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Efficacy of Heat Treatment for the Thousand Cankers Disease Vector and Pathogen in Small Black Walnut Logs

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ABSTRACT Thousand cankers disease, caused by the walnut twig beetle (*Pityophthorus juglandis* Blackman) and an associated fungal pathogen (*Geosmithia morbida* M. Kolařík, E. Freeland, C. Utley, and N. Tisserat), threatens the health and commercial use of eastern black walnut (*Juglans nigra* L.), one of the most economically valuable tree species in the United States. Effective phytosanitary measures are needed to reduce the possibility of spreading this insect and pathogen through wood movement. This study evaluated the efficacy of heat treatments and debarking to eliminate *P. juglandis* and *G. morbida* in *J. nigra* logs 4–18 cm in diameter and 30 cm in length. Infested logs were steam heated until various outer sapwood temperatures (60, 65, and 70°C in 2011; 36, 42, 48, 52, and 56°C in 2012) were maintained or exceeded for 30–40 min. In 2011, all heat treatments eliminated *G. morbida* from the bark, but logs were insufficiently colonized by *P. juglandis* to draw conclusions about treatment effects on the beetle. Debarking did not ensure elimination of the pathogen from the sapwood surface. In 2012, there was a negative effect of increasing temperature on *P. juglandis* emergence and *G. morbida* recovery. *G. morbida* did not survive in logs exposed to treatments in which minimum temperatures were 48°C or higher, and mean *P. juglandis* emergence decreased steadily to zero as treatment minimum temperature increased from 36 to 52°C. A minimum outer sapwood temperature of 56°C maintained for 40 min is effective for eliminating the thousand cankers disease vector and pathogen from walnut logs, and the current heat treatment schedule for the emerald ash borer (60°C core temperature for 60 min) is more than adequate for treating *P. juglandis* and *G. morbida* in walnut firewood.

KEY WORDS debarking, *Geosmithia morbida*, phytosanitary treatment, *Pityophthorus juglandis*, walnut twig beetle

Thousand cankers disease is a progressive, debilitating, and often fatal disease complex affecting eastern black walnut (*Juglans nigra* L.) and certain other *Juglans* species in the United States, as well as wingnut trees in the Asian genus *Pterocarya* Nuttall ex Moquin (Seybold et al. 2013b). The disease develops owing to attacks and gallery formation by a phloem-feeding insect, the walnut twig beetle (*Pityophthorus juglandis* Blackman, Coleoptera: Curculionidae: Scolytinae), and subsequent development of cankers caused by *Geosmithia morbida* M. Kolařík, E. Freeland, C. Utley, and N. Tisserat, a fungus that is carried by the beetle (Tisserat et al. 2009, Kolařík et al. 2011). *P. juglandis* is native to the extreme southwestern United States where it occurs on Arizona walnut, *Juglans major*

(Torrey) A. Heller (Cranshaw 2011, Seybold et al. 2012a), but the beetle and fungus are not known to be significant pests on this host (Moltzan 2011, Utley et al. 2013). In the last two decades, however, the insect has been widely collected outside of its historic range in association with dieback and mortality of *J. nigra* in several western U.S. states, where *J. nigra* is not native but has been widely planted (Cranshaw 2011, Tisserat et al. 2011, Seybold et al. 2012a). Furthermore, from 2010 to 2013, *P. juglandis*, *G. morbida*, and thousand cankers disease were discovered in black walnut trees within the native range of *J. nigra* in Tennessee, Virginia, Pennsylvania, and Ohio, and the pathogen was recently isolated from symptomatic trees in North Carolina (Grant et al. 2011, Seybold et al. 2012a, Hadziabdic et al. 2013, OH Department of Agriculture 2013).

Adult *P. juglandis* introduce spores of *G. morbida* into host trees when they attack branches or stems and begin to construct galleries in the phloem. Beetles initiate attacks on both small and large branches, but attacks are usually observed on branches larger than 2 cm in diameter and the main stem (Tisserat et al. 2009). Small, circular to oblong cankers develop around the galleries and are not visible unless bark is

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Table 1. Sites in Knox County, TN, at which *J. nigra* trees were cut and pheromone-baited logs were deployed for use in heat and debarking experiments, 2011–2012

Sites	Latitude (°N)	Longitude (°W)	No. of source trees cut			No. of baited logs deployed
			June 2011	Aug. 2011	June 2012	June–Aug. 2012
Lakeshore Park	35.9258	–83.9884	3	2	1	42
Beaver Creek Dr.	36.0015	–84.0664		1		
Concord Park 1	35.8616	–84.1385			1	
Concord Park 2	35.8586	–84.1456			1	
Tazewell Pike 1	36.0372	–83.9133			2	36
Tazewell Pike 2	36.0351	–83.9144				12
Kensington Dr.	35.8868	–84.0383				21
Northshore Dr.	35.8775	–84.0416				30
Choto Estates	35.8216	–84.1463				24
Dick Lonas Rd.	35.9477	–84.0277				13
Gibbs Dr.	36.0372	–83.9245				30
Burkhart Rd.	36.0796	–83.8578				18

removed (Tisserat et al. 2009). The pathogen is not systemic, and it appears that large numbers of beetle attacks are necessary to kill branches and stems, and to produce outwardly visible evidence of decline. In advanced stages of the disease, beetle galleries and cankers may occur every 2–5 cm in the bark, eventually coalescing, causing discoloration of the phloem, and girdling branches and stems (Tisserat et al. 2009). Male *P. juglandis* produce an aggregation pheromone that attracts both sexes and a lure has been developed to aid in detection and research (Seybold et al. 2013a). In California, flight occurs primarily at dusk and peak flight occurs in the summer, although adults have been flight trapped in every month of the year except December (Seybold et al. 2012b, 2013a). *P. juglandis* can complete a generation within 7 wk, and there are two or more overlapping generations per year (Tisserat et al. 2009).

