Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

1 **Vacuum steam treatment eradicates viable *Bretziella fagacearum* from logs cut**
2 **from wilted *Quercus rubra***

3

4 Jennifer Juzwik, Northern Research Station, USDA Forest Service, St. Paul, MN; Anna
5 Yang, Department of Forest Resources, University of Minnesota, St. Paul; Zhangjing
6 Chen and Marshall S. White, Department of Sustainable Biomaterials, Virginia
7 Polytechnic Institute and State University, Blacksburg; and Sarah Shugrue and Ronald
8 Mack, Otis Laboratory, USDA Animal and Plant Health Inspection Service, Plant
9 Protection and Quarantine, Buzzards Bay, MA.

10

11 **Corresponding author:** Jennifer Juzwik, jjuzwik@fs.fed.us

12

13 **ABSTRACT**

14 Methyl bromide (MB) fumigation is required for U.S. *Quercus* log exports due to concern
15 over unintentional spread of *Bretziella fagacearum*. MB alternatives are needed due to
16 the chemical's ability to damage the earth's ozone layer. Vacuum steam (VS) is an
17 environmentally-friendly method that was evaluated for its ability to eradicate *B.*
18 *fagacearum* in logs (24 to 61 cm dia; 2.1 m long) obtained from *Q. rubra* that had wilted
19 following natural infection (NI) or artificial inoculation (AI). Five replicate loads of two NI
20 and one AI logs were VS-treated at 56°C for 30 min and 60°C for 60 min (at 5.0 cm
21 sapwood depth). Mean frequencies of pre-treatment fungus colonization ranged from
22 12.5 to 24.4% in NI and 29.4 to 45.6% in AI logs based on isolation from wood chips of
23 inner and outer sapwood, respectively, of two disks per log. Frequencies of pathogen

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

24 DNA detection were similar to those for isolation. No viable pathogen or its DNA were
25 detected in post-treatment logs. Treatment times ranged from 5 to 9 h for the 56°C/30
26 min schedule and 8 to 10 h for the 60°C/60 min schedule. Based on these results, VS is
27 worthy of further development as a MB alternative.

28

29 **INTRODUCTION**

30 Oak wilt, caused by *Bretziella fagacearum* (Bretz) Z.W. deBeer, Marinc., T.A. Duong,
31 and M.J. Wingf. (syn. *Ceratocystis fagacearum* [Bretz] Hunt), is an important disease of
32 *Quercus* species known to occur only in the eastern USA and of regulatory concern
33 within and outside the country. It is not known to occur in any other countries.
34 Fumigation with methyl bromide (MB) is required for oak logs destined for export from
35 the USA due to the concern over potential, unintentional spread of the pathogen. The
36 current fumigation schedule (240 g/m^3 for 72 h) for oak logs is one of the highest
37 recommended schedules for quarantine and pre-shipment (QPS) commodity use
38 (USDA APHIS 2016). MB was officially recognized as a controlled ozone-depleting
39 substance in a 1992 amendment to the Montreal Protocol, an international treaty under
40 the United Nations Environmental Programme (UNEP 2002). Signatories of the
41 agreement recognized two phase-out dates, 2005 for industrialized countries and 2015
42 for developing countries, for most uses of the chemical. However, an exemption was
43 made for the use of MB for QPS purposes. In 2009, the use of MB for QPS was
44 recognized as the largest use category of any halogenated gas known to cause severe
45 damage to the earth's ozone layer (Pizano and Banks 2009). This same review also
46 estimated that 21% of all MB applied for QPS purposes was used for controlling

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

47 quarantine pests in whole logs. Thus, alternatives to MB for oak log treatment are
48 urgently needed.

49 Alternative fumigants and heat treatments have been evaluated for toxicity to the
50 fungus or for their ability to eradicate viable *B. fagacearum* in oak logs for over five
51 decades (Partridge 1961; Jones 1973; MacDonald et al. 1985; Schmidt et al. 1997;
52 Tubajika and Barak 2011; Uzunovic et al. 2017). After oak wilt was first discovered
53 (1942), several countries would not import oak logs from the USA unless they were
54 USDA-certified to be from outside the known disease range or had been kiln-dried so
55 wood moisture was ≤ 20%. Although conventional hot air is useful for phytosanitary
56 treatment of some wood products (e.g. firewood), it is not acceptable for logs due to
57 deleterious degrade issues, e.g. checking and discoloration in veneer logs (Chen et al.
58 2017a). Hot air and hot water immersion treatments were reported to eradicate *B.*
59 *fagacearum* from 0.9 m long main stem sections cut from trees ranging from 28 to 42
60 cm dia at 1.4 m height (dbh) (Jones 1973). Sections were from three different red oak
61 species that had wilted following artificial inoculation with the pathogen. To our
62 knowledge, no further testing with commercial size logs or operational-scale testing
63 using the proposed schedules has been reported. The current MB schedule used for
64 treatment of oak logs for export from the USA is based on a series of trials conducted in
65 the late 1970's and early 1980's (e.g. Schmidt 1983; MacDonald et al. 1985), and was
66 specifically designed to target the sapwood zone where *B. fagacearum* is found.
67 Alternative fumigants have been investigated for their ability to eradicate viable *B.*
68 *fagacearum* from oak logs. However, analysis of fungitoxicity as a function of
69 concentration x time (CT) has often been complicated by issues surrounding

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

70 penetration (e.g. high wood moisture, bark adsorption) (UNEP 2002; Woodward and
71 Schmidt 1995). Sulfuryl fluoride (SF) has been evaluated in both laboratory and larger
72 log studies as an alternative to MB since the early 1990's for the fumigant's ability to kill
73 *B. fagacearum* in colonized oak sapwood (Woodward and Schmidt 1995; Schmidt et al.
74 1997; Tubajika and Barak 2011; Uzunovic et al. 2017). Although SF penetration of wood
75 is superior to MB, higher treatment rates and application at higher temperatures than
76 normally used for MB are required (Scheffrahn et al. 1992). In a recent trial, *B.*
77 *fagacearum* in logs (1.2 m long) from naturally infected and from artificially inoculated *Q.*
78 *ellipsoidalis* E.J. Hill was not completely eradicated by SF treatments (280 g/m^3 and 320
79 g/m^3 for 72 h) (Juzwik et al. 2017). Based on trials with *B. fagacearum* – colonized wood
80 blocks of red oak, maple, poplar, birch and pine, methyl iodide was found to be more
81 effective than SF in killing the pathogen (Tubajika and Barak 2011). Methyl iodide has
82 been shown to kill *B. fagacearum* at rates similar to MB (Tubajika and Barak 2006).

83 Non-chemical alternatives for commercial oak log treatment are also of current
84 interest. Pest and pathogen risks associated with imported hardwood veneer logs have
85 been mitigated in New Zealand using hot water and steam treatment (Pizano and Banks
86 2009). In a recent study, vacuum and steam (VS) processing was used to sanitize low
87 quality ash logs and ash firewood (Chen et al. 2017b). Research has also been
88 conducted on vacuum and steam processing (200 mm Hg vacuum and steam heat of
89 56°C to the log core for 30 min) of three veneer grade red oak logs (mean dia 50 cm;
90 mean length 2.5 m long), which exhibited some end-checking to a depth of 2.5 cm
91 following treatment, but no color change was detected in veneer samples following log
92 slicing (Chen et al. 2017a). The time required for treatment ranged from 15.5 to 26 h,

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

93 which is substantially less than the 72 h required for current commercial treatment with
94 MB (USDA APHIS 2016). Based on results of previously cited studies, outdoor
95 experiments were undertaken to evaluate the ability of the VS process to kill *B.*
96 *fagacearum* in logs from larger diameter *Q. rubra* L. trees using a portable treatment
97 chamber. The specific objectives were to: 1) determine the rate of *B. fagacearum*
98 isolation from two sapwood depths prior to VS treatment for logs from naturally-infected
99 (NI) and artificially-inoculated (AI) *Q. rubra* that had completely wilted by time of tree
100 felling, 2) determine the pathogen isolation rates following treatment at either 56°C for
101 30 min or 60°C for 60 min to 5.0 cm depth into the wood, and 3) document
102 temperatures achieved at selected log depths and time required for each treatment
103 regime. A preliminary report has been published (Yang et al. 2017).
104

