Journal of Building Engineering 50 (2022) 104085

Contents lists available at ScienceDirect

Journal of Building Engineering

journal homepage: www.elsevier.com/locate/jobe

Serviceability limit state model for fungal growth on wood materials in the built environment

Robert Lepage [a,b], Samuel V. Glass [c], Paul de la Bastide [d], Phalguni Mukhopadhyaya [a,*]

[a] Department of Civil Engineering, University of Victoria, 3800 Finnerty Rd, Victoria, BC, V8P 5C2, Canada
[b] RDH Building Science Consulting Inc., 602-740 Hillside Ave, Victoria, BC, V8T 1Z4, Canada
[c] USDA-FS Forest Products Laboratory, 1 Gifford Pinchot Dr, Madison, WI, 53726, USA
[d] Centre for Forest Biology, Department of Biology, University of Victoria, 3800 Finnerty Rd, Victoria, BC, V8P 5C2, Canada

ARTICLE INFO

Keywords:
Biodeterioration
Wood
Fungi
Serviceability limit state

ABSTRACT

Fungal (mould) growth in wood based building components could have significant detrimental effects on the operational and structural requirements of the built environment. Quite naturally, building designers and material scientists are keen to predict mould growth in wood and wood based building materials. Available surface mould growth models primarily predict the levels of mould growth in different environmental and material conditions. However, modern design practices allow acceptable limit of risk in the design process. Hence, a probabilistic mould growth model is required which takes into account risk tolerance thresholds for mould growth. This paper presents a research initiative that shifts the mould growth narrative from predicted levels to probabilistic predictions, which is the foundation block associated with limit state design approach, widely used in structural design codes and standards. More specifically, this paper presents a serviceability limit state mould growth model for wood using jack pine (*Pinus banksiana*) wood and the fungal species *Penicillium chrysogenum*. The outputs from this model were verified using the results obtained from an experimental roof study that investigated the impacts of different surface treatments on mould growth in ventilated roofs.

1. Introduction

Through natural processes, buildings succumb to biotic and abiotic deteriorating forces. Biotic factors, mainly those of bacteria, fungi, and invertebrates, are orders of magnitude more destructive than abiotic factors, and as a result, are keenly researched in efforts to minimize damages. For buildings, the environmental disturbances caused by construction and material manufacturing provide suitable organic substrates sufficient to sustain microbial succession from the more pioneering and damaging of organisms: the fungi.

The damages caused by fungi on buildings can be extensive: they include life-safety risks by way of rot and decay of primary structural members; short- and long-term health impacts from surface moulds that can cause a range of allergic responses, from mild to fatal, or mycoses of immunocompromised persons; monetary damages, from reduced property value and fungal abatement expenses; to psychological symptoms, including psychosomatic effects and concerns for surface fungal contamination and aesthetics. As a result, significant interest is placed in understanding the epidemiology of fungal contamination in buildings, which is reflected by the significant literature that attempts to quantify the risk of building damage due to fungal contamination.

* Corresponding author.
E-mail address: phalguni@uvic.ca (P. Mukhopadhyaya).

https://doi.org/10.1016/j.jobe.2022.104085
Received 3 December 2021; Received in revised form 11 January 2022; Accepted 18 January 2022
Available online 10 February 2022

Currently, most surface mould growth models attempt to quantify or qualify the amount of mould growth that could be expected, given numerous environmental and material parameters over time. However, decision making for remediation of fungal damage occur not based on the quantity of fungi, but rather on its presence or absence. As such, a model that assists in evaluating the risk of fungi is needed, one that provides the range of probabilities of finding growth such that, when taking risk tolerance thresholds into account, appropriate design metrics can be devised. The objective of this research is to shift the mould growth narrative from predicting states and quantities of mould into a probabilistic prediction, which is conceptually aligned with limit-state approach to design. This paper provides a serviceability limit state (SLS) model for surface mould growth on wood building materials.

2. Background

2.1. Fungi growth requirements

The field of mycology has identified many of the fundamental environmental characteristics needed to support the growth of fungi [1–3]. In no particular order, these include.

- Presence of viable fungal spores and/or mycelium
- Adequate temperature,
- Sufficient moisture,
- Nutrient substrate (including trace chemical growth factors),
- Oxygen (species dependent), and
- Lack of biocidal agents (suitable pH, no toxic materials)

Most of these conditions are present in the built environment and so could pose a range of risks to both building and occupants, from potential health impacts to physical damage of the building.

2.2. Fungal growth models

Many existing fungal growth models incorporate a wide-range of techniques and equipment, from long-term in-situ field studies with qualitative evaluations to laboratory studies of fungal growth on agar plates. A summary of mould models and their input assumptions is provided by Refs. [4–6]. However, a pressing need in the building industry is a biodeterioration model that is calibrated to the procedures, equipment, and skillsets available to building professionals.

Building condition assessments frequently use a suite of tools to evaluate the condition of the building, ranging from instantaneous, short-term, or long-term readings that may be qualitative and non-destructive (visual, olfactory, tactile), quantitative and non-destructive methods (RH readings, IR thermography, dielectric surface moisture readings), nominally destructive (electrical resistance moisture readings), to destructive (coring, sampling). Therefore, the methods used to define a fungal growth model should ideally incorporate these same procedures and techniques such that the results may be readily transferable to those building practitioners.

2.2.1. Limit State Design

Limit State Design (LSD) is a design methodology commonly used in structural engineering whereby a limit state (e.g., a potential failure mode), is defined by a probabilistic failure envelope. A serviceability limit state (SLS) is primarily concerned with fungal surface disfigurement and growth, with a focus on the potential health impacts to the occupant. However, for fungal growth, the definition of failure is challenging to identify. The theoretical limiting failure state for surface fungal contamination is the point at which it starts to negatively affect human health. Unfortunately, these limits are highly variable among persons and are thus challenging to use as a quantitative threshold [7–9].

The fields of fungal epidemiology and industrial hygiene have extensively studied the effects of fungi on occupants. These matters are best discussed in the more relevant literature, but in general, it is safe to conclude that the effects of fungi are minimal on healthy occupants [7,10] but may be severe for the immunocompromised and those susceptible to allergic responses with exposure to fungal contaminants [11]. As it is a matter of prudence and good ethical practice to design safe and healthy buildings for all potential occupants, the failure threshold for fungi for this research is defined as the point where it is just visible with the unaided eye. This roughly corresponds to the point at which the pigmented conidia are visible, and dispersed conidia and hyphal fragments are known to cause allergenic responses in persons [12].

Limit state frameworks have been used in the development of other fungal growth models [13,14]; however, their output, as a relative dose or a growth index, is not as conducive towards estimating probabilities of growth. As such, an SLS model for fungal contamination is the point at which the probability of fungal growth detection exceeds the authority having jurisdiction's acceptable risk tolerance threshold.

2.3. Objectives

Following the review of the existing mould model literature, the primary objective of this research is to develop a fungal growth model with the following key attributes:

- Accepts dynamic and varying environmental conditions in multiple time formats
- Yields a probability of mould growth, with guidance on proper interpretation based on risk thresholds
- Provides the flexibility to readily incorporate preservative treatments or a range of different substrates

Table 1
Explanatory and response variables used in fungal modeling.

