ERADICATION OF INSECT PESTS OF SUBTROPICAL AND TROPICAL FOREST PRODUCTS WITH FREEZING STORAGE

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ZAHID MI, GRGURINOVIC CA & ZAMAN T. 2013. Eradication of insect pests of subtropical and tropical forest products with freezing storage. Eradication of insects inside imported timber or forest products remains a challenge for quarantine/plant protection authorities in preventing incursion of exotic pests to a country. Quarantine treatment options such as fumigation are often not effective owing to the presence of impervious layers/finishes (paints). Seventy naturally-infested *Acacia parramattensis* and *Acacia decurrens* timber branches collected during summer from tropical/subtropical Australian region and termite-infested timber and timber blocks inoculated with Bostrichidae beetles were subjected to freezing between -18 and -25 °C for 1 to 14 days. Samples were removed from the freezer at the end of each time interval between 24–360 hours. Removed samples were destructively sampled to determine presence of live/dead insects inside each sample. The insects recovered from the samples belonged to the families Cerambycidae, Bostrichidae and Scolytidae. None of the insects/larvae were found alive after receiving the initial freezing treatment of 24 hours. No adults emerged from the samples that were preserved at room temperature for a further 90 days. All insects in control samples remained alive throughout the experiment. This study has shown that freezing can be used as a quarantine treatment to control or eradicate timber pests present inside imported timber or forest products of tropical and subtropical origin.

Keywords: Timber, wood, cold storage, quarantine, treatment

ZAHID MI, GRGURINOVIC CA & ZAMAN T. 2013. Pembasmian serangga perosak dalam keluaran hutan subtropika dan hutan tropika menggunakan kaedah simpanan sejuk beku. Pembasmian serangga dalam kayu atau keluaran hutan yang diimport merupakan cabaran untuk pihak berkuasa kuarantin atau perlindungan pokok. Pilihan rawatan kuarantin seperti fumigasi selalunya tidak berkesan kerana kehadiran lapisan/kemasan kedap (cat). Sebanyak 70 dahan *Acacia parramattensis* dan *Acacia decurrens* yang secara semula jadi dipenuhi perosak dan dikutip semasa musim panas dari daerah Australia beriklim tropika atau subtropika serta kayu dan blok kayu yang diinokulasi dengan kumbang Bostrichidae didedahkan kepada suhu sejuk beku antara -18 °C hingga -25 °C selama 1 hari hingga 14 hari. Sampel dialih dari penyejuk beku pada akhir setiap sela masa yang ditetapkan iaitu dari 24 jam hingga 360 jam. Sampel yang diasingkan ini dipecah untuk memastikan kehadiran serangga mati atau hidup di dalamnya. Serangga yang jumpai tergolong dalam famili Cerambycidae, Bostrichidae dan Scolytidae. Tiada serangga atau larva hidup diperhatikan setelah rawatan sejuk beku selama 24 jam. Tiada serangga dewasa muncul daripada sampel yang dilanjutkan simpanannya pada suhu bilik selama 90 hari selepas itu. Semua serangga dalam sampel kawalan terus hidup sepanjang eksperimen. Kajian ini menunjukkan kaedah sejuk beku dapat diguna sebagai rawatan kuarantin untuk mengawal atau membasmi serangga perosak dalam kayu atau keluaran hutan yang diimport dari kawasan tropika atau subtropika.

INTRODUCTION

Control of termites and other insects present in imported timber and forest products (e.g. manufactured wooden articles and related products) and their management remain a big challenge for quarantine authorities across the globe. Many destructive forest insects have extended their ranges as a result of increasing global trade (Haack 2006, Peter & Lisa 2006, Smith et al. 2007). Wood borers such as longicorn beetles (Cerambycidae), auger beetles (Bostrichinae), termites, powder post beetles (Lyctinae) and bark beetles (Curculionidae) are commonly found on imported timber and forest products. A diverse range of exotic insects and other pests are routinely intercepted by Australian Quarantine Inspection Service (AQIS) at the border during inspection of imported timber and timber-related products including highly processed furniture, flooring, sporting goods and musical products.

