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Research

Vacuum Steam Treatment Effectiveness for Eradication of the Thousand Cankers Disease Vector and Pathogen in Logs From Diseased Walnut Trees

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Abstract

Logs of high-value eastern black walnut (*Juglans nigra* L.) are commonly exported from the United States for production of veneer and lumber. Veneer logs are not debarked to minimize degradation of wood quality and reduce moisture loss. Thousand cankers disease (TCD) is caused by the walnut twig beetle (*Pityophthorus juglandis* Blackman) and the fungal pathogen, *Geosmithia morbida* M. Kolarik, E. Freeland, C. Utley and N. Tisserat sp. nov., which colonize the inner bark of *Juglans* species. Effective eradication of these organisms by heat or chemical fumigation treatment is required for walnut logs prior to export. Because vacuum steam is an effective and efficient means of heating round wood, its use in eliminating the TCD causal agents was evaluated using *Juglans* logs (12- to 44-cm small end diameter and 1.7- to 1.9-m length) from TCD-symptomatic trees in Oregon and Washington State. Five replicate trials with three logs per load were conducted in a portable vacuum chamber to test two treatment schedules: 60°C for 60 min and 56°C for 30 min. Complete elimination of *P. juglandis* and *G. morbida* was achieved when using a minimum of 56°C at 5-cm targeted depth from bottom of bark furrow into the sapwood and held for 30 min. Treatment cycle time ranged from 298 to 576 min depending on log diameter and initial log temperature. Artificial inoculation of *J. nigra* trees with *G. morbida* within the TCD range in Pennsylvania was minimally successful in producing adequately colonized logs for experimental trials.

Key words: phytosanitary treatment, *Pityophthorus juglandis*, *Geosmithia morbida*, *Juglans nigra*

Eastern black walnut (*Juglans nigra* L.) is one of the most valuable hardwood species in North America (Shifley et al. 2004, Newton et al. 2009). There are approximately 3.4 billion cubic feet of black walnut growing on timber land in the eastern United States, with an estimated value of other $569 billion (USDA Forest Service 2002) and an export market of $325 million (Newton et al. 2009). Although *J. nigra* is native to much of the eastern United States, the Central Hardwood Forest Region is the principal commercial region for this species (American Walnut Manufacturers Association 2017). Both the nuts and timber are of commercial value, and the straight, dark, fine-grained heartwood is prized for use in fine furniture, gunstocks, cabinetry, doors, flooring, and paneling (Newton et al. 2009, American Walnut Manufacturers Association 2017). High-quality veneer also is obtained from high-value eastern black walnut logs that are commonly exported from the United States (US Bureau of Census 2002).

Thousand cankers disease (TCD) is an emerging problem that threatens the health of *J. nigra* and production of nuts and timber from the species. Since discovery of TCD in 2009 (Tisserat et al. 2009), both internal and external quarantines have been established to prevent unregulated export of the canker pathogen, *Geosmithia morbida* Kolařik, Freeland, Utley and Tisserat (Hypocreales: Bionectriaceae), and associated insect vector, *Pityophthorus juglandis* Blackman, (Coleoptera: Curculionidae: Scolytinae) (EPPO 2015, USDA APHIS 2018). To further limit the spread of TCD, fumigation with methyl bromide has been stipulated in state quarantine compliance agreements for domestic movement of walnut products with bark attached from western to eastern states (https://www.thousandcankers.com/industries/). However, concern over methyl bromide use and its destruction of the earth's upper stratosphere ozone layer has led to gradual phase-out of the chemical on a global

scale with certain exemptions (Pizano and Banks 2009). For example, the fumigant has been commonly used for whole log treatment of hardwood logs for export from the United States under quarantine and preshipment use exemption. In a recent ruling, however, the European Union (EU) Commission Decision 2005/359/EC states that the EU will not renew the use of methyl bromide for pre-US export treatment of oak logs for control of oak wilt starting 1 January 2021. Such mandated phase-out deadlines on import restrictions has caused serious concerns for the international log trade industry. A methyl bromide fumigation schedule for *J. nigra* bolts was recently developed and proposed for treatment of walnut for international export (Seabright et al. 2019), but importers of eastern black walnut (e.g., South Korea) do not accept methyl bromide fumigated logs from U.S. states with TCD. Although conventional heat treatment (e.g., kiln-heating) of walnut logs was approved for phytosanitary treatment by South Korea (USDA APHIS 2018), dry heating reduces the quality of the logs, making them unsuitable for veneer. There is an urgent need for an alternative treatment that kills the TCD biotic agents without negatively affecting walnut log quality or the environment.

Steam treatment may provide a viable phytosanitary method for TCD logs but, to date, has only been evaluated using sample blocks of wood and small diameter logs using a heating oven or kiln heat with nearly 100% relative humidity. Oven heating of sample blocks (approximately 25 × 25 × 8 cm in size) from TCD-positive trees in Colorado to 50.1°C at a depth of 3.8 cm beneath the cambium layer for 30 min was sufficient to eradicate walnut twig beetle from the wood (Mackes et al. 2016). Moreover, 2 yr of multiple experiments in Tennessee with small logs (4–18 cm in diameter and 30 cm in length) cut from *J. nigra* trees and colonized by *P. juglandis* and the pathogen, revealed that a minimum temperature of 56°C to a depth of 1 cm below the sapwood surface maintained for 40 min in a high relative humidity chamber (= 'steam-heated') was necessary to kill walnut twig beetle and *G. morbida* (Mayfield et al. 2014). Debarked logs without heat treatment were also included in the 2011 trials, but bark removal failed to eliminate the pathogen from the sapwood surface. Subsequent experiments investigated *P. juglandis* colonization of logs previously steam-heated and natural wane lumber or kiln-dried walnut slabs with bark attached (Audley et al. 2016, Mayfield et al. 2018). In the first case, beetles survived in logs that had been steam-heated, but not in kiln-dried lumber with bark.

Steam treatment evaluated to date involves heating walnut bolts in a kiln to achieve target temperature at the log core while keeping kiln vents closed to create a saturated atmosphere (100% RH) within the chamber (per Audley et al. 2016). Based on results of these studies, we conducted experiments to evaluate the efficacy of vacuum stream (VS) for eradicating walnut twig beetle and *G. morbida* from larger logs of merchantable diameter. In VS, once an initial vacuum (90 mmHg) is established in a VS chamber, saturated steam (85°C) is added and exposure time monitored once target temperature is reached at the predetermined depth (Juzwik et al. 2019). Heat penetration of wood by steam under negative pressure is a more effective and efficient method of heating round wood than steam alone (Simpson 2001).

Heat treatment of oak logs using VS was recently demonstrated to eradicate viable *Bretziella fagacearum* (Bretz) Z.W. deBeer, Marincowitz, T.A. Duong & M.J. Wingfield (Microascales: Ceratocystidaceae), the oak wilt fungus, from large logs obtained from artificially inoculated and naturally infected northern red oak trees (Juzwik et al. 2019). Previous VS trials with logs from five hardwood tree species found minimal end-checking and no discoloration in veneer subsequently cut from the logs following treatment (Chen et al. 2017a). Similar treatment also killed all life stages of the emerald ash borer (*Agrilus planipennis* Fairemaire) in low-quality ash logs and ash firewood (Chen et al. 2017b).

