

# Protocol for Heat Treating Black Walnut Wood Infested with Walnut Twig Beetle

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## Abstract

Black walnut (*Juglans nigra*) is one of the most important tree species in the United States for producing lumber and other forest products. However, a recent outbreak of thousand cankers disease vectored by the walnut twig beetle (*Pityophthorus juglandis*) threatens the population of black walnut in the United States. Infected walnut trees are typically removed to prevent spread of the vector, resulting in large quantities of potential sawlogs that must be sanitized. The objective of this study was to identify the temperature and time combination necessary to heat treat infested materials to 100% beetle mortality. Testing was done on infested sample blocks that contained sapwood and bark. The specimens were heated to various temperatures and examined, both through emergence chambers and destructive sampling, for the presence of *P. juglandis* at any life stage. Results of the study indicate that heat treating black walnut products to 50.1°C at a depth of 3.8 cm (1.5 in.) for 30 minutes will result in 100 percent beetle mortality. As a side product, this study also produced 3,000 board feet of rough-cut lumber. These boards were heat treated and sold, following the protocol developed in this study. This study demonstrates that wood from black walnut trees infested with thousand cankers disease can be effectively heat sterilized and utilized, reducing the need to chip, landfill, or otherwise dispose of the material without economic return.

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Black walnut (*Juglans nigra*) is a highly merchantable timber species. In 2002, the estimated value for the black walnut growing stock in the eastern United States was nearly \$540 billion (Newton et al. 2009). However, outbreaks of thousand cankers disease (TCD) spread by the walnut twig beetle (*Pityophthorus juglandis*) threaten black walnut populations throughout the country. Mortality from the disease complex will likely have significant consequences. For instance, Treiman and Tuttle (2009) estimated that the introduction of this disease in Missouri could cause economic losses that exceed \$850 million by 2030. Because of the value of black walnut wood, some of these costs could potentially be offset by recovery of wood products. However, safe means to sanitize the wood are essential to prevent further spread of *P. juglandis*.

Black walnut is a woodworker's dream; the wood is easy to work with, exhibits very little tendency to split when using fasteners, and absorbs recoil better than any other wood (Alden 1995). The wood's distinctly dark and striped heartwood is tough and prized for its durability under hard use. Primary uses for black walnut include cabinetwork, gunstocks, lumber and veneer, furniture moldings, paneling, and novelties (Rink 1985, Kline 2001).

TCD refers to the disease complex caused by the pathogen *Geosmithia morbida* and its vector the walnut twig beetle (*P. juglandis*). Black walnut tree mortality

results from a combination of the twig beetle's aggressive feeding in the phloem layers and subsequent fungal canker development (Kolarik et al. 2011). The walnut twig beetle exhibits multiple overlapping generations with flight times from April to October. Male beetles colonize trees before females, which are attracted to aggregation pheromones released by the males. The beetles complete their life cycle within 7 weeks, emerging as adults or overwintering in the tree (Tisserat et al. 2009). Adult walnut twig beetles are so consistently associated with the pathogen *G. morbida* that

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the discovery of the beetle can be considered equivalent to a diagnosis of TCD (Cranshaw and Tisserat 2012). After emergence, walnut twig beetles spread fungal spores to new hosts as they tunnel through the phloem, creating brood galleries. *G. morbida* spreads throughout the phloem and outer bark where it causes cankers to develop (Colorado State University Extension 2012). Thousands of cankers coalesce and expand to the vascular cambium, girdling twigs and branches, causing dieback and ultimately tree mortality, and giving the condition its name: thousand cankers disease.

Trees infected with TCD are generally condemned, removed, and taken to “safe” areas or areas where the disease is known to exist or is not in proximity to healthy black walnut trees. The woody biomass from removed trees is typically processed in a chipper, horizontal grinder, or tub grinder and buried in a landfill. However, the beetle can persist in chips for up to 2 weeks, especially in larger chips where the bark remains intact (Sitz 2013). If not stored properly during those 2 weeks, chipping can actually help spread the disease. For example, in 2007 black walnut chips were transported to a landfill in Erie, Colorado. Subsequently, the black walnut trees in the vicinity became infested (K. Alexander, City of Boulder Forestry, personal communication, June 25, 2012).

