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Original Article

Lethal Temperature for the Black Timber Bark Beetle, *Xylosandrus germanus* (Coleoptera: Scolytidae) in Infested Wood Using Microwave Energy

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Abstract The thermal death kinetics of the Black timber bark beetle, *Xylosandrus germanus,* was investigated to develop a heat treatment for control of infested wood packing materials used to export goods. To determine the feasibility of microwave irradiation as an alternative control method, laboratory experiments irradiating wooden blocks of Douglas fir (200 × 200 × 250 mm), which were artificially infested with adults, with 2.45 GHz of microwave energy. All (100%) Ambrosia beetle adults were killed by both hot water treatments and microwave irradiation at 52°C and 58°C, respectively. Probit analyses estimated the internal wood temperature required to produce Probit (0.99) efficacy to be 64.7°C (95% CI 62.4-69.9°C) at one minute after microwave treatment.

Keywords: lethal temperature, *Xylosandrus germanus,* microwave irradiation, wood packing materials

Introduction

The black timber bark beetle, *Xylosandrus germanus* (Blandford) is sexually dimorphic (winged females, flightless males) native species in eastern Asia (Japan, China, and Korea) and one of the most economically important pests of wood packing material throughout much of the northern hemisphere (Nobuchi, 1981). *X. germanus* is extremely polyphagous with a host range of >200 plant species in 52 families, apparently healthy plants and those dying or recently dead (Weber and McPherson, 1983). Although deciduous broadleaf trees and shrubs are preferred, some conifers are attacked (Galko, 2013).

With the increase of industrial development and international exchange, exotic pest invasion is causing problems around the world. Therefore, various methods have been proposed by exporters, importers, and government regulatory agencies to reduce the risk of these invasive pests in wood packaging materials. The International Plant Protection Convention (IPPC, 2009) guidelines provide that wood packing materials should be heat treated (56°C for 30 minutes at the core) or fumigated with methyl bromide. Yet, methyl bromide fumigation has harmful side effects for the environment and humans. Therefore, according to the Montreal Protocol, the use of methyl bromide has been banned, except for a few cases, such as wood packaging materials. In addition, heat treatment involves a long treatment time and can lead to the depletion of resources.

The UN Phytosanitary Commission is seeking alternative technologies, such as microwave energy, which can be used for pest sterilization of wood packing materials. Microwave processes have been used recently to eradicate pests in wood products and lumber (e.g., termites, powder post beetles, woodworms) (Fleming et al., 2003, 2004, 2005; Hoover et al., 2010). The IPPC (2009) proposed the heat treatment of wood packing material using dielectric heat as follows: "… where the application of heat treatment is undertaken using dielectric radiation (e.g. microwaves), wood packing material composed of wood not exceeding 20 cm in cross-section when measured across the smallest dimension of the piece must be heated to achieve a minimum of 60°C for 1 minute throughout the profile of the wood" (ISPM, 2009). Heating to the prescribed temperature must also occur within 30 minutes from ambient temperature (Kim and Suh, 2014).

Controls of wood packing material pests have been dominated by localized chemical treatments, whereas public opinion polls

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over the last several decades have shown an increasing concern and caution towards chemicals for pest management. Interest and the use of less toxic and nonchemical pest control methods by homeowners and pest management firms and agencies are also increasing (Kim and Suh, 2014). However, laboratory and field efficacy data are sporadic or lacking for most less toxic and nonchemical methods that control Black timber bark beetle.

Accordingly, this study attempted to determine the minimum lethal temperature for 100% mortality of Black timber bark beetle using microwave energy as a new treatment for pest control in wood packing materials.

Materials and Methods

Hot water treatment

A hot water treatment, using a digital water bath (300 × 155 × 150 mm, 6 L) (Lab Tech Co., Korea), was used to determine the optimal experimental temperature for microwave irradiation treatments to control the black timber bark beetle in wood packing materials used in the export of goods.

Black timber bark beetle, *Xylosandrus germanus,* infected apple trees were brought into the laboratory and larvae reared to adults at 25°C ± 2°C, 60% RH, and photoperiod of 16:8 h (L:D). Newly emerged adults were placed separately into 15 ml glass test tubes sealed with a cork with a k-type digital thermometer probe with a range of −200°C to 1370°C, ± 0.3% + 1°C (Center Technology Corp, Taiwan) placed in the center. The water bath with 4 L of water was stabilized at 25°C before placing the insects into the container. Once the temperature in the bath was stabilized, two insect containers with the adult beetles were simultaneously immersed in the water bath. The water bath was then programmed to heat up to the target temperature within 15-20 minutes. During the treatment, the temperatures were continuously monitored and recorded inside the insect containers and in the water. After 1 minute of exposure at the critical experimental temperature, the insect containers were removed from the water bath (Kim and Suh, 2014).

