Sanitization of Logs Infested by Exotic Pests: Case Study of the Emerald Ash Borer (*Agrilus planipennis* Fairmaire) Treatments using Conventional Heat and Microwave

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Abstract

Invasive alien pest species periodically infest forests in North America and worldwide, causing the destruction of plant species and decimating populations, resulting in significant economic and ecological losses for areas involved. One common approach used to control pests is the establishment of quarantine zones, which impose limitations on circulation of products and therefore affect the economic viability of goods already affected by the infestation. This project investigated the use of conventional heat and microwaves for the sanitization of emerald ash borer infested logs to allow free circulation and trade, in order to alleviate eradication costs and maintain the economic value of products from infested resources. Four treatment temperatures of 50ºC, 55ºC, 60ºC and 65ºC were used; the logs were later evaluated for insect emergence after appropriate time for insect maturation. Results showed that temperatures of 65ºC were successful for control and sanitization of infested logs. This level is slightly above the Food and Agriculture Organization (FAO) level of 56ºC for 30mn recommended for pallets and wood packaging material. Microwave treatments were not as effective as conventional heat for controlling insect emergence, probably due to uneven distribution of the heat inside the microwave. Approaches to improve the microwave treatment are proposed.

Keywords: Emerald Ash Borer - Heat Treatment - Microwave Treatment – Sanitization – Quarantine - Exotic Insects

Introduction

Invasive alien insects and diseases are a continuous threat for trees and forest ecosystems due to international trade and involuntary movement and spread of vectors. Well known examples include the chestnut blight disease, which fundamentally altered the landscape of temperate eastern North America by completely devastating the chestnut population, the Dutch elm disease, the gypsy moth, the Asian longhorn beetle, the Eurasian pine shoot beetle and the hemlock woolly adelgid (Shea 2006, Fernandez 2003, Haack and Poland 2001, MFS 2007).

More recently, the Great Lakes region was infested with the emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae). This particular insect was discovered in southeastern Michigan in the summer of 2002 (Poland and McCullough 2006). It is largely believed that infested crates, dunnage or pallets were the transportation mechanism that brought larvae or adult insects into the region (Haack et. al. 2002). Since then the beetle has been identified throughout most of Michigan's Lower Peninsula, and in smaller occurrences in Ohio, Illinois, Indiana, Maryland and southern Ontario, Canada. It is estimated that 25 million ash trees have been infected or killed in Michigan alone (roughly 2% of the state's 850 million ash trees)(Poland and McCullough 2006). In the U.S. as a whole, more than 8 billion ash trees, comprising 16 species and approximately 7.5% of the nation's hardwood volume, are at risk (Poland and McCullough 2006). Ash appears to be the only tree species that EAB will infest in North America, though this was not true in its homeland (Anulewicz et. al. 2005).

Similarly to other exotic insect infestations, the initial agency response included imposing strict quarantine regulations on all articles or products capable of spreading the EAB. As of October 31, 2005, quarantine had officially been applied to 21 counties and

31 outlier infestation areas; these do not include a number of more recently discovered sites (Michigan Department of Agriculture 2006). The effectiveness of these restrictions in controlling the spread of the insect is questionable, especially given that enforcement of regulations is hampered by limits in funds and staffing. Nevertheless, the EAB has caused significant economic and environmental losses to private landowners and municipalities who are responsible to pay for the removal of infested trees on their properties. In addition to these direct losses, quarantine restrictions have also undoubtedly caused economic losses by preventing the movement of logs and certain types of valued wood products to their traditional markets.

We have been investigating approaches that can be used to sanitize logs and wood products, which would allow for their free circulation and marketing, and ultimately could maintain their economic value. Having more market opportunities for infested logs will provide addition revenue to non industrial private landowners, municipalities, and states and help alleviate some of the cost of tree removals programs in infested areas.

Several chemical treatments including borate (*Dissodium Octoborate Tetrahydrate*) and Preventol TM (Imidacloprid) were tested and proven effective at reducing insect emergence under laboratory conditions (Nzokou et al. 2006). However, concerns were raised over the environmental and health impact of wood products treated with chemicals. For example, is it appropriate to burn firewood sanitized with borate or imidacloprid? What could be the environmental impact if materials treated with chemicals were exposed to outdoors conditions such as rainfall and sunlight?

In order to alleviate these potential concerns, alternative non-chemical treatments including a conventional heat treatment and a microwave treatment were investigated. The conventional heat treatments were applied using a conventional laboratory kiln.

Treatment temperature levels were selected both to determine the minimum heat level required for complete sanitization and to test the effectiveness of the current FAO standard (Food and Agriculture Organization 2002) for sanitizing wood packaging material (56ºC for 30 minutes) on preventing the spread of the emerald ash borer.

