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Doctoral thesis

Preface

It is my hope that the results of the work presented in this thesis will contribute to increased awareness of the necessity to choose the right materials for various parts of buildings, in order to reduce the problems associated with mould, leading to a better indoor environment and the wellbeing of the residents and users of these buildings. Furthermore, I hope that the results will contribute to the construction of sustainable buildings, as the need to renovate buildings and replace damaged materials can be reduced if materials that can withstand mould growth are chosen already at the design stage of a new building.

The new method presented in this thesis, "Laboratory method for determination of critical moisture level for mould growth on building materials" (SP method 4927) has been remitted to several instances within the Swedish construction sector. I would like to thank everyone who gave their opinion of the method. BOVERKET - The Swedish National Board of Housing, Building and Planning will refer to this work and to the new test method in its general guidelines (BBR 2014 6:52). At the time of printing this thesis, two material manufacturers had allowed their materials to be tested using SP method 4927, and are therefore the first to be able to present results, specific to their products. It is hoped that others will follow so that critical moisture levels can be reported for their materials.

Concerns have been raised that use of the method will disadvantage materials with a low critical moisture level. I am of the opposite opinion – testing according to the new method will instead broaden the area of use of certain building materials that would have failed traditional mould resistance tests. No material is classified as being generally better or worse than another. In the right conditions all materials will be suitable, however, in constructions exposed to high levels of moisture, materials with a high critical moisture level should be used, rather than those with a low level.

The major part of the work presented in this thesis concerns the determination of the critical moisture levels of various materials. A large number of experiments have been performed in the laboratory and in field studies. These studies have been financed by the FORMAS project "Critical Moisture Conditions for Microbiological Growth on Different Building Materials (CRAM)". The formulation of the method has been partially achieved in the VINNOVA project "Wood Build", which also funded the study on the impact of fluctuating climate on mould growth. The last part of this thesis is a meta-study that deals with various parameters affecting mould growth on wood, in

not officially one of my supervisors, you have been an invaluable mentor. Thank you all for your support, advice and good ideas!

I grew up in a home filled with books, from floor to ceiling. My parents have always encouraged me to learn new things. Thank you to my parents, Ingemar and Tove Lundin, you are proof that one is never too old to gain new knowledge and insights.

Finally, my beloved family, Johan, Filippa, Viktor and Joel, thank you for being there and for making my life such fun and so eventful! Thank you for your patience when my thoughts were on my work, rather than what was going on in your lives.

Borås in April 2014

Pernilla Johansson

Abstract

The susceptibility to mould growth varies between building materials. The factors that most affect mould growth, the relative humidity (RH) and temperature also vary in different parts of buildings. One way of preventing the growth of mould in buildings is therefore to choose building materials that can withstand the expected conditions. It is thus crucial that data are available to allow the correct choices of materials to be made, especially information on the critical moisture level, $RH_{crit,}$ is needed. RH_{crit} is the lowest RH at which mould can grow on the building material.

In this work, a variety of laboratory studies on mould growth on building materials at different combinations of RH and temperature were performed. Based on the results, the RH_{crit} for the tested materials were determined. This made it possible to predict the propensity for mould growth on these materials in parts of building subject to known RH and temperature. To validate these predictions, the same materials tested in the laboratory were exposed to the conditions in three crawlspaces and three attics, with varying RH and temperature, for 2½ years. Good agreement was found between the predicted and observed mould growth. A new test method for determining the critical moisture level of a material was therefore developed based on the results of these studies. It was also shown that this method will make better prediction than traditional mould resistance tests, which evaluate the resistance to mould growth in a "worst case scenario", i.e. at relatively high RH and temperature.

The RH and temperature in buildings fluctuate, as does the length of time that the RH_{crit} is exceeded. A simplified approach, considering the cumulative time that conditions had exceeded this level gave sufficient information to validate the laboratory tests. Using this approach will not underestimate the risk of mould growth, but will include a margin of safety. However, it was also shown in this work that to make more precise predictions of the mould growth, the length of the favourable conditions of RH and temperature must also be taken into account. Test specimens of wood were exposed to alternating conditions of favourable and non-favourable RH, either on a short-term basis (12 hours) or a longer term basis (1 week), while maintaining the temperature at a favourable level for mould growth. The results were compared to those obtained following exposure to constant, favourable RH. It was shown that both the cyclic conditions slowed down the process of mould growth on wood; the long-term cycling more than the short term. Fluctuating temperature, while keeping the RH constant at a favourable level, also had an effect on mould growth, as it was slowed down.

In order to determine the RH_{crit} of a material, it is assumed that this property is the same for all samples from that particular material. Wood is a commonly used building material in Sweden. It is an inhomogeneous material and it was shown in a meta-study in this work that several characteristics of wood affect its susceptibility to mould growth; surface structure, wood species, sawing pattern and if the surface was recently planed or sawn. It is therefore not possible to predict the general susceptibility of wood to mould based only on a few data as it is affected by several parameters and it is therefore probably not possible to determine a general value of RH_{crit} for wood. Also, the susceptibility cannot be described by one single parameter, as it depends also on other parameters.

Contents

List of papers

This thesis is based on the following papers, which will be referred to by their roman numerals in the text.

Paper I
Laboratory study to determine the critical moisture level for mould growth on building materials. Johansson P, Ekstrand-Tobin A, Svensson T and Bok G (2012). International Biodeterioration and Biodegradation 73:23-32.
My work input: Main author, planned the study, performed some of the laboratory work and analysis for mould growth, evaluated data statistically.

Paper II
Validation of critical moisture conditions for mould growth on building materials. Johansson P, Svensson T and Ekstrand-Tobin A (2013). Building and Environment 62:201-209.
My work input: Main author, planned the study, performed some of the microbial analysis, evaluated data statistically.

Paper III
Determination of critical moisture level for mould growth on building materials (Laboratory method). SP-Method 4927: 2013.
Work input: Main author.

Paper IV
An innovative test method for evaluating the critical moisture level for mould growth on building materials. Johansson, P, Ekstrand-Tobin A and Bok G. Submitted to Building and environment.
My work input: Main author, performed the literature research on and summary of mould resistance test.

Paper V
The effect of cyclic moisture conditions for mould growth on wood compared to steady state conditions. (2013) Johansson P, Bok G and Ekstrand-Tobin A. Building and Environment 65:178-184.
My work input: Main author, performed the sampling of materials, performed some laboratory work and analysis for mould growth, did the statistical analysis, wrote the paper

Paper VI
Properties of wood that affect mould growth. A meta-analysis. Johansson P, Mjörnell K and Arfvidsson J. Manuscript.
My work input: Main author, performed the statistical analysis.

Other related publications by the author:

Johansson P, Samuelson I, Ekstrand-Tobin A, Mjörnell K, Sandberg, P. I , and Sikander E. (2005). *Microbiological growth on building materials – critical moisture levels. State of the art*. SP RAPPORT 2005:11. SP Swedish National Testing and Research Institute.

Johansson P and Jermer J. (2010) *Mould growth on wood-based materials – a comparative study*. 2010. IRG conference. IRG/WP 10-20455. Biarritz, France

Johansson P, Wamming T, Bok G, Edlund M-L. (2013). *Mould growth on kiln-dried and air-dried timber*. European Journal of Wood and Wood Products 71:473.

Ekstrand-Tobin A and Johansson P. *The effect of surface roughness on mould growth on wood*. Paper accepted to NSB 2014, 10th Nordic Symposium on Building Physics. Lund. Sweden.

Johansson P and Bok G. *Mould growth on timber from three single family house producers*. Paper accepted to NSB 2014, 10th Nordic Symposium on Building Physics. Lund, Sweden.

Abbreviations and terminology

RH	Relative humidity
a_w	Water activity, RH/100
RH_{crit}	Critical moisture level, the lowest RH at which mould growth can grow on a material
Mould resistance	The possibility of a material to withstand mould growth at high moisture levels
Mould	Microscopic fungi growing at the surface of materials, as branching hyphae forming a mycelium. Can be either hyaline or pigmented.
Spores	The reproduction unit of mould fungi
Critical moisture conditions	All the conditions leading to mould growth; temperature, relative humidity and time

1   Introduction

1.1   Context of the study

Mould growth is a common problem in buildings around the world. In Sweden it was estimated that at least 30% of all single family buildings have some form of mould or moisture damage (Boverket, 2010). Mould in buildings can have negative effects on the indoor environment, for example, by the production of odorous substances. Also human health may be adversely affected. The costs associated with this growth, e.g. due to renovation, are substantial. There are, therefore, both economic and health arguments for reducing the risk of mould growth in buildings.

The main environmental factors affecting mould growth in building structures are humidity and temperature; moisture being the crucial factor. Suitable conditions for the growth and reproduction of different mould fungi vary. Some thrive at relatively low relative humidity (RH = 75%), while the majority of fungi require higher values of RH for optimal growth. Different building materials vary in their susceptibility to mould growth; some can withstand high moisture better than others. Mould growth is the result of a complex interaction between all these factors; environmental factors, material properties and the characteristics of mould fungi present. In order to prevent growth in buildings, these interactions must be considered during the design, construction and maintenance of a building.

Mould fungi are a natural part of the organic lifecycle, and spores are present everywhere, both in the air and on material surfaces. It is therefore not possible to protect building materials from contamination by mould spores. It is the germination and growth of these spores that must be prevented. In practice, there are two fundamental ways of preventing the growth of mould in buildings. Either the moisture and temperature conditions should be such that mould growth is not possible, or materials on which mould cannot grow under the prevailing conditions should be chosen.

There is a great deal of knowledge and literature concerning the construction of moisture proof building structures and this will not be discussed in this thesis. Instead the focus is on prevention of mould growth through the choice of materials. In order to make the correct choices it is crucial that there are data available that these choices can be made upon.

Many standardised laboratory test methods are available for determining the mould resistance of building materials at high moisture levels. However, these

methods may not be suitable for predicting mould growth in structures where the moisture conditions are lower. The RH and temperature vary in different parts of a building. Some parts are moist and warm, while others are cold and dryer. The mould resistance tests available today do not provide any information on how a material will perform in a building where the environmental conditions are not as favourable as in those in the laboratory. In addition to the difference in mould resistance at high moisture levels, the "worst case scenario", the susceptibility to mould growth at different values of RH varies between materials; on some materials there will be mould growth at low RH while others can withstand being exposed to higher moisture values without mould growing on them. This is a property called the critical moisture level and is defined as the lowest RH where mould can grow on the material. Therefore, although conventional test methods can be used to discriminate between materials when concerning the mould resistance at a "worst case scenario", those methods do not provide enough information on which the correct choices of building materials can be based.

The Swedish building regulations from 2011 (BFS 2011:6, BBR) states that "Buildings shall be designed to ensure moisture does not cause damage, foul odours or hygienic nuisance and microbial growth, which could affect human health. The requirements … should be verified at the design stage with the aid of moisture safety design … Critical moisture levels shall be used to determine the maximum permitted moisture level…Well-researched and documented critical moisture levels shall be used for materials and material surfaces where mould and bacteria can grow". However, very little data are available on the critical moisture levels for materials. Several studies have attempted to identify the temperature and humidity conditions at which mould begin to grow on different types of building materials e.g.(Nielsen et al., 2000; Ritschkoff et al., 2000; Nielsen et al., 2004; Hofbauer et al., 2008). However, there are differences in methodology and/or variations in evaluation of the data which compiles the comparison between the different studies and the estimation of critical moisture levels. Hence, it is difficult to estimate the critical moisture value of a specific material from these data (Johansson et al., 2005). Also, different brands of one type of material may have different critical moisture levels, for example due to additives. In addition, new products are constantly being developed.

The Swedish building regulations mentioned above prescribe that if there is no data available, 75% RH should be used as the critical moisture level. However, there are materials that can withstand higher levels of RH without mould growth, although not as high as the level tested in the conventional test method (approx. 95%). The lack of methods for testing at this intermittent RH,

between 75% and 95% RH, means that the critical moisture level for many materials is set at a too low value when they have shown mould growth at testing by traditional mould resistance test. Instead of 75%, the critical moisture level might be for example 85% RH.

There is therefore a need of a suitable, new test method for determining the critical moisture value for mould growth on materials. This must be based on biological insight and statistically relevant methods, as well as on the insight of building physics. In the work presented in this thesis, such a method was developed. The work was based on previous findings in the area, new applications of these data and on new data from laboratory and field studies reported in this thesis.

1.2 Aim of the study

The overall aim of this work is to contribute to the knowledge on the conditions leading to mould growth and, in the long term, reduce the growth of mould in buildings.

There were four main objectives in the study:

1. To develop a novel laboratory test method for determining the critical moisture values for mould growth on building materials. It should be possible to use the results obtained using this method when choosing building materials to ensure a minimal risk of mould when the RH and temperature is known.

2. To validate whether results from laboratory studies performed at conditions of constant temperature and RH can be applied to situations in real building parts, where these parameters are varying and affect the conditions for mould growth.

3. To study how mould growth is influenced over time by varying RH and temperature.

4. To study different material characteristics that affect mould growth on wood, a commonly used building material in Sweden. Wood is an in-homogenous material and it is therefore difficult to determinate the general mould resistance of this material.

1.3   Scope and limitations

This thesis concentrates on laboratory testing for mould growth and the assessment of the critical moisture level for various materials. The possible effects of exposure to mould in buildings on human health are not considered.

Also, the results cannot be applied to outdoor building structures, e.g. facades, fences etc., where rain, wind, radiation from the sun etc. may affect mould growth.

No attempt was made to evaluate different building parts, although crawlspaces and attics were used as test environments.

I addition to mould fungi, bacteria also contribute to the microbial fouling of materials in damp buildings. These microorganisms were not studied in this thesis.

1.4   Outline

Chapter 2 provides a general background on the subject of mould growth on building materials and the state of the art. My own research is presented in the following chapters. Chapters 3-7 are methodology chapters. Some methods are the same for all the all laboratory studies in this thesis. These are described in Chapter 3, while each specific study is described more in detail in Chapters 4, 6 and 7. Chapter 5 describes the work of developing the new test method. In Chapter 8 the results from the studies are presented and are discussed in Chapter 9. Main conclusions are given in Chapter 10 and, finally, suggested further studies are given in Chapter 11.

The major part of the work presented in this thesis concerns determination of the critical moisture level of a building material, a laboratory test method for determining this level, and field studies validating the laboratory test (Paper I-Paper IV). The second part concerns the effect of varying the RH and temperature in comparison to constant conditions (Paper V) and mould growth on timber (Paper VI), a very common and important building material in the Swedish building sector. The two parts of this work is connected by issues concerning the effect of RH and temperature on mould growth and issues concerning laboratory testing for mould growth.

2 Background and state of the art

2.1 Definition of mould

Mould is a general term used to describe various microscopic fungi that grows on the surfaces of materials. This chapter presents a summary of the biology of mould, the conditions for mould growth in buildings, the basics of critical moisture levels for mould growth, and methods of testing mould growth on building materials is presented and discussed.

2.2 The biology of mould and the presence of mould growth in buildings

Mould is microscopic fungi, belonging to different biological groups and consisting of many species. In some aspects, they share common traits. They live on the surfaces of materials, use easily-assimilated nutrients for growth and produce airborne spores.

Mould fungi are widely spread across different environments on the Earth and there is no natural place where air and materials are free from spores. When favourable conditions are present, the spores (also called conidia) will germinate and a small germ tube will develop; if the favourable conditions prevail, a hypha will be formed. 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. A schematic illustration of the life cycle is presented in Figure 1.
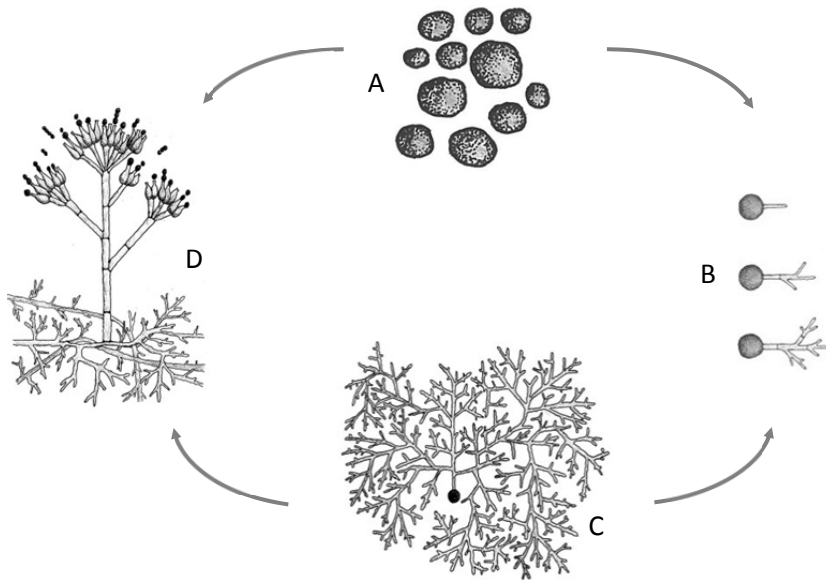
Figure 1   Schematic overview of the asexual life cycle of a typical mould fungus. When suitable conditions are present, spores (A) at the material surface germinate into a germ tube that grows into a hypha (B), which then extends and branches into a mycelium (C). From some of the hyphae, conidiophores are developed (D) and from them, masses of spores are released into the air. Illustration: Agneta Olsson-Jonsson

In the wild, mould fungi act as decomposers of organic debris. In our daily lives, we encounter mould in different ways. Old cheese in the fridge starts to go mouldy; bread in the pantry soon begins to smell and become mouldy; and our facades and patios discolour. The same species of mould fungi and the growth processes are the same inside buildings.

Extended mould growth on building materials may be visible to the naked eye. However, often this fouling cannot be seen by the naked eye. Some fungi produce pigments in their hyphae and spores that can cause discolouration of surfaces on which they grow, while others lack this pigment. The production of pigments by fungi is a species-specific trait, but can also be dependent on the nutrients available, or the growth phase of the fungus (Gadd, 1980; Eagen et al., 1997; Fleet et al., 2001). In addition, especially in houses in Northern Europe, mould usually grows inside sealed building structures. Therefore, extensive mould growth may be present in buildings without anything unusual can be seen.

On outdoor structures, such as façades and fences, mould growth which is not discolouring does not cause any major problems, as it does not affect the aesthetics and usually does not affect the strength properties of the material or the health of those using the buildings. However, inside building structures, the growth of moulds that is not visible to the naked eye may be problematic, as there is a risk of negative effects on the indoor environment, which can pose health risks to those in the building. Mould can theoretically grow anywhere in a building, provided the conditions for growth are suitable. Some types of structures are more favourable than others for mould growth, and some materials are more susceptible to mould growth than others.

When growing on a material, the mould fungi produce a wide range of compounds. The various species produce different compounds, such as volatile organic compounds, of which some cause a mouldy odour, mycotoxins that may affect human health when inhaled and some fungi causes allergy. 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 (Sunesson et al., 1996; Nielsen et al., 2004).

A large number of species of micro-fungi 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 (2011) lists 52 species isolated from building interiors. No matter how big the exact number, one conclusion that can be drawn from these studies is that there is a wide variety of mould fungi in buildings. These fungi represent a broad range of demand for water, as well as other factors affecting growth. Some of the fungi will only grow in very specific environments, while others may be able to colonize more diverse environments (Caddick, 1993). In most cases, several species occur together on a building material (Andersen et al., 2011; Hyvärinen et al., 2002), while sometimes only one species predominates, as reported for *Penicillium corylophilum* in crawl spaces in southern Sweden (Bok et al., 2009). When several species exist together, both interaction and competitive behaviour can be present. This, in turn, may affect several aspects of mould growth, such as growth and which metabolites being produced.

From the above observations it can be concluded that mould growth in different buildings may be very diverse; thus, it is very difficult, 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 the

health of dwellers in a building and/or indoor air negatively. The latter also depends on where in the structure the growth is present and the presence of air movements and ventilations. No dose-response relationship between mould growth and human health/negative effect on indoor air is established. Since the knowledge of this is incomplete it is a necessary precaution to not allow mould to grow at all.

2.3   The moisture requirements of mould fungi

Mould growth on a material is the consequence of a complex interaction between the environmental conditions (RH, temperature, etc.), the characteristics of the material (nutrients, additive components, etc.) as well as the trait of the fungus itself (Blackburn, 2000). In general, the availability of water in the material is regarded as the crucial element for growth to occur. The water available to microorganisms is often referred to as water activity, a_w, which is equivalent to the RH of the air at equilibrium, but is expressed as a fraction instead of a percentage. The mould growth on a building material takes place on the surface. At equilibrium, the RH on the surface is the same as that in the surrounding air.

Each fungal species has a minimum water requirement in order to grow, although the majority of fungi will grow well at high moisture conditions, above a a_w of 0.9. This corresponds to an equilibrium RH of 90%. Fungi that grow below a_w of 0.89 are called xerophilic fungi (Lacey et al., 1980); the most extreme xerophiles may grow at values of a_w as low as 0.62.

Moisture requirements are also related to temperature; at lower temperatures, the fungus requires more 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 (Ayerst, 1969; Magan and Lacey, 1984; Smith and Hill, 1982); examples for two mould fungi are shown in Figure 2 . At an optimum temperature, the required water availability for growth is at minimum.
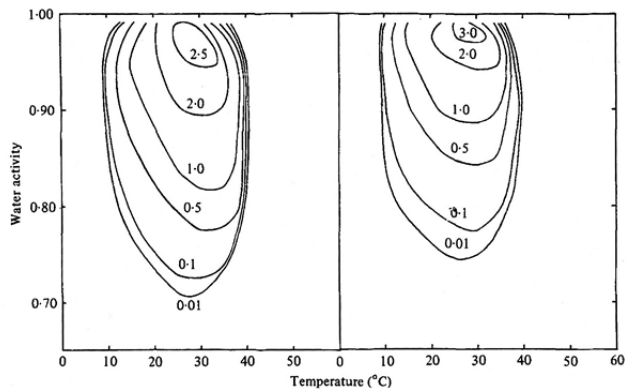
Figure 2   *Effect of temperature and water activity on growth rate of two microfungi;* Aspergillus restrictus *(left) and* Aspergillus versicolor *(right). The numbers on the isoplethes are growth rates in mm/days (from Magan and Lacey, 1984)*

The growth of fungi can be described in different phases. Even under favourable conditions, there is often a latent period before growth takes place. During this time, the spores are taking up water and start to swell, and a germ tube, a pre stadium for a hypha, is beginning to develop. If the favourable conditions are interrupted, the growth may terminate. This has implications for how quickly one humidification, such as water damage in a building or rain on unprotected material during construction, as well as exposure to high relative moisture levels in the air, must be stopped before mould begins to grow on building materials.

2.4   Mould growth on building materials

2.4.1   Variations between materials of susceptibility to mould growth

The susceptibility to mould growth varies between materials. As mentioned in in the introduction, this susceptibility can be defined as mould resistance at high moisture levels or as the critical moisture level for mould growth, RH_{crit}, i.e., the lowest RH at which mould growth can grow on a material. The differences between materials can be explained by differences in the concentration of organic compounds, which are essential nutrients for mould fungi, as well as by differences in other characteristics that may affect growth (such as pH, surface structure, etc).

In general, when there is a high content of organic compounds in a substrate, the water requirement 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, in which the content of organic compounds is expected to be low. Pietarinen et al. (2008) found the highest diversity of microbes on wooden materials.

2.4.2 Mould resistance of building materials

A number of standardized methods are available to test the mould resistance of a material in the laboratory. Most fungi grow well at high RH and if the material is such as to allow mould growth it is expected that mould grow on the test pieces in the laboratory.

The principles of these methods are generally the same: spores from mould fungi are introduced onto the surface of test specimens of the material; which are then incubated in a climate favourable for mould growth (at least 90-95% RH and temperature above 20 °C) and after some weeks, usually four, the surfaces of the test specimens are analysed for mould growth. The methods differ, depending on the species used, the number of spores and how the test specimens are inoculated with the spores, the conditions of RH and temperature at which the materials are tested, the way in which mould growth is assessed and the evaluation criteria. A number of methods are summarized and compared in Table 1 and discussed in Paper IV. For reasons of comparison, the new method presented in this thesis, the CLM-method is also included. Similar comparisons between different methods were made by Adan (1994).

While being able to discriminate between materials in a worst case scenario, these test methods do not provide any information on how a material will perform in a building where the moisture conditions are not that high as those in the test. It may, therefore, be possible to use materials that showed mould growth in the tests where the moisture level was high, in buildings in which the RH is expected to be lower, without risk of mould growth. Furthermore, the design of the tests is not directly applicable to lower humidity levels and can, therefore, not be used to evaluate the critical moisture level of a material.

2.4.3 Critical moisture level for mould growth on building materials

The minimum conditions for mould growth discussed in 2.3 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, 2011).

Block (1953) conducted one of the earliest studies of mould growth on different types of building materials at different moisture levels, and concluded that the most sensitive materials will be subject to mould growth at 80% RH at room temperature. Numerous researchers have since studied mould growth at different conditions, some with the aim of identifying the climates in which different types of building materials begin to mould, some examples are presented in Table 2. 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 also vary between 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.

Table 1 *Comparison between the CML method for testing critical moisture level and five traditional methods used for estimating mould resistance. In the table the short name of each method are used. Complete information of each method is found in the list of references (p.89).*

		The CML method SP MET 4927	ASTM C1338-00	BS 1982: Part 3:1990	ASTM G 21-96	ISO 846:1997	MIL-STD 810 G**
Material		Building materials	Insulation materials and their facings	Panel products, made of or containing materials of organic origin	Synthetic polymeric materials	Plastics	A variety of materials
Purpose of the test		Critical moisture level	Mould resistance	Mould resistance	Mould resistance	Mould resistance	Mould resistance
Specimen	Size	50 x 100 mm	Surface area not defined	40 x 40 mm	50x50mm/ dia. 50 mm/ length 76 cm (rods)	Not defined	Not defined
	Number of test specimens	7	3	Minimum 3	3	5	Not defined
	Number of species	6	5	7	5	5	5
Inoculum	Spore solution	Yes	Yes	Yes	Yes	Yes	Yes
	Concentration of spores in solution	10^6 spores/ml	$10^6 \pm 200\,000$ spores/ml	Not specified	$10^6 \pm 200\,000$ spores/ml	10^6 spores/ml	$10^6 \pm 2\%$ spores/ml
	Inoculation methodology	Spraying	Spraying	Spraying	Spraying	Spraying or pipetting	Spraying
	Amount of solution	0.4 ml	0.5 ml	0.5 ml	Until surface is moistened	0.1	Not specified
	Number of spores/cm²	8000	(a)	(b)	(c)	(a)	(a) and (c)

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	The CML method SP MET 4927	ASTM C1338-00	BS 1982: Part 3:1990	ASTM G 21-96	ISO 846:1997	MIL-STD 810 G**
Incubation Temperature	22 ± 1 °C	30 ± 2 °C	24 ± 1 °C	28-30 °C	24 ± 1°C or 29 ± 1°C	30 ± 1°C
RH	80 ± 2,5% 85 ± 2,5% 90 ± 2,5% 95 ± 2,5%	95 ± 4%	Not specified	>95%*	>95%*	At least 90% but less than 100%
Incubation environment control	Recording of temperature and RH every 10 min	No	No	Automatic recording of wet and dry-bulb temperature recommended	No	Recording of chamber temperature and humidity over time
Incubation time	12 weeks	Min. 28 days	4 weeks	28 days	4 weeks or longer	28 days or up to 84 days
Storage of suspension	Must be used the same day	28 days at 6 ± 4 °C[1]	Must be used the same day	Not more than 4 days at 3-10°C	6 h	6 ± 4 °C for not more than 14 days

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[1] Or until the viability test indicates poor growth or until growth appears in the sealed storage bottle
a) Cannot be calculated since the area of test specimen is unknown
b) Cannot be calculated since the concentration of spores in the suspension is unknown
c) Cannot be calculated since the volume of spore suspension is unknown
* Specimens are placed on solidified nutrient salt agar and RH is according to ISO 846 >95 %.
** although this method is not specified for testing building materials, it is useful and often used for this purpose

Table 2   Examples of laboratory studies on mould growth on various building materials.

Building material	Assessed critical moisture level (Johansson et al 2005).	Reference
Gypsum boards	80-85%	Pasanen, Juutinen et al. 1992; Nielsen, Nielsen et al. 2000; Pasanen, Kasanen et al. 2000; Ritschkoff, Viitanen et al. 2000; Doll and Burge 2001; Horner, Morey et al. 2001; Nielsen, Holm et al. 2004
Wood based boards	75-80%	Pasanen, Juutinen et al. 1992; Wang 1992; Pasanen, Kasanen et al. 2000; Ritschkoff, Viitanen et al. 2000; Nielsen, Holm et al. 2004
Concrete	90-95%	Nielsen, Nielsen et al. 2000; Ritschkoff, Viitanen et al. 2000; Viitanen 2004
Wood	75-80%	Hallenberg and Gilert 1988; Viitanen and Ritschkoff 1991; Pasanen, Juutinen et al. 1992; FNielsen 2002
Mineral wool insulation and other insulation materials	90-95 %	Chang, Foarde et al. 1995; Nielsen 2002; Klamer, Morsing et al. 2004; Viitanen 2004

Sedlbauer (2001) defined a lowest isopleth of mould, the LIM 0 curve, based on measured minimum, optimum and maximum growth conditions for several mould fungi common on building materials, as shown in Figure 3. This curve defines the limiting conditions for mould growth on an optimum medium. Sedlbauer (2001) also developed curves for different building materials categories: Category I: biologically recyclable building materials (wall paper, gypsum boards, building materials made of biologically degradable raw materials material for permanent caulking); Category II: Building materials with porous structure such as renderings,   mineral building materials,

certain wood species as well as insulation material not covered by Category I; and Category III: building materials that are neither degradable nor contain any nutrients. Later, Hofbauer et al. (2008) used laboratory data from extensive tests of mould growth on different building materials at different combinations of RH and temperature and constructed material-specific isopleths from the closest approximation to the LIM 0 curve; as shown Figure 4. These limiting growth curves may be used to assess the risk of mould growth in a building where the expected RH and temperature is known.
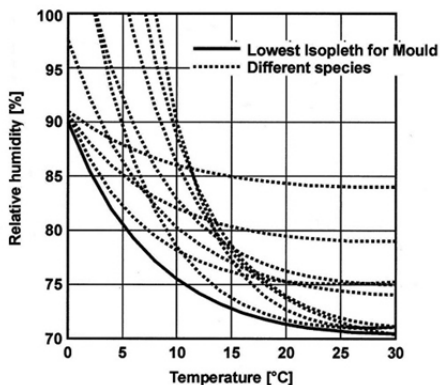
Figure 3   Isopleths for growth of various fungus species and the lowest isopleth for mould (LIM 0) (Sedlbauer 2001).
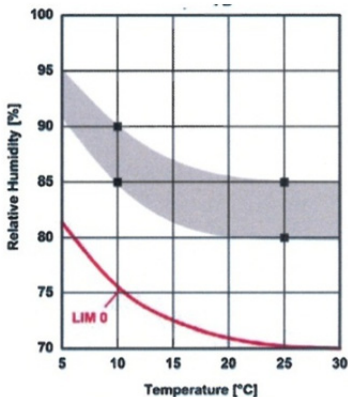
Figure 4   Material specific (Rye fill) isopleth system based on results from laboratory tests and approximation to the LIM 0 curve (Hofbauer et al., 2008).

2.4.4 Wood as a substrate for mould growth

Wood is a commonly used building material in the Swedish construction industry. It has many advantages; one of which is that it is a renewable material. However, it contains a relatively high level of carbohydrates that can be assimilated by mould fungi. Wood is, therefore, often considered to have a low mould resistance and a low critical moisture level for mould growth. However, wood is not a homogenous material and different characteristics of wooden material can affect the mould growth. One such characteristic is variations of the concentrations of available simple carbohydrates on the surface of the timber (Terziev, 1996; Theander et al., 1993).These sugars are formed in the metabolism of the growing tree, and are available in free water as nutrients after the tree has been felled (Schmidt, 2006). During the kiln drying of timber, the free water in the wood cells is relocated from the inner to the outer parts, mainly by capillary actions (Long, 1978). The dissolved carbohydrates are transported with the water and accumulated on the surface of the timber. Increasing the temperature in the kiln speeds up drying, leading to a higher concentration of sugars at the surface, which in turn will increase the risk for mould growth (Terziev, 1996). Wood from different wood drying schemes may therefore differ in mould resistance. This is however not always the fact, as was shown by Johansson, Wamming et al. (2013). Sehlstedt et al. (2011) found that it is possible by direct the migration of sugars towards one side of a board by double stacking during drying, which in turn has an impact on surface mould.

