Industrial Crops and Products 69 (2015) 187–196

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Efficacy of microwave irradiation for phytosanitation of wood packing materials

Martin Payette [a], Timothy T. Work [a], Pascal Drouin [b,1], Ahmed Koubaa [b]

[a] Forest Research Centre, NSERC UQAT-UQAM Chair in Sustainable Forest Management, Université du Québec à Montréal, Département of Biological Sciences, Montréal, (Québec) H3C 3P8, Canada
[b] Forest Research Centre, NSERC UQAT-UQAM Chair in Sustainable Forest Management, Université du Québec en Abitibi-Témiscamingue, Rouyn-Noranda, (Québec) J9X 5E4, Canada

ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 5 January 2015
Accepted 18 January 2015

Keywords:
Phytosanitation
Microwave irradiation
Wood packing materials
Cerambycidae
Decay fungi

ABSTRACT

Wood packing materials (WPM) are important vectors of invasive xylophagous insects and pathogenic and decomposer wood fungi. The International Plant Protection Convention introduced the International Standards for Phytosanitary Measures No. 15 (ISPM No. 15) to regulate the development of treatments to sanitize WPM and prevent the introduction and movement of forest pests. Dielectric heating (e.g., microwave irradiation) has recently been included as an accepted treatment. In this study, the efficacy of microwave irradiation was tested on *Monochamus scutellatus* larvae and on four different pathogenic fungi, *Gremmeniella abietina*, *Heterobasidion annosum*, *Chondrostereum purpureum*, and *Mycosphaerella populorum*, five species of economic significance in Québec, in both jack pine and trembling aspen. We explored different temperature/time combinations on each species in order to accumulate data on the treatment. We irradiated *M. scutellatus* larvae at 56, 61, and 66 °C for 1–3 min and the four fungal species at 50, 55, 60, 65, 70, 75, and 90 °C for 0.5, 1, or 2 min. Fungi were tested at a wider range of temperatures to account for possible higher variation of resistance between species. We obtained 100% mortality in larvae treated at 56 °C for 2 min and at 61 °C for 1 min. The fungi species were much more resistant to the treatment. *G. abietina* was eliminated at 75 °C/0.5 min, *H. annosum* at 90 °C/1 min, *M. populorum* at 90 °C/2 min, and *C. purpureum* was still present at the highest temperature/time combination used. We demonstrated the capacity of microwave irradiation to kill the larvae with similar parameters as IPPC guidelines (60 °C for 1 min), though lethal temperatures for the fungi were very high. As the current ISPM No. 15 standard for microwave irradiation was insufficient to kill all tested fungal species, more work should be done on determining optimal combinations for the greatest number of species. Future studies should test a wider range of treatment times and expand trials to include more insect and fungal species to determine which temperature/time combination will allow us to keep both values as low as possible while assuring complete prevention of adult insect emergence and fungal re-growth.

1. Introduction

Invasive forest insect pests and plant pathogens cost an estimated $2.1 billion US each year in losses and damage within the United States (Pimentel et al., 2005). Wood boring beetles from the family Cerambycidae are among the most frequent and problematic insect groups which are regularly introduced through wood packing material (WPM) (Brockerhoff, 2009; Haack, 2006). Many serious pathogens are transported through dunnage, insect vectors, wood chips, and WPM (Mireku and Simpson, 2003). Large-scale use of microwave irradiation to eliminate wood boring insects and fungal pathogens inside WPM, including wood pallets, may become an important part of the overall effort to minimize the introduction of invasive pests across borders. The International Plant Protection Convention (IPPC) published the International Standards for Phytosanitary Measures No. 15 (ISPM No. 15) which set guidelines for the regulation of WPM in international trade in order to stem the accidental introduction of invasive pests (FAO, 2014). Signatory countries, such as Canada and the United-States, have

Abbreviations: IPPC, international plant protection convention; ISPM No. 15, international standards for phytosanitary measures no. 15; MC, moisture content; LT, lethal temperature.

E-mail addresses: payette.martin.4@courrier.uqam.ca (M. Payette), work.timothy@uqam.ca (T.T. Work), pascal.drouin@uqat.ca (P. Drouin), ahmed.koubba@uqat.ca (A. Koubaa).
[1] Current address: Lallemand Animal Nutrition, 586 Ridge Road, Chazy, NY 12921, USA.

http://dx.doi.org/10.1016/j.indcrop.2015.01.030
0926-6690/

adopted these guidelines and inserted them into their own regulations for the control of out- and in-bound WPM (CBSA, 2012; USDA, 2004). Currently, the ISPM No. 15 standard recognizes several methods for the treatment of wood packing material including methyl bromide fumigation, heat treatment using conventional steam or dry kiln heat chamber and heat treatment using dielectric heating. Methyl bromide fumigation is regulated according to specific temperature and concentration combinations for a treatment period of 24 h, whereas steam and dry kiln heating require that wood core temperature be held at 56 °C for 30 min, a temperature/time combination determined initially to kill the pine wood nematode (*Bursaphelenchus xylophilus* [Steiner and Buhrer] Nickle), the causative agent of pine wilt disease (Ramsfield et al., 2010). Methyl bromide is however a short-lived gas which has been shown to contribute to the depletion of the ozone (WMO, 2006). The Montreal Protocol on Substances that deplete the ozone layer singled out methyl bromide and signatory countries are required to have completely phased out its use by 2013 (Madé, 2012; UNEP, 1994). Contracting parties to the IPPC are therefore encouraged to search for alternatives to fumigation (FAO, 2014). Conventional heating in kilns and with steam can only heat the wood core by transfer of energy from the outside-in and is more time consuming. Dielectric heating, such as occurs during microwave irradiation, affects water molecules more directly and can raise the inner temperature of wood much faster than conventional heating. Dielectric permittivity is a measure of how a medium is capable of being polarized by an electromagnetic field. The relative permittivity of water is very high whereas that of wood is much lower. Organisms within the wood are therefore affected more directly than they would be when treated with conventional heating. Several studies have tested the effects of microwave irradiation on the dielectric properties of wood to test the distribution of heat and the effect of moisture content, and have found that higher moisture contents lower the treatment's capacity to heat wood (Antti and Perré, 1999; Koubaa et al., 2008). The consensus is that higher percentages of moisture absorb more heat and lower the capacity of the microwaves to heat the wood. Heating efficiency also varies according to structural factors, such as the direction of the grain and the heterogeneity of the wood's interior (Antti and Perré, 1999). This does not however cause changes in the structural integrity of the wood and it has been suggested that moving the applicator or rotating the wood could remedy the situation (Antti and Perré, 1999; Fleming et al., 2005). Several studies have tested the efficacy of the treatment on insect pests within the wood for phytosanitation purposes and found it to be effective at temperatures ranging from 60 to 65 °C for much shorter time intervals (<5 min) than the 30 min suggested by the ISPM No. 15 for conventional heating (Fleming et al., 2003, 2004, 2005; Henin et al., 2008; Hoover et al., 2010; Nzokou et al., 2008). Many studies however did not focus on determining the optimal temperature/time combination and mostly tested temperatures for one treatment time or tested much higher times than are necessary to kill many wood pests.

The ISPM No. 15 standard was recently modified to include dielectric heating and requires that wood not exceeding 20 cm in its smallest dimension be heated at 60 °C for 1 continuous min with a maximum period of 30 min to reach the required temperature in the entire volume of the wood (FAO, 2014). As this is a relatively new treatment, the present study has the objective of increasing the amount of data available by testing microwave irradiation as a viable phytosanitary treatment in northeastern North America. We selected larvae from a common species of Cerambycidae, *Monochamus scutellatus* (Say), which is native to North America, as well as four pathogenic fungi, *Gremmeniella abietina* (Lagerberg) Morelet, *Heterobasidion annosum* (Fr.) Bref., *Chondrostereum purpureum* (Pers.) Pouzar and *Mycosphaerella populorum* G.E. Thompson.