The effectiveness of microwaves as a control of pathogens and pests in plants and soils has been known for a number of years (Baugh et. al. 1998). Their use to control insects in particular has also been found to be effective. In the food industry, where there has been considerable pressure to reduce the use of pesticides, microwave treatments have been used as an alternative for the eradication of insect pests with positive results (Phillips et. al. 2001, Sheyesteh and Barthakur 1996, Tang et. al. 2000). Microwave technology was also used successfully in eradicating webbing clothes moths, *Tineola bisselliella* (Humm.), in textiles (Reagan 1982). In addition, a study of heat transfer in spruce wood conducted by Zirlonka and Gierkik (1999) showed that microwaves developede peak temperatures at a layer 3 to 4 centimeters below the wood surface. The actual area of infestation for these insects is immediately below the bark in the cambial layer, near to the peak temperature of the microwave's heating curve proposed by Zirlonka and Gierkik (1999). Such characteristics make microwave treatment theoretically very well adapted to sanitizing ash logs against EAB.

The goal of this study was to investigate the effectiveness of conventional heat and microwave treatments in sanitizing logs infested by EAB. We also evaluated these treatments in relation to the post-treatment handling method by comparing indoor and outdoor rearing. Results obtained will help in the development of an acceptable non-chemical treatment for infested logs, allowing free circulation and marketing of logs harvested from infested areas.

Materials and Methods

Log Preparation

Green logs were prepared from the stems of five ash trees harvested near Ann Arbor, Michigan, one of the most heavily EAB-infested areas in the state. Before harvesting the trees, their levels of infestation were verified and confirmed by removing small portions of the bark at breast height with a bark knife and looking for the presence of galleries and larvae. Log lengths of approximately 0.9 meters (35 inches) were assigned to the different treatments so that all treatments of the same type (heat or microwave) had logs from the same individual trees. The logs were then further divided into two halves; one was assigned to a treatment with logs reared indoors and the other to the exact same treatment with logs reared outdoors. Four logs were assigned to each treatment.

Before applying the treatments, logs were examined and existing EAB exit holes were marked. Log lengths and diameters were measured at three different points on each log. The average of these three measurements was used to compute the total bark surface area.

Treatments

Conventional heat treatments were applied using a 180 cu ft conventional laboratory kiln manufactured by Standard Dry Kiln Co., Indianapolis, IN. Preliminary trials, with the dry bulb and wet bulb adjusted, were conducted to reach a stable kiln temperature of 84ºC. The kiln setting to obtain that temperature was 71ºC for the dry bulb and 65.5ºC for the wet bulb. Once the kiln temperature was stable, logs were inserted and monitored to the desired temperature and kept in for an additional 30 minutes before removing. Microwave treatments were conducted in a conventional kitchen microwave

(2.8 GHz, 1250W) manufactured by Panasonic. The turnstile had to be removed in order to fit the batches into the microwave.

For all treatments, thermocouples were inserted at three different locations in the kiln and in the logs which were wired to dataloggers for continuous monitoring of the kiln air temperature and logs' surface and core temperatures. Logs were removed once the middle log of each pile reached and maintained temperatures of 50ºC, 55ºC, 60ºC or 65ºC for the required 30mn.

Rearing

After treatments, logs were reared indoors and outdoors in structures constructed to allow for insect development to occur, while still trapping them and preventing them from escaping. Logs reared indoors were placed in thick walled cardboard tubes with plastic lids at both ends as described by Nzokou et al. (2006). In one end, a hole roughly half of the radius of the tube was cut into the center of the lid and mesh was glued over the hole. A smaller hole was cut in the mesh and a similar sized hole was cut in the lid of a screw cup. The screw cup lid was glued to the outside face of the mesh so that the two similar sized holes matched . A screw cup was then screwed into each lid (Photo 1). This set up allowed for air circulation and let light into the tube. The cups were used to collect emerging EAB adults.

Logs reared outdoors were placed in individual wooden enclosures covered with a mesh screen (Photo 2). Enclosures were placed outside at Michigan State University's Tree Research Center in East Lansing, Michigan where they were fully exposed to rainfall, sunlight and other weather events during the winter and following spring.

Data collection

For the indoor logs, rearing tubes were checked daily. Insects were collected in separate screw cups and labeled by emergence date and log identifier. Collected insects were fed ash leaves and cups were checked daily to measure the survival longevity of the insects under each treatment. After emergence had ceased, the rearing tubes were opened and the trapped EAB were collected and counted. A total count of emergent adults was calculated for each log by summing the number of EAB previously found in the cups to the number found in the tube. Tubes were then resealed and stored in an unheated storage area for the winter. They will be reared and reevaluated again in the summer of 2007 for further insect emergence.

