# Evaluation of a Heat Treatment Schedule for the Asian Longhorned Beetle, Anoplophora glabripennis (Coleoptera: Cerambycidae)

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## Abstract

The Asian longhorned beetle, *Anoplophora glabripennis*, is an invasive pest that poses a serious threat to many species of North American hardwoods. An efficacious heat treatment schedule for this insect is crucial to allow wood to move from quarantined areas in the United States. A series of experiments were conducted using naturally infested trees to evaluate the International Standards for Phytosanitary Measures Rule No. 15 heat treatment schedule (56°C core temperature for 30 min) on overwintering *A. glabripennis* larvae. Results indicate that this treatment is effective, as no overwintering larvae were observed to survive. Overwintering larval stage, heating rates, oven load factors, and treatments are reported.

L he Asian longhorned beetle, Anoplophora glabripennis, native to China and Korea, was first detected in New York in 1996 (Haack et al. 1997). Additional infestations have since occurred in Illinois, New Jersey, and most recently in Massachusetts in 2008 (US Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine [USDA-APHIS] 2008). Anoplophora glabripennis feeds on and ultimately kills many native hardwoods of economic importance, such as the genera Acer, Betula, Salix, and Ulmus (Hu et al. 2009). Eradication efforts have been successful in Illinois and are ongoing where other established populations are present. These efforts, while successful, have cost millions of dollars annually and highlight the importance of having effective established treatments to mitigate pest risk with wood products as they move across international borders and quarantine boundaries.

Until recently, *A. glabripennis* infestations in the United States have been limited to urban and industrial areas; however, the 2008 detection in Worcester, Massachusetts, has brought the insect closer to hardwood forests in the Northeast that are commercially logged for lumber and firewood (Childs 2009). This presents a new regulatory concern because wood products harvested from these areas have the potential to transport *A. glabripennis* immature stages and infest new areas. To date, control and eradication efforts have focused on the use of insecticides to protect live trees (Poland et al. 2006) and removal and chipping of infested trees to kill all life stages. Phytosanitary treatment

work with *A. glabripennis* has been limited to fumigation of timbers and wood packaging material (Barak et al. 2006a, 2006b).

The objective of this project was to evaluate the efficacy of the current International Standards for Phytosanitary Measures Rule No. 15 (ISPM-15) standard for heat treatment of solid wood packing material for efficacy in killing overwintering A. glabripennis larvae. The ISPM-15 heat treatment standard has been established as a temperature of 56°C maintained throughout the profile of the wood for 30 consecutive minutes (Food and Agriculture Organization 2002). The work to develop and establish this treatment was performed on pinewood nematode Bursaphelenchus xylophilus, a serious pathogen vectored by Cerambycid beetles in the genus Monochamus (EOLAS 1991). Heat treatment studies with various life stages of another exotic Cerambycid, Tetropium fuscum, in Canada yielded complete mortality in 30-minute treatments when temperatures were greater than 50°C (Mushrow et al. 2004). However, recent work with emerald ash borer, Agrilus planipennis, has shown that adults are capable of emerging

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from firewood heated to  $56^{\circ}$ C and above (Nzokou et al. 2008, Myers et al. 2009, Goebel et al. 2010), suggesting this treatment needs to be evaluated for other insects of significant economic importance. We report here on in vitro experiments to evaluate the efficacy of the ISPM-15 heat treatment standard on wood infested with *A. glabripennis* larvae.

## **Materials and Methods**

This work was conducted during the winter and early spring 2010 using naturally infested trees found within the regulated area of the city of Worcester, Massachusetts. The majority of trees ( $\sim 80\%$ ) were red maple (Acer rubrum), although other species also found infested were included in the experiment: boxelder (A. negundo), silver maple (A. saccharinum), and Norway maple (A. platanoides). Trees were harvested during late January 2010, and tree length log sections were stored outdoors and then cut to approximately 50-cm lengths and transferred to a refrigerated shipping container where wood was held at 3°C. Wood sections were examined for oviposition sites, where adult females had chewed into the bark before laying eggs; these appear as  $\sim$ 1-cm round divots in the outer bark. Log sections with evidence of oviposition were selected for treatment to improve the probability of finding larvae in the wood. Pieces without oviposition sites or other signs of infestation were not included in the experiment. Prior to each treatment, wood was removed from the container and held for at least 24 hours at ambient room temperature (22°C to 25°C).

A 0.9-m<sup>3</sup>-capacity Espec environmental heat chamber (Model ESL-4CA; Espec North America) was used to heat wood sections. Wood sections were treated in groups of 16 pieces at a time, and core wood temperature was measured in each piece using two eight-channel thermocouple data loggers (OM-CP-OCTTEMP; Omega Engineering Inc.), with 30 AWG copper-constantan T-type thermocouples. Data loggers have stated accuracy of  $\pm 0.5^{\circ}$ C and were calibrated to National Institute of Standards and Technology specifications prior to the start of the experiment. A batterypowered hand drill and 1.65-mm drill bit were used to drill holes into the center of each log from the end to a depth of 12.5 cm parallel to the grain direction. Thermocouples were secured into each log by inserting round wood toothpicks into the entry hole (Wang et al. 2009). Wood was stacked on two racks positioned at 23 and 50 cm from the floor in the environmental chamber, and the chamber was set to run a constant temperature of 80°C at 70 percent relative humidity.

