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## **NAPPO Science and Technology Documents**

### **ST 03: Review of heat treatment of wood and wood packaging**

**Prepared by the members of the  
NAPPO Forestry Panel<sup>1</sup>**

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## Summary

Heat treatment is an effective method to kill regulated pests that affect forest trees which may be associated with resulting wood commodities. This paper reviews the history of heat as a wood treatment, the scientific basis for its effect on wood pests (including insects, fungi, nematodes and bacteria), the industrial processes by which wood is heat treated and how heat treatment can be incorporated into phytosanitary systems approach. The paper is intended to provide guidance to national plant protection organizations in the use of heat treatment in phytosanitary regulations.

## Historical perspective on heat treatment of wood

Heat has long been used to reduce the moisture content of wood and to kill pests (insects, fungi, nematodes) living in or on wood commodities. Research published in the 1920s and 1930s first documented heat as a treatment to kill insects (Craighead 1920, Snyder 1921) and fungi (Chidester 1937, Snell 1922, 1923, Montgomery 1936) in wood. The use of heat as a method to control pests in grain, fruit and other agricultural commodities is also well documented (Hansen and Johnson 2007, Hansen et al. 2011). Much of the early research on wood treatments focused on quality losses and the reduction of commodity value for domestic markets, but heat treatment for quarantine purposes was mentioned by Snyder (1921):

“Damage of this type [*Lyctus* – powder post beetle infestation] is distributed widely throughout the world, many species of these beetles being carried from one country to another in the commercial products which they infest”.

Quarantine requirements for wood products moving internationally during the first half of the 20th century varied greatly. Some importing countries had virtually no requirements, others a combination of absence of bark, freedom from specified pests and absence of soil.

In the 1980s, European concerns about the potential introduction of pinewood nematode (*Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle) from its native distribution in North America led to joint EU-North American research on lethal heat treatment protocols for the nematode and its insect vector (Smith et al. 1991). The studies indicated that 52.1°C and higher killed all pinewood nematode in wood; following statistical analysis of the temperature required to reach 100% mortality at 99.994% reliability and 95% confidence the final report recommended that wood should be treated to a core temperature of 56°C for 30 minutes (Smith et al. 1991). This time-temperature schedule was incorporated into EU import regulations for wood products originating from pinewood nematode-infested areas (European Commission 1992).

Further heat treatment standards for wood were prescribed in US regulation. Treatments for logs and other wood commodities were reviewed by the US Forest Service in the early 1990s (USDA APHIS 1991) as the international movement of wood products were seen as a major risk to the importation of exotic forest pests (USDA Forest Service 1991, 1992, 1993). A proposed Federal Register Rule was published in 1994 outlining a number of treatments including heat treatment (USDA 1994):

“Heat treatment procedures may employ steam, hot water, kilns, exposure to microwave energy, or any other method that raises the temperature of the center of each treated regulated article to at least 56 °C and maintains the regulated article at that center temperature for at least 30 minutes.”

Following public input, the final rule was modified, specifically the heat treatment requirement:

“Change the standard for heat treatment and heat treatment with moisture reduction from 56 °C for 30 minutes to 71.1 °C for 75 minutes. This change is in response to several commenters who recommended that APHIS use 71.1 °C for 75 minutes as reported in the Forest Service’s Scientific Panel Review of January 10, 1992—Proposed Test Shipment Protocol for Importing Siberian Larch Logs. Upon reviewing this research and our data from the proposal supporting a lesser temperature-time combination, we believe we were in error in believing that the proposed heat treatment would effectively eliminate all plant pests of concern. Specifically, a heat treatment of 56 °C for 30 minutes could allow various harmful fungi to survive. Research reports show that various fungi in wood can survive 1 to several hours of heat treatment at temperatures ranging from 56 °C to 70 °C, but are destroyed by a treatment of 71.1 °C for 75 minutes. The heat treatment required by the regulations must be able to effectively destroy all potentially dangerous fungi.”

Other countries have developed different heat treatment standards for wood commodities. For example, New Zealand’s regulations specify:

“Heat treatment (or kiln drying) at a minimum continuous core temperature of 70°C for more than 4 hours” for sawn wood up to 300 mm in thickness (NZ MPI 2013).

Australia’s import requirements vary with wood species and country of origin specifying different treatment protocols (DAFF 2013). For example, approved treatments for *Fraxinus* L. or *Quercus* L. from all countries include dry heat treatment (Australian authorized treatment - T10025) - 74°C for at least 60 minutes once the core temperature has been reached or a kiln drying option (T9912) that specifies a chamber temperature of 74°C and different treatment times (4 – 18 hours) depending on wood thickness. Treatment time does not commence until the temperature and humidity in the chamber have stabilised and the core temperature of the wood has reached at least 74°C. In contrast, wood imported to Australia from Canada (other than *Fraxinus* or *Quercus*) can be treated under government oversight with heat (T9968): 56°C for 30 minutes, measured at the core of the wood, or the kiln option (T9912) described above.

Through the 1990s wood packaging (pallets, crates, dunnage, etc) was increasingly recognized as an important pathway for alien forest pests (USDA 2000, Allen and Humble 2001). For example, the introductions of the Pine Shoot Beetle, *Tomicus piniperda* L. and the Asian Longhorned Beetle, *Anoplophora glabripennis* Motschulsky, into North America were thought to have occurred via infested crating or ship dunnage (Liebhold et al. 1995). The discovery of an established population of *A. glabripennis* in the US in 1996 and

elsewhere in the world in ensuing years (Haack et al. 2010) motivated some countries to enact import regulations to address the pest risks specifically associated with wood packaging. Various phytosanitary approaches were taken by different countries requiring measures or combinations of measures, e.g. heat treatment (sometimes specifying treatment parameters such as 56°C for 30 minutes core temperature, sometimes referring to kiln schedules), absence of bark and grub holes, kiln drying to a specific moisture content, usually 20%, mandatory phytosanitary certificates. In order to harmonize phytosanitary requirements for wood packaging internationally, the development of a standard that would include globally recognized treatments was undertaken. A regional standard drafted and adopted by the North American Plant Protection Organization (RSPM 11) in 2001 served as a starting point for the development of ISPM 15, “Guidelines for regulating wood packaging material in international trade”, adopted by the International Plant Protection Convention (IPPC) Commission on Phytosanitary Measures in 2002. No specific heat treatment schedule was described in RSPM 11, rather the standard recommended that:

“The wood must be dried by heating in a kiln in accordance with a specific time/temperature schedule, as recommended in a recognized kiln operator’s manual.”