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AQIS pest interception records from January 2007 till January 2009 indicate that the major categories of pests intercepted from imported timber and timber products were ants (Formicidae) (879), flat bark beetles (Cucujidae) (611), powder-posts beetles (Bostrichidae–Lyctinae) or auger beetles (Bostrichidae–Bostrycinae) (621), and various other pests such as thrips, booklice, mites and spiders. In the United States, new species are detected at a rate of more than one species per year (Rabaglia et al. 2008). Some of these species have serious impacts and are potentially capable of virtually eradicating their newly acquired host tree species (Haack et al. 2002). Economic impacts associated with non-native invasive organisms in the United States are estimated to exceed USD136 billion per year (Pimentel et al. 2000). Prevention of the introduction of insect pests in a given country through importation of timber and forest products using a quarantine treatment that is reliable, effective and does not damage the products remains a challenge. Traditional quarantine treatments such as dry heat treatment/kiln drying and methyl bromide fumigation can be effective but not necessarily suitable for all types of finishes (Pinniger 1996, Fields & White 2002) such as painted and coated timber products (e.g. musical instruments, furniture). Painted or lacquered finishes render many items impermeable to methyl bromide fumigation (Hagenmaier & Shaw 1992, Gamliel et al. 1998) while heat treatment can damage such products. Other treatment options such as irradiation may cause damage, are expensive or have gamma facilities/treatment chambers that are not long enough to accommodate the commodity.

There has been an increasing trend around the world for non-chemical and environmentally friendly treatment options such as freezing to replace traditional chemical treatments such as methyl bromide (Castella 1999, Taylor 2000), the use of which is regulated under the Montreal Protocol on Substances That Deplete the Ozone Layer (effective from January 1989). There is widespread acceptance by the forestry sector that the continued use of methyl bromide fumigation for both quarantine and market access treatment of timber and forest products is unsustainable.

Cold treatment (freezing) has been known for a long time to kill insects. Florian (1986) and Berry (2001) studied the mechanism of how freezing affected insects and also timber artefacts. Freezing treatment at a temperature of -18 °C or below has been used to control insect pests of museum products (Strang 1992). Temperature requirements for the eradication of museum insects and proposed appropriate procedures for the application of low temperature/thermal method have also been reported (Strang 1992, 1993, 1995). Museum conservationists have controlled insect pests successfully in wood, hide and leather using extreme low temperatures (Florian 1986, 1990, Gilberg & Brokerhof 1991, Strang 1992). Rapid cooling/freezing using liquid nitrogen has also been reported to control termites in structures (Rust & Kennedy 1993, Lewis & Haverty 1996, Rust et al. 1997). When subjected to freezing temperatures, objects (dried/seasoned timber) do not freeze and ice formation does not occur within them; only the living insects possessing body water are frozen (Anonymous 1994). Alternate cycles of freezing and warming (e.g. room temperature) of infested objects have been reported to be effective in eradicating most insects including termites (Pellitteri 2004). It is the sudden shock of the temperature change rather than the cold that kills the beetles (Anonymous 1998). Some insect species also suffer mortality even from low, non-freezing temperatures (Bale 1993).

Freezing at -18 to -20 °C for over seven consecutive days has been widely reported to kill most pests of museum articles, tapestries and other articles (Strang 1992, Rust et al. 1997, Pinniger 2003) and stored grain (Hunter & Taylor 1980, Jay 1984). However, there is little reliable data for the same treatment on solid timber products and, in particular, coated timber products as most lacquers, paints and varnishes are reported to have varying degrees of gas and water vapour resistance.

Information on the efficacy of exposure time of freezing to kill timber pests including termites will provide additional treatment options for treating imported timber products that are otherwise not suitable for fumigation. This trial using naturally infected branches, artificially inoculated timber blocks and naturally termite-infested timber blocks/pieces was undertaken to evaluate the efficacy of a range of freezing exposure time that could be used to eradicate insect pests of timber products that were not suitable for heat, fumigation or irradiation treatment.

MATERIALS AND METHODS

Seventy tree branches with possible insect infestation, four pieces of timber naturally-infested with termites and six timber cubes artificially infested with either lyctine beetles or termites were subjected to a range of freezing treatments at the AQIS entomology laboratory, Melbourne, Victoria from March till June 2009. The tree branch samples used were a mixture of *Acacia* species, but mostly *Acacia parramattensis* and *Acacia decurrens*. All tree branch samples were collected from natural forests in subtropical New South Wales and Victoria during the summer months. Samples were collected by a forest entomologist and attempts were made during sourcing of samples to collect as many infested samples as possible based on external symptoms such as presence of insect entry/exit holes and swollen galls. Collected tree branch samples were about 25–30 cm long and up to 5 cm in diameter and preserved and transported carefully without exposing to harsh environmental stress. The artificially-infested timber blocks were infested with a mixture of three species of lyctine beetles (*Lyctus brunneus*, *Lyctus discedens* and *Minthea rugicollis*) and were provided by CSIRO Material Sciences and Engineering, Clayton South, Victoria. The beetle cultures had been maintained on solid sterilised (40 °C and -95 kPa) black bean (*Castanospermum australe*), a plant with high starch content. Wood (approximately 8 cm × 8 cm) infested with termite (*Coptotermes* species) were collected from naturally-infested colony.

