with the measured data of RH and temperature for each test site in Figure 5 and Figure 6. Measured relative humidity is plotted against measured temperature, where each point corresponds to one measurement. If points are above the limit lines, growth is expected on the test pieces. Table 3 compares the results expected under this criterion with actual observations of growth on the test pieces at the end of the field study. Limit curves for growth were not
produced for cement-based board, glass fibre board or expanded polystyrene board, since mould did not grow on any of these materials in any of the climates tested in the laboratory and the critical moisture level cannot be estimated and/or the materials are resistant to mould growth.

Figure 5  Growth limit curves for (a) chipboard and (b) thin hardboard and measured temperature and relative humidity in three crawl spaces and three attics over 2.5 years. Each dot represents one measuring point. The circles indicate values where drift of the loggers made it impossible to calculate a “true calibrated value” of RH and are therefore minimum values. The dotted lines are the lower and upper growth limit curves estimated from results in laboratory tests.
Figure 6  Growth limit curves for (a) asphalt paper and two types of plaster boards and (b) plywood and measured temperature and relative humidity in three crawl spaces and three attics over 2.5 years. Each dot represents one measuring point. The circles indicate values where drift of the loggers made it impossible to calculate a “true calibrated value” of RH and are therefore minimum values. The dotted lines are the lower and upper growth limit curves estimated from results in laboratory tests.

Table 4 shows the total time that the climate was over the limit curves for those cases where expected and actual mould growth were not consistent, together with the time when the critical moisture level was achieved in the
laboratory experiments. Since these analyses were carried out once a week, time is reported as an interval.

When both criteria, climate exceeding the limits for growth and cumulative time over this is lower than the time before mould growth is established in the laboratory, there is consistency between actual and expected mould growth.

Table 3  *Expected and actual mould growth for the materials at each test site. If values of RH-temperature in Figure 5 and Figure 6 exceeded the growth limit curves, mould growth was expected (+); otherwise it was not expected (-). Existing growth (+) and non-existing (-) growth are according to Table 1. White boxes are cases where expected and actual growth do not agree based on these criteria.*

<table>
<thead>
<tr>
<th>Material</th>
<th>Crawlspace 1</th>
<th>Crawlspace 2</th>
<th>Crawlspace 3</th>
<th>Attic 1</th>
<th>Attic 2</th>
<th>Attic 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plywood</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chipboard</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Thin hardboard</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Exterior gypsum paper board</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Wet room gypsum paper board</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Asphalt paper</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cement-based board</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Glassfibre board</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Expanded polystyrene board</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*this is based on the findings that there was no mould growth on any of the test pieces in the laboratory
Table 4  Cumulative time that measured climate at different test-sites exceeded growth limit curves for each material, where it was predicted that mould growth would occur, and the time before critical moisture levels was reached in the laboratory tests

<table>
<thead>
<tr>
<th>Material</th>
<th>Crawlspace 3</th>
<th>Attic 1</th>
<th>Attic 2</th>
<th>Attic 3</th>
<th>Time (weeks) in the laboratory before critical moisture level was reached</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (weeks)</td>
<td>Weeks (weeks)</td>
<td>Weeks (weeks)</td>
<td>Weeks (weeks)</td>
<td>10°C</td>
</tr>
<tr>
<td>Plywood</td>
<td>0.3</td>
<td>0.0</td>
<td></td>
<td></td>
<td>11-12</td>
</tr>
<tr>
<td>Chipboard</td>
<td>8.3</td>
<td>3.0</td>
<td></td>
<td></td>
<td>8-9</td>
</tr>
<tr>
<td>Thin hardboard</td>
<td>3.0</td>
<td>0.9</td>
<td>1.9</td>
<td>1.4</td>
<td>10-11</td>
</tr>
<tr>
<td>Exterior gypsum board</td>
<td>0.9</td>
<td>0.3</td>
<td>1.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Wet room gypsum paper</td>
<td>0.9</td>
<td>0.3</td>
<td>1.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Wet room gypsum paper</td>
<td>0.9</td>
<td>0.3</td>
<td>1.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Asphalt paper</td>
<td>0.9</td>
<td>0.3</td>
<td>1.4</td>
<td>0.3</td>
<td>&gt;12</td>
</tr>
</tbody>
</table>