Category	Code	Variable	Description
Explanatory Variables			
Environmental Variables	T	Temperature	Ambient climate chamber set point temperature (°C)
Internal Variables	MC	Moisture Content	Specimen total moisture content as measured gravimetrically
	S/H	Substrate	Jack Pine (*Pinus banksiana*) sapwood (S) or heartwood (H)
	S_f	Fungal Spore Load	Fungal potential, viability, and inoculum spore load, determined through laboratory procedures
Uncontrolled Variables	S_B	Biological Antagonist	Inherent substrate biological resistance
	S_f	Fungal Variability	Inherent variability in fungal growth patterns
	S_n	Substrate Nutrition	Inherent substrate nutritional profile
	S_C	Contamination	Contamination and fungal competition
Response Variable			
	P(G)	Probability of Growth	Probability of detecting growth (0–100%)
	dP(G)/dt	Rate of Change of Probability of Growth	Rate of change of probability of growth per unit time.

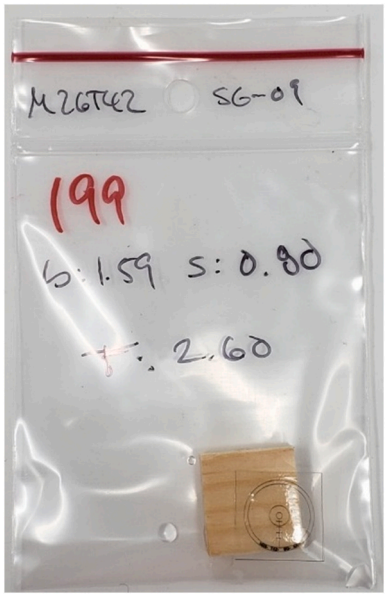


Fig. 1. Individually wrapped specimen in a sealed polyethylene bag, with labels for mass of each component.

- Requires no special training and is intuitive and feasible for use in spreadsheet type software

The underlying research used for the development of the model must also be:

- Founded on the tools and training available to many building practitioners (e.g., specialized equipment should be avoided)
- Readily repeatable by other researchers to expand the model's functionality by controlling as many of the variables as possible

A secondary objective is to develop a database of fungal growth on a North American wood species with a relevant and easily accessible fungus.

3. Methods

Fungal growth is influenced by a number of parameters, many of which cannot be readily identified or controlled. Those controllable and known parameters, as well as a short list of potential unknown effects, are provided in Table 1.

The wood specimens were carefully prepared to control their moisture level; after sterilization in an oven, they were immediately placed in labelled and sterile plastic bags. They were then raised to their target moisture content using sterilized and distilled water and, upon equilibration, were then carefully inoculated with a known fungal suspension. The bags were then placed in temperature controlled climate chambers. The specimens were periodically photo-documented and their moisture contents were adjusted to their target levels.

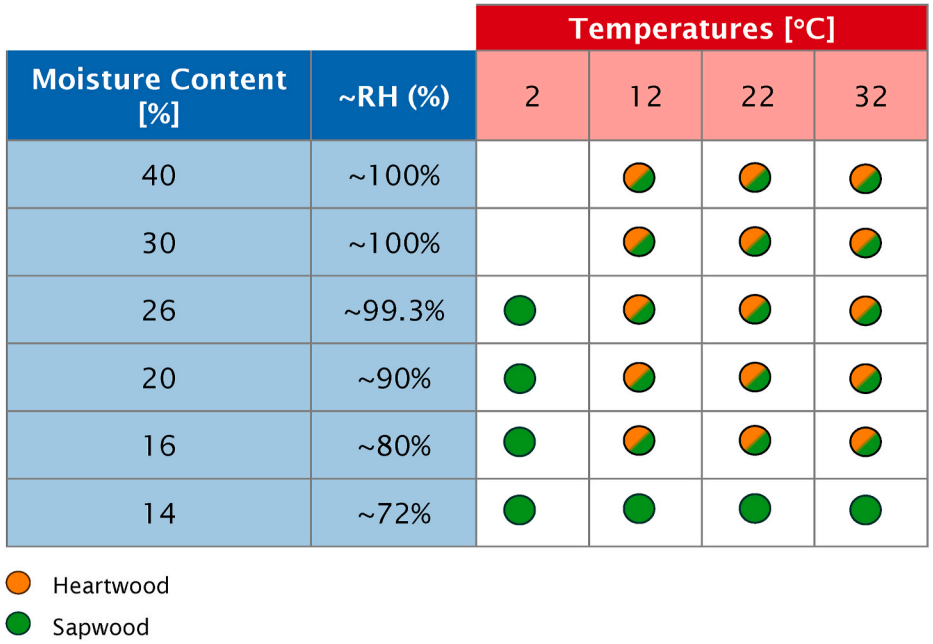


Fig. 2. Temperature and moisture content parameters, with RH approximates, for sapwood and heartwood specimens indicated by green or orange, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.1. Specimen preparation

Small wood chips, 12mmx12mmx6.3 mm, in longitudinal, radial, and tangential orientations respectively, of either sapwood or heartwood were cut and planed from the same jack pine (*Pinus banksiana*) tree. *P. banksiana* was selected due to its widespread geographical distribution and its susceptibility to fungal growth [15]. It is also closely related to lodgepole pine (*Pinus contorta)* and both find use in lumber for rough construction [16,17]. A total of 265 specimens were prepared with 130 sapwood specimens, 108 heartwood specimens, and 27 mixed specimens, which contained both sapwood and heartwood. ASTM D4442, *Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Base Materials* [18], was followed in general conformance in drying the specimens, with the exception of setting the drying temperature to 74±2 °C instead of 103±2 °C, to minimize drying damage to wood cellular structures. This temperature is also aligned with the final drying temperature in the kiln drying process [16] and should therefore be reflective of sterilization potential in store bought kiln dried (KD) lumber. The specimens were verified for heartwood content at the end of the experiments by following AWPA A49-15*, Standard for Determination of Heartwood in Pines and Douglas-Fir* [19].

The dry mass for each specimen was recorded and specimens were sealed in their own individual 6mil (0.15 mm) polyethylene bags (see Fig. 1), to minimize contamination and control the moisture content of the specimen. Prior to placement in each bag, each specimen was subjected to 45 min of UV-C light for additional surface sterilization. The specimens were prepared in accordance with the test protocols; they were brought to their target moisture content using sterilized distilled water on February 7th, 2021 and left to equilibrate until they were inoculated with the fungal suspension on February 15th, 2021. The bags were then placed in their respective climate controlled chamber, set to 2 °C, 12 °C, 22 °C, and 32 °C.

The experiment was terminated on June 20th, 2021, when the specimens were washed in *o*-Anisidine Hydrochloride and Sodium Nitrite heartwood indicator to confirm their conditions, in accordance with AWPA Standard A49-15 [19]. The total duration of the experiment was 126 days.