After the insects were treated, they were placed on filter paper dampened with water inside the bottom of a plastic petri dish. Twenty-four hours after the water bath treatment, the aduls were examined to determine whether they were alive or dead. IBM SPSS Statistics version 19 (2010) was used for the statistical analyses.

Microwave irradiation

A 2.45 GHz microwave oven (Hyeopjin Machineries Co., Korea) with an internal unit chamber dimensions of 100 × 54 × 68 cm (367, 200) and 1-9 kW of power output was used. Douglas fir (*Pseudotsuga menziesii*), a timber that is widely used as an export packaging material in Korea, was specifically selected for this study given its status as a host for the Black timber bark beetle. The harvested logs were subsequently sawn and further processed to prepare final dimensioned wood samples that measured 200 × 200 × 250 mm. Holes were carefully drilled in the wood samples for inserting the adult beetles. Four holes were drilled into each wood sample for the insect galleries, where each hole was 13 mm in diameter and 80 mm deep to maintain an equal temperature in all the holes (Figure 1).

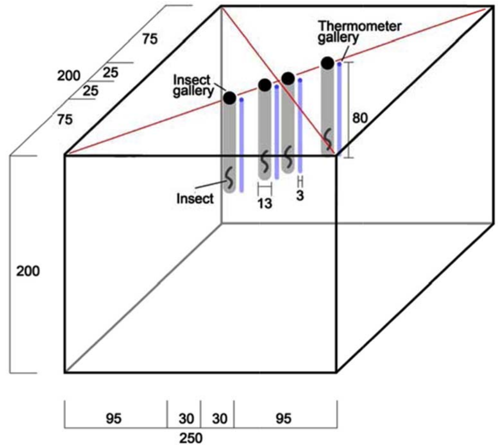

Figure 1. Diagram of wood sample for microwave irradiation treatment (scale unit: mm).

Four secondary holes (3 mm in diameter and 80 mm deep) perpendicular to the insect galleries were also drilled for digital thermocouple probes (k-type, - 200~1370°C, ± 0.3% + 1°C) that were sealed in each hole using a hot melted silicone sealant (Figure 1). K-type thermocouple wires were wrapped around each wire and around the welded tip using a thin layer of aluminum metal tape and threaded through small gaps in the door opening. Each thermocouple was connected to a digital thermometer. During the treatment, the temperatures were continuously monitored and recorded inside the insect containers and recorded at 1-second intervals (Kim and Suh, 2014).

Ten adult beetles per hole with 10 replications were used for this experiment. The entrance holes were subsequently secured with wood plugs. The sample wood with the adult beetles was placed in the microwave oven, which was then programmed to heat up to the target temperature. The microwave treatment was stopped when the last of the four probe galleries in the wood sample reached the target temperature. The treated wood samples were kept in the oven for a 1-minute ‘hold time’, and then immediately removed from the oven depending on the processing temperature that must be maintained for 1 minute, as set forth by the proposal of the International Plant Protection Convention (2011). The temperatures of the four probe galleries were measured in real-time during the treatment at 1-second intervals. The IPPC (2011) proposed heat treatment of wood packing materials using dielectric heat in ISPM 15:2009.

Once the wood samples with the Black timber bark beetles in the galleries were irradiated, they were left at room temperature. Twenty-four hours after the microwave treatment, the insect galleries were examined to determine whether the adults were alive or dead (Kim and Suh, 2014).

The fractional moisture content (MC) was calculated for each wood sample to analyze the impact on mortality. The MC was estimated for each sample using the equation from the American Society for Testing and Materials Standard D2395 following Method A procedures (ASTM, 1996). The final dry weights of the specimens were measured after natural drying for more than three months (Kim and Suh, 2014).

$$MC\ (\%)=100 \times [(I-F)\ /\ F]$$

where, I=initial green weight; F=final dry weight of the specimen.

Results and Discussion

Hot water treatment

To determine the optimal temperature for the microwave treatment, hot water treatment using a digital water bath was carried out (Table 1). These experiments were performed using four target temperatures, 50, 52, 54, and 56°C with 50 adult beetles, respectively, except at 56°C when 150 adult beetles were used, as this is the required processing temperature for phytosanitation heating by the IPPC (2009).

A 100% mortality of the adult was observed with the hot water treatment at 52°C. Probit analyses estimated the temperature required to produce Probit (0.99) efficacy to be 53.0°C (95% confidence interval 51.9-58.4).