105 MATERIALS AND METHODS

106 **Study trees**. Between May and July of 2016, six *Quercus rubra* (43.9 to 54.6 cm dbh)
107 that had been infected naturally with *B. fagacearum* were selected in a 16.23 ha forest
108 stand in East Farmington, WI. The stand was scheduled for harvesting as part of a
109 timber sale during winter 2016. In addition, four healthy *Q. rubra* (41.91 to 55.4 cm dbh)
110 were selected in the same stand for artificial inoculation with *B. fagacearum*. In late April
111 2016, one *B. fagacearum* isolate was obtained from sapwood of northern red oak in
112 East Farmington, WI that had completely wilted during summer 2015 and a pure culture
113 was grown on potato dextrose agar for 14 days under ambient laboratory temperature
114 and lighting. Endoconidia were harvested by flooding the culture surface with sterile
115 dH_2O and filtering the resultant suspension trough sterile cheese cloth. The spore

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

116 suspension was quantified using a hemacytometer and diluted with sterile H_2O to a
117 concentration of approximately 1 x 10^6 conidia/ml. Trees were inoculated in early June
118 2016 using the following protocol. Soil was manually removed to expose three primary
119 roots. One inoculation point was selected per root at approximately 0.3 m distal to the
120 root collar. A 2.0 cm deep hole was drilled into the roots with a sterile 0.64 cm dia drill
121 bit. A 1.0 ml spore suspension was applied through a 5.0 ml pipette tip, which was held
122 in place by using moldable epoxy putty. After uptake of the inoculum by the tree, the
123 holes were sealed with the putty and roots were covered with the original soil. The AI
124 trees were monitored through the summer of 2016 to document crown wilt progression.
125 All study trees were felled in early September 2016. The main stems were cut into 2.1 m
126 long logs and delivered to the treatment location at a biomass processing facility in
127 Shakopee, MN.

128 **Log description and experimental design.** The ends of each log in the designated
129 loads scheduled for treatment on a particular day were trimmed (22 cm length removed)
130 and 8 cm thick disks then taken from each end for pre-treatment evaluation. The bark
131 depth (outer bark plate to cambium), minimum bark depth (bottom of furrow to
132 cambium), and depth from cambium to sapwood-heartwood boundary were measured
133 at eight locations around each disk. Lastly, the length, end diameters, and weight of the
134 now reduced-length logs (< 2 m) from NI trees (n = 20 logs) and AI trees (n = 10 logs)
135 were obtained.

136 The vacuum steam treatment schedules that were evaluated for ability to kill viable
137 *B. fagacearum* in the logs were: 1) 56°C for 30 min, and 2) 60°C for 60 min. Each
138 treatment schedule was replicated five times, with three logs treated in each test load

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

139 (two NI and one AI logs per load). Logs of similar diameter were used in each load. Two
140 AI logs were left untreated to determine whether ambient outdoor conditions reduced
141 fungal viability over the time-course of the trial.

142 **Log sampling and sample processing.** The disks removed from the study logs prior
143 to log measurement and vacuum steam treatment were subsampled for pathogen
144 assay. Bark was removed at locations around the circumference of each log disk using
145 a sterile chisel. Eight locations were opened to the outer sapwood alternating with eight
146 that were opened to the innermost sapwood. Care was taken so that the inner sapwood
147 locations were one or two annual rings distal to the heartwood boundary. Each sapwood
148 location was assayed for viable *B. fagacearum*. Four small (6.4 mm^2) sapwood pieces
149 were excised from vascular-stained sapwood using a sterile wood gouge and inserted in
150 semi-selective medium (Barnett 1953) in Petri plates. Plates containing sapwood chips
151 were incubated up to 21 days at room temperature (approximately 24°C) under ambient
152 lighting and were routinely monitored for presence of the fungus. Positive cultures were
153 identified by the presence of brown to olive-green colonies, characteristic fruity odor,
154 and the presence of endoconidia distinctive of *B. fagacearum* on the medium.

155 Approximately 1.0 ml of drill shaving samples were collected from undisturbed
156 sapwood of the same locations per Banik et al. (2013) to detect fungal DNA using a
157 nested PCR protocol (Yang and Juzwik 2017). This method determined presence of
158 both viable and non-viable *B. fagacearum*. Drill shavings in 2.0 ml microcentrifuge tubes
159 were covered with CTAB lysis buffer (1.4M NaCl, 0.1M Tris-HCl, 20 mM EDTA, 2%
160 CTAB, pH 7.0) and stored at -20°C until further processed. DNA was extracted from the

161 drill shavings following the technique of Banik et al. (2013). Amplification and
162 sequencing of DNA was completed following Yang and Juzwik (2017).

163 Following treatment, logs were removed from the chamber and allowed to cool at
164 ambient outdoor temperatures. When the logs were cool to the touch, disks were
165 removed from the ends of each log as previously described. Isolation of *B. fagacearum*
166 was attempted and collection of drill shavings was repeated in the same manner as
167 described for the pre-treatment samples. Sapwood samples were also removed from
168 pre-treatment and post-treatment sample disks for moisture content determination.
169 Samples were oven-dried (approximately 103°C) to constant weight. The average
170 moisture content from each log (two disks per log) was calculated on a dry weight basis.

171 **Temperature and energy consumption monitoring.** Holes were drilled to different
172 depths along the length of each log and Omega K type thermocouple wires (TT-K-24;
173 260°C max) were inserted to the bottom of each hole and wire sealed with moldable
174 epoxy putty (Chen et al. 2017a) (Supplemental Fig. 1). Real time thermal mapping data
175 of temperature profiles within the logs during treatment was acquired from the
176 thermocouple wire probes and recorded. Energy usage for each test was measured
177 using a line-powered ELITEpro XC (Dent Instruments, Bend, OR) power meter that was
178 installed at the testing location. Energy use was recorded as kilowatt hours (kWh), then
179 converted to kWh per kg of wood treated (kWh/kg).

180 **Vacuum steam treatment.** Logs were end-sealed with liquid paraffin (Anchor Seal, U C
181 Coatings, Buffalo, NY) prior to vacuum steam treatment. The treatment system
182 consisted of an 80 Kw electric boiler (Reimers Electra Steam, Clear Brook, VA), a 5 Hp
183 dry claw vacuum pump (Busch LLC, Virginia Beach, VA) and a custom built vacuum

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.
Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018

184 chamber (Vacutherm Inc., Warren, VT) (1.5 m x 1.5 m x 3.0 m capacity) that was
185 mounted in the rear of a 6.5 m long enclosed trailer as described in White et al. (2017).
186 All treatment replicates began with an initial vacuum of 100 mmHg. Once the initial
187 vacuum was reached, saturated steam (85°C) was added to the chamber. The chamber
188 temperature was monitored and maintained through the entirety of the treatment until
189 target temperature was reached for all probes placed at the 5.0 cm depth (the maximum
190 depth of sapwood for the logs). Each treatment was stopped when the specified
191 treatment time for the target temperature was achieved at the 5.0 cm depth. The steam
192 was then shut off for the specified holding period, then the vacuum was released and
193 the chamber door opened so that steam and condensate could be evacuated.

194 **Data summarization and statistical analysis.** Generalized linear effects models
195 (Agresti 2002) were used to identify differences in detected colonization from the outer
196 and inner sapwood in naturally infected and artificially inoculated trees using isolation
197 and nested PCR. The mixed effects model has the form:

$$Y_{ijkl} \sim Binomial\ (16, P_{ijkl})$$

$$Logit\ (Y_{ijkl}) = \mu + I_i + D_j + ID_{ij} + \alpha_k + \gamma_{l(k)}$$

198 where μ is the overall mean, *I* is the infection type (natural or artificial), *D* is the sapwood
199 depth (outer or inner), α is the error associated with tree number, and γ is the error
200 associated with log number. All calculations were carried out using R (version 1.0.143;
201 R Foundation for Statistical Computing, Vienna). Odds ratios and estimated probabilities
202 of detecting the fungus from the outer and inner sapwood of naturally infected and
203 artificially inoculated trees were conducted using the lsmeans package in R (Lenth

204 2016). A fixed effect for log number was added to both models to determine if the
205 position of a log within tree had an effect on detected colonization.