M. scutellatus lays its eggs beneath the bark of weak and dying conifers (Peddle, 2000). The larvae bore deep into the wood and are often the cause of economic losses in the lumber industry as they attack cut logs in fields and lumberyards, reducing the quality of the wood. Though *M. scutellatus* has not yet been shown to be so, many species in the *Monochamus* genus (*M. carolinensis* [Olivier], *M. altematus* Hope, and *M. salruarius* [Gebler]) are vectors of the pine wood nematode which has caused heavy damages in pine forests in Japan, China, Korea and Taiwan (Fielding and Evans, 1996; Kosaka et al., 2001; Shi et al., 2012; Suzuki, 2002; Zhao et al., 2007) and in Portugal and Spain (Abelleira et al., 2012; Robertson et al., 2011).

Pathogenic fungi capable of killing trees are of great economic significance. Epidemics of Dutch elm disease (Dedic and Zlatanovic, 2001) (*Ophiostoma ulmi*; *Ophiostoma novo-ulmi* [Brasier]) and sudden oak death (*Phytophthora ramorum* [Werres et al., 2001]) are examples of the risks posed by non-indigenous fungi. The criteria in ISPM No. 15 are well adapted to xylophagous beetles and the pine wood nematode but have been shown to be insufficient for several fungi species (Ramsfield et al., 2010). More data shoud therefore be collected to adequetely adjust accepted treatment norms. The four fungi species used in this study were selected based on their presence in the northeastern North America and on their likeliness of selecting hosts commonly used in wood pallet production in this region. In addition, they are minor pests in forests and plantations of certain regions and have all been reported as being found outside North America, meaning that their transport via imported and exported wood is a likely occurrence. Of the four, only species of the *Heterobasidion* genus have been found to have successfully invaded new areas without the help of an insect vector (Gonthier et al., 2012, 2004), though this does not preclude that the others are not capable. *H. annosum* is an economically significant conifer root and butt rot fungus in the Northern Hemisphere (Asiegbu et al., 2005; Lygis et al., 2004). *C. purpureum* is the causal agent of silver leaf disease on many fruit trees and scrubs (Beever, 1970) and is used as bioherbicide against unwanted non-indigenous trees (Pitt et al., 1999; Ramsfield et al., 1996). *G. abietina* is the causal agent of scleroderris canker and attacks mainly pine in North America and sometimes spruce (EPPO, 2009; Wallace, 2012). Finally, *M. populorum* is the causal agent of septoria canker and attacks all species of *Populus* in North America as well as some exotic and hybrid poplars (EPPO, 1980). The accumulation of data on fungal resistance to microwave irradiation will help establish important criteria for effective treatment in case of newly discovered outbreaks.

This study's objective was to determine minimum microwave irradiation combinations of temperature and time required to eliminate cerambycid larvae (*M. scutellatus*) and four different fungal wood pathogens (*G. abietina*, *H. annosum*, *C. purpureum*, and *M. populorum*), all indigenous or established in northeastern North America, in industrial grade wood such as would be found in wood pallets and other types of wood packing materials. Based on the results from previous studies on the heat treatment of wood-boring insect larvae (Fleming et al., 2003, 2004, 2005; Henin et al., 2008; Hoover et al., 2010; Nzokou et al., 2008; Ramsfield et al., 2010) and of fungal pathogens (Ramsfield et al., 2010; Tubajika et al., 2007), we hypothesized that microwave treatments at temperatures of 56 °C or higher at 2 min would be sufficient to kill all larvae and at temperatures of 70 °C or higher would be sufficient to provoke mortality and inhibit re-growth in all fungi species, given the generally higher resistance of fungi to harsh conditions. We also expect that the higher porosity of jack pine than trembling aspen will allow the microwaves to heat more effectively the organisms inside and

Table 1
Preliminary survival results for fungal pathogens with temperatures and times.

	60 °C		65 °C		70 °C		75 °C		80 °C	
	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min
C. purpureum	2/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
G. abietina	0/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
H. annosum	2/2	1/2	2/2	0/2	1/2	0/2	1/2	0/2	1/2	0/2
M. populorum	01/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

therefore decrease the temperature level required to kill them in the softwood blocks versus in the hardwood blocks.

2. Materials and methods

2.1. Equipment

For all experiments, we used a 2.45 GHz BP-111 compact microwave processor with an oven chamber dimensions of 33 by 33 by 20.5 cm (Microwave Research and Applications, Inc. Laurel, MD) to irradiate wood blocks containing insects and fungi. This is the required frequency according to ISPM No. 15 standards (FAO, 2014). The system included an integrated temperature probe and Cal Control® 9500 temperature/process controller. The system is designed to provide a steady rate of temperature increase up to a specified level to which it is then maintained, all the while delivering uniform heating within the chamber. We controlled the program settings and monitored the temperature readings with Cal Control® CALgrafix Controller Software.

The CALgrafix software was also used to record temperature readings for post-treatment evaluation of temperature variation. The readings were used to remove any data-points where the temperature during treatment varied by more than 1 °C over or under the desired value. In all treatments on larvae and several treatments during the trials on fungi species in trembling aspen blocks (see Section 2.6 for specific points), temperature readings recorded with the software were lost.

2.2. Cerambicidea larvae

The *M. scutellatus* larvae were collected from black spruce logs obtained in October 2012 at the Rivière-aux-rats sawmill near La Tuque, Québec. The logs came from a stand in the Mauricie region that had burned the previous spring. The logs were cut to approximately 30 cm. We split logs with an axe until we found sign of a larval gallery and then chiseled the wood carefully until the larvae could be safely extracted. Almost half of larvae found in the logs were already dead or lacking vigor (low mobility, consumption of wood, and/or frass production). Those with obvious signs of low vigor were not kept. We verified both dead and live specimens to species using Craighead (1923). All specimens extracted were *M. scutellatus*. Live larvae were kept separately in indivdual Petri dishes to avoid antagonistic interactions among larvae. Each dish contained a moist towelet, wood shavings and small pieces of wood cut from the logs in which the larvae were found. They were kept at room temperature for no more than three days before treatment. A total of 66 larvae were extracted.

2.3. Wood decomposing fungi

We obtained four species of wood decomposing fungi from the Forest Pathology Laboratory at the Université de Laval: *G. abietina* (strain CFL-87-0064) and *H. annosum* (strain CFL-535), which are associated with conifers, and *C. purpureum* (strain CFL-529) and *M. populorum* (strain CFL-1326), which are associated with deciduous trees. They were originally part of the collection at the Laurentian Forestry Centre and were isolated from trees in the province of Québec.

Cultures were received in June of 2011 and incubated at room temperature. In December 2011, we conducted preliminary mortality tests to establish a range of heat tolerances for each species. Two replicates for each species were subjected to temperatures of 60, 65, 70, 75 and 80 °C for a period of 2 and 5 min (Table 1). Based on these results, the temperatures used in the irradiation treatments were fixed at 24 (control), 55, 60, 65, 70, 75, and 90 °C. We used 10 replicates per temperature/time combination. Instead of 1–3 min for the duration of treatment such as with the larvae, we opted for 0.5, 1, and 2 min to assure there would be enough samples that survived at shorter times to make an adequate regression analysis. *G. abietina* and *H. annosum* were inoculated and irradiated in jack pine blocks and *C. purpureum* and *M. populorum* were inoculated and irradiated in trembling aspen.

2.4. Block preparation

Jack pine, *Pinus banksiana* Lamb., and aspen, *Populus tremuloides* Michx., wood blocks were provided by Tembec Inc., in La Sarre, Québec. All blocks were 3.8 by 6.4 by 20 cm. As the blocks did not exceed 5 cm in thickness on all sides, it was not necessary to use a bidirectional application or multiple waveguides as required by ISPM No. 15 to ensure uniformity of heating (FAO, 2014). All blocks were kiln dried for 24 h at 103 °C and weighed. For the *M. scutellatus* larvae man made tunnel, we used a 1.3 cm (0.5 in) spade bit to drill a 3.8 cm deep hole at the center of one of the narrow longitudinal sides of the blocks. Another hole, approximately 0.6 cm wide and 1.9 cm deep, was drilled into the wide longitudinal surface of the blocks, at the center and approximately 1.3 cm to the side of the larva's tunnel to insert the temperature probe and measure the block's core temperature (Fig. 1).

