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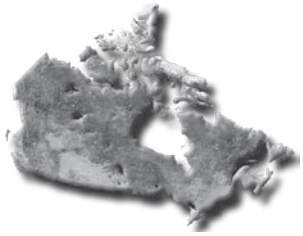

Heat Disinfestation of Mountain Pine Beetle-Affected Wood

Adnan Uzunovic & Lily Khadempour

Mountain Pine Beetle Initiative
Working Paper 2007-14

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Abstract

Exporters of lumber and logs are obligated by Canadian law to apply approved treatments for phytosanitary purposes. Heating to 56°C for at least 30 minutes in a kiln or heating chamber (56/30) is the most common treatment used for lumber and is also an internationally accepted method to treat wood packaging in order to eradicate pests of concern. This time/temperature combination was originally developed to eradicate pinewood nematodes and their insect vectors. Questions have arisen in the marketplace about the efficacy of such treatment against fungal pests, and recently for those associated with mountain pine beetle-affected lodgepole pine. This project looked into the effectiveness of several time/temperature combinations, in particular whether 56/30 kills a selection of mountain pine beetle associated fungi including bluestain fungi (nine isolates), *Ambrosiella* fungi (three isolates) and sap-rot fungi (six isolates). Each isolate was replicated 6 times at all time-temperature combinations. In parallel we tested naturally infested lodgepole pine wood that was confirmed to be colonized with a mixture of bluestain, decay and mold fungi. The heat treatment protocol was developed through international collaboration and used heated water baths where test wood or glass vials (agar slants) with grown fungi were immersed and kept for a specified time after which the revival of fungi was attempted. Additionally, this project determined to what extent air-drying and wood aging reduced the viability of embedded fungi and if slow drying rendered the fungi more heat tolerant.

Leaving wood to air dry up to 4 months (to 15 % EMC.) was insufficient to eradicate fungi present in the wood and cannot be considered a fungal pest-eradication method. Some replicates have shown less heat tolerance if tested on agar slants so wood should be used to do such tests as it better represents a real-life situation. The data showed that 56/30 killed a majority of inoculated fungi grown on agar, inside wood test pieces, and those naturally found in substrate attacked by mountain pine beetle. None of the mixed bluestain fungi and decay fungi present in sapwood of naturally infested mountain pine beetle-affected trees survived 56/30; however, some survived when tested as pure cultures. All isolates and replicates of five of the species tested were killed at 56/30; however a sap-rot fungus *Phellinus chrysoloma* and a few replicates of bluestain fungus *O. montium* survived 56/30 (4 out of 12). All *O. montium* was killed by 56/60 and *P. chrysoloma* was killed at 61/30. These results should be considered to be worst case since the temperature was rapidly raised to the target and dropped rapidly after the target time period. In commercial heat treatments, the inherent tendency of kiln schedules to overshoot the target temperature and the insulating properties of wood will result in higher than target temperatures and the temperature being maintained much longer.

Heat treatment following prior wood air-drying showed some fungi developed heat tolerance when they were allowed to slowly desiccate; however, the evidence for this was limited. In pure cultures 19.0% (8 replicates out of tested 42) survived 56/30 after being air-dried for 90 days. This compares to 0% survival for immediate heat treatment and heat treatment after 15 and 40 days of air-drying. For the naturally infested wood no decay or stain fungi survived 56/30, regardless of prior air-drying; however some aggressive common molds e.g., *Trichoderma* spp showed a significant increase in heat tolerance and resource capture capability, preventing successful isolation of other fungi. We suggest that in a real-life situation where 56/30 will eradicate most of the fungi, these aggressive saprophytic molds (which are also commonly used a biocontrol agents) will most likely outcompete the weakened surviving bluestain or decay fungi, thus minimizing chances of their possible survival and threat of establishment in an importing country.

Résumé

Au Canada, la loi oblige les exportateurs de bois de grume et de bois de sciage à appliquer des traitements phytosanitaires approuvés. Le traitement thermique à 56 degrés Celsius pendant au moins 30 minutes dans un séchoir ou dans une enceinte de chauffage (méthode 56/30) est le procédé le plus couramment utilisé dans le cas du bois de sciage; cette méthode est également acceptée à l'échelle internationale pour le traitement des emballages en bois afin d'éradiquer les ravageurs qui posent problème. À l'origine, cette combinaison temps/température a été mise au point pour éradiquer le nématode du pin et ses insectes vecteurs. Sur le marché, on a soulevé des questions concernant l'efficacité de ce traitement dans le cas des maladies fongiques et, plus récemment, dans le cas des maladies fongiques associées au pin tordu attaqué par le dendroctone du pin ponderosa. Dans le cadre de ce projet, on a examiné l'efficacité de plusieurs combinaisons temps/température; on a notamment cherché à déterminer si la méthode 56/30 permet de détruire une sélection de champignons associés au dendroctone du pin ponderosa, dont les champignons du bleuissement (9 (neuf) isolats), des champignons de l'espèce *Ambrosiella* (3 (trois) isolats) et des champignons causant la pourriture de l'aubier (6 (six) isolats). Chaque isolat a été répliqué six fois dans le cas de toutes les combinaisons temps/température. Parallèlement, nous avons testé du bois de pin de ponderosa naturellement infesté; l'examen a permis de confirmer que ce bois était colonisé par un mélange de champignons du bleuissement, de champignons de carie et de champignons de moisissure. Le protocole de traitement thermique a été élaboré dans le cadre d'une collaboration internationale et comportait l'immersion dans des bains d'eau chauffée d'échantillons de bois ou de fioles de verres (gélose en pente) contenant des champignons de culture; les échantillons ou les fioles ont été maintenus immergés pendant un laps de temps déterminé, après lequel on a essayé de raviver les champignons. En outre, ce projet visait à déterminer dans quelle mesure le séchage à l'air et le vieillissement du bois réduisent la viabilité des champignons incorporés et si le séchage lent rend les champignons plus résistants à la chaleur.

Le séchage du bois à l'air pendant une période de 4 mois au maximum (jusqu'à un degré d'humidité d'équilibre de 15 %) était insuffisant pour éradiquer les champignons présents dans le bois et ne peut être considéré comme une méthode d'éradication des maladies fongiques. Certains réplicats ayant montré moins de tolérance à la chaleur lors des essais en gélose inclinée, il faudrait utiliser du bois pour effectuer ce genre d'essais, étant donné qu'il permet de mieux représenter une situation réelle. Les données ont montré que la méthode 56/30 tue la plupart des champignons inoculés cultivés sur gélose, les champignons contenus dans les échantillons de bois testés et les champignons présents naturellement dans les supports de croissance attaqués par le dendroctone du pin ponderosa. Aucun des champignons de bleuissement et des champignons de carie présents dans l'aubier d'arbres infestés par le dendroctone du pin ponderosa n'a survécu au traitement 56/30; toutefois, certains champignons ont survécu dans les essais effectués sur des cultures pures. Tous les isolats et tous les réplicats de cinq des espèces soumises aux essais ont été tués par la méthode 56/30; toutefois, un champignon causant la pourriture de l'aubier (*Phellinus chrysoloma*) et quelques réplicats du champignon de bleuissement *O. montium* ont survécu à ce traitement (4 sur 12). Tous les champignons *O. montium* ont été tués par le traitement 56/60, et les champignons *P. chrysoloma*, par le traitement 61/30. Ces résultats devraient être considérés comme la pire éventualité, étant donné que la température a été augmentée rapidement jusqu'à la valeur cible et diminuée avec rapidité après le laps de temps prévu. Dans le cas des traitements thermiques commerciaux, la tendance inhérente aux programmes des séchoirs de dépasser la température cible ainsi que les propriétés isolantes du bois expliquent les températures plus élevées et leur maintien plus long.