Timber cubes for artificial inoculation

Timber cubes of 13 cm × 6.5 cm × 6.5 cm were used for artificial infestation. A hole was drilled in the centre of each cube to create space for insects. A second drilled timber cube was used on the top of the first cube to make a sealed chamber (Figure 1). The two timber blocks were joined using paper masking tape to prevent escape of the test insects but allowing room for ventilation. Each cube was infested with 10 active adult lyctine beetles mentioned above. The experiment was replicated twice. Two sets of cubes infested with termites from naturally-infested samples and insects grown on inoculated timber blocks were kept as control samples. All (n = 8 + 8 = 16) control samples were kept at room temperature (21 ± 2 °C).

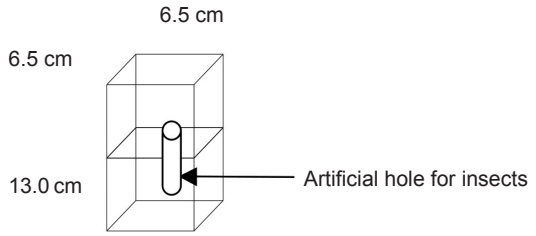

Figure 1 Timber blocks used for artificial inoculation (figure not drawn to scale)

All timber branches in batches of seven, the termite-infested timber blocks and the timber blocks artificially inoculated with lyctine beetles or termite were placed in separate polythene bags, labelled and packed inside a large box freezer with no automatic frost-free options. Samples were kept in the freezer for 24 to 336 hours as outlined in the freezing regime schedule in the appendix. When samples were removed from the freezer at the conclusion of each time period they were placed on a table in the laboratory at room temperature without opening the polythene bag to prevent escape of live insects. Samples were kept in the polythene bag on the table for 48 hours to allow them to achieve room temperature. Samples were then carefully destructively sampled using a chisel and hammer to look for any different life stages of insects. The collected insects/larvae were identified to family level by an AQIS entomologist. All samples in the polythene bag were preserved after sampling in a plastic bag for a further 3 months at room temperature (19–23 °C) to ensure that no larvae hatched from eggs that might have survived the freezing treatment. Number of insect survival at the conclusion of the experiment on inoculated control samples (not receiving freezing treatment) was recorded. Visual observation of insect activity was made on infected wood samples that were not subjected to freezing treatment.

Temperature regime of the freezer

Before the experiment began the temperature was recorded every minute for 5 hours both inside the freezer as well as inside one of the wooden cubes without any insect (data not presented). A temperature probe was attached to a data logger to measure the rate of cooling and the time required to achieve a temperature regime of about -20 °C inside the timber cubes.

Temperature regime inside the freezer during the experimental period starting 10 till 25 March 2009 was also recorded for every alternate minute using a wooden cube as described above.

Data on insect mortality between treatment time and time to achieve a desired core temperature inside the timber pieces were calculated to determine the time required to kill all life stages of insect pests in the timber samples and thus achieving the best control for the target pest present in the timber samples.

RESULTS

No live insects (adult or larvae) were recovered after the first freezing treatment of 24 hours at ≤ -18 °C. No live insects (adult or larvae) were recovered from nine of the remaining longer freezing period used in this study. The number of dead adults/larvae recovered from the naturally-infested samples varied greatly (Figure 2; Appendix) ranging from 1–29. In treatment 9, sample 6 had 29 Bostrichidae insects while sample 7 had 27 insects comprising Bostrichidae and Cleridae. The highest total number of insects was from Cerambycidae followed by Bostrichidae and Hymenoptera (Figure 2).