206 Additional generalized linear models were developed to determine if differences in
207 pathogen detection existed between trees of the same infection type. The models have
208 the form:

$$Logit\ (Y_{kl}) = \mu + T_k + \alpha_k + \gamma_{l(k)}$$

209 where μ is the overall mean, *T* is tree number, *D* is the sapwood depth, α is the error
210 associated with tree number, and γ is the error associated with log number. A Tukey
211 pairwise comparison test was used to control for multiple comparisons between trees
212 within either of the infection types.

213 **RESULTS**

214 **Log characteristics.** Following the removal of pre-treatment disks for measurement
215 and assay, the logs ranged from 1.72 to 1.93 m in length (Supplemental Table S1). The
216 small end diameter (inside bark) ranged from 23.5 to 49.5 cm and the log weights
217 ranged from 86.1 to 408.5 kg. The mean bark depth was 1.57 cm and the mean
218 distance from the bark furrow to the cambium was 0.70 cm. The average width of the
219 sapwood of pre-treatment disks was 0.93 cm.

220 **Pathogen presence in logs.** *B. fagacearum* was isolated from at least one of four
221 chips taken from each assayed location on disks from NI *Q. rubra* logs before VS
222 treatment. The mean rates of isolation were 24.4% of the outer sapwood isolation
223 locations (n = 160) and 12.5% of the inner sapwood locations (n = 160) (Fig. 1; Table
224 1). In logs from AI trees, the fungus was isolated from at least one of four chips from

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

225 each assayed location in 45.6% of the outer sapwood locations (n = 80) and in 29.4% of
226 the inner sapwood locations (n = 80) (Table 1). Frequencies of fungus isolation differed
227 by tree infection type (P = 0.0010) and sapwood location (P = 0.0410) (Fig. 2; Table 2).
228 The estimated probability of isolation was highest for samples taken from the outer
229 sapwood of AI trees and lowest for samples obtained from inner sapwood of NI trees
230 (Table 3). When all other variables were kept constant in each comparison, the odds of
231 success (shown in parentheses) of isolating *B. fagacearum* from study logs were
232 significant for: (a) AI versus NI trees (2.1 to 1; P = 0.0001), (b) inner versus outer
233 sapwood locations (0.6 to 1; P = 0.0003), (c) inner sapwood locations of AI versus NI
234 trees (2.3 to 1; P = 0.0010)), and (d) outer sapwood locations of AI versus NI trees (1.9
235 to 1; P = 0.0035). The position of the sampled log within a tree had no effect on the
236 detected colonization in the isolation assay. No differences (P ≥ 0.2762; Tukey pairwise
237 comparison) were found for pathogen presence among logs within NI trees or within AI
238 trees.

239 *B. fagacearum* was detected in logs of NI *Q. rubra* using the nested PCR assay in
240 one tube of composited wood shavings for each disk location in 21.0% and 11.0% of the
241 samples from the outer (n = 160) and inner sapwood (n = 160) locations, respectively
242 (Table 1). The fungus was detected by nested PCR in 33.0% of the outer sapwood (n =
243 80) locations and from 7.0% of the inner sapwood locations (n = 80), in logs from AI
244 trees. Frequencies of fungus detection differed only by sapwood location (Table 4). The
245 highest estimated probabilities of pathogen detection by nested PCR were similar for
246 outer sapwood locations of both tree infection types and lowest probabilities were
247 similar for inner sapwood of both infection types (Table 5). When all other variables

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

248 were kept constant in each comparison, the odds of success (shown in parentheses) of
249 detecting *B. fagacearum* from oak wilt- killed *Q. rubra* were significant only for inner
250 versus outer sapwood locations (0.3 to 1; $P < 0.0001$). No differences in detection were
251 found for (a) NI versus AI trees ($P = 0.6514$), (b) inner sapwood locations of NI versus
252 AI trees ($P = 0.6961$), and (c) outer sapwood location of NI versus AI tree logs. The
253 position of the sampled log within a tree had no effect on the colonization detected by
254 nested PCR. For the nested PCR assays, no differences ($P \geq 0.1079$; Tukey pairwise
255 comparison) were found for pathogen presence among logs within NI trees or within AI
256 ones.

257 The pathogen was not isolated or detected by nested PCR from any logs after VS
258 treatment of either 60°C for 60 minutes or 56°C for 30 minutes. *B. fagacearum* was
259 isolated from at least one of four chips in 43.8% of the outer sapwood locations (n = 32)
260 and 31.3% of the inner sapwood locations (n = 32) of two untreated (= control) logs for
261 disks taken on the same day pre-treatment samples were obtained for the first logs that
262 were treated. In comparison, the fungus was isolated from one or more wood chips
263 taken from 28.1% of outer sapwood locations (n=32) and 18.8% of inner sapwood
264 locations (n = 32) for disks taken from the same two control logs at the conclusion of the
265 experiments. Thus, fungus isolation rates for the control logs stored outdoors decreased
266 by 15.7 to 12.5% for outer and inner sapwood locations, respectively, over the six days
267 during which the treatments were performed

268 **Temperatures achieved, time and energy required for vacuum steam treatment.**
269 The average time for the 5.0 cm target probes to reach 56°C and hold for 30 min was
270 6.9 h (Table 6) and the average time to reach 60°C and hold for 60 min was 8.6 h.

271 Examples of temperature profiles for each treatment schedule are shown in Fig. 3. The
272 average energy consumption to treat to 56°C for 30 min and 60°C for 60 min was 25.4
273 kWh and 36.7 kWh respectively (Table 6).
274 **Sapwood moisture content.** Moisture content of sapwood in pre-treatment disks
275 ranged from 72.9 to 98.3% with an average of 87.6%, as calculated on a dry weight
276 basis (data not shown). In disks sampled after treatment, sapwood moisture contents
277 ranged from 75.3 to 102.4% with an average of 87.5%. Moisture content of the sapwood
278 from log disks decreased by 0.1% (ave.) following vacuum steam treatment.
279

280 **DISCUSSION**

281 *B. fagacearum*-colonized logs used in this study were obtained from recently wilted,
282 41 to 61 cm diameter *Q. rubra* that had become infected through natural means or from
283 artificial inoculations with the pathogen by the authors. Pathogen-colonized logs in
284 previous log treatment studies were taken from one or the other of these two infection
285 types (Jones 1973; Schmidt 1983; MacDonald et al. 1985; Woodward and Schmidt
286 1995; Schmidt et al. 1997). This study offers a direct comparison of colonization levels
287 and patterns in logs from both types. Furthermore, the presence of the pathogen was
288 assayed using a standard isolation protocol as a measure of viable fungus level and by
289 using a direct pathogen DNA detection technique (extraction from sapwood and
290 amplification using nested PCR) as a measure of both viable and non-viable fungus
291 presence. Previously published studies relied on the standard isolation method only.
292 Pre-treatment isolation rates from our study logs differed by tree infection type and by
293 sampled sapwood depth. Rates were higher for logs from AI trees than those from NI

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

294 trees and higher for outer versus inner sapwood locations within each tree infection
295 type. In addition, more uniform colonization of the sapwood circumference was found for
296 the AI trees based on number of sapwood locations on a disk that yielded the fungus in
297 culture. Tubajika and Barak (2011) speculated that results of efficacy trials for chemical
298 fumigation treatments may be dependent on the level of wood colonization by a target
299 fungus. Our comparative results of pre-treatment colonization of logs from NI and AI
300 oaks suggest that the latter source of logs may be preferable for such tests.