Increased utilization of walnut wood could both help offset the cost of expensive removals and reduce the risk of the beetle being spread in transportation of chips. According to Cranshaw and Tisserat (2012), neither *P. juglandis* nor *G. morbida* have any detrimental effect on the appearance or quality of walnut wood. However, for wood products to be sold and utilized they must first be sanitized to prevent spreading the disease vector. Heat treating beetle-infested wood has been proven to be an effective management technique for emerald ash borer (EAB; *Agrius planipennis* Fairmaire) and Asian longhorn beetle (*Anoplophora glabripennis*; US Department of Agriculture [USDA] Animal and Plant Health Inspection Service [APHIS] Plant Protection and Quarantine [PPQ] 2007). Federal importing standards and regulations, such as USDA APHIS PPQ quarantine regulatory and the International Plant Protection Convention (IPPC) International Standards for Phytosanitary Measures (ISPM)-15 standards give time–temperature requirements necessary for sterilization (IPPC 2009, USDA APHIS PPQ 2011). However, the guidelines in these regulations are not species specific and it is unknown how effective they will be in sanitizing against the walnut twig beetle. This study addresses this issue by testing the time–temperature combination necessary in heat treatment to bring about 100 percent mortality of walnut twig beetles.

### Literature Review: Heat Treatment Protocols

Complete removal of the bark or kiln-drying walnut wood is sufficient to kill the walnut twig beetle (Cranshaw and Tisserat 2012). However, reliable and quick methods of heat treating the wood may be necessary if logs require transportation outside quarantine areas before processing, or if non–kiln-dried lumber includes any wane that could harbor beetles. The ideal time–temperature combination would be effective enough to kill the walnut twig beetle but would not require any more time or energy input than necessary. Generalized sterilization protocols exist to protect against invasive pathogens crossing international borders (IPPC 2009, USDA APHIS PPQ 2011). Addition-

ally, several studies have been done that offer protocols specific to ash (*Fraxinus* spp.) wood infested with the EAB (Nzokou et al. 2008, Myers et al. 2009). With the exception of an article by Mayfield et al. (2014), no protocols exist specifically on sterilization of black walnut wood. This research builds on the existing knowledge base to determine methods appropriate for the sterilization of black walnut.

The ISPM guidelines for regulating wood packaging material in international trade (IPPC 2009) was produced to prevent the international transport and spread of pathogens and insects that could negatively affect native plants or ecosystems. ISPM-15 affects all wood packaging material (pallets, crates, dunnages, etc.), requiring that they be debarked and then heat treated or fumigated with methyl bromide and stamped or branded with a mark of compliance. ISPM-15 standards require that wood packaging materials must be heated in accordance with a time–temperature schedule of 56°C (133°F) throughout the entire profile of the wood including its core for 30 minutes.

APHIS has developed stricter standards than ISPM-15 that regulate imports of plant materials into the United States, or movement within the United States if federal quarantines exist. The USDA APHIS PPQ treatment schedules for miscellaneous plant products list a heat treatment schedule for logs. This document contains a general requirement that logs be heated to 71.1°C (160°F) for 75 minutes. The relevant document is contained in treatment schedule “T300 – Schedules for Miscellaneous Plant Products,” specifically “T314—Logs and Firewood” (USDA APHIS PPQ 2011). Unlike ISPM-15, APHIS requirements do not stipulate that bark be removed before heat treatment.