The types of larvae and insect groups (different life stages) recovered from the samples varied widely (Appendix). Insect families recovered from the naturally-infested samples included Buprestidae, Cerambycidae (*Phoracantha* sp.), Cleridae, Buprestidae, Bothrideridae (*Aeschyntelus* sp.), Bostrichidae (*Xylobosca* sp.) and some families in the order Hymenoptera (e.g. Cimbicidae). No live adults or larvae were recorded from any of the treated samples receiving more than 24 hours of freezing treatment. No live termite (Rhinotermitidae) was recovered from the 12 inoculated timber blocks. There were 33 dead adult workers, 2 dead adults and 1 dead alate recovered after destructive sampling.

Most of the Cerambycidae larvae recovered were 3–10 mm long, Cleridae larvae were up to 8 mm long, Bostrichidae 5 mm while Hymenoptera and Buprestidae 15 mm. None of the lyctine beetles and termites in the artificially-inoculated timber cubes survived the first freezing treatment regime of 24 hours. The temperature record confirmed that the air temperature inside the freezer dropped close to 21 °C within 45 min. However, it took nearly four hours to reach a temperature of close to -18 °C at the core of the wooden block inside the freezer. The temperature of the wooden cube inside the freezer remained steady below -18 °C throughout the experiment (Figure 3).

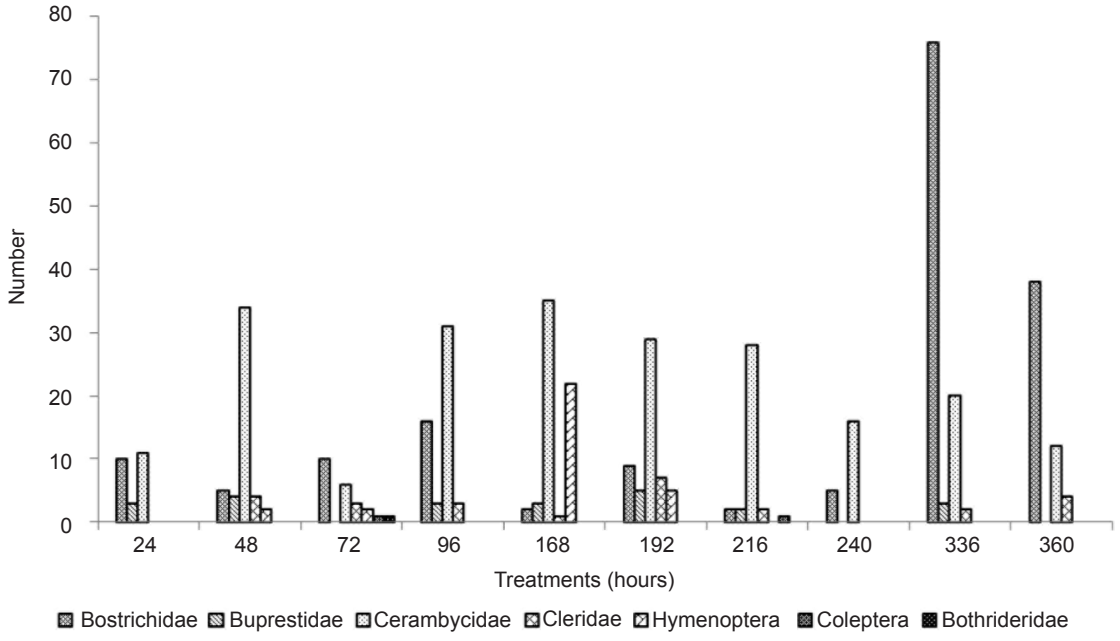

Figure 2 Number of dead adults/larvae under each family recovered after each freezing treatment regime; details of insects recovered from each sample are presented in the appendix

