Phytosanitation Methods Influence Posttreatment Colonization of *Juglans nigra* Logs by *Pityophthorus juglandis* (Coleoptera: Curculionidae: Scolytinae)

J. AUDLEY,[1,2] A. E. MAYFIELD, III,[3] S. W. MYERS,[4] A. TAYLOR,[1] AND W. E. KLINGEMAN III[5]

ABSTRACT Several North American walnut species (*Juglans* spp.) are threatened by thousand cankers disease which is caused by the walnut twig beetle (*Pityophthorus juglandis* Blackman) and its associated fungal plant pathogen, *Geosmithia morbida* M. Kolařík, E. Freeland, C. Utley and N. Tisserat sp. nov. Spread of this disease may occur via movement of infested black walnut (*Juglans nigra* L.) wood. This study evaluated the ability of *P. juglandis* to colonize *J. nigra* wood previously treated with various phytosanitation methods. Steam-heated and methyl bromide-fumigated *J. nigra* logs, as well as kiln-dried natural wane *J. nigra* lumber (with and without bark) were subsequently exposed to *P. juglandis* colonization pressure in two exposure scenarios. Following a pheromone-mediated, high-pressure scenario in the canopy of infested trees, beetles readily colonized the bark of steam-heated and methyl bromide-fumigated logs, and were also recovered from kiln-dried lumber on which a thin strip of bark was retained. In the simulated lumberyard exposure experiment, during which samples were exposed to lower *P. juglandis* populations, beetles were again recovered from bark-on steam-heated logs, but were not recovered from kiln-dried bark-on lumber. These data suggest logs and bark-on lumber treated with phytosanitation methods should not be subsequently exposed to *P. juglandis* populations. Further beetle exclusion efforts for phytosanitized, bark-on walnut wood products transported out of quarantined areas may be necessary to ensure that these products do not serve as a pathway for the spread of *P. juglandis* and thousand cankers disease.

KEY WORDS walnut twig beetle, forest product, quarantine treatment, thousand cankers disease, bark beetle

Several walnut species, particularly black walnut (*Juglans nigra* L.), are currently threatened by thousand cankers disease, caused by an insect–pathogen complex consisting of the walnut twig beetle (*Pityophthorus juglandis* Blackman, Coleoptera, Curculionidae, Scolytinae) and its associated fungal plant pathogen, *Geosmithia morbida* M. Kolařík, E. Freeland, C. Utley and N. Tisserat sp. nov. (Tisserat et al. 2009, Kolařík et al. 2011). Adult male and female *P. juglandis* introduce the pathogen via passive transport to the receptive walnut host as beetles burrow into the bark phloem on tree trunks and branches. *G. morbida* does not move systemically within the host, and repeated introductions are required to produce new cankers (Tisserat et al. 2009). *P. juglandis* is native to the American southwest and historically known from Arizona, New Mexico, and northern Mexico, associated with the range of Arizona walnut *Juglans major* (Torr.) A. Heller (Cranshaw 2011, Seybold et al. 2012a). There is also evidence to suggest the beetle may be native to southern California, where the beetles attack southern California black walnut, *J. californica* S. Watson (Bright 1981, Cranshaw 2011). *J. major* appears to be the least susceptible *Juglans* species (Moltzan 2011, Utley et al. 2013), whereas *J. nigra* and butternut (*J. cinerea* L.) have been shown to be particularly susceptible to the pathogen, developing the largest cankers when inoculated with the fungus (Utley et al. 2013).

In recent decades, the geographic distribution of *P. juglandis* has greatly expanded, with recoveries reported from dead and dying walnut trees, particularly out-planted *J. nigra* throughout the western United States (Cranshaw 2011, Tisserat et al. 2011, Seybold et al. 2012a). *P. juglandis* has also been found in several states in the eastern United States within the native range of *J. nigra* (Cranshaw 2011). Eastern U.S. detections include recovery of both beetle and pathogen in Tennessee (Grant et al. 2011), Virginia, Pennsylvania (Seybold et al. 2012a), North Carolina (Hadziabdic et al. 2014), Maryland (Maryland Department of Agriculture [MDA] 2013), Ohio (Fisher et al. 2013), and Indiana (Marshall 2015). Beetle introductions in areas beyond its native range are hypothesized to have occurred via transport of infested walnut wood and

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[1] Department of Forestry, Wildlife and Fisheries, University of Tennessee, 2506 Jacob Dr., Knoxville, TN 37996.
[2] Corresponding author, e-mail: jaudley@vols.utk.edu.
[3] USDA Forest Service, Southern Research Station, 200 W.T. Weaver Blvd., Asheville, NC 28804.
[4] USDA-APHIS, Center for Plant Health Science and Technology, 1398 W. Truck Rd., Buzzards Bay, MA 02542.
[5] Plant Sciences Department, University of Tennessee, 2431 Joe Johnson Dr., Knoxville, TN 37996.

through the movement of firewood (Newton and Fowler 2009).

In response to the range expansion of *P. juglandis* and threat from thousand cankers disease, states have established quarantines restricting the movement of walnut (Newton and Fowler 2009). For example, in Tennessee, regulated articles restricted from movement out of any county infested with thousand cankers disease include: all plants and parts of plants, except nuts, in the genus *Juglans* including nursery stock, budwood, scionwood, green lumber, logs, stumps, roots, branches, mulch, and any hardwood firewood (TDA 2014). In order to move any of the restricted materials out of a quarantined area, the shipper must obtain a phytosanitation certification or a compliance agreement from the receiving state (Newton and Fowler 2009, TDA 2014).