J. nigra, one of the most economically valuable trees species in the eastern United States, is prized for its timber, nut production, wildlife value, and other commercial and cultural uses (Newton and Fowler 2009, Moltzan 2011). Black walnut lumber and veneer consistently bring a high market price, and *J. nigra* growing stock in the eastern United States was recently valued at more than half a trillion dollars (Newton and Fowler 2009). The spread of thousand cankers disease through the native range *J. nigra* is anticipated to have substantial negative impacts on lumber, veneer, and other wood-related industries owing to excessive tree mortality, premature harvests, and regulations that limit the transport or export of unprocessed walnut wood products (Newton and Fowler 2009). The disease vector and pathogen could be transported easily to new locations through movement of walnut logs, firewood, or other unprocessed wood products, and 16 states in the eastern United States have implemented quarantines regulating walnut wood movement in attempt to slow the spread (Moltzan 2011). Lumber or other processed wood that is squared-edged, kiln dried, and free of bark is not typically regulated under most state quarantines for thousand cankers disease because *P. juglandis* activity is limited only to the phloem. However, phytosanitary treatments for wood

products with bark, including logs and firewood, are desired by the industry to allow safe movement of these products as well.

Heat is a common phytosanitary treatment for eliminating insects, fungi, and nematodes from unprocessed logs, firewood, solid wood packing material, and other wood products (Newbill and Morrell 1991, Dwinell 1997, Myers et al. 2009). The International Standards for Phytosanitary Measures specification for solid wood packing material (ISPM-15) requires wood to be heated to a minimum temperature of 56°C throughout the entire wood profile (including its core) for at least 30 continuous minutes (International Plant Protection Convention [IPPC] 2009). This heat schedule is effective against a number of wood-inhabiting pests (Dwinell 1997, Mushrow et al. 2004), but recent research suggests that others, including the emerald ash borer, *Agrilus planipennis* Fairmaire (McCullough et al. 2007, Myers et al. 2009, Goebel et al. 2010), and some wood-colonizing fungi (Ramsfield et al. 2010), may not be adequately controlled by the ISPM-15 schedule (Haack and Brockerhoff 2011).

The primary objective of this study was to evaluate heat as a phytosanitary treatment to kill the thousand cankers vector (*P. juglandis*) and pathogen (*G. morbida*) in infested *J. nigra* logs, and to determine an efficacious heat treatment schedule. A secondary objective was to evaluate debarking as another potential phytosanitary tool for *J. nigra* logs colonized by these organisms.

Materials and Methods

2011 Heat and Debarking Experiments. *Source Material.* An experiment to evaluate the effect of heat and debarking on *P. juglandis* and *G. morbida* was conducted in June 2011 and repeated in August 2011. In each experiment, logs that were putatively infested naturally with *P. juglandis* and *G. morbida* were obtained by felling three *J. nigra* trees that exhibited crown dieback and bark cankers characteristic of thousand cankers disease from sites in Knox County, TN, where the disease had been previously confirmed (Table 1). In the August 2011 experiment, two of the

three source walnut trees were girdled with a chainsaw at 1.4 m above ground 1 mo before felling in an attempt to increase their attractiveness to *P. juglandis*. For each experiment, 150 logs (7–18 cm in diameter and 30 cm in length) were cut from the felled trees; a wax-water emulsion (Anchorseal, UC Coatings Corp., Buffalo, NY) was applied to both cut ends of each log with a paintbrush to reduce moisture loss, and log diameter was measured at the midpoint with a diameter tape.

Treatments. Thirty logs were assigned to each of the following treatments: 1) control (bark intact and no heat), 2) debarked (no heat), and three heat treatments (bark intact) that achieved the following minimum temperatures measured 1 cm below the sapwood surface for at least 30 min: 3) 60°C, 4) 65°C, and 5) 70°C. Logs from each source tree were evenly distributed among treatments, and there was no significant difference in mean log diameter among treatments in June 2011 (mean = 12.4 cm; $F_{4,145} = 0.1$; $P = 0.973$) or August 2011 (mean = 9.7 cm; $F_{4,145} = 0.1$; $P = 0.966$). Control and debarked logs were kept indoors at laboratory room temperature ($\approx 20^\circ\text{C}$). In the debarking treatment, logs were peeled by hand with a hammer and chisel down to the surface of the sapwood. On logs subjected to heat treatments, bark thickness was measured at two points 90° apart on the largest cut end, and the two values were averaged. Two holes, 3 mm in diameter and 4 cm apart, were drilled into the sapwood near the midpoint of each heat-treatment log with a handheld drill. One hole was drilled to a depth of 1 cm below the cambium (bark thickness + 1 cm) and the other to the log core (outside bark diameter/2). A thermocouple was inserted to the bottom of each hole and secured in place with a toothpick. Chisels and drill bits were cleaned with 95% ethanol between uses on different logs to avoid potential cross-contamination with *G. morbida*.

Logs were placed on wooden racks within a walk-in kiln (SII Dry Kilns, Lexington, NC) at the University of Tennessee, Knoxville, TN. The free end of each thermocouple was fed through a port in the kiln wall and connected to a Keithley model 2700 Multimeter-Data acquisition system (Keithley Instruments, Inc., Cleveland, OH) interfaced with a laptop computer. For each heat treatment, the kiln temperature setting was equal for both the dry bulb and wet bulb with the vents closed (relative humidity approaching 100%). Temperatures at both thermocouple depths (outer sapwood and core) were recorded every 1 min. Logs were heated until the outer sapwood maintained or exceeded the target temperature for at least 30 min. To minimize the number of kiln entries and heat loss through the open door, logs that reached 30 min of treatment exposure within the same 10-min time window were removed from the kiln as a group. Thus, the time each log was exposed to the treatment varied from 30 to 40 min. Logs were removed from the kiln and allowed to cool at laboratory room temperature.