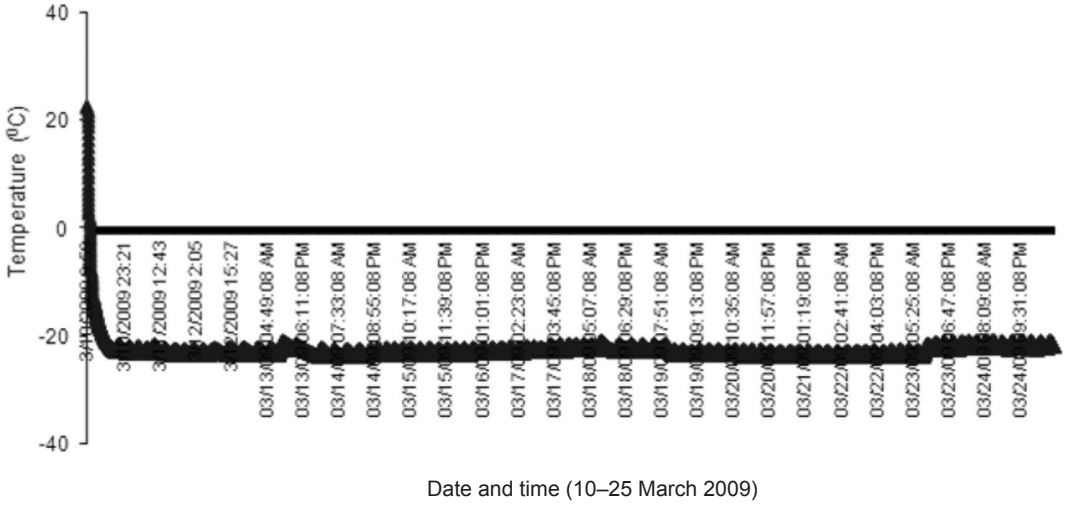

Figure 3 Temperature inside a timber cube during the experiment

All insects in control cubes inoculated with termites/insects and kept at room temperature did not survive until 14 days. No change was recorded on insect activity (free movement) for naturally-infected wood sample or wood samples with established insect colony (i.e. insect activity was normal in naturally-infested termite wood samples as well as on wood samples with established insect colony).

No larvae hatched out from any of the samples that were preserved for the additional 3 months at room temperature after completion of the 14-day trial. Most of the insects (lyctine beetles and termites) were alive in the control samples.

DISCUSSION

This trial using naturally-infested tree branches, termite-infested timber blocks and artificially-infested timber blocks demonstrated that cold treatment using a minimum temperature of -18 °C for 24 hours was sufficient to kill the larvae and adult insects and perhaps any eggs present in the test samples collected from tropical and subtropical regions. Insect activity in the wood samples with established colony remained normal while some insects in the inoculated control samples died most probably due to change in their usual habitat or unavailability of readily available food in the changed environment.

Low temperature or freezing is a technique that has been used for decades to control insects in museum pieces and in food. Generally, rapid change in temperature or cold shock created by freezing kills insect. The water–ice transition can cause cellular dehydration and disrupt chemical gradients, eventually killing organisms (Franks et al. 1990). Freezing was also reported to cause osmotic swelling of cell compartments and eventual rupture of surrounding walls or membranes (Meryman 1971). Freezing may also lead to an elevation of salt concentrations to toxic levels (Lovelock 1953).

This study did not examine the physiological changes that led to the death of insects. However, it is possible that sudden cold shock may have been the cause. Few investigators have examined cellular mechanisms of chilling and freezing injury in insects. Two forms of low temperature injury are evident in insects, namely, injury resulting from freezing and cold shock associated with chilling but without ice formation (Lee 1991). Freezing is triggered by ice-nucleating substances present in the cells or intestines of insects (Zachariassen 1980). It is generally believed that tolerance to freezing from high subzero temperatures with slow cooling rates is possible only if ice formation is restricted to the extracellular spaces (Mazur 1984).

Before freezing can be used as a successful treatment for eradicating timber pests, it is essential to understand the basic survival mechanisms of a range of insects including insects from cooler region. The mechanisms used by insect to survive freezing conditions have been the subject of many investigations. The issue is

very complex and depends on various factors such as habitat, altitude and geographic location, stage of development (egg, larvae, adult) of the insect and its physiology. For example, during winter in the forest litter, snow cover provides a strong buffer against low temperatures (Bale 1991). The flesh fly *Sarcophaga crassipalpis* exposed to -10 °C for 2 hours continued to develop but died in the pharate adult stage just before eclosion (Chen et al. 1987).

This study included only locally-infested timber branches and laboratory-cultured insects and captured data on dead adult and dead larvae/pupae using destructive sampling. It is possible that some unknown number of eggs may have been present inside the timber specimens that did not hatch out from the timber specimens 90 days after freezing treatments. This suggests that the freezing regimes used in this study is either sufficient to kill all stages of insects including eggs or that the eggs present in the samples may have been damaged during the destructive sampling process. Nevertheless, for quarantine purposes, the 100% insect kill rate achieved in this study should not be extrapolated to control insect pests of timber sourced from other geographic regions, e.g. Arctic region or harsh temperate climate.