More prescriptive guidance on heat treatment was included in the original adopted version of ISPM 15:2002. The drafting group evaluated available science information and specified that:

“Wood packaging material should be heated in accordance with a specific time-temperature schedule that achieves a minimum wood core temperature of 56°C for a minimum of 30 minutes. Kiln-drying (KD), chemical pressure impregnation (CPI), or other treatments may be considered HT treatments to the extent that these meet the HT specifications. For example, CPI may meet the HT specification through the use of steam, hot water, or dry heat” (ISPM 15: 2002).

Revised text was adopted by the Commission on Phytosanitary Measures in 2009 recognizing different heat treatment methods and providing specific practical guidance on application.

“Various energy sources or processes may be suitable to achieve the required treatment parameters. For example, conventional steam heating, kiln-drying, heat-enabled chemical pressure impregnation and dielectric heating (microwave, radio frequency) may all be considered heat treatments provided they meet the heat treatment parameters specified in this standard” (ISPM 15: 2009).

## Literature Review - Temperature tolerance of wood-inhabiting organisms

### 1.1 Thermotolerance

Wood-inhabiting organisms are killed at different temperatures; some demonstrating varying levels of thermotolerance. This was acknowledged in the wording of the purpose of ISPM 15:2009 as described in the scope of both the 2002 and 2009-revised text, which was

“to reduce the risk of introduction and/or spread of quarantine pests associated with wood packaging material”

through the application of globally accepted treatments that would address most pests. The 2002 version of the standard recognized the possibility of some pests surviving the approved treatments.

“Approved measures should be accepted by all NPPOs as the basis for authorizing the entry of wood packaging material without further requirements except where it is determined through interceptions and/or PRA that specific quarantine pests associated with certain types of wood packaging material from specific sources require more rigorous measures.”

This wording was revised slightly in 2009:

“These phytosanitary measures should be accepted by all National Plant Protection Organizations (NPPOs) as the basis for authorizing the entry of wood packaging material without further specific requirements. Required phytosanitary measures beyond an approved measure as described in this standard require technical justification.”

The footnote in Annex 1 of the 2002 version of the standard further noted possible thermotolerance; this text was removed in 2009.

“A minimum core temperature of 56° C for a minimum of 30 min. is chosen in consideration of the wide range of pests for which this combination is documented to be lethal and a commercially feasible treatment. Although it is recognized that some pests are known to have a higher thermal tolerance quarantine pests in this category are managed by NPPOs on a case by case basis.”

Some of the variability in experimental results reported in the following sections reflects different experimental approaches. As indicated in section 3, it is critical that standardized methods are used in treatment testing.

## 1.2 Fungi

Although most fungi grow optimally at temperatures between 0°C and 40°C (Seifert 1993) there is considerable variation in the reported temperatures required to kill different fungal species. For example, Lindgren (1942) tested 11 isolates of blue-stain fungi that stopped growth at temperatures between 29-39°C. Most staining fungi can tolerate somewhat higher temperatures and will stop growing at 40-50°C under conditions of high humidity (Seifert 1993). In a survey of 64 species of wood decay fungi, Humphreys and Siggers (1934) showed that 62 of the cultures stopped growth at 46°C. Some species, known as thermophilic fungi, can tolerate and grow at temperatures higher than 50°C (Appendix 2). Jones (1973) demonstrated that the oak wilt fungus (*Ceratocystis fagacearum* (Bretz) Hunt) was killed when logs were treated for 6 hr at >54°C or longer treatment times at lower temperatures. Kappenburg (1998) reported a lethal temperature for *C. fagacearum* of 68°C at high humidity (1998). Jaynes and DePalma (1984) reported that mycelial growth and conidial germination of *Endothia parasitica* were affected by exposure to 50°C or higher for 30 min. Mycelium was generally killed at 53°C or higher but some spores survived 60°C. Chidester (1937) reported that treatment times of 75 min at 66°C or 30 min at 77°C were required to kill three decay fungi (*Lenzites sepiaria* Fr., *Poria incrassata* (Berk. & M.A. Curtis) Burt and *Lentinus lepideus* (Fr.) Fr.). In a more recent study, Newbill and Morrell (1991) found that all test fungi (*Peniophora* spp., *Stereum sanguinolentum* (Alb. & Schwein.) Fr., *Postia placenta* (Fr.) M.J. Larsen & Lombard, and *Antrodia carbonica* (Overh.) Ryvarden & Gilb.) were killed after 75 min at 66°C. Uzunovic and Khadempour (2007) tested bluestain and saprot fungi in naturally-infested and artificially inoculated wood (*Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington, *O. montium* (Rumbold) Arx, *Leptographium longiclavatum* S.W. Lee, J.J. Kim & C. Breuil, and *L. terebrantis* S.J. Barras & T.J. Perry, *Ambrosiella* spp. Arx and Hennebert, *Trichaptum abietinum* (Dicks.) Ryvarden and *Phellinus chrysoloma* (Fr.) Donk). They reported that all fungi in naturally-infested wood were killed at or below 56°C for 30 minutes but that some fungal isolates in artificially-inoculated wood required 61°C or a 60 minute exposure to be killed. Using similar experimental methods, Allen (unpublished) tested a range of fungi: *Phellinus noxius* (Corner) G.H. Cunn., *Heterobasidion annosum* (Fr.) Bref., *Armillaria ostoyae* (Romagn.) Herink, *Gloeophyllum sepiarium* (Wulfen) P. Karst.) *Gloeophyllum striatum* (Sw.) Murrill, *Ceratocystis fagacearum* (Bretz) Hunt, *Ophiostoma wagneri* (Goheen & F.W. Cobb) T.C. Harr., *Ceratocystis polonica* (Siemaszko) C. Moreau, *Leptographium wingfieldii* M. Morelet. All species of test fungi were killed at temperatures at or below 56°C/30 (except for *G. sepiarium* (Wulfen) P. Karst., a known thermotolerant species (Chidester 1939, Kurpik and Wasney 1978) that survived to 71°C. Sapwood-inhabiting fungi have been observed to be more temperature sensitive than heartwood fungi that produce special structures such as chlamydospores (Newbill and Morrell 1991) or arthrospores (Schmidt 2006) facilitating their survival under adverse conditions (Appendix 3).

### 1.3 Insects

Heating wood to 56°C for 30 min will kill most insect life stages. In an early study by Graham (1924) *Ips pini* Say larvae and adults were killed at 49 and 50°C, respectively, and *Chrysobothris dentipes* Germar required treatment for an unspecified time at 52°C. Heat treatment for 1 hr at 50°C was fatal to larvae, pupae and callow adults of *Ips typographus* (Annala 1969). Similar effects were observed in a forest environment where broods on sun-exposed sides of logs were killed and shaded broods survived. Heat treatments using kiln temperatures of 60-71°C for 1 hour were shown to kill *Monochamus* larvae in lumber (Ostaff and Cech 1978). This treatment schedule was refined to 56°C for 30 min for treatment of pinewood nematode-infested wood and the combination has been accepted as a phytosanitary standard for both insects and nematodes (Smith 1991). Mushrow et al. (2004) found that wood-inhabiting *Tetropium fuscum* (Fabr.) larvae, pupae and adults were killed when treated at temperatures less than 50°C for 30 minutes. Egg, larval and adult stages of *Anobium punctatum* De Geer were tested by Hansen and Jensen (1996). Larvae showed 100% mortality at 5 min exposure at 52°C; egg and adult stages were more sensitive to heat treatment. Some insects, such as powder-post beetles (*Lyctus* spp.), have been reported to have a higher temperature tolerance requiring treatment for 30 min at 82°C (Snyder 1923).