In this study, we used large logs of naturally colonized *Juglans* species in the Pacific Northwest, United States, where TCD is widely established and also evaluated the potential for augmenting *G. morbida* colonization to obtain suitable logs for such studies in an eastern U.S. portion of the disease range. Levels of *P. juglandis* and *G. morbida* colonization of tree stems and large diameter limbs are difficult to predict when selecting source trees for logs to use in such studies. Thus, augmentation or artificially inoculation of logs was used in previous studies to increase *P. juglandis* infestation levels and ensure colonization by the pathogen, respectively (Seabright et al. 2019). Stem inoculations of declining *J. nigra* trees with *G. morbida* several months prior to felling and obtaining study logs could also prove useful for such studies.

The objectives of this study were to 1) evaluate canker formation and rate of pathogen reisolation, as well as *P. juglandis* emergence rates, from stems of black walnuts artificially inoculated with *G. morbida* in areas with low, natural levels of TCD, and 2) determine the extent to which two schedules of VS treatment kill *P. juglandis* and *G. morbida* in large logs obtained from moderately to severely symptomatic *Juglans* trees in residential, park, and plantation settings. Trees used in the experiment for the first objective were growing within the known range of TCD in eastern Pennsylvania, although few symptomatic trees were apparent in the area.

Materials and Methods

Artificial Inoculation Experiment

Study Trees

Visual surveys of eastern black walnut trees in Bucks County, PA, were conducted in early June 2016 but failed to identify 10 suitable trees with evidence of crown dieback typical of TCD. Furthermore, no evidence of cankers was found when outer bark was carefully removed from three branches collected from each of four trees with 10–65% crown dieback in New Hope and Buckingham, PA. However, sparse Geosmithia-like cankers and several *P. juglandis* galleries were found on branches of one tree with 30% TCD-like crown decline in Doylestown, PA. Thus, the possibility of obtaining a sufficient number of naturally infested logs from main stems of walnut trees for VS treatment trials was deemed low. Instead, the opportunity was used to evaluate the potential for using standing trees to artificially inoculate the stems with *G. morbida* for such studies. Six trees (26–39 cm diameter at breast height, dbh) on or near a septic system field in a New Hope, PA, wooded residential lot (latitude: 40.3477°N; longitude: –75.1275°W) and one (31 cm dbh) in the fence-row perimeter of a commercial property in Doylestown (latitude: 40.3112°N; longitude: –75.0506°W) were selected for inoculation with landowner approval (Supp Table 1 [online only]).

Fungus Inoculation

Main stems of each candidate tree were marked with a ring of white paint at 2.6-m intervals to delineate where logs would be cut at time of harvest. These stem sections were relatively straight, free of visual defect, and had no branches or only those of small diameter. The number of marked sections varied by study tree (Supp Table 1 [online only]). Colored nylon string was tied around the stem at two different heights (15 and 137 cm) above the lower paint ring on each delineated log section. As needed, ladders or a platform lift were used to access higher stem sections. The 30-cm-long stem sections

centered over each string were designated for either insect emergence (upper string) or fungus isolation (lower string).

In late May 2016, two *G. morbida* isolates were obtained from margins of fresh *G. morbida* cankers on branches of a TCD-symptomatic tree in Doylestown. Pure cultures were grown on half-strength potato dextrose agar (PDA) in Petri plates for 2 wk under ambient room temperature and lighting conditions. The surface of colonized plates was flooded with sterile distilled water, and the resulting suspension was poured into sterile 50-ml tubes. Spores from two isolates were combined and spore concentration was then determined using a hemacytometer and microscope. Appropriate dilution was made to give a final spore suspension of approximately 1×10^3 conidia/ml. Inoculum was stored at 4°C (for ≤6 d) until used. Viability of inoculum was verified by plating 25-µl aliquots on half-strength PDA in Petri plates and confirming growth.

Stem inoculations were performed on 26–28 June 2016. Portable drills were used to make two staggered rings of eight evenly spaced holes (5 mm diameter), above and below marked locations, through a bark furrow to a depth just slightly into the outer xylem. One aliquot (25 µl) of agitated inoculum was placed into each hole using a pipette. Holes were sealed with small amount of petroleum jelly, covered with moldable epoxy putty, and color-coded thumb tacks used to mark hole locations.

Log Harvesting

On 18 October 2016, the delineated stem sections of each study tree were carefully removed by a tree service company that used an aerial lift to access the trees. The crown and upper stem of each tree beyond the uppermost paint ring were removed first. Then, a padded harness on a rope was secured around the center of a marked log section. The bottom of each stem section was then cut at the lower painted ring and the log was carefully lowered into the bed of a transport truck. Multiple logs from each tree were sequentially obtained from the top down in this manner. All logs were taken to the Southeast Agricultural Research and Extension Center of the Pennsylvania State University in Manheim, PA, for processing. End diameters for each log were obtained and recorded upon arrival.

Beetle Emergence

In late October (20–26), the top marked subsection (30 cm long), hereafter referred to as 'section', was taken from each of 22 logs obtained from main stems of seven harvested and fungus-inoculated *J. nigra* trees. Several of the stem sections were split into smaller pieces because their diameter was too large to fit whole into the bucket. The split faces and cut ends of each section were treated with liquid paraffin (Anchorseal, U-C Coatings, Buffalo, NY) to retain moisture. In total, 22 sections were double bagged (5 mil plastic), sealed, placed in buckets with lids, and transported to Purdue University, West Lafayette, IN on 26 October 2016. An Indiana State Regulatory Permit (15-IN-18-012) granted to M.D.G. allowed for interstate transport of infested walnut from Bucks Co., PA, to West Lafayette, IN. Once at the university biocontainment and rearing facility, stem sections for each sample were transferred into emergence buckets and collections were made twice each week. Emergence buckets consisted of a 20-cm-diameter plastic funnel glued to the cut bottom of a ventilated, 19-liter bucket that was suspended from a wooden rack (Reed et al. 2015). All emerged *P. juglandis* were identified (LaBonte and Rabaglia 2012) and numbers recorded.

Pathogen Analysis

Between October 20 and 24, a subsample (30 cm long) centered on the lower band of inoculation holes was cut from each log and moved to an indoor garage area where the bark was peeled to expose cankers. Each section was placed in a wood cradle, outer bark around each inoculated point was carefully removed using a drawknife, and any observed canker measured and marked. In a laminar flow hood in the adjacent research laboratory, four small pieces of inner bark tissue were removed from the margins of each exposed canker or other observed necrosis and placed in Petri dishes on quarter-strength potato dextrose agar amended with streptomycin sulfate (100 µg/l) and chloramphenicol (100 µg/l). The isolation plates were packaged per conditions stated in the USDA APHIS permit granted to J.J. and transported to the USDA APHIS approved containment laboratory in the U.S. Forest Service, Northern Research Station, St. Paul Annex facility in Minnesota. All plates were then incubated on a lab bench under ambient room temperature (~24°C) and lighting conditions, and examined for fungal growth associated with each phloem tissue piece. Mycelium from suspect *G. morbida* colonies were transferred to half-strength PDA in Petri dishes and subsequent transfers made as needed to obtain pure cultures. Initial identifications of *G. morbida* were based on microscopic examination of condiophores and conidia and on colony appearance (size, color, texture) on PDA (per Kolarik et al. 2011). Verification of *G. morbida* was based on the results of DNA sequencing. Briefly, mycelia from colonies of each isolate were placed in cetyltrimethylammonium bromide and DNA extracted following the method of Lindner and Banik (2009). Beta tubulin gene region primer pairs (GmF3/GmR13) were used for PCR amplification of the DNA per protocol in Moore et al. (2019). Visualization of PCR product was done on 1.5% agarose gel with ethidium bromide incorporated under UV light. PCR products were subjected to Sanger sequencing and resulting sequences compared with accessions in GenBank.