The timber produced from circular logs can be divided into centre-boards and side-boards, depending on whether they come from the centre or peripheral parts of the log. The centre-boards will contain heartwood in different amounts, while the side-boards normally contain only sapwood. Heartwood is considered more resistant to mould growth than sapwood, and, centre-boards are therefore expected to have a higher mould resistance than side-boards. Also, the side-boards may be more susceptible to mould growth than centre-boards due to higher concentrations of sugars in the outer part of the tree (Saranpää and Höll, 1898) and as a result of the drying effect mentioned above.

The surface structure of the wood can also affect the mould growth and mould resistance vary between different wooden species (Terziev, 1996;Viitanen 1996; Sehlstedt et al 2011).

2.5 The relevance of laboratory tests in relation to real life conditions.

The conditions under which mould growth is studied in the laboratory will differ from those in practice. Cooke and Whipps, (1993) wrote, "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 also applicable for the conditions encountered in buildings.

In many parts of a structure, there is a fluctuation in RH 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 water activity, 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 micro fungi 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 conditions (Adan, 1994; Viitanen and Bjurman, 1995). In addition, how long these periods last is also of importance.

In addition to the RH and temperature conditions, including duration of favourable or non-favourable conditions, there are other factors that vary between laboratory and natural environments that may affect the mould growth. Some are listed in Table 3. In this thesis, the first four parameters have been evaluated and tested; RH and temperature, duration and impact on fluctuation conditions compared to steady state and effect of spore solution compared to the natural conditions.

Table 3   *Comparison between the conditions in laboratory tests used ti evaluate*
              the resistance of a material to fungal growth and the conditions that will
              be encountered in a building structure

	Laboratory tests	Conditions in a building
Fungal species	A few species (1–6) introduced onto the surface, although other microorganisms may be present if test specimens are not sterilized before testing	Potentially a large number of species
Spore exposure	More or less controlled; high concentration of spores	Unknown, continuous supply, varying between places and time of year
RH and temperature	Constant or varied according to a predefined scheme	Varying over time, sometimes occasionally condensation
Duration of exposure	Limited to some weeks	Many years
Other environmental factors		Presence of other organisms, e.g. mites that eat fungi

3   Materials and Methods

In this chapter, the procedures that are common to all laboratory tests described in this thesis are presented. Each study is described in more detailed in Chapters 4, 6 and 7.

3.1   Building materials and test specimens

Test specimens sized 5x10 mm were prepared for each of the materials that were to be tested. These consisted of different board material (Paper I and Paper II) and of pure wood (Paper V and Paper VI).

3.2   Laboratory studies

Samples of the material to be tested were inoculated with spores from mould fungi. The samples were then incubated in climate chambers under specified conditions of RH and temperature, and inspected at defined intervals for fungal growth. The procedure is illustrated in Figure 5. The RH, temperature, total incubation time, and period of analysis varied between the tests.

1. Cultivation of mould fungi and preparation of standardised spore suspension

2. Inoculation of test specimens by spraying

3. Incubation in moisture chambers
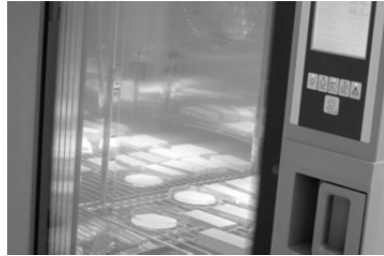
4. Analysing for mould growth on the surface of the test specimens
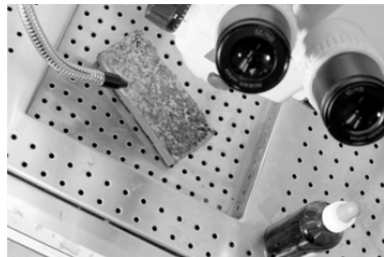
Figure 5   Illustration of the procedure used for the laboratory tests in this thesis.

3.2.1 Inoculation

Spores were added to test specimens by spraying of a spore suspension containing fungi listed in Table 4. The spore suspension was prepared according to MIL-STD 810 F (2010). Spores from each of the fungi were collected from cultures actively growing on agar plates and dissolved in sterile water. The suspension was washed three times, with sterile water, with intermediate centrifugation, in order to avoid contamination of nutrients from the agar on which the fungi were originally grown. The solution was then diluted so that it contained the required amount of spores (10^6 spores/ml), and 0.4 ml of the suspension was sprayed onto one surface of each test specimen, such the spores were more or less evenly distributed over the surface of the test specimens. The procedure is more thoroughly described in Paper I.

Table 4   Mould species used in the study.

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

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

3.2.2 Incubation

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 RH 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 RH 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.

A combined measurement uncertainty was calculated by using the calculated standard deviation and the measurement uncertainty from the calibration of the transmitters, according to Annex B of Paper III and (EA-4/02).

3.2.3 Analysis of mould growth on the test specimens

Mould growth on the inoculated surface of each test sample, excluding the edges, was assessed at defined intervals. Both mould growth visible to the naked eye and that which was only visible under the microscope at 10–40x magnification were rated according to a five-point rating scale (see Table 5).

Table 5   *Rating scale for the assessment of mould*

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

3.2.4 Statistical evaluation of mould growth data

When the mould growth has been assessed according to a rating scale, as in this work, several approaches can be used to describe and evaluate the mould growth on materials. Below, three such approaches are described; all have been applied in various extents in this work or in the related papers.

i.   Visually, in charts, as the median rating of test specimens by time (as shown in Figure 6). This method has been commonly used in published literature to describe mould growth over time. The mean value of ratings is often presented, but this is a mathematically incorrect approach, since rating values are ordinal data. Instead the median value should be used. The evaluation of differences is based on visual impressions of the curves and it is not possible to compare different materials with each other using statistical tests. Therefore, the use of this approach is limited.
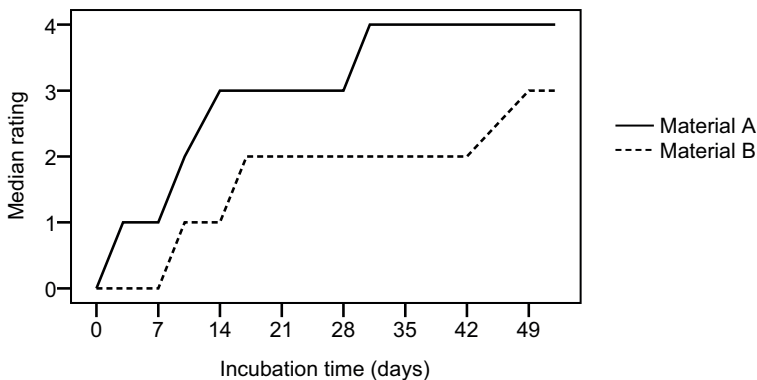
Figure 6   *Mould development over time on two materials, described as median growth, according to the grading scale in Table 5.*

ii. By comparing the distribution of rating at a specific point in time, during or after incubation (as illustrated in Figure 7). With this method it is possible to compare the extent of growth on the different materials using nonparametric methods. A disadvantage of this approach is that evaluation is made at one particular point of time. Since mould growth is an ongoing process, the results may differ depending in the point of time chosen for evaluation.
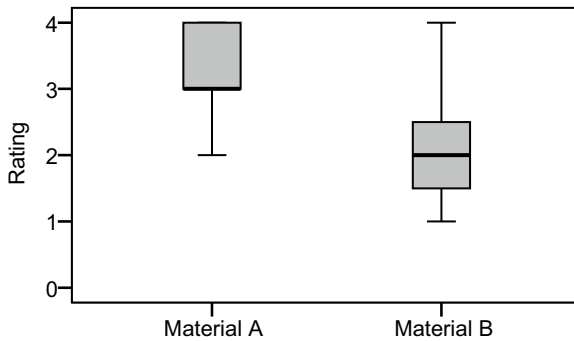
Figure 7   Distribution of rating data for mould growth on two different materials at one point of time.

iii. By using survival analysis, where the "time to event" is considered. In this case the event is the establishment of mould growth on a test specimen and the time is the incubation time started from day 0. Instead of focusing on the extent of mould growth, the event that mould growth established is studied. In practical situations, it is more pertinent to determine if mould growth will become established or not. Therefore, this approach was preferred in this work. Although there is no published study on mould growth on materials that have used this approach, it has been used in many similar fields, i.e. when evaluating the germination of seeds (McNair et al., 2012) and it has many advantages over other methods studying change by time (Singer and Willet, 2003).

A survival function *S(t)* is defined as the probability to survive at least to time t. In this case survival means no established growth on the test specimen. Established growth was defined as rating 2 or higher. The survival function can be plotted against time, and is called the survival curve. The Kaplan-Meier estimation can be used to estimate this curve, as in Figure 8. The more specimens with established growth on them, the lower the proportion of survival at each point of time and the

more to "the left" of the graph the curve. Therefore, when comparing materials (or treatments or another parameter that varies between test specimens), when a Kaplan-Meier curve is "below", or "to the left" of another that indicate that this material is more susceptible to mould growth than the other. Therefore, Figure 8 shows that material A is more susceptible to mould growth than material B. However, the conclusion that these curves are not equal cannot be based solely on visual impression of the look of the curves. Instead, the comparison of the survival function of two, or more, groups should be based on a formal non-parametric statistical test, the log rank test. In this test, the null hypothesis that there is no difference between the population survival curves. Finding significance in this case means that the survival functions were not equal for the groups studied.
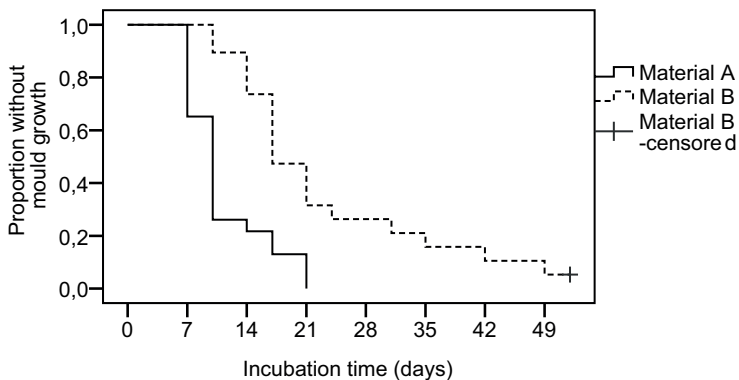
Figure 8   Kaplan-Meier curves of cumulative survival, that is proportion of test specimen without established mould growth (rating < 2), for two materials

The log rank test is suitable to make pairwise comparisons to test whether it is a difference in the survival functions. However, in many cases, several variables affect the survival function and adjustment for variables that can affect survival may improve the precision. Cox's proportional hazard model (Cox regression) investigates the effect of several risk factors on survival at the same time. In Cox regression, the dependent variable is hazard rate. This can be defined as the probability of an individual will experience an event (in this case established growth on a test specimen) within a small time interval, given that it has survived up to a given point of time. In this case survival means there is no growth on the test specimen.

In the end of a study, there will often be individuals that have not encountered the event. These are censored cases. In this work the censored cases consisted of test specimen on which there was no mould growth at the end of incubation. In the survival analysis, these cases are taken into account.

Bewick et al. (2004) and Rich et al. (2010) give good summaries of survival analysis and Kaplan-Meier curves. Singer and Willet (2003) provide a thorough presentation of the hazard/survival models for event occurrence. In this work, survival analysis was performed for data in Paper V and Paper VI. In addition, Kaplan-Meier curves were presented in Paper I.

All statistical analyses in this work were performed using SPSS statistical software.

4 Determining the critical moisture level of building materials (Paper I and Paper II)

4.1 Materials

10 building materials commonly used in new Swedish buildings were investigated: asphalt paper, cement-based board, chipboard, exterior gypsum plaster board, extruded polystyrene board, glass fibre board, pine sapwood, plywood, thin hardboard and wet-room gypsum board. The critical moisture level was expected to vary between the materials. The materials are more thoroughly described in Table 1 of Paper I.

4.2 Laboratory test

4.2.1 Incubation

The materials were tested at 10 combinations of temperature and humidity at 22 °C and 75, 79, 85, 89 and 95% RH, respectively and at 10 °C and 75, 85, 90, 93 and 95% RH. The test period was 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 RH (Johansson et al. 2005) the tests at 75% and 80% RH and 22 °C were continued for 8 respective 7 weeks.

Prior to inoculation and incubation, six of the test specimens from each material were submerged in sterile water for 20 minutes; the other six were simply sprayed with the spore solution before incubation. The purpose of this wetting was to assess the effect of a short period of flood or rainfall on mould growth in general, and on the critical moisture level in particular.

4.2.2 Determining the critical moisture level

The critical moisture level for mould growth was then determined by identifying the lowest RH at which mould growth appeared. Two alternative definitions were 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 exceeded rating 2.

The tests were carried out at constant RH, with RH set at intervals of approximately 5%. The critical moisture level therefore fell into a range, with

the upper limit determined by the case with lowest RH where any of the above criteria were met, and the lower limit by the case with the next-lowest RH tested. For example, if the criteria for critical mould levels were met at 80% RH, the critical moisture level for this particular material was assumed to be in the range of 75% < RH_{crit} ≤ 80%.The principle is illustrated in Figure 9.
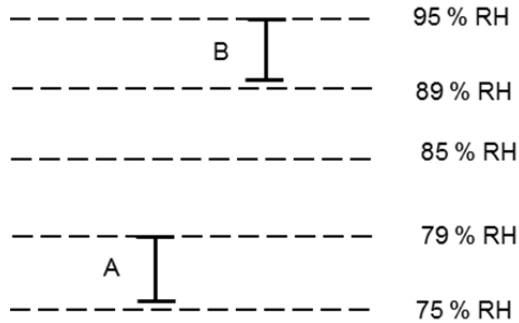
Figure 9   *Principle for the determination of the critical moisture level, RH_{crit}, at 22°C. A is a material on which growth was established at 79% RH but not at 75% RH. The critical moisture level can, therefore, be described as 75% < RH_{crit} ≤ 79%. B is a material on which mould growth was seen at at 95% RH, but not at 89% RH. The critical moisture level is, therefore, 90% < RH_{crit} ≤ 95%.*

In order to estimate the critical moisture levels for temperatures other than 10 °C and 22 °C, growth limit curves were constructed for each material, from the closest approximation to the LIM 0 curve for growth, as shown in Figure 3. This was the same approach as (Hofbauer et al., 2008) used (see Figure 4).

As the equation for the LIM 0 curve had not been published at the time of this study, the data, read from the graph, were fitted to a second-degree polynomial to obtain the parameters describing the curve. This contains three parameters, but in order to simplify the model, only two parameters were wanted. Therefore, the function minimum was fixed at a defined temperature, namely 27 °C. The resulting growth limit curve model could then be described by Equation 1:

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

Where t is the temperature in °C.

The parameters c and a were estimated using Equation 2 and Equation 3, using the data for RH_{crit} and the corresponding temperature from the laboratory tests:

$$c = (RH_{crit1} - RH_{crit2})/(t_1^2 - t_2^2 - 54(t_1 - t_2)) \tag{2}$$

$$a = RH_{crit1} - c(t_1^2 - 54*t_1) \tag{3}$$

Two growth limit curves, one upper and one lower, were produced for each material, as shown in Figure 10. The upper curve was calculated using values from the upper value in the range in which the RH_{crit} is found, and the lower curve by the lowest value. As an example, from $75\% < RH_{crit, 22 °C} \leq 79\%$ and $85\% < RH_{crit, 10 °C} \leq 90\%$, for calculating the lower curve $RH_{crit1} = 75\%$, $RH_{crit2} = 85\%$, $t_1 = 22$ °C and $t_2 = 10$ °C and upper curve $RH_{crit1} = 79\%$, $RH_{crit2} = 90\%$, $t_1 = 22$ °C and $t_2 = 10$ °C. The actual critical moisture level is therefore expected between the two growth limit curves, or above the upper curve (Hofbauer et al., 2008).
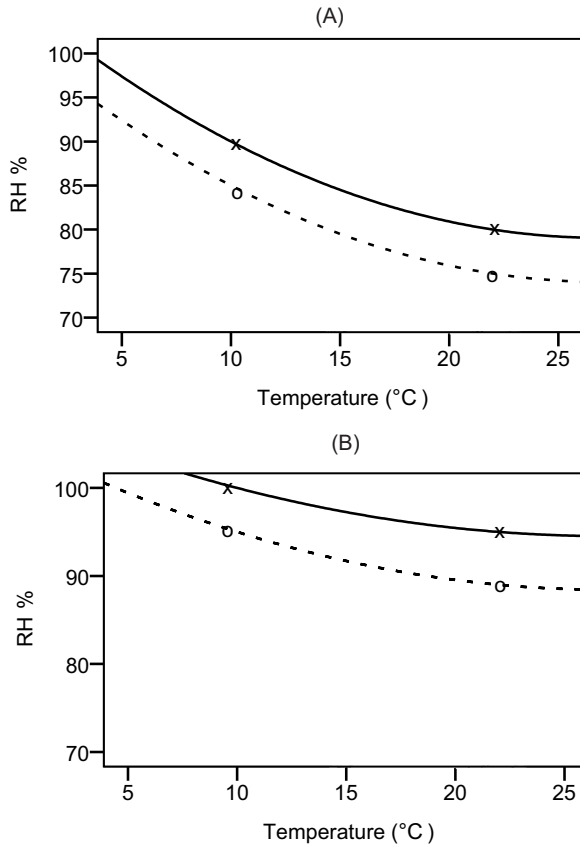
Figure 10   Upper and lower mould growth limit curves for two materials, x is the RH
              where mould growth was found, o is the next lower RH in the test.

(A) $75\% < RH_{crit,\ 22\ °C} \leq 80\%$, $85\% < RH_{crit,\ 10\ °C} \leq 90\%$,

(B) $89\% < RH_{crit,\ 22\ °C} \leq 95\%$, $95\% < RH_{crit,\ 22\ °C}$

4.3   Field test

Test specimens from the same materials as those used in the laboratory tests
were placed in three outdoor ventilated attics and three crawl spaces in houses
with light wooden framework structure, representative of the Swedish housing
stock. These test sites were chosen since they represent structures where the
climate is highly governed by the outdoor climate. Therefore, there is a
seasonal fluctuation in the climate in these structures. 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 of condensation and mould growth. In both types of structure, there is often extensive mould growth on the building materials (Pasanen et al., 2001;Bok et al., 2009; Hagentoft and Kalagasidis, 2010). In addition, the test spaces were easily accessible, and the test specimens could easily be evaluated for mould growth at the defined intervals.

The test specimens were placed in stainless steel spring clips, mounted on aluminium strips (see Figure 11). These were on the blind floor in the crawl spaces and on the inside of the roof in the attic.
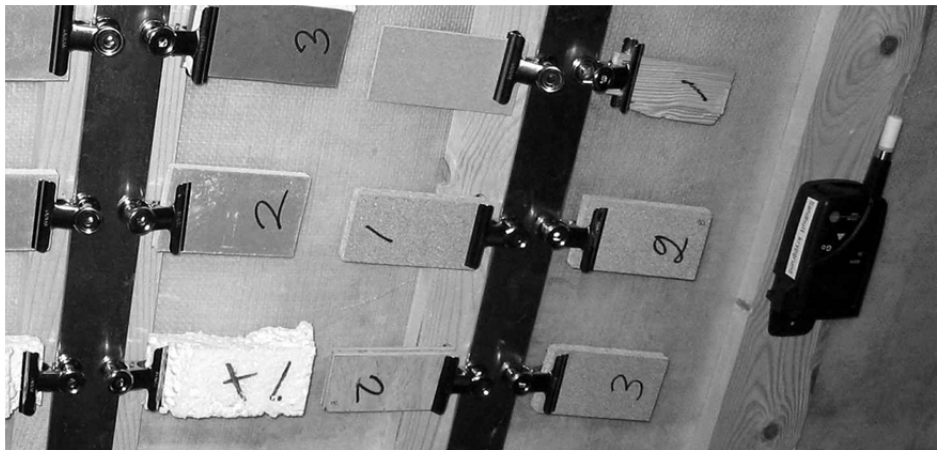
Figure 11   Mounting of the test specimens in the crawl spaces and attics in the field study.

The test specimens were exposed at the test sites for 2.5 years. Every six months, in October and May, the test specimens were removed from the racks, and the surface that had been exposed to the open air in the attic and crawl spaces was studied for mould growth in the same manner as in section 3.2.3. After being analysed, test pieces were replaced in the racks and the exposure continued. In this way, the development of mould growth could be followed at the same test specimen.

The RH 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 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 RH 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 RH were included. In Paper II the procedure is described.

4.4 Comparison of laboratory test results and results from the field study

The monitored data were plotted, as RH versus temperature plots, together with the growth limit curves obtained for each material in the laboratory test, as in. The expected growth on each material was estimated by determining whether the measured data were above or below the growth limit curves (Rowan et al., 1997). If the RH at a specific temperature was below the lowest growth limit curve, no mould growth was expected. If it exceeded the upper limit, mould growth was expected. In between the two curves, there was a zone in which the critical moisture level may fall, and in the present work it was assumed that mould growth would be expected.
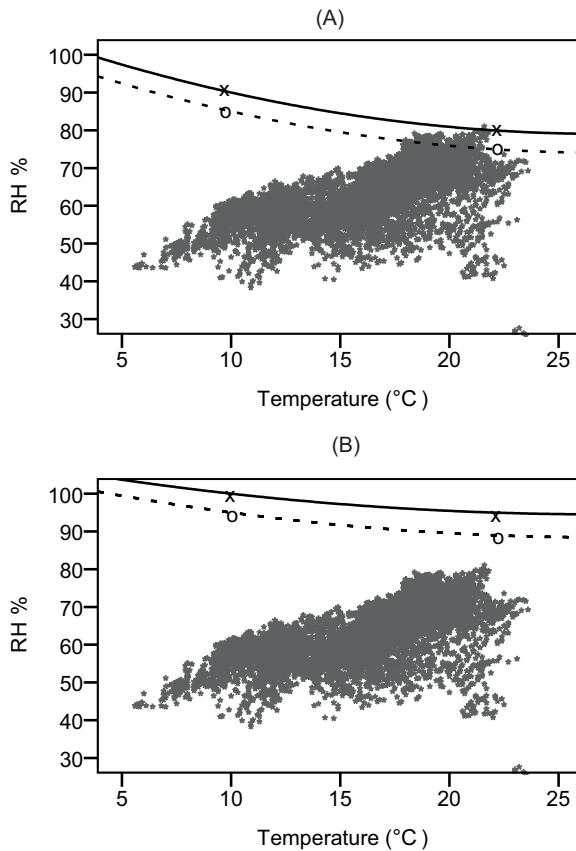
Figure 12   *Illustration of how the results from the laboratory tests were used to predict the possibility for mould growth on test specimens in the field study. The values of RH and temperature measured in the field experiments were plotted against each other, and the growth limit curves obtained from the laboratory tests were added to the graph. This was done for all materials and all test sites in the study. In this example, the growth limit curves shown in Figure 10 are used. For material A, the measured values exceed the growth limit curves and, therefore, mould growth can be expected on that material, while for material B the measured values are well below the growth limit curves and hence no mould growth is expected.*

The results of this analysis (expected mould growth or not) were then compared to the results obtained from the assessment of mould growth on the test samples in the crawl-spaces and attics; observed growth was then defined as at least one test sample of the material showing mould growth corresponding to a rating of 2, 3 or 4. This procedure was repeated for each material tested at each test site.

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 temperature and RH were logged at this interval. The cumulative time (hours) was therefore calculated as the sum of number of occasions over the curves times 4 hours. This cumulative time was compared to the time before the critical moisture level was reached in the laboratory.

5 Test method for determining the critical moisture value of a material

An innovative test method for determining the critical moisture level for mould growth on building materials was developed. The laboratory experiment described in Paper I was the first step in the development of the new method. When designing this test, experience from traditional mould growth tests, the design of existing methods for determining mould resistance and published results were taken into account. The final method is therefore based on the result from Paper I and Paper II.

The species, Table 4, 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 Whipps, 1993). A mixture of spores from six fungal species is, therefore, used in the method. The species varied in their water requirements; between the six species, some require high moisture levels and others only need lower levels for mould growth. This was important as the method includes testing also at lower RHs. The species used 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.

There is no consensus regarding laboratory test methods to evaluate mould growth. A test method that was non-destructive was needed, as the test samples had to be analysed on many occasions. The rating scale used in these studies (Table 5) is somewhat subjective, and it is more difficult to statistically evaluate qualitative data than quantitative data. In order to make the rating more quantitative, the percentage of mould cover on the surface was assessed in the earlier tests, in addition to the rating scale. However, rating was found to be the better approach. The assessment of the percentage of growth was also subjective and the area covered is not a good measure of the amount of bio-mass. Strong, but patchy, well-established growth would yield a low percentage. Therefore, the percentage coverage was not used in later tests.

The rating is subjective, as different raters will vary in their assessment of the extent of mould. To investigate the amount of variation between raters, a comparative study was performed, as described in Paper I. Four trained

individuals with considerable experience in analysing mould growth on materials analysed 63 test specimens, independently of each other. The median value of ratings for each test specimen was considered the "truth". The results from each individual rater were compared to this median value and a probability matrix was constructed. Based on this, simulations were performed to evaluate how many test specimens are needed to ensure the median rating of established growth (rating ≥2) with a level of 95% confidence.

In addition to microbiological issues, practical considerations were also taken into account. The test period, the number of test conditions and the number of test specimens must be limited for a method to be economically feasible in commercial applications.

Testing to find the critical moisture level must be carried out at several RHs, and, to predict the risk at different temperatures according to Section 4.2.2 above, at two temperatures, at least. This entails extensive testing in practice. One way to make the test feasible would be to test at one temperature and to predict the expected critical moisture level at other temperatures. In this case, the parameter c in Equation 1 must be known. In the laboratory test, the values of a , ranged from 102 to 108, with a mean of 105. This value was inserted into Eq. 3, together with the results from the testing at 22 °C. New values of c was obtained and inserted in Equation 1, allowing moisture level to be calculated for any other temperature for the specific material. The RH_{crit} in the temperature interval 0 °C - 40 °C were calculated for each material, using either the calculated a or $a = 105$. The differences in RH_{crit} were calculated.

Theoretically, there is one specific RH (RH_{crit}) for each building material at a given temperature. The lowest RH for mould growth on building materials is often considered to be 75-80% RH at approximately 25 °C (Grant et al., 1989; Adan, 1994; Rowan et al., 1999). Extensive testing at RH levels between 75% and 100% RH would be necessary to determine the exact value. This is obviously not feasible. Neither is it feasible to carry out tests at small intervals. Regardless of the method or equipment used, there will always be measurement uncertainty due to variations in the humidity chambers and calibration of the sensors. The magnitude of the measurement uncertainty therefore limits how narrow these tested intervals may usefully be.

The measured data of RH and temperature of the climate chambers used in the laboratory tests were used to evaluate the measurement uncertainty using this equipment. In the laboratory test, there were some problems with the drift of new loggers, and, therefore, this drift had to be calculated with and the measurement uncertainty was affected. In order to make a second check of the measurement uncertainty during logging in the sort of chambers required in the new test method, these measurements and calculations were performed again at a later point in time. The combined measurement uncertainty was calculated by using the calculated standard deviation and the measurement uncertainty from the calibration of loggers, according to Equation 4. The combined expanded uncertainty was calculated according to Equation 5. The results were then used to determine the intervals.

$$u_c = \sqrt{s^2/\mathrm{n} + u_{cal}{}^2} \qquad\qquad\qquad\qquad (4)$$

$$\mathrm{U}c = k \cdot u_c \qquad\qquad\qquad\qquad (5)$$

where u_c = combined measurement uncertainty
               n   = number of measurements
               s   = standard deviation for measurements
               u_{cal} = measurement uncertainty from calibration of logger
               U   = expanded measurement uncertainty
               k   = 2 (as corresponding to a coverage probability of approximately 95%)

6 The effect of fluctuating conditions on mould growth (Paper V)

6.1 Materials

Side-boards of pine and spruce were used to investigate the effects of fluctuating conditions, compared to steady state conditions, in the laboratory. The materials were collected directly from two sawmills located in south-western Sweden, pine from one sawmill and spruce from the other.

6.2 Incubation and mould analysis

Periods of conditions favourable for mould growth were alternated with periods of less favourable, or non-favourable, conditions. The conditions were varied in a cyclic manner, by changing the temperature or RH periodically, at longer (one week) or shorter (12 h) intervals. Specimens were also incubated under control conditions, during which the RH and temperature were kept constant during the entire incubation period. Figure 13 illustrates the test schemes employed. The maximum incubation time was 84 or 42 days. Total time at 90% RH was 42 days for all test schemes. Mould growth on the test specimens was analysed as described in Section 3.2.3 twice a week on days 3, 7, 10, 14… 42/84.
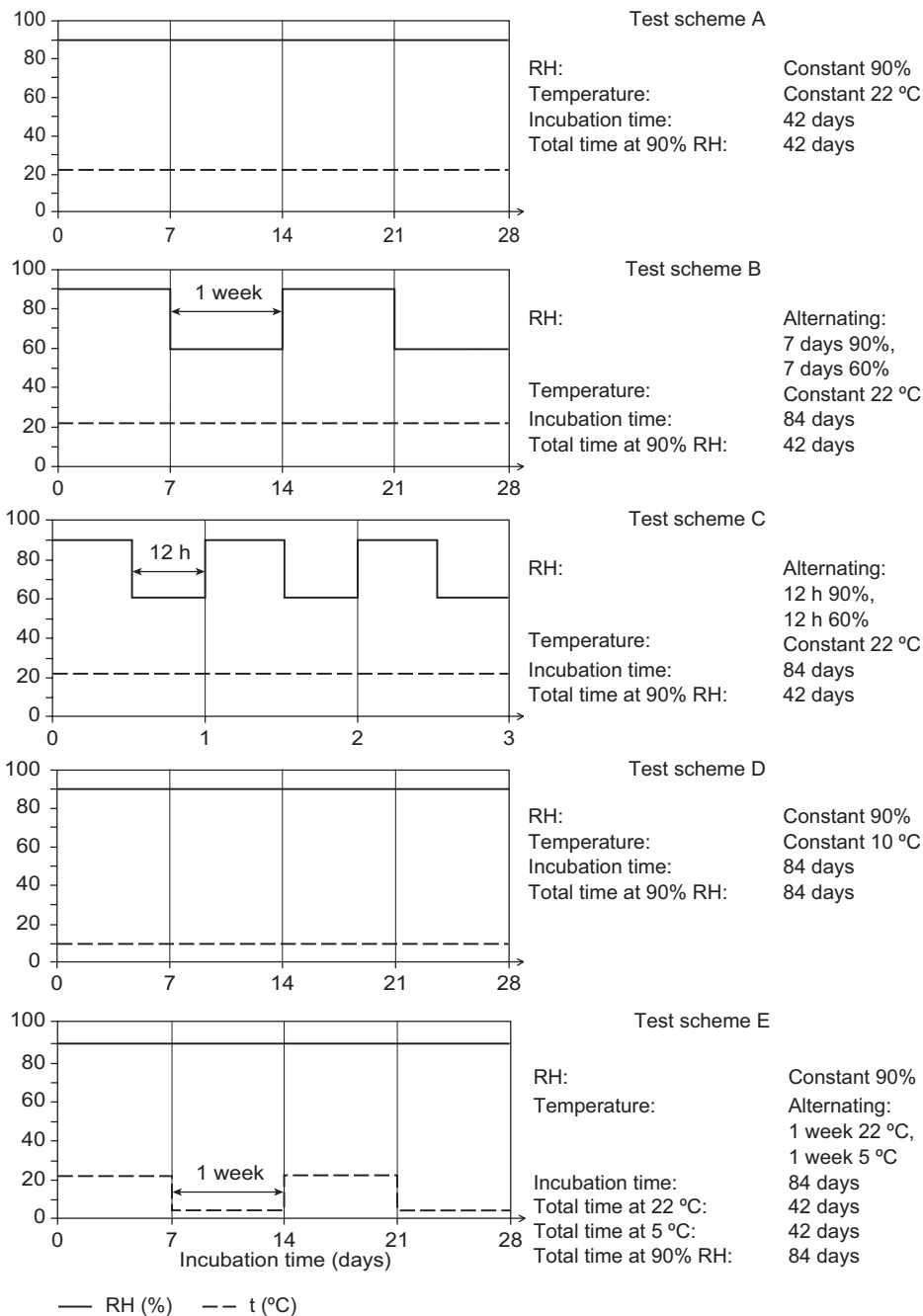
Figure 13   Test schemes in Paper IV.

7 Properties of wood that affect the mould growth (Paper VI)

7.1 Studies

This part of the work involved studies on the effects of several properties of wood on mould growth. The results are based on six separate laboratory studies in which mould growth on wood was studied (Johansson and Jermer 2010; Johansson et al. 2012; Johansson, Bok et al. 2013; Johansson, Wamming et al. 2013; Johansson and Bok 2014; Johansson and Ekstrand-Tobin 2014). In the studies, properties of wood varied between test specimens. All studies were performed in the same way, according to Section 3.2. Test specimens were inoculated with a spore suspension, incubated in moisture chambers, at 22 °C and 90% RH or 95% RH, and at analysed for mould growth. The total length varied between the studies, as did the intervals between inspection of test specimens (once or twice a week), and the number of days from the start of incubation to the first inspection. In Table 6, the design of each study is summarised.