301 In addition, Tubajika and Barak (2011) suggested that results of efficacy trials also
302 may be a reflection of the assay technique used. Detection via isolation has typically
303 been done by plating small wood samples on oak wilt identification agar developed by
304 Barnett (1953) in previously published log treatment studies (Schmidt 1983; MacDonald
305 et al. 1985; Woodward and Schmidt 1995; Schmidt et al. 1997). Both standard isolation
306 and direct DNA detection methods were used in our study for the following reasons. The
307 fungus is not evenly distributed in the sapwood around the circumference of the main
308 stems of recently wilted red oaks, particularly for naturally-infected trees. Rather,
309 vertical strips of vascular discoloration interspersed with clear cambium may be found
310 depending on time since complete tree wilt (Juzwik, *personal observation*). Thus, a true
311 negative isolation or DNA detection may be (but not always) obtained when samples
312 are taken from non-stained tissue. However, difficulty in isolating *B. fagacearum* from
313 diseased sapwood during fall and winter months has been reported anecdotally by plant
314 disease diagnostic laboratories (see Yang and Juzwik 2017) even though such trees
315 are subsequently found to be pathogen-positive. The DNA detection method has proven
316 useful in such cases. Two naturally-occurring conditions in the field that can lead to

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

317 difficulty in consistently isolating *B. fagacearum* from actively wilting or recently wilted
318 oaks are drought and overlap of multiple insect pest and pathogen problems. Reduced
319 sapwood moisture content of living oaks associated with seasonal drought has been
320 anecdotally found to hinder ability to isolate the pathogen from sapwood of suspect
321 oaks in Wisconsin (B. Hudelson, Plant Diagnostic Clinic, University of Wisconsin-
322 Madison *personal communication*). Defoliation by gypsy moth (*Lymantria dispar*) not
323 only increases difficulty in detecting oak wilt symptomatic branches, but often favors
324 extensive colonization by pathogenic *Armillaria* species of oak (Wargo and Harrington,
325 1991). The latter may hamper colonization or detection of *B. fagacearum* in sapwood of
326 the lower stems and root collar area of such trees. Two-lined chestnut borer (*Agilus*
327 *bilineatus*) or the red oak borer (*Enaphalodes rufulus*) followed by extensive root
328 colonization by *Armillaria* species are other pest-pathogen systems that hinder oak wilt
329 detection and collection of *B. fagacearum* – positive sapwood for isolation (Lawrence,
330 Moltzan and Moser, 2002; Wargo and Harrington, 1991). *B. fagacearum* is considered a
331 poor saprophyte as it is relatively quickly replaced in the stems and branches by
332 secondary microbial colonizers in the year following complete crown wilt. Thus, fungus
333 isolation rates for logs taken from NI trees may vary depending on the time of complete
334 wilt in relation to harvest of logs for an eradication treatment trial. *B. fagacearum* has
335 been detected in ≥ 1 year old dead stems of wilted red oaks using the direct DNA
336 detection method, while standard isolation failed to do so (Yang and Juzwik 2017).
337 Similar rates of detection in outer and inner sapwood locations were found for the two
338 techniques in our study for NI trees in situations where source trees had completely
339 wilted in 2015 or early summer 2016 prior to September 2016 tree felling. However,

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

340 actual detection by isolation was higher (but not statistically tested) than detection by
341 PCR for outer and inner sapwood of AI trees. This finding was not anticipated by the
342 authors. In a recently published study (Yang and Juzwik 2017), similar or higher rates of
343 *B. fagacearum* detection were found for nested PCR compared to isolation for assays
344 conducted on sapwood from actively wilting branches of bur, red and white oaks. Thus,
345 the nested PCR protocol appeared to work well for branch samples in comparison to
346 isolation, but not for sapwood of logs cut from main stems of completely wilted AI red
347 oaks in the study reported here. We then hypothesized the “poorer” performance of the
348 PCR protocol compared to isolation could be influenced by time since branch or whole
349 tree wilt and to location source of assay samples (i.e. branch vs. main stem).
350 Furthermore, the subsamples for assaying via PCR and via isolation were collected
351 differently. Specifically, thin (depth-wise) “slivers” of sapwood were removed from the
352 tangential surface of the exposed disk locations for isolation assay, while radial
353 penetration (up to 1.5 cm deep) was done with a 0.64 dia drill bit to collect shavings for
354 the PCR assay. In comparison, findings for *B. fagacearum*-colonized logs in separate
355 2017 chemical fumigation trials with artificially-inoculated red oaks (*Q. ellipsoidalis*)
356 found higher pathogen detection rates for nested PCR than isolation (89 vs. 84% for
357 outer sapwood; 53 vs. 38% for inner sapwood) for 20 to 28 cm dia red oak logs prior to
358 treatment (Yang, et al. 201X). In this case, study trees were artificially inoculated with
359 the pathogen in early June 2016, complete crown wilt occurred in the subsequent two
360 months, and the trees were harvested in late February 2017. We hypothesize that these
361 smaller diameter oaks and the longer time period between inoculation and felling
362 allowed for more extensive sapwood colonization compared to the situation for the

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

363 vacuum steam study trees. Chances for positive pathogen detection from shavings of a
364 radially-penetrating drill bit would be greater in more extensively colonized sapwood.
365 Conversely, frequencies of *B. fagacearum* isolation from naturally-infected trees is
366 inversely related to length of time since complete crown wilt starting at approximately 12
367 months (depending on tree diameter) and beyond. This is due to the relatively quick
368 colonization by secondary microorganisms, as previously mentioned.
369 The vacuum steam treatment (for either schedule tested) to 5.0 cm sapwood depth
370 was apparently sufficient to degrade DNA of *B. fagacearum* based on our failure to
371 detect the fungus in post-treatment logs using nested PCR. Overall, results of our study
372 further validates the use of the standard isolation method of culturing on oak wilt
373 identification agar for evaluation of pathogen presence in log treatment trials.
374 Evaluation of phytosanitary log treatments benefit from reasonable and relatively
375 uniform colonization levels of a virulent pathogen if one is testing the ability of a
376 treatment to essentially eradicate the organism. Results of power analyses for our study
377 based on the total number of sapwood locations sampled found a 99.8% confidence
378 level that *B. fagacearum* could be detected from at least one location (n = 640 attempts)
379 if 1% of the sapwood of NI logs was colonized. Confidence level was lower (95.9%) for
380 detection of the pathogen in at least one location if 1% of the sapwood from AI logs was
381 colonized due to the fewer number of logs with a lower number of detection attempts (n
382 = 320). In contrast, MacDonald et al. (1985) reported a probability of 0.993 of detecting
383 *B. fagacearum* in at least 1 infected log of 18 tested if all test logs were 1% infected.
384 This calculation is based on results for 40 samples (four chips taken from each of eight
385 outer and eight inner sapwood locations). In retrospect for our study, scoring fungus

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

386 presence based on results of individual wood pieces plated would have greatly
387 increased the number of isolation attempts compared to scoring a sapwood location
388 positive if one or more of the four chips taken at a location was positive. This approach
389 will be incorporated in our future trials.

390 The VS treatment for all five log loads exposed to 56°C for 30 min at 5.0 cm
391 sapwood depth were completed in 5 to 9 h (Table 6). Slightly longer treatment times (8
392 to 10 h) were observed for the five log load replicates subjected to the 60°C for 60 min
393 schedule. This treatment at targeted depth resulted in eradiation of viable *B.*
394 *fagacearum* over a shorter time period than Jones (1973) reported for hot water
395 treatment (49°C for 12 h to achieve eradication). Jones (1973) also used logs of smaller
396 length and diameter than those used in this study. The results presented here also
397 compare favorably with the MB schedule (T312) in the USDA Treatment Manual that
398 specifies an extremely high MB dosage (240g/m^3) for 72 h (USDA APHIS 2016).
399 Heating to the log core (as in Chen et al. 2017a and Chen et al. 2017b) is not necessary
400 if the goal is to eradicate the pathogen from the tissues that it is known to colonize (e.g.
401 phloem and sapwood for *B. fagacearum*), and would also unnecessarily burden
402 prospective treatment facilities with added cost.