2017 Vacuum Steam Experiment with Naturally Infested Logs

Study Trees

In late August 2017, plantation-grown *J. nigra* trees in Walla Walla Co., WA, that had exhibited moderate to severe crown dieback (50–80%) typical of TCD were selected for the study. These trees were then felled with a chainsaw in mid-to-late November to obtain 24 logs for the VS trials (Table 1). In addition, nine logs were obtained from a pile of long (>3 m) main stems of *J. nigra* that were harvested in mid-November from a plantation 10 km from the first plantation. The disease had been previously confirmed in both plantations from which trees were obtained. After felling, the outer bark of each tree was carefully peeled off in several locations along the main stem to assess the presence and abundance of *G. morbida*-like cankers. One to four logs (each 2 m long) were cut from stem sections with multiple cankers and transported to the nearby treatment location with the VS equipment trailer. The log ends were sealed with liquid paraffin (Anchor Seal, U-C Coatings) to reduce moisture loss. Short sections (0.3 m long) were cut from each log end for pretreatment assessment of viable *P. juglandis* and *G. morbida*. Length, end diameters, and weight of each log prepared for treatment were measured, and the ends were resealed with liquid paraffin. The pretreatment weight of each log was determined using a digital shipping scale (DYMO, Newell Brands, Hoboken, NJ) and recorded. Immediately following pathogen analysis, small wedges (3 cm thick each, approximately 300 g in weight) were removed from the log sections and oven dried at 103 ± 2°C to constant weight to determine moisture content calculated on a dry weight basis.

Treatments

The harvested logs were lined-up on bolsters based on increasing log diameters. Three logs of similar diameters were then placed in each of ten loads for VS treatment and two logs in the nontreated

Table 1. Sites in Washington and Oregon with *Juglans* species trees exhibiting moderate to severe crown dieback and felled to obtain logs for vacuum steam treatment trials, 2017 and 2018

County/state	Site	Latitude (°N)	Longitude (°W)	Setting[a]	Trees cut		No. of logs obtained
					Date	No.	
Walla Walla, WA	Cottonwood Road	46.0171	−118.2991	Plantation	Late Nov. 2017	12	24
Walla Walla, WA	Gray Lynn Dr.	46.0293	−118.3602	Plantation	Mid-Nov. 2017	Unk[d]	9
Lane County, OR	Alder Street[b]	44.0445	−123.0802	Street	Early Nov. 2018	3	6
Lane County, OR	East 14th Avenue	44.0444	−123.0803	Street	Early Nov. 2018	1	2
Lane County, OR	Friendly Avenue 1	44.0379	−123.1054	Yard	Early Nov. 2018	1	4
Lane County, OR	Friendly Avenue 2	44.0317	−123.1054	Street	Early Nov. 2018	1	1
Lane County, OR	Ferry Street	44.0428	−123.0852	Street	Early Nov. 2018	1	1
Lane County, OR	Monroe Street	44.0274	−123.1040	Street	Early Nov. 2018	1	4
Lane County, OR	Peterson Barn Park	44.0706	−123.1595	Park	Early Nov. 2018	1	2
Lane County, OR	Walnut Grove Park	44.0851	−123.1477	Park	Early Nov. 2018	5	8
Lane County, OR	Unknown[c]	—	—	Yard	Late Oct. 2018	Unk.	4

[a]Logs obtained from long stem sections harvested in late November 2017 from an unknown number of TCD-symptomatic *J. nigra* by plantation owner.
[b]*Juglans regia* trees cut at this site: *J. nigra* harvested at all other sites.
[c]Logs obtained from long stem sections harvested in late Oct. 2018 from an unknown number of TCD-symptomatic *J. nigra* by a commercial arboriculture company.

control load. Loads for VS treatment were randomly assigned to treatment schedule. Two treatment schedules (60°C for 60 min; 56°C for 30 min) were tested with five trials conducted per schedule. Control logs were stored outside (ambient temperatures) on wood bolsters during the eight days of experiments. Three logs were secured on a wood pallet and loaded into the custom-built vacuum chamber (Vacutherm Inc., Warren, VT; 1.5 × 1.5 × 3.0 m capacity) that was mounted in the rear of a 6.5 m long enclosed trailer. The portable treatment system consists of a vacuum source (vacuum pump), the vacuum chamber, a steam generator, and a data acquisition system (see White et al. 2017). Thermocouple wires (Omega K-type, TT-K-24, 260°C max.) were inserted into holes drilled to different depths (surface, 3.2 cm, geometric log center) along the length of each log and were sealed with moldable epoxy putty (as per Juzwik et al. 2019). Depths of the drilled holes were measured from the bottom of the bark furrow into the sapwood. The other end of each wire was fed through a port in the chamber wall and connected to a data acquisition system (software and system from Labview, Laboratory Virtual Instrumentation Engineering Workbench, National Instruments, Austin, TX) on a laptop computer located in the forward compartment of the VS trailer. The vacuum was created using a noncontracting 3.73 kW (5 hp) dry screw vacuum pump (Busch LLC, Virginia Beach, VA) equipped with a coolant recirculating system. The pump was used to generate an initial vacuum of 100 mmHg. Saturated steam (85°C) was generated by an 80 kW electric boiler (Reimers Electra Steam, Clear Brook, VA) and added to the chamber once the initial vacuum was reached. The chamber temperature, as well as that of all probes inserted in logs, was monitored in real-time during the entirety of each treatment. Treatment of a load was stopped when the specified treatment time for the target temperature was achieved for all probes placed at 3.2 cm depth. The steam was cut off prior to the designated 30- or 60-min holding period once the target temperature was reached. At the end of the specified treatment period, vacuum was released, and the chamber door opened. Each log load was removed from the chamber and stored on a pallet at ambient (outdoor) temperature until cool. The weight of each log was then obtained and recorded. The percent change in moisture from VS treatment was calculated based on the pretreatment and post-treatment log weights. End sections (30 cm long) were cut with a chainsaw from the post-VS treatment log and from the control logs. A dedicated 'clean' chainsaw was used for cutting sections from the VS-treated logs.

Beetle Emergence

Individual 0.3-m-long sections were taken from each study log before and after treatment. Both ends of each section were coated with liquid paraffin, and the section was placed in 19-liter rearing buckets (as previously described) that were then hung on a wooden frame within a heated shop building in Walla Walla, WA. Collections of emerged beetles were made every 2 wk. All emerged insects from each bucket per collection period were placed in double zip-locked plastic bags, frozen, and then shipped to Purdue University, West Lafayette, IN (Indiana State Regulatory Permit 15-IN-18-012) where they were identified and numbers of *P. juglandis* recorded.

Pathogen Analysis

For the pathogen assay, individual logs were treated as the experimental unit and the cut log sections were treated as an observational unit. One 30-cm-long section was taken from each pretreatment and post-treatment log to assess viability of *G. morbida*. To account for

any environmental effect on *G. morbida* viablilty, we also assayed cankers from each ambient temperature control log at both the beginning and end of the study. Initial processing of sections occurred in a heated, indoor workshop. Each section was placed in a wood cradle secured to a table with two adjustable clamps. The outer bark was removed from the entire log section using a large drawknife (23 cm blade length), followed by carefully peeling into the inner bark using a small drawknife (15 cm blade length) to expose suspected cankers. Each observed canker was circled with a red paint pen and covered with portion of paper toweling secured with thumb tacks. A maximum of 20 cankers were marked per section. The marked logs were then moved to a clean lab area with laminar flow hoods in a nearby building. The drawknife blades were cleaned with 70% ethanol before peeling each log section. Residual bark shavings on the cradles, supporting table, and surrounding floor were discarded between log sections, and the cradles and table surface were cleaned and sprayed with 70% ethanol.