Some research has demonstrated the survival of some life stages of *Agrilus planipennis* Fairmaire when treated using the time/temperature schedule of 56°/30. McCullough et al. (2007) reported survival of *A. planipennis* prepupae in wood chips (6.5 x 3.1 x 1.5 cm) treated at 60°C for 20 min, but not 120 min. At 55°C, 17% of the prepupae survived; no prepupae survived exposure to 60°C for 120 min; although no pupation of surviving prepupae occurred in chips exposed to 55 or 60°C. This study monitored chamber temperature. Myers et al. (2009) evaluated survival of *A. planipennis* larvae and prepupae in firewood. Temperature monitoring probes were inserted to 3.5 cm (maximum penetration depth of the beetle). Larvae were capable of surviving a temperatures-time combination up to 60°C for 30 min in wood, prepupae up to 55°C for 30 min, 50°C for 60 min and 60°C for 15 min. Adult emergence was observed in firewood in 45, 50, and 55°C treatments for both 30- and 60-min time intervals; no emergence occurred in any of the 60 or 65°C treatments. Nzokou et al. (2008) observed *A. planipennis* adults emerging from logs heated to 60°C for 30 min but not at 65°C. Goebel et al (2010) reported adult emergence from firewood treated at chamber temperatures near 56°C in a small dry kiln. Haack and Petrice (2010) tested survival of *A. planipennis* (as well as ash bark beetle, pine bark beetle and pine weevil) in a 56°C chamber for various lengths of time, measuring temperature at the core and at 1 cm below the surface. No emergence of any species tested was observed in logs treated to a core temp of 56°C. Sobek et al. (2011) tested *A. planipennis* survival in log bolts in an operational heat treatment chamber. They reported complete mortality of all larval instars at 56°C for 30 min. Similarly all pupae died at exposures as short as 10 min at 54°C. They also considered the mechanisms of thermotolerance in EAB. Heat shock proteins were produced when larvae were slowly warmed from room to treatment temperatures; these larvae had higher thermal tolerance. They proposed that this mechanism could result in survival above laboratory tested 56°C for 30 min. However, they argued that heat treatment schedules used under operational conditions in

Canadian HT facilities far exceed the ISPM 15:2009 standard and that even extreme thermal plasticity is unlikely to allow pest insects to survive the heat treatment process. They also considered that sub-lethal impact of treatment that could result in reduced fecundity or sterility might increase the safety margin of existing heat treatments (Sobek et al. 2011 citing Scott et al. 1997, Huang et al. 2007, and Mironidis and Savopoulou-Soultani 2010).

### **IFQRG evaluation of heat treatment to manage the pest risks of *A. planipennis***

The International Forestry Quarantine Research Group reviewed the published literature on tolerance of *A. planipennis* to the heat treatment parameters prescribed in ISPM 15:2009 at IFQRG-8 (2010). The studies were conducted on firewood and wood chips presenting challenges associated with variation in wood size, moisture and the practicalities of heat chamber loading. These studies did not test the ISPM 15:2009 standard and were therefore not valid for consideration in wood packaging. The IFQRG was unaware of any interceptions of *A. planipennis* in wood packaging material in international movement of regulated wood. The EU has not reported any interception of *A. planipennis* in any wood commodity; no US interceptions of *A. planipennis* have been reported in wood packaging. IFQRG participants agreed that the phytosanitary measures applied under normal operating conditions to fulfil requirements of ISPM 15:2009 continue to be appropriate to sufficiently reduce the risk of *A. planipennis*.

#### **1.4 Pinewood nematode**

Pinewood nematode (*Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle) is widely recognized as a serious pest in pine species around the world (Webster 1999). The nematode is exclusively vectored by beetles in the genus *Monochamus* (Mamiya and Enda 1972, Wingfield and Blanchette 1983, Finney-Crawley 1989). Concerns surrounding the movement of the nematode and insect vectors in infested logs and lumber prompted efforts in the 1980's and 1990's to identify treatments that would kill the nematode or its vectors in wood products. A variety of treatments have been evaluated including fumigation, and heat treatment (Ostaff and Cech 1978, Kinn 1986, Smith 1991, Wang et al. 1994, Tomminen and Nuorteva 1992, Soma 2001, Zheng et al. 2001).

Heat treatments using kiln temperatures of 60-71°C for 1 hour were shown to kill *Monochamus* larvae in lumber (Ostaff and Cech 1978). This treatment schedule was further refined in a joint European Union/North American effort to develop a heat treatment protocol for the eradication of pinewood nematode and its vectors (Smith 1991). This study showed that treatment of wood to a core temperature of 56°C for 30 min was highly effective for treatment of pinewood nematode-infested wood. This temperature-time combination has been accepted as a phytosanitary standard for both insects and nematodes and forms the basis for the heat treatment measure in the international wood packaging standard, ISPM 15: 2009. One subsequent study (Qi Longjun et al. 2005) reported lower mortality of nematodes in wood packaging treated at 56/30 but the experimental methods used in the study were difficult to interpret and may not have reflected operational conditions. Studies using microwave and radio frequency as a heating source have also reported 100% mortality (probit-9) at 56°C (Hoover et al. 2010, Lazarescu et al. 2011, Uzunovic et al. 2012).

## 1.5 Bacteria

Little has been published on thermal treatment of wood-inhabiting bacteria. A study by Srivastava and Patel (1970) cites 49°C as the thermal inactivation temperature of *Pseudomonas azadirachtae* a bacterial disease of the Neem tree (*Azadirachta indica* A. Juss.). Keck et al. (1995) reported heat treatment of less than 30 min at 50°C lethal to *Erwinia amylovora* (Burrill) Winslow et al. on living propagation material. *Xanthomonas translucens* pv. *pistaciae* Giblot-Ducray et al. survived in infected wood exposed to 40–55°C for at least 60 min but was killed by exposure to 60°C for 15 min or more (Vu Thahn et al. 2012). Recent studies on *Pseudomonas syringae* pv. *aesculi* (causing bleeding canker of horse chestnut (*Aesculus* spp.)) reported a lethal temperature of 35-40 °C for *in vivo* cultures (Mullett and Webber 2013) and 39 °C for the bacterium on living tree saplings (de Keijzer et al. 2012).

