

Literature Review - Temperature Mortality Thresholds for Insects

by

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Client Report No. 12261

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Ltd**

Date: June 2007
Client: Hosking Forestry Ltd
Contract No:

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BACKGROUND

There is widespread acceptance by the forestry sector that the continued use of methyl bromide fumigation for both quarantine and market access treatment of forest produce is unsustainable. The recent formation of, and support for, Stakeholders in Methyl Bromide Reduction (STIMBR) is testament to the level of this concern and the urgency that is being accorded the search for alternatives. A research strategy developed by STIMBR identifies three broad themes; ecological risk assessment to reduce the need for fumigation, alternative fumigants which do not have the undesirable characteristics of methyl bromide, and alternatives to fumigants, and it is this last theme to which this review contributes.

HEAT AS A QUARANTINE TREATMENT

Heat has long been recognised as an effective and ecologically acceptable treatment of forest produce for quarantine and market access purposes. Kiln dried sawn timber is generally accepted by trading partners as requiring no further treatment. However, determining minimum temperature/exposure time for 100% mortality of specific organisms is essential to determining minimum effective treatment, and hence lowest treatment cost. One of the most significant recent papers on thermal quarantine methods is that of Tang et al (2000), which considers both the theoretical and practical application of heat treatments. The author's propose the development of thermal kinetic models to determine the optimal temperature-time combination to reach the intrinsic thermal mortality of target insects, a concept particularly relevant to the treatment of perishable commodities such as fruit, where unnecessarily high temperatures may cause product damage.

The determination of thermal mortality threshold for as many organisms as possible, may lead to a general rule for temperatures required to treat risks from a wide range of pests and diseases.

Of particular interest is data relating to high risk quarantine pests and diseases, for example Lymantriid moths which are known to lay their eggs on inanimate objects such as containers, vehicles etc. There is however, a lingering concern that there may be insects, which could be transported on New Zealand forest produce which might have a high temperature tolerance, and therefore might survive any proposed treatment. This literature review examines both published and unpublished literature on thermal mortality of insects to determine if this concern is justified.

Past Application of Heat

Heat treatment for insect pests is not new (Fields and White 2002). It has been used extensively for fruit disinfestation, using both forced air and hot water (US EDPA 1996, Hansen 1992). Vapour heat has also been demonstrated as effective for disinfestation of cut flowers and foliage (Hansen *et al.* 1992). These treatments have been primarily aimed at ensuring freedom from fruit flies (Tephritidae). As already noted heat is also used indirectly for quarantine in the case of kiln dried timber which has been exposed to high temperature for quite long periods, up to 48 hours. Heat treatment has also been used against stored products pests in such commodities as grain. The use of radio frequency heating, both with and without additional heating (Janhang *et al* 2005, Wang *et al* 2001, Nelson 1996), has been evaluated for the treatment of insects in rice and nuts. Tang *et al* (2000) discusses the major advantage of radio frequency heating over conventional hot air for walnuts, where air spaces in the shell act to insulate the target kernel, requiring 40 minutes to reach 53°C with hot air but only 3 minutes using radio frequency heating. All of these applications have contributed to our knowledge of the temperature mortality threshold of pests associated with these commodities.

Microbial Growth and Temperature

There are generally accepted rules of thermal mortality for different groups of micro-organisms. Brock (1969) groups them as follows:

Animals including protozoa	45-51°C
Encoryotic micro-organisms	56-60°C
Thermophilic photosynthetic bacteria	60°C
Blue-green algae	73-75°C

He also notes that bacteria which actively grow at over 50°C are uncommon. It should be noted that thermophilic micro-organisms are highly specialize, are usually confined to aquatic environments and generally do not grow at temperatures found in less specialized environments Thermal mortality thresholds for fungi appear quite variable with Ridley and Crabtree (2001) finding a range between 50°C and 70°C for seven fungi, including plant pathogens and wood decay fungi, for a 10 minute exposure time. The most resistant, *Schizophyllum commune* a composting fungi, would be expected to tolerate high temperatures.