3.2. Fungal inoculation

Successful colonization depends on several factors. Pre-colonization time, larger inoculum, and reduced fungal competition all have a strong impact on competitive dynamics when other biodeteriorative agents are introduced [20]. To minimize the variables, it is simplest to use a single fungal species that best represents the most typical conditions experienced. This reduces variability, and thus potential sources of error, while also ensuring greater repeatability of the experimental system for other researchers. Due to its ubiquity in the built environment [21], low health risk (Level 1 biohazard), dark pigmentation for visibility, and ease of conidial harvesting, *Penicillium chrysogenum* was the selected fungus of choice. The culture derives from the UAMH mycological herbarium (UAMH #6525, MycoBank #165757). The process of preparation of the spore suspension closely follows those of Mil-Std-810G Method 508.6 [22]and ASTM C1338, *Standard Test Method for Determining Fungi Resistance of Insulation Materials and Facings* [23].

Penicillium chrysogenum was cultured in accordance with the producer's instructions, on 2% potato dextrose agar (PDA) in 100 mm × 15mm plates and incubated at 20-25 °C. In a sterile environment (biohazard safety cabinet), the cultured plates were flooded with a non-toxic detergent (0.1% Triton X-100) while the culture was scraped with a sterilized glass rod. The suspension was then passed through an autoclaved glass funnel with approximately 6 mm of glass fiber filter placed in the stem. An aliquot of the suspension was

taken and diluted with purified water to obtain a spore count estimate with a haemocytometer. The aliquot was vortexed for approximately 15 s to ensure a homogeneous distribution of the spores and the counted volume was extracted near the middle of the conical tube. A second aliquot was taken to assess the spore viability. This aliquot was diluted with purified water to target approximately 100–200 spores and inoculated on 2% PDA plates. These viability plates were then incubated at 20 °C and counted for Colony-Forming Units (CFUs). The ratio of CFUs to the estimated spore count delivered provides the spores viability ratio.

Once the spore density estimates and viability estimates were completed, the suspension was vortexed again and diluted with purified water to obtain the final spore suspension. The wood specimens were inoculated with identical concentrations of spore suspensions of *P. chrysogenum* one week after they were brought to their target moisture content to enable moisture equilibration. A 10 μL aliquot of the 100x diluted spore suspension was deposited in the middle of each specimen, yielding approximately 1000 ± 100 spores per specimen.

A crucial challenge with growing fungi is controlling contamination from ambient sources, as well as re-colonization from other specimens within the sample set. For repeatability, establishing a single colonization event is essential in determining the net probability of fungal growth. As such, inoculation of a set of specimens that are individually contained, but able to be readily photo-documented to estimate growth parameters is required. The selected solution was to use a sealable transparent polyethylene bag as shown in Fig. 1.

3.3. Environmental parameters

It is known that environmental parameters have a significant impact on fungi. The primary environmental variables of concern are the moisture level of the wood specimens and their ambient temperature. Several experiments have already yielded isopleths of germination time and growth for various species of fungi [24–26]. This experiment uses similar temperature ranges, which also occur commonly in the built environment, but the moisture metric used was water activity. The proposed moisture parameter for this research is moisture content, and the merits are discussed in further detail below. Fig. 2 shows the temperature ranges and moisture content ranges, with the approximate relative humidity value determined from the equilibrium moisture content to relative humidity relation (EMC-RH) of Chapter 4 of the Wood Handbook [16]. The coloured icons represent the specimen substrate type for each permutation.

3.3.1. Moisture

Multiple moisture metrics are available and have been discussed at length in published literatures [27–29]. Generally, water activity (Aw) is used by food scientists, relative humidity (RH) and equilibrium relative humidity (RH and ERH) are used by building scientists and engineers, moisture content (MC) is used by wood scientists, and water potential (Φ) is used by mycologists and plant pathologists. From a cellular metabolism level, water potential appears the most closely related to the relations between the fungal cell wall and the environment, but due to its extreme challenge in direct measurement, an alternative is required. While air humidity levels (Aw and RH) are frequently used in mould models, these have limited resolution as water vapour pressures approach saturation. Moisture content, on the other hand, can account for liquid water effects (condensation, leaks), whereas these would be considered practically the same using an air-based moisture metric (e.g., at or near saturation). Further, moisture content is a direct measurement of the substrate surface and sub-surface conditions; relative humidity characterizes a general area in proximity to the surface but is subject to significant spatial variations due to temperature variations. Lastly, the moisture storing capacity of a porous substrate lends itself for use with a moisture content metric as it retains a degree of memory of prior conditions (e.g., a leak that occurred a few days prior could still be detected). These factors favour moisture content when evaluating fungal growth on wood substrates. Moisture content is also known to influence fungal growth independently from just water activity [30].

3.4. Apparatus

3.4.1. Climate chambers

Climate controlled chambers were designed and constructed to house the specimens. Heat was provided through a large surface area electric resistance mat, and cooling, for those chambers that required it, was provided with Peltier cooling fans. Internal mixing of the airspace was provided with two continuously running fans. The heating and cooling devices were operated by Love Controller switches (TS2 and TSS2). Temperature and humidity sensors (Hobo UX100-01) were placed centrally in each climate chamber. One was also placed within the laboratory space to determine ambient conditions. The sensors have an accuracy of ±0.21 °C and a resolution of 0.024 °C.

3.4.2. Moisture content measurements

The moisture content of the specimens were determined gravimetrically using a Sartorius Quintix scale (Quintix 5102-1 S), with 10 mg resolution. For the average weight of the specimens (2.64g), this provides a moisture content resolution of approximately ±0.4%.

3.4.3. Microscopy

Each specimen was photographed periodically with a digital USB microscope set to a 20× magnification. A total of 24 readings were taken, spaced on average every 6 days, with a standard deviation of 2.1 days, with the shortest elapsed time being 1 day (early in the tests to capture initial growth), and the longest being 10 days, near the end of the testing once the growth rates were plateauing.

A USB microscope (x10-x220) (Dino-Line AM7915MZT) was used to photograph the specimen. At a x20 magnification, it has a field of view of 19 mm × 14 mm and depth of field of 2.5 mm. A jig was devised to ensure uniformity in depth and placement for each specimen shown in Fig. 3. For consistency in lighting conditions, the specimens were photographed in a light-box (see Fig. 3). Each individual specimen bag had a label and target adhered to the surface, with dimension features (circles and bars) to assist in

Fig. 3. Photo documentation set-up, with USB microscope showing individually sealed specimens.

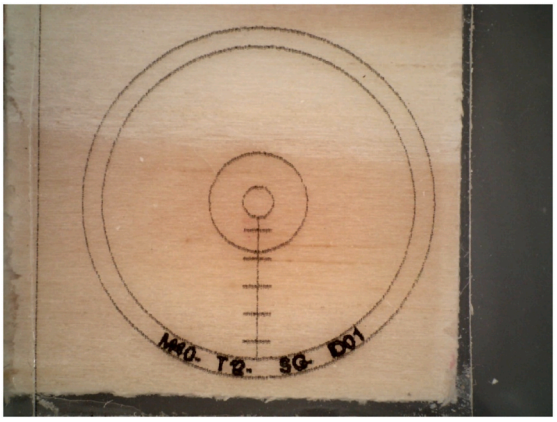


Fig. 4. Specimen target, labelled with MC, Temp, Type, and ID Number. In increasing size, the inner to outermost rings represent 1 mm^2, 10 mm^2, and 100 mm^2 in area; the vertical bars are in units of 1 mm lengths.

measurement of fungi growth characteristics (Fig. 4).