In the laminar flow hood, four small inner bark pieces from the margin of each marked canker were placed on quarter-strength PDA amended with streptomycin sulfate and chloramphenicol. Transport of plates to U.S. Forest Service laboratory in St. Paul, MN, incubation of plates, monitoring for *G. morbida*, subculturing to obtain pure isolates, and identification of the pathogen were conducted as previously described for the 2016 experiment. In addition, any other fungi growing on post-treatment log sections were also transferred to half-strength PDA and subcultured to obtain pure isolates. Identification of these fungi was completed using DNA extraction, PCR, gel imaging, and DNA sequencing of purified products as previously described, except ITS region primers (ITS1F/ITS4) were used for DNA amplification. The number of cankers assayed and number of cankers yielding *G. morbida* were recorded for all VS-treated and control logs.

2018 Vacuum Steam Experiment with Naturally Infested Logs

Study Trees

Four *J. regia* (30–38 cm dbh) and 12 *J. nigra* trees (24–57 cm dbh) in parks and residential areas of the greater Eugene, OR, area that exhibited crown symptoms (30–100% dieback) of TCD in early September 2018, were sourced for VS trials in November 2018 (Table 1). The disease was widespread in Eugene and had been previously confirmed in several of the selected sourced trees. City of Eugene Public Works crews carefully removed major limb and stem portions of multiple trees with a chainsaw for the study. An aerial lift was used to access some trees near utility lines or buildings. Several park trees were felled at their base using a chainsaw and 2.4-m-long logs were cut from the main stems. All logs were loaded into a trailer and transported to the treatment site at Alton Baker Park, Eugene, OR. Four logs were obtained from main stems of TCD-symptomatic trees by a local arboricultural company and delivered to the same site. One 2.4-m section was cut from each of these logs. As in the 2017 experiment, two 30-cm sections were taken from the ends of each log for a pretreatment assay for the pathogen and walnut twig beetle, and wood samples for moisture content determination also were collected. In addition, sapwood width and bark thickness (from outer bark plate and from inner furrow) was measured from one of the walnut twig beetle log sections. The same type of measurements was taken for each now reduced-length log as was done during the 2017 trials. The cut ends were sealed with liquid paraffin before VS treatment.

Experimental Design and Treatments

Logs (n = 30) were sorted by diameter, then three logs of similar diameters were placed in each of 10 loads. As done previously, five loads each were randomly assigned to two treatment schedules: 56°C for 30 min and 60°C for 60 min. Two remaining logs were used as ambient temperature controls and stored outside on wood bolsters. Each log load was inserted into the chamber as previously described. Temperature probes were placed in a similar fashion as previously described with two exceptions; the threshold depth for target temperature was at 5 cm from the bottom of the bark furrow into the sapwood, and an additional probe was inserted into the end grain of the logs at 5 cm depth in the Oregon trial. The latter placement of probes allowed for comparison of thermal gradients at 5 cm depth along the log length to that which was determined using probes inserted through log ends. Specifically, probes were inserted from the log ends at 5 cm below bottom of bark furrow to an interior log depth of 15 cm for 12 logs and 20 cm for 15 logs. These measurements were then compared with the average of the three measurements taken equidistant along log length at the same 5 cm depth.

The same equipment and VS treatment protocol were used in the Walla Walla, WA and Eugene, OR trials. Post-treatment log weights, sampling of log ends for the causal agents, and calculations of percent change in moisture were also conducted in the same manner as previously described. The ends of the 30-cm log sections designated for beetle emergence were coated with liquid paraffin.

Beetle Emergence

In total, 11 pretreatment and 14 post-treatment (56°C for 30 min) log sections and 13 pretreatment and 15 post-treatment (60°C for 60 min) sections were placed in insect emergence buckets, as previously described. All buckets were hung on wooden racks in a heated building at Alton Baker Park (Eugene, OR) and insects collected every other week from 4 December 2018 to 19 February 2019. Emerged *P. juglandis* from each bucket on each collection date were stored in sealed, double-layered plastic bags at –20°C. After being frozen for a minimum of 48 h, collections of emerged beetles from each collection period were shipped in double, zip-locked plastic bags to Purdue University (Indiana State Regulatory Permit 15-IN-18-012) where their identities were confirmed and the number of beetles that emerged from each section per collection period was recorded.

Pathogen Analysis

Similar methods to those described for the 2017 trials were used to assay for viable *G. morbida* with some minor exceptions. Initial processing of 30 cm-long sections was done in a roof-covered (but otherwise open) area outdoors at the treatment site. Sections with exposed cankers (no more than 20 marked for assay) were taken to a heated, indoor park building and attempts to isolate the fungus were made in two, table-top model laminar flow hoods. Isolation plates were packaged as previously described and shipped via courier to the U.S. Forest Service laboratory in St. Paul, MN. Identifications of all suspect *G. morbida* isolates and of all fungi obtained from post-treatment cankers were performed as described for the 2017 trials. Individual logs were treated as the experimental unit and log sections treated as the observational unit.

Data Analysis

Artificial Inoculation Experiment

Regression analysis was used to test the null hypothesis that there was no difference in the mean length of cankers that developed

among inoculated trees. The model was run as a generalized linear model in R (R Core Team 2019), and a means separation test was performed with Tukey's HSD.

2017 and 2018 Vacuum Steam Experiments

For the experiments in Walla Walla County, WA, and Lane County, OR, mixed effects models were used to identify differences in the proportions of cankers yielding *G. morbida* based on plantation location (WA data only) and *Juglans* species (OR data only) prior to VS treatment. Although logs were obtained from TCD-symptomatic *Juglans* trees in multiple types of properties, the log numbers obtained from each type were insufficient to consider location as a variable in the analysis. A generalized linear mixed effects model was also used to determine whether treatment schedule and level of initial pathogen presence had an effect on the number of cankers yielding *G. morbida* following VS treatment (WA data only). Treatment schedule and initial pathogen presence were treated as fixed effects and source tree treated as a random effect. A Tukey pairwise comparison was performed, and odds ratios were calculated for comparing post-treatment isolation rates between treatment regimes. Results of the pre- and post-treatment models were calculated in R using the lme4 package (Bates et al. 2015, R Core Team 2019). Estimated probabilities of detecting the fungus from assayed cankers before and after treatment were calculated using the lsmeans package in R (Lenth 2016). Last, a two-sample *t*-test was used to test the null hypothesis that there was no difference between the side and end temperatures measurements (Forthofer and Lee 1995).

Results

Artificial Inoculation Experiment

Seven *J. nigra* in Bucks County, PA, whose main stems were inoculated with *G. morbida* in mid-June were cut 'stem section by stem section' in October for walnut twig beetle colonization and canker evaluation. Forty-four adult *P. juglandis* were emerged from seven log sections (Table 2).