Effective phytosanitation methods to eradicate the thousand cankers disease causal organisms from infested walnut wood have been reported. Mayfield et al. (2014) demonstrated that heating the outer sapwood (at a depth of 1 cm) of a log to at least 52°C for 40 min killed all life stages of *P. juglandis* and prevented any recovery of *G. morbida*. Because walnut is sometimes comingled with ash in loads of firewood, Mayfield et al. (2014) recommend sanitizing walnut firewood with the same standard (60°C core temperature for 60 min) currently implemented for heat treatment of ash for *Agrilus planipennis* Fairmaire (Myers et al. 2009). Because walnut and ash firewood may be under quarantine in the same area (e.g., much of east Tennessee), utilization of the same standard provides a consistent heat treatment option.

Fumigation with methyl bromide is commonly used as a phytosanitary treatment on logs for export in international trade (Haack and Brockerhoff 2011). Work to develop a methyl bromide treatment schedule for thousand cankers disease has found that treatments of 120 mg/liter at 4.5°C for 48 h are effective in eliminating *P. juglandis* from infested *J. nigra* logs. However, *G. morbida* is capable of surviving this treatment and will require substantially higher rates to achieve quarantine level control (S.W.M., unpublished data).

Although effective phytosanitary measures to eradicate thousand cankers disease have been determined, no work has evaluated the risk of re-infestation of walnut material posttreatment. There is evidence to suggest some beetle species will colonize and persist within previously phytosanitized materials. Haack and Petrice (2009) assessed the ability of bark and wood boring insects (Curculionidae and Cerambycidae) to infest heattreated lumber with bark left on, and found that certain species from both families of wood-attacking insects readily colonized the treated materials.

J. nigra is one of the most economically valuable North American hardwood species, prized for its dark heartwood (Shifley 2004, Newton and Fowler 2009, Moltzan 2011). Total standing U.S. *J. nigra* timber stock for lumber and veneer production is estimated to be >US$500 billion (Newton and Fowler 2009). Continued spread of thousand cankers disease is likely to have significant impacts on lumber, veneer, and other related wood products industries as a result of restrictions on the transport of *J. nigra* and other North American *Juglans* species (Newton and Fowler 2009).

Given the economic, ecological, and cultural importance of *J. nigra* (Newton and Fowler 2009, Moltzan 2011), it is imperative to determine all anthropogenic pathways available to *P. juglandis* and *G. morbida*. Comprehensive assessment of phytosanitation measures can be considered in two parts: First, assessing the efficacy of eliminating a pest or pathogen from infested material, thus rendering material pest free; second, evaluating treatment efficacy in preventing new attack or colonization of the sanitized material. The goal of this study was to assess the latter aspect with respect to the thousand cankers disease system.

In order to determine the risk of colonization of previously treated *J. nigra* wood by *P. juglandis*, two fieldbased experiments were conducted, in which treated samples of walnut were exposed to beetle colonization pressure.

Materials and Methods

Infested Canopy Exposure Experiment. The first experiment involved the exposure of treated samples of *J. nigra* wood ($N = 150$) to *P. juglandis* colonization pressure in the canopies of infested trees in a full factorial experiment. Round-wood logs (bolts; $n = 90$) measuring roughly 61 cm in length and 7–16 cm in diameter and sawn boards ($n = 60$) measuring 61 cm long by 15 cm wide and 5 cm deep, were treated to phytosanitation specifications outlined in the Tennessee Department of Agriculture quarantine regulations for thousand cankers disease (TDA 2014). Bolts were cut on 23 July 2013 from felled *J. nigra* obtained from an orchard in McDowell County, NC (in which no evidence of *P. juglandis* or thousand cankers disease has been reported), and transported to Knoxville, TN, for processing and treatment at the University of Tennessee. Moisture loss was reduced by sealing both ends with paraffin wax (Mayfield et al. 2014). Once sealed, bolts were divided into three groups of roughly equal diameter distributions (visually estimated) and their diameters were measured using calipers and recorded. Groups were then randomly assigned to one of three treatments: steam heat ($n = 30$), methyl bromide fumigation ($n = 30$), or no treatment (control; $n = 30$).

Bolts in the steam heat treatment group were placed on wooden racks and heated in a walk-in kiln (SII Dry Kilns, Lexington, NC). A 4-mm-diameter hole was drilled into each log midpoint and a type K, 22-guage thermocouple was inserted and wedged into the hole with round-wood toothpicks (Taylor and Lloyd 2009). Temperature data were recorded at 1-min intervals using a Keithly Model 2700 multimeter-data acquisition system (Keithley Instruments, Inc., Cleveland, OH) interfaced with a laptop computer (Taylor and Lloyd 2009, Mayfield et al. 2014). The core of each bolt was brought to 60°C and maintained for a duration of 60 min with the kiln vents closed to keep relative humidity near 100% (Mayfield et al. 2014).

Bolts in the fumigation treatment group were placed into two 245-liter, gas-tight sealable steel chambers

housed inside a standard 6.1-m refrigerated shipping container to maintain temperature at 4.5°C. An initial dose of 120 mg/liter methyl bromide (Meth-O-Gas Q, Great Lakes Chemical Inc., West Layfayette, IN) over 48-h exposure period was used based on prior work that had shown this treatment to provide complete control of *P. juglandis*. (S.W.M., unpublished data). Methyl bromide headspace concentrations were monitored during the fumigation using an Agilent 490 micro gas chromatograph (Agilent Technologies Inc.) to ensure target concentrations were maintained throughout the duration of the fumigation. Upon completion of the treatment, the lids to the containers were removed and the bolts allowed to off-gas for 48 h. All treated bolts were housed in the lab for 3–5 d at ~20°C until deployment in the field.