The photographs for each specimen were evaluated for any signs of fungal growth by sequential comparative analysis using Timelapse2.0 software and were exported as a file to be processed in the R programming language (R Studio Version 1.3.1093). The post-hoc tests (final moisture content and heartwood indicator) permitted the samples to be reclassified into respective moisture classifications and labelled into either heartwood, sapwood, or mixed wood.

4. Results

The recorded data from the temperature sensors in the climate chambers and the individual gravimetric moisture readings and mould growth ratings for each specimen are provided in the respective sections below.

4.1. Climate chamber temperature

Temperature control was lost briefly due to a short-duration power outage. The 2 °C and 12 °C climate chamber transformers also failed briefly during the test and were replaced within two days. The replacement transformer on the 2 °C climate chamber failed again at the end of May and so the specimens were relocated into a laboratory refrigerator. The replacement transformer for the 12 °C chamber also failed at the end of June, but as the experiment was nearing its end-date, a replacement was not procured. The qualitative statistical description on the temperature setting for each sensor is provided in Table 2. The 2 °C chamber experienced the largest variations, with a standard deviation of 3.2 °C, with a mean temperature of 2.19 ± 0.12 °C. The standard deviation of the temperature

Table 2
Climate chamber statistical values, including mean, including the 95% confidence interval, and the standard deviation.

Temperature Setting	Mean [°C]	Standard Deviation [°C]
2	2.19 ± 0.12	3.280
12	11.9 ± 0.16	0.157
22	22.7 ± 0.02	0.654
32	30.8 ± 0.01	0.241
Ambient	23.3 ± 0.03	0.868

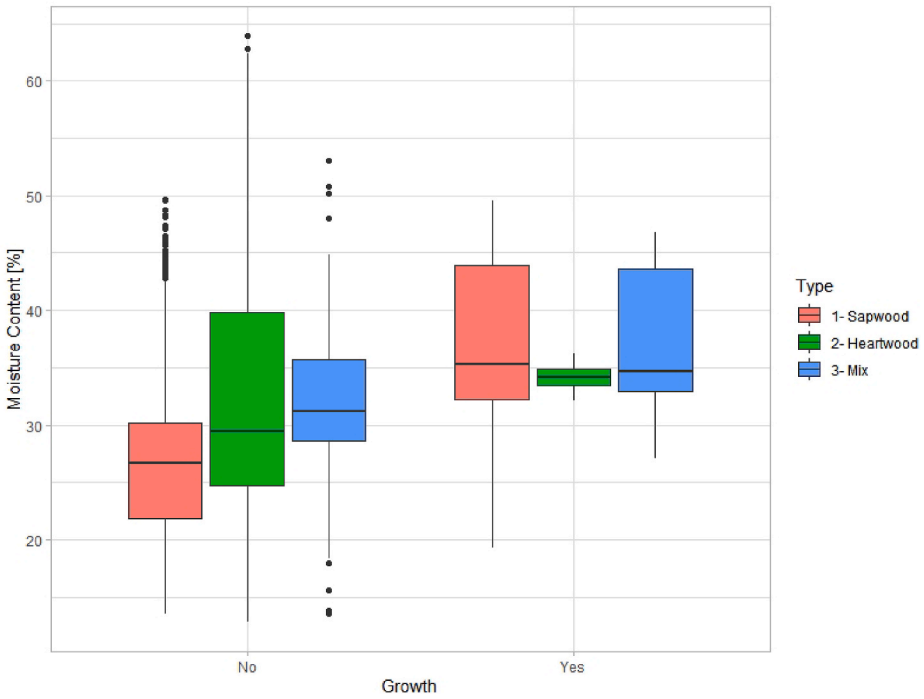


Fig. 5. Boxplot showing range of moisture contents by specimen type as a function of whether growth was observed.

Table 3
–Number of specimens exhibiting fungal growth and cohort replicates by moisture content, temperature, and type, with colour shading into quantiles.

Moisture Class	Type	Temperature (°C)			
		2	12	22	32
<20	**1- Sapwood**	0/1	0/10	0/6	1/11
<20	**2- Heartwood**	0/2	0/9	0/5	0/4
<20	**3- Mix**	0/2	0/2	#N/A	#N/A
~22	**1- Sapwood**	0/5	0/8	4/9	0/8
~22	**2- Heartwood**	0/4	0/4	0/5	0/9
~22	**3- Mix**	0/1	#N/A	#N/A	0/1
~28	**1- Sapwood**	0/2	2/7	6/9	2/9
~28	**2- Heartwood**	0/2	0/8	0/7	0/8
~28	**3- Mix**	#N/A	1/2	1/3	#N/A
~32	**1- Sapwood**	0/1	8/8	6/7	2/6
~32	**2- Heartwood**	#N/A	0/4	1/7	0/7
~32	**3- Mix**	#N/A	3/3	1/4	#N/A
~42	**1- Sapwood**	#N/A	6/6	9/10	3/6
~42	**2- Heartwood**	#N/A	0/8	0/7	0/8
~42	**3- Mix**	#N/A	2/3	1/1	3/5

on the remaining climate chambers were below 1 °C.

4.2. Specimen moisture contents

Each specimen was measured periodically to ensure it remained within the targeted moisture content level. For each specimen, the

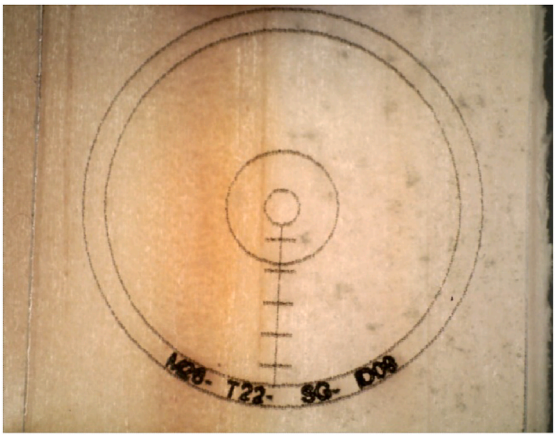


Fig. 6. Mixed sapwood/heartwood specimen at 26% MC and 22 °C after ~3.5 months, exemplifying fungal growth on the sapwood (right side).

95% confidence interval for the targeted MC was ±0.71%, with a mean spread of 2.5%MC over the 135 days that the experiment took place. The moisture content for each specimen is shown in. Only 5 of the 265 specimens had significant short-term deviations from their target MC. During the drying process, there was a delay in measuring some of the specimen dry moisture content mass, and so at the end of the experiment, all specimens were redried and their updated moisture contents were corrected. It is for this reason that not all of the specimens necessarily land near their target, but they were maintained at the same moisture content throughout the duration of the test.

4.3. Surface mould growth

A boxplot showing the relationship between moisture content, specimen type, and whether growth is observed is shown in Fig. 5.

This preliminary visualization of the relationships between the data clearly show that moisture content and type of substrate have a significant impact on growth. For each temperature and moisture content category pairing, the number of specimens showing evidence of fungal growth and the total number of specimens is shown in Table 3, with the intensity of the cell shading indicating the quartile fraction of total specimens experiencing growth (i.e., darkest shade of green indicating 100% growth, no shading indicating no growth).