Necrotic areas were observed at 347 of 352 inoculation points of the lower stem section on the 22 logs. The observed necrotic areas considered as possible *Geosmithia* cankers (n = 329) were characterized as elongate in shape (including elliptical, elongate, oval, rectangular, and long and narrow), either two-toned (brown and dark brown or black) or uniform black in color, and >1 cm in length (7.5 ± 0.18 cm). Five circular cankers were observed but were approximately 1 cm in diameter, black in color, and not considered possible Geosmithia cankers. Black or dark brown tissue ≤1.0 cm in length or diameter and centered over the drilled holes were considered a host response to wounding only.

The percentage of inoculated holes (n = 16) on a log with cankers ranged from 69 to 100, with a median of 94. The mean lengths and SE of these cankers summarized on a tree basis ranged from 3.6 ± 0.2 to 11.2 ± 0.4 cm, with the longest cankers found on logs from tree NH2 based on one-way analysis of variance and Tukey's HSD means separation (Fig. 1).

Of all isolation attempts from necrotic tissue around the holes (n = 347), *G. morbida* was recovered from 10.7% of the inoculated holes representing 21 of the 22 logs (Table 2). More specifically, frequencies of fungus isolation from margins of observed cankers per log ranged from 6.2 to 15.6% for those obtained from New Hope, PA, trees and 19.4% for the logs from the one tree in Doylestown, PA. Pathogen reisolation rate did not differ by tree ($P \geq 0.13$). Eight naturally occurring *Geosmithia*-like cankers associated with walnut

Table 2. *Pityophthorus juglandis* emergence numbers and *Geosmithia morbida* reisolation frequencies for nontreated logs obtained from *Juglans nigra* stems inoculated with the pathogen, 26–28 June 2016, in Bucks County, PA

		Insect emergence[a]			*G. morbida* isolation[b]	
Source tree no.	No. of logs	No. of logs with *P. juglandis*	Total no. of beetles emerged	No. of points assayed	No. of points yielding fungus
DT-1	2	2	20	31	6
NH-1	4	1	17	64	4
NH-2	3	0	0	47	3
NH-3	3	1	1	47	4
NH-4	3	0	0	48	4
NH-5	3	2	6	47	6
NH-6	4	0	0	64	10

Fungus reisolation attempts were done on 20–24 October of the same year, whereas insect rearing occurred between 27 October and 30 December 2016.

[a]Insects emerged from 0.3-m stem sections placed in 19-liter plastic containers with funnel and collection cup.

[b]Only 15 drilled holes were found when evaluating one subsample (30-cm section) of one log from trees DT-1, NH-2, NH-3, and NH-5 in late October. Otherwise, 16 drill holes were assayed for logs from the remaining trees. Attempts were made to isolate the fungus from margin of necrotic tissues surrounding each inoculation hole, regardless of whether a canker was observed or not.

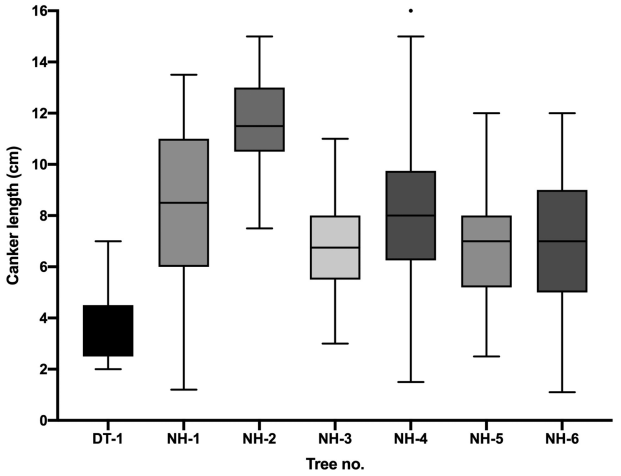

Fig. 1. Mean length of cankers surrounding inoculated points on log sections cut from living *Juglans nigra* main stems 3 mo after treatment with *Geosmithia morbida* in Bucks County, PA. Aqueous suspensions of the pathogen were placed into eight drilled holes in each of two offset rings (7.5 cm apart) on six trees, 26–28 June 2016.

twig beetle galleries (i.e., not associated with systematically inoculated 'rings') were observed on one log from tree DT1 and the pathogen was isolated from the margins of two cankers.

Vacuum Steam Temperature and Moisture Content Statistics

Temperatures documented at different depths for individual logs in each test load of the trials in Oregon and Washington showed expected differences in thermal gradients from bark surface to log core over the treatment time period (see representative profiles in Fig. 2A and B). Because multiple factors (e.g., initial log temperature, log diameter) affect time to reach target temperature at the predetermined threshold depth, individual profiles for each log in each load were documented. For example, the target temperature of 60°C at 5 cm depth at mid-log length was achieved in 370 min for a 28.5 cm small end diameter of one log (Fig. 2B), whereas the 56°C target temperature was achieved in 380 min at mid-log length for a 24.8 cm small end diameter of a different log (Fig. 2A) in the Oregon trials. No differences were found in average temperatures at 5 cm depth (from bottom of bark furrow) for either the three side (i.e., along log length) measurements or the average temperatures at the same 5 cm depth for measurements taken at either 15 or 20 cm distance from end surface into log interior ($P = 0.85$ for 15 cm; $P = 0.37$ for 20 cm; Supp Table 2 [online only]). For example, average end measurement for 15 cm depth ranged from 58 to 66°C compared with average side temperatures that ranged from 57 to 67°C for 15 logs at one time point in the 56°C for 30-min treatment trials.

Treatment cycle time for 56°C for 30 min and 60°C for 60 min at 3.2-cm target depth in Washington trials ranged from 2.9 to 5.5 h and 3.8 to 6.8 h, respectively (Supp Table 3 [online only]). When treating to 5 cm depth in the Oregon trials, the cycle times for 56°C for 30 min and 60°C for 60 min varied from 5.0 to 7.7 h and 7.4 to 10.6 h, respectively (Supp Table 4 [online only]). The rate of heating logs ranged from 2.4 to 12.2 min/°C/kg of log mass depending on log size and initial log temperature. The average energy required to heat to 56°C for 30 min and 60°C for 60 min was 13.45 and 16.31 kWH, respectively, in Washington. For the Oregon trials, average energy consumption was 51.90 and 65.38 kWH for 56°C for 30 min

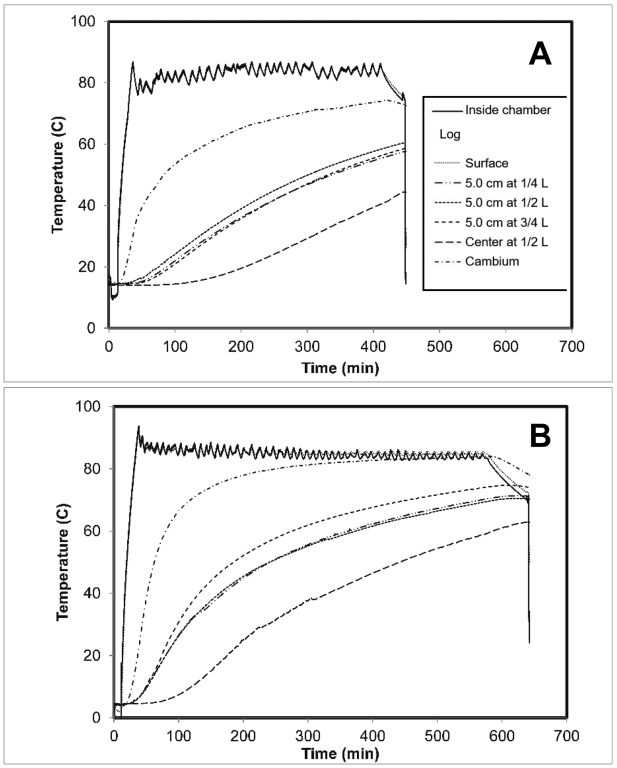

Fig. 2. Temperature profiles of (A) 56°C for 30 min treatment of a *Juglans nigra* log (24.8 cm small end diameter), and (B) 60°C for 60 min treatment of a *J. nigra* log (28.5 cm small end diameter, Lane County, OR, at an initial vacuum of 90 mm Hg. Temperature profiles are based on average temperatures acquired every 5 min during the vacuum steam treatment.

and 60°C for 60 min, respectively. The average increase in moisture content of the treated logs ranged from 1.7 to 4.3% depending on treatment schedule and log size.