Le traitement thermique effectué après le séchage du bois à l'air a montré que certains champignons développaient une tolérance à la chaleur si l'on permettait une dessiccation lente; toutefois, les preuves à cet égard étaient limitées. Dans des cultures pures, 19,0 % des réplicats (8 sur 42 testés) ont survécu au traitement 56/30 après un séchage à l'air de 90 jours. Par comparaison, le taux de survie a été de 0 % dans le cas du traitement thermique immédiat et du traitement thermique après 15 jours et 40 jours de séchage

à l'air. Dans le cas du bois infesté naturellement, aucun champignon de carie ou champignon de bleuissement n'a survécu au traitement 56/30, sans tenir compte du séchage à l'air préalable; toutefois, certaines moisissures communes agressives (par ex., *Trichoderma spp*) ont montré une hausse significative de la tolérance à la chaleur et de la capacité de captage des ressources, ce qui empêchait d'isoler d'autres champignons. Nous suggérons que dans une situation réelle où le traitement 56/30 parvient à éradiquer les champignons, ces moisissures saprophytiques agressives (qui sont également utilisées couramment comme agents de lutte biologique) l'emporteraient sur les champignons de bleuissement ou les champignons de carie survivants et affaiblis, réduisant ainsi les chances que ceux-ci survivent et puissent s'établir dans un pays importateur.

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1 Introduction

The international spread of pest organisms has been increasingly recognized as a major issue by governments, their plant health regulatory agencies and the general public. Questions about such pests carried by wood products often arise in the market and are more likely to be asked when the wood is known to originate from dead trees or when wood is visibly damaged by pests (e.g., bluestain). Pests of phytosanitary concern that could potentially be carried by post-mountain pine beetle wood are a) mountain pine beetles, b) secondary beetles and other wood boring insects, c) bluestain fungi affiliates of mountain pine beetle and secondary insects, d) pinewood nematodes brought by sawyer beetles, and e) wood decaying fungi such as sap-rot and heart-rot fungi. With the exception of the fungi (pests under c) and e)), these organisms are not currently a threat to trade. The heat treatment (HT) stamp borne by exported spruce-pine-fir (SPF) lumber is a guarantee that the core of the wood has been heated to 56^{o}C for at least 30-minutes (56/30) in a kiln or heating chamber and this is known to eradicate most insects as well as pinewood nematodes (Allen 2001a, b).

Earlier research at Forintek facilitated the development of heat treatment as a viable method of disinfesting wood from certain organisms (Smith 1992). Heating the wood at 56°C for 30 minutes (56/30) to the core is now internationally accepted for pinewood nematode control so lumber carrying the HT stamp will also be free of nematodes and the *Monochamus* sawyer beetles known to be their main vector (Allen 2001a, b). It is well known in the scientific community that this will not kill all organisms found on wood. Questions have been raised in the Asian market about whether fungi in this wood are killed during processing of mountain pine beetle-attacked trees. Countries such as Australia and those in the European community are increasingly listing pathogenic fungi in their directives as pests of concern. Mountain pine beetle is associated with several bluestain fungi that are implicated in the rapid loss in vigour and subsequent death of infested trees. Recent University of British Columbia research found a handful of fungi consistently associated with recently attacked mountain pine beetle trees and even more diversity, including wood-decaying fungi, found in longer dead trees (Kim *et al.* 2005). The limited data available for bluestain organisms indicate that some are killed by 56/30, but others might not be (Allen 2001a, b).

There was a need for scientific confirmation of whether or not manufactured products carry live fungi and whether the fungi associated with mountain pine beetle attack will be killed by 56/30. In practice, lumber that is heat-treated will reach a surface temperature somewhere between dry bulb and wet bulb temperatures. For a typical "generic" heat treatment schedule (CFIA 2004 – Option A) for lumber up to 60 mm thick the minimum heat treatment time would be 6.5 hours with at least 2 hours and 3 minutes flow of air at or above 60°C and a final wet bulb temperature of at least 63°C. These conditions ensure that the core of a piece of lumber will exceed the 56/30 criterion in terms of temperature, time or both. For this reason the proposed work will include temperatures and times that exceed 56/30 in practice.

Recent research on the heat schedules required to kill fungi, carried out in several institutions, has caused controversy in the International Forest Quarantine Research Group which is mandated with scientific research and information exchange in support of the International Plant Protection Convention. In particular, research in New Zealand has found that some fungi become more tolerant to heat after being slowly desiccated, probably developing more sturdy resting spores in the process. A standardized test protocol is required to enable corroboration of existing findings and generation of new findings. This project will assist in gaining acceptance of the protocols developed by Forintek and the Pacific Forestry Centre and used by the latter to support 56/30 for disinfestation of fungi in addition to nematodes and insects. The tests will use the fungi isolated by University of British Columbia from mountain pine beetle-affected trees under a Mountain Pine Beetle Initiative-funded project. The project will also determine if

the mountain pine beetle-associated fungi are already dead before the wood is heat treated in the sawmill. Such death may occur from air-drying and/or wood aging such as occurs in standing trees. The fungal associates of mountain pine beetle grow naturally in wet fresh live wood; however similar bluestain fungi have been observed to die back after initial wood colonization.

Overall, this project aims to fill strategic science gaps and will allow regulations applied to lumber that originate from trees attacked by mountain pine beetle to be soundly based on scientific facts. In parallel it will:

- Assess the effectiveness of 56/30 as well as several other selected temperature-time schedules on the mortality of specific fungi;
- Determine if infested wood and fungi grown on agar slants in vials give the same results as when test wood is used;
- Determine if wood air-drying (wood aging) alone reduces the viability of embedded fungi
- Determine if slow pre-drying results in heat tolerance in fungi.

2 Materials and Methods

2.1 Test Material and Test Organisms

We selected and obtained clean and fresh lodgepole pine as the most appropriate wood (host) species substrate. We also obtained naturally infested material (1 m long, stained logs with bark infested with mountain pine beetle) and we confirmed the viability and type of embedded fungi by plating small wood splinters from sapwood onto three types of agar plates: 1% Malt Extract Agar (MEA, Oxoid CM59) augmented with 0.002% benomyl (selective for decay), 0.01% cycloheximide (selective for most ophiostomatoid fungi) and 1% MEA (general medium). The latter two were also augmented with 0.01% chloramphenicol to reduce bacterial growth. The naturally infested material was shipped to Forintek and then stored frozen and cut into sample sizes (30 x 10 x 5 mm) close to the start of the experiment. Clean test samples were cut from green, clean sapwood of lodgepole pines that were not attacked by mountain pine beetle. The sample size was the same as used in previous pinewood nematode studies: 30 x 10 x 5 mm with the grain parallel to the long dimension. These were refrigerated in sealed plastic bags until used in order to preserve green moisture content that was preferred by bluestain fungi. The test fungi were selected based on work by Kim *et al*. (2005) that studied fungi commonly associated with the mountain pine beetle-affected trees and included four species of bluestain, one ambrosia fungus and two sap-rot species. Each fungal species is represented by three isolates supplied from the collections made by University of British Columbia's department of wood science under the supervision of Professor Colette Breuil; the final list is given in Table 1. In a preliminary study, all isolates were checked for stability (no unusual sectoring, free of colony abnormalities e.g., sectored growth and degenerated areas) and vigor (regular growth rate and sporulation) and discussed with other scientists at University of British Columbia to ensure that the morphology was consistent with records of the isolates. This also included measuring their growth rates as described in Brasier (1981) and photographing their colonies to use as a reference to compare with other isolates of the species and to detect changes to recovered fungi. Preliminary tests were done at various moisture contents to determine conditions for optimum growth on test wood. Each isolate was replicated six times for each target time-temperature setting while for the control treatment (water heated at 25°C) three replicates per isolate were used following the required time schedule. For the naturally infested material we used 10 test samples for each treatment. The isolates were maintained in the Forintek Canada Corp. culture collection under sterile water in a fridge running at +4°C and were sub-cultured when needed.