A photograph of Specimen M26-T22-SG-ID09 is provided in Fig. 6 to show a readily visible fungal growth on the sapwood of a mixed specimen, with no growth on the heartwood side.

5. Analysis

The population growth equations describe the relationship between organism abundance to its environmental carrying capacity. Fungi growing on a substrate are subjected to similar growth parameters and can therefore be described by the population growth rate equation (1),

$$\frac{dP}{dt} = r \cdot P\left(1 - \frac{P}{K}\right) \tag{1}$$

where, P is the number of organisms in a given environment, r is the intrinsic growth rate, and K is the environment's carrying capacity. More elaborate models will use a K parameter that is itself a function of other explanatory variables. Integrating equation (1) yields the population growth function (2):

$$P(t) = \frac{K}{1 + \left(\frac{K - P_0}{P_0}\right) e^{-rt}} \tag{2}$$

where, P_0 represents the initial starting conditions. This equation is a variation of the generalized logistic equation (3) [31],

$$f(t) = \frac{L}{1 + e^{-r(t - t_0)}} \tag{3}$$

where, L is the asymptote, r is the growth rate, t is the time-step and t_0 is the translational shift for the $t{=}0$ value. In this application, fungal growth is defined as a binary condition, with 0 representing no observed growth and 1 representing observed growth, and is described by the binomial distribution and modeled using a binomial logistic regression.

A logistic regression function describes the log probability of an occurrence, or logit, providing a continuous probability output as a linear function of a set of predictor variables shown in equation (4).

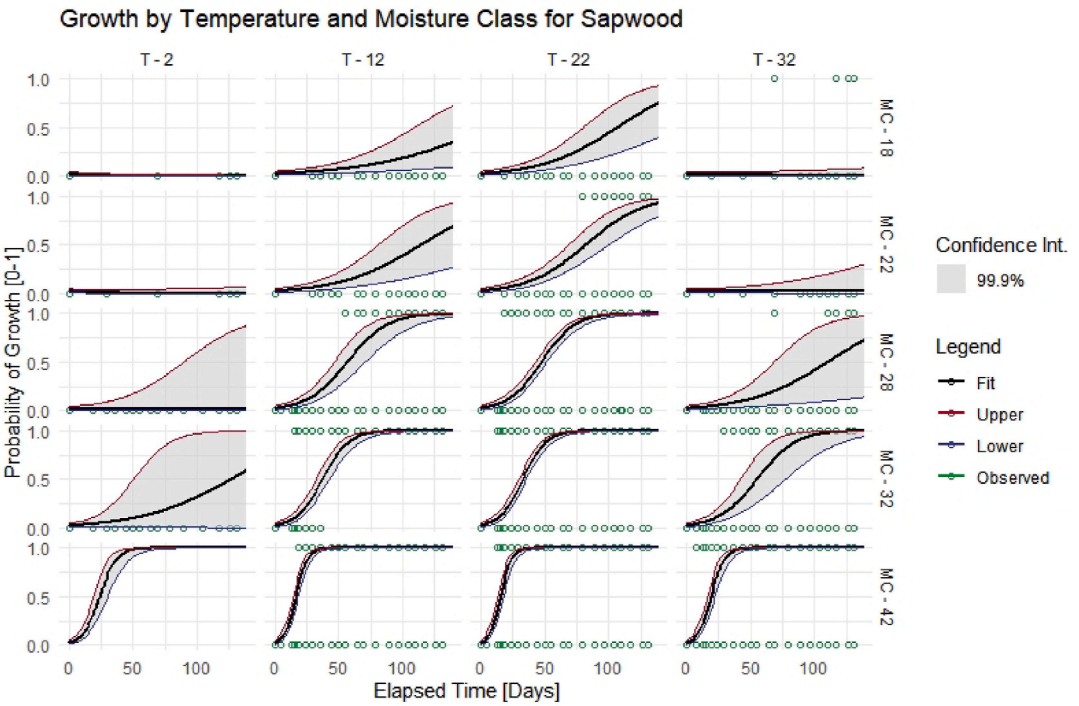


Fig. 7. Predicted and measured growth by elapsed time for temperature and moisture classes. Predicted probability of growth curves are shown in black, with red and blue Lines Representing the 99.9% confidence intervals, whereas empirical binary growth is shown in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$$l = log_b \frac{p}{1-p} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 \ldots \tag{4}$$

where p denotes the probability of occurrence in the form of the logistic equation, x_n are the predictors variables, and β_n are the regression coefficients. The regression coefficients are iteratively determined using the maximum likelihood estimation.

A significant advantage of the logistic equation is that its derivative is a function of the original equation and a condition-dependent growth-rate coefficient, shown in equation (5),

$$f^{'}(t) = r \cdot f(t) \cdot (1 - f(t)) \tag{5}$$

By determining the value of the growth-rate coefficient, r, conversion of steady-state empirical data into a dynamic model is possible. To describe the probability of fungal growth in dynamic conditions, the product of the growth coefficient at a given-time step and the current probability of growth is calculated to yield the rate of growth in probability of fungal detection.

5.1. Binomial logistic regression

Describing fungal growth through a binomial logistic regression requires appropriate parametrization of the predictor variables. From prior research (see: [24,26], we know that the growth rate r varies non-linearly with MC and T; at the extremes of MC and T, the fungi goes dormant or dies. This relationship was therefore described using a second order polynomial for these two parameters. The resulting regression equation takes the form of equations (6) and (7),

$$P(t) = \frac{K}{1 + e^{-(\beta_0 + r \cdot t)}} \tag{6}$$

$$r\,(MC, T) = \left(\beta_1 \cdot MC + \beta_2 \cdot MC^2 + \beta_3 T \cdot + \beta_4 T^2\right) \tag{7}$$

where $P(t)$ is the probability of growth (continuous on [0–1]), t is elapsed time, r is the growth rate coefficient (as a function of MC and T), and K is the upper carrying capacity of the substrate. For this preliminary research, the K coefficient is set to 1, but in future work with fungicide treated substrates, this parameter could be further modified to characterize antagonistic effects. The predicted probability of growth at the temperature and moisture content permutations is provided in Fig. 7, with the points in green representing empirical results and the lines in black representing the predicted growth.

Fungal growth was not conducted or observed in all MC and T classes, and so the projected growth probability requires further confirmation. The experimental limits on moisture content range from 12.7% MC to 63.9%MC and the temperatures from −3.5 to

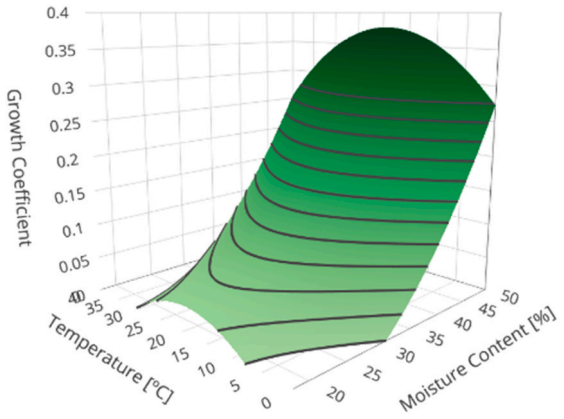


Fig. 8. Calculated growth rate coefficient for given MC and T conditions.