These standards and regulations set a base foundation but in general are not species specific. Much research has been done on utilization of ash wood infested with EAB, including a kiln-heat sterilization study from Michigan State University (Nzokou et al. 2008). The dry and wet bulb settings were calibrated to obtain an ambient kiln temperature of 82°C (180°F). Two k-type thermocouples wired to a data logger were inserted to the center of the logs and 1 cm into the phloem. The logs were then inserted into the kiln and their temperature was monitored until it reached 50°C, 55°C, 60°C, or 65°C (122°F, 131°F, 140°F, or 149°F, respectively). The logs were removed 30 minutes after the core temperature reached the desired level. Results of the study indicated that the kiln heat treatments at a level of 65°C (149°F) or greater were an effective sanitation process for EAB-infested logs and wood materials. However, the authors indicated that further studies using larger sample sizes are needed to determine the actual viability of their results (Nzokou et al. 2008).

Another study on EAB was conducted by Myers et al. (2009). Researchers conducted four separate experiments on EAB larvae and prepupae at the USDA-APHIS Emerald Ash Borer Laboratory in Brighton, Michigan, from December 2006 to January 2008. They studied the thermotolerance of the immature stages of the wood-boring beetles by exposing the larvae and prepupae to a range of temperatures and times using infested ash (*Fraxinus* spp.) logs and samples. The authors concluded that 60°C (140°F) for 60 minutes should be considered the minimum temperature and time combination for the safe heat treatment of firewood infested with EAB.

Both of these EAB studies determined that temperatures above the ISPM-15 standard are necessary to sanitize EAB-

infested ash logs. If ISPM-15 standards don't accurately sanitize ash wood, they should not be assumed to be effective against walnut twig beetle without further testing. This issue was addressed in a study by Mayfield et al. (2014). The authors conducted oven-heating trials of samples infested with walnut twig beetles to determine the temperature necessary to kill the beetles. They also shipped samples from heat treatment to be cultured and analyzed for live *G. morbida*. They found that a temperature of 48°C (118°F), measured 1 cm below the sapwood and applied for 40 minutes, is necessary to kill *G. morbida* and 52°C (126°F) is necessary to kill the walnut twig beetle. The current study complements Mayfield et al. (2014) by offering an independent evaluation of the effect of heat on the walnut twig beetle, using different equipment, procedures, test conditions, and source materials. With so many different standards to choose from, this will help assure wood processors and agency regulators that proper procedures are being followed.

## Study Methods

### Log procurement and processing

The first step of this project was procuring black walnut logs infested with *P. juglandis*. Because of a particularly bad infestation near Colorado State University in the Front Range of Colorado, infested logs were not difficult to find. City foresters and homeowners from Front Range municipalities were contacted to request logs from condemned black walnut trees. To partially offset removal costs, log owners were offered a fair market price for logs based on an estimate of their board footage, scaled using Scribner Decimal C, although most owners agreed to donate logs to the research project.

Tree removals occurred during September 2011. Logs were considered suitable for processing if they had a minimum length of 1.4 m (4.5 ft) and a minimum small-end diameter of 25.4 cm (10 in.). Some logs were stored at collection sites where they were segregated from other disposal piles and others were picked up directly from the removal site. All logs were transported to the Singing Saw Woodworks in Boulder County, Colorado, using a truck with a grapple loader. This site was considered to be safe because it was in an area where the disease was known to exist and was not in proximity to healthy black walnut trees. In early October, a Woodmizer LT40bx horizontal band mill was used for primary breakdown of the logs. Slabs were set aside to be cut into samples for this project, and cants were resawn and edged to be treated and sold.

### Sample preparation and heat treatment

Slabs were cut into sample blocks for the heat-treatment experiment. Lengths of the blocks were  $25.4 \pm 3.8$  cm ( $10 \pm 1.5$  in.), widths were  $15.2 \pm 0.6$  cm ( $6.0 \pm 0.2$  in.), and the heights, measured at the highest point, were  $7.6 \pm 0.3$  cm ( $3 \pm 0.1$  in.). The time and temperature regimes evaluated were adapted from protocols established to sanitize ash logs infested with EAB (Nzokou et al. 2008, Myers et al. 2009). In this experiment, eight temperature treatments were used (42.1°C, 46.1°C, 48.1°C, 50.1°C, 56.1°C, 64.1°C, 71.1°C, and 76.1°C), and room temperature was used as the control treatment (22°C to 24°C). Three durations were evaluated for each temperature: 30, 60, and 120 minutes. The heating oven used was a THELCO GCA