Table 1. Isolates found associated with mountain pine beetle and selected for the study

Fungus	*Geographic origin*	*Isolate letter assigned*	*Isolate*
Ophiostoma clavigerum (Rob.-Jeffr. & R.W. Davidson) T.C. Harr. **(bluestain)**	Manning Park Little Fort Cranbrook	A B C	*M33* S5R131A2-1 S4G11AW2-2
Ophiostoma montium (Rumbold) Arx **(bluestain)**	Manning Park Riske Creek Cranbrook	D E F	MG 8DG2 WG51EW S495EW2-1
Leptographium longiclavatum S.Lee, J.-J. Kim & C. Breuil **(bluestain)**	Manning Park Little Fort Riske Creek	G H I	MG3EW1-2 WG57EG-1 S5R140E2-2
Leptographium terebrantis S.J. Barras & T.J. Perry **(bluestain)**	Riske Creek Cranbrook Little Fort	J K L	WY42EW1-2 MY281W2-1 S5R134A2-1
***Ambrosiella* sp.** Arx & Hennebert	BC BC BC	M N P	*Ambrosiella* sp 3-877EW2-1 *Ambrosiella ferruginea* JB13 *Ambrosiella* sp 2. TR25
Trichaptum abietinum (Dicks.) Ryvarden **(saprot)**	Robson Park Little Fort Manning Park	R S T	848AW1-1 (606) S5Y129A2-1 (526) MY25AW2-1 (120B)
Phellinus chrysoloma (Fr.) Donk **(saprot)**	Radium Manning Park Cranbrook	U V W	MY25AW2-1 (120B) MR17A-HR-3 S4102AW-HR

2.2 Preparation and Inoculation of Sterile Test Material

Clean test wood was sent to be gamma sterilized at 2.5 Mrads. Test cultures for each isolate were first grown on 1% MEA until fungi were well established and covered the plate. Samples were placed in pre-sterilized breathable-patch bags (Western Biologicals Ltd. Cat. No.3TL — they have an air-permeable patch and are designed for mushroom spawn production) with saturated sterilized vermiculite, as a moisture-holding medium, at approximately 50 g of medium-size vermiculite per 250 test pieces (Sexton *et al.* 1993). Liquid inoculum was prepared by first pouring a few millilitres of sterile water into a plate containing 2-week old culture then slowly scraping aerial portions of the fungus into the water. The water containing mycelial fragments was suspended in an additional 50 ml of sterile water. If a test culture readily sporulated on a test plate, then the suspension was used without further preparation. In cases where a test isolate was mostly producing mycelium, the mycelial suspension was blended in a sterile blender jar with three short pulses, or 15 seconds of continuous blending. The mycelial/spore suspension was aseptically sprayed or sprinkled over test wood pieces and vermiculite in a patch bag. After the suspension was added, a separate plate with grown culture was sliced into 0.5 x 0.5 mm or smaller squares that were also added. The bag was closed and then agitated by manipulating it from the outside to increase the chance of all test pieces being coated with inoculum. Bags were not overfilled, so an 8-x 16-inch bag took 250 wood samples (Fig. 1).

For a parallel test where vials were used, 3.5 ml of 1% MEA were added to 7 ml borosilicate glass vials (Fisher 333726) that were kept slanted to allow for the formation of a slanted agar surface. Vials were singly inoculated in the middle of the slanted agar surface with a plug of actively growing mycelium of test fungi. They were incubated at 22°C with caps loosely tightened to allow oxygen exchange until the slanted surface was covered with fungal mat (1 –2 weeks).

Figure 1. *Ophiostoma clavigerum* growing on inoculated wood pieces with vermiculite in a patch bag (left), vacuum sealing (right).

2.3 Incubation, Monitoring and Inspections

Patch bags with inoculated wood were incubated in a clean room at room temperature (close to 22°C) on mite traps to prevent mite infestation. Samples were occasionally (every 3-7 days) turned and slightly manipulated from the outside to shuffle test pieces and to stop fungal growth from sticking the samples together. This also encouraged redistribution of fungal mycelium fragments and faster colonization of all test wood. Moisture in the bags was maintained at the same level by measuring the weight of the bags with samples after the inoculation and aseptically adding more sterile water (by spray and dripping) in the system when there was a >50 g drop in weight due to water loss. There was always a sign of condensation on the inside of the bags. Colonization was also confirmed by sacrificing a few samples to check under the microscope for presence of fungal hyphae deep inside the wood. Samples inoculated with bluestain took around 3-4 weeks before they were heat treated while basidiomycetous fungi took 6-8 weeks. Where appropriate, formation of chlamydospores was confirmed to assure that the most resistant stage of the fungus was tested.

2.4 Heat Treatment

Test samples that were inoculated and fully colonized with test fungi, naturally infested samples, and inoculated vials were heat-treated in a water bath with a matrix of seven different temperatures (41, 46, 51, 56, 61, 66 and 71°C for four different treatment times (up to 1 minute, and for 30, 60 and 120 minutes). The temperature range bracketed the 56/30 criteria by 5°C intervals. The method was a slight modification of the test method developed for testing wood infested with pinewood nematodes (Smith, 1991, see also Newbill and Morrell, 1991) and has also been internationally developed and agreed upon through IFQRG (http://www.forestry-quarantine.org/Documents/ 2006_Uzunovic-HT-protocol.pdf). Controls consisted of a set of three samples for each isolate. Samples were put through the same handling process, but "heat treated" by placing bags in water at 25°C followed by fungal viability test as described below.

Test wood samples were aseptically placed into polyethylene vacuum-sealable bags. For the heat treatment of inoculated pieces, six pieces were used per bag that was cut to 20 x 15 cm. For naturally infested samples, 10 pieces were put in each bag that was cut to 28 x 15 cm. They were aseptically

arranged in a single flat layer, without samples touching. They were held in that position while vacuum sealing took place (Fig 1). The seal around each test piece facilitated heat transfer to the wood, as there was no air insulation in between the bag and the test wood. Bags for all three isolates were labelled then sandwiched together between two mesh grids (metal or plastic). The grid-bag ensemble was then submerged in a heated circulating water bath (Cole Parmer equipped with Polystat® temperature controller) that had been pre-heated to a target temperature (Fig. 2). Submerged bags were kept in the bath 2 cm off the bottom at the desired temperature and for the desired time. For heat treatment testing of vials, the vial lids were first tightened fully then wrapped individually with parafilm to prevent water from getting in. Vials from all three isolates were evenly distributed in a vial rack and submerged in the water. The lids were also kept above the surface to prevent water entry. The temperature of the bath was monitored and recorded during the immersion to assure that there was no significant or prolonged drop in temperature. (*Note: In our preliminary studies the temperature dropped 0.2 C immediately following sample submersion but came back quickly to target temperature within 40 seconds).*

After the target time was met, the bags and vials were removed from hot water and cooled by immersion in 25°C water (*Note: It took a few seconds for temperature to drop 5°C below target temperature and up to 2 minutes to drop 15°C below target temperature).* Immediately following cooling, vial lids were loosened and bags were cut open to allow oxygen to reach the samples. Both wood and mycelium from vials were immediately sub-cultured to assess survival (see the assessment procedure).

Figure 2. Water bath equipped with Polystat® temperature controller, heated at target temperature, test wood enclosed in vacuum-sealed bag and sandwiched together between two mesh grids is being held on the bottom of water bath (left). Heat treatment of fungi grown in vials held in vial racks with the lids remaining above the water line (right).

Before carrying out this experiment, preliminary tests were conducted to determine the required time to reach the target temperature and cool-down times at the core of the wrapped samples at different temperatures. We did this by inserting thermocouples into a 1 m diameter hole drilled 15 mm from the end to the centre of several sacrificial blocks. Holes had to be made in the polyethylene vacuum-sealable bags to allow for the thermocouple wires; these holes were sealed with hot glue. Similarly, the thermocouples were inserted into the vials with agar through a hole made on the vial lids.