Pathogen Analysis. In 2011, 10 of the 30 logs per treatment were used to evaluate *G. morbida* survival. In the June 2011 experiment, each log was visually

examined for cankers by scraping thin layers of bark from about half the log surface with a draw knife, before and after each treatment. Three areas ($\approx 4\text{ cm}^2$) of bark with suspected cankers were removed from each log with a chisel, placed in plastic bags, and transported on ice in an insulated cooler to the USDA Forest Service laboratory in Athens, GA. In the laboratory, the samples were surface sterilized by dipping them in 95% ethanol, briefly shaking to remove excess ethanol, and flaming. The samples were then aseptically cut into smaller pieces and placed in petri dishes with potato dextrose agar (PDA; BD Difco; BD Diagnostic Systems, Sparks, MD) amended with 100 ppm streptomycin sulfate. Three plates with five bark chips per plate were established for each log. Plates were incubated at 25°C for 5–10 d, and fungal growth with conidia and conidiophores typical of *G. morbida* was assessed. In the August 2011 experiment, samples were processed similarly with the exception that all bark was removed from the outer 6 cm of one end of each log with a chisel (opposite ends were used for the before- and after-treatment samples). The bark was dissected and visually examined for cankers, and sample areas $\approx 4\text{--}5\text{ cm}$ in length and $2\text{--}3\text{ cm}$ in width were cut from this material. These samples were then flame sterilized, cut into smaller chips and subsequently plated as previously described.

Beetle Emergence. In 2011, 20 of the 30 logs per treatment were used to evaluate *P. juglandis* adult emergence posttreatment. Each log was fitted with a screw hook inserted into one cut end and placed in a rearing container made from a 19-liter plastic bucket (Encore Plastics Corp., Sandusky, OH). Two 10-cm holes were cut in opposite sides of the bucket and covered with fine (200 micron) mesh screen (Dynamesh, West Chicago, IL) using hot melt glue. The log was suspended within the bucket by fitting the screw hook through an eye-bolt mounted in the inside center of the bucket lid. The bottom of the bucket was cut out and fitted with a 23-cm-diameter (1-liter) plastic funnel (LUBEQ Corporation, Elgin, IL), leading into a 250-ml Nalgene polypropylene straight-sided jar with screw cap (Thermo Fisher Inc.). The collection jar was filled with a small amount of propylene glycol antifreeze to kill and preserve insects entering the jar. Buckets were suspended by their handles on wooden racks at kept at room temperature. An LED string light source (C7, 120V warm white bulb, Sival, Inc., Santa Clara, CA) was illuminated continuously beneath each collection jar. Collection cups were monitored for emerging *P. juglandis* for 2 mo in the June 2011 experiment and for 5 mo in the August 2011 experiment. All *P. juglandis* were counted and expressed as the number of beetles per 100 cm^2 of bark surface.

2012 Heat Experiment. Source Material. In June 2012, five *J. nigra* trees exhibiting crown dieback and branch cankers characteristic of thousand cankers disease were felled from four different locations in Knox County, TN (Table 1). More than 226 logs, 90 cm in length and 4–17 cm in diameter, were cut from these trees and numbered with a metal tag. The cut ends of

each log were sealed with melted paraffin wax, and an eye screw was inserted into one cut end. To help ensure that the source material was infested with *P. juglandis*, logs were hung in clusters of three in a "tripod" arrangement by looping a rope through the eye screws and raising them into the crowns of other symptomatic walnut trees at nine sites in Knox County, TN (Table 1). Ropes were passed through branches of standing walnuts by using a bucket truck (when available) or a throw line with a sandbag and slingshot (Jameson LLC, Clover, SC). A walnut twig beetle pheromone lure (product #300000736, Contech Enterprises Inc., Delta, BC) was stapled to the underside of one log per cluster at its midpoint to attract *P. juglandis*. A sticky card, cut from a quarter-sheet of a clear photocopier transparency and printed with 2-cm grid (L by W = 10 by 8 cm), was covered with a thin layer of insect glue (Stickem Special, Seabright Laboratories, Emeryville, CA) and stapled to the out-facing side of each log at its midpoint. Sticky cards were collected and replaced every 2–3 wk, and the number of *P. juglandis* per card was counted. Bolts were deployed for 2 mo (6 June–6 August 2012) and returned to the laboratory. Logs were sorted by deployment site and by the total number of *P. juglandis* that landed on the corresponding sticky card during the 2 mo of field exposure. Logs with seven or more *P. juglandis* per card were kept for use in experiments, resulting in 157 logs. Starting with the log having highest sticky card catch at each site, logs were alternately assigned either to the heat treatment study reported here (79 logs) or a separate log fumigation study (78 logs; S.W.M., unpublished data).

Treatments. Each of the 90-cm logs was cut into three, 30-cm logs, one of which was randomly chosen for the control treatment ($n = 79$). The remaining logs ($n = 150$) were sorted by deployment site and redistributed into five sets of 30 logs. Deployment sites were distributed as equally as possible among the five sets, and each set was randomly assigned to one of five heat treatments that maintained minimum temperatures of 36, 42, 48, 52, or 56°C (as measured 1 cm below the sapwood surface) for 30–40 min. There was no significant difference in mean log diameter among treatments (mean = 9.0 cm; $F_{5,179} = 0.2$; $P = 0.951$). Heat treatments were conducted in August 2012 by using the same methods described for the 2011 experiments.

Pathogen Analysis and Beetle Emergence. After heat treatment, a cross-sectional disk 3.8 cm in thickness was cut from the end of each log. All the bark and a thin portion of the adjacent sapwood was cut from each disk using a hammer and frow, sealed in plastic bags, placed on ice, and shipped overnight to Athens, GA. The bark was dissected and visually examined for cankers, and sample areas ≈ 4 –5 cm in length and 2–3 cm in width were cut from this material. These samples were then flame sterilized, cut into smaller chips and subsequently plated as described previously. Pathogen isolation was conducted as described previously except that the PDA media was amended with 1,000 ppm of tergitol (Type NP-10, Sigma, St. Louis, MO), which slowed all fungal growth on the plates and

improved the ability to distinguish *G. morbida* from other fungi. The remaining log section was placed in a plastic rearing container as described previously and monitored for emerging *P. juglandis* for 4 mo (August–December 2012). All *P. juglandis* were counted and expressed as the number of beetles per 100 cm² of bark surface.