From the laboratory test six mould growth curves according to 2.5 were calculated. The upper curve of one material is sometimes the same as the lower of another. For the ten materials tested there are therefore a total of six such limiting curves, with values of $a$ ranging from 102 to 108, with a mean value of 105. New growth curves with this value of $a$ were produced, and compared to the ones produced with individual value. Figure 7 shows an example of growth limit curves for the value estimated for the relevant material, in this case chipboard, along with a curve based on the mean of $a$ for all materials in the study. The maximum difference between these two differences in the present case is two percentage points for RH in the 0°C to 40°C interval, and one percentage point in RH in the 10°C to 22°C interval, which represent the values for all materials well.
Discussion

In this study, expected mould growth on building materials, based on laboratory studies of criteria for critical moisture levels, was compared with actual results on test pieces exposed in crawl spaces and attics. If the climate exceeds the limits for growth then mould growth is expected. Length of time above the critical level is also significant. Since both of these criteria are taken into account, the consistency between expected and actual mould growth is good.

The number of test pieces of each material at each test site was relatively low (3 to 5). The number was chosen based on the number of test pieces that are common in testing methods for mould resistance tests. After the start of the study, we found that an appropriate number of test pieces for assessment of mould growth was at least 7 in order to obtain a sufficient level of confidence in the assessment (Johansson et al., unpublished results). With a large expected variation among different test pieces of the same material, the number of test pieces should be as large as possible. After 2.5 years of exposure, however, little variation was found among the test pieces; in 10 of 13 cases where growth was observed, mould grew on all test pieces of the tested materials at each site, see Table, 2 so little variation was found among the test pieces. The number of test pieces is too small to make a Kaplan-Meier estimation; Figure 2 and Figure 4 therefore should be considered empirical data.

In Crawlspace 1, the values for temperature-RH exceeded all growth limit curves on several occasions, and all materials had established growth of mould. Even in Attic 3, measured points for climate were repeatedly above all curves. Established growth was found on plywood, chipboard and thin
hardboard. The reason that mould did not grow on asphalt paper or the two types of plasterboard is probably that the periods of conditions favourable for growth were not long enough, since the cumulative time that the limits for these materials were exceeded was less than one week, which was significantly less than the minimum required for the critical moisture level to be achieved in a laboratory environment. In Crawlspace 3 and Attic 1, growth of mould was expected on all materials on which there was mould growth in the laboratory, since several points of RH-temperature were above the growth limit curves. Although the cumulative time that this occurred was greater than in Attic 3, it was still less than was required for critical mould growth in laboratory conditions, which may explain why mould did not grow on materials other than plywood in Crawlspace 3 or on plywood and chipboard in Attic 1. However, the values for chipboard in Crawlspace 3 are on the boundary of the reference time from the laboratory tests.

In Crawlspace 2, measured values only exceeded growth curves for plywood, which was also the only material on which mould grew. Even in Attic 2, mould was only expected to grow on plywood, though no growth was observed here. Again, the reason might be that the favourable climate did not last long enough; in this case, the cumulative time that the lower growth limit curve was exceeded was less than one week. No values were measured above the upper curve at all. In practice, it is therefore possible that the actual critical value for mould growth was never exceeded, since it may lie somewhere between the upper and the lower curve.