However, heat treatment of 71°C (core temperature) for 60 minutes is accepted by APHIS against fungi and nematodes in imported logs (USEPA 2002), supported by the work of Jones (1973) on oak wilt fungus and Dwinell (1990) on pinewood nematodes.

Insects and Thermal Tolerance

While insects do live in extreme environments such as hot deserts, where boundary layer temperatures may exceed 60°C, they do so through behavioural adaptation. That is they avoid these extreme temperatures rather than endure them physiologically (Cabrera and Rust 2000). For example, desert carabids avoid the extreme boundary layer by remaining beneath the sand surface during the day or by seeking sites out of direct solar radiation. Other beetles practise 'stilting' or walking on tiptoe which lifts their body out of the very hot zone, only a millimetre or two thick, close to the sand surface. A study of desert *Drosophila* species (Stratman and Morkow 1998) did show greater tolerance of high temperature by some species, but it was not consistent across all species and even the most tolerant conformed to the general rule of being within the 45°C to 51°C described above. Even insects

living in the warm aquatic environments, such as the hot pool mosquito *Culex rotoruae*, have a thermal mortality threshold below 50°C (Crabtree *pers com.*). Exceptions to the general rule that this threshold is between 45°C and 51°C for insects (Brock 1969), if they occur, are associated with highly specialised environments and are very uncommon, and could not be construed as posing a serious risk of contamination of forest produce. Fields (1992) reported the following generalised response of stored-product insect pests to elevated temperature:

above 62°C	death in < 1 minute
50 - 62°C	death in < 1 hour
45 - 50°C	death in < 1 day
35 - 42°C	population decline, movement to cooler environment

Temperature effects may vary with individual tissues within an insect such that mortality threshold is determined by effects on critical tissues such that the insect's mortality threshold is determined by the most sensitive individual tissue (Denlinger and Yocum 1998).

Environmental moisture can also affect insect survival at high temperatures. It is suggested that under low air moisture conditions evaporative cooling can lower the target organism's internal temperature giving greater survival rates than in moist air (Gunn and Natley 1936, Beckett 2002). Moist heat is also believed to favour proteins denaturing, while dry heat relies primarily on an oxidation process (Dwinell 1996).

Induced Heat Tolerance

Exposure to elevated temperatures has been shown to induce a greater tolerance to subsequent higher temperatures in some insects (Thomas and Shellie 2000, Dahlgard *et al.* 1998). This phenomenon is of particular interest in the treatment being proposed for forest produce where any surviving insects might show such characteristics. This induced ability to withstand elevated temperatures is known as the heat shock response (Garcia *et al*

2001, Rinehart et al 2006). Insect death by heat shock results from protein denaturing and associated effects on enzymes (Fields 1992) an effect which probably accounts for the relative consistency of the thermal mortality threshold. The heat shock response involves the increased synthesis of heat shock proteins, proteins which protect and repair proteins after heat shock (Petersen and Mitchell 1985, Georgopoulos and Welch 1993). However, the production of such proteins ceases at temperatures above 40°C for *Drosophila melanogaster* and it is unlikely any insects produce them above 50°C (Dahlggaard et al 1998, Lindquist 1986). The production of heat shock proteins also requires slow ramp conditions (Thomas and Shellie 2000) quite different from the rapid heat-up proposed for pre-shipment and quarantine treatment. There is therefore no evidence to suggest induced thermo-tolerance will have any relevance to the use of heat in the present context.

General Theory and Insect Growth and Temperature

A general theory of the relationship between insect development and temperature has been proposed by Gilbert and Raworth (1996) which also identifies the upper limits of growth and survival. They show an almost linear relationship between growth rate and temperature up to about 28°C followed by a very sharp decline. Although some insects show this linearity extending up to 32°C, the theory supports the view that insects, because of their physiology, are animals of environments of 20°C to 30°C, seldom extending into the 40s. These characteristics of insect development and survival have allowed APHIS to approve heat treatment of a wide range of commodities based on internal temperatures for a defined exposure time (Hansen 1992) eg. logs and timber core temperature of 71°C for 60 minutes (USDA 1996). Similarly the recent international standard for the treatment of wood packaging (core temperature of 56°C for 30 minutes) is based on this broadly accepted data and has somewhat refined the log specification (IPPC, 2006).