For fungal irradiation, we drilled four holes equidistant in the forward facing side of each block (same as in Fig. 1). The holes were 1.6 cm wide and 3.8 cm deep. We made them wider than for

Fig. 1. BP-111 compact microwave processor with temperature probe inserted in jack pine block. The hole containing the larva is plugged on the front-facing side.

the larvae to make extraction of the wood pellets easier. A hole, again 0.6 cm wide and 1.9 cm deep, was drilled for the thermal probe through the top of each block between holes 1 and 2, and between holes 3 and 4. Both the holes drilled for the larvae and those drilled for the wood pellets were prepared so as to be facing the microwave door and be perpendicular to the direction of the microwaves within the chamber.

To simulate natural accumulation of moisture in wood, blocks were soaked in water for a minimum of 12 h before treatment. The blocks were weighed immediately before insertion of either larvae or pathogens. Moisture content was calculated by dividing weight difference between wet and dry blocks divided by their dry weight with the following equation: MC = (ww − dw)/dw × 100, where MC = moisture content (%), ww = wet weight and dw = dry weight.

2.5. Treatment of Cerambycidae larvae

We placed the *M. scutellatus* larvae at the center of jack pine blocks and did tests at 24, 56, 61, 66 °C for 1–3 min. A single larva was placed in a block immediately prior to irradiation and the hole was plugged. The plug was made from the same type of wood using a 1.3 cm plug-cutter. There were six replicates per treatment at 56, 61, and 66 °C and four replicates per treatment at room temperature (24 °C, with the power turned off), which served as an experimental control.

Mortality was assessed immediately after treatment based on lack of movement, dehydration, and/or discoloration. In all cases, the larvae were placed back in their respective Petri dishes on a new moist towelet. A second verification was performed 24 h after treatment to see if there was movement with prodding. This approach was based on that of (Fleming et al., 2005, 2003).

2.6. Treatment of pathogenic fungi

All laboratory equipment used to handle fungi was sterilized immediately prior to and following use.

The four fungi were freshly cultivated on malt extract agar incubated at 28 °C prior to starting the liquid culture. An agar pellet aseptically collected of each strain was transfer to a 1 L Malt Extract broth. The bottles were incubated at room temperature under slow agitation (50 rpm) for two weeks prior to the microwave treatments. Hyphal and spores germ count was determined by plate counts on MEA following serial dilution in peptone water buffer. This accounted for 3.4 $\log_{10}$ germs ml^{-1} for *G. abietina*, 3.8 for *H. annosum*, 4.8 for *C. purpureum*, and 3.7 for *M. populorum*.

Wood pellets 1.3 cm wide and 1.3 cm high were prepared with a plug-cutter from the same stock of wood as the blocks. Pellets were heat sterlized, at 103 °C for at least 9 h. Pellets were submerged and kept in a solution of their assigned organism 30 min prior to treatment.

For each temperature/time combination, an inoculated pellet from each of the organisms associated with the same wood species (*G. abietina* and *H. annosum* in jack pine and *C. purpureum* and *M. populorum* in aspen) was placed in either hole no. 1 or 2 of the same block at the same time. The holes were shut with plugs made from the same stock of wood as the blocks. The average time to reach the desired core temperature was 60 s. After treatment, the pellets were removed aseptically as quickly as possible with tweezers. We then cut three to six shavings off of each pellet with a scalpel and placed them on a malt extract agar surface. The block was weighed and then reused, this time placing the inoculated pellets in holes no. 3 or 4. Holes were never used more than once to prevent contamination and to evaluate if the position in the wood block would affect survival.

Wood shavings from each plug were incubated separately on malt dextrose agar (MEA) medium at room temperature for four weeks and inspected visually for presence or absence of growth after 14 days and 28 days. Species isolates were identified based on morphology. The treatment was not considered lethal if the isolates grew on the medium from the wood shavings after irradiation.

In the pilot study examining, *G. abietina* and *H. annosum* in jack pine, we confirmed the presence of both species in control treatments (24 °C with no power). However, in addition to these species, we observed bacterial and/or fungal contamination as evidenced by the presence of different morphotypes in some controls, which competed for space with the study cultures. Furthermore, contamination was not observed in samples that were irradiated with microwaves.

During the second trial, using *C. purpureum* and *M. populorum* in aspen, antibacterial MEA medium (Bengal rose) was used to avoid contamination on either controls or irradiated samples. Though the antibacterial agent was successful in inhibiting contaminants, it also inhibited growth of *M. populorum*. For this reason, we repeated the second trial on *C. purpureum* and *M. populorum* using the same medium as the first trial. There was again evidence of contamination in controls and none in irradiated samples. We therefore considered that the contaminants were most likely killed by temperatures equal to or higher than 50 °C. They then would not have influenced post-treatment growth of fungi samples.

We removed datapoints with temperature readings that varied by more than 1 °C during treatment. Variable readings were likely most often caused by the presence of wood shavings in the holes used to insert the temperature probe as the problem did not re-occur once any leftover shavings were cleaned out with the drill bit.

As temperature readings were not available for some of the treatments on *C. purpureum* and *M. populorum* (50 °C/1 min, 55 °C/2 min, 60 °C/0.5 min, 60 °C/2 min, 65 °C/0.5 min, 70 °C/0.5 min, 70 °C/1 min, 75 °C/1 min, 75 °C/2 min, 90 °C/0.5 min), a separate analysis was made with the original data for comparison.

2.7. Data analysis

For the larvae trials, we used a binomial-response generalized linear model with bias reduction to account for quasi-complete separation of datapoints. This occurs when one or more predictor values are separated by the outcome variable and the model therefore predicts some of the values perfectly, which in turn gives a predicted probability of 1 or nearly 1. The *brglm* package in R version 3.0.0 (Kosmidis, 2013) allowed us to create a generalized linear model and eliminate any small-sample bias by introducing a penalized likelihood (Firth, 1993). The package allows us to have a bias-reduced estimator that is smaller than the maximum likelihood estimator and gives estimates and standard errors that are finite as opposed to maximum likelihood estimates with complete or quasi-complete separation (Kosmidis, 2013). The notation for creating a model is the same, except that the *brglm* notation is used instead of *glm*. We then separated models for each treatment time with only temperature as a fixed effect to determine lethal temperature at a probability of 99% and 99.99%.

For the fungi trials, we analyzed two separate datasets. The first contained all original data and the second was a final adjusted dataset with points removed due to post-assessment of temperature variation higher than 1 °C < 0. Each inoculated plug was considered a separate datapoint with a presence/absence response. All data were analysed with a binomial-response generalized linear model with a logit link function using the *glmer* function in the *lme*4 library with R version 3.0.0 (Bates et al., 2013). Duration of irradiation, temperature and moisture content were used as fixed variables, and position within the block was used as a

Table 2
Survival results of *M. scutellatus* larvae with treatment temperatures and times.

Time	Temperature (°C)	Survivors
1 min	24	4/4
	56	2/6
	61	0/6
	66	0/6
2 min	24	4/4
	56	0/6
	61	0/6
	66	0/6
3 min	24	4/4
	56	0/6
	61	0/6
	66	0/6

random factor. We then separated models for every treatment time using only temperature as a fixed variable in order to predict lethal temperature at 99% and 99.99% at each of these times.

We calculated confidence intervals for all predicted lethal temperatures to verify whether they encompassed the ISPM No. 15 standard of 56 °C.

3. Results

3.1. Cerambycidae larvae

All but two larvae irradiated with microwaves died during or within 24 h of the treatment (Table 2). Both subjects that were still alive after 24 h had been treated at 56 °C for 1 min. All the control treatments (24 °C) survived. The minimum temperature/time combinations at which all subjects were killed were 56 °C held for 2 min and 61 °C for 1 min.

A logit regression showed quasi-complete separation, in which a covariate predicts almost perfectly the outcome of the response variable. In this case, tested temperatures above the control of 24 °C caused the death of all but two larvae whereas all larvae at the control temperature survived. The regression analysis gives a predicted probability close to or equal to 1 and an extremely high standard error. After adjustment through bias reduction of maximum likelihood estimates with the *brglm* package in R version 3.0.0 (Kosmidis, 2013), the probabilities and standard errors for the covariates were much lower and easier to interpret.