Table 4
Regression coefficients and significance level for probability of growth given growth (upper 99% confidence interval and mean), probability of growth for all specimens, and probability of growth for heartwood given growth.

Model	Coefficients				
	Intercept (β0)	MC (β1)	MC^2 (β2)	T (β3)	T^2 (β4)
P(G|G)+	−3.262***	-4.946×10^{-3}***	2.055×10^{-4}***	6.623×10^{-3}***	-1.801×10^{-4} ***
P(G|G)	−3.650***	-8.991×10^{-3}***	2.890×10^{-4} ***	1.090e-02***	-2.835×10^{-4} ***
P(G∀)	−1.883***	-1.711×10^{-3}***	6.485×10^{-5}***	2.252×10^{-3}***	-8.234×10^{-5}***
P(G|G ∈ H)	−2.251***	2.968×10^{-4}	1.072×10^{-5}**	1.753×10^{-4}	-2.336×10^{-5}**

Significance Codes (p-value): p«0.01 (***), p ≤ 0.01(**), p ≤ 0.05(*), p ≤ 0.1('), p ≤ 1.

31.6 °C; any values exceeding these limits should be careful considered on their impact on the growth rates. The growth rate coefficient as a function of T and MC is shown graphically in Fig. 8. As an artefact of the parabolic assumption in the regression formula and limited experimental duration, two unique occurrence are observed near the experimental limits: 1) negative growth rates at extreme temperatures, and 2) non-zero growth-rate at low moisture content.

1) As this experiment was monotonic in nature, any negative growth coefficients have no physical meaning and should be set to zero. Negative probability rates of occurrence can be determined in future research where intermittent inclement conditions are introduced.
2) The local minimum growth coefficient, on the basis of moisture content, occurs around 16% MC and is non-zero. This value corresponds to approximately 80% RH in the EMC-RH relation which describes the lower water activity limit known for many fungi. Until further confirmation on these limiting moisture content values is completed, it is recommended to set the growth coefficient to 0 for any MC values less than 16%MC.

Rate of growth coefficients are provided in Table 4 for four different regression sets. The probability of growth given the specimen will grow mould is the P(G|G) set, whereas the P(G|G)+ represents the 99th percentile growth parameter. The P(G∀) represents the probability of growth for all specimens, including those that do not grow mould, and is therefore more representative of environmental systems which do not necessarily assure growth, such as unsuccessful colonization, predation by fungi consuming organisms, or the stochastic nature of a fungal spore landing in a nutritionally deficient part of wood element. All the regression coefficients are significantly statistically (p«0.01) for the three sets. The last curve, P(G|∈H), provides the coefficients for fungal growth given that the heartwood specimen would grow mould. As this was a rare occurrence, and often found on mixed specimens, the statistical significance of the coefficients for the heartwood regressions are low and should be used with caution.

5.2. Substrate treatments

To control the number of variables, inhibitory substrate effects were limited to heartwood and sapwood from the same boards. Heartwood harbors several extractives (terpenoids, phenolics, etc.) which are antagonistic to fungal growth [32,33] and their presence is used in this study as an analogue for possible preservative treatments. Recalling that the K coefficients in equation (6) represents the upper carrying capacity, it could be modified to suit specific treatments. Equating the P(G|G) and P(G|G ∈ H) models, the required reduction in the K coefficient is represented by the ratios given in (8),

$$K = \frac{1 + e^{-(\beta_{Heart} + r_{Heart} \cdot t)}}{1 + e^{-(\beta_{Sap} + r_{Sap} \cdot t)}} \tag{8}$$

with the β and r variables representing their respective treated or untreated substrates. The K coefficient therefore becomes a

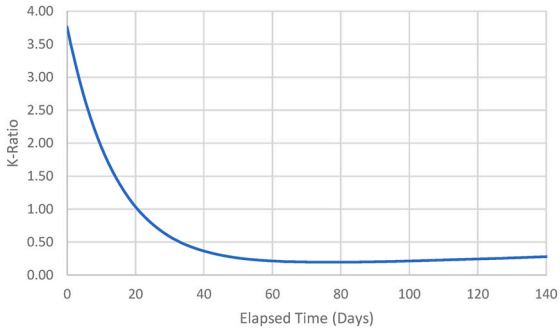


Fig. 9. K capacity reduction coefficient with elapsed time, at MC = 28% and T = 22 °C.

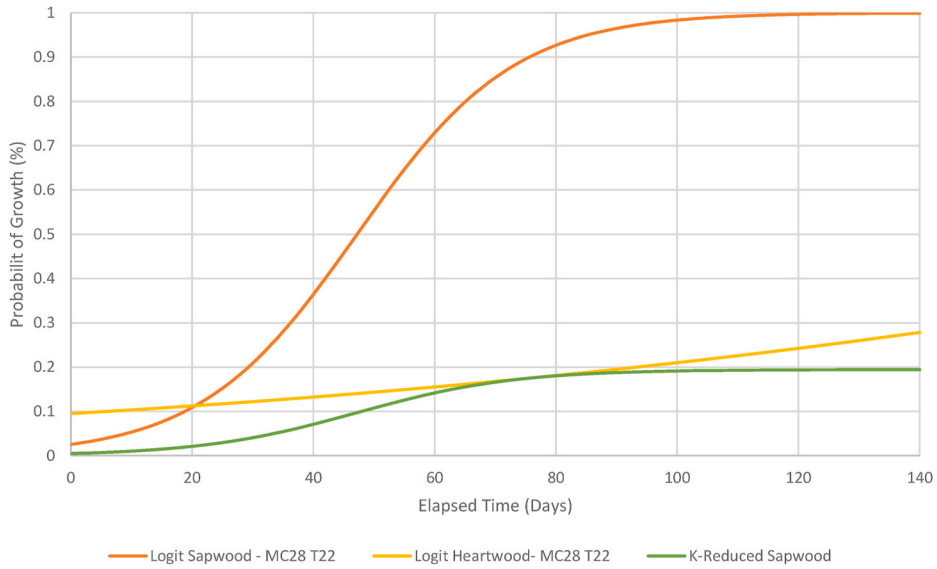


Fig. 10. K-reduced sapwood compared with sapwood and heartwood regressions at MC = 28% and T-22 °C.

function of MC, T, and elapsed time. The values for K with time, for a fixed MC at 28% and T at 22 °C, are shown in Fig. 9.

Extracting the minimum K value and applying it uniformly as a reduction to the probability of growth for the untreated specimen yields the curves found in Fig. 10.

Whereas the results show that the K-Reduced Sapwood and Heartwood Regression predict a 10% difference in the probability of growth, this process is nonetheless suggestive of promising approaches in using the K coefficient for preservative treatments.

6. Discussion

A binomial logistic regression on fungal growth on a North American wood species shows promising results in projecting the probability of fungal contamination as a function of dynamic environmental parameters. Description of the probability failure envelope is a required first step in further development of a limit state design approach to biodeterioration of building materials. Further, identification of risk tolerances and thresholds is also critical in understanding what probability of fungal growth is acceptable. As these tolerance levels may depend on a number of factors, it is important that the model user understand the uncertainty associated with the model. This section discusses how the regression equations can be applied as a tool to evaluate the risks of fungal growth on wood materials. It is followed by a preliminary validation in an attic roof study. Lastly, the caveats of the tool and limits of this research are discussed.