The findings of this study supported those of Teygeler et al. (2004) who reported that material should be frozen quickly and that freezer temperature should reach 0 °C within 4 hours and -20 °C within 8 hours. Although this study did not test rapidity of different cooling regimes, attempts, however, were made to assess the rate of cooling achievable in commercial environments so that cold storage or freezing treatment could be used as a routine option to control timber pests in imported timber products. Data obtained from the electronic data logger showed that the inside temperature of wooden cubes during the experiment dropped to below -18 °C from an air temperature of 29 °C within 4 hours and remained steady at around -24 °C for the rest of the period (Figure 2). This rapid cooling rate may have contributed to the 100% killing of adults, larvae and eggs present in the infested timber samples. It is, however, essential to guard against freeze resistance. Some insects can acclimatise to cold temperatures if they are kept in a cool area before freezing or if freezing happens too slowly (Lee 1991). A few species are known to survive cold temperatures either by supercooling or by freezing tolerance, perhaps depending on conditions in a given year (Sømme 1982, Duman et al. 1991). It has also been reported that cold-hardy insects are usually freeze tolerant (Sømme & Zachariassen 1981, Duman & Montgomery 1991, Sømme 1995) but need an intact metabolic system that prevents them from accumulating polyols (Storey & Storey 1981).

It is also important to note that in a freezer, the temperature at the centre of an object drops as a cooling front moves through the object (Michalski 1991). The centre of a thick wooden object often takes a little longer to reach the ambient temperature inside a freezing chamber. However, there are limits to how quickly the temperature can be lowered. A high rate of cooling decreases the likelihood that insects will be able to adapt for cold temperature survival. As timber samples used in this study were relatively thin (plant branches around 3 cm diameter and wooden blocks up to 13 cm), care must be taken if thicker timber products were frozen. Alternatively, duration of the freezing regime should be longer. It has been reported that thicker objects must be exposed to -30 °C for 3 days or -18 °C for at least 14 days (Pinniger 2003). Rapid cooling of objects infested with dry-wood termites can also be achieved by injecting liquid nitrogen into the walls of objects (Rust et al. 1997) although this method can be impractical.

Failure of any quarantine treatment can result in the introduction of exotic pest to a new country or environment with serious consequence to the native and plantation forests. Quarantine treatments, therefore, always demand a very high level of security to ensure effectiveness against pests from diverse geographical and climatic conditions.

CONCLUSIONS

Freezing of tropical and subtropical timber products for a minimum of 24 hours (or longer depending on thickness of the timber) after achieving a core temperature of -18°C or below can be used as an effective treatment for imported timber products that are not suitable for methyl bromide fumigation or heat treatment. To avoid damage to the wood, freezing treatment should be carried out preferably using a frost-free freezer with a rapid cooling rate.

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Appendix Number of dead adults/larvae recovered from each sample after freezing treatment

Sample number	Sample dimension (length × width (cm)/volume* (cm^3))	Insect group (family)	No of dead insects × length**
Treatment 1	24 hours freezing		
Sample 1	28 × 3	Buprestidae	2 × 2 cm larva 1 × 1 cm larva
Sample 2	25 × 3	Cerambycidae	2 × 1 cm larvae
Sample 3	18 × 1.5	Bostrichidae	3 × 1 mm pupae 1 × 2 mm adult
Sample 4	36 × 6	Cerambycidae	5 × 1 cm larvae 2 × 2 cm larvae
Sample 5	30 × 2	Bostrichidae Cerambycidae	2 × 1 mm larvae 2 × 2 cm larvae
Sample 6	38 × 3	Bostrichidae	4 × 5 mm larvae
Sample 7	28 × 4	–	–
Treatment 2	48 hours freezing		
Sample 1	41 × 1	Cerambycidae	3 × 1 cm larvae
Sample 2	30 × 2	Cerambycidae Cleridae Hymenoptera	8 × 1 cm larvae 1 × 1 cm larva 1 × 1 cm larva
Sample 3	40 × 5	Cerambycidae Cleridae Buprestidae	6 × 1 cm larvae 2 × 1 cm larvae 4 × 2 cm larvae
Sample 4	24 × 2	Cerambycidae Hymenoptera	4 × 1 cm larvae 1 × 2 mm adult
Sample 5	24 × 2	Cerambycidae	5 × 1cm larvae
Sample 6	34 × 4	Bostrichidae Cleridae	4 × 1 mm adults 1 × 2 mm larva 1 × 1 cm larva
Sample 7	40 × 7	Cerambycidae *Phorocantha* sp.	2 × 2 cm larvae 5 × 1 cm larvae 1 × 2.5 cm adult
Treatment 3	72 hours freezing		
Sample 1	33 × 2	Cleridae Cerambycidae Bostrichidae	1 × 1 cm larva 1 × 4 mm larva 1 × 1 cm larva 1 × 2 mm adult
Sample 2	33 × 3	Hymenoptera Cerambycidae	2 × 1 cm larvae 2 × 5 mm larvae
Sample 3	37 × 1	Bothrideridae *Aeschyntelus* sp. Bostrichidae *Xylobosca* sp.	1 × 5 mm adult 6 × 1 mm adult
Sample 4	34 × 2	Coleoptera	1 × 1 mm pupa
Sample 5	35 × 5	Cerambycidae	2 × 1 cm larvae
Sample 6	33 × 2	Bostrichidae Cleridae	2 × 2 mm larvae 1 × 5 mm larva
Sample 7	30 × 2	Cerambycidae	1 × 1 cm larva
Treatment 4	96 hours freezing		
Sample 1	29 × 2	Cerambycidae Buprestidae	11 × 1 cm larvae 1 × 1 cm larva
Sample 2	31 × 3	Cerambycidae	9 × 1 cm larvae

