Phytosanitary treatment of European pallets by microwave: developing a program to ensure compliance with ISPM 15 and monitoring its efficacy on the house longhorn beetle (*Hylotrupes bajulus* L.)

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Abstract

Microwave heating was recently approved by the FAO as a significantly effective phytosanitary treatment for wood packaging material. According to ISPM 15 (FAO 2009), the target organisms are eradicated if a temperature higher than 60 °C is maintained for 60 seconds across the entire profile of the board (i.e. 60 °C/60 s). A study using pallet boards was carried out in order to set up a treatment program that would meet ISPM 15 requirements in terms of wood temperature and insect mortality. A 4 m-long industrial tunnel oven (maximum power of 28.8 kW) was used to carry out the experiments. Temperature was measured by means of a VarioCAM® infrared camera. The most relevant results we found were: (i) achieving a mean temperature of 63.2 °C (*Populus* sp.) or 64.8 °C (*Pinus sylvestris* L.) on the upper surface of 22 mm-thick boards enabled compliance with FAO requirements (i.e. 60 °C/60 s), whatever the moisture content, basic density and initial temperature of the wood (provided the latter exceeded 0 °C); (ii) larvae >150 mg represented the most microwave-resistant life stage of *Hylotrupes bajulus* L.; (iii) the mortality rate of the larvae was influenced by the moisture content of the boards. Using the Gompertz model, the upper surface temperature was estimated that would be needed to achieve a 99.99683% mortality rate (the Probit 9 mortality level of efficacy) for the most microwave-resistant life stage of *H. bajulus*. That temperature was estimated to be 46.8 °C and 57 °C for wood with >50% and <25% moisture content, respectively.

1 Introduction

In order to prevent biological invasions, the Food and Agriculture Organization (FAO) has put in place the International Standards for Phytosanitary Measures (ISPMs). Among these standards is ISPM 15, which specifies the "Regulation of wood-packaging material in international trade". ISPM 15 stipulates that wood used in the international transport of merchandise cannot be allowed to circulate unless it has undergone prior phytosanitary treatment. Only two types of treatment are currently approved: fumigation with methyl bromide and heat treatment (FAO 2009). Use of the former method of treatment is harmful to the ozone layer (Singh and Kanakidou 1993; Fields and White 2002): since January 2005, it has therefore been phased out in line with the Montreal Protocol (UNEP 2000). The most common method of application of heat treatment uses either a conventional steam or a dry kiln heat chamber, requiring in both cases that a temperature of at least 56 °C is maintained across the entire profile of the wood for at least 30 minutes. The main disadvantages associated with this procedure are its duration and the delay caused to the production line due to heat-treatment of the wood in a kiln heat chamber. Another disadvantage previously highlighted is the difficulty of ensuring that the heat-treatment applied is adequate for all the pieces of wood being treated (Henin et al. 2008). In addition, some research has raised doubts about the efficacy of this treatment in certain situations and with regard to some pest species – *Agrilus planipennis* for example (McCullough et al. 2007; Ramsfield et al. 2010; Goebel et al. 2010; Sobek et al. 2011).

However, in April 2013, the FAO approved treatment based on dielectric heating: wood packaging can now be heated using microwaves or radio-frequencies produced in industrial ovens, such as the one described in this study. The heat released due to the irradiation of polar molecules eradicates any organisms that the wood may be harboring. The advantages of this type of treatment are: the rapidity of its action (due to the heat being immediately produced inside the wood), the inability of pests to develop a tolerance to the treatment, its efficacy against all targeted species and the absence of any poison or residues in the wood (Henin et al. 2008).

The lethal effect of microwaves on wood-boring insects can be induced in two ways. First, when the temperature of the environment becomes too high for the insects to survive, this is known as "exogenous" action. The heat produced by this method will not be uniform across the entire profile of the object to be treated. Indeed, the dielectric properties of the wood are influenced by moisture content, basic density, temperature, and the depth and direction of the grain of the wood (Torgovnikov 1993; Hansson and Antti 2008). Secondly, microwaves interact directly with the body fluids of the targeted organisms by raising their temperature ("endogenous" action) (Fleming et al. 2003). In the case of insects, whatever the origin of the heat produced, death almost always occurs when the temperature reaches a threshold of around 60 °C. This threshold corresponds to the coagulation temperature of thermosensitive proteins, which block motor activity and respiratory exchanges (Anglade et al. 1979). However, the effects of temperature can vary according to the tissue. The thermal threshold for the death of the pests is determined by the most sensitive vital tissue (Denlinger and Yocum 1998). Species and stage of development therefore have an indirect influence on the lethal temperature of individuals. This is also influenced by the duration of exposure to the heat, acclimatization and the level of humidity. Fields (1992) and Fields and White (2002) defined ranges of lethal temperatures for insects based on the duration of their exposure: less than a day for 45-50 °C, less than an hour for 50-62 °C and less than a minute if the temperature exceeds 62 °C.

The idea of using microwaves in the context of the sanitary treatment of wood is not new, as attested by the research of Thomas and White (1959). Since that study, research concerning different species has been carried out: Van den Bruel et al. (1960) and Henin et al. (2008) on *Hylotrupes bajulus*, Crocker et al. (1987) on *Curculio sp.*, Kjerulf-Jensen and Koch (1992) on *Serpula Lacrymans,* Lewis et al. (2000) on *Incisitermes minor*, Fleming et al. (2003) on *Anoplophora glabripennis*, Fleming et al. (2004) on *Plectrodera scalator*, Fleming et al. (2005) and Hoover et al. (2010) on *Bursaphelenchus xylophilus*, Nzokou et al. (2008) on *Agrilus planipennis*, Massa et al. (2011) on *Rhynchophorus ferrugineus,* and Bisceglia et al. (2009) on different species of nematodes and insects.

Microwave treatment (MWT) aimed at ensuring the destruction of all wood pests was recently submitted for consideration by the signatory countries to the International Plant Protection Convention (submission period May 15th to September 30th 2012). This method now appears in appendix 1 of the revised version of ISPM 15. The standard stipulates that, during MWT, a minimum temperature of 60 °C should be maintained for sixty seconds across the entire profile of the wood (60 °C/60 s). The thickness of the pieces of wood treated may not exceed 20 cm (FAO 2011). These constraints must be respected whatever the initial characteristics of the boards. It is also necessary to ensure that compliance with the constraint of 60 °C/60 s guarantees the death of the insects with the level of probability required in each specific case, regardless of the characteristics of the wood or the stage of development of the organism.