Samples for the two lumber treatments were cut from a *J. nigra* log purchased from, and sawn into boards roughly 2.4 m by 30 cm by 5 cm at the Oak Ridge Hardwoods mill in Oak Ridge, TN. Boards were then cut to roughly 61- by 15- by 5-cm pieces ($n = 60$). Bark was removed from half of the boards for the kiln-dried lumber no bark (KDNB) treatment ($n = 30$), whereas the remaining samples constituted the kiln-dried lumber with bark (KDB) treatment ($n = 30$). Boards were kiln-dried to~8% moisture content following the standard *J. nigra* drying schedule (Boone et al. 1988), and housed in the lab until field deployment.

Knoxville area field sites were selected based on walnut tree symptom severity, accessibility, or previous trapping successes (Mayfield et al. 2014). Thirty trees at 11 sites were selected in which to hang five treatment bolts per tree. Nylon ropes (1.27 cm diameter) were hung in the infested walnut trees using a weighted line thrown over branches in the canopy to hoist each rope into place as described by Mayfield et al. (2014). One *P. juglandis* pheromone lure (product #300000736, Contech Enterprises Inc., Delta, BC) was stapled midway along the length of each sample and a white 7.6- by 17.5-cm sticky card with a 1-cm^2 grid (AlphaScents Inc., West Linn, OR) was attached to the opposite side to confirm the presence of beetles in flight at each site. Samples hung from eye-bolts inserted into each top side were spaced ≥ 2 m apart in the canopy and were deployed for 30 d (5 August to 5 September 2013).

Upon removal from the trees, each sample was cut in half and either the top half or bottom half was randomly assigned to an emergence chamber (the opposing half not assigned to the chamber was assigned for separate analysis). Samples were brushed vigorously with a handheld broom to remove any beetles residing on the surface prior to placement within the emergence chamber.

Emergence chambers were constructed from 18.2-liter paint mixing buckets and a hook was attached to the inside of the lid. Bucket bottoms were cut off and replaced with a funnel leading to a Nalgene collection cup containing propylene glycol to kill and temporarily preserve emerged beetles (Reed et al. 2013, Mayfield et al. 2014). Emerging beetles were collected using paint strainers to filter insects from the propylene glycol every 4 wk over a 5-mo period. Emerged insects were typed using a dissecting stereoscope and all *P. juglandis* were identified using a screening aid (Seybold et al. 2013) and tallied.

Sample halves assigned for further analysis were assessed for colonization of and reproduction within treated material by measuring total *P. juglandis* gallery lengths and counting life stages. A subsample of the middle $^1/_3$ of bark (10 cm) on each steam-heated, fumigated, and control bolt was removed by scoring the bark with a band-saw and peeling back to the cambial layer using a chisel (Fig. 1). Neither lumber treatment was included in this analysis because the source of the log purchased was not known and may have come from an infested area in TN, potentially confounding results. Galleries of adult *P. juglandis* were measured using a Scalex MapWheel (Scalex Corp. Carlsbad, CA). Counts of *P. juglandis* adults, pupae, and larvae were recorded. Only galleries and life stages that could be observed on the innermost surface of the phloem were measured, as neither the phloem nor the bark was further dissected.

Total emergence was divided by the surface area of bark for each sample and mean emergence (beetles/cm^2 of bark) and mean life stage counts (pooled adult, larvae, and pupae counts) were $\log 10(y + 1)$ transformed to ensure homogeneity of variance based on Levene's test ($\alpha = 0.05$). Analysis of variance (ANOVA) was used to test the null hypotheses of no difference in mean emergence between treatments and no difference in mean life stage counts (PROC ANOVA; $\alpha = 0.05$). Post hoc Tukey's HSD tests were performed to identify any differences between means. All $P < 0.05$ were considered significant for all analyses. Statistics presented represent analysis of transformed data; however, nontransformed data are reported in tables and figures. All analyses were performed using SAS V.9.3 with Enterprise Guide software (SAS Institute 2013).

Simulated Lumberyard Exposure Experiment. The second experiment consisted of treated *J. nigra* samples ($N = 80$) subsequently exposed to *P. juglandis* colonization pressure coming from infested logs placed near the treated material on the ground. Treatments included steam-heated bolts, nontreated (control) bolts, KDB, and KDNB. Methyl bromide fumigation was not included in this experiment based on an observed similar performance to steam-heated material from the first experiment. Twenty replicates of each treatment were split between two blocks; Block A in which all samples received the pheromone lure and Block B in which no samples received the lure. Bolts were cut from felled *J. nigra* in McDowell, NC, to roughly 30.5 cm long and ranged between 6 and 17 cm diameter. Cut ends were sealed in the field using AnchorSeal (UC Coatings Corp., Buffalo, NY) and brought to Knoxville, TN, for treatment. Bolts were randomly assigned to steam heat or control groups. Treated samples were heated until the core temperature was raised to 60°C for 60 min as previously described. Control samples were housed in the lab at ~20°C until deployment.
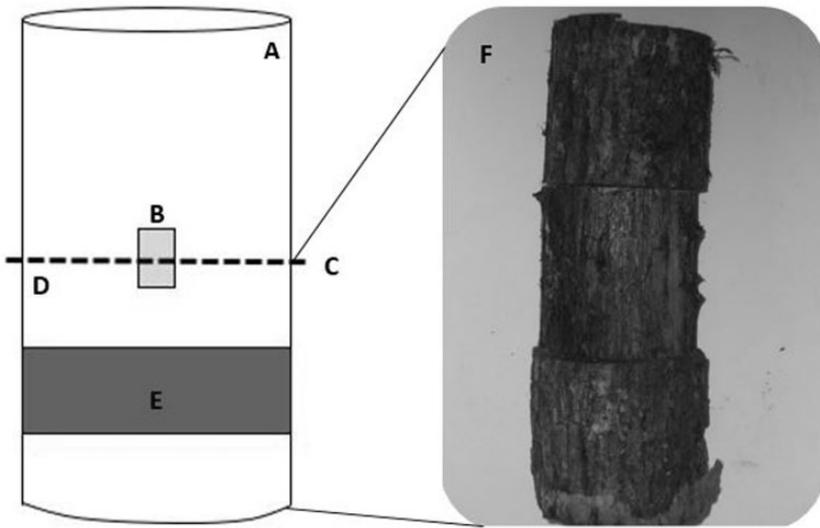
Fig. 1. Diagram of a field-deployed log sample, where (A) is the top half of the log, randomly assigned to an emergence chamber, (B) the box indicates the position at which the pheromone lure was attached, (C) illustrates the cut line dividing the whole log in half, (D) represents the bottom half of the log, assigned to further subsampling, (E) the rectangle indicates the middle third of the lower half where the subsample of bark was taken, and (F) presents the subsample with the bark removed for analysis.