2017 Vacuum Steam Experiments

The logs treated in the Washington trials were approximately 1.8 m long. Diameters (not including outside bark) for the small and large ends of the logs ranged from 11.7 to 22.5 cm and 12.7 to 23.0 cm, respectively, and pretreatment log weights ranged from 18.0 to 61.4 kg (Supp Table 5 [online only]). Mean wood moisture content of pretreatment logs was 73.6 ± 2.5% and was minimally affected by the VS process (decrease of 0.7 ± 0.3% in 56°C for 30 min schedule; increase of 1.4 ± 0.3% in 60°C for 60 min schedule).

In total, 734 adult walnut twig beetle emerged from nine pretreatment log sections. Over 98% were from logs obtained from the Gray Lynn Road site. When pretreatment data were summarized by treatment, between one fifth and one third of the logs yielded walnut twig beetle adults (Table 3). No *P. juglandis* were emerged from VS-treated logs post-treatment, but were emerged from one control log.

The pathogen was obtained from cankers on all study logs in the pretreatment assays. The overall mean frequencies of *G. morbida* isolation from pretreatment log sections were 48.3% ($n = 180$ cankers assayed) for Gray Lynn Road logs and 41.4% ($n = 418$ cankers assayed) for the Cottonwood Road log sections. Data for two logs (one from each VS schedule) were removed from the data set prior to statistical analyses due to low numbers of cankers observed (≤6). There was no difference in the likelihood of isolating the pathogen from cankers on logs obtained from the two locations ($P = 0.35$)

based on expected log odds from model estimates (Supp Table 6 [online only]).

When pretreatment isolation data were summarized by treatment, between 39 and 47% of the assayed cankers yielded *G. morbida* in culture (Table 3). In comparison, less than 2% of cankers assayed following treatment with either VS schedule yielded the pathogen, whereas 30% of assayed cankers on control log sections yielded *G. morbida*. Frequencies of fungus isolation for post-treatment logs were similar for the two VS schedules ($P = 0.3000$), but these differed from those for the ambient temperature control logs ($P < 0.0001$) based on the results of generalized linear mixed effects model analysis (Table 4). Isolation of the pathogen prior to treatment had no effect on the outcome of the post-treatment assay ($P = 0.6260$). The estimated probabilities (logit transformed) of isolating the fungus from these same logs by treatment are as follows: 0.0029 ± 0.0031 SE (95% confidence interval [CI]: 0.0003, 0.02) for 56°C for 30-min schedule; 0.015 ± 0.0083 (95% CI: 0.054, 0.044) for 60°C for 60-min schedule; and 0.27 ± 0.11 SE (95% CI: 0.11, 0.52) for control logs. When all other variables were kept constant, the odds of success (shown in parentheses) of isolating *G. morbida* from cankers on post-treatment logs were significantly lower for 1) 56°C for 30 min VS-treated logs than control logs ($P < 0.0001$, –0.0434 to 1), and 2) 60°C for 60 min VS-treated logs than control logs ($P = 0.0001$, 0.0082 to 1; Supp Table 7 [online only]).

Overall frequency of non-*G. morbida* fungi isolated from canker margins was determined only for post-treatment samples for the Walla Walla VS-treated logs. For the four isolation attempts made from each canker and placed on one agar plate, only 9% ($n = 535$ canker subsamples assayed) yielded any fungal growth following either 56°C for 30-min treatment or 60°C for 60-min treatment. The fungi (identified to genus or general group) were all Ascomycetes (11 genera) belonging to the Sordariomycetes, Dothidiomycetes, Eurotiomycetes, and Saccharomycetes classes. Species in the genus *Cladosporium* (Clapnodiales: Davidiellaceae) were most commonly observed on wood chips following the treatment based on morphological characteristics and DNA sequencing.

2018 Vacuum Steam Experiments

The logs treated with VS in the Oregon trials ranged in length from 1.66 to 1.86 m (1.77 ± 0.01 m). Diameters (not including outer bark) for the small and large ends of the same logs ranged from 16.1 to 44.0 cm and 19.1 to 57.2 cm, respectively, and pretreatment log weights ranged from 27.8 to 169.2 kg for those ($n = 26$) that did not exceed the 181-kg weight limit of the scale (Supp Table 8 [online only]). Bark thickness for the logs ranged from mean maximum of 1.58 ± 0.60 cm to mean minimum of 1.22 ± 0.32 cm. The mean width and SD of the sapwood was 4.13 ± 2.13 cm. Mean wood moisture content of the pretreatment logs was 75.9 ± 1.5%, with a very slight gain associated with the treatments (0.8 ± 0.9 kg for 56°C for 30-min schedule logs and 1.8 ± 0.3 for 60°C for 60-min schedule logs).

In total, 2,397 adult walnut twig beetle emerged from 10 pretreatment log sections and none from the two control logs (Table 5). Of the pretreatment sections that yielded beetles, none emerged from the corresponding post-treatment section of those logs. However, two beetles emerged within the first two collection periods from post-treatment log sections. No beetles emerged from the corresponding pretreatment sections from that tree, suggesting that they were killed by the treatment and were dislodged from the log sections after they were placed in emergence buckets.

Table 3. Summary of *Pityophthorus juglandis* emergence numbers and *Geosmithia morbida* isolation results for logs from two plantations with thousand cankers disease-affected *Juglans nigra* trees before and after vacuum steam (VS) treatment, Walla Walla County, WA, December 2017

		Pre-treatment[c]				Post-treatment[c]			
		P. juglandis		*G. morbida*		*P. juglandis*		*G. morbida*	
Treatment schedule[a]	No. of logs[b]	No. of logs with *P. juglandis*	Total no. of *P. juglandis* emerged	No. of cankers assayed	No. of cankers yielding *G. morbida*	No. of logs with *P. juglandis*	Total no. of *P. juglandis* emerged	No. of cankers assayed	No. of cankers yielding *G. morbida*
56°C/30 min	15	3	216	266	125	0	0	268	4
60°C/60 min	15	5	512	272	107	0	0	270	2
Control	3	1	6	60	28	1	7	60	18

[a]For VS treatments, when target temperature was reached at 3.2 cm depth in all logs in a load, it was held for the prescribed amount of time. Control logs were stored outdoors on bolsters at ambient temperatures over the duration of the trials.

[b]Logs (approximately 1.8 m long; 11.7–22.5 cm diameter of small end) were from 12 trees in one location and an unknown number of trees in a second location. Logs were randomly assigned to treatment loads.

[c]Emergence of *P. juglandis* was conducted with 30-cm-long sections from each log. Isolation of *G. morbida* was attempted from margins of no more than 20 cankers per 30-cm-long section from each log. If fewer than 20 cankers were observed, all cankers present were assayed.