Microwave irradiation

To obtain an estimate of the lethal temperature, an experiment was conducted using 9 kW microwave irradiation at 5 different temperatures: 54, 56, 58, 60°C, and an ambient control (20°C). The control was handled in the same way as the treated samples, although the microwave was not turned on. Among the 1,600 treated adult beetles, 1,565 died showing a mortality of 97.8%, and the mortalities in the center and sub center were similar. However, the completed trials conducted at 58°C showed a 100% mortality. In the case of the surviving adult beetles, the temperature was rapidly decreased and not maintained at the target temperature for one minute after stopping the microwave irradiation. Generally, a higher processing temperature tended to

Table 1. Mortality of *Xylosandrus germanus* adults treated with different temperatures of hot water

Treatment temperature (°C)	No. treated	No. dead	Mortality (%)
50	50	38	76.0
52	50	50	100.0
54	50	50	100.0
56	150	150	100.0
Control	50	–	–

Table 2. Mortality of *Xylosandrus germanus* adults treated at different temperatures using 9 KW microwave irradiation

Treatment temperature[1] (°C)	Internal temperature[2]		No. of trial	Center		Sub-center	
	End of Treatment' (°C)	After 1 minute		No. dead / No. treated	Mortality (%)	No. dead / No. treated	Mortality (%)
54	71.3alit	64.9alit	10	183 / 200	91.5	186 / 200	93.0
56	71.2/ 20	65.9/ 20	10	199 / 200	99.5	197 / 200	98.5
58	75.6/ 20	69.7/ 20	10	200 / 200	100	200 / 200	100
60	76.0/ 20	70.0/ 20	10	200 / 200	100	200 / 200	100
Control	–	–	10	0 / 200	–	0 / 200	–

[1] Lowest internal temperature in sample wood among four temperature probe positions.
[2]Average temperature of four probe positions.

Table 3. Treatment times of target temperatures for *Xylosandrus germanus* adults using 9 KW microwave irradiation

Temperature treated[1] (°C)	Wood weight (kg)	MC[2] (%)	No. trials	Energy density (kcal/kg)		Time required (sec.)	
				Average ± STD	Range	Average ± STD	Range
54	5.1-5.8	23.5-39.6	10	58.5 ± 28.7	21.9-109.4	150.0 ± 79.8	52-295
56	5.2-6.3	23.2-41.3	10	71.1 ± 23.0	33.6-106.5	194.2 ± 71.9	86-312
58	5.1-5.9	21.7-40.9	10	73.3 ± 33.1	32.0-132.1	192.0 ± 89.0	76-344
60	5.5-7.6	34.8-64.3	10	80.4 ± 60.6	26.2-187.6	243.7 ± 207.4	72-663

[1]Lowest internal temperature in sample wood among four temperature probe positions.
[2]Moisture content.
[3]Ratio to wood weight.

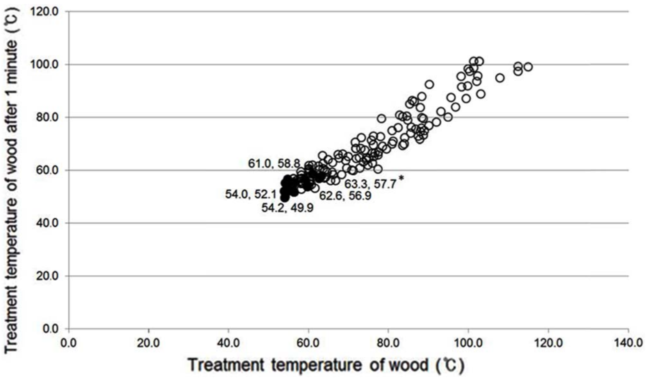

Figure 2. Status of *Xylosandrus germanus* adults after 9 KW microwave irradiation (empty: dead; filled: alive) (n=1,600). *: Data levels for live adults.

increase the mortality of the Black timber bark beetles.

The processing time for heating to the target temperature with 9 KW microwave irradiation ranged from 150.0 to 243.7 seconds (Table 3). It took an average of 194.2 seconds to heat up to 58°C, at which temperature all the treated the Black timber bark beetles died. When comparing the amount of energy with the amount of treated wood, the amount of treatment energy per unit of weight of timber differed depending on the wood characteristics. The average amount of energy was 58.5 kcal/kg at 54°C and 80.4 kcal/kg at 60°C. When increasing the target temperature, the amount of energy also increased, and complete phytosanitation of the Black timber bark beetles required 73.3 kcal/kg of energy.

To estimate the Probit (0.99), the mortality for the actual processing temperature was analyzed, regardless of the target treatment temperature. The highest temperature with surviving adult beetles was 63.3°C, and after one minute it was 58.8°C (Figure 2). The Probit analyses estimated the temperature required to produce Probit (0.99) efficacy to be 64.7°C (95% CI 62.4-69.9°C) at one minute after treatment. These results were similar to a previous report by Fleming et al. (2005) and Kim and Suh (2014), who reported a 100% mortality of cerambycid larvae at 62°C, regardless of the MC.

However, most wood products were not treated at the final target temperature for 1 min because the temperatures rapidly degraded due to the large dimensions of the internal unit chamber. Therefore, 100% mortality of black timber bark beetles could be obtained with lower temperatures than the calculations based on Probit analysis if the final target temperatures are maintained for 1 min after irradiation.

Although further experimental verification is needed, commercial microwave (2.45 GHz) treatment is a feasible alternative to conventional heat treatment to control Black timber bark beetles in wood packing materials used in the export of goods.

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