Lumber was once again procured from a log purchased and cut at a lumber mill in Oak Ridge, TN. Boards were sawn from the log as described above and the bark completely removed from half of the samples. Boards were cut to 30.5 cm in length and kiln-dried to 8% moisture content following the same schedule used in the first experiment (Boone et al. 1988).

To create the simulated lumberyard exposure scenario, ten 6-m-diameter plots, each containing five treatments, were arranged along two 38-m-long east-to-west transects. Each plot was spaced 2 m apart (from edge to edge) and the two transects were arranged so that plot perimeters were 10 m apart (Fig. 2). Plots were established on an open field on the Holston River Farm Research Unit located east of downtown Knoxville, TN, along the Tennessee River (35.959055° N, −83.855090° W). No *J. nigra* were located within 200 m of study area.

Two replicates of each of the four treatments were randomly assigned to each of the 10 plots, one to the northern half and the second to the southern half. An infested source bolt was placed in the center of each plot as the source of potential *P. juglandis* colonizers. To infest source bolts, 40–70-cm-long bolts were cut from a single tree at Nebo, NC, and from three trees at the University of Tennessee Arboretum in Oak Ridge, TN. Each bolt was baited with pheromone lure and hung in known infested trees as described in the previous experiment. Bolts were hung for 30 d prior to deployment in the experiment. After 30 d, a 10-cm-long subsample was cut off of the top of each and placed into a rearing chamber in order to estimate the total beetle emergence. Beetle emergence for each subsample was monitored for 8 wk and extrapolated to estimate the total emergence per source log. A source log was randomly assigned to each of the 10 exposure plots and placed on-end in the center.

Samples were exposed to emerging beetles at the field plots for 57 d from 20 August to 16 October 2014. Exposure time was originally planned for 30 d, but was extended based on low beetle emergence from the infested source bolt subsamples. Supplemental beetles (10 M, 10 F beetles at each plot) were released on two occasions to increase the number of potential colonizers. Supplementary release beetles were reared from infested bolts in the lab, transferred to plastic Petri dishes for transport to the field site, and placed onto a small wooden platform set atop each source bolt. Beetles were released between 5:30 and 6:30 pm EST based on reported peak flight activity (Seybold et al. 2012b, Chen and Seybold 2014). After exposure concluded, all samples were placed into emergence chambers for 5 mo.

Total *P. juglandis* colonization pressure per cluster was estimated by calculating the number of beetles that emerged per unit surface area of bark as previously described from each of the 10-cm-long source bolt subsamples (Table 1). The subsample emergence was then extrapolated to the source bolt by multiplying the total number of beetles per subsample by the total surface area of bark for each source log. The two supplemental releases totals (20/cluster/release) were added to the extrapolated beetle emergence to get the total per plot colonization pressure (Table 1).

Data were analyzed using nonparametric ANOVA due to multiple treatments with zero values. The Kruskal–Wallis test was used to test the null hypothesis that there was no difference in mean beetle emergence (number of beetles/100 cm^2 of bark) among treatments within each block, and the Dwass, Steel,
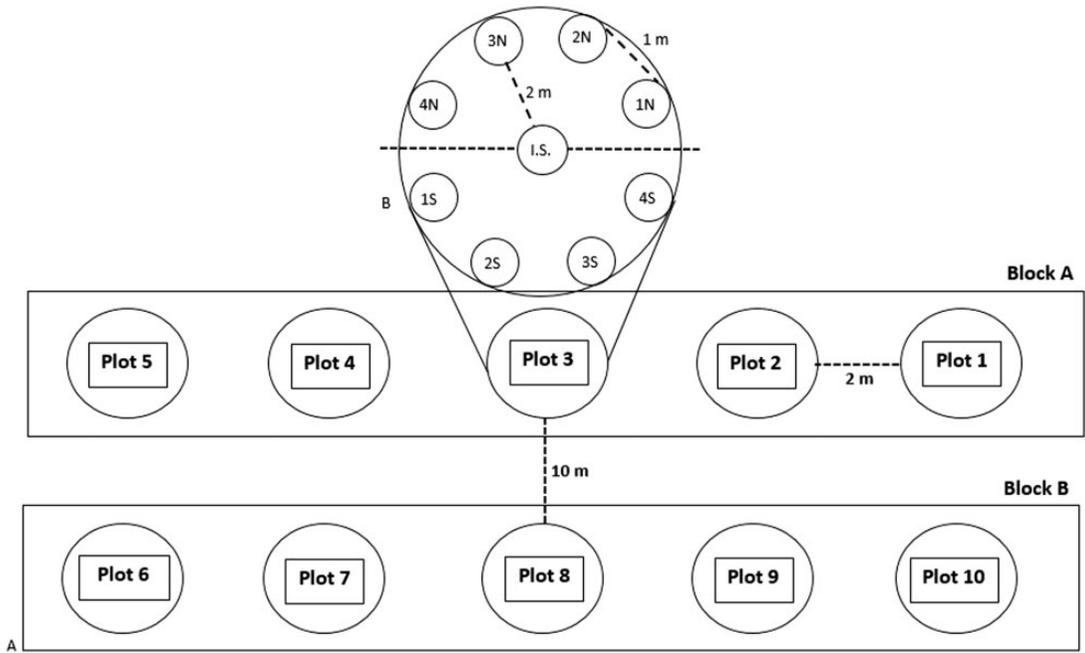
Fig. 2. Diagram of the circular plots arranged on two transects Block A and Block B, each with five plots (A). Detailed setup within a single plot (B) shows the division between the four positions on the northern half of the plot and the four positions on the southern half of the plot. I.S. indicates the location where infested source material was introduced.