### Treatment testing protocols

Currently, ISPM 15: 2009 recognizes three treatments, heat, dielectric heating and methyl bromide fumigation. As new treatments are developed, for wood packaging or other wood commodities, it is critical that they are shown to be effective against the wide range of pests that may be found associated with wood. The IPPC standard ISPM 28: 2007, *Phytosanitary Treatments for Regulated Pests*, describes the data requirements for submission of a phytosanitary treatment in order to be recognized internationally as a phytosanitary measure (ISPM 28: 2007). One of the most important components of this data is the “proof” that the treatment is:

“effective in killing, inactivating or removing pests, or rendering pests infertile or for devitalization associated with a regulated article.”

The standard further requires that treatments:

“be well documented to show that the efficacy data has been generated using appropriate scientific procedures, including where relevant an appropriate experimental design. The data supporting the treatment should be verifiable, reproducible, and based on statistical methods and/or on established and accepted international practice”.

While ISPM 28: 2007 does not require specific efficacy targets, experimental methods used to determine experimental doses for treatments as well as the statistical level of confidence supporting efficacy claims must be reported. Where experimental data is unavailable or insufficient, other evidence that supports the efficacy (i.e. historical and/or practical information or experience) is required.

Much of the data on heat treatment of wood is sourced from older studies that follow many different experimental approaches. In some studies ambient kiln temperatures were monitored rather than temperatures at the core of the wood. Few organisms have been studied in detail. Standardized test protocols were rarely used and data must be drawn from studies that may have tested pests on a variety of substrates including *in vitro*

experiments, tests on agar (in the case of fungi), lumber, wood chips and whole logs. Most of the experimental work was done with very small specimen sample sizes and little if any statistical confidence information was provided. The pinewood nematode work carried out in the 1990s was the first to apply a rigorous statistical approach (Smith et al. 1991). This work concluded that temperatures of 52 °C and higher killed all pinewood nematode in *in vivo* experiments. Following a statistical adjustment of the data (a probit-like analysis with a gompit transformation) to establish the temperature required to reach 99.994% mortality reliability at 95% confidence the recommended treatment temperature was determined to be 56.1°C. More recently, statistical analyses of dose-response data on fungi (Ramsfield et al. 2010) and *Agilus planipennis* (EFSA 2011) demonstrate that recommended treatment temperatures are influenced by parameters set by risk managers including desired control levels (i.e. mortality rate, often 99%, 99.9% or 99.99683), models used to analyse data and the choice of statistical confidence level (e.g. 90%, 95%, or 99%). Such statistical manipulations are useful in providing quantifiable reliability of experimental data. However, the resultant treatment doses may be unnecessarily high, possibly overlooking biological considerations that limit survival such as temperature thresholds related to the denaturing of proteins or irreversible sub-lethal effects on reproductive capacity. New approaches to designing treatment testing protocols that consider the challenges related to wood pests are being developed (Haack et al. 2011, Schortemeyer et al. 2011) and are planned as an annex to ISPM 15:2009.

### How organisms respond to heat treatment; mortality - survival physiology

There is a considerable body of knowledge, mostly on fruit and stored product pests, on the physiological responses of insects and fungi to heat (Crisan 1973, Denlinger and Yocum 1998, Neven 2000, Maheshwari et al. 2000, Fields and White 2002, Rangel et al. 2005).

**Table 1: Response of stored-product insect pests to temperature** (after Fields and White 2002)

Effect	Temperature range (°C)	Effects
Lethal	Above 62	Death in <1 min
	50–62	Death in <1 h
	45–50	Death in <1 day
	35–42	Populations die out, mobile insects seek cooler environment
Suboptimum	35	Maximum temperature for reproduction
	32–35	Slow population increase
Optimum	25–32	Maximum rate of population increase
Suboptimum	13–25	Slow population increase
Lethal	5–13	Slowly lethal
	3–5	Movement ceases
	-10 to -5	Death in weeks, or months if acclimated
	-25 to -15	Death in <1 h

Exposure to high temperature has been shown to affect the synthesis and structure of cellular macromolecules (e.g. proteins, DNA, RNA, lipids, carbohydrates) and cellular

structures (e.g. membranes, ribosomes, mitochondria). Exposure to heat may cause immediate death or result in sub-lethal damage to normal developmental and reproductive success, expressed as reduced fecundity or sterility of insects (Denlinger and Yocum 1998, Neven 2000) or fungal propagules (Lifshitz et al. 1983, Freeman and Katan 1988, Aurora et al. 1996, Assaraf et al. 2002). In cases where a wood treatment does not result in complete mortality of pathogenic fungi, colonization by saprophytic organisms following treatment has been shown to outcompete the pathogens. Uzunovic et al. (2008) noted in laboratory tests of heat treated wood, saprotrophic mold fungi (e.g., *Trichoderma*, *Zygomycetes*, *Penicillium* and *Aspergillus*) colonized the wood very quickly precluding successful isolation of blue-stain or decay fungi and that the saprophytes would be likely to kill or outcompete any surviving pathogenic fungi, preventing their spread from the treated wood under real-life situations. Similarly Munnecke et al. (1976) reported that *Armillaria mellea* (Vahl) Quel. “stressed” by sublethal doses of chemicals, heat or drying were subsequently killed by antagonistic soil micro-organisms, primarily *Trichoderma* spp.

Sometimes organisms can be “pre-conditioned” to heat treatment through the production of heat shock proteins that confer a level of thermotolerance when organisms are subjected to sub-lethal temperatures (Lindquist and Craig 1988, Sienkiewicz et al. 1997). For example Yocum and Denlinger (1992), studying the flesh fly *Sarcophaga crassipalpis* Macquart, showed that a 2 h exposure to 40°C resulted in subsequent survival to an otherwise lethal heat treatment of 90 min at 45 C. In *Agrilus planipennis*, Sobek et al. (2011) reported increased levels of the heat shock protein hsp70 and suggested a link with thermotolerance to temperatures exceeding the ISPM standard of 56°C.

Heat shock protein responses have been described in wood-decay fungi, *Serpula lacrymans* (Wulfen) P.Karst. (Sienkiewicz et al. 1997) and the pinewood nematode, *Bursaphelenchus xylophilus* (Xie et al. 2009).

### **Variability in thermotolerance among life stage**

The effect of heat on physiological processes may vary among life stages of pest organisms. Identification of “the most resistant” life stage is important in treatment testing; ISPM 28: 2007 recommends that “where several life stages may occur on the regulated article, the most resistant life stage of the pest should be used for testing a treatment”. This guideline presents a significant practical challenge in treatment development given the limited published data available regarding relative thermotolerance and the difficulties in manipulating organisms to produce specific life stages “on command”.