A single treatment based on the core wood temperature as measured by the thermocouple at a depth of 12.5 cm from the cut end of each piece of wood. Temperatures in each piece were monitored at 1-minute intervals during the heating process, and individual pieces were removed from the chamber after they had reached 56°C for 30 minutes. Fourteen replicates for a total of 224 log sections were treated. Untreated controls consisted of 75 log sections selected using the same criteria as above.

Treatments were evaluated for larvae 1 to 4 days from the time of treatment by physically extracting larvae from the logs posttreatment rather than waiting for larvae to complete development and emerge as adults. This method was chosen, as it allowed us to determine the rate of infestation in each log and avoid the year or longer wait for larvae to complete development. Following treatment, each log was initially examined for oviposition sites, and hand chisels were then used to remove bark around the egg site and inspect for early instar larvae. Wood was then split into small, ~3-cm-thick pieces, using an electric-powered hydraulic log splitter (Model UT49102; Homelite Co.) and carefully examined for larvae residing in the wood. The efficacy of the treatment was determined by visually evaluating larvae posttreatment. Larvae found to be turgid and active after removal from the wood were counted as live, while flaccid and unresponsive larvae were counted as dead. The length and diameter at both ends of each piece of wood was measured to estimate total volume of wood. The load factor in the chamber was calculated using the measurements from each of the log sections treated to determine their volume, then summing the log volumes from each replicate and dividing by the total volume of the chamber. Heating rates, time to 56°C, and load factors for each replicate were calculated. Log sections that contained no larvae were excluded from calculations of heating parameters and infestation rate. Head capsule widths were measured posttreatment, and larval instars were estimated using the relationship developed by Keena and Moore (2010) to determine the distribution of overwintering stages in the treated and untreated wood. Summary statistics and frequency distributions for both treatments were calculated using SAS v9.2 statistical software (SAS Institute Inc. 2008).

### **Results and Discussion**

A total of 302 *A. glabripennis* (Cerambycidae) larvae were extracted from the 99 treated and 29 control logs in the experiment that contained larvae, for a mean of  $2.40 \pm 0.20$  (standard error of the mean) larvae per wood section. No larvae were found in the remaining 163 pieces. The larva frequency distribution in treated and untreated log sections is shown in Figure 1. The distribution of larval instars, as estimated from head capsule widths, was similar between control and treated groups (Fig. 2). While all instars 1 through 9 were represented in the log sections treated, the majority of larvae were second instar or younger (50.4%) or sixth or later instars (36.5%), suggesting that later instars were likely to pupate and emerge as adults in the coming



Figure 1.—Number of Anoplophora glabripennis larvae recovered per log in treated (n = 244) and untreated (n = 58) sections.



Figure 2.—Distribution of Anoplophora glabripennis larval instars in winter-harvested wood from treated (n = 244) and untreated (n = 58) log sections. Instars 6 and 7 and 8 and 9 are grouped, as the variability in head capsule widths of these instars prevents an exact determination of larval instar.

summer, while the first and second instars may require an additional year or longer in the tree to reach pupation. This is consistent with other reports that indicate that *A. glabripennis* requires 1 or 2 years to develop (Hu et al. 2009, Keena and Moore 2010). No pupae were found in any of the logs, indicating that it is uncommon for *A. glabripennis* to overwinter in the pupal stage. Haack et al. (2006) observed similar seasonal development patterns with larvae typically pupating in late spring or summer.

The treatment temperature of the infested log sections during the 30 minutes of the treatment was  $57.8^{\circ}C \pm 0.3^{\circ}C$ (mean  $\pm$  standard error). All 244 larvae found in the heattreated log sections were dead, while mortality in the control logs was 1.7 percent (1 of 58 larvae). Moisture content, measured from the surrogate log group, was  $36.4 \pm 0.4$ percent. The mean load factor by volume in the chamber was  $12.67 \pm 0.93$  percent, heating times to reach 56°C ranged from 73 to 454 minutes, and the mean heating rate was  $0.20 \pm 0.01^{\circ}$ C/min at 80°C and 70 percent relative humidity. Under these conditions, the heating rate was largely a function of wood volume and density rather than loading factor, as loads were relatively small relative to the heating capacity of the chamber. Typical heat treatment times for firewood are 12 to 36 hours with load factors in the range 30 to 50 percent (Goebel et al. 2010), although some may take as long as 3 or 4 days during winter months (S.W.M., personal observation). The heating rates used in this experiment produced a mean treatment time of 205.8  $\pm$ 8.2 minutes, which more closely resembles treatment durations for ISPM-15 heat treatment of pallets. These are generally much shorter, typically requiring 3 to 5 hours (Bond 2005), as the wood is thinner and airspace in the kiln is abundant.

The number of insects tested does not produce statistical models with high a degree of confidence; however, our results yield a probability of success for this treatment of 0.996 and, while not at the Probit9 level that is often required for phytosanitary treatments (Follett and Neven 2006), still provides good evidence that the schedule of 56°C for 30 minutes is adequate as a mitigating treatment for *A. glabripennis* host material as logs or firewood.

Since eradication efforts are under way at all know infested sites, all host trees in the generally infested area are immediately felled and ground into chips or mulch to mitigate the risk of further proliferation. Future detections may allow for continued testing, although the likelihood of large stands of heavily infested material is slim. In the meantime, this study provides evidence that the ISPM-15 is an adequate phytosanitary treatment for this insect.

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