It is in this context that the present study, focusing on the pallet-making industry, aimed at addressing the following questions:

1. Is it possible to guarantee compliance with the 60 °C/60 s constraint through the surface temperature of the wood when measured during treatment by microwave, regardless of the initial wood characteristics such as species, density, moisture content and temperature?

2. Do the conditions defined by the standard (60 °C/60 s) guarantee the mortality of the pests with the required probability level (i.e. 99.997%: see 2.7) even under the most unfavorable conditions for their eradication (characteristics of the wood and stage of development of the pests)?

2 Material and methods

2.1 Wood material

The wood species chosen for the experiments were *Pinus sylvestris* and *Populus* sp. as they are frequently used by the European pallet industry (Moncel and Chanrion 2004). Pine is also the reference species for testing preservation products with regard to wood-boring insects (CEN 2009) and wood-decaying fungi (CEN 2008). Poplar has a lower density in comparison with pine, thereby providing an opportunity to assess the impact of this factor. The dimensions of the boards treated were 80 cm x 10 cm x 2.2 cm. These dimensions correspond to the standard values for boards used in the manufacture of EUR EPAL® pallets (Chanrion and Level 1999). European pallets are made up of only boards and blocks (no stringers); the latter are extruded and thus do not harbor pests. Hence, this study focused on 22 mm-thick boards. For the different tests, the boards were conditioned to reach the required values of temperature and moisture content, aiming to encompass the variability these characteristics present within the pallet-making industry (see Table 1). Moisture content was expressed in percentage on a dry mass basis (see also § 2.6).

2.2 Tunnel and batch microwave ovens

Two types of microwave oven were used:

- The first was an industrial “tunnel-type” microwave oven. This oven was equipped with a 45 cm-wide conveyor belt enabling a continuous supply of wood for uninterrupted treatment. Its speed was adjustable from 1 to 10 m/min and the speed was calibrated. The oven contained 16 magnetrons of 1.8 kW (maximum power=28.8 kW), which could be controlled individually. The magnetrons were distributed along the four meter long irradiation tunnel. The frequency of the microwaves was 2.45 GHz. The waves of each magnetron were spread throughout the tunnel by means of a waveguide. At the entrance to the oven, a detection system made it possible to program the automatic activation and deactivation of the magnetrons as the boards passed through their zone of action. This automated management meant that the electricity consumption of the oven could be minimized. Sixty centimeter-long wave-traps were positioned in front of the entrance and beyond the exit of the tunnel. The total length of the oven exceeded 7 m;

- The second type of oven, the “batch type”, was a classical domestic oven provided with a turning plate (aiming to homogenize irradiation of the issues) and generating waves of 2.45 GHz for a maximum power of 900 W. The dimensions of the chamber were 34 x 33 X 20.5 cm.

2.3 Insect species

In the context of European standardization, the house longhorn beetle (*Hylotrupes bajulus* L.) is the reference species with regard to the preventive or curative biocidal treatment of wood. The beetles used in this study were bred and supplied by the Laboratory of Wood Technology of the Public Service of Wallonia (SPW): the breeding chamber was managed in accordance with the recommendations decreed in the appendix to Standard EN 1390 (2009). Samples of imagos, larvae and eggs were supplied in accordance with the terms of the above standard. The samples were selected at a maximum of three days before being used in the experiments. During this period, they were kept individually in plastic vials and their vitality was controlled before the experiment (in accordance with EN 1390).

2.4 Temperature measurement

The temperature increase during MWT is not uniform (Antti and Perré 1999; Rattanadecho 2006; Hansson and Antti 2008). Consequently, estimates based on spot measurements of the temperature of a treated piece of wood are not accurate. In the present study, it was therefore chosen to use a VarioCAM® thermal camera (JENOPTIC, Germany) to measure the temperature of the boards. This camera has a resolution of 1.280 x 960 infrared pixels and allows for the measurement of temperatures varying from -40 to 1200 °C. The precision of the camera is ±1.5 °C for temperatures ranging from 0 to 100 °C. This technology offers the advantage of performing rapid measurements and simultaneously recording numerous temperatures along every point of a surface with great precision. Photos taken were analyzed with the IRBIS®3 software (InfraTec, Germany). The temperature of an object (a board or a specimen of *H. bajulus*) corresponded to the average of the temperature values associated with all the pixels that made up the image of the object. The emissivity values of pine and poplar wood were previously determined (0.924 and 0.816, respectively) in order to ensure that the temperature would be estimated as accurately as possible. The values were corrected by taking into account the room temperature.

2.5 Statistical analysis

Data were analyzed with the Minitab 16 statistical software. First of all, raw data were analyzed with boxplot and other basic statistical procedures, in order to identify outliers. Afterwards, the main analysis involved linear, nonlinear and binary logistic regression procedures, as well as analysis of variance (ANOVA) procedures. Statistical requirements on raw data were assessed through the Kolmogorov-Smirnov normality test and the homoscedasticity F-test where appropriate.

2.6 Experiment 1: Ensuring compliance with ISPM 15

Overall, 17 series of 20 boards were irradiated. Whilst the series exhibited contrasting basic density (BD), initial moisture content (MC_i) and initial temperature (T_i), these characteristics were as homogeneous as possible within each series (Table 1). However, the MC_i of green planks in a given batch presented some variability depending on the species (mainly for poplar), radial position or wood density, for instance. The dry board series were conditioned for four weeks in order to obtain homogenous temperatures and moisture content within each board.

No board with a T_i < 0 °C was used, given their specific behavior with regard to microwaves (Rattanadecho 2004; Hansson et al. 2005; Henin et al. 2012). Just before treatment, the boards were weighed and a thermal photo of the surface was taken in order to determine the initial temperature (T_i).

The power supplied by the oven was adjusted from one series of boards to another in order to obtain post-treatment mean core temperatures of between 35 and 85 °C. The speed of the conveyor belt was 1.5 m/min for all the series of boards. Power and speed were constant while each series of 20 boards was passing through the oven.