#### **1.6 Insects**

In the stored products literature reported differences in response to heat among eggs, young larvae, old larvae, pupae and adults of the beetle *Tribolium confusum* (Jacquelin du Val) (Boina and Subramanyam 2004, Maroof et al. 2004). Differences among life stages of wood-inhabiting organisms have also been reported, for example *Neolyctus erythrocephalus* Fab. (Snyder 1923), *Anobium punctatum* (DeGeer) (Hansen and Jensen 1996), *Tetropium fuscum* (Fabr.) (Mushrow et al. 2004), *Agrilus planipennis* (Sobek et al. 2011). Studies examining temperature effects on bark beetle development and population dynamics touch on the subject (Wermelinger and Seifert 2008).

## 1.7 Fungi and oomycetes

Fungi and oomycetes may be present in and on wood in a number of different morphological forms, for example mycelium, different spore states: basidiospores, ascospores, oospores, conidia, sporangia, zoospores. Fungal structures such as sclerotia, chlamydospores, and ascospores that sometimes form in response to physical, chemical, nutritional or biological conditions have been reported to show heat tolerance relative to other fungal cell types (Seifert et al. 2004, Dijksterhuis 2007, Suryanarayanan et al. 2011). Few studies are specific to thermotolerant structures associated with fungal pests of wood; Widmer (2011) showed that oospores of *Phytophthora kernoviae* Brasier, Beales and Kirk. survived 30°C treatment longer than sporangia and mycelium.

## 1.8 Pinewood nematode

Differences in population responses among larval stages of the pinewood nematode, *Bursaphelenchus xylophilus*, have been correlated with desiccation, availability and storage of nutrients (Ishibashi and Kondo 1977, Maehara and Futai 1996). The third larval stage (J<sub>III</sub>) is sometimes referred to as the “resting stage” (Mamiya 1984) and has the thickest cuticle of all life stages (Kondo and Ishibashi 1978) but it is not clear whether this feature is related to thermotolerance. Tomminen and Nuorteva (1992) were not able to show the J<sub>III</sub> to be more resistant to heat in comparison with other developmental stages. Nevertheless, Magnusson and Schröder (2009) considered the J<sub>III</sub> stage important to include in mortality testing for treatment development. Ensuring that all larval stages are present in wood at the time of testing addresses this concern.

## How wood is heated

### 1.9 Types of HT chambers and dry kilns

Various types of equipment are used for the heat treatment of wood. Some are designed specifically for phytosanitary purposes (e.g. heat chambers), others incorporate lethal treatment conditions as part of another industrial process (e.g. dry kilns, chemical pressure impregnation). Some equipment is specially designed to accommodate certain wood commodities, e.g. treating wood chips (Dwinell et al. 1994).

A heat treatment (HT) chamber only provides and controls heat and, in some cases, air circulation for the proper treatment of wood. HT chambers are not typically designed to dry wood but simply to heat the entire profile of the wood at a certain temperature for a period of time (e.g. 56°C/133°F for at least 30 minutes).

A lumber dry kiln consists of a chamber that provides and controls heat, humidity and air circulation necessary for the proper drying or seasoning of wood. Dry kilns are designed to dry wood to a specified moisture content with minimum drying defect following a kiln schedule, a series of temperatures and humidity applied at various stages of the drying or seasoning process. Kiln dried lumber can be considered heat treated when the schedule used includes time temperature combinations that meet specific phytosanitary requirements.

### 6.1.1 Temperature of operation

HT chambers and dry kilns are designed to operate within specified temperature ranges. The following are common classification of chambers and kilns based on maximum operating temperatures:

- Conventional-temperature – operate in the 43 to 82°C (110 to 180°F) range.
- Elevated-temperature – operate in the 43 to 99°C (110 to 211°F) range.
- High-temperature – most of the drying schedule is above 100°C (212°F), usually in the 110 to 138°C (230 to 280°F) range.

Thermocouples or temperature probes may be utilized to determine the core temperature of the wood. When thermocouples or temperature probes are used, multiple probes or thermocouples should be inserted into the most difficult to heat treat piece of wood and be located in the coolest part of the chamber. The thermocouples or temperature probes should be sealed with non-conductive material to prevent air infiltration.

Most North American heat chambers or “dry kilns” generally utilize both dry-bulb and wet-bulb temperature measurement as an alternative to measuring internal wood core temperatures. Dry-bulb temperature reflects the ambient or operating temperature of the kiln. Wet-bulb temperature measures the cooling effect of evaporation and estimates the temperature of wood as it is affected by moisture reduction. “Wet-bulb depression” is the difference between the wet- and dry-bulb measurements and is used to determine the relative humidity from a standard hygrometric chart. Initial testing of wood using probes or thermocouples inserted into the wood is standardized against the wet- and dry-bulb measurements to develop a standardized schedule that may be used for ongoing treatment applications.

### 6.1.2 Heat Source

Most heat chambers and conventional dry kilns use moist or dry air to heat the wood commodity through a combination of conduction, convection and radiant heat transfer (Tschernitz 1991). The temperature of the air is raised by electrical heaters or by burning waste wood, oil, propane or natural gas. Recently systems have been developed that utilize dielectric energy (microwave or radio frequency) to heat wood for pests found in the wood (Fleming et al. 2003, 2005, Hoover et al. 2010, Lazarescu et al. 2011, Uzunovic et al. 2012).

### 6.1.3 Heating medium

Wood that is being treated is exposed to a liquid, high humidity (steam) or dry air environment which has a significant effect on heating times; heat is transferred faster in steam saturated air. HT chambers and dry kilns use dry air or steam; liquid media might be used as part of a chemical impregnation process (Taylor and Lloyd 2009).

#### 1.10 Temperature and moisture

It is very important, when considering the treatment of wood to kill insects and other microorganisms, not to confuse kiln drying (moisture reduction) with heat treatment.

Although most kiln drying schedules include heat, moisture reduction targets can be achieved without the application of lethal temperatures. Moisture reduction by itself is not sufficient to meet phytosanitary goals. Some species of fungi can withstand air drying (Uzunovic and Khadempour 2007) and can survive up to 10 years in wood stored at 30-40% RH (Wilcox 1973). Similarly, some insects can survive long periods of time in low moisture wood. Moisture levels during treatment are also critical. Most bluestain fungi will be killed at temperatures of 40-50°C when the RH is 100% (Seifert 1993). The wood decay fungus *Lenzites trabea* (Pers.) Fr. was killed with a 3 hour treatment at 70°C in wet conditions but required 96-120 hours at the same temperature in dry conditions (Cartwright and Findlay 1958).