Data Analysis. Kiln Treatment Statistics. In each experiment, one-way analysis of variance (ANOVA) was used to compare mean kiln treatment statistics (average time at or above target temperature, average and maximum outer sapwood temperature, and rate of outer sapwood temperature increase) among the treatments. In the August 2011 experiment, temperature data for the 60°C heat treatment were lost owing to a malfunction in the data acquisition software and thus descriptive temperature statistics for this treatment could not be summarized. ANOVA was followed by Tukey's Honestly Significant Difference mean comparison procedure by using Statistica v. 9.1 (StatSoft 2008) with a threshold for significance of $\alpha = 0.05$.

Data from three kiln treatments (52°C August 2012, 56°C August 2012, and 60°C June 2011) were used to illustrate the relationship between outer sapwood and core temperature. The mean difference between the two temperature measurements (outer sapwood and core) during the 30- to 40-min treatment exposure period was calculated for each log, and the data were regressed against log diameter by using simple linear regression in Statistica v. 9.1 (StatSoft 2008).

2011 Heat and Debarking Experiments. Because all heat treatments (60, 65, and 70°C) in the two 2011 experiments affected posttreatment recovery of *G. morbida* identically (no pathogen recovery), pathogen data from both experiments were combined for statistical analysis and the three heat treatments were combined into a single "heat" variable. For both pretreatment and posttreatment sampling periods, logistic regression was used to test the null hypothesis that the proportion of logs from which *G. morbida* was recovered did not differ by treatment (control, debark, and heat) or experiment (June 2011 and August 2011). Pairwise treatment comparisons were made by using the Conditional Exact Test (CET) of the PROC LOGISTIC procedure in SAS v. 9.2 (SAS Institute 2008), and *P* values were Bonferroni-corrected by multiplying initial *P* values by 3 (the number of possible two-way treatment comparisons). Corrected *P* values <0.05 were considered significant. In the 2011 experiments, mean *P. juglandis* emergence (number of beetles emerged/100 cm² of bark surface) and the proportion of logs from which *P. juglandis* emerged were zero in all but one treatment (control, August 2011), so these variables were not analyzed statistically and only means (SE) are reported.

2012 Heat Experiment. For the 2012 experiment, the Cochran–Armitage test for trend was used to test for an association between increasing treatment temperature and the proportion of logs from which *G. morbida* or *P. juglandis* were recovered posttreatment. Tests were conducted on *G. morbida* and *P. juglandis* data sets separately. Pairwise treatment comparisons

Table 2. Mean (SE) temperature statistics for *J. nigra* logs subjected to various heat treatments in 2011–2012

Exp	Treatment ^a	Kiln temp (°C)	Avg time at or above target (min.)	Avg treatment temp (°C)	Max treatment temp. (°C)	Rate of temp increase (°C/min.)
June 2011	60°C	80	37.2 (0.7)a	63.8 (0.4)a	66.4 (0.7)a	0.17 (0.02)a
	65°C	80	35.2 (0.7)a	68.0 (0.4)b	70.0 (0.5)b	0.14 (0.01)a
	70°C	80	35.7 (0.8)a	71.6 (0.2)c	73.0 (0.3)c	0.09 (0.01)b
			$F_{2,80} = 2.0$ $P = 0.144$	$F_{2,80} = 136.9$ $P = 0.000$	$F_{2,80} = 36.7$ $P = 0.000$	$F_{2,80} = 9.5$ $P = 0.000$
Aug. 2011	60°C ^b	80				
	65°C	80	35.2 (0.7)a	67.5 (0.3)a	69.2 (0.3)a	0.12 (0.01)a
	70°C	80	36.8 (0.8)a	70.9 (0.2)b	72.1 (0.2)b	0.08 (0.01)b
			$F_{1,54} = 2.1$ $P = 0.149$	$F_{1,54} = 120.9$ $P = 0.000$	$F_{1,52} = 49.4$ $P = 0.000$	$F_{1,52} = 8.4$ $P = 0.005$
Aug. 2012	36°C	60	34 (0.6)a	42.1 (0.6)a	45.1 (0.7)a	0.26 (0.02)a
	42°C	66	34.2 (0.6)a	47.9 (0.7)b	51.0 (0.9)b	0.25 (0.02)a
	48°C	72	34.4 (0.7)a	54.4 (0.6)c	57.7 (0.8)c	0.27 (0.02)a
	52°C	76	35.3 (0.6)a	60.0 (0.7)d	63.3 (0.9)d	0.30 (0.02)a
	56°C	76	35.1 (0.6)a	61.5 (0.6)d	64.2 (0.7)d	0.23 (0.02)a
			$F_{4,145} = 0.9$ $P = 0.486$	$F_{4,142} = 166.2$ $P = 0.000$	$F_{4,142} = 102.8$ $P = 0.000$	$F_{4,142} = 1.8$ $P = 0.139$

Note: Within an experiment and column, means with the same letter are not significantly different ($\alpha = 0.05$).

^a Control and debarking treatments were conducted at room temperature (20°C). Heat treatments indicate the minimum target temperature maintained for the outer sapwood (30–40 min).

^b In the August 2011 60°C treatment, an error in the temperature data acquisition software prevented capture of temperature data, so summary statistics could not be calculated.

were made by using Fisher exact test. Analysis of variance could not be applied to the quantitative *P. juglandis* emergence data (beetles/100 cm² of bark surface) owing to heterogeneity of variance (that could not be corrected by transformation) and zero or nearly-zero values in multiple treatments, so pairwise treatment comparisons were made by using Pitman randomization T-tests. For Fisher exact tests and Pitman randomization T-tests, *P* values were Bonferroni-corrected by multiplying initial *P* values by 15 (the number of possible two-way treatment comparisons). Corrected *P* values <0.05 were considered significant. The Cochran–Armitage test and Fisher exact test were computed by using the PROC FREQ procedure and the Pitman randomization T-test was computed using the PROC NPAR1WAY procedure in SAS v. 9.2 (SAS Institute 2008).