The 20 boards of each series were introduced into the oven in single file (± 2 cm of space between each board). They were systematically positioned and elevated to a height of 4 cm above the conveyor belt by means of boxes made of polyethylene terephthalate. The first and last five boards of the series had the role of maintaining a constant load of wood in the oven during the passage of the other 10 boards, referred to as “central boards”, in order to ensure they had a constant energy density. Only the data relative to these 10 central boards were retained for analysis of the results.

When exiting the oven, a thermal photo of the upper surface of the boards was taken in order to measure the surface temperature after treatment ($T_{Surface}$). One minute after exiting the oven, the 10 central boards were cut at 20 cm from their ends, and a thermal photo of the section ($T_{Section}$) was immediately taken. The $T_{Sections}$ were not measured at the extremities of the boards because these areas generally reach higher temperatures (Antti and Perré 1999; Henin et al. 2008). This was also found to be the case during preliminary experiments where it was established that $T_{Sections}$ at 2 cm from the end of boards of the same dimensions were significantly higher than at 20 and 40 cm. The boards were then weighed and a sample was taken from each board and oven-dried in order to calculate the MC_i and BD as follows:

[1] $$MC_i = 100 * \frac{Mb_i - Mb_f * Ms_0 / Ms_f}{Mb_f * Ms_0 / Ms_f}$$

[2] $$BD = \frac{Ms_0}{Vs_g}$$

where

Mb_i is the initial mass of the board before MWT,

Mb_f is the (final) mass of the board after MWT,

Ms_f is the mass of the sample after MWT,

Ms_o is the oven-dry mass of the sample,

Vs_g is the green volume of the sample.

2.7 Experiment 2: *H. bajulus* resistance to microwave temperatures

In order to be able to meet the second objective of our study, we first analyzed the literature for information on the most MWT-resistant life stage of the house longhorn beetle. Using a conventional hot water bath test, Andreuccetti et al. (1994) found that the highest resistance was displayed in larvae with a mass in excess of 100 mg. From preliminary MW tests performed in the authors' laboratory (not documented here), larvae with a mass in excess of 150 mg showed the highest resistance to the treatment. Those observations led to hypothesize that the heavier the larvae, the more heat-tolerant they are. For this reason, the samples in the present study were divided into four groups: eggs, larvae with a mass of less than 150 mg, larvae with a mass in excess of 150 mg, and adults (table 2). The number of eggs treated was quite high because they were firmly attached to a Petri dish and could not be separated from each other without the risk of damage; eggs also needed a longer period of treatment than larvae or adults in order to reach the target temperatures. The number of larvae used with a mass in excess of 150 mg was higher than that of lighter larvae, in order to determine with accuracy the lethal temperature for the heavier larvae. Individuals of each stage of development were sampled at random and used as controls.

For the tests on the adults and the larvae, four individuals at the same stage of development were placed in a Petri dish. Concerning the eggs, they were kept and tested in the Petri dish in which they had been laid. The dishes were treated one by one in the "batch-type" microwave oven at 900 W, in random order.

Immediately after treatment, a thermal image was taken in order to determine the average body temperature of each individual. The larvae and adults were then observed for seven days in order to determine their vital state (dead or alive). A larva was considered to be alive if it reacted to tactile stimuli (movement of body and/or mandibles); eggs were considered to be alive if larvae emerged from them after being returned to normal breeding conditions.

The temperature of the most resistant stage of development for which a mortality rate of 99.99683% (Probit 9) could be achieved was then estimated. This level is stipulated in the provisional version of appendix 1 of the revised ISPM 15 and is currently being used by the majority of countries as the level at which treatment of pests is considered to be effective (Haack et al. 2011). The provisional version of appendix 1 of ISPM 15 stated: "*Efficacy testing can be completed either directly, using the numbers of test individuals required to statistically demonstrate the efficacy level, or by extrapolation by fitting dose-response data to a known theoretical dose-response curve (e.g. normal (i.e. Probit), logistic, Gompertz, Weibull)*" (FAO

2010). In our study, the Gompertz model was fitted with the Minitab 16 software program. This model presents the best statistical adjustment for the extreme values and was previously used by Witten and Satzer (1992) and Hoover et al. (2010) for similar data to our own. Because the distribution of errors deviated from a normal distribution, especially at the tails, confidence interval of the 99.99683% death probability was computed based on the bootstrap percentile interval corresponding to 2000 prediction replicates (Davison and Hinkley 1997; Canty and Ripley 2012).

2.8 Experiment 3: Testing ISPM 15 constraints on *H. bajulus* infested boards

Larvae with a mass in excess of 150 mg were inserted into boards and treated in the industrial oven. Fourteen of the 17 series of boards involved in experiment 1 were prepared in this way (all the series except for PIGL 2, PODL 2 and PIDH 3). Two cavities with a diameter of 9 mm and 3 or 6 cm in depth were drilled into the center of the 10 central boards of the different series. The cavities were drilled at 20 and 40 cm from the extremities in order to avoid the superheated areas (Figure 1). Just before the treatment of the boards, one larva was delicately introduced headfirst into the end of each cavity with the help of a small soft-brush, and the openings were sealed with a cotton wad (in compliance with the EN 1390 standard).

A preliminary experiment, carried out under the same conditions with 70 larvae, showed that the temperature of the larvae one minute after the treatment was lower than the T_{Section} in 96% of cases (average difference of 9.7 °C). The heat transfer from the board to the larvae remained after the board was taken out of the oven. However, we realized the importance of not checking on the state of the larva too early after removing the board from the oven, as this could result in the survival of some individuals (Henin et al. 2008). The temperature inside the wood decreased slowly as the board cooled down, and larvae that had spent a long time inside the wood would be more likely to die under these conditions than if they were removed early for examination. For this reason, the larvae were kept in the cavities after treatment until the boards had cooled completely (between 2.5 and 3.5 h). During this period, the boards were left cooling individually on a grid enabling air circulation. This was in order to reproduce the least favorable conditions likely to apply in an industrial context, where boards might be directly assembled into pallets after treatment. After this period, the larvae were carefully removed from the boards and the lethality of the treatment was determined in accordance with the procedure defined for experiment 2. The lethality of MWT was modeled by binary logistic regression (the Gompertz model) in order to identify the T_{Surface} for which a mortality rate of 99.99683% could be reached (as for experiment 2, the confidence interval of this value was assessed through a bootstrap procedure). Six larvae were used as controls.