## 1.11 Problems-challenges with heat treatment of wood

### 6.3.1 Physical properties of wood

Wood is a cellular structure composed of cellulose, hemicellulose and lignin. Variations in the proportion of these components and difference in structure make woods heavy or light, stiff or flexible. Wood properties (i.e. specific gravity, density, moisture content, etc.) of a single wood species are relatively constant but still vary within and between trees or pieces of lumber.

Important thermal properties of wood that affect the dynamics of heating include thermal conductivity, heat capacity, and thermal diffusivity. Thermal conductivity is the measure of the rate of heat flow through one unit thickness of a material when subjected to a temperature gradient. It is affected by the wood properties such as density, specific gravity, moisture content, extractive content, grain direction, fibril angle, and temperature. Thermal conductivity increases as specific gravity, density, moisture content, and temperature of the wood increases. Heat capacity is the amount of energy needed to increase one unit of mass by unit in temperature. It is dependent on temperature and moisture content of the wood but independent of specific gravity, density or species. The heat capacity of green (wet) wood is greater than dry wood. Thermal diffusivity measures how quickly a material can absorb heat from its surroundings; it is the ratio of thermal conductivity to the product of density and heat capacity. Due to low thermal conductivity, moderate density and heat capacity of wood, thermal diffusivity of wood is lower than that of other materials (e.g. metal, brick).

The moisture content of wood is the amount of water in wood expressed as a percentage of its oven dry weight. Many properties of wood (i.e. weight, shrinkage, strength, etc.) are dependent on its moisture content. In softwoods, the moisture content of the heartwood is usually lower than the sapwood. In hardwoods, the difference in moisture content between the heartwood and sapwood is dependent on the wood species. Simpson et al. (2005) reported that the differences in heating times among hardwood species (red maple, sugar maple, red oak, basswood and aspen) was not large and was based on the natural variability between individual boards. They concluded that there is no practical reason to heat treat different hardwood species separately.

#### 1.11.2 Size and configuration

Size of the wood and its configuration affect the heat treatment process. Heating time increases with thicker and bigger wood products (Table 1). Cants and logs will obviously take longer periods to heat treat than sawn wood with smaller dimensions. Odd shapes and varying dimensions of firewood mean different heating times for individual pieces resulting in a more demanding treatment schedule that accommodates the worst-case scenario (Wang et al. 2009, 2010).

**Table 1: Heating time curves of boards with thicknesses ranging from 0.75 to 12 in (from Wang 2010)**

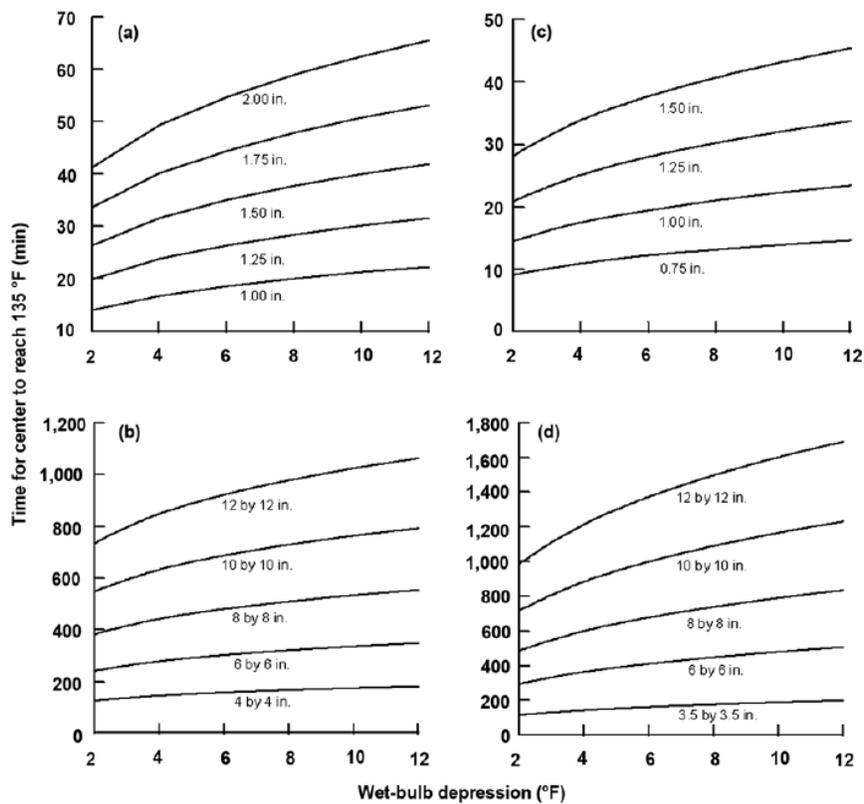


Figure 20-1. Dependence of heating time on wet-bulb depression for (a) 1- to 2-in.-thick ponderosa pine boards; (b) 4- to 12-in. ponderosa pine timbers; (c) 3/4- to 1-1/2-in.-thick Douglas-fir boards; and (d) 3-1/2- by 3-1/2-in. Douglas-fir timbers (initial temperature: 60 °F). ( $^{\circ}\text{C} = (^{\circ}\text{F} - 32)/1.8$ ; 1 in. = 25.4 mm)

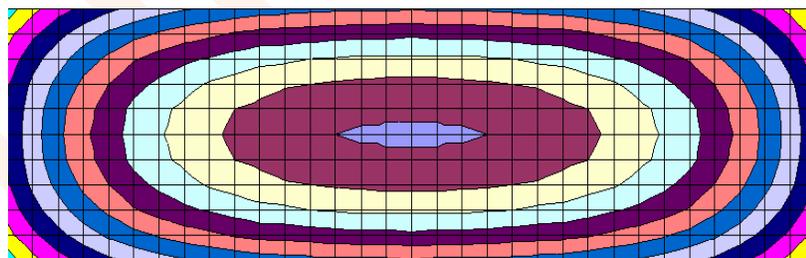
## 1.12 Thermal penetration models – temperature gradients

Currently most heat treatment is achieved through the use of existing moisture reduction wood kilns (where controlled application of heat is a part of the drying process) or chambers specifically designed for heat treatment. There are a number of considerations that need to be addressed in order to achieve a core temperature of 56°C for every piece of wood in a large load; e.g. wood species, variability in wood density, moisture and piece size, initial temperature, and evenness of heat distribution in the chamber. In order to compensate for these variables, temperature monitoring probes are placed strategically in sentinel pieces of wood or temperature time schedules are developed. In either case, ambient chamber temperatures are set higher than 56°C, often 70-90°C. Since the heat treatment process requires many hours for all wood pieces to reach 56°C, much of the wood, in particular the outer “skin” and corners of each wood piece is heated to temperatures higher than 56°C for times far in excess of 30 min. For organisms like *Agrilus planipennis* therefore,

“Heating the wood core to 56°C will result in a mass and size-dependent temperature gradient across the logs, and species dwelling in the outermost layers, such as EAB, will be exposed to considerably higher temperatures for longer periods of time than species dwelling in the core. After termination of the treatment, thermal inertia means the wood will remain at higher temperatures for some time, which gives reassurance that the current standard (as implemented in the facility we investigated) is sufficient in exterminating EAB.” (Sobek et al. 2011).