Results

Kiln Treatment Statistics. Within each experiment, the mean amount of time that logs spent at or above the target temperature (≈ 35 min) did not differ significantly among treatments (Table 2). Within experiments, all heat treatments differed significantly with respect to mean and maximum outer sapwood (1 cm below cambium) temperatures, except for the 52 and 56°C treatments in August 2012 (Table 2). Furthermore, within experiments, the rate of temperature increase in the outer sapwood did not differ significantly among treatments, except that logs heated more slowly in the 70°C treatments relative to other treatments in the 2011 experiments (Table 2).

The mean difference in temperature between the two depth locations (outer sapwood and core) in each log during the 30- to 40-min exposure period increased with increasing log diameter, as illustrated in the 52°C treatment ($R^2 = 0.61$; $F_{1,28} = 43.5$; $P < 0.001$), 56°C

treatment ($R^2 = 0.82$; $F_{1,27} = 125.5$; $P < 0.001$), and 60°C treatment ($R^2 = 0.50$; $F_{1,25} = 24.5$; $P < 0.001$; Fig. 1). As expected, the outer sapwood consistently

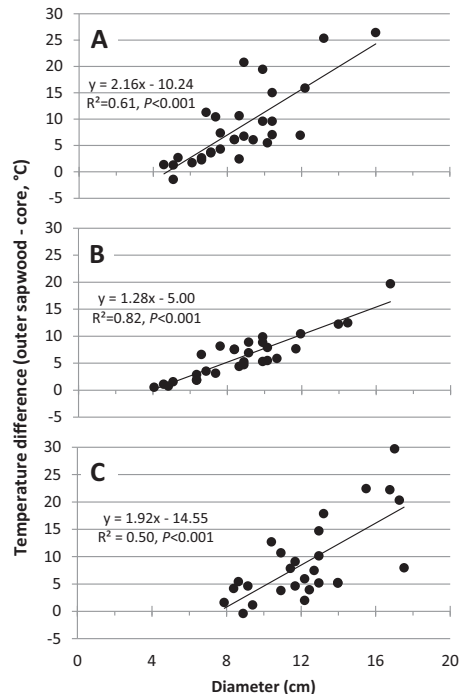


Fig. 1. Scatterplots of the mean difference between *J. nigra* outer sapwood (1 cm below cambium) and core temperature during the 30- to 40-min treatment period versus log diameter for the (A) 52°C treatment (August 2012), (B) 56°C treatment (August 2012), and (C) 60°C treatment (June 2011). Temperature difference = outer sapwood – core (°C). Thresholds for each treatment were based on outer sapwood temperature.

Table 3. Mean (SE) emergence of *P. juglandis* and number of logs from which *P. juglandis* and *G. morbida* were recovered in heat and debarking experiments in June and August 2011

Exp	Treatment ^a	<i>P. juglandis</i>			<i>G. morbida</i>		
		No. logs total	No. logs w/adult beetles	Adults emerged per 100 cm ²	No. logs total	No. logs positive	
				Pretreatment			
				Posttreatment			
June 2011	Control	20	0	0.0 (0.0)	10	4	6
	Debarked	20	0	0.0 (0.0)	10	10	1
	60°C	20	0	0.0 (0.0)	10	7	0
	65°C	20	0	0.0 (0.0)	10	10	0
	70°C	20	0	0.0 (0.0)	10	8	0
Aug. 2011	Control	20	5	1.4 (1.0)	10	6	5
	Debarked	20	0	0.0 (0.0)	10	5	0
	60°C	20	0	0.0 (0.0)	10	3	0
	65°C	20	0	0.0 (0.0)	10	6	0
	70°C	20	0	0.0 (0.0)	10	3	0

^a Control and debarking treatments were conducted at room temperature (20°C). Heat treatments indicate the target minimum temperature at or above which the outer sapwood was maintained for 30–40 min.

heated more quickly than the log core. Outer sapwood and core mean temperatures were fairly similar (difference <5°C) at the smallest log diameters (e.g., 4–9 cm), whereas mean outer sapwood temperature was frequently 12–25°C higher than mean core temperature in the larger diameter logs (e.g., 15–18 cm; Fig. 1).

2011 Heat and Debarking Experiments. When analyzing data combined from the two 2011 experiments, pretreatment recovery of *G. morbida* did not differ among the control, debarked, and heat treated (60, 65, and 70°C combined) groups (CET score = 2.9; $P = 0.260$), but the pathogen was recovered pretreatment from a significantly higher proportion of logs in the June 2011 experiment than in the August 2011 experiment (CET score = 10.8; $P = 0.002$). *G. morbida* was detected pretreatment from 4/10 to 10/10 of the logs in the June 2011 experiment and 3/10 to 6/10 of the logs in the August 2011 experiment (Table 3). In contrast, posttreatment recovery of *G. morbida* differed significantly by treatment (CET score = 43.4; $P < 0.001$). *G. morbida* was recovered posttreatment from 5/10 and 6/10 of the control logs in the June and August 2011 experiments, respectively, but from none of the heat-treated logs (Table 3). *G. morbida* was

recovered from one debarked log posttreatment in the June 2011 experiment (Table 3).

No *P. juglandis* emerged from any of the control, debarked, or heat-treated logs in the June 2011 experiment (Table 3). In the August 2011 experiment, 252 *P. juglandis* emerged from 25% (5 out of 20) of the untreated control logs at a mean density of 1.4 beetles per 100 cm², but no beetles emerged from heated or debarked logs (Table 3).