Using the cumulative time for climate that was favourable is a simplified method of considering the conditions that affect mould growth. These favourable conditions were followed by less favourable, and how long they last, how far they are from the limits, and how rapidly the climate changes between favourable and unfavourable conditions are significant to the risk that mould growth will occur, as well as to its scope and its growth rate (Viitanen and Bjurman, 1995; Adan, 1994; Pasanen et al., 2000). This may explain why mould did not grow on chipboard in Crawlspace 3, even though the cumulative time that favourable conditions were present was about the same as the reference values from the laboratory tests.

It is difficult to predict, based on the material data from tests in the laboratory, how long a material can withstand a particular environment without risk of mould growth (i.e. in addition to the time tested in the laboratory). This testing was conducted over 2.5 years. It is possible that testing for a longer time would increase the chance that mould could develop even on the material where no growth was observed, since the cumulative time during which
favourable conditions are present would be longer. A material ages over time, and contamination of the material can reduce resistance to mould growth (Grant et al., 1989; Chang et al., 1996).

Assessing the critical moisture level of a material requires testing it in a number of climates. The test period and the number of test climates must be limited in order for a commercial method to be economically justifiable and the results to be delivered within a reasonable amount of time. The model used to produce growth limit curves for different materials requires testing to be carried out at two temperatures. However, an adjustment was made in this study so that when testing at only one temperature, critical limits could be estimated for other temperatures within a maximum error of 2% RH. To reduce the time before mould growth occurs, the temperature should be relatively close to the optimum temperature for growth.

5 Conclusion

Overall, critical moisture levels, as determined in accelerated laboratory experiments under constant climatic conditions, match the results in real conditions where both temperature and relative humidity vary. If the combination of relative humidity and temperature exceeds the growth limit curves calculated from the critical moisture levels, mould growth is expected. Further, when the duration of favourable conditions is not considered, the laboratory test results will not underestimate the risk of mould growth. Instead, the results will entail a certain margin of safety.

If the expected climate in a construction is known, knowledge of the materials’ critical moisture levels and the calculated growth limit curves may therefore be used as a tool when choosing materials for the construction, with a minimum risk of mould growth.
6 References


Test Method for Determining Critical Moisture Level for Mould Growth on Building Material

Pernilla Johansson, Annika Ekstrand-Tobin, Gunilla Bok
SP Method 4927, draft (February 23, 2012)

Test Method for Determining Critical Moisture Level for Mould Growth in Building Material

Pernilla Johansson, Annika Ekstrand-Tobin, Gunilla Bok

1 Scope

SP Method 4927 determines critical moisture conditions for mould growth in building material. It is valid for new, clean material that has not previously been exposed to conditions that will enable mould growth. It can also be used for testing the effect of a treatment of material.

The tests are carried out under constant RH and temperature. In buildings, these factors fluctuate more or less, which affects mould growth. Therefore, the results 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.

2 Principle

Specimens of the material to be tested are inoculated with a suspension of spores from mould fungi and are then incubated in climate chambers under specified conditions. At defined time intervals, the samples are inspected for fungal growth, which is assessed according to a rating scale. The critical moisture level for mould growth is determined by considering the lowest RH at which mould growth appears during the present test period.

3 Significance and Use

Building materials that are stored or used in damp climates may be subject to mould growth. The susceptibility of building materials to mould growth varies. Some materials withstand high relative humidity without mould growth occurring, while on others mould can grow at as low as 75% RH. In a building, different construction parts are exposed to various humidity and
temperature conditions. To minimise the risk of microbial growth, materials should be chosen that can tolerate the climate in question.

This test method can be used to determine a material's critical moisture level regarding mould growth related to a specific incubation period; that is, a specified moisture level at which there is a risk of developing mould growth.

4 Climates

The test is performed at reference values 22°C and RH of 95%, 90%, 85% and 80%.

5 Apparatus

All apparatus must be serviced and calibrated at regular intervals.

5.1 Climate chambers. Climate chambers that can provide the required climate are used. They must maintain a climate of temperature and humidity with good homogeneity; the fluctuations must not be bigger than 3% RH. The required climate must be restored within eight hours after opening and closing of the chambers. Also, the climate must be the same in all parts of the chambers.