403 VS is an effective and efficient method of heating round and rectangular sections of
404 wood (Simpson 2001). Furthermore, heat penetration of wood by steam under negative
405 pressure is superior when compared to the questionable and limited penetration of logs
406 by chemical fumigants such as sulfuryl fluoride and methyl bromide (Tubajika and Barak
407 2011). Other advantages for considering use of VS compared to chemical fumigation
408 include the minimal level of harm to the environment, reduced human health concerns,

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

409 and potentially faster treatment times. There are significant advantages to decreased
410 treatment time that have direct and positive impact on business profitability and overall
411 efficiency of port operations (e.g. reduced congestion). We found VS able to eliminate
412 viable propagules of *B. fagacearum* from well-colonized oak logs, but the treatment
413 would likely be effective for eradication of other pathogens and insect pests of logs (e.g.
414 *Geosmithia morbida* and walnut twig beetle associated with thousand cankers disease)
415 as well. Furthermore, the target depth of treatment and the treatment schedule could be
416 developed specifically ("tailored") for other pests and pathogens. The flexibility provided
417 by VS treatment for logs would fit the current model in log trade, e.g. a bilaterally
418 negotiated phytosanitary treatment schedule between trade partners that protects the
419 importing country from unwanted phytosanitary risk.
420 Practical considerations for the continued development and potential use of VS for
421 phytosanitary treatment include the fact that commercial VS chambers are already
422 available in commerce for a variety of applications. These include devitalization of weed
423 seeds in spices and bird seed upon import, conditioning of cotton bales for export,
424 sterilization of mushroom substrates, and coloring of textiles among others. Vacuum
425 steam treatment application for logs would likely be conducted at log holding yards or at
426 shipping ports prior to movement. However, cost comparison studies for commercial
427 scale up (e.g. energy required for VS versus cost of chemical fumigation; chamber cost
428 versus tarp treatment for fumigants) are needed if vacuum steam is to be considered a
429 viable commercial option for the treatment of logs.
430 In summary, we report here the results of initial trials with VS for targeted treatment
431 of oak logs to kill viable *B. fagacearum* propagules. We plan to conduct a second set of

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

432 VS trials with logs from diseased red oaks in central or southern USA where sapwood
433 depth is greater (and thus, target treatment depth is likely greater than that used in this
434 study). To further position VS treatment of logs as a viable commercial entity, future
435 tests will also consider a number of key operational aspects. Evaluation of numerous
436 logs at scale in a fixed, commercial chamber is needed, as are considerations regarding
437 log grouping by diameter. Optimal chamber design to accommodate the logistics of
438 loading, treatment, and unloading should be thoroughly discussed with developmental
439 partners in the treatment business community to enable a successful transition from
440 methyl bromide to VS treatment for logs.
441

442 **Acknowledgements**

443 We thank J. Glaeser, J. Koch and S. Pfister for helpful comments on the manuscript;
444 P. Castillo, M. Moore, D. Iverson, and M. McDermott-Kubeczko for technical assistance;
445 S. Nelson for locating site with study trees and providing logging industry contacts; and
446 the Shakopee Mdewakanton Sioux Community for providing space, utilities, staff help,
447 and forklift use for conduct of the study. The Statistical Consulting Center at the
448 University of Minnesota and, in particular, H. Nilakanta helped with the analysis of this
449 study. This research was partially funded by the USFS FHP Washington Office.

450 **LITERATURE CITED**

451 Agresti, A. 2002. Categorical Data Analysis. John Wiley & Sons, Inc. Hoboken, New
452 Jersey.
453 Banik, M.T., Lindner, D.L., Juzwik, J., and Glaeser, J.A. 2013. Use of DNA sequencing
454 to detect pathogenic, saprotrophic, and stain fungi in sapwood of declining red pine

455 (*Pinus resinosa*) in the Upper Midwest. Pages 101-110 in: Proc. 2012 Western
456 International Forest Disease Work Conference. Browning, John; Palacios, Patsy,
457 comps.
458 Barnett, H. L. 1953. Isolation and identification of the oak wilt fungus. W. V. Exp. Sta.
459 Bull. 359T. 15 pp.
460 Chen, Z., White, M., and Mack, R. 2017a. Evaluating vacuum and steam process on
461 hardwood veneer logs for export. Eur. J. Wood Prod. 75: 911-918.
462 Chen, Z., White, M., and Qiu, Z. 2017b. Investigation of vacuum and steam treatments
463 to heat treat and sanitize firewood-grade ash logs and ash firewood. For. Prod. J.
464 67: 258-265.
465 Jones, T. W. 1973. Killing the oak wilt fungus in logs. For. Prod. J. 23: 52-54.
466 Juzwik, J., Yang, A., Myers, S., Furtado, M., and Taylor, A. 2017. Survival of
467 *Ceratocystis fagacearum* following red oak log fumigation with sulfuryl fluoride.
468 (Abstr.) Phytopathology 107:S5.45. https://doi.org/10.1094/PHYTO-107-12-S5.45
469 Lawrence, R., Moltzan, B., and Moser, K. 2002 (rev. 2010). Oak decline and the future
470 of Missouri's forests. Missouri Conservationist Magazine, July 2002. (accessed 23
471 July 2018). https://mdc.mo.gov/conmag/2002/06-0/oak-decline-and-future-Missouri-
472 s-forests
473 Lenth, R. V. 2016. Least-squares means: the R package lsmeans. J. Stat. Softw. 69:1-
474 33.
475 MacDonald, W. L., Schmidt, E. L., and Harner, E. J. 1985. Methyl bromide eradication
476 of the oak wilt fungus from red and white oak logs. Forest Prod. J. 35: 11-16.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

477 Partridge, A. D. 1961. Fumigants kill the oak wilt fungus in wood. Forest Prod. J. 11: 12-
478 14.
479 Pizano, M. and Banks, J. 2009. Montreal protocol substances that deplete the ozone
480 layer and methyl bromide in the forest industry – use and alternatives. UNEP QPS
481 Taskforce, Technology and Assessment Panel. Retrieved 5 April 2018 from
482 http://www.uneptie.org/ozonaction/information/mmcfiles/7464-e-Mbr_Pizano.pdf
483 Scheffrahn, R. H., Su, N. Y., and Hsu, R. C. 1992. Diffusion of methyl bromide and
484 sulfuryl fluoride through selected structural wood matrices during fumigation. Mater.
485 Org. 27:147-155.
486 Schmidt, E. L. 1983. Minimum temperatures for methyl bromide eradication of
487 *Ceratocystis fagacearum* in red oak log pieces. Plant Dis. 67: 1388-1399.
488 Schmidt, E., Juzwik, J., and Schneider, B. 1997. Sulfuryl fluoride fumigation of red oak
489 logs eradicates the oak wilt fungus. Eur. J. Wood Prod. 55: 315-318.
490 Simpson, W. T. 2001. Heating times for round and rectangular cross sections of wood in
491 steam. Gen. Tech. Rep. FPL-GTR-130. Madison, WI: U.S. Department of
492 Agriculture, Forest Service, Forest Products Laboratory, 103 pp.
493 Tubajika, K. M. and Barak, A. V. 2006. Methyl iodide and sulfuryl fluoride as quarantine
494 treatments for solid wood packing material. In: 2007 Annu. Int. Res. Conf. on Methyl
495 Bromide Alternatives and Emissions Reductions, Jan. 2007, San Diego, CA.
496 Tubajika, K. M. and Barak, A. V. 2011. Fungitoxicity of methyl iodide, sulfuryl fluoride,
497 and methyl bromide to *Ceratocystis fagacearum* in red oak, maple, poplar, birch,
498 and pine wood. Am. J. Plant Sci. 2: 268-275.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