Precision Scientific Model 17 (Chicago, Illinois) with a 2.77-ft<sup>3</sup> capacity. A trial consisted of a single temperature–time combination. There was one replication of each trial, except in the case of 50.1°C, which had three replications at each duration, and 56.1°C, which had two replications at each duration. Three sample blocks were placed in the oven in each trial, totaling 102 samples for the study. All samples contained natural layers of heartwood, sapwood, cambium, and bark.

Temperatures were monitored and readings were recorded using Watchdog B-series data loggers (Spectrum Technologies, Plainfield, Illinois) with an accuracy of  $\pm 1.1^\circ\text{C}$ . The button-type data loggers were inserted into the samples 3.8 cm (1.5 in.) beneath the cambium layer using a battery-powered hand drill and 0.75-in. (1.91-cm) drill bit. The hole was then filled with a plug made of black walnut sapwood to seal in heat. The data loggers recorded temperatures at 5-minute intervals. First, test trials were run to determine the length of time required to accurately heat the samples. This was done by setting the oven to the desired temperature, inserting a sample complete with data logger, and leaving it for several days. Afterward, data were downloaded and the time required to reach the desired temperature was recorded to be used as a baseline during the heat treatments. Once this baseline information was known, the individual heat treatments could begin. A data logger was installed in each sample before it was placed in the oven. After the general warming time was completed, the button was checked to ensure that the sample had reached the desired temperature. This required removing the button from the sample and replacing it with a new one to continue with uninterrupted measurements. Once it was confirmed that the sample had reached the desired temperature, it was left in the oven for the desired amount of time and then removed and placed into emergence chambers.

### Posttreatment monitoring

After each trial, all three sample blocks were removed from the oven and placed in cardboard boxes that served as emergence chambers to capture any remaining live *P. juglandis*. Boxes measured 7.6 cm (3.0 in.) wide by 46 cm (18 in.) long by 32 cm (13 in.) deep and were modified by incorporating a 45° polypropylene fixture that was fitted to a 2.54-cm (1-in.) hole in the end of each box. A 50-mL clear Falcon plastic tube was screwed into the attached fixture. The inside surface of the tube was roughened to provide beetles with a surface to gain traction. Beetles were attracted to the light from the hole where the Falcon tube was attached, as a result of positive phototaxis. Box seams were sealed with standard gray duct tape to prevent beetles from escaping. Emergence chambers were monitored daily for 21 days and data were recorded for presence or absence of beetles. The 21-day time period was chosen because beetles steadily emerged for the first week or two in trials where they survived. After that, emergence drastically fell and a full week went by without any beetles being observed. Emergence chambers remained in place for the full duration of the experiment but were not actively checked after 21 days.

In each emergence chamber, one of the three samples was removed after 48 hours for destructive sampling. Therefore, a total of 34 samples were destructively evaluated. During destructive sampling, the outer bark was removed using a scalpel to examine the cambial layers and inner bark using a

dissecting microscope. In both the emergence chambers and destructive sampling, efficacy of the treatments was determined by whether live beetles emerged from or were present in treated samples. A treatment resulting in 100 percent mortality of beetles was considered an effective treatment. Therefore, any sample where beetles emerged was considered ineffective.

### Data analysis

A logistic regression was run in SAS v. 9.4 (SAS Institute, Cary, North Carolina) to test the null hypothesis that treatment temperature had no effect on beetle survival. The independent variables of duration, temperature, and observation method were used to predict the response. Because duration and observation method ended up with a *P* value of 1.0, a reduced model was run with temperature as the sole predictive variable. Additionally, a post hoc Fisher's exact test was run on the data with 50.1°C as the separation point.