During the heat treatment we used immersion times based on the preliminary tests. The pre-heating and cooling time differed for different wood species, temperatures, wood moisture contents, as well as the type of material (wood versus glass versus plastic vials) and needed to be calibrated for each case. From the earlier pinewood nematode work it was found that required heating time is ~4 minutes. From earlier Canadian Forest Service/Forintek work that studied eight pathogenic fungi, there was some variation at different temperatures between different species of wood (e.g., pine took 1.8-3 minutes while oak took 3-3.8 minutes to heat to the target temperature). In our preliminary tests it took 1.6 minutes for completely dried lodgepole pine wood to reach the target temperature. For freshly inoculated wood it took 2.7 minutes at temperatures 66°C and below and 3.6 minutes at 71°C. Thus, in our experiment 4 minutes was selected for pre-heating before starting to count time. Agar dispensed in Simport (T 406-2 12 ml) polystyrene vials took longer to heat (8.5 minutes) compared to agar in Fisher (333726) 7 ml borosilicate glass (4.5 minutes). Consequently, 4.5 minutes was allowed for pre-heating of agar in the glass vials used throughout the main experiment.

For some of the fungi, the testing procedure was streamlined so that all treatment times could be run simultaneously. In order to do this, stainless steel racks were made that would hold the bags under water. The same preliminary test on core temperature change was performed and there was no significant difference so the pre-heating time of 4 minutes was maintained. In addition, the bag size was reduced from 15 cm to 9 cm, with no significant difference.

2.5 Air-drying of Wood and Fungal Survival

Samples for air-drying studies were prepared and incubated at the same time and in the same way as samples for the heat-treatment studies. After incubation, for each isolate, six sample pieces were immediately plated (day 0 AD) and 66 samples were put into sterile Mason glass jars. The jars had a piece of stainless steel mesh grid placed inside and were covered tightly with cotton flannel fabric (Fig. 3). This entire assembly was autoclaved and then, once cooled, was filled with the wood pieces. The jars were laid horizontally with the wood pieces suspended on the stainless steel mesh. This prevented the samples from touching the glass bottom and allowed sufficient air circulation around them. During the experiment the jars were kept in an environmental chamber set to obtain equilibrium moisture content of 15% (78% RH at 20°C at sea level). The atmosphere from the chamber was reflected inside the jar as the fabric allowed unhindered exchange of humidity and air temperature while preventing contamination of test samples. The viability of fungi inside the samples was checked by destructive sampling after 0, 4, 8, 12, 16 days, and 3, 4, 5, 6, 7, and 8 weeks and 4 months, where at each time a set of six replicates per isolate were aseptically removed and fungal recovery was attempted. The same was done for naturally infested material where replication was 10 pieces for each sampling time.

Figure 3. Air-drying of samples placed on stainless steel mesh inside Mason® jar that is closed with metal ring and a piece of sterile cotton flannel fabric (left). Fungi growing out of air-dried naturally infested material at the time of assessment (right).

2.6 Air-drying (Desiccation) and Heat-Tolerance

To determine whether slow desiccation changed the heat tolerance of the test fungi, the same jar method was used to air-dry a required amount of samples (six replicates for each time-temperature treatment and three for the control for inoculated wood and 10 replicates for each time-temperature treatment for naturally infested material). The jars were kept in an environmental chamber set to obtain an equilibrium moisture content of 15% (target T 20°C and 79%RH) and samples were stored under these conditions for 15, 40 and 90 days after which they were removed and subjected to heat treatment as described above, followed by viability checking. A maximum of 200 wood pieces were put in each jar so that three jars were required per isolate.

2.7 Assessment of Survival and Recovery Procedure

Immediately following the heat treatment or after the samples from air-drying experiments were removed from the jars, test samples were placed aseptically on 1% MEA Petri-plates to monitor if test fungi would grow. (*Note: In preliminary tests there was no difference in recovery of fungi on regular 1% MEA compared to moist host sawdust agar*). The assessment of fungal survival from vials was done by collecting and plating two mycelial plugs taken from across the colony onto fresh 1% MEA and monitoring the growth of fungi. Slight modification of the method was employed for the naturally infested test wood samples. As the intent was to assess the survival of any type of fungi present in the wood the three types of agar media were used, as previously described. Each test piece following the treatment was aseptically split into three pieces and each piece was plated on these three different media.

Sub-cultured samples were monitored regularly for fungal growth and marked as survived (1) or not survived (0) so numbers presented in the tables represent total number of samples (out of total number of tested replicates) where fungus survived. For the naturally infested materials, the assessments were done in parallel on three types of media and marked as survived (1) regardless of whether its growth occurred on one, two or all three media. Sub-cultured samples were assessed after 7 and 21 days for any growth and any unusual features of growth were noted. Plates without any growth (0) were checked once more after additional 30 days of incubation following the 21-day assessment. Unusual samples were checked under a microscope or sub-cultured for later assessment of growth rate and morphology. Subcultures were compared to the original culture to determine whether the test time/temperature affected the fungus.

Due to the large volume of work, molecular methods or vegetative compatibility (VC) trials could not be employed to prove the origin of recovered isolates. This was also deemed unnecessary as in the majority of cases the identity of the isolate was obvious based on observed morphology and experience and familiarity with the test fungi.

Likewise, when naturally infested material was assessed, neither the time nor resources were available to check the identity of every single isolate that grew out of wood. Based on experience with these fungi, a dissecting microscope was used to assess the isolates in situ and group them under the following categories: bluestain (with *Sporothrix*, *Pesotum* or *Leptographium* anamorphs), Yeast/Bacteria, *Zygomycetes* and several main genera of molds (*Aspergillus*, *Paecilomyces*, *Penicillium* and *Trichoderma)*. The category "Others" was comprised of other mold-stain genera that occurred in very low frequency and included *Acremonium*, *Aureobasidium/Hormonema*, *Botrytis*, *Curvularia, Ulocladium* and a few sterile mycelia. Whitish non-sporulating mycelia of a particular shape, consistency and morphology were grouped separately under "White mycelia". These isolates were considered likely to be basidiomycetes and some therefore potentially decay fungi. Approximately 10% of these were checked under the microscope and all were confirmed to have clamp connections, which is a typical morphological feature found in basidiomycetes. Furthermore, many "White isolates" were found only on benomyl-amended medium, which is selective for basidiomycetes. The majority of unchecked "White mycelia" looked alike and most likely represented basidiomycetes, although this was not definitively shown.

3 Results

3.1 Heat Treatment - Test Wood Inoculated with Test Isolates

All replicates of all isolates of five of the species tested were killed at 56/30 (Table 2). *Ophiostoma montium,* for which only two isolates were tested, had four out of six replicates of isolate E survive 56/30, while all other replicates of the second isolate were eradicated at 56/60. *Phellinus chrysoloma* proved to be the most resistant of the tested fungi as none of the isolates were killed by 56/30. This fungus required 61/30 for 100% eradication (for all 18 replicates), but showed some signs of beginning to succumb at 56/120. All three isolates (18 replicates) of *Leptographium terebrantis* were killed by 56/30 while a few replicates (5 out of 18) survived short exposure of 1 minute at 61°C. A number of replicates were killed at even lower temperatures and shorter exposure times. For example, Isolate I of *Leptographium longiclavatum* and isolates R and S of *Trichaptum abietinum* had complete eradication at 51/30.