Table 6   *Test design in the different studies, all carried out at 22 °C.*
A=(Johansson et al. 2012), B=(Johansson, Bok et al. 2013),
C=(Johansson and Ekstrand-Tobin 2014), D=(Johansson and Jermer
2010), E=(Johansson and Bok 2011), F=(Johansson, Wamming et al
2013)

	Study					
	A	B	C	D	E	F
Set relative humidity (%)	90	90	90	90	90	95
Maximum incubation time (days)	84	63	70	42	57	21
Frequency of analysis	Once a week	Twice a week	Once a week	Twice a week	Twice a week	Once a week
No of days after start of incubation when first analysis was performed	7	4	7	4	4	7

Five of the studies were performed at 90% RH, although on different occasions. By combining the results from all these studies, the sample size and thus the power of the studies of the effect of the parameters studied could be increased. This is an analytical technique called Meta-analysis. The sixth study was performed at a higher RH, and the results were therefore not included in the meta-analysis. The results from this study were compared to those obtained from the meta-analysis.

7.2   Materials

Test specimens of wood with different properties varied in the different studies. Table 7, lists the various properties of the samples, together with the hypothesis tested.

Table 7   The characteristics of the wood investigated, together with the hypothesis tested

Parameter	Sample	Hypothesis
Wood Species	Pine Spruce	Pine wood is more susceptible to mould growth than spruce wood.
Surface structure	Planed Sawed	A sawn surface will have a higher susceptibility to mould growth than a planed surface, due to rougher surface and greater area, and the microclimate is expected to differ.
Sawing pattern	Centre-board Side-board	Centre-boards are less susceptible to mould growth due to higher amount of heartwood and/or smaller amount of easy assimilated nutrients.
Face of specimen	Outside face (sapwood side) Inside face (pith side)	The outside face of the boards and planks will be more susceptible to mould growth due to increasing amounts of carbohydrates that serve as nutrients towards the periphery of the stem.
Age of surface	Original/"old" Newly prepared	A newly prepared surface will have higher mould resistance due to a "cleaner" surface with no contamination and possibly emission of substances inhibits mould growth.

The purpose of the studies was not to evaluate all of the various parameters and, therefore, it varied between the studies which parameters that were represented, as shown in Table 8. The combinations of parameters varied as well.

Table 8   Numbers of specimens of each parameter in the various test schemes. A=(Johansson et al. 2012), B=(Johansson, Bok et al. 2013), C=(Johansson and Ekstrand-Tobin 2014), D=(Johansson and Jermer 2010), E=(Johansson and Bok 2011), F=(Johansson, Wamming et al 2013)

| | | Test scheme | | | | | |
		A	B	C	D	E	F
Wood species	Spruce	6	12	27	6	42	40
	Pine	-	12	-	6	-	40
Surface structure	Planed	4	18	7	12	36	40
	Sawn	2	6	20	-	6	-
Sawing pattern	Centre-board	-	0	27	6	19	40
	Side-board	6	24	-	6	23	40
Face of specimen	Inside face	2	12	-	-	21	-
	Outside face	4	12	27	12	21	40
Age of surface	New	-	12	21	-	-	40
	Original	6	12	6	12	42	40
Total number of specimens		6	24	27	12	42	80

7.3   Statistical analysis

In order to investigate the influence of the different parameters on mould growth, Cox regression was performed of the data in the meta-analysis and of the data from the test at 95% RH. In the meta-study, sawing pattern, face of specimen, surface structure, age of surface, wood species and original study were included as factors. Cox regression of data from the test at 95% RH included sawing pattern, age of surface and wood species as factors. Pairwise comparisons of survival functions of each of the parameters were also performed by log rank test.

7.4 Selection of data

The original purpose of the six studies used for this analysis differed. Therefore, there is more data than needed for this study. The first selection for the meta-study was to choose only data from laboratory studies performed at 90% RH and 22 °C. All parameters that are evaluated in this work were not represented in all original studies. Initial Cox regression analysis showed that the studies were a significant parameter in the regression model, meaning that this difference in representation of parameters affected the outcome. Therefore, it was not possible to evaluate the data as planned. Instead, only pairwise comparisons of each of the parameters were performed, but in each such comparison only data from the studies that contained such information were used. Also, there were some additional selections of data to obtain a relevant sample. The factors and test schemes analysed are given in Table 9.

Table 9   Selection of data for pairwise comparison in the meta-analysis (Studies at 90% RH and 22 °C). A=(Johansson, Ekstrand-Tobin et al. 2012), B=(Johansson, Bok et al. 2013), C=(Johansson and Ekstrand-Tobin 2014), D=(Johansson and Jermer 2010), E=(Johansson and Bok 2011)

Parameter studied	Studies included in the analysis.	Additional selection
Wood species	B and E, tested separately	Wood species
Surface structure	A, B, C, E	Only original surfaces were included
Sawing pattern	E	Only original surfaces were included
Face of specimen	A, B, E	Only side-boards were included, since only the outer face is represented on centre-boards
Age of surface	B, C	

The data for the second analysis, of data from testing at 95% RH, were taken from Johansson et al., (2013). The original purpose of that study was to investigate whether different kiln drying schemes would have different effect on mould growth, and the results were compared with air dried test specimens. Test specimens were either sawn, re-sawn or remained with the original surface after drying. Also, surfaces at or between stickers were studied separately. No significant difference was found between the different kiln

drying methods, but there was difference between air dried specimens and kiln dried specimens. For the analysis presented here, data from kiln dried specimens, from the surface between stickers and specimens with either planed surface or original surface were selected.

8 Results

8.1 Determining the critical moisture level

In the laboratory test in the first section of this study, Paper I, the materials most susceptible to mould growth were pine sapwood and plywood, followed by chipboard, thin hardboard, plaster boards, and asphalt paper. No growth was detected on any samples of glass fibre board, cement-based board, or extruded polystyrene boards at any of the RH tested, either at 10 °C or at 22 °C. In addition, at 10 °C, there was no growth on asphalt paper, wet-room gypsum board, or exterior plaster board at any of the RH tested. On many of the test specimens there was extensive mould growth although there was no discolouration that was visible to the naked eye. In Figure 14, some examples of the difference in visual appearance from test specimens with rating 4 are shown.
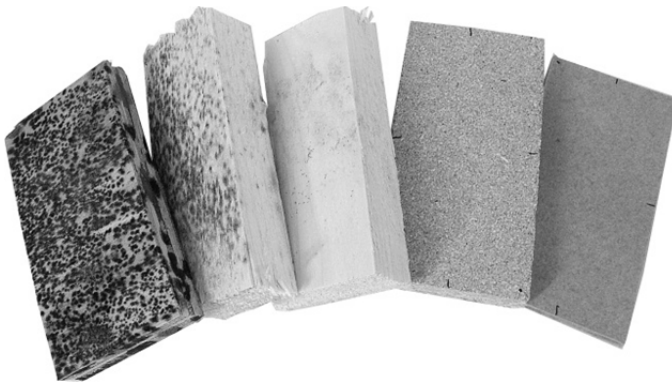
Figure 14   Test specimens from materials in Paper I. Photographed by Annika Ekstrand-Tobin.

Figure 15 and Figure 16 presents the results obtained for mould growth at 22 °C and 10 °C as Kaplan-Meier curves. No data are shown for materials on which there was no growth in any of the RHs tested.
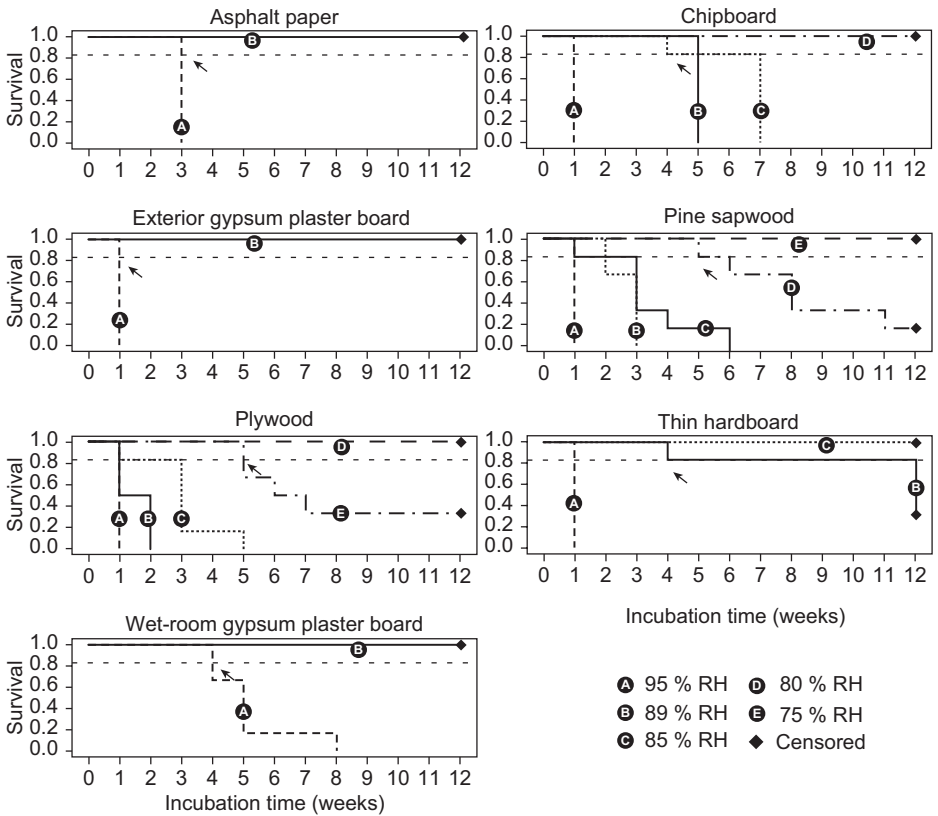
Figure 15   Cumulative survival, that is proportion of test specimes (n=6) on which there was no established mould growth (rating <2), over time (12 weeks) at 22°C and different values of RH. The critical moisture level is reached when at least one of the test pieces shows mould growth ≥2, represented as a horizontal dotted line. The arrow indicates the time when this is reached.
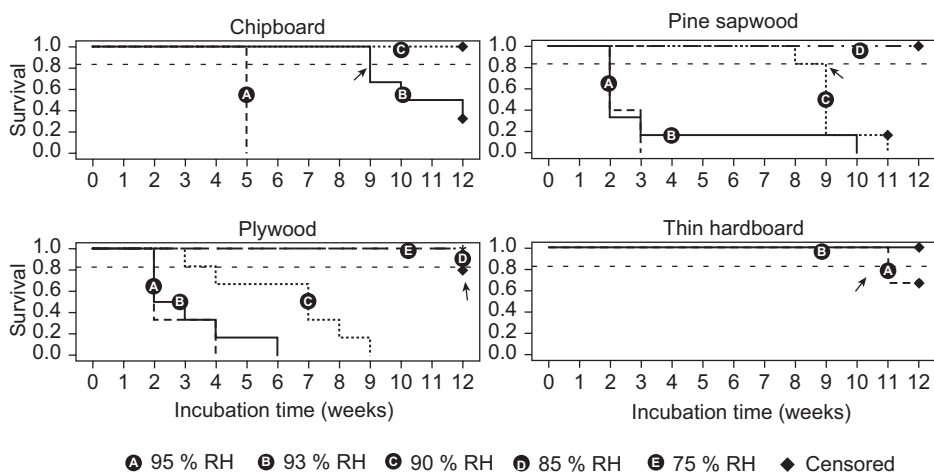
Figure 16   *Cumulative survival, that is proportion of test specimens(n=6) on which there was no established mould growth (rating <2), over time (12 weeks) at 10°C and different values of RH. The critical moisture level is reached when at least one of the test pieces shows mould growth ≥2, represented as a horizontal dotted line. The arrow indicates the time when this is reached.*

As can be seen from Figure 15 and Figure 16, the time at which the critical moisture level was reached varied for the different materials. For example, the criterion for critical moisture value of exterior gypsum plaster board was reached during the first week, while for wet-room gypsum plaster board, it was not reached until the fourth week of incubation.

Table 10 presents the estimated critical moisture levels, based on the curves in Figure 15 and Figure 16 and the criteria given in Section 4.2.2. The maximum RH used was 95%, so the critical level for materials that did not show any mould growth during the test period was above this level. The lowest RH at which mould growth appeared was 80%. Some test pieces that showed no growth during 12 weeks of incubation did show some mould growth when incubated for additional time at 75% or 80% RH and 22 ºC, for an additional period of 20 respectively 7 weeks. Submerging of test specimens prior to incubation did not affect the critical moisture level of the materials, as shown in Paper I.

Table 10 *Range in which the critical moisture level is expected, based on the results of 12 weeks' incubation and the criteria that the mould growth on at least one test specimen was assessed as rating ≥ 2.*

	22 °C	10 °C
Pine sapwood	$75<RH_{cr12w}\leq80$	$85<RH_{cr12w}\leq90$
Plywood	$75<RH_{cr12w}\leq80$	$75<RH_{cr12w}\leq85$
Chipboard	$80<RH_{cr12w}\leq85$	$90<RH_{cr12w}\leq93$
Thin hardboard	$85<RH_{cr12w}\leq89$	$93<RH_{cr12w}\leq95$
Wet-room gypsum plaster board	$89<RH_{cr12w}\leq95$	$95<RH_{cr12w}$
Exterior gypsum plaster board	$89<RH_{cr12w}\leq95$	$95<RH_{cr12w}$
Asphalt paper	$89<RH_{cr12w}\leq95$	$95<RH_{cr12w}$
Cement-based board	$95<RH_{cr12w}$	$95<RH_{cr12w}$
Glass fibre	$95<RH_{cr12w}$	$95<RH_{cr12w}$
Extruded polystyrene	$95<RH_{cr12w}$	$95<RH_{cr12w}$

The temperature and RH measurements from the field tests are presented in Figure 17 and Figure 18. There was a both short term, daily, variation, and a long term, seasonal, variation. The daily variation was greatest in the attics. In the crawl-spaces, the RH was highest in the summertime, while in RH was highest during the winter months, when also the temperature was low, in the attics.
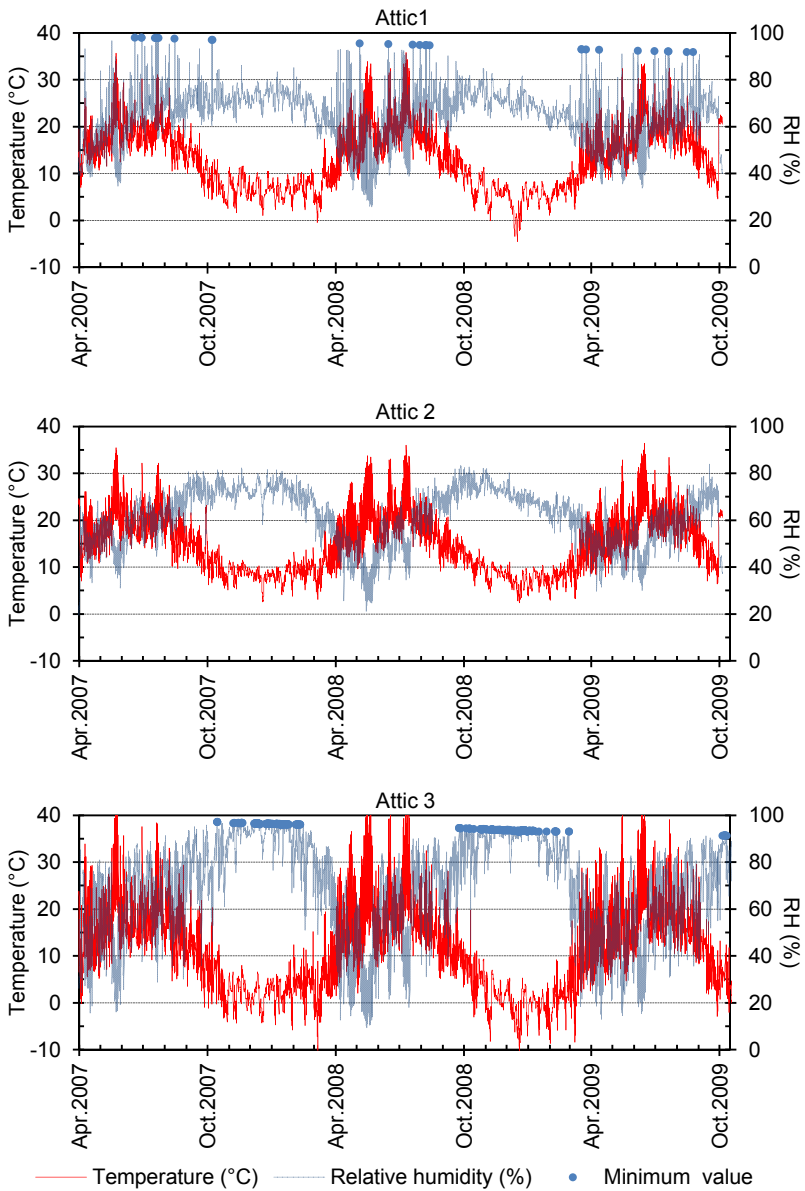
Figure 17   Measured RH and temperature in three attics in Sweden. Data given as circles refer to data where the drift of the loggers was such that the correct value could not be estimated and represents the minimum value that could be expected. The dates refer to the time when the analysis for mould growth was performed.
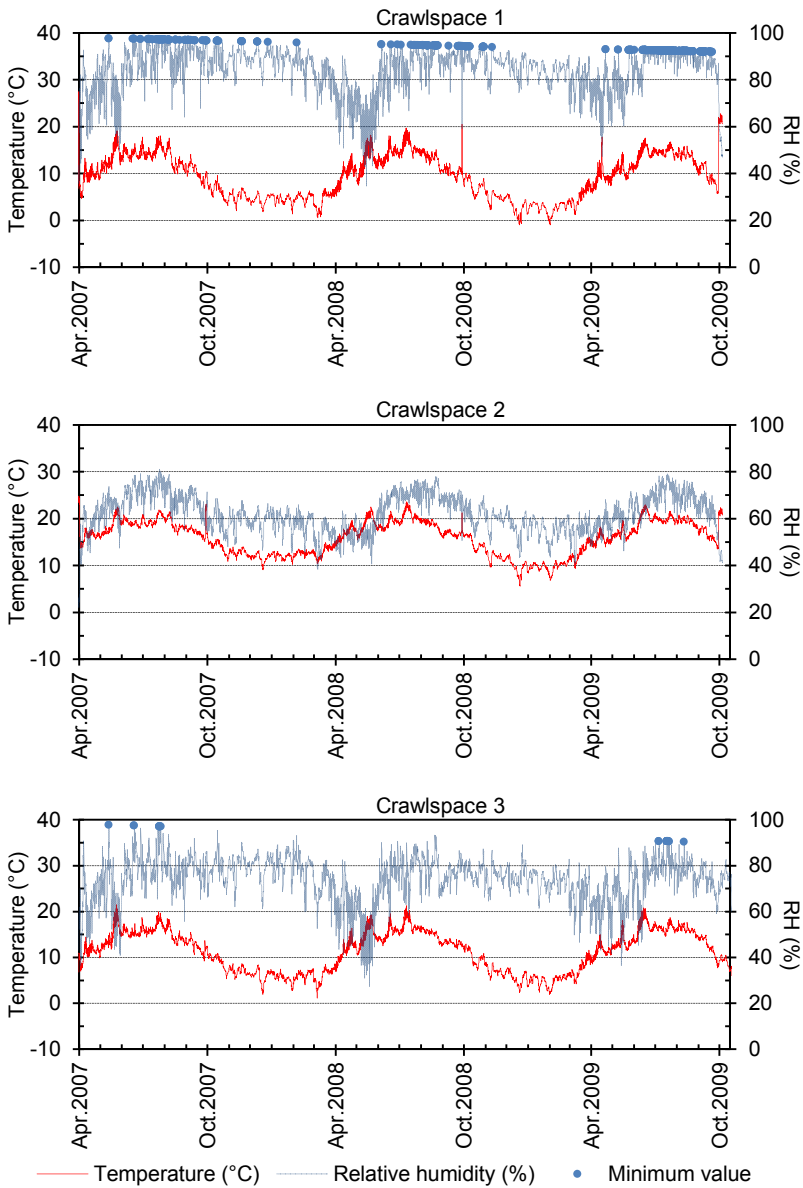
Figure 18   Measured RH and temperature in three attics in Sweden. Data given as circles refer to data where the drift of the loggers was such that the correct value could not be estimated and represents the minimum value that could be expected. The dates refer to the time when the analysis for mould growth was performed.

There were mould growth on many of the test specimens exposed in the crawl-spaces and attics after 2 ½ years. However, differences were observed between the sites regarding which materials became mouldy, how quickly growth started, and the extent of growth on test specimens at each site. No growth was detected on any of the test specimens of cement-based board, glass fibre board or extruded polystyrene board.

When comparing the results obtained from the field tests with the expected outcome, in the way described in Section 4.4, there was a good agreement in most cases, as can be seen from Table 3.

Table 11 *Predicted and observed mould growth on the materials studied at each test site. Predicted growth is denoted + if conditions was such as exceeding the critical moisture levels, as described in Section 4.4,while – means that no mould growth was predicted. Observed growth on at least one test specimen in the field study is denoted +, - means that no mould growth was found on any of the test specimens.*

	Crawl-space 1		Crawl-space 2		Crawl-space 3		Attic 1		Attic 2		Attic 3	
	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
Plywood	+	+	+	+	+	+	+	+	+	-	+	+
Chipboard	+	+	-	-	+	-	+	+	-	-	+	+
Thin hardboard	+	+	-	-	+	-	+	-	-	-	+	+
Exterior gypsum plaster board	+	+	-	-	+	-	+	-	-	-	+	-
Wet room gypsum plaster board	+	+	-	-	+	-	+	-	-	-	+	-
Asphalt paper	+	+	-	-	+	-	+	-	-	-	+	-
Cement-based board	-*	-	-*	-	-*	-	-*	-	-*	-	-*	-
Glassfibre board	-*	-	-*	-	-*	-	-*	-	-*	-	-*	-
Extruded polystyrene board	-*	-	-*	-	-*	-	-*	-	-*	-	-*	-

*Based on the observation of no mould growth on any of the test specimens in the laboratory test (Paper I)

In cases where there was a discrepancy between the expected and the observed values, the model had predicted mould growth while none was detected on the test specimens in the field study. Table 12 shows the total time that the temperature and RH were over the limit curves for those cases where expected and actual mould growth were not consistent; it also shows 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.

Table 12   Cumulative time that measured conditions 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

	Crawlspace 3		Attic 1		Attic 2		Attic 3		Time (weeks) in the laboratory before critical moisture level was reached	
	Time (weeks) over lower curve	Time (weeks) over upper curve	Time (weeks) over lower curve	Time (weeks) over upper curve	Time (weeks) over lower curve	Time (weeks) over upper curve	Time (weeks) over lower curve	Time (weeks) over upper curve	10 °C	22 °C
Plywood					0.3	0.0			11-12	4-5
Chipboard	8.3	3.0							8-9	3-4
Thin hardboard	3.0	0.9	1.9	1.4					10-11	3-4
Exterior gypsum board	0.9	0.3	1.4	0.3			0.4	0.0	12	0-1
Wet room gypsum paper board	0.9	0.3	1.4	0.3			0.4	0.0	>12	3-4
Asphalt paper	0.9	0.3	1.4	0.3			0.4	0.0	>12	2-3

Comparisons were also made between the results from the field studies and the results from the laboratory tests as if they had been performed according to conventional tests for the evaluation of the mould resistance of building materials. Therefore, results after incubation for 28 days and 95% and 22 °C were considered, as representing a "general" mould resistance test (Table 2). Mould growth was found on test samples of seven of the tested materials: asphalt paper, wet-room gypsum plasterboard, exterior gypsum plasterboard, plywood, thin hardboard, chipboard and pine sapwood in those conditions.

However, in the field studies, mould growth was not found on some of these materials at some test sites (Table 11). For example, on asphalt paper and exterior gypsum paper board, mould growth was found on all the test samples in the laboratory test, within three weeks and one week, respectively, of incubation in the laboratory test at 95% and 22 °C (Figure 15). Mould growth was only found on these materials at one test site (Crawlspace 1). At lower RH tested (at 22 °C) none of these materials showed any mould growth even after 12 weeks. The first situation therefore overestimate the risk for mould growth in some building parts, while if had been tested only at the lower RH, the risk had been underestimated.

8.2 The new test method for determining the critical moisture level

8.2.1 Testing at one temperature

The maximum difference in RH_{crit} when using a mean value of 105 for the constant *a* instead of different values calculated from eq. 2 was at maximum approx. 1.5 percentage points in RH in the temperature interval 0°C - 40 °C. Therefore, to perform the test for critical moisture value at 22 °C and estimate the critical moisture value also for other temperatures in the way described in Section 5 was considered a feasible way.

8.2.2 Measurement uncertainty of RH and intervals tested

The maximum expanded measurement uncertainty in the RH in the studies of Paper I was 2.5 percentage points. Later measurements showed a lower measurement uncertainty, see Table 13.

Table 13   Results from measurement of RH in the test chambers prescribed in the CLM method (Paper III)

Set RH	Mean RH	Standard mean Deviation	Expanded measurement uncertainty	Lower limit	Upper limit
95	95.6	0.2	1.4	94.2	97.0
90	90.3	0.3	1.3	89.0	91.6
85	85.4	0.3	1.3	84.1	86.7
80	80.8	0.3	1.2	79.6	82.0

8.2.3 Number of test specimens

Generally, the more test specimens that are used in a study the better. However, for practical reasons, the number of test specimens in a commercial test method must be limited. In the evaluation of measurement uncertainty between raters, it was shown that using the median of seven the test specimens provides a 95% level of confidence for the rating. Therefore, in SP method 4927, the number of test specimens in the study is prescribed as seven, at a minimum. However, the criterion for the critical moisture level is reached when two out of seven test specimens has a rating higher or equal to 2. Based on the same matrix as used above, the probability to correctly detect failure (rating >2) is 99.9%.

8.2.4 Summary of the test method (CLM method)

The new test method is presented in Paper III and is discussed in Paper IV.

Specimens of the material to be tested are inoculated by a spore suspension according to Section 0. They are then incubated at 22 °C and at 80 % RH, 85 % RH, 90 % RH and 95 % RH, seven test specimens are used in each RH tested. Every second week the specimens are inspected for mould growth as in Section 3.2.3. The critical moisture value is determined after 12 weeks incubation as in Section 4.2.2. At least two of the test specimens must show mould growth of rating 2 or higher for the critical moisture level criteria to be met.

In Paper I it was shown that mould could grow at 75% RH at 22 °C. No examples could be found in the literature of established mould growth on building materials at lower RH. Therefore, this value is considered to be the lower bound of the test. Consequently, if there is mould growth at 80% RH, the critical moisture value will be expressed as $75\% \leq RH_{crit22°C} \leq 80\%$.

To ensure test reproducibility, it is important to control the testing procedure. In the test method, Paper III, there are several routines that must be followed. In order to distinguish the different incubation RH from each other, there are specified limits, ± 2.5 % of each of the set values for RH and $\pm 2°C$ for the temperature, within which the mean value during incubation, together with the measurement uncertainties must fall. Figure 19 and Table 14 illustrates this. These values were based on the results of Table 13.

Specified upper limit of
incubation condition

Specified lower limit
of incubation condition

(A)   (B)
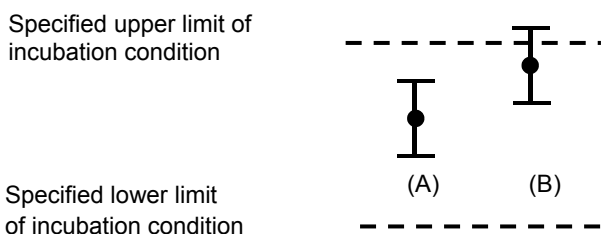
*Figure 19   Illustration of the evaluation of incubation criteria. The specified limits of
incubation condition are described in Table 2. The dots represent mean
measured values of RH or temperature and the whiskers represent the
calculated expanded uncertainty. In case (A) the mean value with
uncertainties fall into allowed limits. In case (B) it is not, since the upper
limit is exceeded. Therefore, the test is not valid.*

Table 14   The set points of each incubation condition of the test method

Incubation condition	Set point	Specified lower limit	Specified upper limit
T	22.0 °C	20.0 °C	24.0 °C
RH 1	80.0%	77.5%	82.5%
RH 2	85.0%	82.5%	87.5%
RH 3	90.0%	87.5%	92.5%
RH 4	95.0%	92.5%	97.5%

Other routines in the CML method comprises the preparation and inoculation
of spores on test samples, requirements on the performance, service and
calibration of equipment (test chambers, device for recording incubation
conditions, inoculation device, stereo microscope, laminar air flow bench,
autoclave and centrifuge) handling of test specimens and assessment of mould
growth. There are also routines for the measures to be taken if failure is
detected in any stage of the test. The last part of the test method contains
instructions of how to determinate and report the critical moisture value.

8.3 The effect of fluctuating conditions in comparison to steady state conditions

The results of the tests obtained when varying the relative humidity are shown in Figure 20 as Kaplan-Meier curves. When alternating the RH between favourable conditions and unfavourable conditions (too low RH for mould fungi to grow), the mould growth rate differed from that seen under the steady state conditions (test scheme A). The duration of low RH conditions had obvious effect; a longer period of unfavourable conditions (test scheme B) affected the mould growth more than the shorter periods (test scheme C). The differences are supported by the log-rank test (p<0.001 for all comparisons).

Figure 20   *Proportion of test specimens on which there was no established mould growth (rating < 2) over time (days) when the RH was changed cyclically (test schemes B and C); compared with the reference steady state conditions (test scheme A). Samples that did not show any established growth during the test period are censored in the plots.*

The results obtained when varying the temperature during incubation are shown in Figure 21. The time until mould growth established on the test specimens was longer when the temperature was reduced from 22 °C (test scheme A) to 10 °C (test scheme B) (p<0.001). When the temperature fluctuated between 22 °C and 5 °C (test scheme E), the time for mould growth to establish was also lowered, as than in the test scheme in which the temperature was constant at 22 °C (p<0.001). There was no clear difference between test schemes D and E (p=0.261).

A. Constant 22°C, 90 % RH
D. Constant 10°C, 90 % RH
E. Cyclic 22/5°C (1 week), 90%
Censored
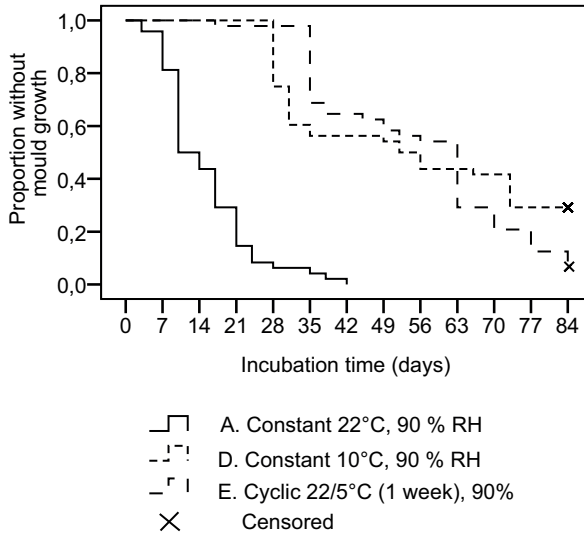
Figure 21   Proportion of test specimens on which there was no established mould growth (rating < 2) over time (days) at different temperatures, constant (Test schemes A and D) and cyclic (Test scheme E). Samples that did not have any established growth during the test period are censored in the plots.

8.4 Parameters affecting mould growth on wood

The results from the tests at 22 °C and 90% show that the time before the for growth to establish varies between the test specimens, ranging from 3 to 63 days, as shown in Figure 22.

× Test specimens with established mould growth
○ Test specimens with no mould growth (censored)
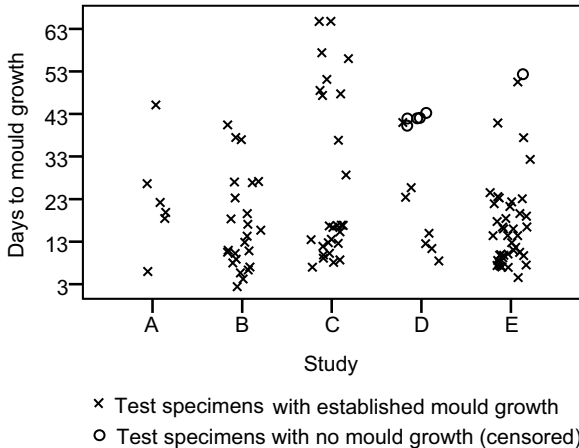
Figure 22   Days before mould growth was observed in each individual test specimen in 5 different studies at 22 °C and approx 90% and. x=specimens with mould growth, o=specimens where no mould could be detected during the test (censored cases).