Table 1. Number of potential *P. juglandis* colonizers for each circular plot from the simulated lumberyard replication experiment

Circular plot	Block	No. from subsample	Estimated no. from source bolt[a]	Supplemental releases	Total potential colonizers[b]
1	A	24	82	40	122
2	A	14	46	40	86
3	A	15	48	40	88
4	A	344	1,686	40	1,726
5	A	15	56	40	96
6	B	2	7	40	47
7	B	42	137	40	177
8	B	35	112	40	152
9	B	8	26	40	66
10	B	60	210	40	250
				Total	2,810

All samples in plots assigned to Block A were baited with a pheromone lure whereas samples in plots assigned to Block B were left unbaited.

[a] Estimated numbers of potential colonizers were estimated by extrapolating the number of beetles per total surface area of bark emerged from a 10-cm-long subsample of each bolt, multiplied by the total surface area of the source bolt. All beetle estimates are rounded to the nearest whole beetle.

[b] Total estimated number of potential colonizers.

Critchlow-Fligner (DSCF) multiple comparisons procedure was used to identify means that differed between groups in pairwise tests (Hollander and Wolfe 1999). The Wilcoxon two-sample Z-test was used to test the null hypothesis that mean beetle emergence was not different between treatments across blocks (with lure and without lure). All *P* values < 0.05 were considered significant. Nonparametric ANOVA was performed using PROC NPAR1WAY in SAS V.9.3 with SAS Enterprise Guide software package (SAS Institute 2013).

Results

Infested Canopy Exposure Experiment. A total of 43,455 *P. juglandis* emerged from 105 wood samples following posttreatment field deployment (Table 2). The majority, 64%, of beetles were collected from the control samples. Four beetles were recovered in the collection cup of two samples from the KDNB treatment; however, a *t*-test indicated that the mean emergence from this treatment was not significantly different from zero ($t_{1, 29} = 1.28$; $P = 0.21$), and thus,

Table 2. Total number of adult *P. juglandis* emerged from each of the five treatments ($n = 30$) from 5 September 2013 to 5 January 2014 and the number of samples colonized per treatment tested in the infested canopy exposure experiment

Treatment	Total *P. juglandis*	Mean ($\pm$ SE) surface area bark[a]	No. of beetles/100cm² bark	Number of samples[b]
Steam heated	9,509	1,160.6 $\pm$ 42.0	788	29
Fumigated	6,022	1,230.5 $\pm$ 34.7	468	30
KDB[c]	63	79.7 $\pm$ 0.8	81	14
KDNB[d]	4	0 $\pm$ 0	NA	2
Control	27,857	1,094.5 $\pm$ 33.2	2,578	30
Total	43,455		3,914	105

Samples were deployed in 30 trees across 11 sites in the greater Knoxville, TN, area from 5 August to 5 September 2013.
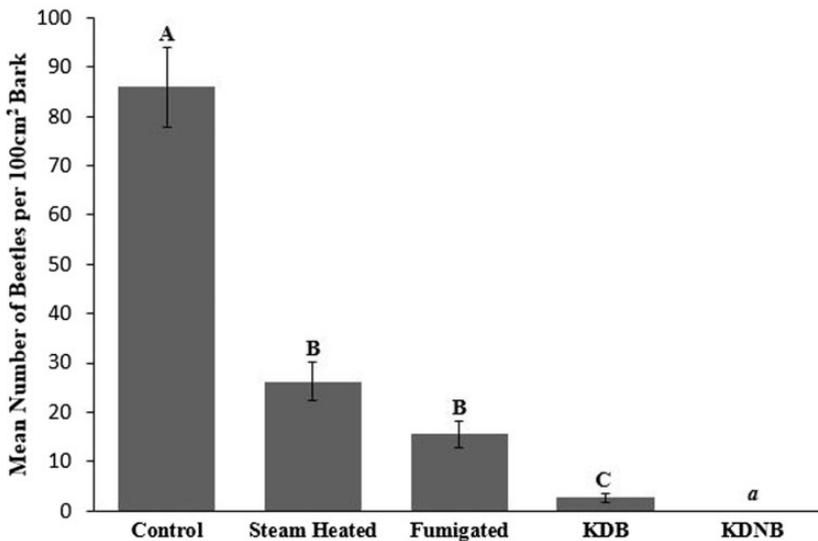Note: all numbers were rounded to the nearest whole beetle.
NA: Not applicable, as there was no bark on these samples.
[a] Surface area of bark in cm².
[b] Number of samples with $\geq$1 beetle emergence.
[c] Kiln-dried lumber with bark intact.
[d] Kiln-dried lumber with no bark.

Fig. 3. Mean number of adult *P. juglandis* beetles/100cm² of bark ($\pm$SE) emerged from *J. nigra* samples treated with various phytosanitation methods ($n = 30$), baited with pheromone lures and hung in the canopies of infested *J. nigra* trees at 11 sites throughout Knoxville, TN, from 5 August to 5 September 2013 during the infested canopy exposure experiment. Emergence was monitored for 20 wk from 5 September 2013 to 5 January 2014. Means labeled with the same letter are not significantly different ($\alpha = 0.05$). [a]Kiln-dried No Bark samples were not included in the ANOVA analysis.

the KDNB treatment was excluded from ANOVA. The mean number of emerging beetles differed significantly by phytosanitation treatment ($F_{3, 116} = 60.23$; $P < 0.01$). Emergence was greatest from the control samples, followed by emergence from steam-heated and fumigated bolts. KDB samples yielded the lowest emergence (Fig. 3).