### Results

Results from the posttreatment monitoring are shown in Table 1. No walnut twig beetles survived when oven temperatures were 50.1°C (122.2°F) or higher. Duration of the treatment had no effect on the samples; at each temperature setting the results were the same regardless of duration. After implementing the heat treatments, beetles continued to emerge from samples that were heated to 48°C (118°F), but did not emerge from blocks heated at higher temperatures. Furthermore, through destructive sampling, no live beetles, larvae, or eggs were found in specimens heated to 50.1°C or above.

Results from the logistic regression were inconclusive because of the complete separation in the data. This resulted from the logistic model perfectly predicting the response, because the binary response cleanly switched at 50.1°C. The reduced model that was run had a *P* value of 0.583, which is insignificant. Because of this, a post hoc Fisher's exact test was run with emergence at temperatures below 50.1°C (*n* = 3) versus temperatures above 50.1°C (*n* = 8). Not surprisingly, this test returned a significant *P* value of 0.006.

### Discussion

Findings from this research have been corroborated by other studies, including Mayfield et al. (2014) and Peachy (2012). Mayfield et al. (2014) determined that a temperature of 52°C (126°F) for a duration of 40 minutes was necessary for 100 percent mortality of *P. juglandis*. In the Mayfield study, the temperatures were taken at a 1-cm depth beneath the cambium, which is naturally hotter than the 1.5-inch depth of this study. Additionally, the authors were targeting 30-minute durations but had up to a 10-minute time lag before retrieving samples. In the Peachy (2012) study, exposed *P. juglandis* adults and larvae were heated to various temperatures to determine the lethal temperature. Study results showed that 50.2°C (122.4°F) was the critical temperature for reaching complete mortality of these life stages, which is only one-tenth of a degree different from the 50.1°C result of this study.

Because there was considerable variation in emergence frequency between replications of the experimental trials, the total numbers of adult beetles that emerged versus those killed were not tallied. In hindsight, this is one area where the study could have been improved. Although beetles emerged in all trials when the maximum temperature was less than 50.1°C (122.2°F), an increase in temperature may have caused increased walnut twig beetle mortality and subsequently fewer beetles to emerge as temperatures approached the 50.1°C threshold. This would also have helped in the statistical analysis. Having binary results that split exactly at 50.1°C caused a complete separation that reduced the effectiveness of a logistic regression. If the data were continuous rather than binary, the results may have been significant. Another way to address this would be running further experiments designed to test emergence at temperatures very close to and surrounding 50.1°C.

Another opportunity for improvement in this study's methods would be a longer monitoring period for the emergence chambers. The monitoring period was 21 days, but the *P. juglandis* life cycle can take 7 weeks to complete. Theoretically, it could have been possible for an immature stage of the beetle to still be present during emergence trials. However, this is unlikely because the time between tree felling and emergence observations was much longer than a generation. Additionally, destructive sampling would have

Table 1.—Results from walnut twig beetle (*Pityophthorus juglandis*) posttreatment monitoring.<sup>a</sup>

| Temp. (°C) | 30 min            |                    | 60 min            |                    | 120 min           |                    |
|------------|-------------------|--------------------|-------------------|--------------------|-------------------|--------------------|
|            | Emergence chamber | Destructive sample | Emergence chamber | Destructive sample | Emergence chamber | Destructive sample |
| 22–24      | Y                 | Y                  | NA                | NA                 | NA                | NA                 |
| 42.1       | Y                 | Y                  | Y                 | Y                  | Y                 | Y                  |
| 46.1       | Y                 | Y                  | Y                 | Y                  | Y                 | Y                  |
| 48.1       | Y                 | Y                  | Y                 | Y                  | Y                 | Y                  |
| 50.1 (1)   | N                 | N                  | N                 | N                  | N                 | N                  |
| 50.1 (2)   | N                 | N                  | N                 | N                  | N                 | N                  |
| 50.1 (3)   | N                 | N                  | N                 | N                  | N                 | N                  |
| 56.1 (1)   | N                 | N                  | N                 | N                  | N                 | N                  |
| 56.1 (2)   | N                 | N                  | N                 | N                  | N                 | N                  |
| 64.1       | N                 | N                  | N                 | N                  | N                 | N                  |
| 71.1       | N                 | N                  | N                 | N                  | N                 | N                  |
| 76.1       | N                 | N                  | N                 | N                  | N                 | N                  |