Treatment time was not significant. Larval weight, ranging from 0.1089 g to 0.9549 g and averaging 0.4714 g, showed no significant effect or relationship to mortality. Wood moisture content, which varied between 16.42% and 73.54% with an average of 36.24%, was also not significant (Table 3).

Predicted lethal temperature (LT) and their confidence intervals are presented in Table 4. The LT values at a probability (p) of 99.99% are higher by a difference of 35.5 °C at 1 min and of 28.6 °C at 2 and 3 min versus those at a probability of 99%. The predicted LT at p = 99% are higher by a difference of 24.5 °C at 1 min and of 10.1 °C at 2 and 3 min versus the observed LT. The large discrepancy between the predicted LT at 99% and 99.99% probability as well as with observed LT is probably due to the small sample size. We can

Table 3
Outcome estimates (including β estimate, standard error, Wald statistic [z value], P-value [P]) for binomial generalized linear model of *M. scutellatus* larvae mortality following microwave irradiation according to temperature, time, moisture content (MC), and larval weight (LW).

	β estimate	Standard error	z value	$P(>\|z\|)$	
Intercept	12.358	4.983	2.48	0.013	*
Temperature	−0.240	0.072	−3.334	0.001	***
Time	−1.915	1.173	−1.633	0.103	
MC	0.070	0.045	1.562	0.118	
LW	−1.946	3.485	−0.558	0.577	

Note: Significant results at $P \leq 0.05$. $P < 0.1$, '.' $P < 0.05$, '•' $P < 0.01$, '••' $P < 0.001$; '•••'.

nevertheless note that 60 °C falls within the confidence intervals of all predicted lethal temperatures except one (1 min; p = 99.99%), though by less than 1 °C.

3.2. Pathogenic fungi

Observable LT were different for each of the four fungal species in either the original datasets or in the final adjusted datasets where samples with temperature variability were removed (Fig. 2). *H. annosum*, *C. purpureum* and *M. populorum* still showed signs of growth after being treated at 90 °C for 0.5, 1 and 1 min respectively, while for *G. abietina* there is no growth as of 75 °C/0.5 min in the original dataset and as of 70 °C/2 min in the final adjusted dataset. For *G. abietina*, *H. annosum* and *M. populorum*, however, there is a wide gap that separates the few cases of growth at higher temperatures from the cluster of growths at lower ones. All observed results are presented in Table 5.

For both the original and final datasets, a generalized linear mixed model for binomial data revealed significant effects of treatment temperature and time for *G. abietina* and *H. annosum* (Table 6). Both variables had negative effects on post-treatment regeneration of each species. For *C. purpureum* and *M. populorum*, only temperature treatment had a significant negative effect on post-treatment regeneration (Table 6). For all four species, there was no significant effect of wood moisture content, which ranged from 14.21% to 25.72% and averaged 20.49% in the blocks of jack pine and from 4.14% to 78.43% and averaged 30.09% in the blocks of aspen. In the original data, variances and standard deviations for holes as a random effect were all 0 for *H. annosum* and *C. purpureum* and below 0.00001 for *M. populorum*. They were higher for *G. abietina* (variance = 2.82; SD = 1.68). In the final adjusted dataset, the variances and standard deviations for *H. annosum*, *C. purpureum* and *M. populorum* were all non-equal to 0 but below 0.00001. They were again higher for *G. abietina* (variance = 0.54; SD = 0.74). The variances and standard deviations are small enough that we can interpret that there is little to no difference between holes and within the same hole, indicating that sample location in the oven and in the block did not overly affect estimates for fixed effects.

Predicted lethal temperatures with their confidence intervals at each treatment time are shown in Table 7. Differences in predicted LT between the original and final adjusted datasets vary in most cases between 0.2 °C and 5.5 °C, though in three instances

Table 4
Predicted lethal temperatures of *M. scutellatus* larvae with 99% and 99.99% probability for each treatment time with standard errors and confidence intervals (CI).

M. scutellatus	1 min		2 min		3 min	
	p = 99%	p = 99.99%	p = 99%	p = 99.99%	p = 99%	p = 99.99%
Lethal temperature (°C)	80.5	116.0	66.1	94.7	66.1	94.7
Standard error	12.6	28.2	11.1	21.1	11.1	21.1
CI	24.6	55.3	21.8	41.4	21.8	41.4
CI lower limit	55.9	60.7	44.2	53.3	44.2	53.3
CI upper limit	105.1	171.3	87.9	136.1	87.9	136.1

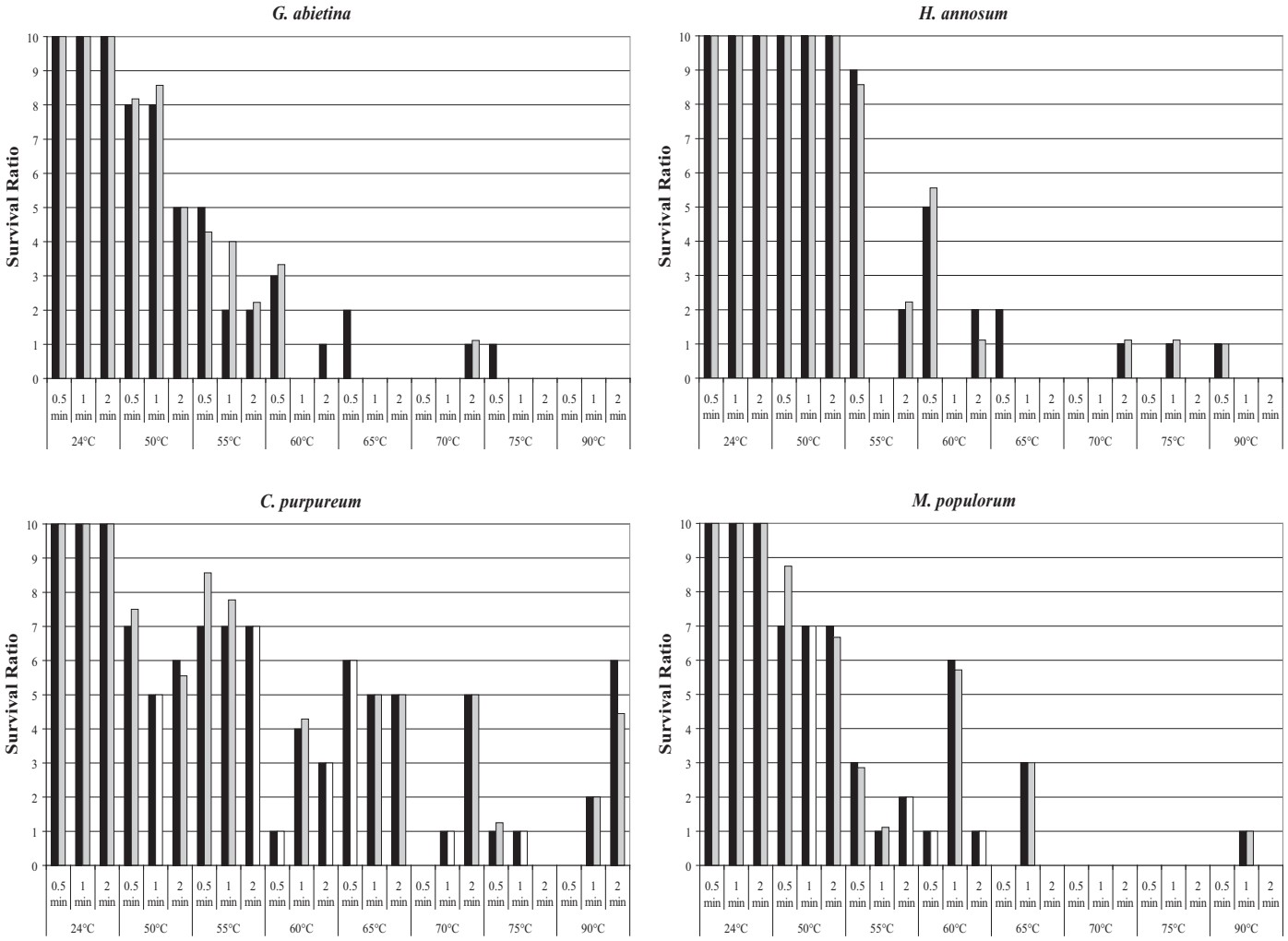

Fig. 2. Survival ratios for the four pathogenic fungi with temperature-time combinations. Ratios for the final dataset adjusted for temperature variations were all brought to an equivalent denominator (10). Bars in black represent data with datapoints from the original datasets, bars in gray represent data with the final adjusted data points, and bars in white represent series that could not be adjusted.

the variation is greater than 23 °C (*G. abietina*/0.5 min/p = 99.99%; *C. purpureum*/2 min/p = 99% and p = 99.99%). As with the larvae, there is a large discrepancy between the predicted temperatures at 99% and 99.99%. This is again probably due to the small sample size. None of the predicted LT/time combinations for any of the species had a predicted LT lower or equal to the ISPM standard of 60 °C. Several points, however, did fall into the confidence intervals (*G. abietina*: 0.5 min/99%, 1 min/99.99%, 2 min/99%; *H. annosum*: 1 min/99%; *M. populorum*: 0.5 min/99%, 2 min/99%).