499 UNEP DTIE Energy and OzonAction Branch. 2002. The Montreal protocol control
500 schedule and its evolution. Information paper. DTIE OzonAction Programme,
501 OzonAction Information Clearinghouse. Accessed 5 April 2018 from
502 http://www.unep.fr/ozonaction/information/mmcfiles/3326-e.pdf
503 USDA Animal and Plant Health Inspection Service (APHIS). 2016. Chemical
504 Treatments: fumigants: methyl bromide. 2 TM. In: Treatment Manual. USDA APHIS
505 PPQ. Accdssed 5 April 2018 from
506 http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment
507 .pdf
508 Uzunovic, A., Mukherjee, A., Elder, P., and Myers, S. W. 2017. Rapid screening of
509 sulfuryl fluoride as a potential phytosanitary treatment for a broad selection of fungi
510 relevant to forestry. For. Prod. J. 67:4-12.
511 Wargo, P.M. and Harrington, T.C. 1991.Chapter 7 – Host stress and susceptibility.
512 Pages 88-100 in Armillaria Root Disease. C.G. Shaw III and G.A. Kile, eds. Agric.
513 Handbook No. 691. U.S. Dept. of Agric. For. Serv., Washington, DC.
514 White, M.S., Chen, Z., and Mack, R. G. 2017. The effect of non-chemical phytosanitary
515 treatment on the packaging integrity of unitized products to prevent the international
516 spread of invasive plant and animal species. In: 2017 Proc. of ISTA TransPack
517 Forum, Orlando, Florida.
518 Woodward, R. P. and Schmidt, E. L. 1995. Fungitoxicity of sulfuryl fluoride to
519 *Ceratocystis fagacearum* in vitro and in wilted red oak log sections. Plant Dis. 79:
520 1237-1239.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

521 Yang, A. and Juzwik, J. 2017. Use of nested and real-time PCR for the detection of
522 *Ceratocystis fagacearum* in the sapwood of diseased oak species in Minnesota.
523 Plant Dis. 101:480-486.
524 Yang, A., Juzwik, J., White, M., Chen, Z., Shugrue, S., and Mack, R. 2017. Vacuum
525 steam treatment as a promising alternative to methyl bromide for killing *Ceratocystis*
526 *fagacearum* in *Quercus rubra* logs for global export. (Abstr.) Phytopathology
527 107:S5.47. https://doi.org/10.1094/PHYTO-107-12-55.47
528 Yang, A., Seabright, K., Juzwik, J., Myers, S.W., and Taylor, A. 201X. Survival of the
529 oak wilt fungus in logs fumigated with sulfuryl fluoride and methyl bromide. For.
530 Prod. J. XX: 000-000. (submitted)
531

532 **Supplemental Table 1.** Characteristics of logs from *Quercus rubra* trees that were naturally
533 infected or four artificially inoculated with *Bretziella fagacearum* prior to vacuum steam
534 treatment.

Test[a]	Log characteristics				
	Log no.	Weight (kg)	Length (cm)	Small end diameter[b] (cm)	Large end diameter[b] (cm)
1	11	347.1	175.3	44.5	57.2
	81	350.8	175.3	49.5	53.3
	31	334.6	175.3	47.0	53.3
2	82	292.6	175.3	45.7	48.5
	12	218.9	175.3	39.4	41.9
	32	265.7	177.8	43.2	45.7
3	51	203.6	170.2	36.8	42.5
	71	244.5	177.8	39.4	45.7
	61	200.8	182.9	36.8	39.4
4	72	171.0	172.7	35.6	36.8
	62	154.1	170.2	33.7	35.6
	52	176.7	175.3	35.6	37.5
5	54	146.5	177.8	32.4	33.7
	64	86.1	180.3	23.5	26.7
	74	86.1	180.3	23.5	26.7
6	91	255.1	175.3	41.3	46.4
	60	262.6	182.9	40.6	46.4
	41	231.5	177.8	36.8	45.7
7	21	310.4	175.3	44.5	52.1
	101	408.5	175.3	49.5	61.0
	13	230.4	190.5	39.4	40.6
8	22	253.1	190.5	40.6	43.2
	14	205.5	190.5	37.5	38.1
	102	234.1	179.1	41.3	41.9
9	63	153.6	182.9	33.0	33.7
	104	168.0	172.7	35.6	36.2
	34	228.9	180.3	39.4	42.5
10	33	296.6	193.0	44.5	45.7
	103	213.3	175.3	36.8	43.2
	23	240.4	186.7	40.6	41.9

535

536 [a] Two logs per test were obtained from *Quercus rubra* naturally infected with *B. fagacearum* and
537 one log obtained from *Q. rubra* artificially inoculated with *B. fagacearum*.
538 [b] Small and large end diameters measured inside of the bark.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

539 **Table 1.** Number of locations yielding *Bretziella fagacearum* in culture or via nested PCR from

540 the outer and inner sapwood of logs taken from naturally infected and artificially inoculated

541 *Quercus rubra* trees prior to vacuum steam treatment.

542

Tree infection type	Tree no.	No. of logs	Isolation[a]				Nested PCR[a]			
			Outer		Inner		Outer		Inner	
			(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
Natural	1	4	23	35.9	9	14.1	27	42.2	15	23.4
	2	3	8	16.7	6	12.5	7	14.6	2	4.2
	3	4	6	9.4	10	15.6	24	37.5	12	18.8
	4	1	4	25.0	1	6.3	1	6.3	0	0.0
	5	4	11	17.2	3	4.7	3	4.7	1	1.6
	6	5	26	32.5	11	13.8	5	6.3	3	3.8
Artificial	7	3	27	56.3	20	41.7	24	50.0	5	10.4
	8	2	17	53.1	10	31.3	10	31.3	3	9.4
	9	1	3	18.8	2	12.5	3	18.8	0	0.0
	10	4	26	40.6	15	23.4	15	23.4	3	4.7

543 [a] Two disks taken per log and eight locations per disk were used for isolation (four wood

544 chips/location) and nested PCR (composited shavings from four to five drill penetrations) assays

545 per disk at each sapwood depth (n=16).

546

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

547 **Table 2.** Coefficients of the generalized linear mixed effects model fitted to the *Bretziella*
548 *fagacearum* isolation data for locations of disks sampled prior to vacuum steam treatment. Disks
549 were from logs obtained from wilted *Quercus rubra* trees, six naturally infected and four
550 artificially inoculated with *B. fagacearum*. Artificially inoculated and inner sapwood are the
551 reference levels.

Variable	Coefficient estimate	SE	P - value
Intercept	-1.2587	0.1884	< 0.0001
Natural infection	-0.8479	0.2581	0.001
Outer sapwood	0.4478	0.2191	0.041
Natural infection x outer sapwood	0.2155	0.3038	0.4781

552
553
554

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

555 **Table 3.** Estimated probabilities of *B. fagacearum* detection by isolation based on logistic
556 regression of the interactions of infection type (naturally infected or artificially inoculated) and
557 sapwood location (outer sapwood or inner sapwood).

Tree infection type	Sapwood location	Probability	
		Estimated ± SE[a]	Actual ± SE[b]
Natural	Outer	0.1910 ± 0.0224	0.2437 ± 0.0240
Natural	Inner	0.1085 ± 0.0175	0.1250 ± 0.0184
Artificial	Outer	0.3077 ± 0.0353	0.4563 ± 0.0393
Artificial	Inner	0.2212 ± 0.0324	0.2938 ± 0.0360

558 [a] Estimated probability based on logit transformation from model estimates.

559 [b] Actual probability based on calculated proportions from samples used in this study.

560

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

561 **Table 4.** Coefficients of the generalized linear mixed effects model fitted to the *Bretziella*
562 *fagacearum* nested PCR detection data for locations of disks sampled prior to vacuum steam
563 treatment from logs obtained from wilted *Quercus rubra* trees, six naturally infected and four
564 artificially inoculated with *B. fagacearum*. Artificially inoculated and inner sapwood are the
565 reference levels.