5.2 Device for monitoring the climate. A separate humidity and temperature transmitter with a data logger is used in each climate chamber to monitor the stability of the temperature and relative humidity every tenth minute. From these sampled data, the means and standard deviations for each climate are calculated during incubation. The transmitter is calibrated once a year if continuously used; otherwise it may be calibrated prior to the time of use. The expanded uncertainty of measurement according to EA-4/02 must not exceed 3% RH.

5.3 Spraying device. An airbrush connected to a mini-compressor, or to small cans with compressed air, is used to spray the spore suspension onto the surface of the test pieces. The most effective way is to use a model where the cup for the liquid is open and placed directly on the airbrush. A filter/regulator that removes moisture from the compressed air is connected to the compressor.

5.4 Stereomicroscope with 40x magnification with an adjustable light source makes it possible to light the whole surface of the item at a low angle.
5.5 *LAF bench* is needed to provide biological safety throughout the process of making the spore suspension as well as during the assessments.

5.5 *Autoclave* is used to sterilize reagents, materials and other utensils for the test.

5.6 *Centrifuge*.

### 6 Reagents and materials

6.1 *Purity of water*. The water used is deionized, with a minimum degree of purity of 0.2 µS/cm. This water is also autoclaved at 121°C for 15 minutes.

6.2 *Nutrient media*

   a) A minimum of 10 Petri dishes are prepared with malt agar (20 g agar and 20 g malt extract to 1000 ml water) for cultivation of fungi and for viability controls.

   b) A 250 ml malt solution (20 g malt extract to 1000 ml water) is prepared for climate control.

6.3 *Test organisms*

<table>
<thead>
<tr>
<th>Organism</th>
<th>CBS number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eurotium herbariorum</em></td>
<td>115808</td>
</tr>
<tr>
<td><em>Aspergillus versicolor</em></td>
<td>117286</td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>401.92</td>
</tr>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td>101160</td>
</tr>
<tr>
<td><em>Cladosporium sphaerospermum</em></td>
<td>122.63</td>
</tr>
<tr>
<td><em>Stachybotrys chartarum</em></td>
<td>109.292</td>
</tr>
</tbody>
</table>

The CBS numbers refer to strains maintained by Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands and can be ordered from their website [www.cbs.knaw.nl](http://www.cbs.knaw.nl).

6.4 *Cultivation of fungi*. Prepare freeze dried strains according to instructions from the supplier. Cultivate each species on separate Petri dishes with malt agar (20 g agar and 20 g malt extract to 1000 ml water) until heavy sporulation
has been developed. Usually this will occur within four weeks. Prepare two to three Petri dishes for each strain.

6.5 Control glass microfiber filters. Glass fiber filters, binder-free and not containing any cellulose, 55 mm in diameter are used for controls. These are autoclaved at 121°C for 15 minutes.

6.6 Glass beads. About 100 cl of glass beads, about 5 mm in diameter, are used in the spore solution production process.

6.7 Flasks. Six glass flasks, each with volume 250 ml, are needed in the spore solution production process.

6.8 Glass funnels. Six glass funnels are used in the spore solution production process.

6.9 Washed glass wool is used as a filter in the glass funnels.

6.10 Counting chamber or equivalent equipment is used when calculating the concentration of spores in the solution. Handling and calculation is done according to the manufacturer’s instructions.

6.11 Burner or spirit lamp is used to sterilize the preparation needle when spores are harvested from the different cultures.

6.12 Additional supplies necessary during production of spore solution: Pipettes, pipette tips, centrifuge tubes, Petri dishes, latex or plastic gloves, face masks.

7 Test specimens

Prepare 7 test specimens of the material to be tested for each of the four climates (4x7 samples). Each piece should be 50x100 mm. The sample sets should consist of samples originating from different batches in the production line of the material. If the test material consists of boards with two different sides, duplicate sample sets are required as each side will be tested separately.