3 Results and discussion

3.1 Experiment 1: Ensuring compliance with ISPM 15

The objective of this experiment was to determine how to guarantee that the temperature across the entire piece of wood was higher than 60 °C for 60 seconds by monitoring the surface temperature of the boards. The T_{Section} was higher than the T_{Surface} in 91% of cases (*N*=170) (Figure 2). This clearly shows that there are exceptions to the statement: “When using microwaves as a heating source, the coldest part of the wood is the surface” (initial version of annex 1 of ISPM 15; FAO 2011). The results of this study demonstrate that compliance with the standard is not guaranteed even when the $T_{Surface}$ reaches 60 °C for 60 seconds.

The $T_{Surface}$ makes it possible to explain 91.6% of the variability of the $T_{Sections}$ of the two wood species studied here ($T_{Section} = 5.171 + 1.001 * T_{Surface}$; N = 170). The addition of T_i, MC_i or BD to the explanatory variables of the relationship between $T_{Section}$ and $T_{Surface}$ slightly improved the coefficient of determination R^2 (91.9, 91.6 and 91.7%, respectively). According to Antti (1993) and to Zielonka and Gierlik (1999), increasing the moisture content of the wood reduces the depth of penetration by the microwaves and causes a less uniform distribution of temperature across the profile of the board. The absence of impact of moisture content on the relationship between mean $T_{Surface}$ and mean $T_{Section}$ observed in this experiment was probably due to the reduced thickness of the boards (22 mm). However, the moisture content of the boards obviously influences the time needed to reach a given mean surface (or core) temperature; the moisture content of the boards also influences the pattern of the $T_{Section}$.

The $T_{Sections}$ were significantly different according to the wood species (ANOVA relating to the variable $T_{Section}$, with the wood species as a fixed factor and $T_{Surface}$ as a covariate; p-value of 0.000 and 0.042 for the $T_{Surface}$ and the wood species, respectively). The regression equation between $T_{Surface}$ and $T_{Section}$ can therefore be reformulated as follows for pine [3] and for poplar [4]:

$$[3]\ T_{Section} = 5.72 + 0.983 * T_{Surface}\ (R^2 = 90.0\%;\ N = 90)$$

$$[4]\ T_{Section} = 4.89 + 1.017 * T_{Surface}\ (R^2 = 93.4\%;\ N = 80)$$

These equations are only valid for the ranges of $T_{Surface}$ and $T_{Section}$ between 40 and 80 °C.

The use of confidence intervals (estimated for individual values) with different levels of confidence made it possible to calculate the $T_{Surface}$ that warrants $T_{Sections}$ of higher than 60 °C for a period of at least one minute (Table 3). It should be noted that for the same confidence level, the $T_{Surface}$ of the pine boards needed to be slightly higher than that of poplar.

Room temperature during the experiments fluctuated between 15 and 23 °C. The cooling of the boards would have been slightly quicker at a lower room temperature. It should be remembered that our results are valid for boards with a thickness of 22 mm and that are not frozen.

The application of this type of monitoring in an industrial context would require an accurate estimate of the $T_{Surface}$. Should the temperature be measured with infrared sensors, data would need to be recorded at several spots along each board. Figure 3 shows two examples of thermal photographs of the upper surfaces of pine boards taken during the experiment, revealing a large temperature heterogeneity.

During the experiments, measurement of the $T_{Surface}$ could not be carried out immediately at the end of the MWT for practical and safety reasons (exposure of the operator to microwaves). The thermal photos were taken around 30 seconds after the treatment finished (corresponding to the time necessary for the board to pass through the microwave filters). In an industrial context, the $T_{Surface}$ would have to be measured during the irradiation process, so that the treatment might be stopped and energy saved when the desired temperature limit was reached. As a consequence, the calculated $T_{Section}$ estimate would have to be corrected.

3.2 Experiment 2: *H. bajulus* resistance to microwave temperatures

Table 4 presents the number of living and dead individuals seven days after their irradiation, per stage of development and per class of average body temperature. During this same period, no deaths were observed among the group of control larvae.

A 100% mortality rate was obtained from 45 °C, 51 °C, 57 °C and 66 °C for the adults, eggs and larvae with a mass of less than 150 mg and in excess of 150 mg, respectively. The adults constituted the group most sensitive to microwaves, while the larvae with a mass in excess of 150 mg represented the most resistant stage of development. This result is consistent with the experiment of Andreuccetti *et al.* (1994). In their study of *Leptinotarsa decemlineata*, Pelletier and Colpitts (2000) found that the egg and adult stages of development showed the least resistance to MWT. Adjustment of our data by logistic regression according to the Gompertz model (Figure 4) showed that the instantaneous temperature that is theoretically required to reach a mortality rate of 99.99683% for larvae with a mass in excess of 150 mg is 70 °C (confidence interval of the probit value corresponding to 70° C is [99.94%; 100%]). The probability of their death is determined by the following equation [5]:

$$[5] \quad Death\_probability\ (\%) = 1 - e^{\left[-e^{(-8.0012+0.14783*T_l)}\right]}$$

where T_l is the body temperature of the larvae at the end of the MWT.

The lethal temperature would be expected to be lower for larvae present in a board. As explained by Fields (1992), the lethal temperature depends (apart from the species and the stage of development) on the duration of exposure to heat and the level of humidity. Larvae inserted into a board will cool more slowly and will often be exposed to a more humid environment than if out in the open air.

3.3 Experiment 3: Testing ISPM 15 constraints on *H. bajulus* infested boards

The analysis of this experiment focused on different factors likely to influence the mortality rate of the larvae. These factors were: **(i)** the position at which the larvae were inserted into the board, **(ii)** the $T_{Section}$ and $T_{Surface}$ and **(iii)** the initial characteristics of the boards. Based on the results of these experiments, the last step was **(iv)** the estimate of the $T_{Surface}$ for which the level of mortality corresponds to Probit 9. Results obtained within this framework are summarized below:

(i) Of the 34 larvae found to be still alive after the treatment, 20 had been introduced into the 3-cm deep cavities and 14 into the 6-cm deep cavities (see Figure 1). It is therefore not possible to conclude that the positioning of the larvae had a conspicuous influence on lethality.

(ii) Logically, we concluded that the temperature of the boards directly influenced the mortality rate of the larvae (Figure 5). The maximum $T_{Section}$ and $T_{Surface}$ at which a larva was found to be still alive were 57 °C and 49 °C, respectively.