Variable	Coefficient estimate	SE	P - value
Intercept	-2.8492	0.4578	< 0.0001
Natural infection	0.2204	0.5644	0.6961
Outer sapwood	1.5814	0.3514	< 0.0001
Natural infection x outer sapwood	0.2155	0.3038	0.4781

566
567

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

568 **Table 5.** Predicted probabilities of *B. fagacearum* detection by nested PCR based on logistic
569 regression of the interactions of infection type (naturally infected or artificially inoculated) and
570 sapwood depth (outer sapwood or inner sapwood).

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

Tree infection type	Sapwood location	Probability	
		Estimated ± SE[a]	Actual ± SE[b]
Natural	Outer	0.1276 ± 0.0348	0.2125 ± 0.0229
Natural	Inner	0.0673 ± 0.0212	0.1063 ± 0.0172
Artificial	Outer	0.2196 ± 0.0635	0.3250 ± 0.0370
Artificial	Inner	0.0547 ± 0.0236	0.0688 ± 0.0366

571

572 [a] Estimated probability based on logit transformation from model estimates.

573 [b] Actual probability based on calculated proportions from samples used in this study.

574

575 **Table 6.** Total vacuum steam treatment time for either 56°C for 30 min or 60°C for 60 min to 5.0

576 cm depth in sapwood of *Quercus rubra* logs. Initial 200 mmHg pressure and saturated steam at

577 90°C.

Treatment regime[a]	Test[b]	Average initial log temp. (°C)	Total cycle time (min)	Heating rate (min/°C)	Energy use (kwh)	Energy use (kwh/kg)
56°C/	1	23.0	547	16.6	n/a[c]	n/a
30 min	4	20.5	326	9.2	26.54	0.053
	5	20.5	354	10.0	21.01	0.066
	8	14.0	437	10.4	29.33	0.042
	9	15.0	402	9.8	24.81	0.045
60°C/	2	23.5	617	16.9	37.23	0.048
60 min	3	19.0	478	11.7	34.25	0.053
	6	17.5	498	11.7	36.51	0.049
	7	19.0	493	12.0	38.11	0.040
	10	16.5	509	11.7	37.52	0.050

578

579 [a] When temperature at target depth (5 cm into sapwood) reached 56°C for all 3 logs per test,

580 treatment was continued for 30 min (at 200 mHg) for 56°C for 30 min treatment. Similarly, when

581 temperature reached 60°C for all three logs per test, treatment was continued for 60 min for

582 60°C for 60 min treatment.

583 [b] Two logs per test were obtained from *Q. rubra* naturally infected with *B. fagacearum* and one

584 log obtained from *Q. rubra* artificially inoculated with *B. fagacearum*.

585 [c] Energy use data collector was not until after test 1 of treatment.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

586 **FIGURE CAPTIONS**

587

588 **Figure 1.** Proportion of subsamples yielding *Bretziella fagacearum* per disk from
589 naturally infected *Quercus rubra* trees based on total number of subsamples assayed
590 using (A) laboratory isolation techniques and (B) nested PCR prior to vacuum steam
591 treatment.

592 **Figure 2.** Proportion of subsamples yielding *Bretziella fagacearum* per disk from
593 artificially inoculated *Quercus rubra* trees based on total number of subsamples
594 assayed using (A) laboratory isolation techniques and (B) nested PCR prior to vacuum
595 steam treatment.

596 **Figure 3.** Temperature[a] profiles of (A) 56ºC for 30 min treatment of *Quercus rubra* log
597 31 and (B) 60ºC for 60 min treatment of *Quercus rubra* log 21 at an initial vacuum of
598 100 mmHg. Temperature profiles based on the average temperature for every 5 min
599 duration during vacuum steam treatment.

600 **Supplemental Figure 1.** Schematic diagram illustrating the placement and depth of
601 Omega K type thermocouple wire probes along the length (L) of logs from *Quercus*
602 *rubra* trees that were naturally infected or artificially inoculated with *Bretziella*
603 *fagacearum*. Point locations were used to map temperature profiles during vacuum
604 steam treatment.

605

606

607

608

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

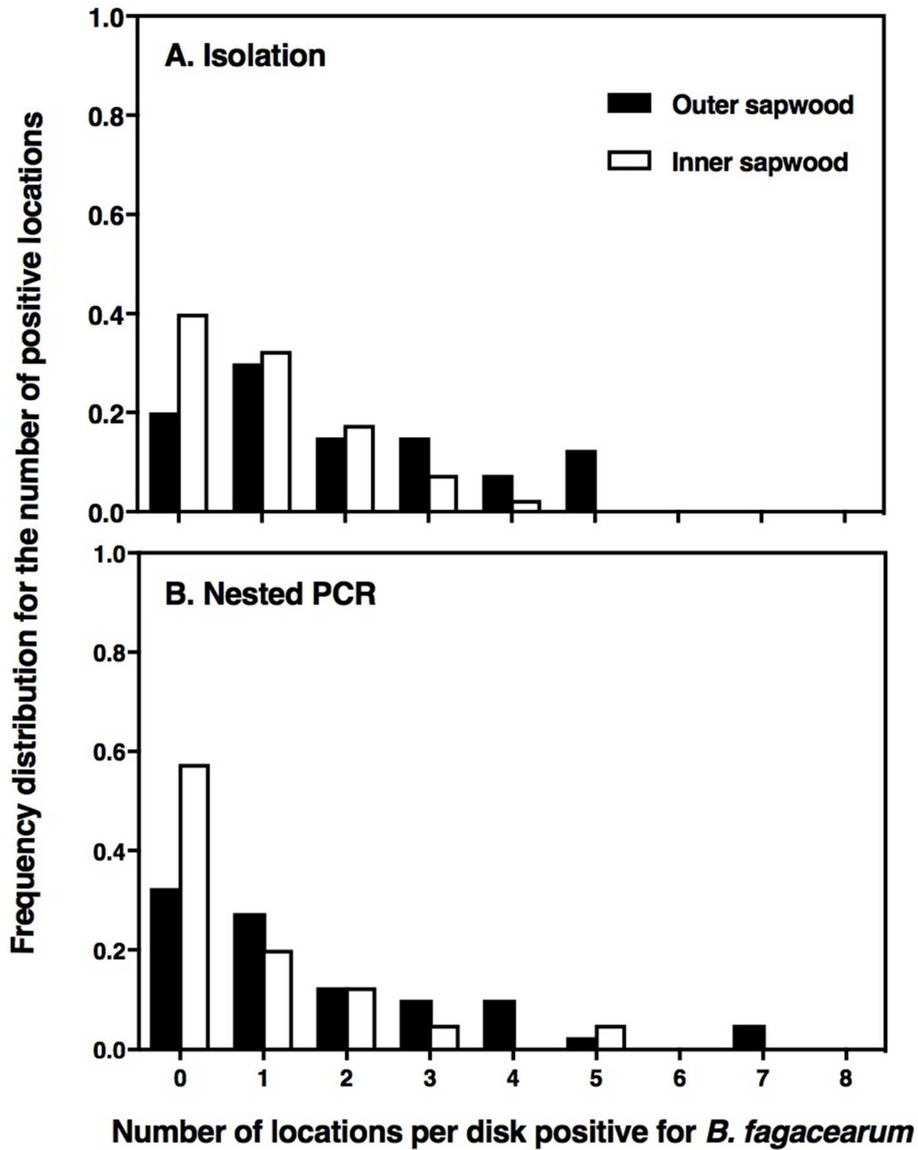


Figure 1. Proportion of subsamples yielding *Bretziella fagacearum* per disk from naturally infected *Quercus rubra* trees based on total number of subsamples assayed using (A) laboratory isolation techniques and (B) nested PCR prior to vacuum steam treatment.