Mean emergence was plotted over the monitoring period to detect temporal patterns among the treatments (Fig. 4). A similar pattern was observed for the control, steam-heated, and fumigated bolts. Peak emergence occurred within 8 wk and was followed by a steady decline during the remaining 12 wk. Conversely, 87% ($n = 55$) of emergence from the KDB samples occurred within the first 4 wk, followed by an immediate decline (Fig. 4).

P. juglandis gallery lengths differed significantly between phytosanitation treatments ($F_{2, 87} = 44.7$; $P < 0.01$). On average, galleries were twice as long in control samples as in steam-heated samples, and four times longer in steam-heated samples than in fumigated samples (Fig. 5). Mean counts of all *P. juglandis* life stages also differed by treatment ($F_{2, 87} = 6.3$; $P < 0.01$), with control logs harboring more life stages than fumigated samples (Fig. 5).

Simulated Lumberyard Exposure Experiment. Despite an average estimated number of potential colonizers of 281 beetles per plot, only 288 *P. juglandis* emerged from all samples in all four treatments. Approximately 80% of beetles emerged from control bolts and the remaining 20% emerged from steam-heated bolts. Of the beetles emerged from control
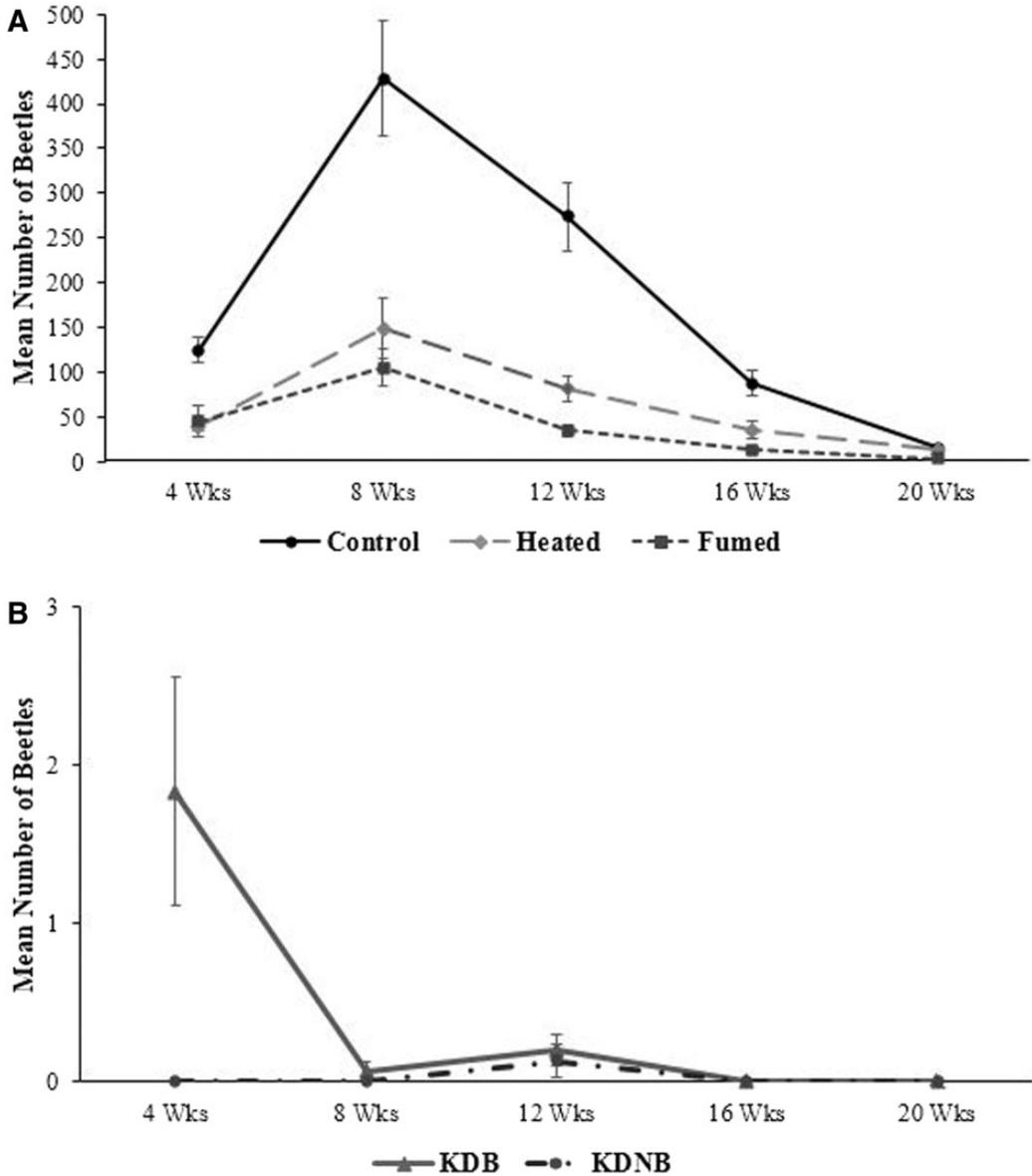
Fig. 4. Mean number (±SE) of *P. juglandis* adults emerged by treatment (*n* = 30) and collection time plotted over 20 wk (5 September 2013 to 5 January 2014) of emergence following deployment in the field at locations around Knoxville, TN, from 5 August to 5 September 2013. (A) Top plot depicts emergence from the control, steam heated, and methyl bromide fumigated treatments. (B) Bottom plot depicts emergence from two kiln-dried lumber treatments, and was plotted on a smaller scale to improve visibility of the emergence trends.

bolts, 80% were collected from bolts in Block A (in which samples were baited with pheromone). Of the 57 beetles collected from steam-heated samples, 46% were collected from samples in Block A and 54% were collected from Block B. All collected beetles were recovered from just eight bolts (10%), representing five controls and three steam-heated samples.