2012 Heat Experiments. In the August 2012 experiment, there was a significant negative association of heat treatment temperature with both the proportion of logs from which *G. morbida* was recovered (Cochran–Armitage $Z = 6.89$; $P < 0.001$) and the proportion of logs from which *P. juglandis* emerged (Cochran–Armitage $Z = 12.14$; $P < 0.001$). In the control treatment, *P. juglandis* emerged from 95% (75/79) of the logs at a mean density of 51 beetles per 100 cm² of bark surface, and *G. morbida* was recovered from 41% (32/79) of the same logs (Table 4). Beetle emergence density and pathogen recovery from logs exposed to treatments of 42, 48, 52, and 56°C were significantly lower than emergence and recovery from logs in the control and 36°C treatment groups (Table 4). The

Table 4. Mean (SE) emergence of *P. juglandis* and number of logs from which *P. juglandis* and *G. morbida* were recovered in the August 2012 heat experiment

Exp	Treatment ^a	<i>P. juglandis</i>			<i>G. morbida</i>	
		No. logs total	No. logs w/adult beetles	Adults emerged per 100 cm ²	No. logs total	No. logs positive, posttreatment
Aug. 2012	Control	79	75a	51.0 (5.7)a	79	32a
	36°C	30	26a	51.3 (10.5)a	30	11a
	42°C	30	10b	8.9 (4.1)b	30	1b
	48°C	30	1b	0.01 (0.01)b	30	0b
	52°C	30	0b	0.00 (0.00)b	30	0b
	56°C	30	1b ^b	0.00 (0.00)b ^b	30	0b

Note: Values in the same column followed by the same letter are not significantly different ($\alpha = 0.05$). Pairwise treatment comparisons are based on Fisher's exact test for the number of logs with *P. juglandis* or *G. morbida*, and Pitman's randomization *t*-test for mean *P. juglandis* emergence per 100 cm², followed by Bonferroni adjustment of *P* values.

^a Control logs were maintained at room temperature (20°C). Heat treatments indicate the target minimum temperature at or above which the outer sapwood was maintained for 30–40 min.

^b One dead *P. juglandis* was recovered from the collection cup from one sample log in the 56°C treatment, but the beetle is not believed to have survived the treatment (see Discussion).

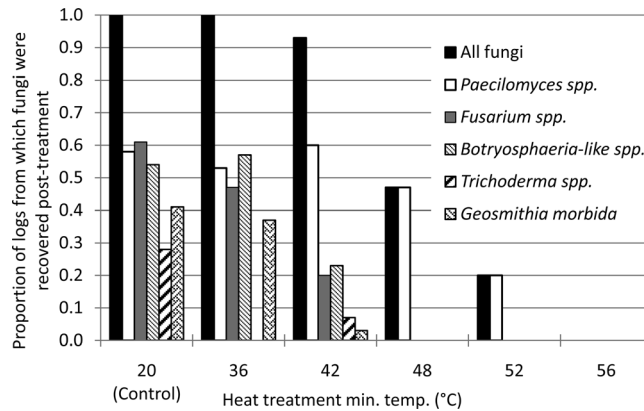


Fig. 2. The proportion of *J. nigra* logs from which *G. morbida* and other fungi were positively isolated after various heat treatments in August 2012. Total number of logs in sample: $N = 79$ for control, $N = 30$ for each of the heat treatments.

total number of *P. juglandis* that emerged from logs of the 36, 42, 48, and 52°C treatments were 14,665, 3,130, 3, and 0, respectively. One dead *P. juglandis* adult was found in the collection cup from one log from the 56°C treatment (Table 4); it is suspected that this beetle did not survive the treatment (see Discussion). *G. morbida* was not recovered from any logs subjected to a target minimum temperature treatment of 48°C or higher (Table 4). Several other fungi (identified only to genus or general group) were isolated frequently from posttreatment bark samples, including species of *Paecilomyces*, *Fusarium*, *Botryosphaeria*-like fungi, and *Trichoderma*. A *Paecilomyces* sp. was the only fungus recovered from logs exposed to the 48 and 52°C treatments; no fungi were recovered from logs in the 56°C treatment (Fig. 2).

Discussion

The results of this study demonstrate that heat treatment is an effective means of eliminating *P. juglandis* and *G. morbida* from infested logs of *J. nigra*. In the 2012 experiment, increasing temperature had a significant negative effect on *P. juglandis* emergence and pathogen recovery from logs. *G. morbida* did not survive in logs exposed for 30–40 min to treatments in which the minimum outer sapwood temperature was 48°C or higher (Tables 3 and 4; Fig. 2), and mean *P. juglandis* emergence decreased steadily to zero as minimum threshold temperature increased across the range from 36 to 52°C (Table 4).

Treatments in this study were implemented under high relative humidity (approaching 100%) with the kiln temperature set above the target minimum temperature to bring the outer sapwood (monitored 1 cm below the cambium) to the target temperature quickly. For example, in the lowest temperature treatment that eliminated both the beetle and the pathogen (minimum 52°C), the kiln temperature was set to 76°C, the outer sapwood temperature increased at a rate of 0.3°C/min during the 30- to 40-min treatment window, and the average outer sapwood temperature during the treatment window was 60°C (Table 2).

Although this approach makes it difficult to determine the exact temperature at which mortality of the pest organism occurs, it is similar to the approach likely used by commercial kiln operations that wish to maximize the number of loads of wood treated in a given time frame, and to the wet-heat treatment option for ash firewood described by Wang et al. (2009). An alternative approach would be to set the kiln temperature at or near the minimum target threshold, but this would result in much longer total treatment times. Treatment time and rate of temperature increase have been shown to influence mortality in heat treatment of insects (Neven 1998, Whiting and Hoy 1998). Slow rates of warming may allow for enhanced development of heat shock proteins and better insect tolerance of elevated temperature (Sobek et al. 2011).

The elimination of *P. juglandis* with the 52°C minimum threshold treatment in this study is consistent with temperature tolerance thresholds determined for *P. juglandis* in other recent studies. When exposing *P. juglandis* to various temperatures in vitro (beetles placed in test tubes and heated in a thermal cycler), Peachy (2012) observed 100% beetle mortality at 50.2°C, and predicted upper lethal temperatures (LT_{99}) of 53 and 48°C for adults and larvae, respectively. After heating small slabs of infested wood in a drying oven to various sapwood temperatures measured ≈ 1.3 cm below the cambium, Costanzo (2012) observed no emergence or live life stages of *P. juglandis* in pieces heated to core temperatures of 50.1°C or higher for 30 min, but did observe survival at temperatures of 48.1°C and lower. Although the experimental conditions in these two previous studies differ from those of the current study, all three studies suggest that exposure to 53°C or higher for at least 30 min is lethal to all *P. juglandis* life stages.