This logic is in line with a modelling analysis conducted by Forintek and CFS in 2007 that demonstrated that ash wood treated under hardwood treatment schedules in the CFIA manual PI-07 received exposure to temperatures in excess of 60°C for several hours (Figure 1).

Figure 1: Temperature profile at end of heat treatment through cross-section of 51mm thick by 152mm wide ash wood of 56°C (time to reach 56°C /30 = 314 minutes)



■ 55-56 ■ 56-57 □ 57-58 □ 58-59 ■ 59-60 ■ 60-61 ■ 61-62 □ 62-63 ■ 63-64 ■ 64-65 ■ 65-66 □ 66-67 |

The generic schedule approach adopted by PI-07 contains sufficient safeguarding measures to ensure that all wood products treated according to the schedule will meet the phytosanitary standard, a minimum wood core temperature of 56°C for a minimum of 30 minutes. It provides a minimum standard to achieve the treatment target. Where moisture reduction is also a goal, wood is subjected to heat for much longer periods of time. For

example, in a typical charge of 5/4 (32 mm thick) ash sawn wood dried to 7% MC at a Canadian hardwood mill, the wood were exposed to ambient temperatures exceeding 90°C for 232 hours (more than 9 days). The wet-bulb temperature during the same time period exceeded 73°C. In this example the phytosanitary requirement was met within the first 11 hours.

### **Heat treatment as a component of an integrated measures approach**

Various mechanical processes that are used in the manufacture of wood products from trees including harvesting practices, wood storage, milling and post-milling processes result in the reduction of associated pests (FAO 2011). These processes transform the structure and physical properties of the wood, generally reducing the quality of the substrate for the successful survival of pest organisms that may have inhabited the living tree. Each of these steps reduces pest prevalence in the wood and can be considered independent phytosanitary measures. In keeping with international principles of integrated measures, the cumulative effect of these processes results in greater pest risk reduction than by a single measure. In this context, heat treatment is a part of a greater risk reduction exercise, not the sole opportunity for pest mitigation.

The effectiveness of these risk reduction processes is based on the understanding of the biology of the pest, how and where it lives in the bark and woody tissues of the host tree. For example, life stages of *Agrilus planipennis*: eggs, larvae, prepupae, pupae and adults live in the bark or in the cambial or sapwood tissue just underlying the bark. In merchantable log sizes the beetle life stages rarely, if ever penetrate the sapwood and are effectively removed during debarking. This single production process removes most pest risk. The next major production process, where logs are sawn into boards removes a significant portion of the outer sapwood where prepupal chambers are formed in smaller diameter logs. Finally, heat treatment of sawn wood that ensures that the wood has reached a core temperature of 56°C for 30 minutes kills virtually all *A. planipennis* life stages that may still be present. In combination these independent phytosanitary measures reduce pest risk more effectively than implementation of any one measure alone (ISPM 14: 2002)

Set in the context of risk reduction through multiple integrated measures, it may therefore not be necessary for a single component measure, heat treatment for example, to result in near-100% mortality. Haack et al. (2011) indicated that biological factors also come into play suggesting that

“the focus on mortality as the sole criterion for evaluating quarantine security disregards risk-based factors along the pathway, such as the likelihood of infestation, natural survival, reproductive potential and establishment potential, as well as processing parameters such as packaging and shipping practices and distribution times”.

Changing the expectation that a treatment need to provide very high levels of mortality on their own should allow greater flexibility in developing effective phytosanitary risk reduction systems. Recognizing the quantifiable risk reduction value of a treatment in conjunction with other quantified measures will permit the design of such systems that meet specified phytosanitary risk reduction targets.

DRAFT

## Appendix 1: Examples of heat treatment import requirements from various countries

### NZ Ministry for Primary Industries

<http://www.biosecurity.govt.nz/imports/forests/standards/non-viable-forest-produce/sawn-wood.htm> (accessed June 2013)

#### Treatment options

**Sawn wood:** Heat treatment (or kiln drying) at a minimum continuous core temperature of 70°C for more than 4 hours

**Poles, piles, rounds, and sleepers:** Heat treatment for more than 4 hours at a minimum continuous core temperature of 70°C or kiln dried to less than 20% moisture content at temperatures exceeding 56°C.

### Australia Department of Agriculture Fisheries and Forestry (DAFF) 2013

<http://www.daff.gov.au/aqis/import/timber/approved-treatments-timber/heat-treatments> (accessed June 2013)

Wood packaging/dunnage that is free of bark and has undergone a DAFF approved heat treatment within 21 days of export, is considered to be effectively treated for quarantine pests exotic to Australia, except where DAFF has identified a specific quarantine concern.

The DAFF approved wood heat treatments are:

1. Kiln drying for quarantine purposes (T9912)
2. Heat treatment: 56°C for 30 minutes (T9968)
3. Heat treatment of wood packaging and dunnage in accordance with ISPM 15:2009

Whilst the time-temperature schedule for the T9968 heat treatment is the same as the time-temperature schedule for the heat treatment specified in ISPM 15:2009, DAFF documentary requirements for validating each of these heat treatments are different.

Please see the Minimum Documentary Requirements Policy for further details.

The DAFF Heat Treatment Standard (Australia DAFF 2013) is a general methodology for performing dry heat treatment to meet Australian quarantine requirements.

## Appendix 2: Examples of wood-inhabiting thermophilic fungi

Fungus	Fungal group	Lethal temperature	Reference
<i>Aspergillus fumigatus</i> Fresen.	anomorph ascomycete	of >82 °C	Tansey 1971
<i>Chaetomium thermophile</i> La Touche var. <i>coprophile</i> Cooney & R. Emers.	ascomycete	>60 °C	Tansey 1971
<i>Dactylomyces thermophilus</i> Sopp	ascomycete	>52 °C	Guilmo et al. 1998
<i>Humicola lanuginosa</i> (Griffon & Maubl.) Bunce	ascomycete	>83 °C	Tansey 1971
<i>Penicillium bacillisporum</i> Swift	anomorph ascomycete	of >52 °C	Guilmo et al. 1998
<i>Rhizomucor</i> sp	zygomycete	>52 °C	Guilmo et al. 1998
<i>Sporotrichum thermophile</i> Apinis	anomorph basidiomycete	of >55 °C	Semeniuk and Carmichael 1966
<i>Thermoascus aurantiacus</i> Miehe	anomorph ascomycete	of >82 °C	Tansey 1971