4. Discussion

All *M. scutellatus* larvae were killed at 56 °C for 2 min and at 61 °C for 1 min or higher. Since we did not observe any abnormalities in the temperature readings for the two surviving larvae at 56 °C for 1 min, we must conclude that this is not a sufficient amount of time to guarantee 100% mortality in treated material. These results are close to the ISPM No. 15 standard of 60 °C for 1 min. Other studies that have tested microwave irradiation on xylophagous insect larvae showed varying results. In dry wood, it was only necessary to irradiate Asian longhorn beetle larvae for 5 s at 60 °C to obtain 100% mortality in treated larvae (Fleming et al., 2003). In the same study, larvae treated in green wood blocks at 60 °C had 100% mortality when the treatment time was three min or over. A later study obtained 100% mortality on cottonwood beetle (Plectrodera scalator [Fabricius]) larvae by heating wood up to at least 62 °C before removing them from the wood (Fleming et al., 2005). Other researchers found that microwave irradiation for 30 min on wood infested with the emerald ash borer did not prevent adult emergence in treated wood at any temperature (Nzokou et al., 2008). They proposed that uneven heat distribution might be the cause of the treatment's ineffectiveness. As they used recently cut log segments still covered in part with bark, we hypothesize that the heterogeneity of the wood due to the bark and the probably high moisture content may have affected the treatment's effectiveness versus cut wood which has had time to dry and contains no bark. Henin et al. (2008) irradiated larvae of the cerambycid *Hylotrupes bajulus* (L.) and found that a wood core temperature exceeding 55 °C and a surface temperature of 60 °C were enough to obtain 100% mortality. In all studies, except that of Nzokou et al. (2008), larvae were placed in wood blocks. A summary of findings indicate that temperatures around 60 °C are enough to kill Cerambycidae larvae in wood blocks with microwave irradiation, though the present study and those mentioned above set various starting conditions and do not follow a single standard method. The present study did however use wood dimensions and microwave applicator settings that followed ISPM No. 15 guidelines.

This study has shown that microwave irradiation can kill wood infecting fungi at time intervals much shorter than the

Table 5
Survival results for all four pathogenic fungi with temperatures and times after removal of errors due to measurement inaccuracy. In bold are results for the original datasets and in normal font are results for the datasets with datapoints removed because of high variation in temperature measurements. Results in italic were not corrected due to unavailability of temperature measurements.

		Jack pine				Trembling aspen			
Time (min)	Temperature (°C)	*G. abietina*		*H. annosum*		*C. purpureum*		*M. populorum*	
0.5	24	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10
	50	**8/10**	9/11	**10/10**	11/11	**7/10**	6/8	**7/10**	7/8
	55	**5/10**	3/7	**9/10**	6/7	**7/10**	6/7	**3/10**	2/7
	60	**3/10**	3/9	**5/10**	5/9	**1/10**	***1/10***	**1/10**	***1/10***
	65	**2/10**	0/5	**2/10**	0/5	**6/10**	***6/10***	**0/10**	***0/10***
	70	**0/10**	0/6	**0/10**	0/6	**0/10**	***0/10***	**0/10**	***0/10***
	75	**1/10**	0/7	**0/10**	0/7	**1/10**	1/8	**0/10**	0/8
	90	**0/10**	0/10	**1/10**	1/10	**0/10**	**0/10**	**0/10**	**0/10**
1	24	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10
	50	**8/10**	6/7	**10/10**	7/7	**5/10**	**5/10**	**7/10**	**7/10**
	55	**2/10**	2/5	**0/10**	0/5	**7/10**	7/9	**1/10**	1/9
	60	**0/10**	0/10	**0/10**	0/10	**4/10**	3/7	**6/10**	4/7
	65	**0/10**	0/10	**0/10**	0/10	**5/10**	5/10	**3/10**	3/10
	70	**0/10**	0/9	**0/10**	0/9	**1/10**	**1/10**	**0/10**	**0/10**
	75	**0/10**	0/9	**1/10**	1/9	**1/10**	**1/10**	**0/10**	**0/10**
	90	**0/10**	0/10	**0/10**	0/10	**2/10**	2/10	**1/10**	1/10
2	24	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10
	50	**5/10**	3/6	**10/10**	6/6	**6/10**	5/9	**7/10**	6/9
	55	**2/10**	2/9	**2/10**	2/9	**7/10**	**7/10**	**2/10**	**2/10**
	60	**1/10**	0/9	**2/10**	1/9	**3/10**	**3/10**	**1/10**	**1/10**
	65	**0/10**	0/10	**0/10**	0/10	**5/10**	5/10	**0/10**	0/10
	70	**1/10**	1/9	**1/10**	1/9	**5/10**	5/10	**0/10**	0/10
	75	**0/10**	0/9	**0/10**	0/9	**0/10**	**0/10**	**0/10**	**0/10**
	90	**0/10**	0/10	**0/10**	0/10	**6/10**	4/9	**0/10**	0/9

30 min (Table 4) suggested by ISPM No. 15 for conventional heat treatments. According to our results, however, the minimum temperature of 60 °C with a heating time of 1 min for dielectric heating is unlikely to be sufficient as a standard temperature/time combination for fungi as all four species showed re-growth at higher combinations, even though 60 °C did fall within the lower range of confidence intervals in predicted data at times equal or under 1 min for some of the points (*G. abietina*: 0.5 min/99%, 1 min/99.99%; *H. annosum*: 1 min/99%; *M. populorum*: 0.5 min/99%)

We have also determined that different species vary in susceptibility to heat treatment (Fig. 1). This corroborates the results from a study that tested conventional heating using heated water on 11 wood-decay fungi species, with three strains per species, in wood blocks (Ramsfield et al., 2010). They found that lethal temperatures varied between species of wood fungi. In all but one species, exposure to 61 °C for less than 1 min was sufficient to prevent re-growth. In the resistant species, *Schizophyllum commune*, 76 °C for less than 1 min was lethal. They suggested that *S. commune*'s resistance was due to its being able to form chlamydospores, a thick-walled structure produced by many fungi species which increases tolerance to heat.

Table 6
Outcome estimates (including β estimate, standard error, Wald statistic [z value], P-value [P]) for binomial generalized linear mixed model of fungi mortality following microwave irradiation according to temperature, time, and moisture content (MC). In bold are results for the original datasets and in normal font are results for the datasets with datapoints removed because of high variation in temperature measurements.