The outdoor enclosures were checked for adult EAB weekly. Once emergence had commenced, a rough weekly count was made to track the progress. After emergence had ceased and all adult beetles were dead, the enclosures were opened and the number of dead were counted and recorded. Enclosures were then resealed, and will be reevaluated in the spring and summer of 2007. Logs will subsequently be stripped examined for remaining viable larvae.

Data analysis

For each type of heat treatment, temperature level, and rearing scenario, the collected data was used to calculate the mean emergence density (MED) as the mean number of emerged EAB adults per square meter of bark. Due to large differences in variance, a Log10 transformation was applied to the data. The computer software, SAS version 9.1, was used to perform one-way ANOVA tests. Conventional and microwave treatments, with logs reared both indoors and outdoors, were separately compared. If one or more of the means was found to differ from the rest at a multiple comparison adjusted significance level of 0.01, Dunnett's procedure was then used to determine which treatments were significantly different from their control using the same significance level. In order to compare the indoor-reared treatments to the outdoor-reared treatments, the mean difference between corresponding indoor and outdoor-reared logs was calculated for each treatment using the untransformed data. This value was then compared to an expected value of zero using a paired t-test at a significance level of 0.01.

Results and Discussion

Emergence Density

Average MED and standard errors for all treatments are summarized in Table 1.

The data presented shows that the conventional heat treatment was very effective in reducing insect emergence both for logs reared indoors and outdoors. For indoor-reared logs, the average MED of treated logs ranged from 0 to 1.77 adults per square meter compared to 58.73 adults per square meter for control logs. Both the lowest (50ºC) and highest (65ºC) temperature trials resulted in zero emergence. Similar trends were observed for logs reared outdoors with MED ranging from 0 to 1.23 adults per square meter for treated logs compared to 47.17 for control logs. Treatments at 50ºC, 60ºC and 65ºC resulted in full control of the insect emergence and only the 55ºC treatment logs differed with a MED value of 1.23. Treatments at the 50ºC temperature produced no insect emergence; however the next higher temperature tested of 55ºC failed to completely eradicate the insect in both the indoor and outdoor rearing methods. The 65ºC treatment offered complete control for both indoors and outdoors rearing, indicating that it might be safer to go to this higher temperature to ensure killing of insect larvae from infected logs.

The one-way ANOVA tests showed that indoor and outdoor-reared conventional heat trials both produced a highly significant difference in MED ($P < 0.0001$ in both cases). The Dunnett's procedure showed that the MED for all temperature treatments were significantly lower than those of the control treatment in both indoor and outdoor rearing. While more extensive testing should be done to determine the exact sufficient temperature requirements for treating EAB infested logs, temperatures of 60ºC or below should no longer be considered as options.

The current FAO standard for treatment of packaging material is 56ºC for half an hour. This level is between the test temperatures of 55º and 60ºC, neither of which completely eliminated the infestation in these trials. This clearly shows that to be on the safe side in controlling the spread of EAB through the movement of wood products, heat levels above the recommended FAO standards should be applied.

The microwave treatments produced inconsistent reductions in MED. Indoor-rearing treatments resulted in average MED varying from 1.35 to 37.33 insects per square meter, compared to the control treatment MED of 36.29. When the logs were reared outdoors, average MED values ranged from 9.95 to 20.71, compared to a control treatment MED of 64.26. The statistical analysis shows that the microwave treatments failed to significantly reduce the MED in both indoors rearing ($P = 0.1320$) and outdoors ($P = 0.1382$). These results were surprising because similar temperature treatments were successful at controlling insect emergence using conventional heat sources. We are hypothesizing that the results for microwave treatment must have been caused by the uneven distribution of heat in the microwave during the treatment. Similar temperature distribution problem has been reported before (Zhao and Turner 2000).

With the high costs of energy, and the level of energy needed to thoroughly heat logs to the desired 60-65ºC, microwave technology is still a very attractive solution for rapid heat sterilization of infested wood materials. However, the problem of ensuring appropriate temperature distribution needs to be addressed in the design and operation of the microwave.

There are several other ways to streamline the microwave heating process. Water greatly affects the dielectric properties of a substance (Zielonka and Gierlik 1999). Decreasing the moisture content of ash logs would allow microwaves to penetrate deeper into the wood. However, achieving a given moisture content for logs before treatment may be impossible and impractical in applications outside of the laboratory. Another avenue that may be used to improve microwave treatments involves radio wave frequency. It has been shown that for a given substance, dielectric properties are dependant upon the frequency of the microwaves used. These properties have been closely examined for the food and agriculture industries. Documented dielectric property curves have been developed that describe the function of wave frequencies in microwave sterilization for a number of foods and their common insect pests (Wang et. al. 2003, Wang and Tang 2004, Nelson 2005). If these curves could be estimated for both ash wood and the EAB, it may be possible, with a known moisture content of the wood, to choose a frequency that increases the treatment efficiency and decreases the risk of damaging the wood by more accurately targeting the insects for heating.