3.2 Heat Treatment - Agar Slants Inoculated with Test Isolates

Agar slant tests were performed for three fungi: *Ophiostoma clavigerum*, *Leptographium terebrantis* and *Trichaptum abietinum,* so these data are compared with those of the same species tested on wood. Some replicates showed less heat tolerance if grown and treated on agar slants compared to the same isolates grown on wood (Tables 4, 5 and 6). For example, all 18 replicates of *Ophiostoma clavigerum* grown on wood survived HT at 46°C for 60 and 120 minutes while none of the same isolates grown on agar survived the same treatment. A similar trend was observed for the other two fungal species. For example, heat treatment of isolate L inoculated on wood showed six survivors at 56/1, but only one surviving replicate for the agar slant. *Trichaptum abietinum* exhibited similar mortality, except in the case of isolate T where two out of six replicates survived 56/30 when grown on agar compared to on wood, which was eradicated at that temperature/time. All *Ophiostoma clavigerum* isolates grown on agar were killed at 51/30 compared to *O. clavigerum* grown on wood where isolates survived 51/30 (Table 4).

3.3 Air-drying at 15% EMC - Test Wood Inoculated with Test Isolates

All fungi and all replicated isolates survived the entire 4-month testing period and have been readily re-isolated on all sampling dates (Table 3). Data for *Ambrosiella* sp. are not yet available. These data indicated that wood air-drying at 15% EMC was insufficient in eradicating tested fungi. During these tests humidity and temperature inside the jar assemblies was monitored and confirmed to be the same as those in the environmental chamber (20°C and 78 %RH) where the jars were stored.

3.4 Heat Treatment after Air-drying for 15, 40 and 90 days - Test Wood Inoculated with Test Isolates

As this treatment introduced a huge amount of work only three species were subjected to it: one decay fungus *Trichaptum abietinum* (three isolates) and two stain fungi: *Ophiostoma clavigerum* (three isolates) and *Leptographium terebrantis* (one isolate). After the inoculated and colonized test wood pieces were subjected to air-drying (target EMC of 15%) for 15, 40 and 90 days, they were heat treated following the matrix. Interestingly, there appeared to be limited evidence for an increased heat tolerance when isolates were subjected to air-drying prior to heat treatment (Tables 4, 5 and 6). Two out of six replicates of the single tested isolate (K) of *Leptographium terebrantis* survived heat treatment at 56/30 that followed 90 days of air-drying, while in immediate heat treatment studies all six replicates were killed by 56/30. All replicates of isolate K air-dried for 90 days were killed at the next longer time period 56/60. Likewise, isolate R of *Trichaptum abietinum* showed an increase in heat tolerance. All six replicates of isolate R survived 56/1 and 56/30 and were finally eradicated at 56/60, when air-dried for 90 days prior to heat treatment. This shows considerably higher heat tolerance than this isolate exhibited with no prior drying, where eradication occurred at 51/1. The other isolates and replicates of *Trichaptum abietinum*, however, responded in largely the same way regardless of whether they were air-dried prior to treatment or heat treated immediately after incubation. When observed under the microscope, there was no significant difference in morphology between samples that received different treatments.

3.5 Heat Treatment - Naturally Infested Material

Although bluestain fungi and decay fungi were confirmed to be alive and readily found in naturally infested material, none survived 56/30 treatment (Table 7). There were only six colonies of common fungi (three yeast, one *Aspergillus* sp and two *Penicillium* spp) that were found on wood treated with temperature/time combinations exceeding 56/30. This is a miniscule number compared to the plethora of fungal colonies and types coming out of the same (control) wood that had been heat-treated at 25°C.

3.6 Air-drying - Naturally Infested Material

Following air-drying of naturally infested material a number of different fungal groups were isolated at all sampling dates and all survived air-drying for the 4-month duration of the experiment (Table 8). Fungi that were isolated throughout the experiment included bluestain anamorphs *Sporothrix* and *Leptographium*, Yeasts/Bacteria, *Zygomycetes*, *Penicillium* and *Trichoderma*. Basidiomycetes (white mycelia) were not readily isolated beyond 3 weeks of air-drying. On the other hand, molds like *Trichoderma*, *Zygomycetes* and *Penicillium* were isolated from a majority of samples in the later stages of the experiment. These data also confirmed that air-drying to 15% EMC was insufficient to eradicate fungi in colonized wood, although the data suggest a shift towards prevalence of molds in later stages of drying, replacing bluestain and decay-like fungi.

3.7 Heat Treatment after Air-drying for 15, 40 and 90 days - Naturally Infested Material

As with the straightforward heat-treatment of naturally infested material, no surviving bluestain or decay fungi were found following 56/30 treatment (Table 9). There was only one isolate of white sterile mycelia growing from wood on benomyl-augmented media after 61/60 with prior drying for 40 days. This isolate

did not show clamp connections so it is uncertain if this represented a decay fungus or something else. In straightforward heat treatment of naturally infested material at temperatures below 56/30, bluestain was observed with *Leptographium* but none with *Sporothrix* anamorph. However, in material that had been air-dried prior to heat treatment, especially for 90 days, bluestain was observed with *Sporothrix* anamorph more often (Fig. 4). This particular species, having white powdery and slow growing mycelium and *Sporothrix* anamorph, was identified by University of British Columbia's Wood Science Department as *Ophiostoma nigrocarpum*. A few yeast colonies, *Zygomycetes*, or *Aspergillus* did survive on wood treated beyond 56/30, but these isolations were sparse and without any obvious trends. There was, however, a substantial presence of a few genera of common saprophytic molds on heat-treated and aged material (dried for 40 or 90 days prior to heat treatment). These included *Penicillium* and, in particular, *Trichoderma*. In the majority of cases, when air-dried for 15, 40 or 90 days prior to treatment, *Trichoderma* survived 56/120 and 61/30 (in a few pieces *Trichoderma* even survived 71/30 and 71/120). This was in sharp contrast to direct heat treatment of naturally infested material where *Trichoderma* did not survive beyond 51/1. Furthermore, the test wood with *Trichoderma* was completely covered with dense mycelial mat of this genus and no other fungi could be seen underneath that mat (Fig. 4). *Paecilomyces* (which is a known thermotolerant mold) was also found to survive treatment at 71/60 in a few cases.

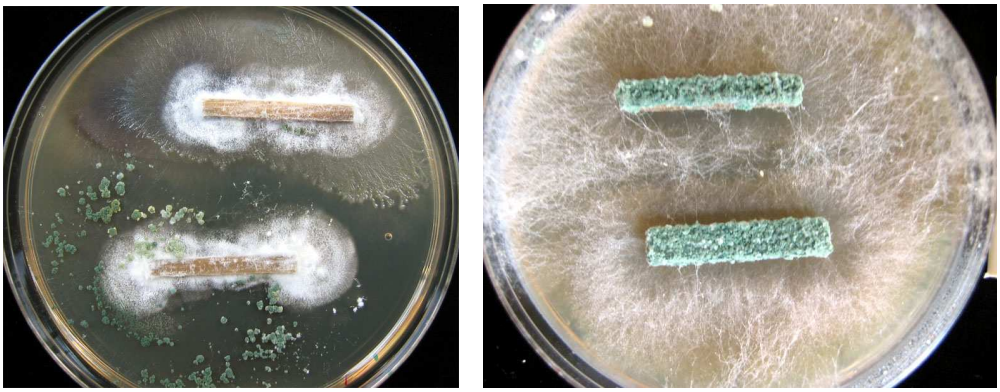

Figure 4. Bluestain with *Sporothrix anamorph* (white powdery mycelium shown in the left photo) growing out of naturally infested test wood after heat treatment. The picture on the right shows characteristic green and white mycelium of *Trichoderma* completely covering both wood and the agar plate after surviving heat treatment at target temperatures. Its grip on the wood sample is complete and no other fungi can be seen on the wood or on the agar.