	β Estimate		Standard error		*z* value		*P* (>|*z*|)			
G. abietina										
Intercept	**−20.947**	−12.461	**5.225**	2.598	**−4.009**	−4.796	**<0.001**	***	<0.001	***
Temperature	**0.370**	0.219	**0.089**	0.043	**4.172**	5.112	**<0.001**	***	<0.001	***
Time	**0.021**	0.015	**0.010**	0.007	**2.165**	2.296	**0.030**	*	0.022	*
MC	**0.260**	0.190	**0.390**	0.255	**0.667**	0.742	**0.505**		0.458	
H. annosum										
Intercept	**−13.290**	−13.227	**2.553**	2.311	**−5.206**	−5.725	**<0.001**	***	< 0.001	***
Temperature	**0.221**	0.221	**0.041**	0.037	**5.323**	5.896	**<0.001**	***	< 0.001	***
Time	**0.016**	0.014	**0.007**	0.006	**2.300**	2.290	**0.021**	*	0.022	*
MC	**0.448**	0.381	**0.259**	0.231	**1.725**	1.651	**0.084**	.	0.099	.
C. purpureum										
Intercept	**−3.997**	−3.825	**0.806**	0.766	**−4.962**	−4.997	**<0.001**	***	< 0.001	***
Temperature	**0.075**	0.074	**0.012**	0.012	**6.188**	6.297	**<0.001**	***	< 0.001	***
Time	**−0.006**	−0.007	**0.004**	0.004	**−1.400**	−1.802	**0.162**		0.072	.
MC	**−0.113**	−0.190	**0.161**	0.155	**−0.704**	−1.223	**0.481**		0.221	
M. populorum										
Intercept	**−8.562**	−9.621	**1.633**	1.757	**−5.243**	−5.477	**<0.001**	***	< 0.001	***
Temperature	**0.160**	0.179	**0.027**	0.030	**5.900**	6.017	**<0.001**	***	<0.001	***
Time	**0.004**	0.004	**0.006**	0.006	**0.768**	0.713	**0.443**		0.476	
MC	**−0.100**	−0.149	**0.200**	0.197	**−0.501**	−0.755	**0.616**		0.450	

Significant results at $P \leq 0.05$. $P < 0.1$, '.' $P < 0.05$, '•' $P < 0.01$, '••' $P < 0.001$; '•••'.

Table 7
Predicted lethal temperatures for the four pathogenic fungi with 99% and 99.99 % probability for each treatment time with confidence interval value. In bold are results for the original datasets and in normal font are results for the datasets with datapoints removed because of high variation in temperature measurements.

	0.5 min		1 min		2 min	
G. abietina						
p = 99 %	65.4 ± 8.7	**79.9 ± 11.7**	59.0 ± 5	**59.4 ± 4.8**	70.8 ± 12.1	**73.7 ± 12.1**
p = 99.99 %	75.2 ± 14.3	**103.9 ± 23.2**	64.2 ± 8.5	**66.3 ± 9.1**	92.9 ± 26.6	**98.3 ± 26.6**
H. annosum						
p = 99 %	81.4 ± 11.7	**79.9 ± 9.1**	68.5 ± 9.1	**65.9 ± 7.8**	69.9 ± 9.3	**70.1 ± 8.2**
p = 99.99 %	102.0 ± 22.3	**99.0 ± 17.7**	84.2 ± 18.9	**79.3 ± 15.9**	86.2 ± 19.3	**85.7 ± 16.4**
C. purpureum						
p = 99 %	88.3 ± 14.9	**89.4 ± 15.1**	115.8 ± 29.2	**114.8 ± 28.4**	146.5 ± 52.3	**170.9 ± 75.6**
p = 99.99 %	118.1 ± 30.4	**121.6 ± 30.8**	173.6 ± 59.3	**172 ± 57.6**	232 ± 104.2	**278.2 ± 149.4**
M. populorum						
p = 99 %	64.1 ± 6.6	**65.9 ± 7.8**	93.8 ± 19	**94.1 ± 18.4**	65.1 ± 8.1	**64.8 ± 7.7**
p = 99.99 %	74.5 ± 12.9	**79.3 ± 15.9**	135.3 ± 40	**134.7 ± 38.5**	78.5 ± 16.9	**77.6 ± 15.8**

H. annosum is a chlamydospore-forming fungus which may explain why its observed and predicted LT in this study showed higher heat resistance than *G. abietina*, which was treated in the same jack pine blocks. Inactivation of mycelia of *H. annosum* is reportedly 35 °C, while conidia and ascospores, more resistant structures, are inactivated at 45 °C for 60 min (Otrosina and Cobb, 1989). Drier climates and warm weather favor its development (Kliejunas et al., 2010) and adhesion to conifer tissues is enhanced at temperatures of 30–70 °C, though germinability of the spores is not (Kliejunas et al., 2010).

G. abietina development is optimal at high humidity and at temperatures of 10–15 °C, with the capacity to grow at or near 0 °C (Barklund and Unestam, 1988). It is intolerant to higher temperatures and manifests rapid suppression of growth from 20 to 25 °C Dorworth and Krywienczyk (1975). This may be due to its range being confined to parts of the northern temperate zone and points to its low resistance to heat relatively to the other three species. It is also notable that its growth was much slower than the other three and only presented small clusters of hypha at mid-level or at the bottom of the aqueous solutions before treatment. The development of spores in its case must take more time and may explain its low resistance to the treatment.

Results showed a high temperature tolerance from *C. purpureum* compared to *M. populorum*, which was treated in the same aspen blocks. A growth at the surface of the aqueous solution in which it was kept before treatment indicates that there may already have been presence of spores. *C. purpureum*, however, grows in temperate climates and does not seem to present a particularly high tolerance to heat. The optimal temperature for basidiospore release is reported to be 18 °C and sporophores have a high affinity for moisture (Spiers, 1985). Germtube development is optimal at 25–27 °C with an upper limit of 37 °C (Spiers and Hopcroft, 1988). In *M. populotum*, ascospores are released between 3 and 30 °C with the optimal temperature being 27 °C (Thompson, 1941).

The short literature review above on the four species would indicate that they can be inactivated at temperatures much lower than those at which they were treated during this study (75 °C for *G. abietina* and 90 °C or more for *H. annosum*, *C. purpureum*, and *M. populorum*). This may be due to the short duration during which they were irradiated though, as mentioned, Ramsfield et al. (2010) found that 61 °C for less than 1 min was sufficient to kill all but one of 11 species of fungi. Another study did the same on four species of pathogenic fungi with radio-frequency and found that temperatures between 60 and 70 °C for 2 min were enough to kill between 98% and 100% of all cultures (Tubajika et al., 2007). These two studies had similar results to one another using two different modes of heating. As radio-frequency is a form of dielectric heating, we would have expected our results to be similar. We have found no mention in the literature pertaining to any particular tolerance to heat of the four species tested in the present study. All we can do is speculate as to the possibility of cross-contamination between samples during post-treatment manipulation of the cultures, causing sterilized mediums to be accidentally inoculated.

It is difficult to interpret variation in heat resistance between species that were irradiated in different types of wood since differences in the microstructure of wood cause differences in its dielectric properties (Duchow and Gerhardt, 1996). Generally, softwoods have a much more ordered and open microstructure with longer and larger cells than hardwoods. This means that the volume porosity of softwoods is usually higher than hardwoods. The permittivity of air is 1, which means that higher porosity in wood decreases its permittivity. Irradiation treatments are therefore likely to be more efficient when the permittivity of the medium surrounding unwanted organisms is lower. In one study, it was indeed shown that microwave drying for fir blocks was much more intensive than for beech blocks (Dedic and Zlatanovic, 2001). We would therefore expect microwaves to have a stronger effect on fungal pathogens in the jack pine blocks than in aspen. However, there was no clear demarcation in heat resistance between species in aspen or in jack pine blocks. In both the observed and predicted LT, *C. purpureum* and *M. populorum* (both species in aspen blocks) showed more heat resistance than *G. abietina*, though *M. populorum* did not show higher resistance than *H. annosum*. This is hard to interpret, however, since predicted LT are much higher for *M. populorum* than *H. annosum* at a treatment time of 1 min but lower at a time of a 2 min.

It was expected that LT would descend with each increase in time. The species whose reaction to the treatment resembled this pattern most was *H. annosum* (Table 7, Fig. 2). The predicted lethal dose for the final adjusted datasets dropped by 12.9 °C at p = 99% and by 17.8 °C at p = 99.99% from 0.5 to 1 min and then only varied by 1.4 °C and 2.0 °C from 1 to 2 min. The three other species reacted differently. There was a 6.4 °C drop at p = 99% and 11.0 °C drop at p = 99.99% for the lethal dose from 0.5 to 1 min for *G. abietina*, but at 120 °C it increased to 5.4 °C and 17.7 °C higher than the lethal temperature for 0.5 min. For *C. purpureum*, LT increased considerably with every increase in time. Those for *M. populorum* varied very little for 0.5 and 2 min, but were almost 30 °C higher than both these times at 1 min. The unexpected increase of predicted lethal temperatures at longer treatment times may simply indicate that 2 min are not sufficient with the current temperatures tested to insure that these three species are completely incactived and unable to regenerate from their more resistant structures and life stages.