After selection of cases, as described in Section 0, a pairwise comparison of the different parameters tested was performed. In several of these comparisons, data originated from two or more studies. The test schemes had in neither of the cases any significant effect on the hazard as determined by Cox-regression, except in the wood species case. Therefore, the effect of this parameter was analysed separately for both studies.

The survival functions shown as Kaplan-Meier curves are presented in Figure 23 together with the p-values from the pairwise comparisons.
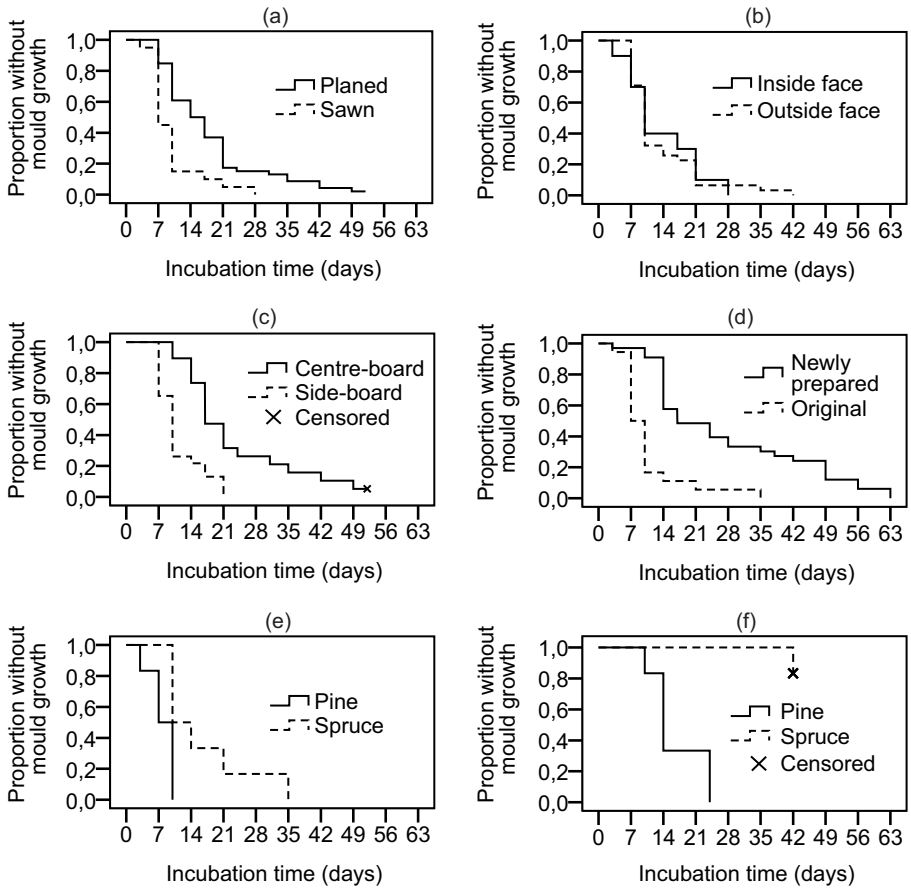
Figure 23   Proportion of test specimens on which there was no established mould growth (rating < 2) over time (days)at 90 % RH and 22°C, for the selected cases described in Table 2.; a=surface structure (p<0.001), b=face of specimen (p=0.996), c=sawing pattern (p<0.001), d=age of surface (p<0.001), e=wood species Study B (p=0.018) f=wood species Study D (p<0.001).

Figure 24 shows the results from testing at 22 °C and 95% RH. Pairwise comparison revealed statistically significant differences between the survival functions for the three parameters evaluated as illustrated by the p values.
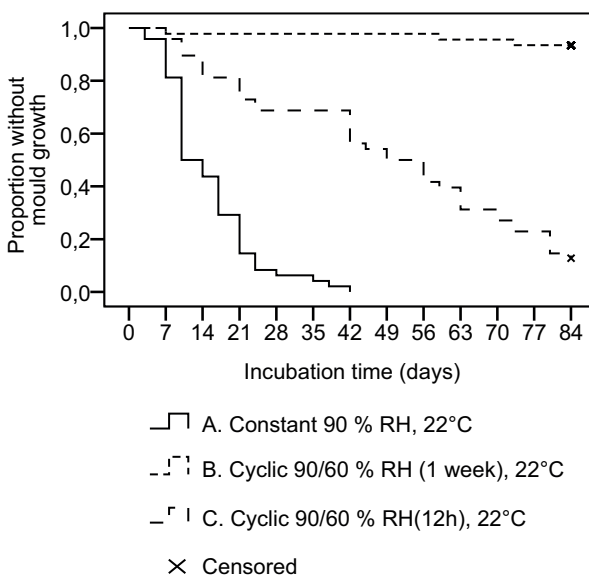
73
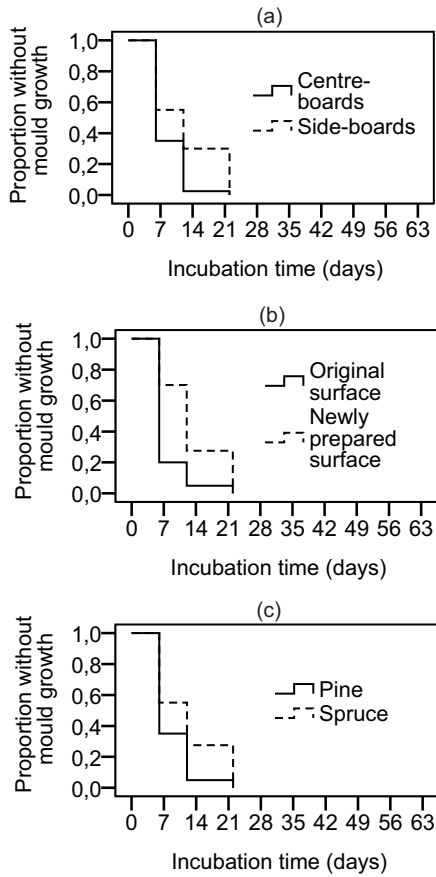
Figure 24   Proportion of test specimens on which there was no established mould growth (rating < 2) over time (days) 95% RH and 22 °C. (a)=Sawing pattern (p=0.002), (b)=age of surface (p<0.001), (c)=wood species (p=0.008).

The output of the Cox-regression applied to the data from testing at 95 % RH is shown in Table 15. The p values indicate that the age of the surface and wood species have an impact on mould growth. However, the effect of sawing pattern was not statistically significant in this model. The exponent (B) values indicate that mould growth at an original surface is 1.9 times as likely as on a newly prepared surface and mould growth on pine is 1.6 times as likely as mould growth on spruce.

Table 15   Application of Cox's regression data, using age of surface, sawing
            pattern and wood species as explanatory variables

	Coefficient (B)	Standard error	Sig.	Exp(B)	95,0% CI for Exp(B)	
					Lower	Upper
Age of surface	0.623	0.240	0.009	1.865	1.165	2.987
Sawing pattern	0.430	0.238	0.070	1.537	0.965	2.450
Wood Species	0.472	0.238	0.047	1.602	1.006	2.553

9 Discussion

9.1 General

In this work it was shown that results from laboratory testing at several values of RH can be used to predict the possibility for mould growth in buildings. Therefore, a new test method for determining the critical moisture level for mould growth on building materials was developed and presented based on the results.

9.2 The incubation time

The duration of a laboratory test designed to investigate established growth of mould fungi on building materials is important. It must be long enough for mould spores to germinate and for growth to start and become established. The longer the incubation period, the higher the probability of mould growth on a material. This raises the question of whether mould will grow on all materials when the conditions are favourable if the time is sufficiently long. If this were the case, all materials would eventually mould given sufficient time. However, testing in the laboratory to determinate the critical moisture level of a material or mould resistance assumes that there is a difference between different materials. Therefore, mould is not expected to grow on all materials under conditions generally favourable to mould growth, no matter how long they are incubated. However, this does not mean that time can be overlooked.

Viitanen et al. (2010) found mould growth on concrete after many months of testing. However, it is not practically feasible to carry out tests in the laboratory for such long periods. An upper limit for when the inoculated spores cease to be viable must also be assumed. According to Hofbauer et al. (2008), 100 days is a reasonable upper level for a single test. In MIL STD 810 G (2008), the maximum incubation time is 84 days. In the laboratory tests described in Paper I and the new method presented in Paper III, an incubation time of 12 weeks was used, and was shown to be suitable for real conditions.

At lower levels of RH, a longer incubation time is required for mould growth to become established. It was not possible 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 was material-specific. For example, for chipboard, the time required for moisture growth at 22 °C was about 5 times longer at 90% RH than at 95% RH. The corresponding

value for pine sapwood was 3 times, while no difference was found between 90% and 95% RH for plywood. The incubation period was thus set to 12 weeks for all levels of RH, as was the period used in the laboratory study described in Paper I.

9.3 Validation of and application of the data for real life constructions

In general, the critical moisture levels determined in accelerated laboratory experiments under constant climatic conditions, agree with the results obtained under real conditions, where both temperature and RH vary. If the combination of temperature and RH exceeds the growth limit curves calculated from the critical moisture levels, mould growth should be expected, provided that the cumulative time above the mould growth curves is long enough.

However, mould growth is also expected to be influenced by the magnitude and frequency of changes in RH and temperature (Adan, 1994; Viitanen and Bjurman, 1995; Pasanen et al., 2000). In Paper V it was shown that the period of favourable RH, when interrupted by periods of conditions were mould growth is not possible, is crucial for mould growth. The longer periods of non favourable conditions slowed down the mould growth more than the shorter periods. Variation of higher and lower temperatures also effected mould growth, as the time to mould growth was longer in comparison to when the temperature were constant and high. Using the cumulative time during which conditions were favourable for mould growth, as was done when validating the results from the laboratory test in real buildings, is therefore a simplified approach. In this case it was, however, useful for the prediction of mould growth. By using this approach, the results of laboratory test of the critical moisture level will not underestimate the risk of mould growth; the results will instead include a certain margin of safety. However, for making more precise predictions, the effect of fluctuation of RH must be taken into consideration.

If the results from Paper V are interpreted in terms of critical moisture level of a material, this would mean that the length of periods during which the RH is above the RH_{crit} is decisive. This may explain why mould did not grow on chipboard in Crawlspace 3 (Paper II), despite the fact that the cumulative time of favourable conditions was about the same as the reference values in the laboratory tests. The amount by which the actual values exceed the critical values is probably also of importance.

9.4 Effect of wetting on the critical moisture level

It has been reported previously, that when a wet building material, for example, after flooding or in a bathroom after showering, is dried out to at an RH in the surrounding air not suitable for mould growth, there is still a risk that mould growth will appear on the surface (Horner et al., 2001; Menetrez et al., 2004, Adan, 2011), indicating that the critical moisture level can be lower for wetted materials. However, this was not confirmed by the results of the present study (Paper I). One reason for this could be that the wetting period was too short. A more likely explanation is that the high air exchange rate in the climate test chambers led to rapid equilibrium between the surface of the wetted samples and the conditions prevailing in the chamber. The surfaces would, thus, be comparable to the surfaces of samples that were not subjected to wetting. Furthermore, measurements of the moisture content of test samples of wood incubated under constant RH and temperature in the same chambers, but in another study, showed that the entire test sample was in equilibrium with the surrounding air in the chamber within one week of incubation, regardless of whether it was wetted or not (Johansson and Bok, 2014). Therefore, when using climate chambers with the prescribed performance (Paper III), there is no need for preconditioning of test specimens at certain conditions, as the initial moisture content will not affect the outcome of the test.

However, when using a climate chamber with no air exchange over the surface, wetting was shown to have an effect on mould growth (Johansson and Bok, 2011), and it may, therefore, have practical implications in real structures and any water damage must be dried out immediately, even if the materials in the water damaged building have documented high RH_{crit}.

9.5 How well does the results of critical moisture levels agree with earlier findings?

Critical moisture levels for different groups of building materials have been proposed in on a literature review by Johansson et al. (2005). The agreement between the critical moisture levels presented in Paper I and the results of the studies that formed the basis for this review varied. This may be due to methodological differences, or because it is not possible to compare the results obtained for a particular material manufactured by different companies. It may also be difficult to define the group to which a certain material belongs. For example, chipboard, plywood and thin hardboard are all wood-based materials, but the values of critical moisture level obtained for these materials in the

present work differed. The results presented in Paper I can, therefore, not be used to estimate RHcrit for a similar material. It follows from this, that the results published by Johansson et al. (2005) cannot be used as general critical moisture values, although at that time, this was the only knowledge available.

9.6 The new method compared to conventional mould resistance tests

As was shown in this study, when evaluating the risk for mould growth on a building material in any building part, the test result from a traditional mould resistance test may not be suitable, since it overestimates the risk for some structures. In addition, if the tests had been performed only at lower RH, the risk had been underestimated for some materials.

When evaluating the possibility for mould growth, the conditions of the RH and temperature in the building part where the material is to be placed must also be considered. Even by doing so, the test from one specific RH and temperature did not give the right predictions; it is difficult to make predictions based on just one test result. Therefore, to test at several levels of RH and construct mould limit curves is the more appropriate the approach.

9.7 The mould growth - its appearance and the analysis

The analytical method used to study mould growth in this thesis is common to many test methods and previously published research. It was chosen because it can be used to follow mould on a specific test sample growth over time, without affecting the mould growth itself. In many standard methods and previous studies on mould resistance, only mould growth that can be seen with the naked eye is considered. In some cases, non-visible growth is also considered, but the rating scales used assume that extensive growth causes discolouration, and that if there is no visible growth, there is very little growth at all. The work presented here shows that this conclusion is incorrect; there may be extensive growth despite the fact that nothing can be seen with the naked eye. Consequently, the mould growth on a test sample in the present studies could have been rated high (rating 3 or rating 4), even though the sample seemed unaffected. Therefore, both mould growth visible to the naked eye and that only visible under the microscope at a magnification of 40 x were assessed in the same way in all the studies in this work and this is also prescribed in the CML method (Paper III).

One reason for differences in discolouration between the test samples may the presence of different species of fungi, as some species produce dark pigments.

Although fungal species growing on the samples were not identified in the present studies, it was found that there was a variation of species on different specimens. Different species of fungi have a higher propensity to grow on different building materials, even under the same conditions. Moreover, different species have different moisture requirements (Block, 1953) and their growth is expected to vary under the same incubation conditions. In the spore suspension used in the studies, *A. pullulans, S. chartarum* and *C. sphaerospermum* represent discolouring mould fungi. These species are secondary colonizers on building materials and may, therefore, have a limited ability to compete with the primary colonizers *A. versicolor, E. herbariorum* and *P. chrysogenum* (Grant et al. 1989) during the incubation period. The difference found among materials if the growth vas visible or not may therefore be the result of different species growing on the specimens. In addition to the variation between species, the production of pigment can also vary within one species depending on which nutrients are available, or on the growth phase of the fungus (Eagen et al. 1997; Fleet et al. 2001; Gadd 1980). It should also be borne in mind that the ability to discern discolouration is related to the contrast with the underlying surface; dark mould growth will not be as visible on a dark surface as on a pale surface.

9.8 The homogeneity of materials and the consequences for mould growth tests

It is expected that there will be a variation in the critical moisture level of a material when evaluating individual test samples. The greater the variations, the larger the sample size must be in order to accurately represent the material. It was assumed in the development of the new CML test method that the material to be tested is reasonably homogeneous. In the first study (Paper I), the different test samples originated from different boards, however the RH_{crit} did not differ between the boards.

Timber is a common building material in Sweden. It is, therefore, important to know the critical moisture conditions for wood, in order to reduce the risk of mould in Swedish buildings. However, timber is an inhomogeneous material and, although mould growth was seen at a RH as low as 75-80% RH at room temperature (Paper I), some of the test samples in a later study (Paper VI) showed no mould growth, despite the fact that they had been incubated at a higher RH. This indicates that the critical moisture level may be higher for some wooden materials, and that the inhomogeneity makes it impossible determining a general RH_{crit} for mould growth on wood.

9.9   Mould growth on wood

Paper VI highlights the complexities associated with the prediction of mould growth on wood. In this study the mould resistance at 22 °C and 90% RH or 95% for different wood was studied. In both RH tested there was a significant difference in mould growth between the wood species investigated; pine being more susceptible to mould growth than spruce. This has also been reported in other studies (e.g Sehlstedt, 2011; Viitanen, 1996).

Centre-boards were less susceptible to mould growth than were side-boards. This may be an effect of higher amounts of nutrients on the surface of side-boards. In the living, growing tree, sugars from photosynthesis are transported from the leaves along the branches and into stem. Therefore, the concentration of sugars is higher in the side-boards than in the centre-boards. Some centre-boards will contain some heartwood, which is generally considered to be less susceptible to mould growth than sapwood. No statistically significant difference in survival functions was found in the test at 90% RH when considering the face (inner or outer) of the test samples of side-boards, which indicates that there was no difference in sugar content that affects the mould growth on these both sides. However, neither the sugar content nor the presence of heartwood was analysed in the present study, and no conclusions can thus be drawn regarding their effects.

In the tests at 90% RH (Paper VI), the survival functions for planed and sawn surfaces differed significantly on original surfaces, which indicates that surface roughness has an effect on mould growth; sawn surfaces being more susceptible to mould growth than planed surfaces, which is in agreement with previous findings (Terziev, 1996). In one of the studies included in the meta-analysis of Paper VI, (Johansson and Ekstrand-Tobin, 2014), no difference in mould growth was found among test samples that were planed, fine-sawn or coarse-sawn. All the surfaces were then newly prepared, and it was concluded that the lack of any difference could be the consequence of only small differences in roughness, or the consequence of the surface treatment itself. A newly prepared surface may changes in the chemical composition at the surfaces, which may affect the mould growth. This is supported by the other results in Paper VI, since statistically significant differences were found between the original surfaces and newly prepared surfaces; the new surfaces were less susceptible to mould growth. However, this parameter may not have any effect on the mould growth in actual buildings as newly prepared timber is not used in construction. This issue must however be considered when testing different sorts of wood in the laboratory.

The CML method (Paper III) can be used to assess the impact of different kinds of treatments of wood, assumed to give better resistance to mould growth, for example chemical treatments. The same basic materials and treatment must be used as a reference. Otherwise, there is a risk that the characteristics of different types of wood will be studied, and not the effect of the treatment. Furthermore, the results from Paper VI show that if generalised conclusions are to be drawn regarding the susceptibility of common wood to mould, a large number of samples must be used when testing each property and that recent changes in the surface, planing or sawing, must be taken into consideration.

10 Conclusions

Many factors affect the critical moisture level of a building material. In this thesis, temperature, relative humidity, incubation time, and assessment criteria for mould growth were identified as important parameters. In order to compare the results from different tests, it is important that such factors are carefully controlled and the same test method used. An innovative method of determining the critical moisture level, the CML method, was presented in this thesis.

The results obtained in this work, only apply to the materials tested here, and they cannot be used to draw general conclusions about a certain type of building material, for example "gypsum plasterboard" or "wood-based board."

As in all modelling and testing, every possible eventuality cannot be taken into account. However, the critical moisture levels determined in accelerated laboratory experiments under constant climatic conditions, generally agreed with the results obtained under real conditions, where both temperature and relative humidity vary.

If the expected temperature and RH in a structure are known, the results from the CLM method can be used to select materials with a minimal risk of mould growth for that structure. When the duration of conditions favourable for mould growth is not considered, the laboratory test results will not underestimate the risk of mould growth, but include a certain margin of safety.

Although the simplified approach of considering the cumulative time over the growth limit curves gave sufficient information to validate the method, more precise predictions could be performed using more sophisticated models. Instead of using the cumulative time of favourable RH or the mean relative humidity, the length of periods of favourable conditions should be used, since this is decisive for mould growth. The results of critical moisture level testing should also be included in those models. The effect of fluctuating temperature is not as apparent when it lies in the range that supports fungal growth. Based on the results presented in this thesis, it appears that the mean temperature can be used in predictive models.

Several material parameters affect the time required for mould growth on wood. It is not possible to predict the general susceptibility of wood to mould based only on a few data as it is affected by parameters as surface structure, wood species and sawing pattern. Also, the susceptibility cannot be described by one single parameter, it depends on several parameters.

11 Future studies

Further knowledge is needed to understand the effect of variations in relative humidity and temperature around the critical moisture level of a material on mould growth. The results presented in this thesis can be used as the basis for further studies in the laboratory and in real buildings.

In the present work, one parameter, either RH or temperature, was kept constant at a favourable level. Future studies should include test schemes in which temperature and RH are varied simultaneously. Furthermore, the activity of the fungal cells is likely to be affected by the cycling of favourable and less favourable conditions, and it would be interesting to further investigate the possible physiological processes taking place.

In order to validate the method of assessing critical moisture levels for temperatures other than those tested further tests should be performed at different temperatures and the results compared to the predicted outcome.

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

Pernilla Johansson, Annika Ekstrand-Tobin, Thomas Svensson and Gunilla Bok (2012)

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ABSTRACT

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

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1. Introduction

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

Conditions for mould growth include nutrient availability, temperature, pH, and moisture. In general, the availability of water in the material is regarded as the crucial element for growth to

occur. The water available to microorganisms is often referred to as water activity, A_w. It is defined as the vapour pressure in the substrate divided by that of pure water at the same temperature.

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

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

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

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

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

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

2. Materials and methods

2.1. Building materials

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

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

2.2. Fungal species

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

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

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

Table 2
Mould species used in the study.

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

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

2.3. Inoculum preparation

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

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

2.4. Inoculation of test specimens

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

2.5. Incubation

2.5.1. Incubation chambers

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

2.5.2. Registration of incubation conditions

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

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

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

2.5.3. Incubation conditions

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

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

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

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

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

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

2.6. Assessment of mould growth

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

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

2.7. Validation of ratings

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

test pieces, independently of each other. Mould growth from all of the rating grades in Table 4 was expected to be represented on the test pieces.
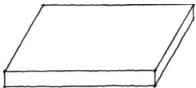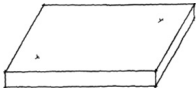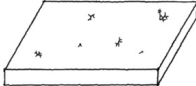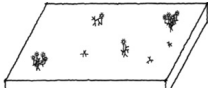
Since the classification was based on human judgement, the obtained values cannot be regarded as numerical values, and so statistical measures such as average and standard deviation are not appropriate for analysis. In order to still control the measurement uncertainty, a new idea was implemented, based on simulations from a calibration matrix: Each judgement in the calibration procedure was compared to a "true" value, and the relative frequencies of the judgements given for each true rating were collected in a matrix, i.e. the number in the matrix position ij represents the probability of judging a rating j when the true rating is i. Simulations from this matrix then allowed estimation of the overall measurement uncertainties. The calibration indicated that the variation among operators was negligible, and therefore all observations were regarded as independent. As the "true" rating was unknown, the median of the four assessments of each test piece was defined to be "the truth". Since an even number (six) of assessments was performed, there was a problem with defining the median in cases with two non-equal middle values. The usual way of taking the average is not possible with non-numeric values. The problem was solved in this particular case by taking the average value of a large number of relative frequency matrices, each generated by taking random choices of truth in cases of ambiguity.

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

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

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

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

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

2.8. Definition of critical mould growth and critical moisture level

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

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

2.9. Description of mould development by time

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

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

3. Results

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

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

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

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

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

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

4. Discussion

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

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

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

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

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

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

Duration of incubation will influence the critical moisture level of the material being tested. In our study, no growth was found on any of the materials tested after 12 weeks at 75% RH, but mould began to grow on plywood after 16 weeks and on pine sapwood after 32 weeks. The critical moisture level was thus reduced to below 75%, from having been between 75% and 80% RH at 22 °C. Had the test been allowed to continue for longer than 12 weeks in
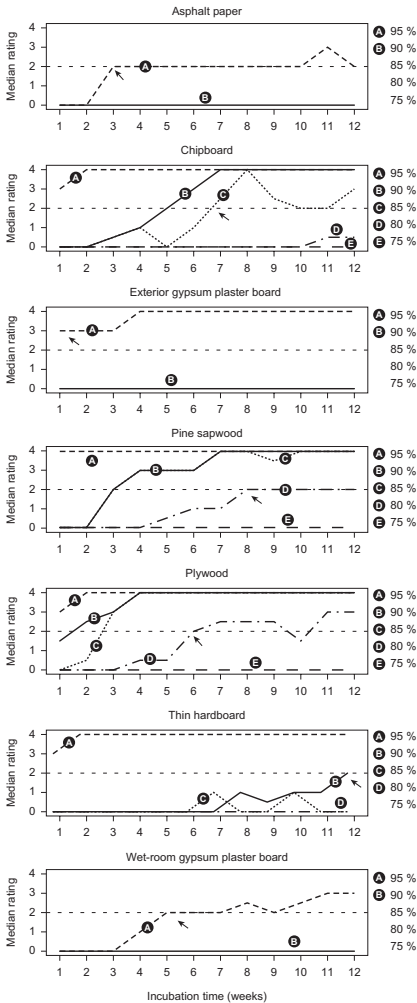
Fig. 3. Median value of mould growth on test pieces ($n = 6$) of building materials at different RH at 10 °C during 12 weeks. The critical moisture limit is reached when the median ≥ 2, represented as a horizontal dotted line. The arrow indicates the point when this is reached.
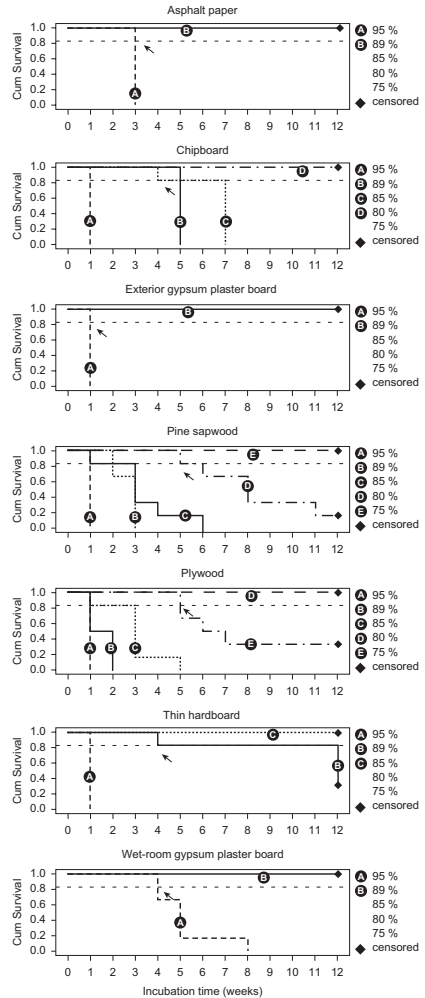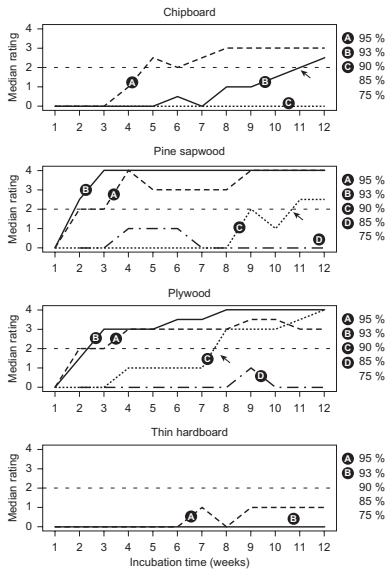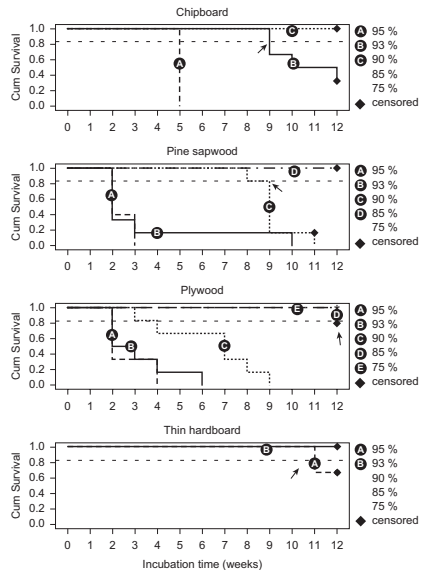

Fig. 4. Survival functions of mould growth on test pieces ($n = 6$) of building materials at different RH at 10 °C during 12 weeks. The critical moisture limit is reached when at least one of the test pieces reaches mould growth ≥ 2, represented as a horizontal dotted line. The arrow indicates the point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

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

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

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

explanation is that the individual fungal species in the spore suspension differ in their ability to germinate and grow at different temperatures, and that these species differ regarding growth rate.

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

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

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

[a] This is based on the criterion (b).
[b] When using the criterion (a) concerning median rating ≥ 2, the result was $85 < RH_{cr12w} \leq 90$.
[c] When using the criterion (a) concerning median rating ≥ 2, the result was $95 < RH_{cr12w}$.
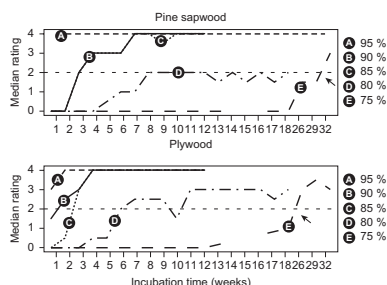
Fig. 5. Median value of mould growth on test pieces ($n = 6$) of building materials at different RH at 22 °C when incubation was extended to more than 12 weeks. The critical moisture limit is reached when the median ≥ 2, represented as a horizontal dotted line. The arrow indicates the point when this is reached.

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

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

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

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

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

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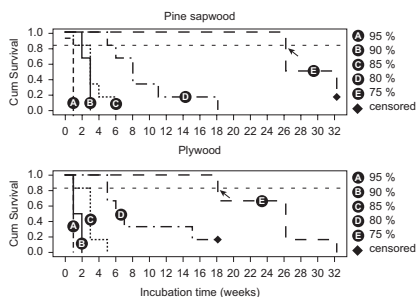
a theoretical limit for how much growth is acceptable. This threshold is influenced by where in the building growth can be found, which reflects the risk of affecting the indoor environment. No consensus currently exists on how much mould growth should be allowed and still considered acceptable. In this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

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

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

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

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

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

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

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

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

5. Conclusions

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

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

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

Acknowledgements

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

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

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Validation of critical moisture conditions for mould growth on building materials

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Validation of critical moisture conditions for mould growth on building materials

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ABSTRACT

Materials that are stored or used in damp conditions 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 tests, at constant temperatures and relative humidity (RH) favourable to mould growth. Within a building however, these parameters are expected to vary from one part of the construction to another, and are seldom constant; there is fluctuation in temperature and RH 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 temperature and RH varied, 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 temperature and RH. When the actual conditions of RH and temperature exceeded these 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 serve well to indicate mould growth within a building construction.

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1. Introduction

Different parts of a building are exposed to different temperatures and relative humidity (RH), e.g. as a result of construction design and moisture produced by human activity. Materials used in construction are affected by the ambient conditions, and if these are 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 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 as isopleths, e.g. [1–3]. Some of these isopleths have been adapted to predict the risk of mould growth on building materials [4–6]. By calculating the expected conditions of a construction or part of a building, these limiting growth curves may be used to assess the risk of mould growth. These curves should also be based on critical moisture values and be material specific, since the value for one material cannot be used to predict the properties of another, although they may seem similar [7]. Also, different treatments of a material may alter resistance to mould growth [8].

Although there are optimal and minimal growth conditions for the different microfungi that are described as mould fungi, the organisms can survive periods of unfavourable conditions. How well they can tolerate fluctuating periods varies from species to species [9]. On building materials, the rate and extension of mould growth have been shown to be lower when favourable conditions alternate with less favourable [10]. In addition, how long these periods last is also of importance. In constructions, the conditions that building materials are exposed to are seldom constant; there are variations in both temperature and RH. These variations can be long-term, such as seasonal variation, or shorter-term, due to human activity or local climatic conditions, for example. Therefore, the critical moisture level for a building material may be exceeded

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for a shorter or a longer period, while at other times the level may not favour mould growth. It is therefore expected that the risk of mould growth occurring is low if the variation is such that the moisture levels are substantially lower than the critical limits over a sufficiently long period and the critical limits are exceeded only for short periods of time.

Ventilated crawl spaces and attics are constructions where the temperature and RH are predominantly governed by outdoor conditions. There is therefore seasonal fluctuation in these structures. In Scandinavia, the RH 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 RH will increase. In outdoor ventilated attics, however, the RH is usually at its highest during the winter months. There is also short-term variation, e.g. during clear nights when heat radiates from the roof to the sky, leading to a reduced temperature at the interior surface of the roof, which in turn enhances the risk of condensation and mould growth. In both crawl spaces and ventilated attics, there is often extensive mould growth on the building materials e.g. [11–13].