Table 4. Coefficients of the generalized linear mixed effects model fitted to the *Geosmithia morbida* isolation data for *Juglans nigra* logs treated with either 56°C/30 min or 60°C/60 min vacuum steam (VS) for post-treatment log sections in the Walla Walla County, WA, 2017 experiments

Coefficient	Estimate	SE	*P*-value
Intercept	−4.3607	0.6344	<0.0001
60°C/60 min. VS	−0.9383	0.9046	0.3000
Control	3.2493	0.7470	<0.0001
Pretreatment *G. morbida*	0.2411	0.4880	0.6260

The 56°C/30 min is the reference for both variables in the model. Pretreatment *G. morbida* isolation level is a covariate.

Geosmithia morbida was isolated from at least one canker on the pretreatment sample sections taken from all study logs. The overall mean frequencies of pathogen isolation from cankers were 60% (n = 480) for *J. nigra* logs and 70% (n = 160) for *J. regia*. Although one log of each *Juglans* species was used for the two controls, different ratios of *J. nigra* to *J. regia* were used in the five experiments for each of the VS treatment schedules because similar log diameter was desired for all logs within a three-log load. On a treatment basis, the mean frequencies of pathogen isolation from cankers were 68.7% (n = 300) for 56°C for 30 min schedule, 56.0% (n = 300) for 60°C for 60 min schedule, and 65% (n = 40) for the ambient temperature controls. Data for two logs in two different 60°C for 60 min loads were removed from the data set prior to statistical analyses due to low number (<5) of pathogen-positive cankers. Removal of these outliers improved fit of the model and regression residuals. There were no differences in the likelihood of isolating *G. morbida* from cankers on pretreatment *J. nigra* versus *J. regia* logs (P = 0.71) based on log odds ratio analysis.

Geosmithia morbida was not isolated from any cankers on post-treatment sections of logs subjected to 56°C for 30 min (n = 300 cankers assayed) or 60°C for 60 min (n = 300 cankers assayed) VS treatment at the 5-cm target depth (Table 5). In contrast, the pathogen was isolated from a similar number of cankers on each of the control logs that were sampled at the beginning and end of the experiment.

Frequency of non-*G. morbida* fungi isolated from canker margins were documented only for post-VS treatment logs in Oregon. For the four isolation attempts made from each canker and placed on one agar plate, 23.9% (n = 590 cankers) produced non-*G. morbida* fungi. These latter fungi were all Ascomycetes (five genera) belonging to the Dothidiomycetes, Eurotiomycetes, Saccharomycetes, and Sordariomycetes classes. Of the species present on wood chips following treatment, species in the genera *Cladosporium*, *Penicillium* (Eurotiales: Trichocomaceae), and *Aspergillus* (Eurotiales: Trichocomaceae) were the most abundant.

Discussion

Main stems of living *J. nigra* that were inoculated to obtain suitable pathogen-colonized logs for VS heat treatment trials in Pennsylvania were marginally useful. Although several morphological types of small cankers developed around most of the inoculation holes within 4 mo, *G. morbida* was reisolated from canker margins at a low rate (<11%). Therefore, subsequent treatment trials were scheduled for geographical locations where TCD was moderate to severe in intensity. Interestingly, freshly cut *J. nigra* bolts (9 cm diameter ×

Table 5. Summary of *Pityophthorus juglandis* emergence numbers and *Geosmithia morbida* isolation results for logs from thousand cankers disease-affected *Juglans nigra* and *J. regia* trees before and after vacuum steam (VS) treatment, Lane County, OR, November 2018

		Pre-treatment[c]				Post-treatment[c]			
		P. juglandis		*G. morbida*		*P. juglandis*		*G. morbida*	
Treatment schedule[a]	No. of logs[b]	No. of logs with *P. juglandis*	Total no. of *P. juglandis* emerged	No. of cankers assayed	No. of cankers yielding *G. morbida*	No. of logs with *P. juglandis*	Total no. of *P. juglandis* emerged	No. of cankers assayed	No. of cankers yielding *G. morbida*
56°C/30 min	14/15[c]	4	1,491	300	206	1	1	290	0
60°C/60 min	15	7	906	300	168	1	1	300	0
Control	2	0	0	40	26	0	0	40	28

Source trees were in park, residential, and street locations of the greater Eugene area.

[a]For VS treatments, when the target temperature reached at 5.0 cm depth in all logs in a load, it was held for the prescribed amount of time. Control logs were stored outdoors on bolsters at ambient temperatures over the duration of the trials.

[b]Logs (1.66–1.86 m long; 16.1–44.0 cm diameter of small end) were randomly assigned to treatment loads.

[c]Emergence of *P. juglandis* was conducted with 30-cm-long sections from each log (n = 14). Isolation of *G. morbida* was attempted from margins of no more than 20 cankers per 30-cm-long section from each log (n = 15). If fewer than 20 cankers were observed, all cankers present were assayed.

60 cm length) have been successfully inoculated with *G. morbida* in another study that evaluated methyl bromide fumigation, but these small diameter bolts were not representative of commercial-size material typically considered for treatment and trade (Seabright et al. 2019).

In both Washington and Oregon trials, both treatment schedules were effective at eradicating *P. juglandis* from naturally infested, larger logs of *J. nigra* and *J. regia*. For pretreatment log sections that yielded beetles, no beetles emerged from corresponding post-treatment sections, suggesting that walnut twig beetle is killed by VS treatment. Two dead adult walnut twig beetles were collected from post-treatment in the Oregon trial; however, there was no corresponding pretreatment section for those logs with which to compare. An examination of temperature records for each of these logs revealed that the beetles likely experienced lethal temperatures reported in Colorado studies, i.e., 50.1°C (Costanzo 2012, Peachy 2012). These beetles may have been on the bark surface at the time of treatment and then killed by the VS treatment and dislodged from the bark surface after the log sections were placed in emergence buckets. In an evaluation of heat treatment to eliminate *P. juglandis* and *G. morbida* in small diameter logs, Mayfield et al. (2014) collected one dead adult walnut twig beetle from a log that experienced a treatment temperature of 56°C and similarly concluded that this beetle was likely killed by the treatment and then fell passively from the log after it was placed in an emergence bucket.

Elimination of viable *G. morbida* was achieved only for Oregon logs (100% for both schedules). The Oregon logs experienced temperature thresholds at a depth of 5 cm from the bottom of bark furrow compared with Washington logs where target threshold temperature reached a depth of only 3.2 cm. In the latter trials, the pathogen was isolated from 2.5 to 5% of cankers on three logs subjected to the 56°C for 30-min schedule and 2.5% of cankers on two logs subjected to the 60°C for 60-min schedule. It was clear that the greater target depth in the Oregon trials provided the necessary combination of time and temperature to mitigate *G. morbida* to a quarantine-safe level.

In comparison to other studies on TCD using some form of heat to kill *G. morbida* and walnut twig beetle, complete elimination of live *P. juglandis* was achieved when infested sample blocks of walnut (sapwood with bark attached) were placed in a dry heating oven and exposed to a temperature of 50.1°C at a 3.8 cm depth beneath the cambium layer for 30 min (Mackes et al. 2016). In a series of Tennessee trials, Mayfield et al. (2014) used heat treatment in a dry kiln with vents closed (relative humidity approaching 100%) for small bolts of *J. nigra*. This saturated steam-heat treatment of the bolts to a minimum outer sapwood (1 cm below cambium) temperature of 56°C that was held for 30 min resulted in complete elimination of both *P. juglandis* and *G. morbida*. In our study, complete elimination of both fungus and beetle was achieved with vacuum and steam combination when a minimum of 56°C was achieved at 5 cm depth (from bottom of bark furrow into the sapwood) and held for at least 30 min. Conventional dry heat would not be used on high-quality logs due to negative effects resulting from drying and checking. The data presented in this study support the development of a VS treatment schedule that specifically targets the causal organisms (*P. juglandis* and *G. morbida*) of TCD in the inner phloem region of *J. nigra* logs.