There is a large discrepancy between observed LT and predicted LT as well as between predicted LT at p = 99% and p = 99.99%. Values at p = 99.99% also seem very high when compared to other stud-

ies (Ramsfield et al., 2010; Tubajika et al., 2007). The gaps between predicted values at 99% and 99.99% probabilities in both larvae and fungi may be due to lack of precision caused by the small sample size. This also probably explains the large confidence intervals, as there is an inverse square root relationship between sample size and confidence intervals. For the difference between observed results and predicted LT, another study dealing with microwaves had a similar problem when testing microwaves on the pine wood nematode and suggested that it was due to variability in the data used for the analyses (Hoover et al., 2010).

Moisture content in the jack pine blocks for the treatments on *H. annosum* and *G. abietina* only varied between 14.71% and 25.72% while variation was much higher in the trembling aspen blocks (4.14–78.43%) for treatments on *C. purpureum* and *M. populorum* and in jack pine blocks (16.42–73.54%) for treatments on *M. scutellatus* larvae. Even in the cases of higher variation, moisture content did not significantly affect mortality. Microwaves penetrate deeper into wood when moisture content is lower, which should have inhibited their capacity to cause mortality in organisms in the wood (Zielonka et al., 1997). However, the temperature probes were placed in such a fashion that they were never more than 1.5 cm away from the organisms in the wood, making it unlikely that high variations in temperature occurred between the probes and the organisms.

In the present study, despite the fact that placement of the inoculated wood samples in the blocks did not show any significant effects on mortality of fungi, the small dimensions of the wood blocks may have eliminated the problem of unbalanced heating within the wood sample. Fleming et al. (2005) showed that trials on cerambycid larvae yielded nearly 100% mortality on tested larvae when the samples were set on a turntable and rotated during treatment compared to when samples stayed in a fixed position. Sample blocks in these experiments were at least four times larger than those in the present study. It stands to reason that in larger industrial scale samples, homogeneity of heat distribution will be an important issue to consider. We have not found any other studies than Fleming et al. (2005) looking at wood sample rotation as a means of equalizing heat distribution in the context of phytosanitation. Rotation of the wood samples may reduce the required lethal temperature and time and circumvent the problem of wood heterogeneity such as was encountered in Nzokou et al. (2008).

5. Conclusion

We found that *M. scutellatus* larvae were eliminated from jack pine blocks at an observed temperature/time combination of 56 °C for 2 min or of 61 °C for 1 min and at a predicted value of 66 °C for 2 min at 99% probability. In the experiments on pathogenic fungi, results were variable. The fungi species were much more resistant to the treatment. *G. abietina* was eliminated at a temperature/time combination of 75 °C/0.5 min, *H. annosum* at 90 °C/1 min, *M. populorum* at 90 °C/2 min, and *C. purpureum* was still present at the highest temperature/time combination used. These values are surprisingly high but seem nevertheless encouraging as they indicate that the current ISPM No. 15 standard of 60 °C for 1 min for microwave irradiation is most likely sufficient for *M. scutellatus* larvae (FAO, 2014). We have also shown that three of the four pathogenic fungi (except *C. purpureum*) treated in this study are susceptible to microwave irradiation at short treatment times, albeit with higher temperature values than required by ISPM No. 15. In the event that industrial-scale microwave equipment would entail more or less the same costs as conventional ovens, microwave irradiation could considerably reduce time and increase cost efficiency for phytosanitary treatments in Québec. More data however is still necessary in order to adapt ISPM No. 15 standards to the higher resistance of pathogenic fungi transported in the wood. Future experiments should take into account longer treatment times and a wider range of temperatures to increase predictive power of analyses. It will also be necessary to evaluate and compare the resistance to temperature of additional pathogenic fungi species to give a better idea of how high the temperature would need to be to eliminate a much wider spectrum of species. To determine the optimal temperature/time combination from an economic standpoint, we suggest a future study to evaluate the cost and benefits of reducing treatment time and increasing temperature.

Acknowledgements

We present our thanks to the Fonds Québécois de la Recherche sur la Nature et les Technologies for providing the necessary funding for the project.

We would also like to thank Gilles Villeneuve and Ayoub Hanzouli for assistance with the microwave processor, Louis Bernier for providing access to fungi samples from the Forest Pathology Laboratory, Marc Mazerolle and Jenna Jacobs for help in statistics and coding, Luana Gragam-Sauvé and Simon Paradis for field assistance, the Rivière-aux-Rats sawmill for access to infested logs, Dan Kneeshaw, Christian Messier, Sheila Tenneson, Daniel Payette, Sibandith Sitthivong, Mathieu B. Morin, Simon Legault, Fanny Maure, Valérie Robichaud, Stephen Method, Ludivine Litzler and Alexandra Abboud for proof-reading and writing assistance.

References

Abelleira, A., Picoaga, A., Mansilla, J.P., Aguin, O., 2012. Detection of *Bursaphelenchus xylophilus*, causal agent of pine wilt disease on *Pinus pinaster* in Northwestern Spain. BioControl 95, 776.

Antti, A.L., Perré, P., 1999. A microwave applicator for on line wood drying: temperature and moisture distribution in wood. Wood Sci. Technol. 33, 123–138.

Asiegbu, F.O., Adomas, A., Stenlid, J., 2005. Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. *s. l.* Mol. Plant Pathol. 6, 395–409.

Barklund, P., Unestam, T., 1988. Infection experiments with *Gremmeniella abietina* on seedlings of Norway spruce and Scots pine. Eur. J. For. Pathol. 18, 409–420.

Bates, D., Maechler, M., Bolker, B., 2013. Package 'lme4', Linear mixed-effects models using S4 classes. Version 0.999,999-2.

Beever, D.J., 1970. The relationship between nutrients in extracted xylem sap and the susceptibility of fruit trees to silver-leaf disease caused by *Stereum purpureum* (Pers.) Fr. Ann. Appl. Biol. 65, 85–92.

Brockerhoff, E.G., 2009. Wood Borer and Bark Beetle Risk Analysis. Scion report.

2012. Wood Packaging Material and ISPM 15 Requirements. Canadian Border Services Agency.

Craighead, F.C. 1923. North American Cerambycid Larvae: A Classification and the Biology of North American Cerambycid Larvae Ottawa.

Dedic, A., Zlatanovic, M., 2001. Some aspects and comparisons of microwave drying of beech (*Fagus moesiaca*) and fir wood (*Abies alba*). Eur. J. Wood Wood Prod. 59, 246–249.

Dorworth, C.E., Krywienczyk, J., 1975. Comparisons among isolates of *Gremmeniella abietina* by means of growth rate, conidia measurement, and immunogenic reaction. Can. J. Bot. 53, 2506–2525.

Duchow, K.J., Gerhardt, R.A., 1996. Dielectric characterization of wood and wood infiltrated with ceramic precursors. Mater. Sci. Eng.: C 4, 125–131.

EPPO, 1980. *Mycosphaerella populorum* G.E. Thompson Ascomycetes: Dothideales *Mycosphaerella populorum* G.E. Thompson Ascomycetes: Dothideales. EPPO Bull. 10, 13–17.

EPPO, 2009. PM 7/92(1): *Gremmeniella abietina*. EPPO Bull. 39, 310–317.

FAO, 2014. International Standards for Phytosanitary Measures (ISPM): Guidelines for Regulating Wood Packaging Material in International, No. 15. Food and Agriculture Organization of the United Nations.

Fielding, N.J., Evans, H.F., 1996. The pine wood nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (equals B-lignicolus Mamiya and Kiyohara): an assessment of the current position. Forestry 69, 35–46.

Firth, D., 1993. Bias reduction of maximum likelihood estimates. Biometrika 80, 27–38.