The purpose of this study was to investigate whether results from laboratory testing of materials for critical moisture levels in which constant conditions are maintained can predict mould growth in constructions where the temperature and RH fluctuate. The same materials that were tested in a laboratory environment [7] 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. Mould growth limit curves for the materials tested were produced based on known critical moisture levels and risk for mould growth in each construction were then expected if the RH and temperature exceeded these. Expected and observed growth was then compared.

Not all materials used in the study were intended to be used in crawl spaces and/or attics, but because the temperature and RH conditions of these constructions were expected to favour mould growth and the conditions expected to fluctuate, they were considered to be suitable test environments. In addition, the materials were readily available and 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 purchased from a local building supply store. The materials are commonly used in Swedish building construction sector, and have different critical moisture limits, as determined in an earlier laboratory study involving the same materials [7]. One material was asphalt paper, and the remaining eight were boards. Three individual boards of each type were used, and from each a single test piece, 50×100 mm, was prepared for exposure at each test site. For plywood and chipboard, an additional test specimen was prepared from two of the boards; consequently, there were five replicates of those materials. From the asphalt paper, all test pieces were prepared from one roll, and three pieces were placed at each test site. The choice of the number of test pieces was based on the number that are common in standardised methods for testing mould resistance of materials.

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 easily dismantled from the clips for the analysis of microbial growth. All materials were handled in such a way (e.g. using plastic gloves) as to minimise risk of contamination that could have led to mould growth.

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 structures 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 constructions at both high and low risk of mould growth.

2.2.1. House A: attic 1 and crawl space 1

This house, the oldest in the study, was built in 1923. It 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. The roof material (from the outside inwards) comprised concrete tiles, thin hardboard and wooden rafters. The foundations were of stone; the floor of the crawl space was of soil and stone, and the height about 1 m. The joists were insulated with approx. 10 cm of wooden chips.

2.2.2. House B: attic 2 and crawl space 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

Table 1
Building materials used in the study.

Building material	Description	Range in which the critical moisture level is expected (Johansson et al., unpublished results)	
		22 °C	10 °C
Pine sapwood	19 mm tongued and grooved board	$75 < RH_{crit} \leq 79$	$85 < RH_{crit} \leq 90$
Plywood	12 mm softwood plywood	$75 < RH_{crit} \leq 79$	$85 < RH_{crit} \leq 90$
Chipboard	12 mm particle board	$79 < RH_{crit} \leq 85$	$90 < RH_{crit} \leq 93$
Thin hardboard	3.2 mm high density hardboard made of wood fibres and lignin	$85 < RH_{crit} \leq 89$	$93 < RH_{crit} \leq 95$
Wet-room gypsum plaster	13 mm gypsum board with cardboard surfaces	$89 < RH_{crit} \leq 95$	$95 < RH_{crit}$
Exterior gypsum plaster	13 mm gypsum board with cardboard surfaces	$89 < RH_{crit} \leq 95$	$95 < RH_{crit}$
Asphalt paper	1.5 mm windproof barrier of asphalt-impregnated cellulose paper	$89 < RH_{crit} \leq 95$	$95 < RH_{crit}$
Cement-based board	8 mm cement based board consisting of cement, limestone and cellulose fibres, covered with a plastic dispersion	$95 < RH_{crit}$	$95 < RH_{crit}$
Glassfibre board	15 mm rigid glass wool insulation board	$95 < RH_{crit}$	$95 < RH_{crit}$
Expanded polystyrene board	50 mm expanded polystyrene insulation board	$95 < RH_{crit}$	$95 < RH_{crit}$

space under two-thirds of the house. The remainder consisted of laundry room and boiler room. Heating was by pellet boiler. The foundations were of stone; the floor of the crawl space was of soil and stone; and the height about 0.5 m. The joists were insulated with approx. 10 cm of wooden chips.

2.2.3. House C: crawl space 3

This house was built around 1980. There was a crawl space under two-thirds of the house. The remaining one-third consisted of a cellar including laundry and boiler room. The house was heated from a wood furnace in this basement. The crawl space section, where the test was carried out, was ventilated with outdoor air and unheated. The foundations were of concrete blocks, and the floor of the crawl space was crushed rock/gravel. The joists were insulated with mineral wool insulation.

2.2.4. House D: attic 3

This 1½-storey house was built in 1982 and had 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 investigation, fires were frequently lit in a centrally located wood-burning stove. The roof was built, from the outside inwards, of concrete tiles, roofing felt, roof trusses and hard fibre board.

2.3. Measurement of temperature and RH at the test-sites

Temperature and RH at each test-site were registered every fourth hour by a single data logger with internal sensors (Testo 177-H1). Each logger was placed in close proximity to the specimens to ensure that the conditions logged were as close as possible to those that specimens were exposed to.

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%. After exposure in the field, a new calibration was performed in moisture chambers with a calibrated reference moisture and humidity sensor at temperatures of 22 °C and 15 °C and RH of 90%, 85% and 60%. The calibration made it possible to adjust the measured RH values with the correction factor from calibration (reference minus measured RH). However, since this factor was expected to be dependent on temperature [14], and the temperature and RH were not constant in the field measurements, the second calibration procedure made it possible to apply a more accurate adjustment of data. A multiple regression was performed in which both temperature and RH were included. Each sensor was then given an equation which was used to adjust the measured values.

Comparing the results of RH from the initial and final calibrations, it was concluded that all sensors had drifted, to various extents, so that they showed higher values after exposure than 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 between the calibration errors before and after measurement in the field. By dividing this difference by the total number of times measurements were taken (which was about 5500, i.e. six times each day for 2.5 years), we obtained a value for the drift at each logged time. We called this the "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.

$$RH_{adjusted} = a_i + b_i * RH_{instrument} + c_i * t_{instrument} - \gamma * T \ [\%] \qquad (1)$$

where

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

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. The uncertainty of drift can be considered negligible since it was very small in comparison.

2.4. Analysis of mould growth on test pieces

Every April and October, i.e. five occasions in all, the test pieces were removed from the racks where they were exposed; they were then analysed for mould growth. The surface that had been exposed to the open air in the attics and crawl spaces was examined at $10\times–40\times$ magnification under the microscope. Both mould growth visible to the naked eye and that which was only visible under the microscope 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 most or all of the surface. A test piece was considered to have failed when the rating of mould growth first reached 2 or higher, we define this as when mould growth was clearly established [7].

2.5. Description of mould growth with time

The percentage of test pieces that were not failed (3.4) at each analysis time was expressed as Kaplan–Meier curves. This is an approach that has many advantages [15]. On each occasion that a test piece failed, the percentage of test pieces without no mould growth decreased.

2.6. Creation of mould growth limit curves

To create growth limit curves for each material, we used the same technique as Hofbauer et al. [16], where material-specific isopleths were constructed from the closest approximation to LIM 0 curve for mycelial growth [5].

As the equation for the LIM 0 curve had not been published at the time of our study, we carried out curve fitting to obtain parameters for such an equation. We then fitted 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. In order to be able to get such an equation, we fixed a function minimum at a defined temperature. We chose 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 [3]. Above and below 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 according to Sedlbauer [5] lies between 22° and 30 °C, with a mean value of 27 °C. However, testing with different values of min temperature revealed that the model was not sensitive for

which value between 25 °C and 30 °C that were used in the temperature range between 0 °C and 30 °C.

The resulting growth limit curve model can then be described by Equation (2):

$$RH = a + c\left(t^2 - 54*t\right) [\%] \tag{2}$$

where t is the temperature in °C.

The parameters c and a were estimated by using Equations (3) and Equation (4), where the data for RFcrit and corresponding temperature come from the results in the laboratory tests [7], Table 1:

$$c = (RH_{crit1} - RH_{crit2})/\left(t_1^2 - t_2^2 - 54(t_1 - t_2)\right) \tag{3}$$

$$a = RH_{crit1} - c\left(t_1^2 - 54*t_1\right) \tag{4}$$

Two growth limit curves, one upper and one lower, were produced for each material. The laboratory tests were carried out in test chambers with constant RH, with RH set at intervals of 5%; therefore the critical moisture level fell within a range. The upper limit was determined by the case with lowest RH where any of the above criteria were met, and the lower limit by the case with the next-lowest RH. The actual critical moisture level may therefore lie between the two growth limit curves, or above the upper curve [16].

2.7. Comparison of results from laboratory and field studies

In order to find out if the conditions in the test-sites had exceeded the growth limit curves, these were drawn into plots of monitored temperature and RH. Mould growth was then expected when the combination of temperature and RH exceeded the growth limit curves [4]. This expected growth was then compared to the results from the assessment of mould growth on the test pieces at each test site.

Fig. 1. Monitored relative humidity and temperature in three crawl spaces in Sweden. Values marked "RH$_{min}$" refer to data where the drift of the loggers was such that the correct value could not be estimated. The specified dates refer to when microbiological analyses were performed.
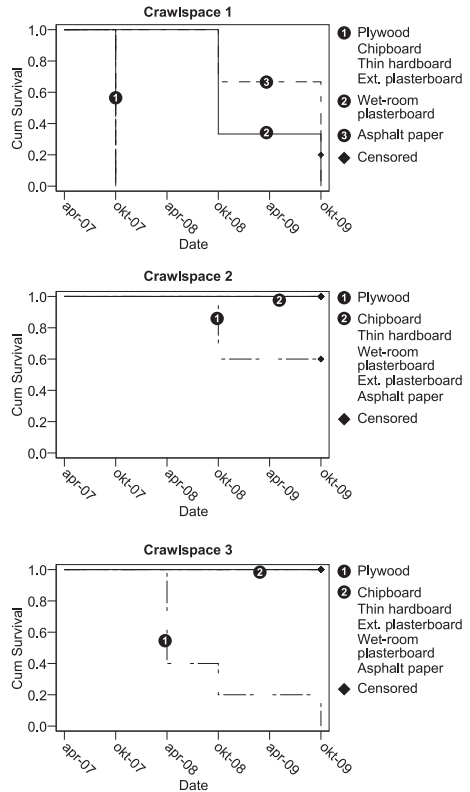
Fig. 2. Proportion of test pieces of six building materials, exposed to conditions in three crawl spaces, with no established growth at different times of analysis.

In those cases where expected and observed mould growth was 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 temperature and RH 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.

2.8. Prediction of critical moisture levels for different temperatures

Testing to find the critical moisture level must be carried out at several RHs, and, to predict the risk at different temperatures according to Section 2.6 above, at two temperatures, at least. 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, the parameter or c needs to be known. Based on the values of a that were estimated for each material in the study, an average was therefore calculated and then used to calculate parameter c for each material. New growth limit curves could then 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 temperature and RH measurements from April 2007 to October 2009 are presented in Figs. 1 and 3. Variation during the day was greatest in the attics, with the RH being 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. Measurement uncertainty for RH was maximum 2% for each location.

Mould grew on several of the test pieces. However, among the sites, we observed differences in which materials became mouldy, how quickly growth arose and the extent of growth at test pieces at each site. Figs. 2 and 4 present the percentage of test pieces that had not failed, that is had no established mould growth on them, at each analysis time. Pieces that did not fail during the entire 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.

Figs. 5 and 6 show the limit curves for mould growth for each material, together with the measured data of temperature and RH for each test site. Measured RH is plotted against measured temperature, each point corresponding to one measurement. If points were above the limit lines, growth was 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 tests in
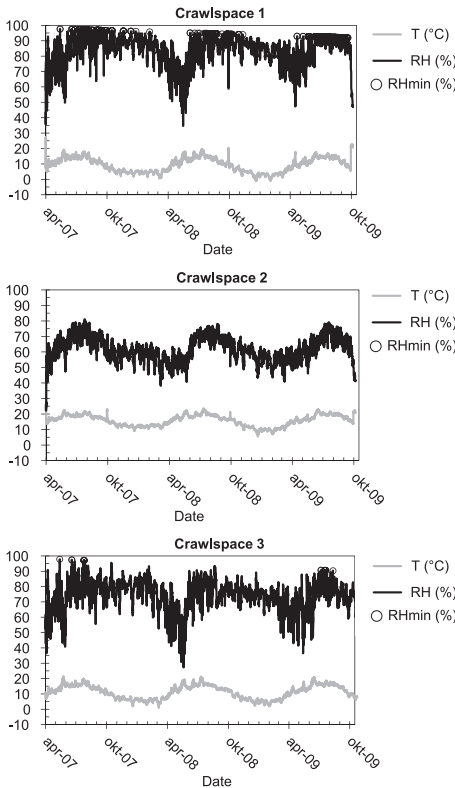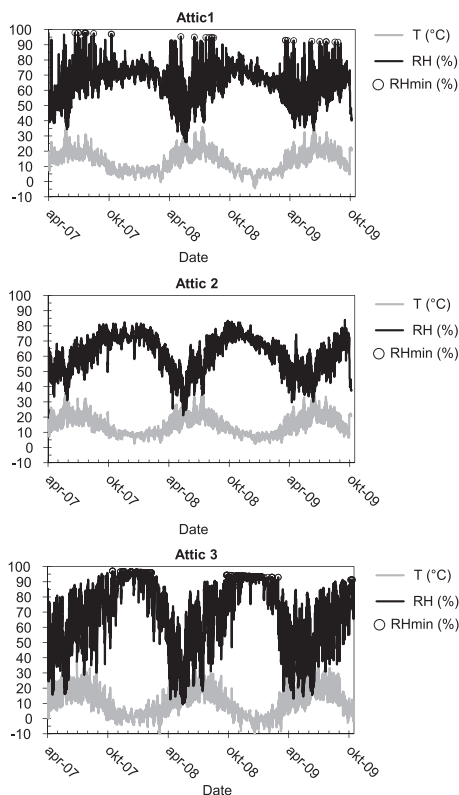

Fig. 3. Monitored relative humidity and temperature in three attics in Sweden. Values marked "RH$_{min}$" refer to data where the drift of the loggers was such that the correct value could not be estimated. The specified dates refer to when microbiological analyses were performed.
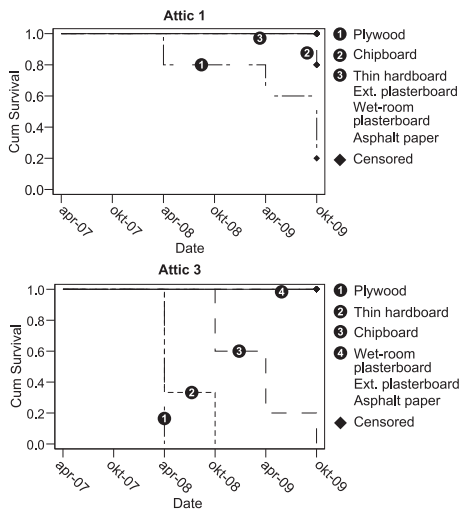
Fig. 4. Proportion of test pieces of six building materials, exposed to conditions in three attics, with no established growth at different times of analysis. In Attic 2 there was no growth on any of the test specimens during the test period.

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.

	Chipboard (N = 5)	Plywood (N = 5)	Thin hardboard (N = 3)	Wet room plaster board (N = 3)	Exterior plaster board (N = 3)	Expanded poly-styrene (EPS) board (N = 3)	Asphalt paper (N = 3)	Glass fibre board (N = 3)	Cement based board (N = 3)
Crawl space 1	5	5	3	3	3	0	3	0	0
Crawl space 2	0	2	0	0	0	0	0	0	0
Crawl space 3	0	5	0	0	0	0	0	0	0
Attic 1	1	4	0	0	0	0	0	0	0
Attic 2	0	0	0	0	0	0	0	0	0
Attic 3	5	5	3	0	0	0	0	0	0
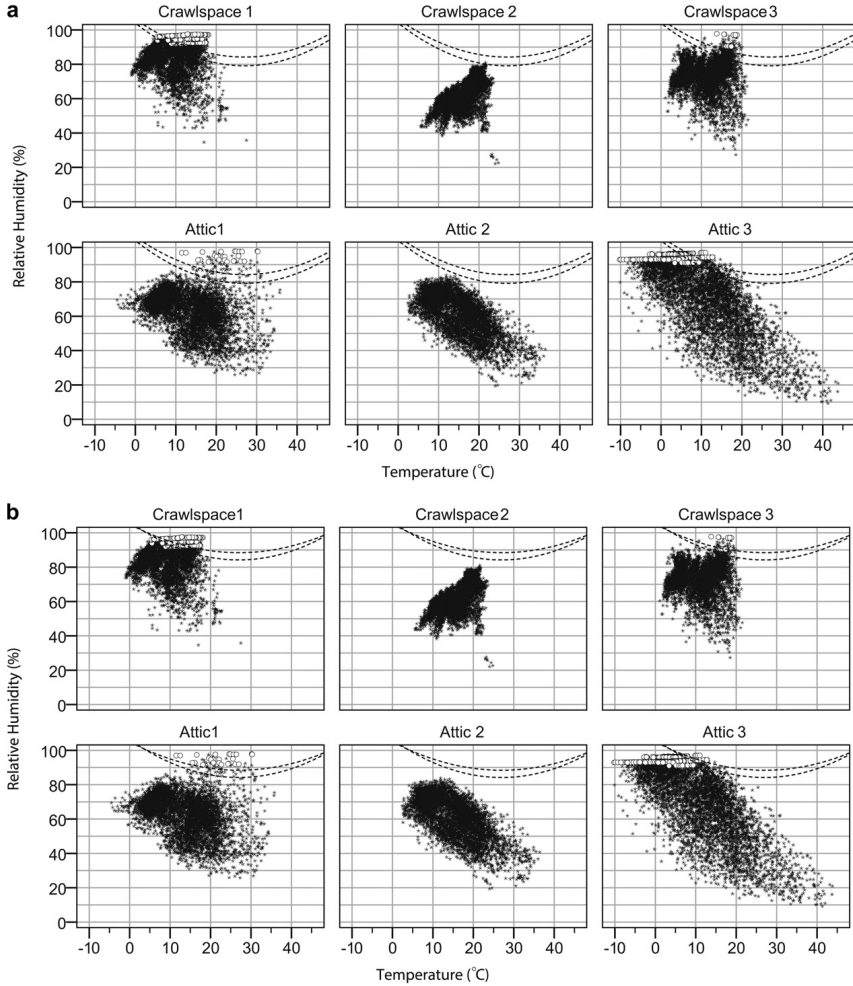
Fig. 5. Growth limit curves for (a) chipboard and (b) thin hardboard and measured temperature and relative humidity in three crawl spaces and three attics over 2.5 years. Each dot represents one measuring point. The circles indicate values where drift of the loggers made it impossible to calculate a "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.
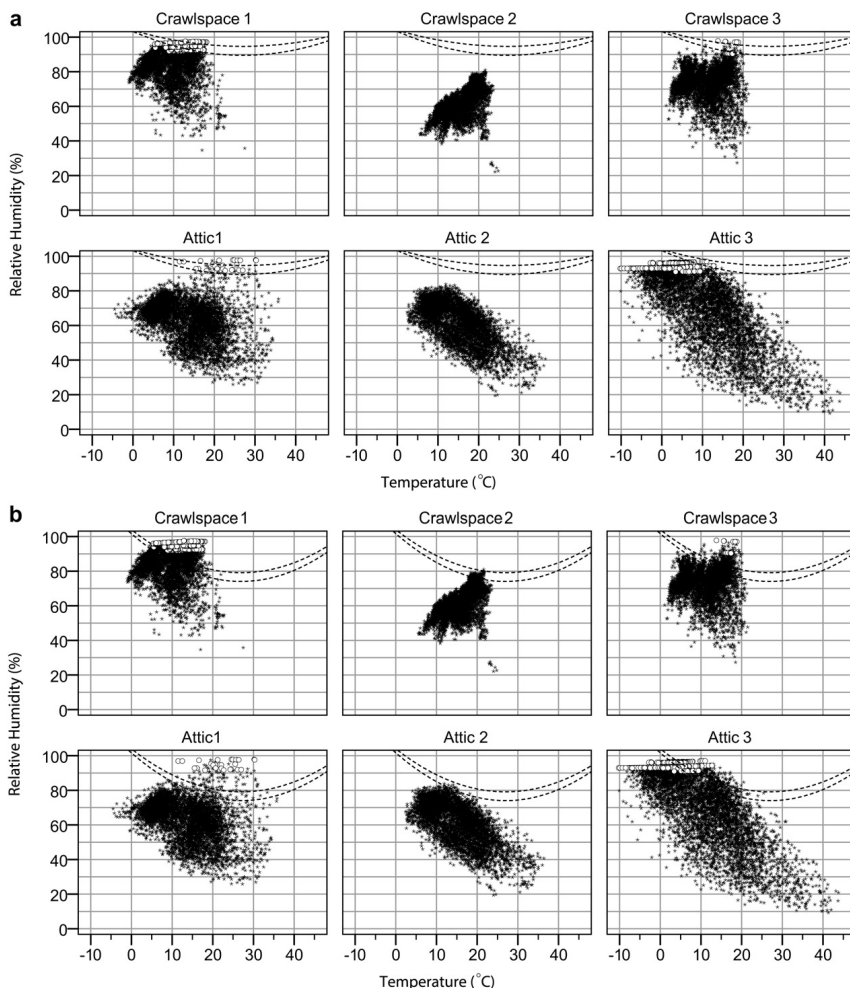
Fig. 6. Growth limit curves for (a) asphalt paper and two types of plaster boards and (b) plywood and measured temperature and relative humidity in three crawl spaces and three attics over 2.5 years. Each dot represents one measuring point. The circles indicate values where drift of the loggers made 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.

the laboratory and the critical moisture level could not be estimated and/or the materials were resistant to mould growth.

Table 4 shows the total time that the temperature and RH were over the limit curves for those cases where expected and actual mould growth were not consistent; it also shows 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.

There was consistency between actual and expected mould growth when both criteria — (a) conditions exceeding the limits for

growth and (b) cumulative time over the limits being lower than the time before mould growth were established in the laboratory — were considered.

From the laboratory tests, six mould growth curves (see Section 2.5) were calculated. The upper curve of one material was sometimes the same as the lower curve of another. For the nine materials tested, there were therefore a total of six such limit curves, with values of a ranging from 102 to 108, with a mean of 105. New growth curves with this value were produced and compared to those produced with individual values. Fig. 7 shows an example of

Table 3
Expected and actual mould growth for the materials at each test site. If values of RH-temperature in Figs. 5 and 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.

	Crawl space 1		Crawl space 2		Crawl space 3		Attic 1		Attic 2		Attic 3	
	Expected	Existing	Expected	Existing	Expected	Existing	Expected	Existing	Expected	Existing	Expected	Existing
Plywood	+	+	+	+	+	+	+	+	+	−	+	+
Chipboard	+	+	−	−	+	−	+	+	−	−	+	+
Thin hardboard	+	+	−	−	+	−	+	−	−	−	+	+
Exterior gypsum paper board	+	+	−	−	+	−	+	−	−	−	+	−
Wet room gypsum paper board	+	+	−	−	+	−	+	−	−	−	+	−
Asphalt paper	+	+	−	−	+	−	+	−	−	−	+	−
Cement-based board	−[a]	−	−[a]	−	−[a]	−	−[a]	−	−[a]	−	−[a]	−
Glassfibre board	−[a]	−	−[a]	−	−[a]	−	−[a]	−	−[a]	−	−[a]	−
Expanded polystyrene board	−[a]	−	−[a]	−	−[a]	−	−[a]	−	−[a]	−	−[a]	−

[a] This is based on the findings that there was no mould growth on any of the test pieces in the laboratory.

growth limit curves for the value estimated for the relevant material together with a curve were the value of *a* was 105. The maximum difference between these two curves, in this case for chipboard, is two percentage points for RH in the 0°−40 °C interval, and one percentage point in the 10°−22 °C interval. These values are similar for all materials tested.

4. 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 temperature and relative humidity conditions exceeded the limits for growth, then mould growth was expected. Length of time above the critical level is also significant. When both of these criteria were taken into account, the consistency between expected and actual mould growth was good.

The number of test pieces of each material at each test site was relatively low (three to five). After the start of the study, we found that in order to obtain a sufficient level of confidence in the assessment, the appropriate number of test pieces for assessment of mould growth was at least seven [7]. 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 exposure for 2.5 years, however, little variation was found; 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). The number of test pieces was too small to make a correct Kaplan−Meier estimation; Figs. 2 and 4 therefore should be considered empirical data.

In Crawl space 1, the values for temperature and RH exceeded all growth limit curves on several occasions, and on all materials there

was mould growth. Even in Attic 3, measured points were repeatedly above all curves. Established growth was there found on plywood, chipboard and thin hardboard. The reason that mould did not grow on asphalt paper or the two types of plaster board 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 Crawl space 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 temperature and RH 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 Crawl space 3 or on plywood and chipboard in Attic 1. However, the values for chipboard in Crawl space 3 are on the boundary of the reference time from the laboratory tests.

In Crawl space 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 conditions 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 conditions that was favourable is a simplified method of considering the conditions that affect

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.

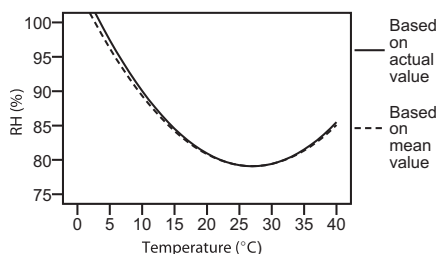|	Crawl space 3		Attic 1		Attic 2		Attic 3		Time (weeks) in the laboratory before critical moisture level was reached	
	Time (weeks) over lower curve	Times (weeks) over upper curve	Time (weeks) over lower curve	Weeks (weeks) over upper curve	Time (weeks) over lower curve	Weeks (weeks) over upper curve	Time (weeks) over lower curve	Weeks (weeks) over upper curve	10 °C	22 °C
Plywood					0.3	0.0			11−12	4−5
Chipboard	8.3	3.0							8−9	3−4
Thin hardboard	3.0	0.9	1.9	1.4					10−11	3−4
Exterior gypsum board	0.9	0.3	1.4	0.3			0.4	0.0	12	0−1
Wet room gypsum paper board	0.9	0.3	1.4	0.3			0.4	0.0	>12	3−4
Asphalt paper	0.9	0.3	1.4	0.3			0.4	0.0	>12	2−3

Fig. 7. Comparison between growth limit curves based on actual values of parameter *a* for chipboard and based on values of parameter a calculated from the mean value of parameters a from all materials tested.

mould growth. These favourable conditions were followed by less favourable. How long they last, how far they are from the limits, and how rapid the changes between favourable and unfavourable conditions are affects the risk that mould growth will occur, as well as its extent over the surface and its growth rate [10,17,18]. This may explain why mould did not grow on chipboard in Crawl space 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 from laboratory tests on the material 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. The testing here was conducted over 2.5 years, but it is possible that testing for a longer time would increase the chance of mould developing (even on the material where no growth was observed in this study), since the cumulative time during which favourable conditions were present would be longer. Also, a material age with time, and contamination of the material can reduce resistance to mould growth [19,20].

Assessing the critical moisture level of a material requires testing in a number of temperature and RH conditions. The test period and the number of test conditions must be limited for a commercial method to be economically justifiable and the results delivered within a reasonable 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 with 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. Conclusions

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 temperature and RH 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; the results will instead include a certain margin of safety.

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

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Determination of critical moisture level for mould growth on building materials (Laboratory method)

Pernilla Johansson, Annika Ekstrand-Tobin and Gunilla Bok (2013)

SP Method 4927:2013 (Ver. 2)

Determination of critical moisture level for mould growth on building materials (Laboratory method)

Bestämning av kritiskt fukttillstånd för påväxt av mögel på byggnadsmaterial (Laboratoriemetod)

Contents

Introduction

Building materials that are stored or used in damp environments 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% relative humidity. In a building, different building parts are exposed to various humidity and temperature conditions. To minimize the risk of microbial growth, materials should be chosen and handled in a way so they can tolerate the conditions in question.

This SP Method 4927 describes a method for determining the critical moisture level of mould growth on the surface of a building material. The tests are performed during constant incubation conditions; at 22 °C and 80% RH, 85% RH, 90% RH and 95% RH. The results can be used to estimate critical moisture level at other temperatures than tested.

When designing this method a number of standardized methods were taken into consideration [1], [2], [3], [4], [5], [6], [7] together with data and practical experience from several Swedish research projects [8], [9] and [10]. The method provides conditions for good repeatability by the requirements which describe technical equipment, incubation and material handling.

1   Scope

SP Method 4927 describes a laboratory test method for determining critical moisture levels for mould growth on building materials. It is applicable for clean materials that have 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, e.g. chemical treatments.

SP Method 4927 is not applicable for prediction of how long a material may be exposed beyond the time tested in the laboratory without risk of mould growth, as relative humidity and temperature in buildings are fluctuating, which affects the mould growth rate.

2   Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EA-4/02 M: 1999, *Expression of the Uncertainty of Measurement in Calibration. Guidance document to ISO/IEC 17025 and ISO 15189. Application and Technical/Advisory documents,* European co-operation for Accreditation, www.european-accreditation.org

SS-ISO 3696:1987, *Water for analytical laboratory use – Specification and test methods*

3 Terms and definitions

For the purposes of this Technical Specification, the following terms and definitions apply:

3.1 Expanded uncertainty

The expanded uncertainty in this standard is a quantity defining an interval about the results of measurements that can be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurands [11]. In this test method the measurands are temperature and relative humidity.

3.2 RH

The relative humidity is a measure of the amount of water vapour in the air (at a specific temperature) compared to the maximum amount of water vapour air could hold at that temperature and is given as a percentage value.

3.3 RH$_{crit}$

The critical moisture level RH$_{crit}$ (%) is defined as the lowest RH at which mould growth appears on a material at a certain temperature.

4 Principle

Specimens of the material to be tested are be inoculated with a suspension of a mixture of spores from each of six species of mould fungi and are then incubated in test chambers at specified temperature and relative humidity conditions during 12 weeks. At defined time intervals, the specimens are inspected for fungal growth at a magnification of 40x. The fungal growth is assessed according to a rating scale. The critical moisture level for mould growth is determined by considering the minimum tested relative humidity at which established growth of mould is detected during the test period.

5 Apparatus

5.1 General

All apparatus shall be serviced and calibrated at regular intervals.

5.2 Test chambers

Incubation chambers that can provide the required conditions shall be used. They shall maintain temperature and humidity with good homogeneity in all parts of the chambers. The fluctuations shall not be larger than ±2 °C and ±2.5% RH during operation. The required conditions shall be restored within two hours after opening and closing of the chamber.

A test chamber with salt solutions shall not be used as it may affect the mould growth, since the salt can migrate.

NOTE   Lighting in the chambers is not required.

5.3   Device for recording incubation conditions

Each test chamber shall be equipped with a separate transmitter with a data logger for recording temperature and relative humidity.

The transmitter shall be calibrated at least once a year if continuously used. Otherwise it shall be calibrated prior to the time of use.

The expanded uncertainty of measurement of the relative humidity and temperature shall be considered according to 10.8 and Annex B.

5.4   Inoculation device

An airbrush connected to a compressor, or to small cans with compressed air, shall be used to spray the spore suspension onto the surface of the specimens to be tested. A filter or regulator that removes moisture from the compressed air should be connected to the compressor.

NOTE   The most effective way is to use an airbrush where the cup for the liquid is open and placed directly on the airbrush.

5.5   Stereo microscope

A stereo microscope with magnification of 40x shall be used in all analyses. An external, adjustable light source shall be used to illuminate the whole surface of the specimen at a low angle.

5.6   Laminar air flow bench

A laminar air flow bench shall be used to provide biological safety throughout the process of making the spore suspension.

NOTE   A continuous unidirectional horizontal air flow prevents contamination from operator and the environment.

5.7   Autoclave

An autoclave shall be used to sterilize reagents, materials and other utensils for the test.

5.8   Centrifuge

A centrifuge shall be used for separating spores from the water solution when cleaning the spores in the process of preparing the spore suspension.

6   Reagents and laboratory material

6.1   Purity of water

The water used shall be according to the classification grade 3 in SS-ISO 3696:1987.

6.2   Nutrient media

6.2.1   Malt extract agar

A minimum of 25 Petri dishes shall be prepared for cultivation of fungi and for viability controls. The culture medium shall be a malt agar medium with the following recipe:

20 g Agar agar (purified and free of inhibitors) mixed with 20 g malt extract to 1000 ml water and autoclaved at 121 °C for 15 minutes.

6.2.2   Malt solution

A 250 ml malt solution shall be prepared for incubation environment control with the following recipe:

20 g malt extract per 1000 ml water, autoclaved at 121 °C for 15 minutes.

6.3   Test organisms

6.3.1   Test fungi

The test fungi in Table 1 shall be used.