(continued)

Appendix (continued)

Sample 3	28 × 2	Cerambycidae Cleridae Bostrichidae	5 × 1 cm larvae 2 × 8 mm larvae 1 × 5 mm larva
Sample 4	21 × 2	Cerambycidae	2 × 5 mm larvae 1 × 5 mm adult 2 × 1 cm larvae
Sample 5	28 × 2	Bostrichidae Cleridae	4 × 5 mm pupae 3 × 5 mm larvae 1 × 1 cm larva 1 × 5 mm larva
Sample 6	35 × 1	Cerambycidae	6 × 1 cm larvae
Sample 7	47 × 4	Buprestidae Cerambycidae Bostrichidae	2 × 2 cm larvae 4 × 1 cm larvae 3 × 1 cm larvae
Treatment 5	168 hours freezing		
Sample 1	33 × 2	Cerambycidae Buprestidae Hymenoptera Cleridae	4 × 1 cm larvae 2 × 1 cm larvae 2 × 1 cm larvae 1 × 1 cm larvae
Sample 2	28 × 3	Cerambycidae Bostrichidae	1 × 1 cm larva 1 × 5 mm larva
Sample 3	39 × 2	Cerambycidae Hymenoptera	3 × 1 cm larvae 1 × 15 mm larva
Sample 4	22 × 2	Cerambycidae Bostrichidae	4 × 1 cm larvae 1 × 5 mm larva 1 × 5 mm larva
Sample 5	21 × 3	Hymenoptera Cerambycidae Cleridae Hymenoptera	1 × 5 mm adult 20 × 1 cm larvae 1 × 1 cm larva 1 × 1 cm larva
Sample 6	22 × 1	Cerambycidae	5 × 1 cm larvae 1 × 8 mm pupa 1 × 5 mm larva
Sample 7	37 × 5	Hymenoptera Cerambycidae Buprestidae	6 × 1 cm larvae 1 × 15 mm larvae 7 × 1 cm larvae 2 × 5 mm larvae 1 × 3 mm larva 1 × 15 mm larvae
Treatment 6	192 hours freezing		
Sample 1	34 × 1	Cerambycidae Cleridae Bostrichidae	1 × 1 cm larva 1 × 1 cm larva 1 × 5 mm larva 2 × 3 mm adults
Sample 2	38 × 3	Cerambycidae Cleridae Hymenoptera	9 × 1 cm larvae 1 × 1 cm larva 1 × 8 mm larvae
Sample 3	28 × 2	Cerambycidae Hymenoptera Buprestidae	1 × 5 mm larva 1 × 1 cm larva 2 × 8 mm larvae 1 × 8 mm pupa 1 × 1 cm larva
Sample 4	30 × 4	Cerambycidae Hymenoptera Buprestidae Cleridae	12 × 1 cm larvae 1 × 15 mm larva 1 × 1 cm larva 1 × 1 cm larva

(continued)

Appendix (continued)