82x101mm (300 x 300 DPI)

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

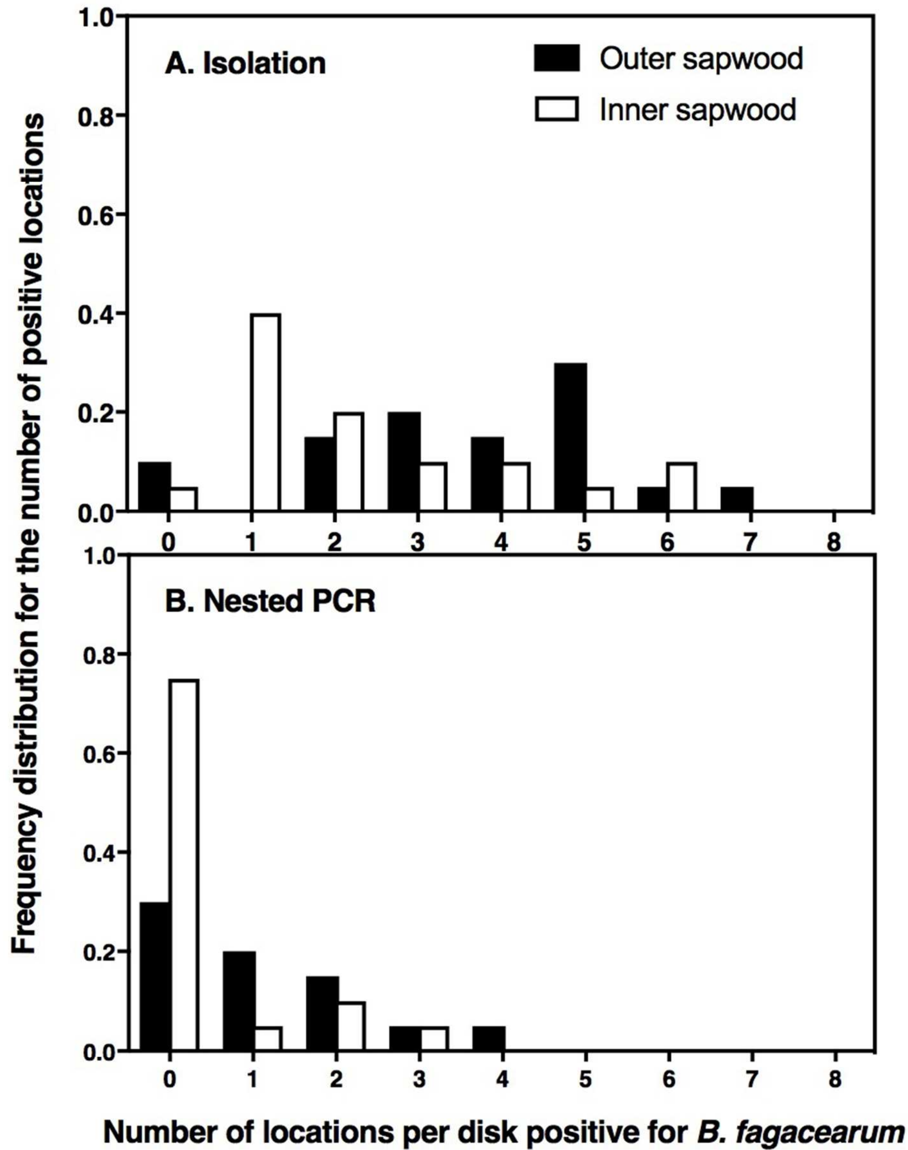


Figure 2. Proportion of subsamples yielding *Bretziella fagacearum* per disk from artificially inoculated *Quercus rubra* trees based on total number of subsamples assayed using (A) laboratory isolation techniques and (B) nested PCR prior to vacuum steam treatment.

82x102mm (300 x 300 DPI)

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

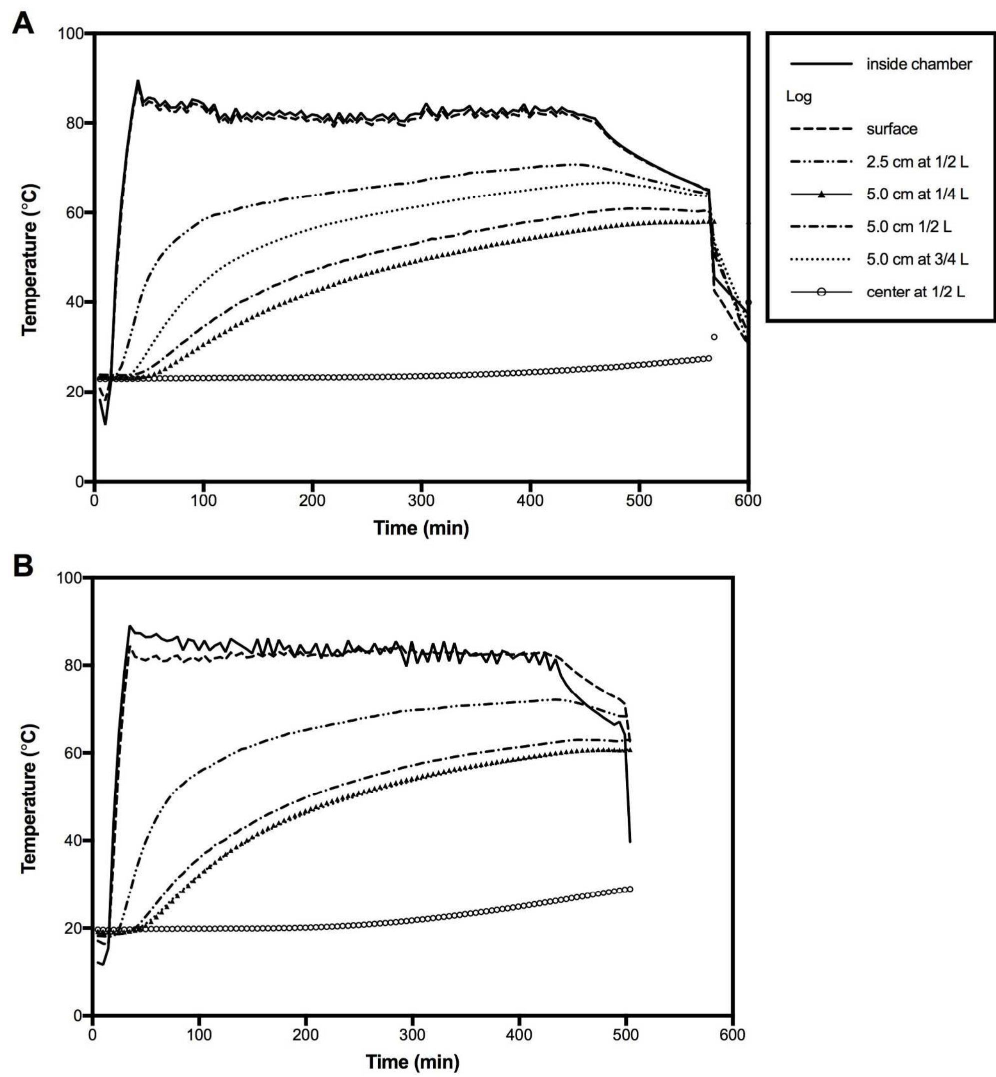
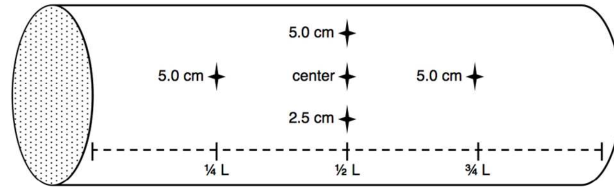


Figure 3. Temperature[a] profiles of (A) 56ºC for 30 min treatment of *Quercus rubra* log 31 and (B) 60ºC for 60 min treatment of *Quercus rubra* log 21 at an initial vacuum of 100 mmHg. Temperature profiles based on the average temperature for every 5 min duration during vacuum steam treatment.

196x214mm (300 x 300 DPI)

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-07-18-1252-RE • posted 08/04/2018
This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.



Supplemental Figure 1. Schematic diagram illustrating the placement and depth of Omega K type thermocouple wire probes along the length (L) of logs from *Quercus rubra* trees that were naturally infected or artificially inoculated with *Bretziella fagacearum*. Point locations were used to map temperature profiles during vacuum steam treatment.

279x215mm (150 x 150 DPI)