4 Discussion

The results of these tests show that leaving wood to air dry up to 4 months is insufficient to eradicate fungi present in the wood and cannot be considered a fungal pest-eradication method. The decision was made to dry wood to 15 EMC (by keeping it at 20°C and 79% RH); however the question remains if there would be any significant changes in survival if wood was dried to even lower EMC, for example drying the wood below 10% EMC (by keeping it at 55% RH or less). Heat treatment at specific temperatures and times is needed in order to eradicate fungi.

There was a difference in results for the heat treatment for some isolates when grown and tested on wood versus on agar slants in glass vials. Some replicates have shown less heat tolerance if tested on agar slants and, although the vial method is easier and faster to set and handle, it should not be used for large-scale studies. Tests using wood better represent a real-life situation and the organisms that might be found imbedded in traded wood that would normally be heat-treated at 56/30 prior to export.

The data show that 56/30 kills a majority of inoculated fungi grown on agar and inside wood test pieces and those naturally found in substrate attacked by mountain pine beetle. None of the bluestain fungi and decay fungi present in sapwood of naturally infested mountain pine beetle-affected trees survived 56/30. The survival exceptions occurred for several pure cultures, grown on the test wood and tested under laboratory conditions. These included ll isolates of a sap-rot fungus *Phellinus chrysoloma* and a few replicates of bluestain fungus *O. montium* survived 56/30. *P. chrysoloma* was 100% killed a the next highest temperature and *O. montium* was 100% killed at the next longest time. In some earlier work it was shown that some decay fungi are heat tolerant. In particular, Newbill and Morrell (1991) showed that heartwood-colonizing basidiomycetes were more tolerant to heat and regularly survived 56/30. Heat tolerant fungi commonly produce chlamydospores or other similar resting/surviving features. However, *P. chrysoloma* is not on the list of basidiomycetous fungi that produce chlamydospores (Stalpers 1978), although updated information on the CBS fungal database (CBS, 2006) mentions that chlamydospores are scattered in the tramal tissue of some specimens, we did not see any such structures when examining the fungus under the microscope. The heat tolerance of this sap-rot fungus is, therefore, somewhat surprising.

Heat treatment that followed 15, 40 and 90 days of air-drying showed that some fungi developed heat tolerance when they are slowly desiccated. Slow drying stresses the fungi and it is believed that such stress can induce production of survival structures in the fungal mycelium; these structures may encourage survival of fungi at temperatures where they would normally not survive in a freshly grown state (Joost Stalpers, CBS-Netherlands, personal communication). It is not surprising then, that some replicates survived 56/30 after being subjected to drying before treatment. This presents a potential threat that some fungi may develop heat resistance in wood that would normally lie around before it is commercially heat-treated and prepared for export. However, evidence for this increased heat tolerance due to air-drying was sparse. A total of eight replicates out of tested 42 survived 56/30 after being air-dried for 90 days. This compares to 0% survival for immediate heat treatment and heat treatment after 15 and 40 days of air-drying.

These results should be considered to be worst-case scenarios in terms of fungal survival since the temperature was rapidly raised to the target and dropped rapidly after the target time period. In commercial heat treatments, the inherent tendency of kiln schedules to overshoot the target temperature and the insulating properties of wood will result in higher than target temperatures and the temperature being maintained much longer.

In the more realistic context of naturally infested wood, no decay or stain fungi survived 56/30, regardless of prior air-drying. In the natural context, the interaction and competition from multiple fungi will most likely reduce the overall survivability of the fungi of interest so air-drying does not increase their tolerance to 56/30 heat treatment. Below 56/30 a bluestain fungus with *Sporothrix* anamorph was encountered more often than bluestain with *Leptographium* anamoph and the former would be considered more saprofitic rather then pathogenic, e.g., primary colonizer able to grow in fresh and live host tissue. Bluestain with *Leptographium*-like anamorph, which would normally include *Grosmannia clavigera* and *Ophiostoma montium* are common associates of mountain pine beetle in its early stages of colonization and primary colonizers that prefer fresh and uncolonised substrates. Other saprofitic species, especially some molds, commonly follow the primary colonizers and in these experiments were found in increasing numbers in naturally infested wood that was air-dried prior to heat treatment. There was a noticeable and increased heat tolerance and survival of several common wood molds, in particular the genera *Penicillium* and *Trichoderma*. *Trichoderma* was found on almost all test-wood pieces that were treated at or beyond 56/30, with some even surviving 71/30 and 71/120.

Trichoderma densely covered the test wood in a large number of cases. Where *Trichoderma* was present there was virtually no other fungi seen and *Trichoderma* was the only genus isolated. Primary colonizers, which prefer fresh wood, display poor saprophytic survival and it has been confirmed that these fungi often do not maintain occupation of their initial territory (Strong et al. 1998). This is particularly the case for the majority of bluestain fungi that are associated with beetles or that colonize fresh wood. Gibbs (1993) reported *Trichoderma* spp racing through blue-stained sectors of logs preventing successful isolation of bluestain. Extensive culturing using selective media from bluestained areas of naturally dried fresh wood, recovered *Trichoderma* in 88% of cases and live ophiostomatoid fungi from only 29%. *Trichoderma* is a well known, aggressive, fast growing and vigorous genus used as a biocontrol agent. It has mycoparasitic ability and produces a number of secondary metabolites that may inhibit other fungi (Morris et al. 1992, Schoeman et al, 1996, Score 1998, Bruce et al. 2000, Crozier et al. 2000, Humphris et al. 2001, Phillips-Laing et al. 2003. More than half of the registered and available fungal products (to control various plant diseases) are *Trichoderma*- or *Gliocladium*-based preparations (Butt et al. 2001, Mason & Huber 2002).

Using our experience and common knowledge, we suggest that in a natural setting, where wood would be left to air dry while lying around prior to heat treatment, aggressive molds will take over the substrate. These aggressive saprophytes (which also sometimes act as mycoparasites) will influence survival of other fungi e.g., bluestain or decay (some that may be considered pests and may survive 56/30). The practical implication of this is that commercially used 56/30 treatment will eradicate most fungi of phytosanitary concern and will predispose the substrate to saprophytic fungi with greater competitive ability. Their presence will reduce, to an acceptable minimum, the survival of phytosanitary pests that could otherwise pose a risk and potential threat.

5 Conclusions

A heat treatment schedule of 50°C for 30 minutes successfully controlled all the organisms present in naturally infested post-mountain pine beetle material.

A heat treatment schedule of 50°C for 30 minutes successfully controlled the majority of organisms tested in artificially inoculated material.

Species that were not controlled under these conditions were killed at the next highest temperature, 61°C, or the next longest time, 60 minutes.

Slow air-drying increased resistance to heat treatment in some species, but again 56/60 or the 61/30 provided 100% kill; however the most resistant species were not tested after air-drying.

Leaving wood to air dry up to 4 months (to 15 % EMC) was insufficient to eradicate fungi present in the wood and cannot be considered a fungal pest-eradication method.

In slowly air-dried naturally infested wood, aggressive common molds such as *Trichoderma* spp showed a significant increase in heat tolerance, thoroughly colonized the substrate and prevented growth of other fungi weakened by heat treatment.

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Table 2. Fungal isolation following heat treatment of test wood inoculated with test isolates

Temp.	Time	Species and different isolates (A-W)																		
		Ophiostoma clavigerum			*Ophiostoma montium*		*Leptographium longiclavatum*			*Leptographium terebrantis*			*Ambrosiella* sp.		*Trichaptum abietinum*			*Phellinus chrysoloma*		
(°C)	(min)	A	B	C	D	E	G	H	I	J	K	L	M	P	R	S	T	U	V	W
25	<1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	30	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	60	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	120	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
41	<1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	60	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	120	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
46	<1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6	6	6	6	6
	60	6	6	6	6	6	6	6	6	6	6	6	6	6	1	6	6	6	6	6
	120	6	6	6	6	6	6	6	6	6	6	6	4	4	0	2	1	6	6	6
51	<1	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6
	30	3	3	4	4	6	1	6	0	6	6	6	1	0	0	0	0	6	6	6
	60	0	1	1	0	6	0	6	0	5	5	6	0	0	0	0	0	6	6	6
	120	0	0	0	0	1	0	4	0	0	2	2	0	0	0	0	0	6	6	6
56	<1	4	4	5	3	5	2	6	0	6	6	6	0	0	0	0	2	6	6	6
	30	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	6	6	6
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6	6
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	5	1
61	<1	0	0	0	0	0	0	0	0	1	3	1	0	0	0	0	0	6	6	6
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Numbers in table represent number of recovered isolates/replicates after heat treatment. There were always six replicates apart from the control at 25°C when there were three replicates.