### Appendix 3: Basidiomycete fungi that produce chlamydospores (from Stalpers 1978)

- Abortiporus biennis* (Bull. ex Fr.) Sing.  
*Amylocystis lapponica* (Romell) Bond. & Sing.  
*Anomoporia bombycina* (Fr.) Pouzar  
*Antrodia carbonica* Overh.  
*Antrodia malicola* (Berk. & Curt.) Murr.  
*Antrodia oleracea* Davidson & Lombard  
*Antrodia serialis* (Fr.) Murr.  
*Antrodia sinuosa* (Fr.) Sarkar  
*Antrodia vaillantii* (DC. ex Fr.) Cooke  
*Antrodia xantha* (Fr. ex Fr.) Cooke  
*Bjerkandera adusta* (Willd. ex Fr.) P. Karst.  
*Bjerkandera fumosa* (Pers. ex Fr.) P. Karst.  
*Bondarzewia berkeleyi* (Fr.) Bond. & Sing.  
*Bondarzewia montana* (Quel.) Sing.  
*Ceraceomyces borealis* (Romell) J. Erikss. & Ryv.  
*Ceriporia alachuana* Murr.  
*Ceriporiopsis rivulosa* (Berk. & Curt.) Cooke  
*Climacocystis borealis* (Fr.) Imaz.  
*Climacodon septentrionalis* (Fr.) P. Karst.  
*Veluticeps fimbriata* (Pers. ex Fr.) Pouzar  
*Daedalea quercina* (L.) ex Fr.  
*Dichomitus squalens* (P. Karst.) D. Reid  
*Dichostereum effuscatum* (Cooke & Ellis) D.P. Rogers & H.S. Jacks.  
*Dichostereum pallescens* (Schw.) D.P. Rogers & H.S. Jacks.  
*Diplomitoporus lindbladii* (Berk. & Br. ex Berk.) Cooke  
*Echinodontium tinctorium* Ellis & Everh  
*Fistulina hepatica* (Schaeff.) ex Fr.  
*Fomitopsis cajanderi* (P. Karst.) Kotl. & Pouzar  
*Fomitopsis meliae* Underw.  
*Fomitopsis officinalis* (Vill. ex Fr.) Donk  
*Fomitopsis palustris* Berk. & Curt.  
*Fomitopsis pinicola* (Schw. ex Fr.) P. Karst  
*Fomitopsis spraguei* Berk. & Curt.  
*Ganoderma colossum* (Fr.) C.F. Baker  
*Ganoderma lucidum* Boud. apud Pat.  
*Gloeocystidiellum porosum* (Berk. & Curt.) Donk  
*Gloeophyllum abietinum* (Bull. ex Fr.) P. Karst.  
*Gloeophyllum odoratum* (Wulf. ex Fr.) Imaz  
*Gloeophyllum protractum* (Fr.) Imaz  
*Gloeophyllum sepiarium* (Wulf. ex Fr.) P. Karst.  
*Gloeophyllum striatum* (Sw. ex Fr.) Murr.  
*Gloeophyllum trabeum* (Pers. ex Fr.) Murr.  
*Grifola frondosa* (Dicks. ex Fr.) S. F. Gray  
*Hapalopilus croceus* (Pers. ex Fr.) Donk  
*Hapalopilus mutans* Peck  
*Hericium coralloides* (Scop. ex Fr.) S.F. Gray  
*Hericium erinaceus* (Bull. ex Fr.) Pers.  
*Hymenochaete rubiginosa* (Dicks. ex Fr.) Lev.  
*Hyphodermella corrugata* (Fr.) Bres.  
*Hypochnicium vellereum* (Ell. & Cragin) Parm.  
*Hypochnicium vellereum* (Ell. & Cragin) Parm.  
*Inonotus rickii* (Pat.) D. Reid  
*Laetiporus sulphureus* (Bull. ex Fr.) Bond. & Sing.  
*Laxitextum bicolor* (Fr.) Lentz  
*Megalocystidium lactescens* (Berk.) Boidin  
*Melanoporia nigra* (Berk.) Cooke  
*Microporellus obovatus* Berk. & Curt.  
*Mycoacia fuscoatra* (Fr.) Donk  
*Osteina obducta* (Berk.) Donk  
*Perenniporia compacta* Overh.  
*Perenniporia fraxinophila* (Peck) Ryv.  
*Perenniporia robinophila* (Murr.) Lloyd  
*Phaeolus schweinitzii* (Fr.) Pat.  
*Phanerochaete chrysosporium* Burds. & Eslyn  
*Phanerochaete sordida* (P. Karst.) Burt  
*Phlebia merismoides* Fr.  
*Phlebia subserialis* H. S. Jacks. & Dearden

*Phlebia subserialis* (Bourd. & Galz.) Donk  
*Phlebia tremellosus* Schrad. ex Fr.  
*Phlebia chrysocreas* (Berk. & Curt. apud Berk.) Burdsall  
*Piptoporus betulinus* (Bull. ex Fr.) P. Karst  
*Polyporus brumalis* (Pers. ex Fr.) Fr.  
*Polyporus mori* (Bosc.) ex Fr.  
*Poria aurea* Peck  
*Postia amara* (Hedgec.) Lowe  
*Postia balsamea* (Peck) Murrill  
*Postia placenta* (Fr.) Cooke  
*Postia salmonicolor* (Berk. & Curt.) Pouzar  
*Postia sericeomollis* (Rom.) Bond. & Sing.  
*Postia tephroleuca* (Fr.) Donk  
*Punctularia atropurpurascens* (Berk. & Br.) Petch  
*Pycnoporus cinnabarinus* (Jacq. ex Fr.) P. Karst  
*Pycnoporus sanguineus* (L. ex Fr.) Murr.  
*Radulodon casearium* Ryv.  
*Schizophyllum commune* Fr.  
*Sparassis crispa* (Wulf. ex Fr.) Fr.  
*Spongipellis delectans* (Peck) Murr.  
*Spongipellis pachyodon* (Pers.) Kotl. & Pouzar  
*Spongipellis unicolor* (Schw.) Murr.  
*Sporotrichum pruinosum* Novobranova  
*Trametes cubensis* (Mont.) Sacc.  
*Trametes pubescens* (Schum. ex Fr.) Pilat  
*Trametes suaveolens* (Fr.) Fr.  
*Trametes versicolor* (L. ex Fr.) Pilat  
*Tryomyces chioneus* (Fr. ex Fr.) P. Karst  
*Tryomyces fissilis* (Berk. & Curt.) Donk  
*Tryomyces fumidiceps* Atk.  
*Vararia granulosa* (Fr.) Laurila  
*Veluticeps berkeleyi* (Berk. & Curt.) Cooke

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