A single *P. juglandis* adult was found dead in the collection cup of one log from the 56°C treatment group (August 2012 experiment, Table 4). Given the trend of decreasing emergence with increasing temperature, no *P. juglandis* emergence from the 52°C treatment, the complete absence of any other insects in the collection cups from all other logs in the 56°C

treatment group, and the temperature tolerances reported for *P. juglandis* in other studies (Costanzo 2012, Peachy 2012), we strongly suspect that this beetle did not survive the 56°C treatment, but rather died at or near the surface of the log and was dislodged after the log was placed into the rearing container. Because the collection cup was located directly beneath the suspended log, the beetle likely fell passively from the log surface into the collection cup.

P. juglandis creates egg and larval galleries in the phloem, and at its greatest depth only slightly engraves the surface of the sapwood. Similarly, *G. morbida* is routinely isolated from the phloem rather than within the sapwood. The treatment threshold temperatures in this study were based on thermocouples placed in the outer sapwood (1 cm below the cambium) to determine effective temperatures in the region of the log near the pest organisms. However, federal and international heat treatment guidelines for wood products are typically based on temperature thresholds measured at the center of the wood (IPPC 2009, U.S. Department of Agriculture–Animal and Plant Health Inspection Service [USDA–APHIS] 2012); therefore, core temperature was also monitored and compared with outer sapwood temperature in this study. These comparisons demonstrated that, as expected, logs heated from the outside toward the center, with outer sapwood temperature generally exceeding core temperature in proportion to the diameter of the log (Fig. 1).

The USDA–APHIS heat treatment schedule for another invasive wood-inhabiting insect, the emerald ash borer, *A. planipennis*, requires logs and firewood to be heated to a minimum of 60°C (measured at the center of the log) for 60 min to achieve complete control (Myers et al. 2009, USDA–APHIS 2012). The results of the current study demonstrate that the current treatment schedule for emerald ash borer is more than adequate to provide phytosanitary control of *P. juglandis* and *G. morbida* in *J. nigra*. Emerald ash borer and the thousand cankers disease causal organisms now co-occur in several U.S. states including Pennsylvania, Ohio, Virginia, Tennessee, and North Carolina, and government quarantines regulating the movement of ash and walnut out of these states are in place. Currently, commercial movement of firewood out of emerald ash borer quarantine areas requires a compliance agreement certifying that the wood has received at least one form of phytosanitary treatment, of which heat treatment is a standard option. The ranges of both emerald ash borer and thousand cankers disease continue to expand and the number of states in which they co-occur is likely to increase. *J. nigra* grows in association with both white ash (*Fraxinus americana* L.) and green ash (*Fraxinus pennsylvanica* Marshall) in certain mixed hardwood and floodplain forests (Williams 1990), and both walnut and ash wood are considered of high quality for firewood (Kuhns and Schmidt 2013) and are used by commercial firewood producers (Newton and Fowler 2009). Thus, it is reasonable to assume that walnut could be comingled with ash in a load of firewood in

the eastern United States. Because many states are regulating all hardwood firewood (regardless of tree species) in response to one or both of these pest problems, a single firewood heat treatment standard that is effective against both pests would be beneficial from a regulatory standpoint. We therefore recommend a minimum temperature of 60°C at the wood center for 60 min as a logical, conservative heat treatment schedule for walnut firewood coming from thousand cankers disease-affected areas.

Although a core temperature-based schedule makes sense for walnut firewood that could be potentially comingled with ash and other species, a heat standard based on outer sapwood temperature should be considered for treatment of large diameter walnut sawtimber and veneer logs before movement across state or county borders. The largest logs in this study (16–18 cm in diameter) were relatively small compared with logs used for lumber or veneer (typically >30 cm in diameter) and yet core temperature in our largest logs lagged behind outer sapwood temperature by as much as 20–30°C at the end of the treatments (Fig. 1). Larger diameter logs destined for lumber or veneer would require substantially longer treatment times and increased energy inputs to bring the log core to 60°C for 60 min. Given the biology and relatively superficial location of the thousand cankers disease causal organisms, a core-temperature threshold seems unnecessary. Rather, these data indicate that achieving a minimum outer sapwood temperature (measured at least 1 cm below the cambium) of 56°C for 40 min would be an effective treatment schedule for *P. juglandis* and *G. morbida* in walnut logs. Although we did not evaluate the commercial acceptability of steam-heating walnut logs under this schedule, walnut lumber is routinely steamed in industrial operations to darken the sapwood, and walnut flitches (half-logs) are heated to soften the wood before slicing veneer (Cassens 2004). If steam-heating logs does not excessively degrade log quality for lumber or veneer, it may be an acceptable phytosanitary measure by which walnut logs could be safely transported from locations affected by thousand cankers disease to mills located outside the quarantine area.