HEAT TREATMENT OF FOREST PRODUCE

A series of risk assessments undertaken by USDA APHIS for the importation of logs from Siberia (USDA 1991), New Zealand (USDA 1992) and Chile (USDA 1993) led to the development of mitigation measures by APHIS that would ensure negligible risk of insects and fungi entering the USA through these commodities. Heat treatment was considered a viable method of control, and specifications for steam and dry heat for both logs and lumber have been accepted (USEPA 2002), with heat treatment being considered more effective than methyl bromide fumigation (Morrell 1995). The major practical constraint for logs is the requirement of a core temperature of 71°C. Typically pressurised steam is introduced into a treatment chamber and thermocouples used to monitor core temperature. For lumber kiln drying is the most commonly used heat treatment. As discussed later in this review moist heat is more effective in killing insects, and it also provides more rapid heat penetration of logs and lumber. A comparison of costs between methyl bromide and heat treatment for lumber (USEPA 2002) shows that while treatment cost of methyl bromide is lower, the added value of kiln dried timber far outweighs the additional treatment cost.

Heat treatment of wood chips is effective against pinewood nematode which has a thermal death point of 46°C (Dwinell 1990). However, although internal temperatures of chip piles reach 60°C through spontaneous heating, outer layers remain close to ambient and it is not considered economically feasible to actively heat wood chips as a quarantine treatment (Dwinell 1996). Similarly while heat treatment of logs is effective, the economics of treatment solely for quarantine purposes are not viable.

THERMAL MORTALITY BY INSECT GROUP

Fruit fly (Diptera: Tephritidae). By far the insect group which has received the most attention in the use of heat for quarantine treatment, is the fruit flies. Research has focused on both the insect (Gazit et al 2004, Hansen and Sharp

2000) and the commodity (Shellie and Mangan 1998, Birla et al 2004), while quarantine organizations and government agencies, such as US Environmental Protection Agency (1996), have produced comprehensive reviews integrating both. All these studies show the lethal temperature to be below 50°C and treatment specifications focus on a core temperature of between 44°C and 46°C, irrespective of the type of heat treatment used. Thermo tolerance has been shown for fruit fly larvae with mortality time being extended from 41 minutes to 61 minutes with a slow ramp up time (120 mins) compared with a fast ramp time (15 mins) at 44°C (Thomas and Shellie, 2000). Jong et al (1999) showed a rapid decrease in exposure time required to achieve 100% mortality (60 min. to 15 min.) with a temperature increase from 43°C to 47°C for eggs and larvae of *Bactrocera latifrons*.

Lepidoptera. Lepidoptera have received attention because of their contamination and infestation of fruit and also because some representatives are stored products pests. Whiting *et al.* (1995) examined the mortality of six tortricid species in both controlled atmospheres and in air. They showed that no species, or their life stages, could survive exposure to an air temperature of 40°C. While 5 hours exposure gave on LT₉₉ for 5 species, *Epiphyas postvittana* required 20 hours for 5th instar larvae. Diapausing codling moth larvae were shown to require significantly higher temperatures (LT₉₅ = 16 hours at 45°C) but that low RH almost doubled the effectiveness (Soderstrom *et al.* 1996). Similar temperature requirements were found by Neven *et al.* (1996) for core temperatures of apples and pears against codling moth (*Cydia pomonella*). They compared the efficacy of vapour forced air and water bath and found 44°C for 120 minutes effective in both cases. Tang et al (2000) presents a thermal kinetic model for 5th instar codling moth larvae based on a thermal-death-time curve for a 20°C/min. heating rate which shows 100% mortality after 11 minutes at 48°C, 5 minutes at 50°C and 1 minute at 53°C. The model is supported by the findings of Yin et al (2006) that despite thermal conditioning 5th instar codling moth larvae, the most heat resistant stage, suffered 100% mortality after only 3 minutes exposure to 52°C Eggs of the Indian meal moth (*Plodia interpunctella*), a stored products pest, were found not to survive 48°C for 34 minutes (Lewthwaite *et al.* 1998). (Yin et al 2006).