(iii) Initial moisture content was found to play a part in the survival of the larvae (Figure 6). For similar $T_{Sections}$, the proportion of living larvae was higher when the boards were dry. Regarding the $T_{Sections}$ of higher than 50 °C, living larvae were only found when the corresponding MC_i was lower than 16%. This could be explained by the process causing lethality. Humid heat causes death by the coagulation of proteins within the insects' body, while a dry heat involves an oxidation process, which is activated at a higher temperature (Dwinell 2001).

(iv) Following our finding that mortality was influenced by MC_i, the data set was then divided into two, distinguishing, on the one hand, the dry boards (with an MC_i of lower than 25%) and, on the other hand, the green boards (with an MC_i of higher than 50%). The choice of these values can be justified in two ways. First, there is a clear difference between the distributions of mortality on either side of these values. Secondly, the pallet industry generally uses green wood (or wood that has been stored in the open air for a few months), for which the MC_i is higher than 25%. These two categories included 140 larvae each, of which 18 and 14, respectively, emerged alive from the dry and the green boards after MWT. The probability of death can be deduced from binary logistic regression equations based on the Gompertz model by distinguishing the dry [6] and the green [7] boards:

$$[6] \qquad Death\_probability\ (\%) = 1 - e^{\left[-e^{\left(-7.8362+0.17851*T_{Surface}\right)}\right]}$$

$$[7] \qquad Death\_probability\ (\%) = 1 - e^{\left[-e^{\left(-22.998+0.54184*T_{Surface}\right)}\right]}$$

These models are only valid for mortality rates of higher than 50%. According to these models, and as illustrated in Figure 7, the theoretical $T_{Surface}$ necessary to reach a mortality rate of 99.99683% is 57.0 °C for the dry boards and 46.8 °C for the green boards. The confidence intervals of the probability of death associated with those temperature values are [99.50%; 100%] and [99.34%; 100%], respectively. These two values are somewhat higher than the maximum $T_{Surface}$ values at which a larva was found to be still alive (48.7 °C and 40.9 °C, respectively for the dry and the green boards). However, these two theoretical values are still lower than 60 °C. It can thus be inferred that, whenever $T_{Surfaces}$ reach 63.2 or 64.8 °C for poplar and pine boards, respectively, the treatment will guarantee that i) the $T_{Section}$ will remain above 60 °C for 60 seconds (with a confidence level of 99%, as demonstrated in experiment 1), and ii) all *H. bajulus* individuals will be eliminated with a probability higher than 99.99683%. The FAO requirements (60 °C/60 s) therefore guarantee an effective biocidal treatment for both poplar and pine boards.

4 Conclusion

The present study showed that when the $T_{Surface}$ of a non-frozen 22 mm-thick board, irrespective of all other initial characteristics, reached 65 °C (63.2 and 64.8 °C for poplar and pine wood, respectively) through MWT, the FAO requirements (60 °C/60 s) were satisfied with a probability in excess of 99%. It also showed that, in these conditions, treatment guaranteed the lethality of *H. bajulus* individuals*,* whatever their stage of development, with a probability higher than the Probit 9 level. The use of MWT could therefore be envisaged with a view to meeting the requirements of ISPM 15.

In an automated production line, measurement of $T_{Surface}$ must take into account the variation in the values observed for the same board. If the average temperature cannot be determined by infrared surface analysis, it will be necessary to multiply the number of spot values identified by an infrared sensor.

In order to meet the requirements for the industrial production of pallets, the oven used for treatment must provide a power supply suited to the speed of production. For example, a power supply of 800 kW is necessary to treat boards needed for the production of a pallet every seven seconds from poplar wood with an MC_i of 45% and an initial temperature of 10 °C.

Compared with a conventional heat treatment, MWT could be very useful when applied to specific areas of industry, due to the rapidity of the treatment (one to three minutes) and the comparatively small quantity of material required. It also makes it possible to guarantee the effectiveness of treatment for each individual board. It might therefore be useful if the standards applicable to phytosanitary treatment became more restrictive. A specific system of individual marking could be envisaged to ensure the traceability of each board treated in compliance with the requirements of ISPM 15.

Several decades after research dedicated to the possible use of microwaves had been initiated, aiming at the control of biological agents, the findings of this study provide guidelines allowing for the transfer of this technology to the wood industry. Further experiments are needed in order to extend the application field of microwave phytosanitary treatment, notably by providing treatment parameters for thicker boards.

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Table 1 Characteristics of the 17 series of boards irradiated in the industrial microwave oven[a]

Species	Set ID	T_i (°C)	MC_i (%)	BD (kg m^{-3})	Delivered power (kW)
Poplar	POGH 1	16.2 (0.1)	163 (48)	370 (55)	19.8
	POGH 2	21.9 (0.1)	184 (29)	363 (38)	12.6
	POGL 1	15.7 (1.1)	189 (74)	340 (50)	14.4
	POGL 2	10.5 (0.6)	225 (34)	340 (42)	10.8
	PODH 1	17.4 (0.1)	15 (3)	382 (47)	7.2
	PODH 2	23.2 (0.2)	15 (0.1)	374 (28)	3.6
	PODL 1	16.6 (1.1)	18 (1)	391 (31)	5.4
	PODL 2	15.8 (2.2)	16 (1)	389 (30)	10.8
Pine	PIGH 1	17.2 (0.6)	90 (16)	407 (49)	18.0
	PIGH 2	22.9 (0.1)	102 (13)	410 (43)	10.8
	PIGL 1	16.7 (1.0)	125 (40)	442 (69)	16.2
	PIGL 2	13.1 (0.7)	169 (18)	409 (29)	23.4
	PIDH 1	17.8 (0.4)	16 (2)	433 (50)	9.0
	PIDH 2	23.4 (0.1)	15 (0.4)	423 (35)	3.6
	PIDH 3	23.1 (0.3)	15 (1)	418 (64)	12.6
	PIDL 1	16.4 (0.9)	18 (1)	430 (30)	5.4
	PIDL 2	11.8 (1.1)	21 (1)	401 (29)	3.6

[a] ID = identifier, based on the species (Poplar or Pine), moisture content (Green or Dry) and initial temperature (Lower or Higher). Initial temperature (T_i), initial moisture content (MC_i) and basic density (BD) of the boards represent the average values of the 20 boards. Numbers in brackets are the standard deviations.