When testing in situ formed loose-fill thermal insulation or similar materials, each material sample is placed in a cage of fine stainless steel mesh (autoclaved) with an internal volume of approximately 0.05 litres.

The density in the cage should be the declared bulk density.
Risk of contamination that might lead to mould growth by handling all materials with care, for example, is minimised by using gloves and clean equipment.

8 Procedure

8.1 Aseptic work conditions

To ensure aseptic conditions during the preparation of spore suspension and inoculation of test pieces, sterilise all equipment, such as forceps, funnels, flasks, etc. before use. Perform the work with clean gloves in a laminar air flow (LAF) bench.

8.2 Preparation of spore suspension

a) Prepare six flasks with 45 ml distilled water and a layer of glass beads and autoclave at 121°C for 15 minutes. Allow to cool.

b) Put a thin layer of glass wool in six glass funnels and autoclave at 121°C for 15 minutes. Allow to cool.

c) Verify the purity of each culture.

d) Pour 10 ml of distilled, autoclaved water onto each of the Petri dishes with subcultures and scrape the surface of the fungal colonies gently and carefully with an inoculation spreader.

e) Pour the liquid into the prepared sterile flasks containing glass beads and autoclaved water, one for each species. Shake the flasks vigorously to liberate the spores from conidiophores and to break up any large spore clumps.

f) Filter the liquid through the sterile glass wool in the glass funnel into a centrifuge tube, one for each species.

g) Centrifuge the tubes with suspension until a spore pellet is formed. Then pour off the supernatant, fill the tubes with new water and centrifuge in the same manner as before. Repeat this procedure three times, so that any nutrients from the agar that could affect the test results are washed out and hyphae are avoided in the final solution.
h) Determine the spore concentration in the final washed residue for each species using a counting chamber under the microscope, then dilute this residue so it contains approximately $10^6$ spores per ml.

i) Prepare the final spore suspension by mixing equal volumes of suspension from each species.

8.3 *Inoculation of test specimens.*

Spray 0.4 ml of the spore suspension onto one surface of each test specimen and the controls (sec.7) by using an airbrush. During spraying, sweep the airbrush along at an even speed so the spores will be distributed more or less evenly over the surface of the test pieces.

8.4 *Inoculation of positive control #1: viability of the spore suspension and each of the species.*

Spray 0.4 ml of the spore suspension onto two Petri dishes with malt agar. Inoculate separate Petri dishes with one or two droplets of each of the spore suspensions from the individual species. Incubate the Petri dishes, with lid on, in room temperature or higher, maximum 30°C.

8.5 *Inoculation of positive control #2: climate chamber environment.*

Dip four glass microfiber filters (sec. 6.5) into malt solution (sec. 6.2b). Then spray 0.2 ml of the spore suspension onto each filter paper and place in separate Petri dishes, with the lid off. Incubate together with inoculated test pieces in each climate chamber.

8.6 *Inoculation of negative control: purity of spore suspension.*

Inoculate one microfiber filter by spraying with 0.2 ml of the spore suspension. Place it in a sterile Petri dish and incubate in the test chamber, with the lid off, at 95% RH, 22 °C.

8.7 *Non-treated reference.*

When evaluating the efficiency of a treatment, for example, a coating or added fungicide, use non-treated test pieces from the same material as reference.
8.8 Incubation.

Following inoculation, incubate the test specimens horizontally on cleaned racks, sprayed surface up, in the dark in the climate test chambers. Maximum time for incubation is 12 weeks, or until median rating of the six test pieces equals or exceeds rating 2, according to section 8.9.

8.9 Analysis of microbial growth.

Every second week, remove the test pieces from the moisture chambers for analysis of mould growth under the microscope at 10–40 x magnification by an experienced analyst. Mould growth on the inoculated surface of each test sample, excluding the edges (0–5 mm), are assessed according to the rating scale shown in Table 1. During this procedure, it is important to use low-angle light to detect hyaline as well as dematiaceous hyphae.