Table 1 – Test fungi

Test fungus	CBS number
Eurotium herbariorum	115808
Aspergillus versicolor	117286
Penicillium chrysogenum	401.92
Aureobasidium pullulans	101160
Cladosporium sphaerospermum	122.63
Stachybotrys chartarum	109292

NOTE   CBS number refers to strains maintained by Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

6.3.2   Maintenance of test fungi

Subsequent inoculation from the original prepared cultivation of test fungi on new Petri dishes (see 10.2) shall be repeated no more than 12 consecutive months in order to avoid degeneration of the fungi. Each Petri dish with prepared stock culture shall not be kept for more than 4 months at 8 ± 2 °C before it is used in a test.

6.4   Laboratory material

6.4.1   Culture vessels

Petri dishes shall be used as culture vessels. They shall have a diameter about 90 mm and a height of about 15 mm.

6.4.2   Flasks

Six glass flasks, each with a volume of 250 ml, shall be used for the spore solution production process.

6.4.3   Glass beads

About 100 cl of glass beads, about 5 mm in diameter, shall be used in the spore solution production process.

6.4.4   Control glass microfiber filters

Glass fibre filters, binder-free and not containing any cellulose, 55 mm in diameter shall be used for controls.

6.4.5   Glass funnels

Six glass funnels shall be used in the spore solution production process.

6.4.6   Washed glass wool

Washed glass wool for laboratory use shall be used as a filter in the glass funnels.

6.4.7   Counting chamber

A counting chamber or equivalent equipment shall be used when calculating the concentration of spores in the suspension. Handling and calculation shall be done according to the manufacturer's instructions.

6.4.8   Burner

A burner or a spirit lamp shall be used to sterilize the preparation needle during the process of preparing the spores suspension.

6.4.9   Additional supplies for production of spore solution

Additional supplies that shall be used for the production of spore solution are: Automatic pipettes, pipette tips, forceps, inoculation needle or spreader, centrifuge tubes, Petri dishes, stainless steel mesh, latex or plastic gloves, face masks.

7   Test specimens

7.1   General

To minimize organic contamination, all handling of the material to be tested shall be carried out by using clean gloves and clean tools.

7.2   Preparation of test specimens

The material selected for the test should be representative, clean and previously unused material. Preferably, the material should originate from different batches in the production. This is particularly important if the quality is expected to vary between batches.

Prepare seven specimens of the material for each of the four incubation sets. Each specimen should be about 50 mm x 100 mm. If the two surfaces of the test material have had different treatments, consists of different materials or structures, duplicate specimen 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 shall be placed in an autoclaved cage of fine stainless steel mesh with an internal volume of approximately 50 ml. The density in the cage should be according to the stipulated density for the integrated product or the density recommended in any applied standard.

NOTE   The method does not require pre-conditioning of the test specimens. It has been validated in separate experiments for different building materials that the initial moisture content of test specimens has no impact on the critical moisture level assessed after 12 weeks [8] when using test chambers with the specified properties in section 5.

7.3   Reference specimens

When evaluating the efficiency of a treatment, seven untreated specimens of the material for each of the four incubation sets shall be prepared from the same material and used as references.

NOTE   A treatment such as a detergent, a coating or added fungicide.

8 Procedure

8.1 General

To ensure aseptic conditions during the preparation of spore suspension and inoculation of test specimens, all equipment shall be sterilized before use. Perform the work with clean gloves in a laminar air flow bench. The relevant procedures for good biological practise in [7] should be applied.

8.2 Cultivation of test fungi

Prepare strains according to instructions from the supplier. Cultivate each species on separate Petri dishes with malt agar until heavy sporulation has been developed. Usually this will occur within four weeks. Prepare a minimum three Petri dishes for each strain. Previously prepared cultures may be used according to 6.3.2.

8.3 Preparation of spore suspension

The spore suspension shall be inoculated on the test specimens immediately after the production or within 12 hours after preparation. The following steps shall be performed in successive order:

a) Prepare six flasks with 35 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, i.e. that each Petri dish contains the correct mono-culture.

d) Pour 10 ml of distilled, autoclaved water onto each of the Petri dishes with one of the subcultures and scrape the surface of the fungal colonies gently and carefully with a clean inoculation spreader or inoculation needle. Repeat for each subculture.

e) Pour the liquids 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.

h) Pour off the supernatant, fill the tubes with new water and centrifuge in the same manner as before. Repeat this washing procedure after the first centrifugation 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.

i) Determine the spore concentration in the final washed residue re-suspended in about 20 ml sterile deionized water for each species using a counting chamber under the microscope. Adjust the spore concentration with sterile deionized water to approximately 1×10^6 spores per ml.

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

8.4   Inoculation

8.4.1   Inoculation of test specimens

Spray 0.4 ml of the spore suspension onto one surface of each test specimen by using an airbrush (see 5.3). During spraying, sweep the airbrush along at an even speed so the spores become evenly distributed over the surface.

8.4.2   Inoculation of positive control #1 – Viability of spore suspension

In order to check the viability of the spore suspension and each species of fungi, inoculate separate Petri dishes (see 6.2.1) with one or two droplets of each of the spore suspensions from the individual species. Spray 0.4 ml of the mixed spore suspension onto one Petri dish with malt agar (see 6.2.1). Incubate the Petri dishes, with lid on, in room temperature or higher, maximum 30°C.

8.4.3   Inoculation of positive control #2 – Test chamber environment

In order to check the test chamber environment in each cabinet, dip four glass microfiber filters (see 6.4.4) into malt solution (see 6.2.2) and allow them to dry. Then spray 0.2 ml of the spore suspension onto each filter paper and place in separate, empty, Petri dishes, with the lid off. Incubate one filter together with inoculated test specimens in each test chamber.

NOTE   A second positive control (without lid) may be placed in each test chamber to check possible influence to the incubation environments from materials treated with volatile inhibitors (see 9.2). The double positive controls should have equal growth rate.

8.4.4 Inoculation of negative control – Nutrient purity of spore suspension

In order to check the nutrient purity of the spore suspension, inoculate one sterile microfiber filter (see 6.4.4) by spraying with 0.2 ml of the spore suspension. Place it in an empty sterile Petri dish and incubate in the test chamber, with the lid off, at 95% RH and 22 °C.

8.5 Incubation

The test shall be performed at 22 °C and for in total 12 weeks at four levels of relative humidity: 80% RH, 85% RH, 90% RH and 95% RH. The temperature and relative humidity shall be recorded every 10 minutes during the test period. Evaluate the incubation conditions throughout the test according to 8.8.

The test shall be performed in one of the following ways: by starting all four tests at different levels of humidity using the same spore solution to all specimens and separate incubation cabinets or by starting each case of RH condition successively in the same incubation cabinet. Consequently in the latter case: each start shall then include a freshly produced spore solution. All test specimens shall originate from the same material in order to be comparable.

Following inoculation, place the test specimens horizontally on racks (cleaned with 70% ethanol) in the test chambers, sprayed surface up. Incubate in the dark in the test chambers for 12 weeks.

8.6 Assessment of test

8.6.1 Examination of test specimens

Every 14 days, replace the test specimens from the test chambers and analyse for mould growth with a microscope at 40x magnification (see 5.4). Mould growth on the inoculated surface of each test specimen, excluding the surfaces 0 mm to 5 mm from the edges, shall then be assessed according to the rating scale shown in Table 2. During this procedure, low-angle light shall be used to detect hyaline as well as pigmented hyphae.

If there is mould growth only on the edges of the test specimen at the end of the test period, this shall be noted.

NOTE 1   Contamination from operator and the environment during examination can be prevented by providing a working surface with exhaust ventilation or in a biological safety chamber, Biosafety level 1 [13].

NOTE 2   To streamline the analysis and limit its cost, the scheme described in Annex A may be used.

Table 2 - Assessment scale of mould growth extent

Rating	Description of extent of growth
0	No mould growth.
1	Initial growth: One to a few hyphae with none to a few branches, scattered on the surface, no conidiophores present.
2	Clearly established but sparse growth scattered on the surface: Branched hyphae occasionally with few conidiophores.
3	Patchy, heavy growth: Mycelia often with many well-developed conidiophores.
4	Heavy growth over more or less the entire surface.

8.6.2 Evaluation of incubation chamber conditions

The set points of each incubation condition of the test method are specified according to Table 3 below.

Table 3 – Set points for the incubation conditions

Incubation condition	Specified lower limit	Set point	Specified upper limit
T	20.0°C	22.0°C	24.0°C
RH 1	77.5%	80.0	82.5%
RH 2	82.5%	85.0	87.5%
RH 3	87.5%	90.0	92.5%
RH 4	92.5%	95.0	97.5%

In order to establish the specific critical moisture level of a material, it is important to be able to distinguish each of the four different incubation conditions at which the test are carried out. Therefore, the registrations of temperature and relative humidity for each incubation condition shall be sampled and calculations shall be produced according to Annex B. The following criteria shall be met throughout the test:

The mean value for all records of relative humidity and temperature together with the expanded uncertainty for each parameter (calculated in accordance with Annex B) shall not exceed the specified limits of incubation (see 8.6) including allowable fluctuations (see 5.1). See Figure 1.

Specified upper limit of incubation condition

(A) (B)
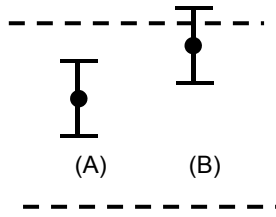
Specified lower limit of incubation condition

Figure 1 – Illustration of the evaluation of incubation criteria. The specified limits of incubation condition are described in Table 3. The dots represent mean measured values of RH or temperature and the whiskers represent the calculated expanded uncertainty. In (A) the mean value with uncertainties fall into allowed limits. In case (B) it is not, since the upper limit is exceeded. Therefore, the test is not valid.

9   Disruptions and deviations

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

Check the agar dishes (see 8.5.2) after one week. If there is a lack of heavy growth, a new solution shall be prepared and the test shall be repeated with new test samples.

9.2   Test chamber conditions (positive control #2)

All positive controls shall be analysed in all incubation chambers every 14 days. Note the results and record when growth appears. If no growth is seen on the malt prepared glass fibre filter (see 8.5.3) in after 12 weeks of incubation, the test shall be repeated.

When more than one material is tested in the same chamber, attention shall be given to the possibility of mutual influences through volatile substances. An unexpectedly low amount of mould growth on the positive controls according to 8.5.3 may be an indication of such influences, which may invalidate the test. When a possibility is identified for inhibition of growth due to influence from another set of samples, separate chamber tests should be considered.

9.3   Purity of spore suspension (negative control)

Analyse all the negative control samples (see 8.5.4) every 14 days. If growth appears on the untreated filter paper controls a new solution shall be prepared and the test be restarted with new test specimens since it might indicate the presence of nutrients in the spore solution.

9.4   Deviation of temperature and relative humidity

If both the measurement results and its uncertainties fall inside the specified limits the test is valid (see 8.8). If not, the test shall be considered as failed and the test shall be restarted.

10 Expression of results

A test specimen shall be defined as positive when the mould growth equals or exceeds rating 2. The critical moisture level shall be considered reached at the RH level when at least two test specimens are positive and when no growth is detected on the test specimens at the next lower RH level tested. The actual critical moisture level is then expected between these two values or at the RH level when the test specimen is positive. The critical moisture level ($RH_{crit, 22°C}$) shall be reported as a range:

$$RH_B \quad < RH_{crit, 22°C} \leq RH_A$$

Where RH_A is the incubation condition with more than 2 specimens positive after 12 weeks;

RH_B is the incubation condition 5% lower than in RH_A, with less than 2 specimens positive after 12 weeks;

The incubation conditions A and B are defined as in 8.6.

EXAMPLE   90% RH < $RH_{crit, 22°C}$ ≤ 95% RH.

If mould is detected at 80 % RH, then the critical moisture level shall be estimated to 75% < $RH_{crit, 22°C}$ ≤ 80% RH, since the lowest RH for mould growth on building materials is considered to be 75%.

In case of mould growth on edges but not on the surface, this might indicate that the mould resistance differs between the inside of the material and the surface. Extended testing of the material should then be considered, in which the properties inside the materials are evaluated.

The results of this testing of critical moisture level shall only be valid for the tested material and the specifically analysed surfaces of these samples.

NOTE   In Annex C it is demonstrated how results achieved for 22 °C can be extrapolated to other temperatures.

11 Test report

The report shall include at least the following information:

a) a reference to this Technical Specification;

b) the name of the client;

c) identification of the material;

d) details of sampling regime;

e) species and strain numbers of the fungi used for the test;

f) start and termination date of incubation;

g) mean values, standard deviations and expanded measurement uncertainty, of the temperature and relative humidity for each test condition period;

h) any deviations from the method;

i) status of controls;

j) median assessment grade for each material and analysis week;

k) report if mould growth has been stated at the edges;

l) the critical moisture level at 22 °C, 12 weeks;

m) a statement that the results are valid only for the tested material;

n) name of the organization responsible for the test report and the date of issue;

o) name and signature of the person(s) in charge of testing;

Annex A
(informative)

Analysis for mould growth

A.1 General

If the test is performed in several incubation conditions simultaneously, the scheme described below may be used to streamline the analysis process and limit its cost.

Begin the analyses fourteen days after the incubation start. The test specimens (see 8.5.1) and controls (see 8.5.2 to 8.5.4) are then to be analysed for mould growth every 14 days.

Mould is not expected to grow at one level of relative humidity until the same week as the next highest level of relative humidity.

A.2   Key to analysis scheme

Follow through this analysis scheme every 12 week until median growth assessment on each separate set of 7 specimens is ≥ 2. When the criterion is fulfilled for a set of specimens, continue to incubate this specific set without further analyses until the final analysis at 12 weeks of incubation.

I.   Analyse all the test specimens in test chambers at 95% RH. Is there mould growth on at least one of the test specimens with rating ≥ 2?

- Yes: go to II
- No: continue to analyse each test specimen in test chambers at 95 % RH every 14 days. If there is mould growth on at least one of the test specimens with rating ≥ 2, then go to II.

II.   Analyse all the test specimens in test chambers at 90 % RH. Is there mould growth on at least one of the test specimens with rating ≥ 2?

- Yes, go to III
- No, continue to analyse each test specimen in test chambers at 90% RH every 14 days. If there is mould growth on at least one of the test specimens with rating ≥ 2, then go to III.

III.   Analyse all the test specimens in test chambers at 85 % RH. Is there mould growth on at least one of the test specimens with rating ≥ 2?

- Yes, go to IV
- No, continue to analyse each test specimen in test chambers at 85% RH every 14 days. If there is mould growth on at least one of the test specimens with rating ≥ 2, then go to IV

IV.   Analyse all the test specimens in test chambers at 80% RH

A.3 Interruption criteria

If growth rating 4 is observed before the end of the 12 week test period, those test specimens can be removed from the cabinet in order to minimize an exaggerated load of new spores on the remaining specimens.

Annex B
(normative)

Calculation of means with expanded uncertainty

The results of T_{min}, T_{max}, RH_{min}, RH_{max} calculated in Equation B.3 to B.6 below, shall fall within the specified limits according to Table 3 in 8.8. This is consistent for each incubation chamber throughout the test period.

I. Calculate preferably every 14 days, the day before each analysis session, the mean values and standard deviations from all sampled data (sampled every 10 minutes) for each incubation chamber.

II. Have at hand calibration uncertainties (unexpanded) given in the calibration protocols for the transmitters.

III. Calculate the combined standard uncertainty according to Equation B.1

$$u_c = \sqrt{s^2 + u_{cal}^2} \qquad\qquad\qquad (B.1)$$

where
u_c is combined standard uncertainty
s is calculated standard deviation for the sampled series of measurements
u_{cal} is the measurement uncertainty from the calibration of the transmitters

Perform the calculations for the measurement series of relative humidity and temperature respectively.

IV. Calculate the combined expanded uncertainty according to Equation B.2

$$U_c = k \cdot u_c \qquad\qquad\qquad (B.2)$$

where
U_c is the combined expanded uncertainty
k equals 2, corresponding to a coverage probability of approximately 95 %
u_c is the combined standard uncertainty calculated in B.1

Perform the calculations for the measurement series of relative humidity (RH) and temperature (T) respectively.

V. Calculate the minimum and maximum results of the incubation conditions by subtracting and adding the expanded uncertainty calculated in B.2 to the mean values for relative humidity and temperature according to Equation B.3 to B.6

$$T_{min} = T_{mean} - U_{cT} \qquad\qquad (B.3)$$
$$T_{max} = T_{mean} + U_{cT} \qquad\qquad (B.4)$$
$$RH_{min} = RH_{mean} - U_{cRH} \qquad\qquad (B.5)$$
$$RH_{max} = RH_{mean} + U_{cRH} \qquad\qquad (B.6)$$

Annex C
(informative)

Extended use of the results

RH$_{crit}$ at another temperature than the tested

In this technical specification, a method is described to measure the two levels of relative humidity, RH_A and RH_B (see 10), between which the critical moisture level $RH_{crit\ 22}$ at the specific tested temperature (22 °C) is found. The results can be applied to extrapolate a theoretical critical moisture level ($RH_{crit\ T2}$) at another temperature (T_2) according to reference [9] by using equation C.1 and C.2.

$$RH_{crit\ T2} = 105 + c(T_2{}^2 - 54T_2) \tag{C.1}$$

where

$RH_{crit\ T2}$ is the critical moisture level (%) at temperature T_2
c is given from equation (C.2)
T_2 is the chosen temperature (°C) for the extrapolation

$$c = (RH_{crit\ 22} - 105) / (T_1{}^2 - 54T_1) \tag{C.2}$$

where

c a constant
T_1 is 22 °C (the temperature at which the test was performed)
$RH_{crit\ 22}$ is the resulting critical moisture level (%) from the test at temperature T_1

As the test results are defined by RH_B and RH_A (see 10), Equation C.2 must be used to calculate two sets of c. Then, the new range for RH_{critT2} is estimated at the chosen temperature T_2.

The procedure above may be repeated for a series of temperatures, in the interval 0-30 °C according to reference [10]. These calculated values may be presented as growth limit curves.
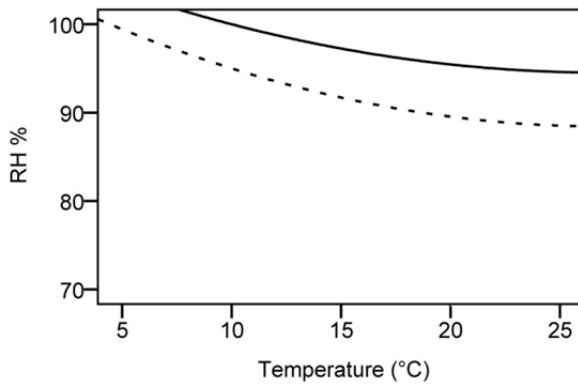
Figure C1 - Example of mould growth limit curves for a material calculated by equation C.1 and C.2. The input where the results from test at 22 °C of critical moisture level: $90\% < RH_{crit\ 22} \leq 95\%$.

In building parts where the relative humidity at a specific temperature 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 level may fall. To be on the safe side, if the relative humidity is in this zone, mould growth should be regarded as possible.

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An innovative test method for evaluating the critical moisture level for mould growth on building materials

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1   Introduction

Mould growth on building materials is the consequence of an interaction between environmental factors (temperature and humidity), material properties and the characteristics of the mould fungi. In general, the availability of water in the material is regarded as the crucial element for growth to occur. It is then the surface of the material that is of interest, since the mould fungi are growing at the surface of solid material. At equilibrium, the moisture at the surface is the same as in the surrounding air. Generally, the higher the moisture availability, the higher is the risk for mould growth. However, building materials differ in their mould resistance; some materials can withstand high moisture conditions better than others. Also, while some materials are susceptible to mould growth at low levels of humidity, down to 75 % RH, others can tolerate high moisture levels, above 95% RH, without mould growing on them (Ritschkoff et al. 2000) (Nielsen et al. 2004; Hofbauer et al. 2008; Johansson et al. 2012). There is a theoretical level of RH for each material, above which mould growth is possible, the critical moisture level for mould growth. This is dependent on the temperature; the lower the temperature, the higher is the critical moisture level (Johansson et al. 2012).

Traditionally, mould resistance is evaluated by exposing test specimens of the material to spores of mould fungi and then incubating the specimens at a relative humidity and temperature that are favourable to mould growth. The principle behind the test is that most fungi grow well at high RH and if the material is such as to allow mould growth, then it should also grow on the test pieces in the laboratory. Several standardised test methods are available; some are presented in (Adan 1994b) and in Table 3 of this paper. While being able to discriminate between materials in a general way, these test methods do not

provide any information on how a material will perform in a building where the moisture conditions are not that high. It may, therefore, be possible to use materials that have been subject to mould growth in the tests in constructions where the moisture load is lower.

In this paper, we present a new test method (SP-Method-4927 2012), the Critical Moisture Level method, for evaluating the critical moisture level of a material. Instead of completely excluding the use of certain materials that have failed existing mould resistance tests, the CML method can differentiate mould susceptibility at several different moisture levels. With the introduction of this newly developed and validated method, the field for testing materials' susceptibility to mould has widened. It also makes possible a practical application for use in situations with known lower RH. This in turn provides the basis for material choice in designs where moisture and temperature conditions are known.

The CML method is a result of a range of tests conducted in the laboratory over several years. These tests have been based on routines from several of the existing testing methods; with some modifications to fit the purpose. The method was validated by field studies in buildings.

The aim of this paper is to provide a basic understanding of the CML method, considering both the field of application and the repeatability of the tests performed according to the method. In the paper, we compare some parts of the method to existing standardised methods for testing mould resistance by highlighting some parameters that are crucial for the quality and reproducibility of the new test method.

2   The CML method

The principle underlying the new CLM methodology is the same as in most of the previous test methods of mould resistance: that is, spores from mould fungi are applied to the surface of test pieces of the material, and these are then incubated in conditions of temperature and relative humidity favourable for mould growth. After some weeks of incubation, the surfaces of the test pieces are analysed for mould growth. There are differences among the methods in terms of which species are used, the number of spores and how they are introduced to the test pieces, the incubation environment in which the materials are tested, the assessments of growth of the test pieces, and the evaluation criteria. Table 1 compares some of these parameters of the new CML method with those of five selected test methods for determining mould resistance.

In the CML method, a spore solution is sprayed onto one surface of the material. Four sets of test specimens are then incubated in four different RH (80%, 85%, 90% and 95%) at 22°C. The test may be performed in parallel in separate moisture chambers with the different RH levels. The specimens are incubated for 12 weeks in each RH and are then visually inspected for fungal growth at x40 magnification; growth is assessed according to a rating scale.

The critical moisture is determined by considering the RH at which mould growth is established on the test specimens and the next lower RH where no growth can be detected during analysis. The actual critical moisture level is then expected to be somewhere between these two values, or at the RH when the test pieces failed. The actual critical moisture level is therefore reported as a range. The principle is illustrated in Figure 1.
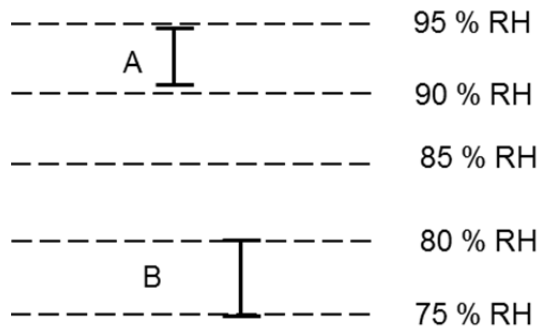
Figure 1   *Principle of determination of critical moisture level. The numbers of RH represent the tested RH, except for 75% which is used as the lowest limit for mould growth based on literature. In case A, there is mould growth at 95% RH but not at 90% RH and the critical moisture level is therefore 90 %< RHcrit, 22°C ≤95 %. In case B there is mould growth at 80% RH and the critical moisture level will consequently be determined as 75% < RHcrit, 22°C ≤ 80%.*

As the RH_{crit} is temperature-dependent, the test results are only valid for the temperature tested, that is, 22°C. However, to be able to use the results for applications in buildings, it is important also to assess the critical moisture levels at other temperatures. In the CML method, an equation is given where the critical moisture level can be calculated for any temperature commonly found in actual buildings (between 0°C and approximately 30°C); if the

calculations are repeated for a series of temperatures, the critical moisture levels can be expressed as growth limit curves (see Figure 2). The equation and procedure for calculating the growth limit curves has been presented by (Johansson et al. 2013a) and is based on both our own (Johansson et al. 2012) and other studies (Sedlbauer 2001; Hofbauer et al. 2008).
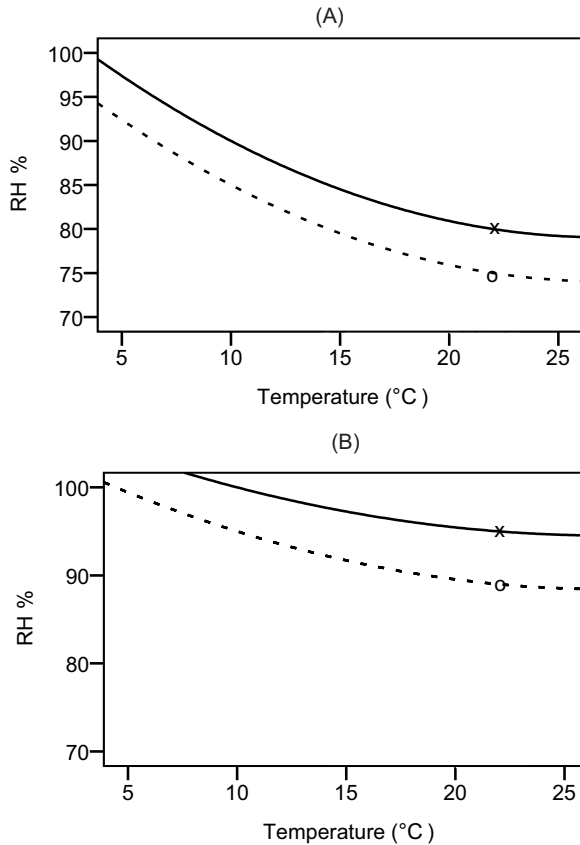
Figure 2   *Mould growth limit curves for materials with 75% < RHcrit, 22°C ≤ 80% (A) and 90%< RHcrit, 22°C ≤ 95% (B). x is the RH where mould growth is found, o is the next lower RH in the test.*

Table 1   Comparison between the CML method for testing critical moisture level and five traditional methods used for estimating mould resistance.

		The CML method SP MET 4927	ASTM C1338-00	BS 1982: Part 3:1990	ASTM G 21-96	ISO 846:1997	MIL-STD 810 G**
Material		Building materials	Insulation materials and their facings	Panel products, made of or containing materials of organic origin	Synthetic polymeric materials	Plastics	A variety of materials
Purpose of the test		Critical moisture level	Mould resistance	Mould resistance	Mould resistance	Mould resistance	Mould resistance
Specimen	Size	50 x 100 mm	Surface area not defined	40 x 40 mm	50x50mm/ dia. 50 mm/ length 76 cm (rods)	Not defined	Not defined
	Number of test specimens	7	3	Minimum 3	3	5	Not defined
	Number of species	6	5	7	5	5	5
Inoculum	Spore solution	Yes	Yes	Yes	Yes	Yes	Yes
	Concentration of spores in solution	10^6 spores/ml	$10^6 \pm 200\,000$ spores/ml	Not specified	$10^6 \pm 200\,000$ spores/ml	10^6 spores/ml	$10^6 \pm 2\%$ spores/ml
	Inoculation methodology	Spraying	Spraying	Spraying	Spraying	Spraying or pipetting	Spraying
	Amount of solution	0.4 ml	0.5 ml	0.5 ml	Until surface is moistened	0.1	Not specified
	Number of spores/cm²	8000	(a)	(b)	(c)	(a)	(a) and (c)

		The CML method SP MET 4927	ASTM C1338-00	BS 1982: Part 3:1990	ASTM G 21-96	ISO 846:1997	MIL-STD 810 G**
Incubation	Temperature	22 ± 1 °C	30 ± 2 °C	24 ± 1 °C	28-30 °C	24 ± 1°C or 29 ± 1°C	30 ± 1°C
	RH	80 ± 2,5% 85 ± 2,5% 90 ± 2,5% 95 ± 2,5%	95 ± 4%	Not specified	>95%*	>95%*	At least 90% but less than 100%
	Incubation environment control	Recording of temperature and RH every 10 min	No	No	Automatic recording of wet and dry-bulb temperature recommended	No	Recording of chamber temperature and humidity over time
	Incubation time	12 weeks	Min. 28 days	4 weeks	28 days	4 weeks or longer	28 days or up to 84 days
	Storage of suspension	Must be used the same day	28 days at 6 ± 4 °C[1]	Must be used the same day	Not more than 4 days at 3-10°C	6 h	6 ± 4 °C for not more than 14 days

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[1] Or until the viability test indicates poor growth or until growth appears in the sealed storage bottle
a) Cannot be calculated since the area of test specimen is unknown
b) Cannot be calculated since the concentration of spores in the suspension is unknown
c) Cannot be calculated since the volume of spore suspension is unknown
* Specimens are placed on solidified nutrient salt agar and RH is according to ISO 846 >95 %.
** although this method is not specified for testing building materials, it is useful and often used for this purpose

3   Assuring quality control and test reproducibility

To ensure test reproducibility, it is important to control the testing procedure. The CML method provides routines that make this possible. Among these routines are procedures for the preparation and inoculation of spores on test specimens, incubation conditions (including specifications for the test chamber), handling of test specimens and assessment of growth on the test pieces. Also, there are instructions for the action to be taken if deviation occurs.

These quality assurance routines are based on our own test results and experiences in research and commissions over more than 15 years. During the process, other test methods were also taken into consideration and relevant parts were integrated. With this background, we now discuss the quality-critical sections of the method.

3.1   Inoculum

In the CML method, as in many other test methods, spraying of a water solution of spores is used to apply the spores to test specimens. The advantage of using a spore suspension instead of, for example, natural contamination or application of dry spores is that the number of spores and the species are controlled and remain the same for each application, since the concentration of spores in the solution is known and the amount of spore solution, as well as the surface area of the test piece, is prescribed in the method. Also, by spraying, the spore solution will be more or less evenly distributed over the surface of the test specimen. This is of importance when it comes to evaluating the extension of growth over the surface.

In this way, by following the routines of the CML method for the production and application of spores to the samples, repeatability becomes possible. This includes the procedures for preparation of the spore suspension, which ensures that no added nutrients that would influence the test results are present in the solution. The routines are more or less the same as in (MIL-STD-810G 2010).

One argument against using spores in aqueous solution is that they can swell, which may affect the start of growth (Dantigny et al. 2006). In the CML

method, this risk is reduced since the solution must be used directly upon production – the same day.

Another argument against using a liquid solution is that the water may influence the critical moisture level. This is, however, not a problem since the CML method prescribes the use of test chambers where equilibrium is reached quickly between the air with the desired relative humidity and temperature and the surface of the material. In the laboratory tests (Johansson et al. 2012), 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.

In real-life situations in buildings, different species of fungi often occur together on the building materials, and various fungi occur to different extents on different materials (Hyvärinen et al. 2002; Andersen et al. 2011). To emulate real conditions, spores from six of the fungi most commonly found on many building materials were used in the spore solution (*E. herbariorum, A. versicolor, P. chrysogenum, A. pullulans, C. sphaerospermum, S. chartarum)*.

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 90% RH. However, some species cannot grow at lower RH. Since the purpose of the method is to find the lowest RH where a material will mould, both xerophiles (fungi that grow at lower RH) as well as more moisture-loving fungi are present in the spore suspension. (Johansson et al. 2012) summarize the moisture requirements of the fungal species and the origin of the particular strains used in the method.

3.2   Test specimens

The CML method is valid for new material; it must not have been exposed to conditions that would enable mould growth. Also it must be clean, since soiling of material may lower the critical moisture level (Chang et al. 1996). Preferably, the test specimens come from different production batches, which is particularly important if the quality is expected to vary among batches.

3.3   Incubation environment

The purpose of the CML method is to find the critical moisture level for mould growth on a material. It is essential to have control over the particular values of RH at which the test has been performed. The incubation is carried out at four levels of RH, which are set at 80%, 85%, 90% and 95%, all at 22°C. Temperature and RH are logged and recorded every ten minutes with calibrated sensors. The precise mean RH and temperature that the specimens are exposed to will never be exactly the set values. This is because of the expanded measurement uncertainties caused, for example, by variation in each chamber and by measurement error from calibration of the sensors.

In order to distinguish the different incubation RH from each other, there are specified limits, ± 2.5 % of each of the set values for RH and ±2°C for the temperature, within which the mean value together with the measurement uncertainties must fall. The procedure for calculating this is given in the CML method. Figure 3 and Table 2 illustrate the principle.