Table 2 Number of individuals used (controls and those irradiated) per stage of development

Stage of development	Controls	Individuals treated	Irradiation period (s)
Eggs	10*	542	20
Larvae with a mass of less than150 mg	18	46	10
Larvae with a mass in excess of 150 mg	33	442	10
Adults	10	20	10

*10 eggs were randomly chosen in 3 egg masses laid by 3 different females

Table 3 Values of $T_{Surface}$ per wood species and according to three confidence levels making it possible to ensure a $T_{Section}$ of at least 60 °C one minute after the treatment had finished (22 mm-thick boards)

Level of confidence (%)	$T_{Surface}$ (°C)	
	Pine	Poplar
95.00	62.5	61.0
99.00	64.8	63.2
99.99	69.9	68.1

Table 4 Number of dead or alive individuals per stage of development and in relation to average body temperature of the individuals after microwave treatment

Stage of development	Vital state	Classes of average body temperature (°C)																
		25[a]	27.1-30	30.1-33	33.1-36	36.1-39	39.1-42	42.1-45	45.1-48	48.1-51	51.1-54	54.1-57	57.1-60	60.1-63	63.1-66	66.1-69	69.1-72	72.1-75
Eggs	Dead	-	-	-	-	-	-	-	21	110	6	52	-	81	126	-	-	-
	Alive	10	-	-	-	-	-	53	88	5	-	-	-	-	-	-	-	-
Larvae < 150 mg	Dead	-	-	-	-	-	-	-	1	1	1	5	8	4	4	2	-	-
	Alive	18	-	1	5	2	3	2	1	2	-	4	-	-	-	-	-	-
Larvae > 150 mg	Dead	-	-	-	-	1	-	3	6	11	12	16	29	35	71	67	41	11
	Alive	33	8	20	17	21	26	15	6	4	10	3	5	3	1	-	-	-
Adults	Dead	-	-	-	-	-	1	-	2	4	3	4	2	1	1	1	-	-
	Alive	10	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-

[a] Corresponds to individual controls

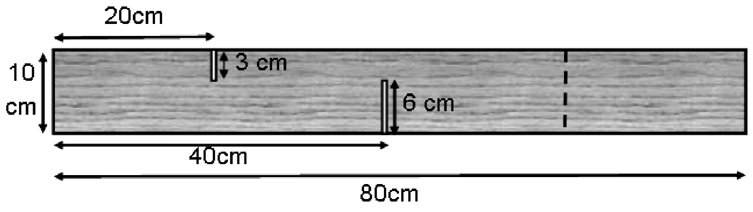

Fig. 1 Diagram of a board and the positioning of the two openings allowing for insertion of the larvae. The dashed line represents the location of the cut for measuring the $T_{Section}$ and taking a sample of about 20 cm long.

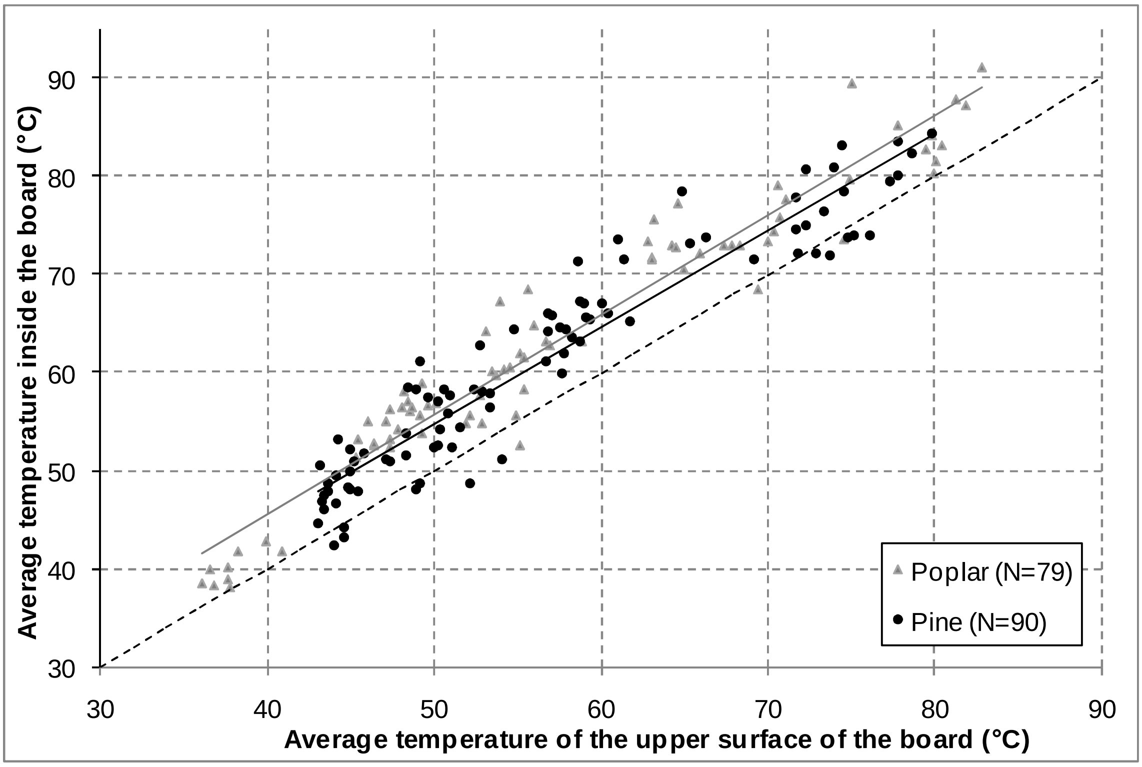

Fig. 2 Relationship between $T_{Section}$ and $T_{Surface}$ according to wood type. Each dot represents the data relative to one board. The linear equation $T_{Section} = T_{Surface}$ is represented by a dashed line.