Of particular interest to the forestry sector are the findings of Hosking (2001) that diapausing eggs of gypsy moth (Lymantriidae) suffered 100% mortality after 5 minutes exposure at 55°C. The thermal death point is likely to be lower than this the shortest exposure period and lowest temperature tested.

Unpublished data by Paszek and Tardif (1985) suggest the mortality threshold to be closer to 50°C.

Preliminary exposure trials of 2nd instar gum emperor moth (*Opodiphthera eucalypti*) larvae (Ridley and Crabtree 2001) found 100% survival at 40°C for 10 minutes but complete mortality at 50°C for 10 minutes.

The thermal mortality thresholds in these studies are consistent with Brock's (1969) general theory for all invertebrates falling well within the 45°C to 51°C envelope, given a suitable exposure period.

Longhorn Beetles (Cerambycidae). Like termites, many longhorn beetles are wood borers and hence of particular interest to forestry quarantine. These groups of insects are likely to be found both in and on export and import forest produce. Dentener *et al.* (1999) found that although huhu (*Prionoplus reticularis*) larvae took approximately 10 days to die at an air temperature of 35°C, this was reduced to 3 hours at 45°C. While the LT₉₉ for well developed larvae was as above, it was only one hour for neonate larvae. Trials with logs (Dentener *et al.* 2001) showed that a 45°C core temperature reached by a 3.5 hour ramp and held for 2.5 hours was 100% effective against huhu larvae up to 10cm deep in the wood. Dwinell (2001) reported that reaching a core temperature of 50°C would kill the cerambycid vector of pinewood nematode, *Monochamus* spp. in pine logs, while eggs and pupae in sawn timber required only 40°C. Once again the general envelope of 45°C to 51°C is supported by research data.

Termites (Isoptera). Heat treatment has long been used against drywood termite infestations in structural timber (Horner and Bowe 1934, Ehrhorn 1934). Woodrow and Grace (1998a) found *Cryptotermes brevis* was controlled by in-wood exposure to 49°C for 30 minutes (Woodrow and Grace

1998b). For operational applications a treatment temperature of 55°C for 60 minutes is recommended (Ebeling 1997).

Hymenopteron parasitoids. Studies by Maisonnoute *et al.* (1999) on the influence of heat shock on the parasitoid *Trichogramma brassicae* showed that a heat shock of 44°C on the pupal stage increased mortality, decreased fecundity and induced male sterility. These findings suggest such temperatures are approaching the lethal temperature for the species. In a study of the effect of temperature on the development of the lepidopteran parasitoid *Cirrospilus* sp. Urbaneja *et al.* (1999) found that development ceased above 35°C. Yet another very different insect group that conforms to the general theory of temperature mortality thresholds.

Other Hymenoptera. Desert dwelling ants are known to be amongst the most tolerant of all insects to high temperatures with critical thermal maximums exceeding 55°C (Gehring and Wehner 1995). However, such tolerance is based on both physiological and behavioural characteristics and can only be sustained for very short periods of time (10 – 30 seconds). Continuous exposure to temperatures over 51°C was lethal to desert ants (March 1985).

Other Beetles (Curculionidae, Bostrychidae, Laemophloeidae). The stored grain pests *Rhyzopertha dominica* (Bostrychidae) and *Sitophilus oryzae* (Curculionidae) are shown to have mortality thresholds of 42°C to 48°C and 45°C to 53°C respectively (Beckett *et al.* 1998, Evans 1986), while Jian *et al.* (2002) showed adult *Cryptolestes ferrugineus* (Laemophloeidae) were unable to move away from a heat source in wheat at 50°C, with 100% mortality after 3 hours. These results are consistent with insect groups in general.