Specified upper limit of
incubation condition

a   b

Specified lower limit
of incubation condition

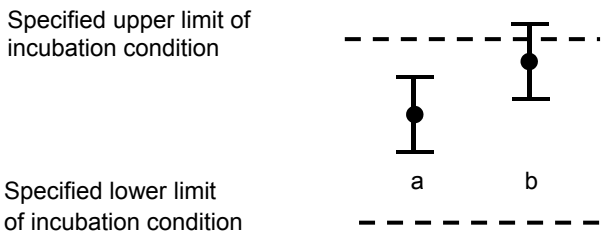*Figure 3*   *Illustration of the evaluation of incubation criteria. The specified limits of incubation condition are described in Table 2. The dots represent mean measured values of RH or temperature and the whiskers represent the calculated expanded uncertainty. In case I the mean value with uncertainties fall into allowed limits. In case II it is not, since the upper limit is exceeded. Therefore, the test is not valid.*

Table 2   The set points of each incubation condition of the test method

Incubation condition	Set point	Specified lower limit	Specified upper limit
T	22.0 °C	20.0 °C	24.0 °C
RH 1	80.0%	77.5%	82.5%
RH 2	85.0%	82.5%	87.5%
RH 3	90.0%	87.5%	92.5%
RH 4	95.0%	92.5%	97.5%

Fewer percentage points between two tested humidity levels would narrow the interval for RH_{crit}. However, measurement uncertainty limits how narrow these intervals may usefully be. Our laboratory study showed that the uncertainty was at most 2.5 percentage points RH, so settings of RH in ranges smaller than 3 percentage points are not possible with the settings used in the CML method (Johansson 2012).

To ensure that the incubation environment will not deviate more than allowed from the prescribed, the CML method specifies the homogeneity and stability of the test chambers and of the loggers used to monitor the conditions in the chambers. Also, instructions and guidelines for calibration, maintenance and service are provided.

This control of RH and temperature is a major difference from other test methods for testing mould resistance. In most of those methods in no monitoring of RH and temperature is prescribed. The purpose of these methods is to test resistance to mould at climates that are optimal for mould growth. It is therefore of minor importance to measure the exact climate in which the test pieces are exposed.

3.4   Methodology of analysis and rating of mould growth

In the CML method, the surfaces are studied in 40x magnification in order to find mould growth, classified according to a rating scale (Table 3).

Table 3   *Rating scale for the assessment of mould growth on the test*
              specimens

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

Although there may be extensive growth on a material, it might not be seen by the naked eye. This is because there is a variation in pigmentation among species or across stages in their life cycle. In addition, the discoloration is perceived differently, depending on the contrast with the underlying colour of the surface. Figure 4 shows an example of different visual appearance to the naked eye, demonstrated for the same extent of growth according to the rating scale.
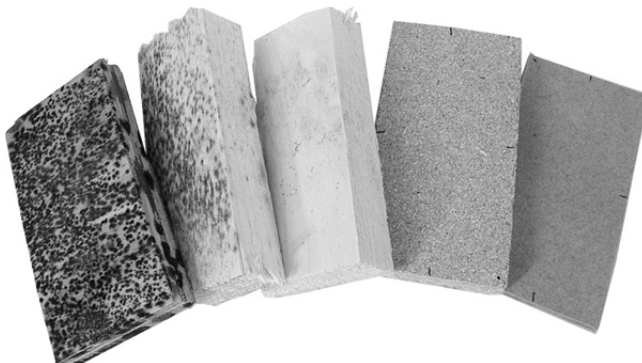

Figure 4   *Test specimens with mould growth corresponding to rating 4 in*
               Table 3 with different visual appearance.

In most of the mould resistance methods in Table 1, a stereomicroscope or a hand lens is used only if found necessary or to confirm sparse growth. We interpret this to mean that it is primarily the growth that can be seen with the naked eye that is analysed. In this area, therefore, there is considerable difference between those methods and the CML method . For the reasons given, we believe the latter is a better method.

It is important that those performing the analysis are experienced in analysing mould growth. It is also critical 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; we have determined that a minimum of seven samples provides a 95% level of confidence (Johansson et al. 2012).

4   Application of the method

4.1   Relevance for real-life conditions

The test is performed in a laboratory at constant conditions of temperature and relative humidity. These conditions will differ from what the materials encounter in 'real life'. (Cooke and Whipps 1993) write, '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 fluctuation 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 moisture conditions but have an excellent ability to adjust to fluctuating water and temperature conditions (Deacon 2005).

On building materials, the rate and extension of mould growth have been shown to be lower when favourable conditions alternate with less favourable (Johansson et al. 2013a; Adan 1994a; Viitanen and Bjurman 1995). In addition, how long these periods last is also of importance.

Factors other than environmental conditions vary among laboratory and natural environment, and some are listed in Table 4.

Table 2   *Comparison between a general laboratory test for evaluating the resistance of a material to fungal growth and the natural conditions that a material will encounter in a building construction*

	Laboratory tests	Conditions in a building
Fungal species	A few species (1–6) introduced onto the surface, although other microorganisms may be present if test specimens are not sterilized before testing	Potentially a large number of species
Spore exposure	More or less controlled; high concentration of spores	Unknown, continuous supply, varying between places and time of year
RH and temperature	Constant or varied according to a predefined scheme	Varying over time, sometimes occasionally condensation
Duration of exposure	Limited to some weeks	Several years
Other environmental factors		Presence of other organisms, e.g. mites that eat fungi

In spite of all the limitations of and simplifications in the accelerated laboratory method compared to real-life situations, the CML method serves well to predict the mould growth in buildings, as has been shown in (Johansson et al. 2013b). In the present study, the same materials as had been tested in the laboratory test (Johansson et al. 2012) with the CML method was exposed in three crawlspaces and three attics for 2.5 years. If the actual RH and temperature was above the critical moisture level for the material, as had been tested in the laboratory with the routines as prescribed in the CML

method, there was growth on the test pieces if the time was long enough. If the RH and temperature did not exceed the critical moisture level, there was no mould growth on the material.

4.2 Use of the results

By using the growth limit curves constructed according to Figure 2 and on the basis of the results from the testing by the CLM method, it can be estimated whether there is risk for mould growth in a building part where the relative humidity and temperature is known, either by measurements or by hygrothermal calculations.

The results from one of the measurements in our field study (Johansson et al. 2013b) serves as an example of how results may be used. Figure 5 shows the measurements of temperature and relative humidity in a crawlspace for 2.5 years. Each value of measured relative humidity is plotted against the temperature, and the growth limit curves for materials are plotted as in Figure 6. If the relative humidity at a specific temperature is 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 level may fall. To be on the safe side, if the relative humidity is in this zone, mould growth should be regarded as possible.
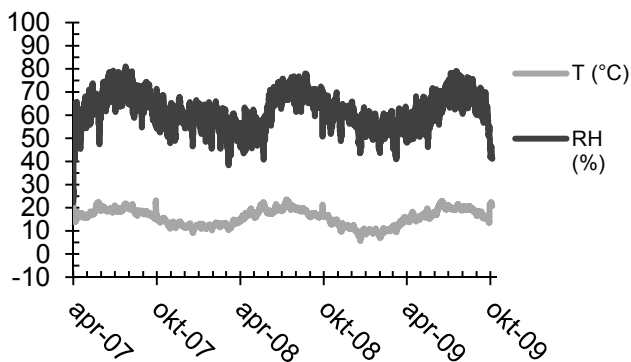
Figure 5   Measured relative humidity and temperature in a crawlspace (Johansson et al. 2013b).
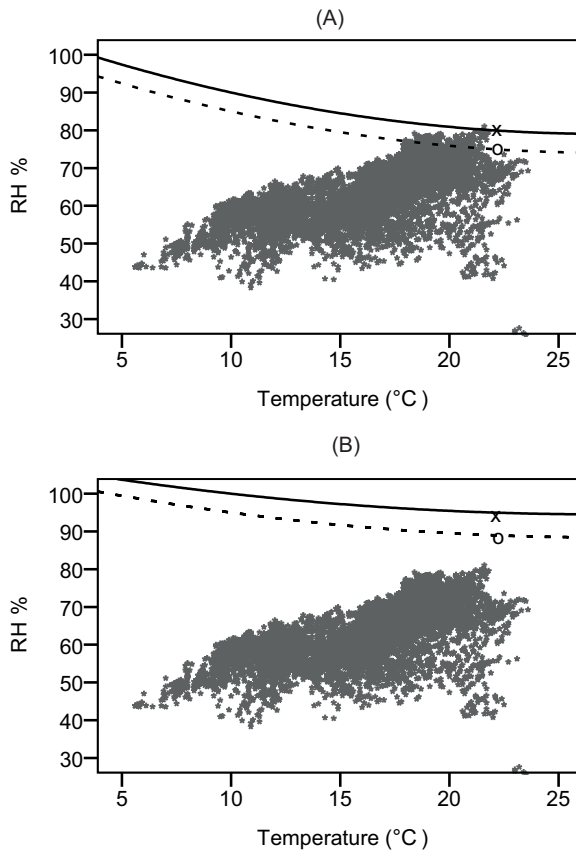
Figure 6   *Example of how to use the results from the test. The measured RH conditions in Figure 5 are plotted as a function of measured temperature. The growth limit curves according to Figure 2 have been introduced to the plots. In case A, no mould growth is expected, since the growth limit curves is well above measured values. In case B, there are values in the critical moisture level interval and hence there is a possible risk for mould growth. If the measured or calculated conditions are expected to be more humid/warm this risk increases since the limit curves then are well exceeded.*

One limitation of the method is that the results cannot be used to predict how long the material can withstand the actual conditions in buildings where the temperature and relative humidity fluctuate. The combined RH and temperature conditions must be above the critical moisture level for a sufficient time (Johansson et al. 2013b) for mould growth to develop. If only

exceeded occasionally, there is probably no enhanced risk for mould growth. Also, it is the duration of the favourable and unfavourable conditions that is decisive (Johansson et al. 2013a). Based on the field test, we find that when the duration of favourable conditions is not considered, the laboratory test results will not underestimate the risk of mould growth; the results will instead include a certain margin of safety (Johansson et al. 2013b).

Several mathematical models for assessing the risk for mould growth have been or are being developed, and some of them are reviewed in (Vereecken and Roels 2012). In the further development of such methods, the critical moisture condition should be considered. Results of the test according to the CLM method can then be used as input in the calculations.

5 Conclusions

The CML method for testing the critical moisture level of a material provides a new and enhanced tool to assess the susceptibility of a material for mould growth in buildings in a way that has not been possible earlier. The method contains quality-assured routines and has been validated in real-life conditions.

If the expected temperature and RH in a building part is known, either by measurements or by using heat-and-moisture simulation software, knowledge of the critical moisture levels of a material, as determined by the CML method and the calculated growth limit curves may be used as tools when choosing materials for construction with minimum risk for mould growth.

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The effect of cyclic moisture conditions for mould growth on wood compared to steady state conditions

Pernilla Johansson, Gunilla Bok and Annika Ekstrand-Tobin (2013)

The effect of cyclic moisture and temperature on mould growth on wood compared to steady state conditions

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ABSTRACT

Moisture and temperature are the two key environmental parameters that determine the possibility of mould growth on building materials. The time that the material is exposed to these elements is also crucial. The natural environmental conditions of relative humidity and temperature are seldom constant over prolonged time periods in a building. Instead, they vary over time, with fungi encountering both favourable and unfavourable conditions; such variable conditions affect mould growth. This paper reports findings from a laboratory study in which mould growth at alternating RH (between 90% and 60%) or temperature conditions (between 22 °C and 5 °C) was studied and compared to steady state conditions. Fluctuating RH led to slower mould growth, and when the period of unfavourable/favourable conditions was longer (1 week), growth was affected more than if the duration of these conditions was short (12 h). When alternating the temperature weekly between 22 °C and 5 °C, with a mean of 13 °C, the mould growth rate was lower compared to when the temperature was held constant at 22 °C. The mould growth under fluctuating temperature conditions was comparable to when the temperature was kept constant at 10 °C.

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1. Introduction

Mould fungi play a special role in moisture-damaged buildings. Some species can cause minor degradation of the material that they grow on. A greater problem, however, is the negative impact on the indoor environment that mould causes, e.g., the production of substances that cause bad odours and possible adverse health effects on people who live or work in the building [1]. One way to achieve a better indoor environment for residents and users is therefore to prevent mould growth in buildings.

Moisture and temperature are the two key environmental parameters that determine the possibility of mould growth on building materials, with the effect of moisture being more important than temperature when it comes to preventing mould growth [2]. Without enough available moisture, fungi cannot grow at all. All cellular processes require water to function and there is a minimum amount required for these to work. Water also acts as a solvent when transporting substances into and out from the cell.

The water available in the material for microbial growth can be expressed as water activity, a_w. It is not possible to measure the water activity within a material; instead, it can be determined from the relative humidity of the air surrounding the material when the air and the sample are at equilibrium.

At room temperature, relative humidity below 75–80%, which corresponds to $a_w = 0.75$–0.8, is considered safe with respect to the risk of mould growth on building materials [3–8]. At RH at or above this value, there is a possible risk of mould growth on the most susceptible building materials. However, some building materials can be exposed to relatively high moisture loads without becoming subject to mould growth [6]. Each brand of material has a specific critical moisture level. If this is exceeded, there is a risk that mould fungi will develop on the material.

Fungal growth can take place over a wide range of temperatures. Provided that the moisture conditions favour mould growth, the temperature normally encountered in buildings is not a limiting factor for growth. Most fungi grow well at temperatures from 5 to 35 °C [9]. However, cellular processes are slowed down as temperature is reduced [10]. A consequence of this is that the growth rate is lowered and that the moisture content required for mould growth is increased [6].

It should be noted though, that the amount of time that the material is exposed to these conditions is of crucial importance. Exposure for a short time may not lead to mould growth, while longer exposure leads to a greater risk of mould developing on the material [6].

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The natural environmental conditions of RH and temperature are seldom ideal for fungal growth. Nevertheless, fungi exist even where conditions are far from optimal. For example, there are fungi that can live in relatively dry conditions or at low temperatures. These conditions can prevail for continuous periods of time or appear intermittently. Compared to the more ideal conditions that are studied in the laboratory, these conditions put stress on the organisms. A stressful environment can be defined as the abiotic conditions under which the growth and reproduction of an organism are restricted or prevented [9]. However, different species vary in their adaptability to the environment that they are exposed to; for example, whether a fungus actually grows or just survives the stressful conditions depends on the species. In addition, species differ in their ability to withstand extreme conditions, e.g., exposure to low relative humidity for prolonged periods of time, and have different characteristics that allow them to survive [11].

In building construction, the temperature and RH often fluctuate, which also means that the growth conditions change with time. These variations can be long term, such as seasonal variation in attics and crawlspaces, or short term, e.g., as a result of moisture production due to human activity, daily fluctuations on external walls or condensation on the inner roof at night due to night radiance. In the laboratory, the rate and extent of mould growth has been shown to be lower when RH and temperature conditions alternate between favourable and unfavourable conditions for mould growth [12].

To ensure that no mould will occur in buildings it is important to keep track of RH and temperature and to use materials with higher critical moisture levels than expected. By using models for predicting mould growth, in which fluctuating conditions and the time factor are considered, an even more realistic prediction can be made.

Several published studies have examined how mould fungi grow under constant conditions, e.g. [6,13–15] while fewer have described the same situation during fluctuating stressful conditions [12,16]. In this study, we analysed the effect of short and long variations of moisture and temperature on mould growth compared to steady state conditions of favourable RH and temperature. Our hypothesis was that cycling would lower fungal growth and that longer periods of unfavourable (temperature) or inhibitory (RH) conditions would affect mould fungi more than brief periods. Furthermore, a second hypothesis was that variations in temperature between optimal and suboptimal values would affect the growth less than fluctuations in moisture.

The object of this study was to increase our knowledge about the effects of environmental stress on the growth of mould fungi and to facilitate the modelling of mould growth in buildings.

2. Materials and methods

2.1. General setup of the test

Test specimens of wood were inoculated with mould spores and incubated in incubation chambers at the given relative humidity and temperature. They were then analysed for surface mould growth at regular intervals of time.

This approach has previously been used with success to make each test repeatable and comparable [6].

2.2. Wood material and test specimens

Because we expected that mould growth would differ among different qualities of wood, wood species and surface treatments, we used a selection of different wood materials in order to represent the wood encountered on the market. Two qualities of pine

and spruce were used. Both consisted of sapwood; quality I was from the outermost sapwood and quality II was sapwood originally closer to the pith. The newly dried materials were collected directly from two sawmills located in southwest Sweden, pine from one sawmill and spruce from the other. The surface of half of each test boards was planed off.

In the laboratory, the boards and planks were cut into smaller test specimens that were 100×50 mm in size. Six replicates of each board with the original, sawn surface were used for each test condition of temperature and relative humidity. In addition, six test specimens with newly sawn surfaces were used in each Test scheme. In all, 48 test specimens were tested for each condition (Table 1).

2.3. Inoculation with mould spores

Prior to incubation, one surface of each test specimen was inoculated with 0.4 ml of a spore suspension by spraying. The suspension contained spores of *Eurotium herbariorum*, *Aspergillus versicolor*, *Penicillium chrysogenum*, *Aureobasidium pullulans*, *Cladosporium sphaerospermum* and *Stachybotrys chartarum*. Preparation of the spore suspension and spraying of the test specimens followed the protocols described in Ref. [6].

2.4. Incubation

The test specimens were incubated in climate chambers (CTS C-20/350, CTS GmbH, Hechningen, Germany) under different test conditions (Fig. 1). Periods of conditions favourable for mould growth (RH and temperature) were alternated with periods of less favourable (temperature) conditions or such conditions where growth is not possible (RH). Changes were made regularly in a cyclic manner. For the control conditions, the RH and temperature were held constant during the entire incubation period. For the cyclic Test scheme, either the temperature or relative humidity was changed periodically, at longer (about one week) or shorter (approx. 12 h) intervals (Fig. 1).

The duration of the incubation differed among the different Test schemes. In this paper, we chose to analyse the results after 42 days (Test scheme A) and 84 days (Test schemes B–E). After 42 days at 90% RH and 22 °C (Test scheme A), there was established mould growth on all test pieces. This time point was therefore chosen for analysis for the other test schemes. The total time that the test specimens were exposed to 90% RH (42 days) was then the same in Test schemes A, B and C.

The lowest critical moisture limit (RH_{crit}) for mould growth on wood is generally considered to be 75% at room temperature, although there might be qualities of wood that can withstand mould growth at higher moisture levels. In this study, 60% RH was used as the test level at which mould growth is not possible.

Generally, mould growth can occur at temperatures as low as 0 °C. At lower temperatures, the process is slower, however. When

Table 1
Materials and surface treatments used in the study.

Wood species	Quality	Surface characteristics	Number of test specimens
Spruce	I	Original, planed in sawmill	6
(*Picea abies*)		Original, sawn in sawmill	6
	II	Planed in laboratory	6
		Planed in laboratory	6
Pine (*Pinus*	I	Original, sawn in mill	6
sylvestris)		Original, sawn in mill	6
	II	Planed in laboratory	6
		Planed in laboratory	6
			$\sum$**48**
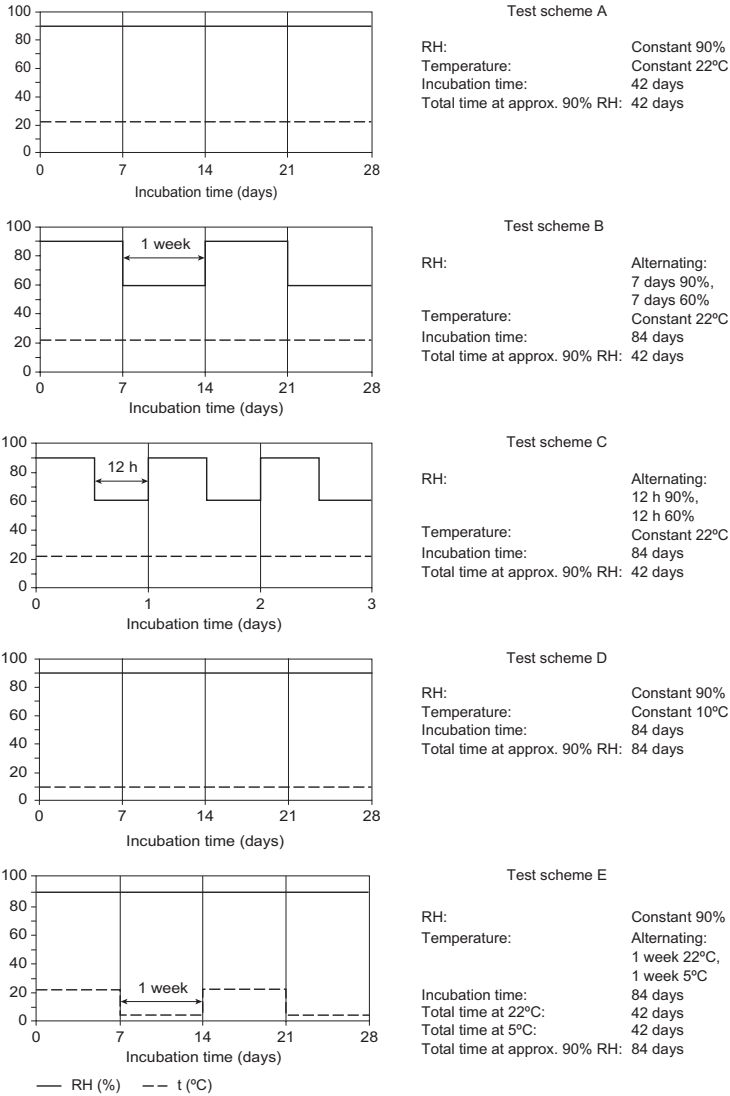
Fig. 1. Test schemes.

the test scheme contained cycling temperature periods, 22 °C was chosen as the favourable condition, while 5 °C was considered less favourable.

To monitor and record the RH and temperature that the test specimens were exposed to, an external humidity and temperature transmitter (Vaisala HUMICAP® HMT330, Helsinki, Finland) were mounted in the test chamber. The temperature and relative humidity values were saved in a computer-based program (Exomatic) every 5 min. The transmitter was calibrated regularly at an accredited laboratory (SP Technical Research Institute, Energy Technology, Borås, Sweden). The recorded data were adjusted according to calibration results.

Table 2
Rating scale for assessment of fungal growth by microscope at 40× magnification.

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

The test chamber was cleaned with 70% ethanol after each completed test scheme.

2.5. Analysis of mould growth

Mould growth on the surface of each test specimen was analysed with a stereomicroscope at 40× magnification and assessed according to Table 2. This analysis was conducted twice a week, at 3, 7, 10, 14… 42/84 days. The method of analysis is a good way to observe mould growth over time on the same test specimen because it is non-destructive.

Mould growth was considered established on each test specimen when the rating was equal to or greater than 2 for the first time.

3. Results

The measured RH and temperature of the incubation conditions for each test scheme are presented in Table 3. The measurements show that for the cyclic test schemes, the desired RH or temperature value was achieved within 30–60 min at maximum; the same was true after opening and closing of the test chambers when the test specimens were taken out of the cabinet for analysis.

Mould growth as a median rating over time for each Test scheme is presented in Figs. 2 and 3. In Figs. 4 and 5 the results are shown as Kaplan–Meier charts [17] and show the percentages of test specimens that have not yet reached a rating 2 or higher, meaning that there is no established growth. Samples that did not reach a

Table 3
Mean values with standard deviations for RH and temperature in each test scheme.

Test scheme	Description of test scheme	Period	Mean (Std. deviation)	
			RH (%)	t (°C)
A	Constant RH 90% Constant t 22 °C	Whole period	88.6 (1.1)	22.4 (0.2)
B	Cyclic RH 90%/60% (1 week) Constant t 22 °C	Whole period	74.9 (15.2)	22.3 (0.2)
		Period with "higher" RH	90.2 (1.2)	22.3 (0.1)
		Period with "lower" RH	60.0 (1.8)	22.4 (0.2)
C	Cyclic RH 90/60% (12 h)[a] Constant t 22 °C	Whole period	74.4 (15.7)	22.4 (0.2)
		Period with "higher" RH	90.0 (1.9)	22.3 (0.2)
		Period with "lower" RH	58,6 (1.8)	22.5 (0.2)
D	Constant RH 90% Constant t 10 °C	Whole period	88.7 (1.1)	10.4 (1.1)
E	Cyclic t 22 °C/5 °C (1 week) Constant RH 90%	Whole period	87.9 (3.5)	13.1 (8.3)
		Period with "higher" temperature	89.5 (4.1)	22.3 (0.4),
		Period with "lower" temperature	86.6 (2.0)	5.5 (0.5)

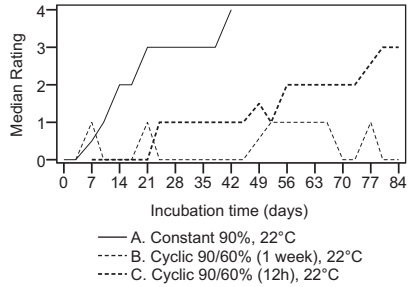[a] For the first 48 h, the RH was constant at 95%.

Fig. 2. Median mould growth rating (according to Table 2) over time (days) when the relative humidity alternates. The results include all tested surfaces.

Fig. 3. Percentage of test specimens on which there was no established mould growth over time (days) when the temperature alternates. The results include all tested surfaces. Samples that did not reach rating 2 during the test period are censored in the plots.

rating 2 during the test period were censored in the plots. In Figs. 6 and 7 the results are separated by surfaces and quality.

When alternating the relative humidity between favourable conditions and low RH (too low for mould fungi to grow), the mould growth rate differed from that seen under the reference
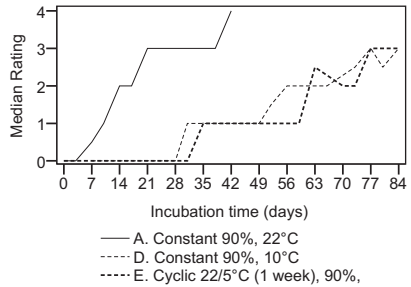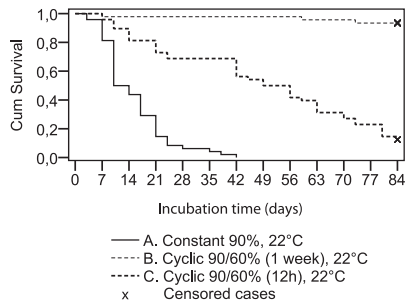

Fig. 4. Percentage of test specimens on which there was no established mould growth over time (days) when the relative humidity alternates. The results include all tested surfaces. Samples that did not reach rating 2 during the test period are censored in the plots.
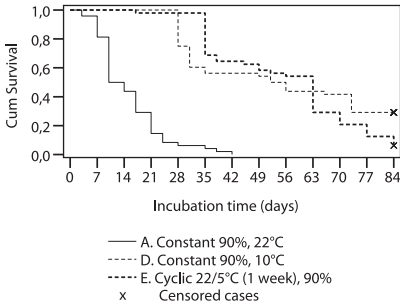
Fig. 5. Median mould growth rating (according to Table 2) over time (days) when the temperature alternates.

steady state conditions of Test scheme A. There was also an obvious effect of the duration of low RH conditions; a longer period of unfavourable conditions (Test scheme B) decreased the growth more than the shorter periods (Test scheme C).

When the temperature was lowered from 22 °C (Test scheme A) to 10 °C (Test scheme D), the mould growth rate slowed down. Additionally, when the temperature fluctuated between 22 °C and 5 °C (Test scheme E), the rate of mould development was lowered compared to the test scheme in which the temperature was constant at 22 °C. There was no obvious difference between test schemes D and E, though the results from these test schemes clearly deviated from the constant, favourable conditions of Test scheme A.

The growth pattern was observed to be more or less the same for all qualities of wood and all surfaces tested. What did differ, however, among these groups of test specimens were how long it took for mould growth to appear and to what extent the mould grew on the surface (Fig. 7).

Fig. 6. Median mould growth rating (according to Table 1) over time for all surfaces and qualities tested when the relative humidity alternated, divided by the surfaces and qualities tested.

Fig. 7. Median rating of mould growth rating (according to Table 1) over time for all surfaces and qualities tested when the temperature alternated.
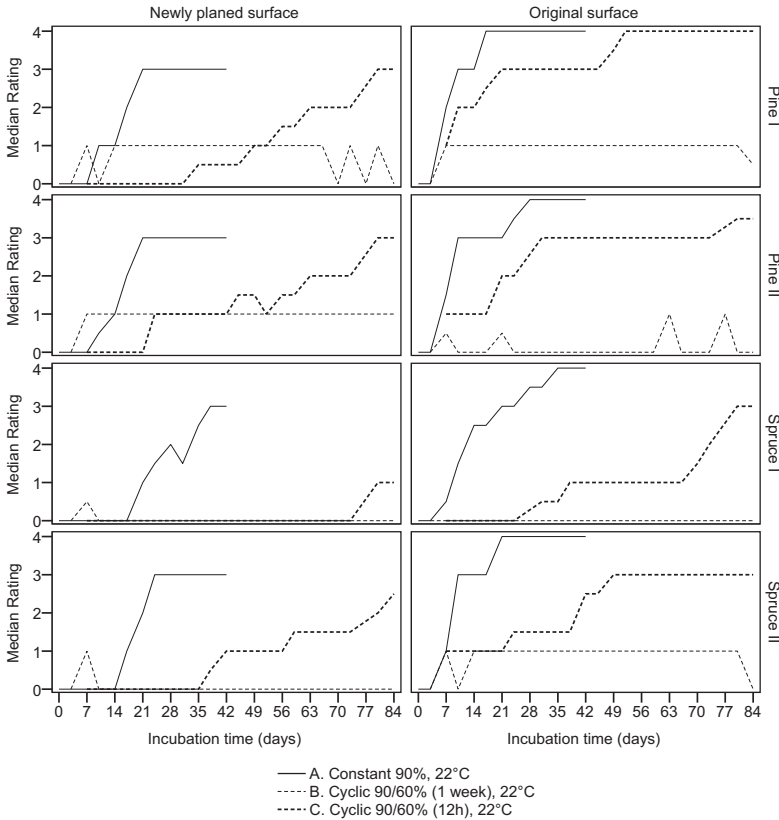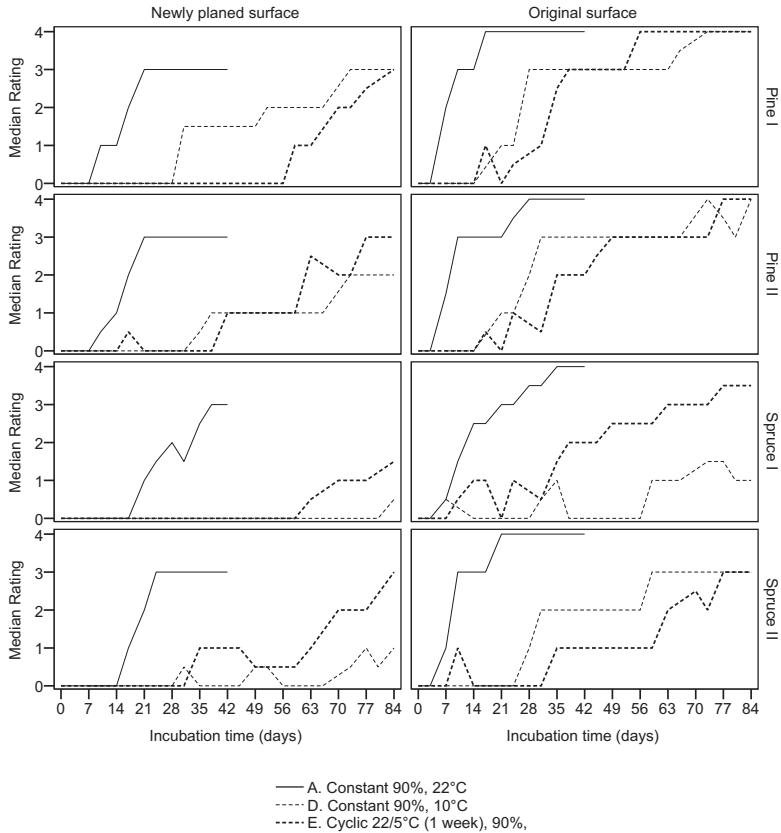
4. Discussion

There are different ways to analyse mould growth. In this study, we used a fairly rough method to evaluate fungal growth, which was to measure the extent of growth over the surface of test specimens, assessed according to a rating scale. This method was chosen because it can be used to follow mould growth over time on a specific test specimen, without affecting the mould growth itself. When the mycelium has established on a surface, it will not physically disappear over the test period, even if the conditions for growth are poor, and it will be rated according to the appearance on the test specimen. However, while cessation of growth may have taken place, this cannot be observed by the method used in the study. The activity of the fungal cells has most likely been affected by the cycling of favourable and less favourable conditions, but this was not observed in this study.