6.1. Application of tool

The model provides a range of probability outputs for differing assumptions. Conversion of these outputs into actionable recommendations by building practitioners requires translation into language that is understandable by laypersons. The process whereby this model can be incorporated with spreadsheet data, which may originate from either empirical records from a data logger or an output from a hygrothermal model, is shown conceptually in Fig. 11.

The process follows:

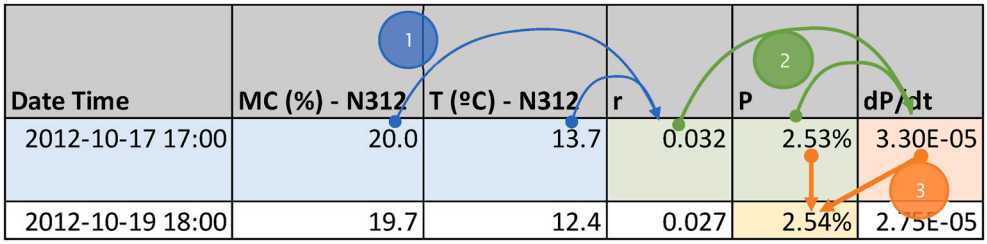


Fig. 11. Application of P(G|G) model in spreadsheet given hourly input data.

Table 5
Likelihood scales for terminology on probability of growth.

Classification	Term	Likelihood of Outcome
1	Very Likely	90–100%
2	Likely	66–90%
3	More likely than not	50–66%
4	Less likely than not	33–50%
5	Unlikely	10–33%
6	Very unlikely	0–10%

Step (1) – The growth rate coefficient (r) is calculated from the environmental parameters (MC and T) using Equation (7). The probability of growth (P) for the first time step is calculated using Equation (6).

Step (2): the growth rate coefficient and the current time-step's probability of growth value are then used to compute the rate of probability growth (dP/dt) with Equation (5). The dP/dt value is normalized according to the time-step length (e.g., divided by 24 should the values be hourly, or by 1, if average daily values are used).

Step (3): the probability of growth for the next time-step is calculated as P + dP/dt from the previous time-step.

Depending on the audience, translation of probability into common language may be preferred. Table 5 provides guidance on mould growth classification level, the range of likelihood outcomes, and a plain language equivalent to assist in communications with building decision makers. The table is based off of the likelihood scales from the [34].

The combination of the P(G|G) and P(G|G)+ models can be used to describe upper limit and most likely mould growth occurrences, whereas the P(G∀) can be used to describe lower-limits of anticipated growth on suitable substrates.

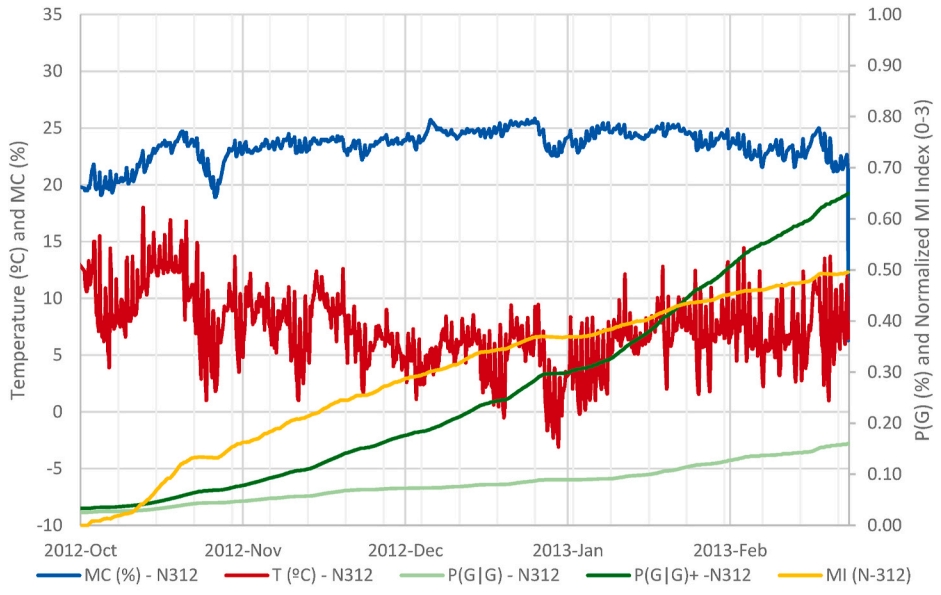
6.2. *Preliminary validation*

The model was compared with an experimental roof study that investigated the impacts of different surface treatments on mitigating mould growth in ventilated roofs. Further detail may be found in Ref. [35]. Two ventilated attic roofs were constructed in Vancouver, BC, and were instrumented with temperature, relative humidity, condensation, and moisture content sensors and connected to a data logging system that made hourly recordings. The test roofs were constructed in September 2012 and preservative treatments were applied on October 15th, 2012. This also coincides with the activation of the meteorological station that recorded ambient humidity, and so therefore is considered the start of this test. The substrate conditions depart from the original assumptions in this model on three fronts: 1) the substrate is a Douglas-fir plywood, not jack pine, 2) an application of bleach was applied to disinfect the plywood of any fungus at the start of the test, and 3) fungal inoculation occurred naturally with ambient spores. However, these three conditions are assumed to have a smaller impact relative to the contribution of the environmental effects, the salient concern for this model.

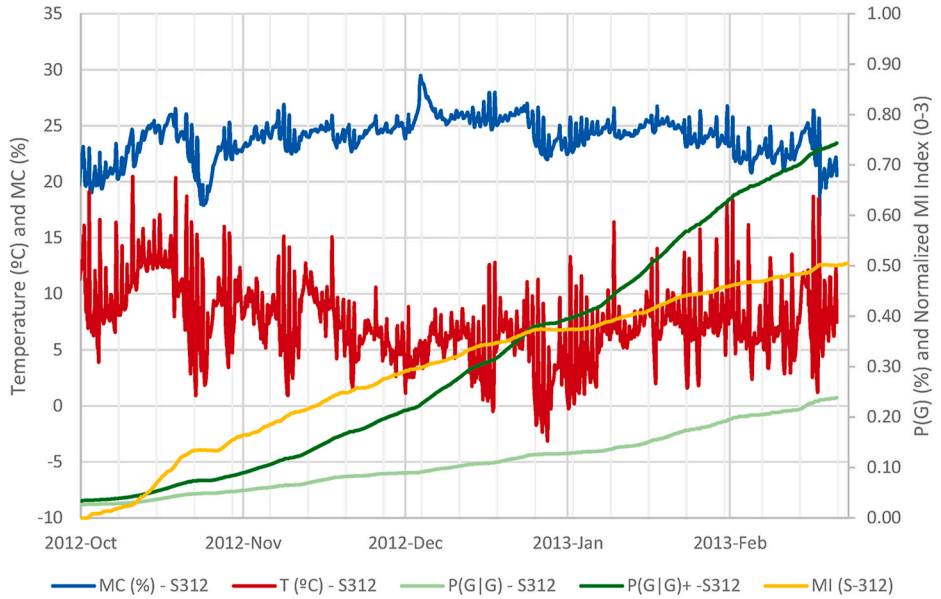
The moisture content and temperature values were used as input variables into the model, and the resulting probability of growth output was compared with the photographed results. The hourly input data (MC and T) and the projected P(G|G) and P(G|G)+ models are shown in Fig. 12. The model is contrasted with the VTT model [36,37], with a mould index (MI) rating scale of 0–6, with a value of '3' representing visual mould growth. For ease of plotting the VTT and P(G|G) models, the VTT model was normalized to an MI of 3. A sensitivity class of "Sensitive" and a decline coefficient C_{eff} was selected for the VTT model. The relative humidity input was from the nearby meteorological station and may pose some variance from the surface relative humidity of the sheathing.