Figure 3 present a visual comparison between indoor and outdoor rearing. There is no apparent difference between the two rearing methods for both heat and microwave treatments. This was confirmed by the statistical analysis that showed no significant difference between logs reared indoors and logs reared outdoors for either the conventional heat treatment (P = 0.2903) or the microwave treatment (P = 0.4662). These results indicate that the rearing method and post-handling conditions of the logs did not affect the efficacy of the treatments.

Survival Longevity

Table 2 shows the mean number of days that the insects survived after emergence. Of course, the data presented reflect only those treatments where some insects emerged, were captured, and reared. No data is available for the 65ºC microwave treatment or for any of the conventional heat treatments. There is no apparent trend and the treatments do not seems to have affected the survival longevity of EAB adults after emergence. No statistical analysis was performed due to large differences in sample sizes and insufficient replication.

Conclusions

Emerald ash borer-infested logs were treated using conventional heat treatments (with a laboratory kiln) and microwave treatments (with a commercial microwave) to identify sanitization methods that would allow for the safe circulation and marketing of these wood materials.

The conventional heat treatments produced highly significant reductions in the mean adult insect emergence density on logs reared both indoors and outdoors. However, only the highest tested temperature of 65ºC appears to produce complete control of insect emergence. This temperature is higher than the FAO standard of 56ºC for wood packaging materials, which suggests that the current regulations may not be adequate to prevent the transport of viable emerald ash borers in wood products.

Microwave treatments did not provide significant reductions in insect emergence for all temperatures tested. We attributed the low effectiveness of microwave treatments to uneven heat distribution inside the microwave. As expected, no significant difference in treatment effectiveness was found between logs reared indoors and the logs reared outdoors for either microwave heating treatments or conventional heating treatments; indicating that the post-treatment handling method will not have any influence on insect survival and emergence. Rough data of the survival longevity showed no apparent difference between temperature treatments, indicating that the insects will do well if they survive the treatment.

Data from this study suggests that conventional heat treatment at a level of 65ºC or greater is an effective sanitization process for EAB-infested logs and wood materials. By using this standard, more free movement of logs, firewood and other wood products infested by exotic insects could occur, thereby creating more valuable market opportunities for these resources without compromising forest health.

Additional trials using larger log sizes are needed using temperatures in the 56-65ºC to determine the actual viability cutoffs and validate the preliminary results obtained in this study.

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Photo 1. *Cardboard rearing tube with collection cup for containment and collection of emerald ash borer adults in indoor treatments.*

Photo 2. *Mesh enclosure set up for containment and collection of emerald ash borer adults in outdoor treatments.*

Table 1 - *Mean emerald ash borer emergence densities (with standard error in parentheses) from logs treated with conventional and microwave heat and subsequently reared indoors and outdoors. Mean emergence densities that are significantly lower than their control at a significance level of 0.01 are marked with an asterisk (*).*

Treatment	Time	Mean Emergence Density (insects/m^2)	
		indoors	outdoors
Heat	control	58.73(17.09)	47.17(24.95)
	50ºC	0.00(0.00)*	0.00(0.00)*
	55ºC	1.77(1.77)*	1.23(1.23)*
	60ºC	0.96(0.96)*	0.00(0.00)*
	65ºC	0.00(0.00)*	0.00(0.00)*
Microwave	control	36.29(19.34)	64.26(19.46)
	50ºC	37.33(9.24)	15.15(8.76)
	55ºC	21.89(10.94)	9.95(7.47)
	60ºC	26.82(15.06)	19.99(13.82)
	65ºC	1.35(1.35)	20.71(2.78)

<u>Table 2</u> – *Survival longevity of adult emerald ash borers, in number of days, after emerging from logs heat treated using microwaves to eliminate infestation.*

Treatment[a]	Time	Sample size	Mean survival period (# of days)
	Control	10	13.70(2.14)
	50º	12	12.75(1.71)
Microwave	55º	8	17.13(2.36)
	60º	11	14.82(2.45)
	65º	0	---

[a] Conventional heat treatment excluded due to lack of adequate sample sizes

<u>Figure 3</u> – *Mean emergence densities (MED) of emerald ash borer adults from logs heat treated to different temperatures and subsequently reared either indoors or outdoors.*

Standard error bars are included. (a) Conventional heat treatment (b) Microwave heat treatment