Table 3. Fungal isolation following air-drying at 15% EMC for test wood inoculated with test isolates

Duration	Species and different isolates (A-W)																		
	Ophiostoma clavigerum			*Ophiostoma montium*		*Leptographium longiclavatum*			*Leptographium terebrantis*			*Ambrosiella* sp.		*Trichaptum abietinum*			*Phellinus chrysoloma*		
	A	B	C	D	E	G	H	I	J	K	L	M	P	R	S	T	U	V	W
0 Days	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
4 Days	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
8 Days	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
12 Days	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
16 Days	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
3 Weeks	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
4 Weeks	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
5 Weeks	6	6	6	6	6	6	6	6	6	6	6			6	6	6	6	6	6
6 Weeks	6	6	6	6	6	6	6	6	6	6	6			6	6	6	6	6	6
7 Weeks	6	6	6	6	6	6	6	6	6	6	6			6	6	6	6	6	6
8 Weeks	6	6	6	6	6	6	6	6	6	6	6			6	6	6	6	6	6
4 Months	6	6	6			6	6	6	6	6	6			6	6	6	6	6	6

Numbers in table represent number of recovered isolates/replicates after the treatment. Total number of treated isolates is always six.

Table 4. Survival of O*phiostoma clavigerum* following HT of isolates grown on test wood and agar slants following HT of inoculated wood after 15, 40 and 90 days of prior air-drying

Ophiostoma clavigerum

Temp.	Time	Isolate A					Isolate B					Isolate C				
		Test	Agar	Wood dried prior to HT			Test	Agar	Wood dried prior to HT			Test	Agar	Wood dried prior to HT		
(°C)	(min)	Wood	Slant	15 Days	40 Days	90 Days	Wood	Slant	15 Days	40 Days	90 Days	Wood	Slant	15 Days	40 Days	90 Days
25	<1	3	3	3	3	3	3	3	3	3	3	3	3	3	3[a]	3
	30	3	3	3	3	3	3	3	3	3	3	3	3	3	3[a]	3
	60	3	3	3	3	3	3	3	3	3	3	3	3	3	3[a]	3
	120	3	3	3	3	3	3	3	3	3	3	3	3	3	3[a]	3
41	<1	6	6	6	6	6	6	6	6	6	6	6	6	6	6[a]	--
	30	6	6	6	6	6	6	6	6	6	6	6	6	6	4	--
	60	6	6	6	6	6	6	6	6	6	6	6	6	6	6	--
	120	6	6	6	6	6	6	6	6	6	6	6	6	6	6	--
46	<1	6	6	6	6	6	6	6	6	6	6	6	6	6	6[b]	--
	30	6	6	6	6	6	6	3	6	6	6	6	6	6	6	--
	60	6	0	6	6	6	6	0	6	6	6	6	0	6	4	--
	120	6	0	6	5	6	6	0	3	5	6	6	0	6	2	--
51	<1	6	3	6	6	6	6	1	6	6	6	6	3	6	6	6
	30	3	0	4	3	6	3	0	3	1	6	4	0	6	2	3
	60	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1
	120	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
56	<1	4	0	5	5	4	4	0	4	2	3	5	0	6	2	4
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
61	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
66	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
71	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--

Numbers in table represent number of recovered isolates/replicates after heat treatment. There were always six replicates apart from the control at 25°C when there were three replicates.

[a] all replicates had a phenotypic fluffy white growth

[b] four out of the six replicates had a phenotypic fluffy white growth

-- these replicates were not performed due to an insufficient number of infected wood pieces

Table 5. Survival of *Leptographium terebrantis* following HT oF isolates grown on test wood and agar slants following HT of inoculated wood after 15, 40 and 90 days of prior air-drying

Leptographium terebrantis

Temp. (°C)	Time (min)	Isolate J		Isolate K					Isolate L	
		Test Wood	Agar Slant	Test Wood	Agar Slant	Wood dried prior to HT			Test Wood	Agar Slant
						15 Days	40 Days	90 Days		
25	<1	3	3	3	3	3	3	3	3	3
	30	3	3	3	3	3	3	3	3	3
	60	3	3	3	3	3	3	3	3	3
	120	3	3	3	3	3	3	3	3	3
41	<1	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6	6	6
	60	6	6	6	6	6	6	6	6	6
	120	6	6	6	6	6	6	6	6	6
46	<1	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6	6	6
	60	6	5	6	6	6	4	6	6	5
	120	6	3	6	3	6	0	6	6	3
51	<1	6	6	6	6	6	5	6	6	6
	30	6	1	6	2	6	0	6	6	1
	60	5	2	5	0	5	0	6	6	2
	120	0	0	2	0	2	0	5	2	0
56	<1	6	1	6	0	6	0	6	6	1
	30	0	0	0	0	0	0	2	0	0
	60	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0
61	<1	1	0	3	0	0	0	0	1	1
	30	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0
66	<1	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0
71	<1	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0

Numbers in table represent number of recovered isolates/replicates after heat treatment. There were always six replicates apart from the control at 25°C when there were three replicates.

Table 6. Survival of *Trichaptum abietinum* following HT oF isolates grown on test wood and agar slants following HT of inoculated wood after 15, 40 and 90 days of prior air-drying

Trichaptum abietinum

		Isolate R					Isolate S					Isolate T				
	Time	Test	Agar	Wood dried prior to HT			Test	Agar	Wood dried prior to HT			Test	Agar	Wood dried prior to HT		
Temp. (°C)	(min)	Wood	Slant	15 Days	40 Days	90 Days	Wood	Slant	15 Days	40 Days	90 Days	Wood	Slant	15 Days	40 Days	90 Days
25	<1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	30	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	60	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	120	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
41	<1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	60	6	3	6	6	6	6	6	6	6	6	6	6	6	6	6
	120	6	0	6	6	6	6	6	0	6	6	6	6	6	6	6
46	<1	6	5	6	6	6	6	6	6	6	6	6	6	6	6	6
	30	5	0	6	6	6	6	6	0	6	6	6	6	6	6	6
	60	1	0	0	6	6	6	6	0	6	6	6	6	3	4	6
	120	0	0	0	4	6	2	6	0	4	6	1	4	0	0	6
51	<1	6	0	3	6	6	6	6	0	6	6	6	6	4	3	6
	30	0	0	0	0	6	0	0	0	0	0	0	0	0	0	1
	60	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
56	<1	0	0	0	0	6	0	1	0	0	0	2	1	0	0	0
	30	0	0	0	0	6	0	0	0	0	0	0	2	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Numbers in table represent number of recovered isolates/replicates after heat treatment. There were always six replicates apart from the control at 25°C when there were three replicates.