<sup>a</sup> Infested samples were heated in a scientific oven for the displayed time–temperature combination and then monitored. Both evaluation methods (emergence chamber and destructive sampling) were conducted for each time–temperature combination. Y's indicate that beetles were found in the sample and N's indicate that no beetles were seen. NA = not applicable.

likely turned up any live immature beetles that remained in the wood, had they existed.

The cants produced in preparing the samples were resawn, edged, and sold by Singing Saw Woodworks. Results from this study were used to guide heat treatment of the product, and the minimum effective heating temperature reported here was applied to a load of 3,000 board feet of black walnut lumber. Seasoned boards were then sold or utilized. These boards were seasoned in a Koetter TTF4150 kiln, which is constantly vented (as opposed to the typical condensation style) and has a maximum drying temperature of 71.1°C (160.0°F). Kiln capacity is 3,000 board feet. Most of the lumber was used for furniture and display shelving. Some lumber also went to the Red Rocks Community College's woodworking program in the Denver metropolitan area for use by the program's students. As a precaution, all wood utilized had the bark removed, which eliminated the potential for beetle reinfestation after treatment.

Another important component of the production process is the disposal of infested slabs, small logs, and other woody biomass unsuitable for lumber production. As past research and case studies have shown, if this material is simply chipped and taken to a landfill, beetles may survive and spread. Therefore, material from this project was transported to a local facility that burned it in a boiler to produce heat. All unused woody residues, including bark, were transported to Gilpin County Public Works by the Colorado State Forest Service's Wood Utilization and Marketing Program specialists and used to heat the Gilpin County Public Works building near Black Hawk, Colorado. Residues were chipped and burned in a wood-fired boiler. A stack analyzer was used to monitor air emissions regulated by the State of Colorado and no emissions issues were encountered. Plant operators reported no problems with introducing the black walnut residues into the feedstock stream, adding that the black walnut chips burned well and emanated a pleasant smell.

## Conclusions

Results of this study show that black walnut wood infested with walnut twig beetles can be successfully sterilized with heat treatment. Heating the interior wood to a minimum temperature of 50.1°C (122.2°F) for a period of at least 30 minutes causes 100 percent walnut twig beetle mortality. In this experiment, temperature was measured at a depth of 3.8 cm (1.5 in.) below the cambium. Therefore, measuring temperature at this depth or greater will ensure that the outer layers, where walnut twig beetles feed, are hot enough to cause mortality. The findings from this experiment are supported by other recent research on the subject, including studies by Peachy (2012) and Mayfield et al. (2014). By sanitizing the wood in this way, a marketable product can be produced to help offset black walnut removal costs. Lumber quality is not affected by either the vector or the pathogen of TCD (Cranshaw and Tisserat 2012). Additionally, heating to the proposed temperature does not damage any strength properties of the wood. Only temperatures above 66°C generally cause permanent strength reduction in wood, and typically prolonged exposure is required up to 93°C (Denig et al. 2000).

The real-world aspect of this project helps support this notion. Over 3,000 board feet of black walnut lumber was processed from TCD-infected logs, heat treated in a kiln, and sold on the open market. The lumber outlet allows for

higher value recovery. Residues from the board manufacturing process are still suitable for coproducts, increasing the recovery yield and potential for revenue and profit. Finally, all remaining material—including slabs, cull logs, and trim—was used as fuel in a biomass boiler. This material was harvested, transported, and utilized without spreading TCD and without causing harm to the end user, in this case the Gilpin County Road and Bridge Facility. Removing infested black walnut trees can be expensive and generate large amounts of waste. The implication of this research is that logs of suitable size can be heat treated and used to make higher-value, solid-sawn wood products. These products can reduce waste and help offset the cost of removals.

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