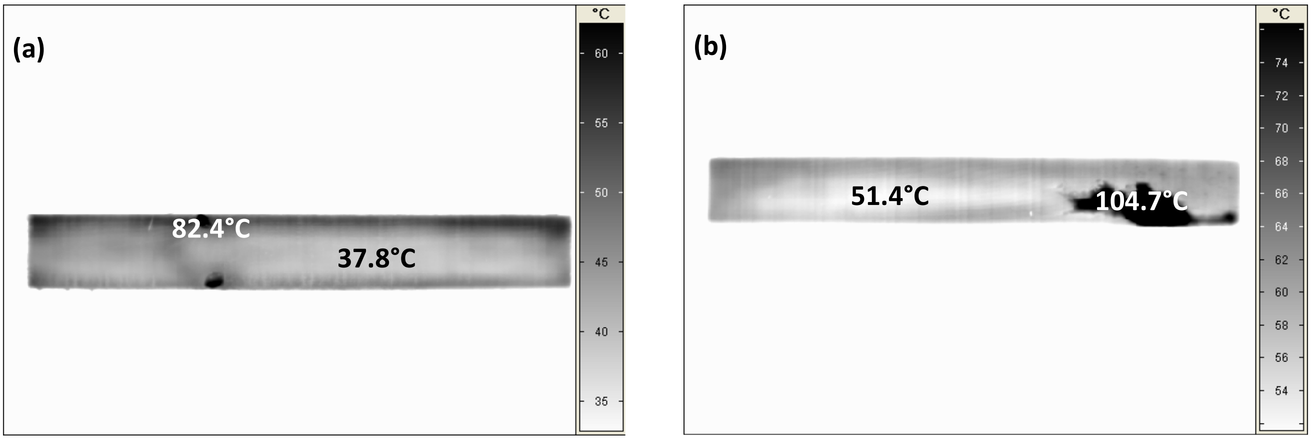

Fig. 3 Thermal photos of the upper surface of two pine boards after MWT. The temperature scales are shown to the right of the images. Maximum and minimum temperatures are reported. The first board (a) has two knots that have a significantly higher temperature than the rest of the board. The upper edge of the board is also hotter. On the second board (b), a resin stain with a higher temperature than the rest of the board can be observed.

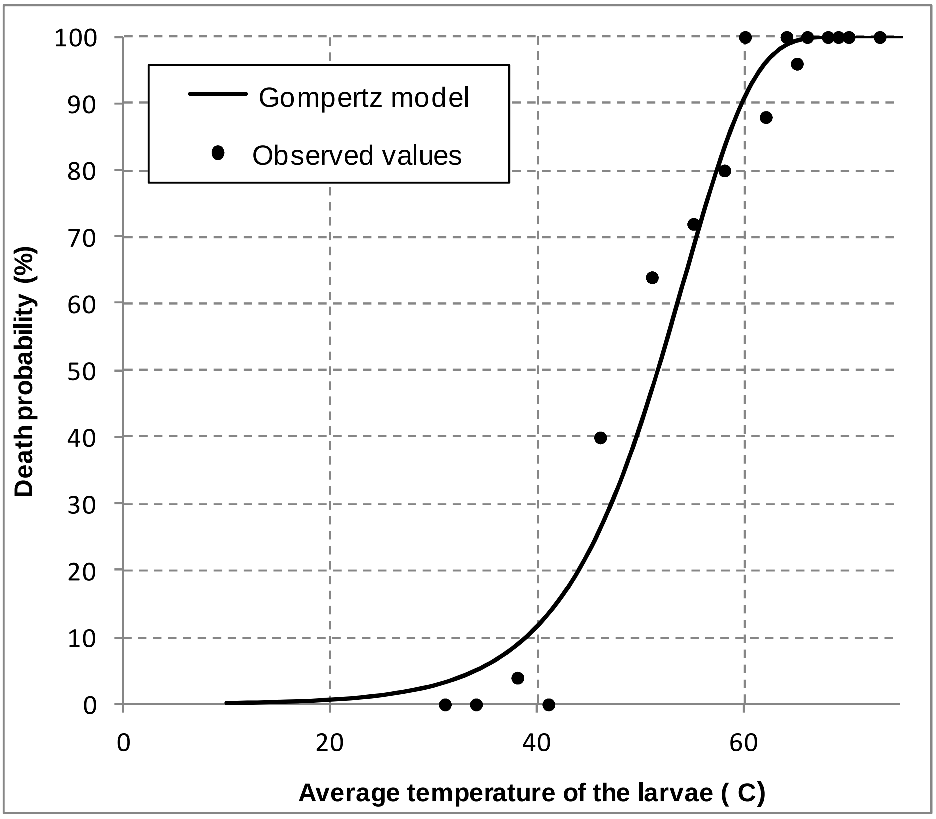

Fig. 4 Percentage of deaths according to the average temperature of the larvae modeled according to the Gompertz model. The percentages of deaths actually observed are also represented (each black dot corresponds to 25 larvae treated, with the exception of the dot at the highest temperature point, which corresponds to 17 larvae).

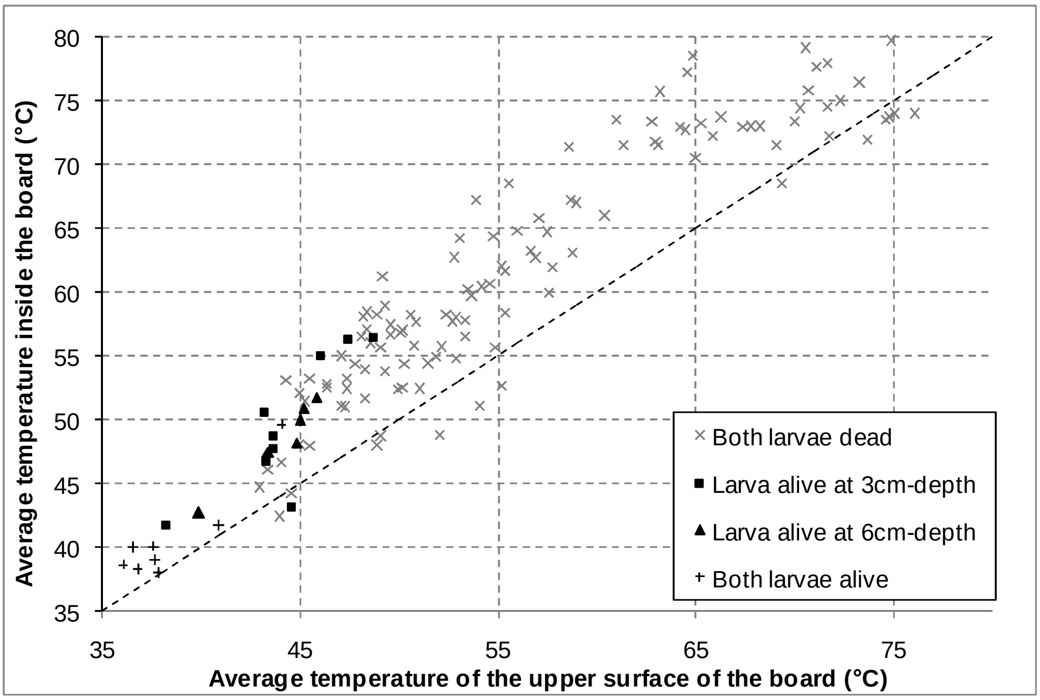

Fig. 5 $T_{Section}$ and $T_{Surface}$ of the 10 central boards of each of the 14 series (140 boards). The linear equation $T_{Section} = T_{Surface}$ is represented by a dashed line.