Other Flies (Diptera). A study by Stevens (1998) of the chironomid *Chironomus tepperi* with a focus on temperature and rate of development found failure of adult emergence at 37.5°C. This development threshold is similar to that described by Urbaneja *et al.* (1999) and suggests the thermal

mortality for Chironomidae is unlikely to differ significantly from the general theory.

Mites (Acari). The lethal temperature for *Dermanyssus gallinae* was found to be 45°C by Nordenfors *et al.* (1999). At lower temperatures, but still at the upper and lower limits, high RH was found to enhance survival, contrary to that found for other insect groups where moisture lowered the lethal temperature.

Aphids and Mealybugs (Homoptera). Gould and McGuire (2000) found hot water immersion at 49°C for 20 minutes was lethal to the mealybugs *Phalococcus citri* and *Pseudococcus odermatti* on limes. They also noted that the treatment also killed all other arthropods found externally on limes, in a study involving 7200 fruits. Kersting *et al.* (1999) found that continuous exposure to 35°C was lethal for the aphid *Aphis gossypii*. It is reasonable to assume these groups will conform to the 45°C to 51°C mortality threshold.

CONCLUSIONS

For the application of heat to be a viable disinfestation technique for forest produce it is necessary to demonstrate its efficacy against the risk organisms being targeted, at temperatures which are well below those that might damage the produce. The current practice of fumigation with methyl bromide is primarily against insects, which have been the focus of this review.

Product temperature resilience. Forest produce, including logs, sawn timber, and paper pulp, is relatively robust and is unlikely to suffer any damage from short term exposure to temperatures below 100°C. Longer term exposure (hours rather than minutes) may need further evaluation for temperatures over 80°C.

Efficacy – fungi. As briefly discussed fungi show greater variability in temperature mortality thresholds than insects. Limited data on New Zealand

fungi suggests 10 minutes direct exposure at 60°C would be effective against perhaps 80% of species but temperatures would need to exceed 70°C to achieve 100%. It is possible resting stages such as spores might be even more resistant. However, current practice using methyl bromide is known to have poor efficacy against fungi.

Efficacy – insects. Two separate areas need to be considered in the application of heat against insect contamination of forest produce, those involving insects within the product eg. huhu larvae in logs or sawn timber, *Hylastes* life stages beneath the bark of logs, and those that are surface contaminants.

Heat treatment against insects within produce relies on the interior temperature in the vicinity of the insect exceeding the lethal level. In practical terms this means longer treatment time depending on the rate of heat penetration. The international standard for wood packaging requires a core temperature of 56°C for 30 minutes, a similar specification for a softwood log could require several hours ramp time before this temperature could be attained. However, heat treatment of logs is unlikely to be viable from either an operational point of view or economically. Kiln drying of sawn timber is already accepted as an effective disinfestation treatment as well as adding value to the product, and is likely to remain the most effective method of meeting market access biosecurity requirements. The international standard for wood packaging covers most other products such as pallets, and treatment time will depend on the dimensions of the timber involved.

It is the area of surface insect contamination of forest produce that heat offers greatest promise, in particular against the burnt pine longhorn beetle *Arhopalus ferus*. Surface insects are to a large extent directly exposed to heated air and so laboratory determinations of mortality thresholds can be directly applied. The present review shows the mortality threshold for insects directly exposed to heat to be remarkably consistent, with even those showing the greatest tolerance only extending the threshold by a few degrees. The lack of resistant stages, such as seen in fungi, and the physiological changes

to insect protein which leads to death, account for this consistency. Few if any insects can survive even short term exposure to temperatures above 50°C. In very hot environments such as deserts insects survive higher temperatures through behavioural response rather than physiological tolerance. All available data supports a general theory of an upper thermal mortality threshold of between 45°C and 51°C for insects, and is likely to include the wider terrestrial arthropods such as spiders, mites, etc.

The published literature strongly supports direct exposure to 55°C for 10 minutes as a minimum heat treatment specification for forest produce. However, this heat exposure must be delivered to the site of the insect, and actual treatment time will depend on packaging, heat permeability within timber packets, etc.

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