The Kruskal–Wallis test indicated there was evidence of a variation across the treatments in Block A ($\chi^2 = 9.52$; $P = 0.02$); however, the DSCF multiple comparisons did not indicate any pairwise differences.

There was no evidence to indicate a difference among treatments in Block B ($\chi^2 = 2.05$; $P = 0.56$). Similarly, the Wilcoxon two-sample Z-test indicated there was no evidence of variation in mean beetle emergence between any of the treatments across the two blocks.

Discussion

Infested Canopy Exposure Experiment. *P. juglandis* readily colonized the phloem of steam-heated and methyl bromide-fumigated *J. nigra* logs
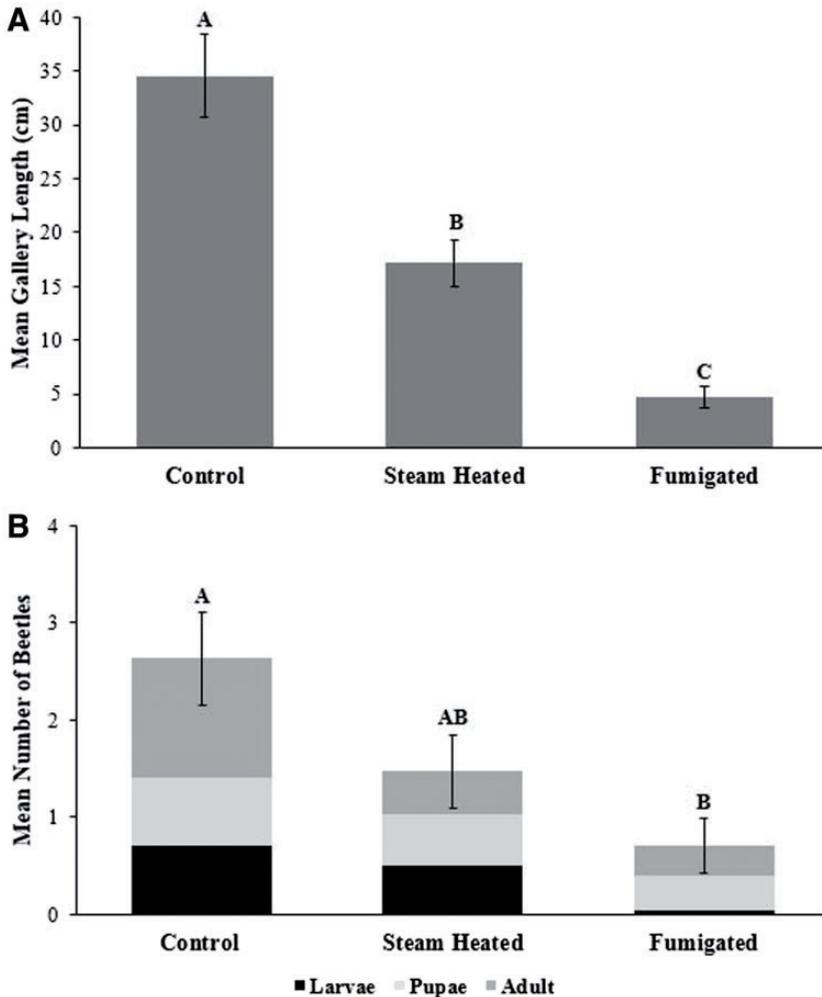
Fig. 5. Mean gallery lengths (± SE) measured from subsample of bark for heated, fumigated, and control samples (A). Different letters indicate different means based on Tukey's HSD test (α = 0.05). Mean number of aggregated *P. juglandis* larvae, pupae, and adults (±SE) by treatment (B). The average number of individuals for each life stage within each cohort is indicated by different colored bars. Different letters indicate different means based on Tukey's HSD test. Means labeled with the same letter are not significantly different (α = 0.05).

that were baited with a pheromone lure. Although treatments reduced the number of beetles that emerged compared with the untreated samples (Fig. 3), residual effects from the steam heat and methyl bromide fumigation sanitation treatments did not prevent beetles from attacking and reproducing within the material. Both mean beetle emergence and mean gallery length of the inner phloem layer are well above zero (Figs. 3 and 5). Thus, the steam heat and methyl bromide fumigation treatments reduce beetle performance in the material but do not preclude subsequent colonization and reproduction.

Recovery of all three life stages from subsamples of the steam heat and methyl bromide fumigation treatments, as well as the timing of peak emergence (Fig. 4) indicates that *P. juglandis* reproduced in the steam-heated and methyl bromide-fumigated logs. Emergence trends for these two sanitation treatments are

very similar to the control logs (Fig. 4), and likely indicate production of subsequent *P. juglandis* generations within the rearing container (Peachey 2012, Nix 2013, Mayfield et al. 2014). Emergence in all three treatments peaked within 8 wk of the beginning of emergence monitoring and is consistent with the previously reported generation time of 7 wk (Tisserat et al. 2009).