The VTT model with the sensitive material class (representative of some plywood) suggests that no visible mould should be present on either the North or South elevations. In the P(G|G) model on the other hand, suggests that it is unlikely to find mould on either elevation, on average, but that the maximum probability of finding mould is in *more likely than not* on the North elevation, and *likely* on the South elevation, 99 times out of 100.

Review of the attic plywood sheathing photographs in Table 6 shows that the North elevation has a few patches of fungal growth in the treated area, with virtually no growth around its perimeter. The South elevation shows no fungal growth within the treated area but some staining around its perimeter. In both cases, the VTT model suggests no observable fungal growth, which is not accurate. The P(G|G) models suggest a large range of probabilities of detecting fungal growth. On the North and South elevation, there's only about a 15% and 25% chance of mould growth, on average, with the 99% confidence interval suggesting possibilities as high as 65% and 75%



(a)

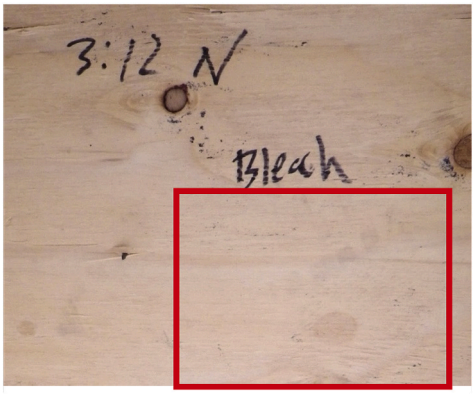
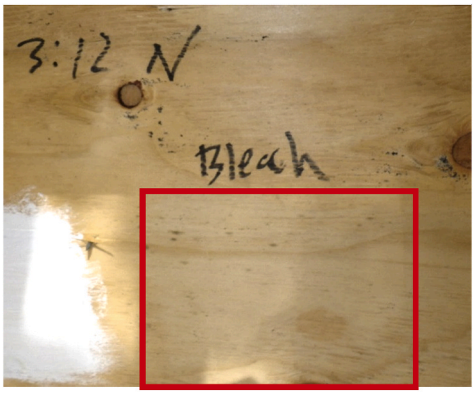
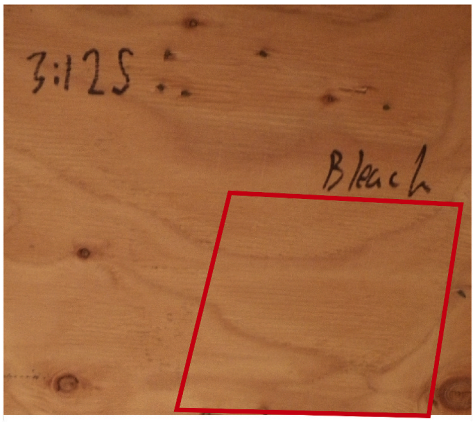


(b)

Fig. 12. Moisture content and temperature for ventilated attic on North (a) and South (b) orientation, with normalized VTT mould index (relative to an MI = 3) in yellow, P(G|G) projection in light green and P(G|G)+ in dark green. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

chance of detecting growth, respectively. The large variability in probability encompasses both low and high likelihood of growth conditions, which is supported by the visual evidence. Some areas grew mould, others did not, due to unknown biological and substrate factors. This is an improvement over deterministic models, which provide a fixed estimate of a biological growth model, which is not fully represented in this test.

Table 6
Surface mould photographs for North and South elevation, bleach treated attic roofs, treatment area outlined in red, on 2012/10/15 and 2013/02/15.

	2012/10/15: When Applied	2013/02/15: 4 Months Later
North		
South		

7. Recommendations

This research indicates that a logistic regression to determine the probability of finding mould growth is an appropriate method for use with limit state design concepts. This research could be further enhanced in three ways: 1) improvements to the range and duration of the underlying dataset, 2) investigation of delaying or reducing impacts from inclement conditions and treated substrates, and 3) verification of the effects of natural sources of inoculum and fungal competition.

7.1. Model caveats

There are several important caveats in these models. The most important is the assumption of monotonic growth. As this model does not currently incorporate delays or reductions in probability of growth, over long enough time horizons, some degree of growth is guaranteed. Despite observance of negative growth rates, this may be an artefact of the regression and may not necessarily be reflective of actual delay in probability of detecting growth. Until such experiments are conducted, it is instead suggested to set all negative growth coefficients to a value of 0. This approach yields conservative growth estimates but may be justifiable as once fungal resting spores are developed, the fungi can quickly resume growth upon resumption of adequate environmental condition. Investigations into delaying effects, as identified by Ref. [38]; may be a method to counteract this limitation until further research is completed.

The second important caveat is experimental limitations. The experimental duration was limited to 4 months. As a result, some of the specimens that did not grow mould could have been prematurely terminated. This applies both to limiting experimental environmental conditions (low moisture or temperature) as well as those specimens in optimal growing conditions that did not yield growth.

The third caveat lies within incorporation of the model within simplified spreadsheets. The experiment carefully controlled for contamination and is therefore only reflective of a single colonization event. In reality, the substrate may be continuously inoculated from local spores. Initial germination of early colonizers may be interrupted or terminated with inhospitable environmental conditions, which, upon resumption of suitable conditions, subsequent spores may be more successful. An iterative approach, whereby a continuously running 4-month period of growth check over longer-durations, in which only the highest amount of growth at the end of the 4 month running period, may be a suitable work-around in the short-term. Further investigation is required.

The last caveat lies within the use of a single fungus species, *P. chrysogenum*. Many other fungi have varying environmental and substrate preference, different growth rates, and abilities to compete with other organisms. Further investigation into probability of

growth rates with other fungi, including potential competition effects with antagonizers is warranted.

CRediT authorship contribution statement

Robert Lepage: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Samuel V. Glass:** Writing – review & editing, Supervision, Resources. **Paul de la Bastide:** Writing – review & editing, Supervision, Resources. **Phalguni Mukhopadhyaya:** Supervision, Funding acquisition, Writing – review & editing, Project administration, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge Dr. William Hintz (Department of Biology, University of Victoria) for providing access to his laboratory for this testing. We would also like to acknowledge the Canadian Wood Council, the Canadian Wood Preservation Association, the American Wood Protection Association, and NSERC for their contributions.

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