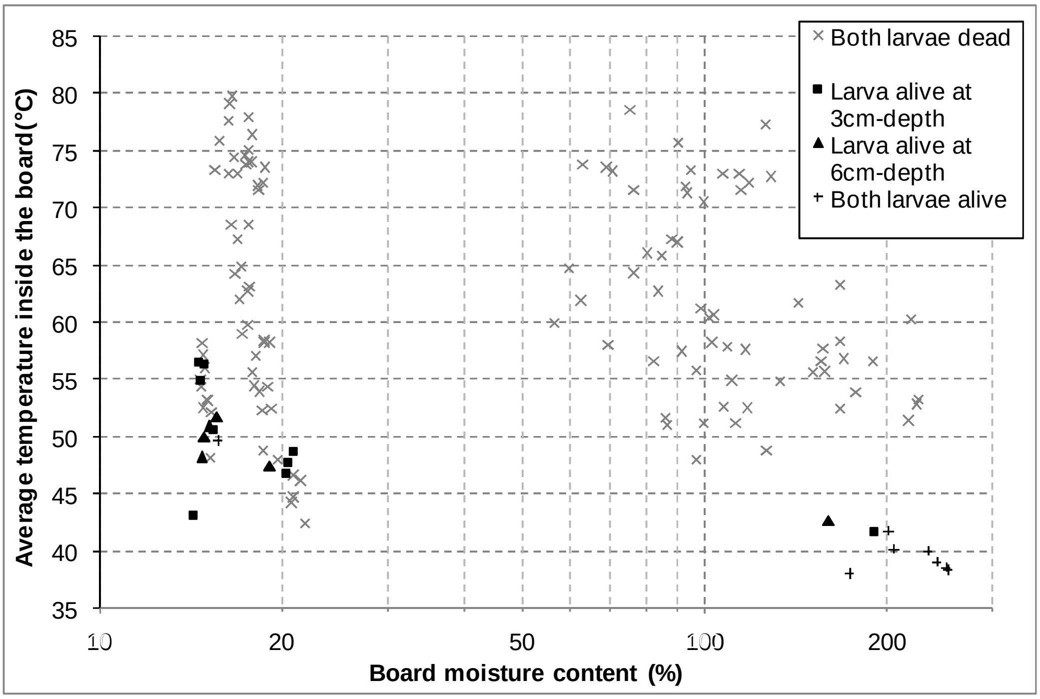

Fig. 6 $T_{Section}$ according to the MC_i (10-base logarithmic scale) of the 10 central boards of each of the 14 series (140 boards)

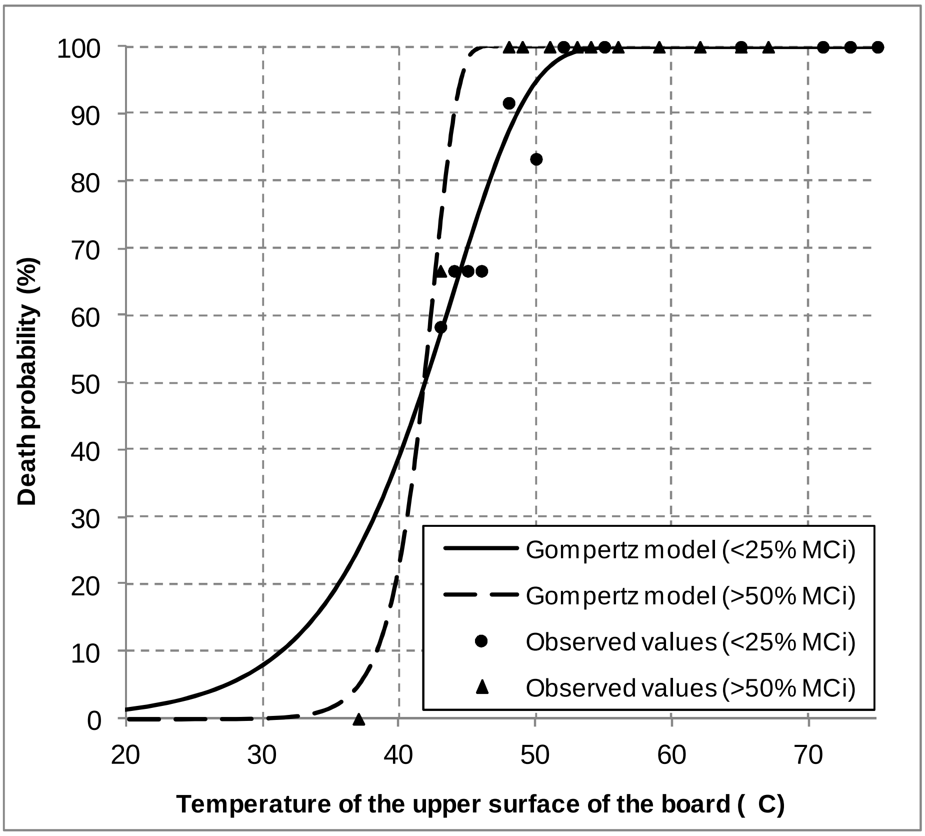

Fig. 7 Relationship between the percentages of deaths and $T_{Surface}$. The logistic binomial regression curves were calculated according to the Gompertz model for the boards with an MC_i lower than 25% (N = 140) and higher than 50% (N = 140). The percentages of deaths actually observed are also shown (each black dot corresponds to 12 larvae, with the exception of the highest temperature dot for both types of board, which corresponds to 8 larvae).