Though we cannot identify the possible physiological process changes at the organismic level, we can draw some conclusions about the appearance of fungal growth on the somewhat more "macroscopic" level.

The time period for which the RH was approximately 90% was the same for Test schemes A, C and E (Fig. 1). Nevertheless, the mould growth differed markedly among these Test schemes. Altering RH between dry and humid levels while keeping the temperature constant at an optimal level (22 °C) had a reducing effect on growth. In the two Test schemes for which the RH altered (C and E), the mean RH value over the incubation period was approximately the same, 75% (Table 2). However, when the alternating period was short (12 h), development of mould growth was not affected as much as when the unfavourable conditions lasted for longer periods of time (7 days). These results suggest that when predicting mould growth, using the total time of exposure to favourable conditions or the mean RH value will lead to an incorrect estimation of the risk for mould growth over a specified time.

The mould growth rate was lower on the test specimens at 10 °C compared to 22 °C, while the moisture conditions were constantly favourable for fungal growth. The same tendency was observed when temperature was varying between 22 °C and 5 °C. When the temperature is lowered, the rates of biological processes in the

fungal cell are expected to be reduced [18]. This could be the reason for the slower mould growth rate observed.

Varying the temperature did not have the same effect on mould growth as alternating the relative humidity; it did not differ much between those conditions and the situation when the temperature was kept constant at 10 °C. This result is not surprising because fungi can grow at the lower temperature used in the study (5 °C), though it is not the optimum temperature for most fungi. When considering the mean temperature value (Table 2), it seems that the mean value that a material will be exposed to can be used when modelling growth.

The main conclusions from the study are drawn from results from mould growth on all qualities and treatments of wood that were used. The growth patterns for the different Test schemes were similar when all qualities, wood species and surface treatments were studied separately. There were nevertheless some differences; pine was slightly more sensitive to mould growth than spruce, original surfaces were more sensitive than newly planed surfaces, and boards originated from the outermost part of the sapwood (quality I) were more sensitive than boards closer to the pith side (quality II). These results are also consistent with earlier findings, e.g. [19–21]. However, these results, which reveal very small differences, must not be over-interpreted. Not only is the sample size small, but all test samples were also taken from the same original board. Based on our data from other, unpublished studies, the time at which mould growth first appears varies considerably among different boards, and there are differences due to the origin of the board.

Wood is a material for which there are plenty of easily accessible nutrients for mould growth. On building materials where these nutrients are expected to be lower, different patterns of mould growth may be observed due to the additional stress to the fungi. Moreover, in this study, either the RH or temperature was kept constant at favourable conditions. In actuality, both RH and temperature fluctuate simultaneously which also causes additional stress and may lead to different growth patterns.

The total incubation time is also of importance. For some of the Test schemes presented in this paper, the incubation continued for more than 84 days, up to 119 days. The percentage of test specimens with established mould growth was then slightly higher, but the pattern described above remained the same.

Different fungal species, as well as different strains of species, have different capacities to adapt to stressful environments. In this study, we used only six of the hundreds of fungal species that are common in the environment. These species were chosen to represent both fungi that demanding high amounts of water for growth as well as xerophile fungi [6]. However, how the different species would handle fluctuating water or temperature conditions was not considered. At least two of the species, *A. pullulans* and *C. cladiosporiodes*, are common on leaf surfaces or on facades [9] where these parameters fluctuate highly.

We have chosen to present our data in two ways: by describing the development of mould on the material as the median of growth on the samples and as a survival function, where the time before established growth occurs is described. We prefer the latter measure, but for the sake of comparison, we have also presented the first method because similar ways have previously been used to study the growth of mould.

5. Conclusions

It is not the mean relative humidity that a building material is exposed to that governs the growth rate of mould fungi, nor is the total time that a material is exposed to favourable conditions (when it fluctuates) the critical factor. Instead, the duration of both favourable and unfavourable conditions is decisive. Therefore, using the sum of periods favourable for growth, or the mean RH value, will not give a realistic prediction of mould growth in predictive models.

The effect of fluctuating temperatures is not as apparent when the temperature values lie in the range that supports fungal growth. From our results, it seems that the mean temperature value may be used in predictive models.

The patterns observed here were the same no matter which wooden quality or surface was studied, and the substratum does not affect how mould fungi react to stressful environmental conditions in terms of relative humidity and temperature. However, it would be interesting to further investigate whether the pattern is the same on materials that are not as nutrient-rich as wood.

In this study, one parameter, either RH or temperature, was kept constant at favourable conditions. Future studies should contain test schemes in which temperature and RH vary simultaneously.

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Properties of wood that affect mould growth. A meta-analysis.

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Properties of wood that affect mould growth. A meta-analysis.

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1   Introduction

The susceptibility to mould growth varies between materials. The differences between materials can be explained by differences in the concentration of organic compounds, which are essential nutrients for mould fungi, as well as by differences in other characteristics that may affect growth (such as pH, surface structure, etc).

In general, when there is a high content of organic compounds in a substrate, the water requirement 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, in which the content of organic compounds is expected to be low. Pietarinen et al. (2008) found the highest diversity of microbes on wooden materials.

Wood is a commonly used building material in the Swedish construction industry. It has many advantages; one of which is that it is a renewable material. However, it contains a relatively high level of carbohydrates that can be assimilated by mould fungi. Wood is, therefore, often considered to have a low mould resistance. In laboratory studies it was shown that the lowest relative humidity for mould growth, also called the critical moisture level (RH_{crit}) was lower for wood than other building materials (Johansson et al., 2012, Nielsen 2002, Viitanen and Ritschkoff 1991). However, wood is not a homogenous material and different characteristics of wooden material can affect the mould growth. Johansson and Bok (2011) found that some types of wood could withstand mould growth even after exposure to high moisture

levels for several weeks, while on others there was mould growth already after some days of exposure to the same conditions. People working in the construction industry often have different opinions regarding how common mould growth is on wood as a building material. This could be explained by differences in the use of materials.

Possible properties of wood that may affect the resistance to mould growth are wood species, drying method, felling time (winter or summer), width of annual rings and sawing pattern (the part of the cross-section of the log). Also, the surface structure may affect the mould growth. Some of these parameters have been studied (e.g. Terziev et al 1996, Johansson and Ekstrand-Tobin 2014, Johansson et al. 2013, Viitanen 1996, Sehlstedt 2011, Terziev and Boutelje 1998), however in some studies only the discolouring fungi have been taken into consideration in the evaluation. There may be extensive growth on a piece of wood although not visible by the naked eye, as shown by Johansson et al. (2013). Although the "invisible" growth do not cause any aesthetic problems on outdoor building structures, as facades or fences, mould growing inside a building may have a negative impact on the indoor air and on the wellbeing of the residents and users of the building.

The present paper compiles results from a number of studies on the effects of several properties of wood on mould growth, also such growth not visible to the naked eye. The results are based on six separate laboratory studies in which mould growth on wood was studied (Johansson and Jermer 2010; Johansson et al. 2012; Johansson, Bok et al. 2013; Johansson, Wamming et al. 2013; Johansson and Bok 2014; Johansson and Ekstrand-Tobin 2014). In the studies, properties of wood varied between test specimens. The aim of the study was to highlight the complexity of predicting the risk for mould growth on wood due to the big variation in the properties that can affect this growth among different sets of timber.

2   Materials and methods

2.1   Studies

The six separate laboratory studies in which mould growth on wood was studied (Johansson and Jermer 2010; Johansson et al. 2012; Johansson, Bok et al. 2013; Johansson, Wamming et al. 2013; Johansson and Bok 2014; Johansson and Ekstrand-Tobin 2014) were performed in the same way. Test specimens were inoculated with a spore suspension, incubated in moisture chambers, at 22 °C and 90 % RH or 95 % RH, and were analysed for mould

growth after a certain time. The procedure followed the method described in Johansson et al. (2012). The suspension containing spores from six mould fungi, *E herbariorum, A versicolor, P chrysogenum, A pullulans, C sphaerospermum* and *Stachybotrys chartarum*, was sprayed onto the surface of each test specimen. The total length of incubation varied between the studies, as did the intervals between inspection of test specimens (once or twice a week), and the number of days from the start of incubation to the first inspection. In Table 1 the design of each study is summarised.

Table 1   *Test design in the different studies, all carried out at 22°C. A=(Johansson et al. 2012), B=(Johansson, Bok et al. 2013), C=(Johansson and Ekstrand-Tobin 2014), D=(Johansson and Jermer 2010), E=(Johansson and Bok 2011), F=(Johansson, Wamming et al 2013)*

	Study					
	A	B	C	D	E	F
Set relative humidity (%)	90	90	90	90	90	95
Maximum incubation time (days)	84	63	70	42	57	21
Frequency of analysis	Once a week	Twice a week	Once a week	Twice a week	Twice a week	Once a week
No of days after start of incubation when first analysis was performed	7	4	7	4	4	7

Although the set temperature in each test was 22°C and the set RH was 90 % or 95 %, variations in the actual measured values in the different studies are expected. This is due to differences in the different chambers being used, since the same chamber was not used for all tests, and also due to measurement uncertainty in the loggers. A combined measurement uncertainty was calculated by using the calculated standard deviation and the measurement uncertainty from the calibration of loggers, according to (EA-4/02).

Both mould growth visible to the naked eye and that which was only visible under the microscope at 10–40x magnification was assessed and rated according to a five-point rating scale, see Table 2. Although the exact

formulation of description of extent of growth varies some among the published studies the criteria underlying the evaluation was the same. In Johansson, Wamming et al. (2013) there was also a class 5. In the other studies, rating 4 and 5 were merged into rating 4 since no actual differences between the two ratings could be found (Johansson 2012).

Table 2   Rating scale for the assessment of mould

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

2.2   Materials

Test specimens sized 5x10 mm were prepared for each of the materials that were to be tested. These consisted of wood with different properties, as listed in Table 3, together with the hypothesis tested.

Table 3   The characteristics of the wood investigated, together with the hypothesis tested

Parameter	Sample	Hypothesis
Wood Species	Pine	Pine wood is more susceptible to mould growth than spruce wood.
	Spruce	
Surface structure	Planed Sawed	A sawn surface will have a higher susceptibility to mould growth than a planed surface, due to rougher surface and greater area, and the microclimate is expected to differ.
Sawing pattern	Centre-board Side-board	Centre-boards are less susceptible to mould growth due to higher amount of heartwood and/or smaller amount of easy assimilated nutrients.
Face of specimen	Outside face (sapwood side) Inside face (pith side)	The outside face of the boards and planks will be more susceptible to mould growth due to increasing amounts of carbohydrates that serve as nutrients towards the periphery of the stem.
Age of surface	Original/"old" Newly prepared	A newly prepared surface will have higher mould resistance due to a "cleaner" surface with no contamination and possibly emission of substances inhibits mould growth.

The purpose of the studies was not to evaluate all of the various parameters and, therefore, it varied between the studies which parameters that were represented, as shown in Table 4. The combinations of parameters varied as well.

Table 4   *Numbers of specimens of each parameter in the various studies.*
A=(Johansson et al. 2012), B=(Johansson, Bok et al. 2013),
C=(Johansson and Ekstrand-Tobin 2014), D=(Johansson and Jermer
2010), E=(Johansson and Bok 2011), F=(Johansson, Wamming et al
2013)

		Test scheme					
		A	B	C	D	E	F
Wood species	Spruce	6	12	27	6	42	40
	Pine	-	12	-	6	-	40
Surface structure	Planed	4	18	7	12	36	40
	Sawn	2	6	20	-	6	-
Sawing pattern	Centre-board	-	0	27	6	19	40
	Side-board	6	24	-	6	23	40
Face of specimen	Inside face	2	12	-	-	21	-
	Outside face	4	12	27	12	21	40
Age of surface	New	-	12	21	-	-	40
	Original	6	12	6	12	42	40
Total number of specimens		6	24	27	12	42	80

2.3   Statistical analysis

Mould growth is an ongoing process on a material when exposed to conditions favourable to growth. No dose-response relationship between the extent of mould growth and human health/negative effect on indoor air is established. A precaution is not to allow established mould growth at all. In practical situations, it is more pertinent to determine if mould growth will become established or not. Instead of focusing on the extent of mould growth, the event that mould growth established was therefore studied.

Survival analysis was used to evaluate the mould growth data. A survival function $S(t)$ is defined as the probability to survive at least to time t. In this case survival means no established growth on the test specimen. Established growth was defined as being assessed according to rating 2, 3 or 4 (Johansson et al., 2012). The survival function can be plotted against time, and is called

the survival curve. The Kaplan-Meier estimation was used to estimate this curve, and curves were produced for mould growth on specimens for each of the parameter, in the example of sawing pattern; survival functions were produced for side-boards and center-boards. The equality of the two functions was tested by a log rank test, where the null hypothesis that there was no difference between the population survival curves was tested. Finding significance in this case means that the survival functions were not equal for the groups studied.

The log rank test is suitable to make pairwise comparisons to test whether it is a difference in the survival functions. However, in many cases, several variables affect the survival function and adjustment for variables that can affect survival may improve the precision. Cox's proportional hazard model (Cox regression) investigates the effect of several risk factors on survival at the same time. In Cox regression, the dependent variable is hazard rate. This can be defined as the probability of an individual will experience an event (in this case established growth on a test specimen) within a small time interval, given that it has survived up to a given point of time. Again, in this case survival means there is no growth on the test specimen. In order to investigate the influence of the different parameters on mould growth, Cox regression was performed of the data in the meta-analysis and of the data from the test at 95 % RH. In the meta-analysis, sawing pattern, face of specimen, surface structure, age of surface, wood species and original study were included as factors. Cox regression of data from the test at 95 % RH included sawing pattern, age of surface and wood species as factors. Pairwise comparisons of survival functions of each of the parameters were also performed by log rank test

In the end of a study, there will often be individuals that have not encountered the event. These are censored cases. In this work the censored cases consisted of test specimen on which there was no mould growth at the end of incubation. In the survival analysis, these cases are taken into account.

Bewick et al. (2004) and Rich et al. (2010) give good summaries of survival analysis and Kaplan-Meier curves. Singer and Willet (2003) provide a thorough presentation of the hazard/survival models for event occurrence.

2.4 Selection of data

The original purpose of the six studies used for this analysis differed. Therefore, there is more data than needed for this study. The first selection for the meta-study was to choose only data from laboratory studies performed at 90 % RH and 22 °C. All parameters that are evaluated in this work were not represented in all original studies.

A major assumption of the Cox proportional hazards model is that the effect of a given covariate does not change over time, meaning that the hazard function of two individual specimens must be the proportional at any point of time. If the assumption is violated, the Cox model is not appropriate. When considering the proportional hazards of the different studies in this study, this assumption of PH was not evident, as confirmed by crossing hazard ratio curves. The cox regression was performed anyhow, with studies as covariates. This showed that these had a significant effect, both when all parameters were used as covariates and when only one parameter at a time was considered. Therefore, it was not possible to evaluate the data as planned. Instead, only pairwise comparisons of each of the parameters were performed, but in each such comparison only data from the studies that contained relevant information were used. Also, there were some additional selections of data to obtain a relevant sample. The factors and studies analysed are given in Table 5.

Table 5   Selection of data for pairwise comparison in the meta-analysis (Studies at 90% RH and 22 °C). A=(Johansson, Ekstrand-Tobin et al. 2012), B=(Johansson, Bok et al. 2013), C=(Johansson and Ekstrand-Tobin 2014), D=(Johansson and Jermer 2010), E=(Johansson and Bok 2011)

Parameter studied	Studies included in the analysis.	Additional selection
Wood species	B and E, tested separately	Wood species
Surface structure	A, B, C, E	Only original surfaces were included
Sawing pattern	E	Only original surfaces were included
Face of specimen	A, B, E	Only side-boards were included, since only the outer face is represented on centre-boards
Age of surface	B, C	

The data for the second analysis, of data from testing at 95 % RH, were taken from Johansson, Wamming et al (2013). The original purpose of that study was to investigate whether different kiln drying schemes would have different effect on mould growth, and the results were compared with air dried test specimens. Test specimens were sawn, re-sawn or remained with the original surface after drying. Also, surfaces at or between stickers were studied separately. No significant difference was found between the different kiln drying methods, but there was difference between air dried specimens and kiln dried specimens. For the analysis presented here, data from kiln dried specimens, from the surface between stickers and specimens with either planed surface or original surface were selected.

3   Results

3.1   Incubation conditions

The mean value of measured RH and temperature during the incubation is shown in Table 6.

Table 6   Mean incubation RH (%) and temperature (°C) for each study.

Test scheme	RH (%)		95 % CI for mean RH (mean ± expanded measurement uncertainty)		Temperature (°C)	
	Mean	Standard deviation	Upper bound	Lower bound	Mean	Standard Deviation
Study A	89.4	0.7	90.8	88.0	22.2	0.2
Study B	88.8	1.5	90.2	87.4	22.5	0.2
Study C	91.4	1.2	92.8	90.0	22.1	0.2
Study D	88.8	1.5	90.2	87.4	22.5	0.2
Study E	91.7	1.4	93.1	90.3	22.1	0.2
Study F	95[1]				22[1]	

[1] There is no data logged for this test scheme and therefore no statistics can be calculated. Manual readings from the display of the transmitters during incubation ensured that the actual values were approximately in accordance to the set values.

3.2   Mould growth

3.2.1   Mould growth on test specimens in the different studies

The median time before mould established on test specimens on wood at constant conditions at approx. 90 % RH and 22 °C was 14 (CI 95 %. 11.5 to 16.5) days. Within one week. 20 % of all test specimens had mould growth that equalled or exceeded rating 2. In Figure 1, the survival function is described as a Kaplan-Meier curve.
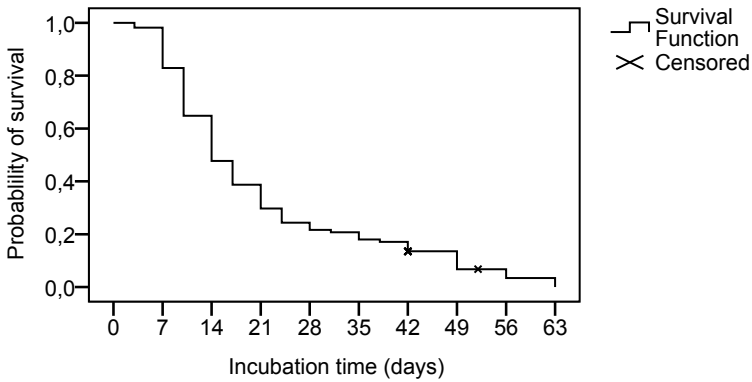
*Figure 1   Kaplan-Meier plot of all test specimens in all studies with set RH 90%
            and set temperature 22°C.The censored cases refers to the termination of
            some studies were there was no mould growth on the censored cases*

The survival time, that is incubation time before mould growth established,
varied both within each test scheme and among the different studies as shown
in Figure 2.
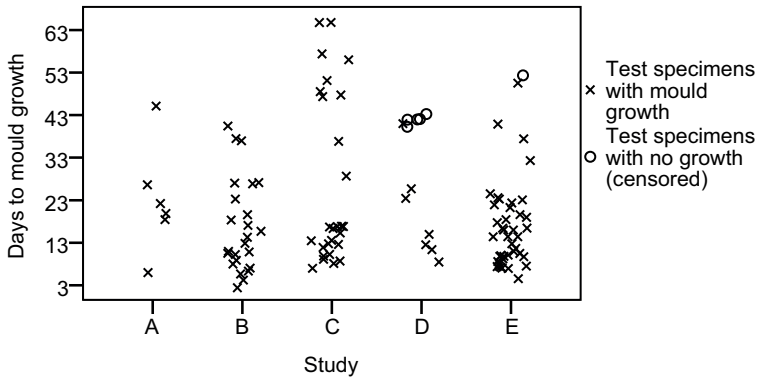

*Figure 2   Incubation time to mould growth for all test specimens in the different
            studies. The censored cases are specimens where there was no
            established growth at the termination of incubation in that particular
            study.*

The minimum survival time was 4 days, the maximum 63. The survival
functions of the five test were not equal, as determined by the log-rank test,
Chi^2 (4)=14.9, p=0.005. Log-rank pairwise comparison was run to determine
which studies had different survival distribution. A Bonferroni correction was
made with statistical significance accepted at the p<0.005 level (p value at
0.05 level divided by 9 comparisons). There was statistically significant

difference between Test schemes D and E ($Chi^2(1)=9.9$. p=0.002) and between Test schemes B and D ($Chi^2(1)=10.3$. p=0.001). No statistically significant difference was found between the other tests in this pairwise comparison.

The median survival time for test specimens incubated at 95 % RH was 7 days. The survival function is shown in Figure 3.
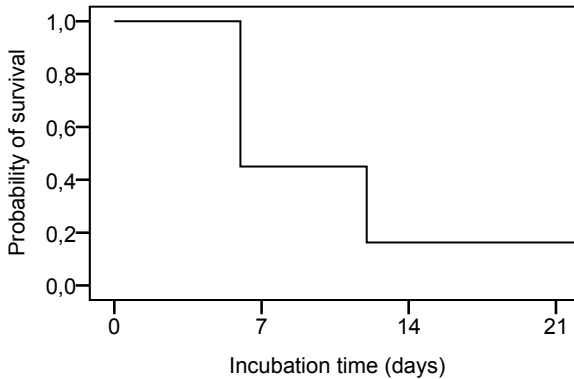

Figure 3   Kaplan-Meier plot of all test specimens in all studies with set RH 95% and set temperature 22°C.

3.2.2   The effect of different characteristics of wood on mould growth

After selection of cases, as described in Section Table 5, a pairwise comparison of the different parameters tested was performed. In several of these comparisons, data originated from two or more studies. The studies had in neither of the cases any significant effect on the hazard as determined by Cox-regression, except in the wood species case. Therefore, the effect of this parameter was analysed separately for both studies.

The survival functions shown as Kaplan-Meier curves are presented in Figure 4. In Table 7, the results from the comparisons of survival functions are shown, together with the median survival time. Significant differences were found for all parameters except for face of specimen.

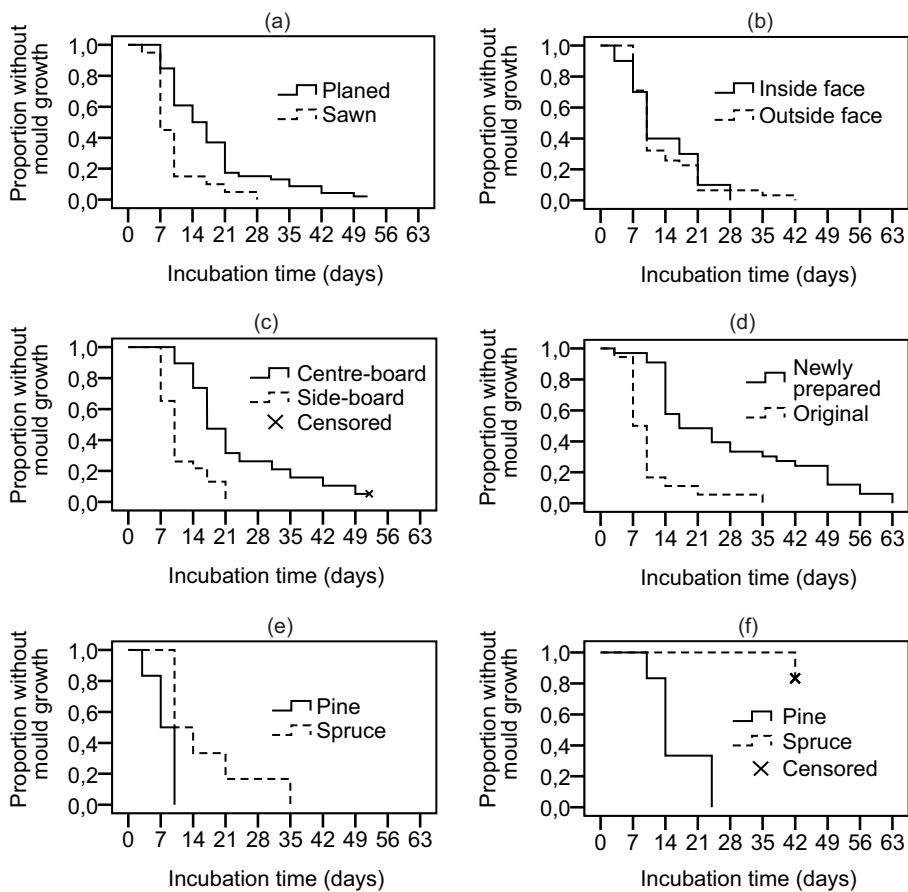*Figure 4   Proportion of test specimens on which there was no established mould growth (rating < 2) over time (days)at 90 % RH and 22°C, for the selected cases described in Table 2.; a=surface structure (p<0.001), b=face of specimen (p=0.996), c=sawing pattern (p<0.001), d=age of surface (p<0.001), e=wood species Study B (p=0.018) f=wood species Study D (p<0.001).*

Table 7   *Medians for survival time (days) and results from comparison of survival functions. Study B=(Johansson, Bok et al. 2013), Study D=(Johansson and Jermer 2010)*

Parameter			Number of test specimens	Median	95% Confidence Interval for median		
					Lower Bound	Upper Bound	Sign.
Surface structure	Planed		46	14	9.7	18.2	<0.001
	Sawn		20	7	5.3	8.7	
Face of specimen	Inside face		10	10	6.9	13.0	0.996
	Outside face		31	10	8.7	11.3	
Sawing pattern	Centre-board		19	17	13.3	20.7	<0.001
	Side-board		23	10	8.6	11.4	
Wood species	Study B	Pine	6	7	3.7	10.4	0.018
		Spruce	6	10	-	-	
	Study D	Pine	6	14	11.0	17.0	0.001
		Spruce	6	[a]	[a]	[a]	
Age of surface	Newly prepared surface		33	17	7.6	26.4	<0.001
	Original surface		18	7	4.9	9.1	

[a] no statistics could be computed due to the presence of too many censored cases at the end of incubation

Figure 5 shows the results from testing at 22°C and 95 % RH. Pairwise comparison revealed statistically significant differences between the survival functions for the three parameters evaluated as illustrated by the *p* values.
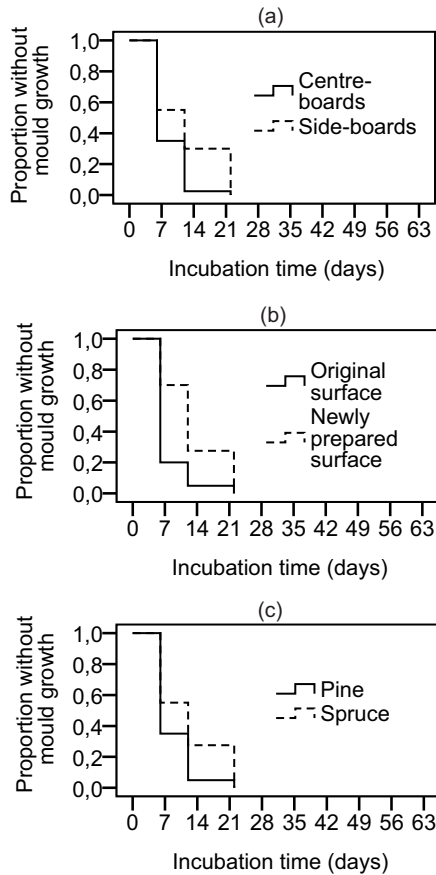
Figure 5   Proportion of test specimens on which there was no established mould growth (rating < 2) over time (days) 95% RH and 22 °C. (a)=Sawing pattern (p=0.002), (b)=age of surface (p<0.001), (c)=wood species (p=0.008).

The output of the Cox-regression applied to the data from testing at 95 % RH is shown in Table 8. The *p* values indicate that the age of the surface and wood species have an impact on mould growth. However, the effect of sawing pattern was not statistically significant in this model. The exponent (B) values indicate that mould growth at an original surface is 1.9 times as likely as on a newly prepared surface and mould growth on pine is 1.6 times as likely as mould growth on spruce.

Table 8   Application of Cox's regression data, using age of surface, sawing pattern and wood species as explanatory variables

	Coefficient (B)	Standard error	Sig.	Exp(B)	95,0% CI for Exp(B)	
					Lower	Upper
Age of surface	0.623	0.240	0.009	1.865	1.165	2.987
Sawing pattern	0.430	0.238	0.070	1.537	0.965	2.450
Wood Species	0.472	0.238	0.047	1.602	1.006	2.553

4   Discussion

At 90 % RH and 22 °C, mould growth on wooden material is likely to occur. The longer the wood is exposed to this condition, the higher the hazard for mould growth. However, even in as short time period as within a week. mould growth will appear at some test specimens. Differences in time to established growth were found among the five studies at 90 % and 22 °C. One possible reason is that actual incubations condition varied between the different studies; the mean RH was in some of the tests higher than the others. When considering the 95 % confidence interval for the mean of each test scheme it appears that there were no actual differences in incubation RH, since the confidence intervals overlapped. Further, it was assumed, based on earlier findings (Johansson et al. 2012) that the small differences found would not have extensive effect on mould growth. An additional result that indicates that the RH levels involved an equal risk of mould is that there was a significant difference in mould growth between D and E, although the incubation conditions were identical. Another difference among the tests schemes were the total incubation time and the difference in time interval of analysis. However, the analysis used are robust for those changes, as was tested by using 42 days as an upper incubation limit in analysis. The results then showed the same pattern.

Based on the above discussion, the differences among the studies may be due to the different properties of wood, and it was found relevant to perform a meta-analysis and a Cox regression of the data.

In the original setup of the individual studies, there was no intention to compare the results between the studies. Therefore it varied among the studies which parameters that were studied and all factors were not represented in all

cases. Although this meant that it was not possible to perform the meta-analysis that was originally planned, i.e. analysing all test results in the same analysis, pairwise comparisons were performed, using data only from relevant studies for each factor analysed. At both RH tested, there was a significant difference in mould growth between the wood species investigated; pine being more susceptible to mould growth than spruce. This has also been reported in other studies (Viitanen 1996).

Centre-boards were less susceptible to mould growth than were side-boards. This may be an effect of higher amounts of nutrients on the surface of side-boards. In the living, growing tree, sugars from photosynthesis are transported from the leaves along the branches and into stem. Therefore, the concentration of sugars is higher in the side-boards than in the centre-boards. Some centre-boards will contain some heartwood, which is generally considered to be less susceptible to mould growth than sapwood. No statistically significant difference in survival functions was found in the test at 90 % RH when considering the face (inner or outer) of the test samples of side-boards, which indicates that there was no difference in sugar content that affects the mould growth on these both sides. However, neither the sugar content nor the presence of heartwood was analysed in the present study, and no conclusions can thus be drawn regarding their effects.

In the tests at 90 % RH, the survival functions for planed and sawn surfaces differed significantly on original surfaces, which indicates that surface roughness has an effect on mould growth; sawn surfaces being more susceptible to mould growth than planed surfaces, which is in agreement with previous findings (Terziev et al. 1996). In one of the studies included in the meta-analysis, (Johansson and Ekstrand-Tobin 2014), no difference in mould growth was found between test specimens that were planed, fine-sawn or coarse-sawn. All the surfaces were then newly prepared, and it was concluded that the lack of any difference could be the consequence of only small differences in roughness, or the consequence of the surface treatment itself. A newly prepared surface may changes in the chemical composition at the surfaces, which may affect the mould growth. This is supported by the other results in, since statistically significant differences were found between the original surfaces and newly prepared surfaces; the new surfaces were less susceptible to mould growth. However, this parameter may not have any effect on the mould growth in actual buildings as newly prepared timber is not used in construction. This issue must however be considered when testing different sorts of wood in the laboratory.

The results from the meta-analysis cannot be used to draw definite conclusions of the general susceptibility to mould growth on wood. Representation of parameters in the different studies varies and the number of test specimens for each parameter is unbalanced. The study highlights the complexities associated with the prediction of mould growth on wood and can be considered exploratory. The results can therefore be used when designing future studies and in selection of materials to be tested.

In this study the tests were performed at conditions which are very favourable to mould growth and there was mould growth on all (at 95 % RH) or on the majority (at 90 % RH) of the test specimens in the end of each study. At conditions less favourable, it is possible that the effects are even more evident. Future tests should therefore include also testing at lower RH, for example following the Critical Moisture Level (CML) method described in (Johansson 2014).

5   Conclusions

Several material parameters affect the time required for mould growth on wood. It is not possible to predict the general susceptibility of wood to mould growth based only on a few data as it is affected by parameters as surface structure, wood species and sawing pattern. Also, the susceptibility cannot be described by one single parameter, but depends also on other parameters.

If predictions are to be made of the mould susceptibility of wood in general, a large number of samples with different properties must be tested, as well as any recent altering or preparation of the surface.

When testing any treatment of a wooden material, it is important that untreated material originating from the same basic material is used as a reference. Otherwise, there is a risk that the characteristics of different types of wood will be studied, and not the effect of the treatment.

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