If there is mould growth at the edges of the test piece at the end of the test period, this must be noted. Also, if the material tested is porous and mould growth might be expected in the material, slit the test pieces and analyse the new surfaces. If there is mould growth, note this.

In order to minimise further contamination with spores and dirt, which could increase the risk of mould growth, perform the analyses in a laminar air flow (LAF) bench and handle the test pieces with gloves.

Mould growth is not expected to grow at one RH until the same week as the next highest RH. To streamline the analysis process and limit its cost, the scheme described in Annex A may be used.
Table 1  Assessment scale of mould growth extent

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

9 Disruptions and deviations

9.1 Viability of the spore suspension and each of the species (positive control #1).

Check the plates after 5–7 days. If there is a lack of heavy growth, a new solution must be prepared and the test repeated with new test pieces.

9.2 Climate chamber environment (positive control #2).

If no growth is seen on the treated filter paper after 12 weeks of incubation, the test must be repeated.

9.3 Purity of spore suspension (negative control).
If growth appears on the untreated filter paper controls before or at the same time as on the treated filter papers, it might indicate the presence of nutrients in the spore solution. A new solution must then be prepared and the test restarted with new test pieces.

9.4 Non-treated reference.

If no growth is seen on the untreated reference material at the end of the test, the effect of the treatment cannot be evaluated. A new reference material must then be used and the test restarted.

10 Report and evaluation of results

A test piece is considered failed when the mould growth equals or exceeds rating 2. The critical moisture level is considered reached at the RH when at least two test pieces have failed and when no growth is detected on the test pieces at the next lower RH tested. The actual critical moisture level is then expected between these two values or at the RH when test pieces failed. The value is therefore reported as a range.

In the test report, it must be clearly stated which method has been used and at which temperature it is valid. The limits of the range are reported as mean value of the test climate, together with measurement uncertainty, not the reference values (80%, 85%, 90%, 95% RH).

If mould is detected at 80% RH, then the critical moisture level is estimated to be $75\% \leq \text{RH}_{\text{crit}} < 80\%$ RH.

To estimate the critical moisture limit for other temperatures than $22^\circ\text{C}$, two growth limit curves are calculated according to Equation 1. The parameter $c$ is then calculated according to Equation 2. An example is given in Figure 1.

$$\text{RH} = 105 + c(t^2 - 54t) \quad (\%) \quad (\text{Equation 1})$$

$$c = (\text{RH}_{\text{crit}_t} - 105)/(t^2 - 54t) \quad (\text{Equation 2})$$

where $t$ is the temperature ($^\circ\text{C}$) at which the test is performed, and $\text{RH}_{\text{crit}}$ is the value of the critical moisture level at this temperature.

In constructions where the climate is expected to be below the lowest growth limit curve, no mould growth is expected. If it exceeds the upper limit, mould growth is possible. In between the two curves, there is a zone in which the
critical moisture limit may fall. To be on the safe side, if the climate is in this zone, mould growth should be regarded as possible.

![Figure 1](image)

**Figure 1**  Example of mould growth limit curves, where critical moisture level at 22°C is $90 < R_F^{\text{crit}} < 95$

### 11 References

Standard Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions

Annex A

Incubation and analysis key at 2+2n weeks after incubation, n = 0 to 5

Start analysing the samples in the most humid climate two weeks after incubation. Beginning with the most humid climate, continue the analysis every second week as follows:

I. If mould growth is assessed \( \geq 2 \) according to Table 1 in any of the seven samples, this indicates that the critical moisture level is reached. Continue to analyse the seven samples in the less humid climate at the same analysis occasion with the same criterion described in I and II. If the criteria are not met, continue the incubation and await the next analysis occasion.

II. If the median of the ratings equals or exceeds 2 according to Table 1, then the incubation in this climate may be stopped.