Probing temperature at a depth of cm at the cut ends of the log and again at least 15 cm into log interior from the cut ends of the logs allowed us to predict the temperature at that depth along the length of the log. Thermal gradients within logs during heat treatment are significant from the surface to log centers. However, there are no consistent or significant temperature gradients along the log length at the same depth from the log surface, when temperatures are uniform throughout the chamber based on earlier studies (Chen et al. 2017a, Juzwik et al. 2019). Based on the results of the current Oregon study, internal log temperatures could be reliably monitored by inserting probes into the end of the log at the target depth from the log surface. In a future commercial application of VS, the ends of logs will be more accessible for probing than the sides of logs because of the way logs would be loaded into treatment chambers or freight containers. In addition, temperature probing of logs from the end would allow access to all logs in a stack.

As expected, the total time required for treatment cycles was greater for 60°C for 60-min schedule than for 56°C for 30 min and for loads with larger diameter logs (Supp Tables S3 and S4 [online only]). Although not directly compared, we expect that treatment time would increase as depth of the target point for threshold temperature increases.

Overall, VS treatment resulted in slightly higher wood moisture content than in pretreatment logs. It is likely that most of this moisture was absorbed by the thick bark on walnut logs. Though negligible, this change in wood moisture content would likely be desired for logs destined for veneer. In fact, logs are customarily placed in hot water 'cooking vats' for several hours prior to processing to increase the moisture content of the wood and making it easier to slice (A.D., personal communication). As further evidence that steam heating is an accepted and desirable practice on walnut and will not negatively affect the quality, premium lumber from walnut saw logs is often steamed to invoke heartwood pigment migration through the inner sapwood in an effort to improve commercial width category and subsequent value (M.W., personal observation). As previously mentioned, traditional kiln-heating accompanied with drying cycles leads to reduced wood moisture content in round wood and would not be suitable for veneer logs. Kiln-heating, however, of rough-cut lumber is an effective means used for killing the walnut twig beetle and *G. morbida* in walnut without reducing its commercial value (Mackes et al. 2016). In contrast, the vacuum and steam process is an effective and efficient method of heat-treating both rectangular and round wood (Simpson 2001). Steam-heat treatment (kiln heat applied in chamber with near 100% relative humidity) as described by Mayfield et al. (2014) and Audley et al. (2016) differs from the VS process. In the former, the steam is produced as heat drives water out of the logs in the chamber and the closing of vents prevents escape of the endogenously generated steam. With this scenario, the mechanism of heat transfer and distribution using steam at atmospheric pressure is conduction. In comparison, VS involves the introduction of exogenously generated steam into a negative atmosphere (i.e., under vacuum). The heat transfer or distribution mechanism using a vacuum is via pressure gradients, which is considerably faster and distributes heat more uniformly than the previously described method. Furthermore, steam saturation occurs at lower temperatures with VS process compared with the steam-heat treatment. Chamber temperatures are lower (80–90°C) in a vacuum compared with 100°C at atmospheric pressure with steam-heat treatment. Thus, the adverse effect of temperature on log quality is less when heating with steam in a vacuum. Based on the above discussion, vacuum and steam would be the preferred method of heating when considering a commercially viable mitigation method for logs. The vacuum-facilitated heating of round wood is also superior to penetration by chemical fumigants such as sulfuryl fluoride (Tubajika and Barak 2011).

Schedules for effective methyl bromide fumigation of *P. juglandis* and *G. morbida* infested *J. nigra* bolts were recently published (Seabright et al. 2019). The authors from that study qualified their

findings as requiring additional data on full-size, commercial logs. Even with supporting efficacy data on TCD causal organisms, methyl bromide is undergoing a substantial phase-out among countries that are actively involved in log trade with the U.S. market. Most recently, the EU deadline on accepting methyl bromide treated oak logs from the United States has prompted a coordinated effort to identify treatment solutions for immediate placement (R.M., personal observation). Efforts are focusing on methyl bromide alternatives for logs to maintain bi-lateral trade between trading partners into the foreseeable future. Vacuum steam treatment offers the advantages of minimal environmental harm, eliminates human health concerns during treatment and during treatment chamber opening, and could require fewer hours for treatment compared with methyl bromide. Furthermore, production and availability of methyl bromide is decreasing over global concerns and agreements to eliminate or greatly curtail the use of this ozone-depleting chemical (Pizano and Banks 2009).

In summary, subjecting larger *J. nigra* and *J. regia* logs to either 56°C for 30 min or 60°C for 60 min VS treatment at a 5-cm targeted treatment depth into the sapwood eliminated *G. morbida* and *P. juglandis* from infested logs. The potential for shorter treatment times and minimal environmental and human health impacts compared with methyl bromide fumigation warrants further evaluation of VS process, including scaled-up trials in operational-level settings that lead to test shipments with industry and trading partners. The process is particularly useful for veneer logs of species such as walnut and oak where bark is preferentially retained but can also apply to high-value saw logs of these and other species.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

Supp. Table S1. Descriptions of *Juglans nigra* trees selected for artificial inoculation of main stems with *Geosmithia morbida* in Doylestown and New Hope, PA, in mid-June 2016.

Supp. Table S2. Correlation between the average of three side temperature measurements and measurements from the end of the log and a depth of 5 cm from the log side surface during the heat treatment of *Juglans* logs with vacuum steam in November 2018, Lane County, Oregon.

Supp. Table S3. Total vacuum steam treatment time for either 56°C for 30 min or 60°C for 60 min to 3.2 cm depth from bottom of bark furrow into sapwood of *Juglans nigra* logs for Walla Walla County, WA, experiments, December 2017. Initial 90 mm Hg pressure and saturated steam at 90°C.

Supp. Table S4. Total vacuum steam treatment time for either 56°C for 30 min or 60°C for 60 min to 5.0 cm depth from bottom of bark furrow into sapwood of *Juglans nigra* and *J. regia* logs for Lane County, OR, experiments, November 2018. Initial 90 mm Hg pressure and saturated steam at 90°C.

Supp. Table S5. Characteristics of logs cut from *Juglans nigra* plantation trees selected for vacuum steam treatment in Walla Walla County, WA, November 2017.

Supp. Table S6. Coefficients of the generalized linear mixed effects models fitted to the *Geosmithia morbid*a isolation data for cankers from *Juglans* species logs sampled prior to vacuum steam treatments in Walla Walla County, WA, in November 2017 and Lane County, OR, in November 2018.

Supp. Table S7. Estimated odds ratios from model of *Juglans nigra* logs yielding viable *Geosmithia morbida* in post-treatment samples of the two vacuum steam treatments () and the ambient temperature control for Walla Walla County, WA, 2017 experiments. Odds ratios calculated using Tukey method for comparing treatment levels. Values are averaged for two location sources of the logs.

Supp. Table S8. Characteristics of logs from *Juglans nigra* and *J. regia* street, park and yard trees selected for vacuum steam treatment in Lane County, Oregon, in November 2018.

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