Fleming, M.R., Hoover, K., Janowiak, J.J., Fang, Y., Wang, X., Liu, W.M., Wang, Y.J., Hang, X.X., Agrawal, D., Mastro, V.C., Lance, D.R., Shield, J.E., Roy, R., 2003. Microwave irradiation of wood packing material to destroy the Asian longhorned beetle. For. Prod. J. 53, 46–52.

Fleming, M.R., Janowiak, J.J., Kearns, J., Shield, J.E., Roy, R., Agrawal, D.K., Bauer, L.S., Miller, D.L., Hoover, K., 2004. Parameters for scale-up of lethal microwave treatment to eradicate cerambycid larvae infesting solid wood packing materials. For. Prod. J. 54, 80–84.

Fleming, M.R., Janowiak, J.J., Kimmel, J.D., Halbrendt, J.M., Bauer, L.S., Miller, D.L., Hoover, K., 2005. Efficacy of commercial microwave equipment for eradication of pine wood nematodes and cerambycid larvae infesting red pine. For. Prod. J. 55, 226–232.

Gonthier, P., Lione, G., Giordano, L., Garbelotto, M., 2012. The American forest pathogen *Heterobasidion irregulare* colonizes unexpected habitats after its introduction in Italy. Ecol. Appl. 22, 2135–2143.

Gonthier, P., Warner, R., Nicolotti, G., Mazzaglia, A., Garbelotto, M.M., 2004. Pathogen introduction as a collateral effect of military activity. Mycol. Res. 108, 468–470.

Haack, R.A., 2006. Exotic bark- and wood-boring Coleoptera in the United States: recent establishments and interceptions. Can. J. For. Res. 36, 269–288.

Henin, J.-M., Charron, S., Luypaert, P., Jourez, B., Hebert, J., 2008. Strategy to control the effectiveness of microwave treatment of wood in the framework of the implementation of ISPM 15. For. Prod. J., 58.

Hoover, K., Uzunovic, A., Gething, B., Dale, A., Leung, K., Ostiguy, N., Janowiak, J.J., 2010. Lethal temperature for pinewood nematode, *Bursaphelenchus xylophilus*, in infested wood using microwave energy. J. Nematol. 42, 101.

Kliejunas, J.T., Brian, W., Geils, S.J., Glaeser, Jessie Micales, Goheen, Ellen Michaels, Hennon, Paul, Harry Kope, Mee-Sook Kim, Stone, Jeff, Sturrock, Rona, Frankel, S.J., 2010. Review of Literature on Climate Change and Forest Diseases of Western North America. US Department of Agriculture Forest Service Pacific Southwest Research Station.

Kosaka, H., Aikawa, T., Ogura, N., Tabata, K., Kiyohara, T., 2001. Pine wilt disease caused by the pine wood nematode: the induced resistance of pine trees by the avirulent isolates of nematode. Eur. J. Plant Pathol. 107, 667–675.

Kosmidis, I. 2013. Package 'brglm', Bias reduction in binomial-response GLMs. Version 0. 5–7.

Koubaa, A., Perré, P., Hutcheon, R.M., Lessard, J., 2008. Complex dielectric properties of the sapwood of aspen white birch, yellow birch, and sugar maple. Drying Technol.: An Int. J. 26, 568–578.

Lygis, V., Vasiliauskas, R., Stenlid, J., Vasiliauskas, A., 2004. Silvicultural and pathological evaluation of Scots pine afforestations mixed with deciduous trees to reduce the infections by *Heterobasidion annosum ss*. For. Ecol. Manag. 201, 275–285.

Madé, B., 2012. Avis à toute personne qui utilise le bromure de méthyle. Environnement Canada – Division de la production des produits chimiques.

Mireku, E., Simpson, J.A., 2003. Fungal and nematode threats to Australian forests and amenity trees from importation of wood and wood products. Can. J. Plant Pathol. 24, 117–124.

Nzokou, P., Tourtellot, S., Kamdem, D.P., 2008. Kiln and microwave heat treatment of logs infested by the emerald ash borer (*Agrilus planipennis* Fairmaire)(Coleoptera: Buprestidae). For. Prod. J. Index 58, 68–72.

Otrosina, W.J., Cobb Jr, F.W., 1989. Biology, ecology and epidemiology of *Heterobasidion annosum*. In: Symposium on Research and Management of Annosus Root Disease in Western North America, US Department of Agriculture, Forest Service Monterey, California, pp. 26–33.

Peddle, S.M., 2000. Host selection, oviposition behavior, and inter- and intra-specific competition in the white-spotted pine sawyer beetle. In: *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae), Faculîy of Forestry. University of Toronto.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288.

Pitt, D.G., Dumas, M.T., Wall, R.E., Thompson, D.G., Lanteigne, L., Hintz, W., Sampson, G., Wagner, R.G., 1999. *Chondrostereum purpureum* as a biological control agent in forest vegetation management. I. Efficacy on speckled alder, red maple, and aspen in eastern Canada *Chondrostereum purpureum* as a biological control agent in forest vegetation management. I. Efficacy on speckled alder, red maple, and aspen in eastern Canada. Can. J. For. Res. 29, 841–851.

Ramsfield, T.D., Ball, R.D., Gardner, J.F., Dick, M.A., 2010. Temperature and time combinations required to cause mortality of a range of fungi colonizing wood. Can. J. Plant Pathol. 32, 368–375.

Ramsfield, T.D., Becker, E.M., Rathlef, S.M., Tang, Y., Hintz, W.E., Vrain, T.C., Shamoun, S.F., 1996. Geographic variation of *Chondrostereum purpureum* detected by polymorphisms in the ribosomal DNA. Can. J. Bot. 74, 1919–1929.

Robertson, L., Cobacho Arcos, S., Escuer, M., Santiago Merino, R., Esparrago, G., Abelleira, A., Navas, A., 2011. Incidence of the pinewood nematode *Bursaphelenchus xylophlius* Steiner and Buhrer, 1934 (Nickle, 1970) in Spain. Nematolog 13, 755–757.

Shi, J., Chen, F., Luo, Y.Q., Wang, Z., Xie, B.Y., 2012. First isolation of pine wood nematode from *Pinus tabuliformis* forests in China. For. Pathol. 43, 59–66.

Spiers, A.G., 1985. Factors affecting basidiospore release by *Chondrostereum purpureum* in New Zealand. Eur. J. For. Pathol. 15, 111–126.

Spiers, A.G., Hopcroft, D.H., 1988. Factors affecting *Chondrostereum purpureum* infection of *Salix*. Eur. J. For. Pathol. 18, 257–278.

Suzuki, K., 2002. Pine wilt disease – a threat to pine forest in Europe. Dendrobiology 48, 71–74.

Thompson, G.E., 1941. Leaf-spot diseases of poplars caused by *Septena musiva* and *S. populicola*. Phytopathology 31, 241–254.

Tubajika, K., Jonawiak, J., Mack, R., Hoover, K., 2007. Efficacy of radio frequency treatment and its potential for control of sapstain and wood decay fungi on red oak, poplar, and southern yellow pine wood species. J. Wood Sci. 53, 258–263.

UNEP, 1994. Montreal Protocol On Substances that Deplete the Ozone Layer: UNEP 1994 Report of the Methyl Bromide Technical Options Committee. United Nations Environment Programme.

Rules and Regulations: Importation of Wood Packaging Material, in: Agriculture, U.D.o. (Ed.). Animal and Plant Health Inspection Service.

Wallace, S., 2012. *Gremmeniella abietina* (Scleroderris Canker) – Fact Sheet.

WMO, 2006. Scientific Assessment of Ozone Depletion: 2006. World Meteorological Organization.

Zhao, L.L., Wei, W., Kang, L., Sun, J.H., 2007. Chemotaxis of the pinewood nematode, *Bursaphelenchus xylophilus*, to volatiles associated with host pine, *Pinus massoniana*, and its vector *Monochamus alternatus*. J. Chem. Ecol. 33, 1207–1216.

Zielonka, P., Gierlik, E., Matejak, M., Dolowy, K., 1997. The comparison of experimental and theoretical temperature distribution during microwave wood heating. Holz Roh Werkst. 55, 395–398.