Table 7. Fungal isolation from naturally infested wood following heat treatment at different time/temperature exposures

Temp. (°C)	Time (min)	Bluestain		Yeasts/Bacteria	Zygomycetes	Aspergillus	Paecilomyces	Penicillium	Trichoderma	White Mycelia (Decay)	Others
		Sporothrix	Leptographium								
25	<1	0	**10**	**7**	**1**	0	0	**6**	**7**	**9**	0
	30	0	**10**	**7**	**3**	0	0	**4**	**5**	**6**	0
	60	0	**10**	**8**	**3**	0	0	0	0	**5**	0
	120	0	**10**	**6**	**1**	0	0	**5**	0	**8**	0
41	<1	0	**10**	**8**	**1**	0	0	**5**	**2**	**8**	0
	30	0	**10**	**10**	0	0	0	**4**	**3**	**2**	0
	60	0	**7**	**10**	0	**1**	0	**3**	**4**	**3**	0
	120	0	**7**	**10**	0	**1**	0	0	**3**	**6**	0
46	<1	0	**10**	**10**	0	0	0	0	0	0	0
	30	0	**2**	**6**	0	**1**	0	**2**	**1**	**3**	0
	60	0	**2**	**4**	0	0	**1**	**1**	**3**	**2**	0
	120	0	0	**2**	0	0	0	**1**	**1**	0	0
51	<1	0	**2**	**8**	0	0	0	0	**4**	**2**	0
	30	0	0	**1**	0	0	**1**	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0
	120	0	0	**1**	0	0	0	0	0	0	0
56	<1	0	0	**2**	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	**1**	0	0	0	0	0
61	<1	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	**1**	0	**1**	0	0	0
	60	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0
66	<1	0	0	**1**	0	0	0	**1**	0	0	0
	30	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0
71	<1	0	0	**2**	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0

Numbers in the table represent number of test wood pieces (out of 10 replicates for each temperature/time combination) from which a particular fungus grew out and was identified.

Table 8. Fungal isolation from naturally infested wood following air-drying at 15% EMC for different times.

Duration	Bluestain		Yeast/bacteria	Zygomycetes	Aspergillus	Paecilomyces	Penicillium	Trichoderma	White mycelia (Decay)	Others
	Sporothrix	Leptographium								
0 Days	1	10	9	1	0	0	7	1	6	2
4 Days	0	10	10	2	0	0	1	1	7	0
8 Days	1	10	6	1	0	0	7	5	8	0
12 Days	4	10	8	0	0	0	6	8	7	0
16 Days	4	10	2	0	0	0	6	8	8	2
3 Weeks	3	9	8	2	0	0	7	8	5	3
4 Weeks	3	7	7	0	0	0	1	10	0	0
5 Weeks	10	3	0	9	0	0	6	10	0	0
6 Weeks	10	0	2	10	0	0	10	10	2	0
8 Weeks	10	2	0	9	0	0	10	10	0	2
4 Months	7	1	1	6	0	0	7	10	0	0

Table 9. Fungal isolation from naturally infested wood that was first air-dried at 15% EMC for 15, 40 or 90 days then heat-treated at different temperature /time exposure

Treatment		Bluestain																														
		Sporothrix			*Leptgraphium*			Yeasts/Bacteria			*Zygomycetes*			*Aspergillus*			*Paecilomyces*			*Penicillium*			*Trichoderma*			White Mycelia (Decay)			Others		
Temp.	Time	Drying prior to HT			Drying prior to HT			Drying prior to HT			Drying prior to HT			Drying prior to HT			Drying prior to HT			Drying prior to HT			Drying prior to HT			Drying prior to HT			Drying prior to HT		
		Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
(°C)	(min)	15	40	90	15	40	90	15	40	90	15	40	90	15	40	90	15	40	90	15	40	90	15	40	90	15	40	90	15	40	90
25	<1	1	4	10	9	2	1	5	4	0	2	0	2	0	1	0	0	0	0	4	9	6	6	10	10	10	0	0	0	1^{U}	9^{A}
	30	5	9	10	10	2	0	7	1	1	3	7	1	0	0	0	0	0	0	6	10	7	2	10	10	5	0	0	0	0	7^{A}
	60	1	10	10	10	2	1	5	3	0	6	2	1	0	0	0	0	0	0	4	10	9	0	10	10	4	0	0	0	0	10^{A}
	120	5	10	10	10	1	3	10	1	0	3	1	1	0	0	0	0	0	0	0	8	9	8	10	10	8	0	0	0	0	10^{A}
41	<1	2	3	8	10	4	0	6	4	1	0	1	4	0	0	0	0	0	0	3	4	1	8	10	10	9	0	0	0	0	0
	30	0	6	8	10	3	0	6	2	0	6	3	6	0	2	0	0	0	0	8	7	2	0	10	10	4	0	0	0	0	0
	60	4	6	6	10	3	0	9	3	1	0	0	8	0	0	0	0	0	0	4	8	3	8	10	10	8	0	0	0	1^{Ap}	0
	120	2	5	3	8	2	0	6	1	0	0	2	8	0	1	0	0	0	0	3	9	1	8	10	10	8	0	0	0	0	0
46	<1	4	7	5	5	0	0	2	2	0	6	0	8	0	0	0	0	0	0	1	7	0	10	10	10	5	1	0	0	1^{C}	0
	30	2	10	5	4	0	0	4	2	0	2	2	8	0	0	0	0	0	0	1	5	1	10	10	10	5	1	0	0	0	0
	60	2	6	3	5	0	0	5	1	0	2	8	4	0	1	0	0	0	0	0	6	0	10	10	10	5	0	0	0	0	0
	120	3	8	6	4	1	0	4	6	0	0	0	10	0	0	0	0	0	0	1	10	1	10	10	10	6	0	0	0	0	0
51	<1	0	9	10	0	2	0	1	1	0	1	5	10	0	0	0	0	0	0	0	10	4	10	10	10	1	0	0	0	0	10^{A}
	30	0	3	4	0	1	0	0	9	0	0	2	8	0	0	0	0	0	0	0	8	9	10	10	10	0	0	0	0	0	2^{U}
	60	0	0	3	0	0	0	0	6	0	0	0	10	0	1	0	0	0	0	0	8	6	10	10	10	0	0	0	0	0	1^{A}
	120	5	0	1	0	0	0	4	0	0	1	1	10	0	1	0	0	0	0	0	1	10	10	10	10	0	0	0	0	0	2^{A}
56	<1	0	2	9	0	0	0	0	1	0	2	10	10	0	2	0	0	0	0	0	2	5	10	10	10	0	0	0	0	0	1^{W}
	30	0	0	0	0	0	0	0	1	0	0	4	3	0	0	0	0	0	0	0	0	10	10	10	10	0	0	0	0	0	3^{U}
	60	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	9	10	10	10	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	3	9	8	9	10	0	0	0	0	0	0
61	<1	0	0	0	0	0	0	4	2	0	0	0	0	0	1	0	0	0	0	0	0	10	10	10	10	0	0	0	0	0	0
	30	MIS	0	0	MIS	0	0	MIS	1	0	MIS	0	0	MIS	1	0	MIS	0	0	MIS	0	2	MIS	9	10	MIS	0	0	MIS	0	0
	60	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4	7	0	1	0	0	0	0
	120	0	0	0	0	0	0	4	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	6	0	0	0	0	1^{W}	1^{B}
66	<1	MIS	0	0	MIS	0	0	MIS	2	0	MIS	0	0	MIS	0	0	MIS	0	0	MIS	0	1	MIS	10	10	MIS	0	0	MIS	0	0
	30	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
	60	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
	120	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
71	<1	0	0	0	0	0	0	3	0	0	0	1	0	0	1	0	0	2	2	0	0	0	3	4	3	0	0	0	0	0	0
	30	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	2^{W}
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0
	120	0	0	MIS	0	0	MIS	0	0	MIS	0	0	MIS	0	0	MIS	0	0	MIS	0	0	MIS	0	1	MIS	0	0	MIS	0	0	MIS

Numbers in the table represent number of test wood pieces (out of 10 replicates for each temperature/time combination) from which a particular fungus grew out and was identified.

MIS - Missing data
[A] *Acremonium*
[Ap] *Aureobasidium pullulans*
[C] *Curvularia*
[U] *Ulocladium*
[W] Sterile white mycelium

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