Sample 5	35 × 2	Cerambycidae Cleridae Bostrichidae	1 × 5 mm larva 1 × 1 cm larva 4 × 2 mm larvae 1 × 4 mm adult
Sample 6	29 × 2	Bostrichidae	5 × 4 mm larvae
Sample 7	35 × 3	Cerambycidae Hymenoptera Buprestidae	1 × 5 mm larva 2 × 5 mm larvae 3 × 1 cm larvae
Treatment 7	216 hours freezing		
Sample 1	43 × 3	Cermabycidae Bostrichidae	3 × 1 cm larvae 2 × 2 mm larvae
Sample 2	20 × 1	Cerambycidae	1 × 1 cm larvae
Sample 3	30 × 1	Cerambycidae Coleoptera	2 × 1 cm larvae 1 × 1 cm pupa
Sample 4	30 × 1	Cerambycidae	1 × 1 cm larvae
Sample 5	28 × 3	Cerambycidae	4 × 1 cm larvae
Sample 6	23 × 3	Cermabycidae Buprestidae	5 × 1 cm larvae 1 × 1 cm larva
Sample 7	24 × 3	Cerambycidae Buprestidae Cleridae	13 × 1 cm larvae 1 × 1 cm larva 2 × 1 cm larvae
Treatment 8	240 hours freezing		
Sample 1	41 × 3	Cerambycidae	6 × 1 cm larvae
Sample 2	39 × 1	Cerambycidae	2 × 1 cm larvae
Sample 3	60 × 3	Bostrichidae *Xylobosca* sp.	2 × 2 mm adults
Sample 4	41 × 2	–	0
Sample 5	28 × 2	Cerambycidae	5 × 1 cm larvae
Sample 6	32 × 2	Bostrichidae *Xylobosca* sp.	2 × 2 mm adults 1 × 1mm larva
Sample 7	27 × 2	Cerambycidae	3 × 1cm larvae
Treatment 9	264 hours freezing		
Sample 1	29 × 3	Cerambycidae Cleridae	8 × 1 cm larvae 1 × 5 mm larva 1 × 1 cm larva
Sample 2	33 × 4	Cerambycidae Buprestidae	11 × 1 cm larvae 3 × 15 mm larvae
Sample 3	26 × 1	Bostrichidae	19 × 5 mm larvae
Sample 4	33 × 2	–	0
Sample 5	33 × 3	–	0
Sample 6	42 × 2	Bostrichidae	19 × 5 mm larvae 10 × 4 mm adults
Sample 7	20 × 3	Bostrichidae Cleridae	23 × 5 mm larvae 3 × 4 mm adults 1 × 1 cm larva
Treatment 10	336 hours freezing		
Sample 1	25 × 2	Bostrichidae Cleridae	16 × 4 mm larvae 2 × 4 mm adults 2 × 1 cm larvae

(continued)

Appendix (continued)

Sample 2	26 × 1	Cerambycidae	1 × 1 cm larva
Sample 3	35 × 3	Bostrichidae Cleridae	7 × 4 mm larvae 3 × 4 mm adults 2 × 4 mm pupae 2 × 1 cm larvae
Sample 4	28 × 2	Cerambycidae	3 × 1 cm larvae
Sample 5	35 × 2	Bostrichidae	6 × 4 mm larvae 2 × 4 mm adults
Sample 6	52 × 4	Cerambycidae	4 × 1 cm larvae
Sample 7	21 × 3	Cerambycidae	4 × 1 cm larvae
Wooden blocks	360 hours freezing		
Sample 1 (control)	6.5	–	0
Sample 2 (inoculated)	6.5	Bostrichidae *Lyctus* sp.	9 × adults
Sample 3 (termite)	6.5	Rhinotermitidae *Coptotermes* sp.	7 × workers
Sample 4 (termite)	6.5	Rhinotermitidae *Coptotermes* sp	5 × workers
Sample 5 (inoculated)	6.5	Bostrichidae *Lyctus* sp.	7 × adults
Sample 6 (inoculated)	6.5	Rhinotermitidae *Coptotermes* sp.	2 × adults
Sample 7 (control)	3.5	–	0
Sample 8 (control)	3.5	–	0
Sample 9 (termite)	3.5	Rhinotermitidae *Coptotermes* sp.	4 × workers
Sample 10 (termite)	3.5	Rhinotermitidae *Coptotermes* sp.	7 × workers 1 × alate
Sample 11 (control)	3.5	–	0
Sample 12 (termite)	3.5	Rhinotermitidae *Coptotermes* sp.	10 × workers

*For wooden blocks; **there were no live insects observed