In 2011, emergence of *P. juglandis* from control logs was either absent (June experiment) or very low (August experiment), despite the fact that cankers characteristic of thousand cankers disease were present and *G. morbida* was isolated from pretreatment samples of logs in every treatment group (Table 3). It appears likely that the logs cut from these trees for the study were not well infested with *P. juglandis*, despite thousand cankers disease symptom expression in the source trees. One possible explanation for the lack of beetles recovered in June 2011 is that the emergence monitoring period (2 mo) was not long enough. Preliminary information suggests that the *P. juglandis* life cycle can be completed in ≈7 wk (Tisserat et al. 2009), only slightly shorter than our monitoring period in the June 2011 experiment. Furthermore, *P. juglandis* may have emerged and then recolonized logs within the containers without entering the collection cups by the

end of the monitoring period. However, the bark of logs assigned to pathogen evaluation in the June 2011 experiment were carefully scraped with a draw knife both before and after treatment to identify cankers, and little to no evidence of *P. juglandis* gallery formation was evident. Because these logs were from the same source material as the logs used to monitor beetle emergence, we would have expected to encounter some evidence of *P. juglandis* during this process if the material had been infested. Rather than continue a long experiment with potentially uninfested material, we ended the June 2011 experiment after 2 mo and repeated it in August 2011 and 2012 using a longer emergence monitoring period (4–5 mo). In the latter experiments, beetles began to appear in the collection cups for logs of the control and lower-temperature treatments (36 and 42°C) almost immediately after being containerized and continued to emerge between September and December. It is therefore unlikely that the lack of emergence from high-temperature treatments in these experiments was owing to adult recolonization of logs within the container.

Another possibility for the presence of *G. morbida* without *P. juglandis* in certain sample logs of this study is that beetles may have initiated attacks on branches and stems, penetrated the bark sufficiently to allow entry of the pathogen, and then vacated those stems before creation of egg galleries and production of brood. It is uncertain whether this type of behavior is characteristic of *P. juglandis*, but it has been observed or suspected in other bark and ambrosia beetles such as vectors for pitch canker (*Pityphthorus setosus* Blackman and *Pityphthorus carmeli* Swaine, Sakamoto et al. 2007), Dutch elm disease (*Scolytus scolytus* (F.), *Scolytus multistriatus* (Marsham), and *Hylurgopinus rufipes* (Eichhoff), Webber and Gibbs 1989), and laurel wilt disease (*Xyleborus glabratus* Eichhoff, Fraedrich et al. 2008). Alternatively, *G. morbida* may have been present in the tree by some other means than *P. juglandis* transmission. However, *G. morbida* does not move systemically through the tree, is thought to be tightly associated with *P. juglandis*, and has been isolated only from this insect or from the cankers associated with its attacks and galleries (Kolařík et al. 2011).

Baiting freshly cut logs of *J. nigra* with the *P. juglandis* pheromone lure and hanging them in the crowns of trees symptomatic of thousand cankers disease, as was done in the 2012 experiment, was a much more reliable method for obtaining material infested with *P. juglandis* than simply using wood cut from symptomatic trees. In contrast to the poor recovery of beetles from control logs in 2011, *P. juglandis* adults emerged from 95% of the 79 control logs in the 2012 experiment at an average density of 51 beetles per 100 cm² (Table 4). Our experience felling trees with thousand cankers disease in known infested or infected areas of eastern Tennessee suggests that the spatial distribution of *P. juglandis* within a tree is not uniform and there may be long sections of stems or branches that do not harbor beetles. We therefore recommend

use of the baited bolt method for studies of *P. juglandis* that require acquisition of infested material.

Wood-inhabiting fungi can vary widely in their ability to tolerate elevated wood temperatures (Ramsfield et al. 2010). The results presented here suggest that *G. morbida* is relatively sensitive to elevated temperature and is unlikely to survive any heat treatment lethal to *P. juglandis* (Tables 3 and 4; Fig. 1). In contrast, a *Paecilomyces* sp., most likely *P. variotii* which is known to be heat-tolerant (Wang 1990), was isolated from some logs at temperatures as great as 52°C in this study. The specific biological roles of the *Paecilomyces* sp. and other fungi isolated from *J. nigra* bark samples in this study (including an unidentified *Fusarium* sp., *Botryosphaeria*-like fungi, and an unidentified *Trichoderma* sp.) are uncertain. In the nonnative range of *J. nigra* in Colorado, Tisserat et al. (2009) isolated *Fusarium solani* (Martius) Saccardo from the margins of trunk cankers on *J. nigra* affected by thousand cankers disease, demonstrated its ability to cause cankers in inoculation studies, and suggested it may have a minor role in *J. nigra* mortality associated with thousand cankers disease. There may be other potential plant pathogens besides *G. morbida* associated with cankers on *J. nigra* in its native eastern United States range, and studies to discover, identify, and examine the pathogenicity of such potential fungi seem warranted. Although some of the fungi in our samples are not known plant pathogens (e.g., *Trichoderma* and *Paecilomyces* spp.), all of the fungi we detected competed with the growth of *G. morbida* on the PDA plates, presenting challenges to isolation of the target pathogen and prompting our addition of tergitol to the agar in 2012 to slow competing fungal growth. Others who attempt to isolate *G. morbida* from *J. nigra* in the eastern United States should be aware of the possibility of encountering these and other fungi during the isolation process.

Interestingly, the thousand cankers disease pathogen was recovered posttreatment from the sapwood surface of one log in the debarking treatment of the June 2011 experiment (Table 3). This suggests that the *G. morbida* pathogen can occur in the extreme outer sapwood and can survive, at least temporarily, on the surface of a freshly debarked walnut log. Furthermore, although the debarking treatment was implemented by peeling off all phloem with a hand chisel as thoroughly as possible, it was not possible to remove 100% of the bark even on small diameter logs owing to knots, branch stubs, and other forms of included bark. This suggests that debarking alone is not a fully effective phytosanitary treatment for thousand cankers disease. Because *G. morbida* was recovered in the initial experiment, we did not continue to evaluate debarking as a phytosanitary treatment.

In closing, heating *J. nigra* logs to a minimum outer sapwood temperature of 56°C (measured at least 1 cm below cambium) for 40 min is an effective phytosanitary treatment for eliminating the thousand cankers disease vector and pathogen. The current treatment schedule for the emerald ash borer in firewood (60°C core temperature for 60 min) is also more than ade-

quate to control *P. juglandis* and *G. morbida* in walnut firewood. It is important to note that this study did not evaluate the potential for *P. juglandis* to reinfest *J. nigra* logs after they have been heat treated; this question is being addressed in a separate study by the authors. Additional research is needed to build on the treatment efficacy information presented here and to increase the diversity of phytosanitary treatment options available to the walnut wood industry.

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