While control samples had three times as many beetles emerge as the steam-heated samples (Table 2), the mean number of all *P. juglandis* life stages collected from control and steam-heated bolts was not different (Fig. 5). Another surprising trend observed was the mean gallery length in steam-heated samples was more than four times longer than the mean gallery length in fumigated samples (Fig. 5), despite similar mean beetle emergence in these two treatments (Fig. 3). Once possible explanation for the discrepancy between gallery lengths and emergence when comparing steam-heated

and fumigated bolts may be an uneven distribution of *P. juglandis* colonization throughout a log. Beetles likely congregate in spots along the profile of a log, perhaps in response to specific cues, for example, the male aggregation pheromones (Seybold et al. 2013). If this is the case, the subsample of bark taken to measure gallery lengths may have missed the activity of beetles that were otherwise accounted for in the emergence sample.

Sixty-three beetles were recovered from 14 KDB samples (Table 2) indicating that *P. juglandis* can enter bark dried to 8% moisture content when baited with a pheromone lure. Although there is evidence that beetles can enter and persist for some time within the dried bark, there is no evidence of successful reproduction within the material. Unlike the heated, fumigated, and control bolts, peak emergence occurred within the first 4 wk in the KDB samples then, rapidly decreased to zero (Fig. 4). Despite not being viable material for reproduction, kiln-dried bark may provide a temporary refuge allowing for accidental transport of adult *P. juglandis*.

On the other hand, the KDNB samples were not successfully attacked by the beetles, even when baited with pheromone lure. Four beetles were recovered from the emergence container collection cup for two KDNB samples during a single collection (Table 2); however, it is unlikely that these beetles indicate successful colonization of walnut wood. Carful inspection of both boards following the conclusion of the emergence monitoring did not yield any evidence of beetle entrance holes or galleries. We suspect that beetles landed on the wood in response to the pheromone bait in the field, and then crawled into crevices created from splits within the boards. Both of these samples had >5-cm-long splits that occurred as lumber dried, and beetles may have remained wedged in the crevices, eventually falling into the respective collection cups during the emergence monitoring period. It is also possible that beetles managed to escape from adjacent emergence chambers and either found a way into the two chambers (perhaps at a weak point in the lid–funnel interface which was hot glued together), or into the two collection cups while cups were removed, prior to straining the sample. Inclusion of de-barked lumber was, in part, to act as a negative control, as there is no reason to believe *P. juglandis* would excavate galleries into xylem tissue.

Simulated Lumberyard Exposure Experiment. A majority of samples in both the control and steam-heated groups, and all of the kiln-dried lumber samples were devoid of *P. juglandis* emergence, likely confounding true differences that may exist among the treatments tested. The large number of zero values likely also explains why no difference was detected within treatments across the two blocks (with lure A and without lure B).

Although we estimated an average of >200 potential colonizers per plot (Table 1), these estimations may have been artificially inflated by the method used to predict the number of beetles to emerge per source log. Several researchers observed a later start to the

P. juglandis flight season and lower than expected numbers of *P. juglandis* trap catch across east Tennessee for 2014 compared with 2013 field observations (W. E. K., personal communication). We suspect that the source material used in the lumberyard exposure study was not heavily infested with beetles due to relatively low *P. juglandis* populations in the area in 2014, thus inhibiting the ability to draw conclusions from this experiment.

An alternative explanation could be that the source bolts were actually acting as a beetle sink. Emerging *P. juglandis* may have been more attracted to the source bolt in a neighboring cluster or perhaps were not compelled to leave the source material at all.

Conclusions

Understanding potential pathways of introduction of a quarantined pest or pathogen is a crucial aspect of effective management. Effective phytosanitation treatments that eliminate *P. juglandis* from *J. nigra* logs have been determined (Mayfield et al. 2014, S.W.M., unpublished data). Data from this current study provide evidence that *P. juglandis* can colonize steam-heated and methyl bromide-fumigated bolts when baited with a pheromone lure and exposed to extreme beetle colonization pressure. There does not appear to be a residual treatment effect strong enough to prevent successful colonization or reproduction within these treated materials. We also demonstrated that *P. juglandis* can enter and persist within kiln-dried bark for a short time, though reproduction likely did not occur. These results support Haack and Petrice's (2009) findings that numerous species of bark and ambrosia beetles can colonize previously steam heat-treated logs and wood packing materials.

The ability of the pathogen *G. morbida* to colonize the treated wood material was not addressed in this study. Presence of the pathogen is a key component to the presence of thousand cankers disease. If the fungus cannot develop in the phloem of steam-heated, fumigated logs, or kiln-dried lumber with bark intact, the threat of spreading thousand cankers disease may be reduced even if *P. juglandis* can still colonize the material. Colonization of treated walnut wood should be tested through inoculation studies. However, if beetles can still emerge from the material carrying viable *G. morbida* spores, it will likely not matter from a regulatory perspective. Thus, live fungal recovery from beetles that have emerged from the treated material should also be examined.

Many biological and ecological questions remain for how *P. juglandis* interacts with its host plant and habitat. It is still unclear how the beetle finds and selects a suitable host tree. Bark beetles are known to use host plant volatile cues (Wood 1982), visual cues (Mayfield and Brownie 2013), and even land-and-"taste" cues (Raffa and Berryman 1982) for identifying potential hosts prior to selection. Understanding the combination of cues utilized by *P. juglandis* would provide researchers and forest managers with a better understanding of how host trees are located and selected for

colonization, which in turn would facilitate protection of living walnut trees and commercially transported walnut wood.

These findings indicate that logs and lumber (bark on) treated by approved phytosanitary measures should not be exposed to *P. juglandis* posttreatment. However, results suggest that the risk of *P. juglandis* colonizing kiln-dried bark is significantly less than steam-heated or methyl bromide-fumigated bark, and that this material may represent a low-risk pathway. Further testing is required to determine whether or not *P. juglandis* can colonize kiln-dried lumber with bark left intact when not facilitated with the pheromone lure. Preventing further anthropogenic spread of *P. juglandis* is crucial to protecting *J. nigra* from further damage by thousand cankers disease.

Acknowledgments

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