Oak Wilt Fungus (*Bretziella fagacearum*) Survival in Logs Following Fumigation with Ethanedinitrile

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Abstract

The phaseout of methyl bromide (MB) fumigation creates an urgent need for an alternative phytosanitary treatment to limit the risk of international spread of the oak wilt fungus, *Bretziella fagacearum*. Fumigation with ethanedinitrile (EDN) is considered a potential alternative to MB fumigation to eradicate wood-inhabiting pests and pathogens. We evaluated the efficacy of EDN fumigation by comparing the rate of *B. fagacearum* isolation before and after fumigation of red oak (*Quercus rubra* or *Quercus ellipsoidalis*) log sections from oak wilt–affected trees. Logs (range 15.2 to 98.0 cm long; diameter 9.1 to 46.1 cm) were obtained from red oak trees that were naturally infected (NI) or artificially inoculated (AI) with *B. fagacearum*. The logs were fumigated for 24, 48, and/or 72 hours with 120 g/m^3 EDN. Frequencies of pathogen isolation from the sapwood before treatment were higher for AI logs than for NI logs. EDN treatments greatly reduced the frequency of viable pathogen recovery, but eradication occurred only in experiments using the smallest log diameters (9 to 14 cm). Our results suggest that EDN may have limited penetration in oak logs with intact bark, similar to fumigants currently used on wood products, such as MB and sulfuryl fluoride. Results of future work may help in the understanding of the limitations for consistent and full efficacy of EDN against *B. fagacearum* in logs harvested from diseased trees.

Oak wilt is a vascular wilt disease that is one of the most significant threats to oak (*Quercus*) species in the eastern United States (Juzwik et al. 2011). The fungal pathogen that causes oak wilt, *Bretziella fagacearum* (syn. *Ceratocystis fagacearum* [Bretz] Hunt), is known to exist only in the United States; however, all *Quercus* species are suspected to be susceptible to infection and mortality (Bretz 1955). As such, the disease is of high regulatory concern for countries importing US logs. Phytosanitary treatment using methyl bromide (MB; 240 g/m^3 for 72 h) can be required for oak logs with bark intact exported from the United States (Liese and Ruetze 1985, US Department of Agriculture Animal and Plant Health Inspection Service 2016). As a Class I ozone-depleting substance, MB has been phased out for most uses in accordance with the Montreal Protocol (United Nations Environment Programme 2014). However, veneer logs harvested from species that are hosts to quarantine pathogens (e.g., *Quercus* sp.) maintain an exemption that allows for MB treatment in quarantine and preshipment (QPS) applications. Despite existing QPS exemptions for MB use, the US export industry is increasingly challenged by air quality restrictions that limit the use of MB (Bragard et al. 2020).

Evaluation of alternative fumigants to replace MB to treat oak logs began in the mid-1990s (Woodward and Schmidt 1995, Schmidt et al. 1997), but a globally accepted replacement for MB has not yet been adopted. Uncertainty regarding international fumigation requirements threatens the US oak export industry, which is valued at greater than $200 million annually (Simoes and Hidalgo 2011, Luppold et al. 2022). In 2020, the European Union ended its exemption for MB-treated logs in favor of alternative phytosanitary approaches. A proposed alternative is sulfuryl fluoride (SF) fumigation as one step in an integrated systems

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approach to reduce the risk of the accidental spread of *B. fagacearum* to Europe and other countries that import oak logs (Bragard et al. 2020). However, phytosanitary trials found that SF, while demonstrating high efficacy against *B. fagacearum*, did not fully eliminate the pathogen in logs harvested from diseased trees (Yang et al. 2019). Similarly, SF fumigation of pine (*Pinus* sp.) products (e.g., blocks, lumber, and logs) has mixed efficacy against the pinewood nematode (*Bursaphelenchus xylophilus*), which also poses a regulatory concern (Buckley et al. 2010, Bonifácio et al. 2013, Seabright et al. 2020). Although it is not an ozone-depleting substance, SF is a greenhouse gas that contributes to global warming and may not be an ideal replacement for MB (Mühle et al. 2009, Tsai 2010).

Ethanedinitrile (EDN; C_2N_2) is a candidate to replace MB to treat wood products and does not contribute to ozone depletion or greenhouse gas emissions (Commonwealth Scientific and Industrial Research Organization [CSIRO] et al. 1996). EDN was recently registered for log fumigations in New Zealand, Australia, the Czech Republic, South Korea, Malaysia, and Russia, and registration is pending in several other countries (Hall 2022, Uzunovic et al. 2022, Hall and Adlam 2023). Experiments testing the efficacy of EDN fumigation on softwood logs infested with quarantine pests at various life stages showed promising results (Najar-Rodriguez et al. 2020, Seabright et al. 2020, Park et al. 2021). However, evaluations against wood-inhabiting pathogens have generally been limited to laboratory screenings using fungi inoculated on barley grain, including *B. fagacearum*, which was killed at all test parameters (Uzunovic et al. 2022). To date, phytosanitary evaluation of EDN has not been conducted on pathogenic fungi in logs. It follows that the next step to validate laboratory screenings is to test EDN fumigation using logs colonized with the oak wilt pathogen. If successful, EDN could be a practical replacement for MB, requiring no substantial changes to existing fumigation infrastructure or processes.

Developing effective fumigation treatments is particularly challenging because pathogens are often in the matrix of the wood tissue rather than in insect tunnels and galleries where fumigants can easily reach insect pests. One of the main obstacles to replace MB is that many fumigants, including MB, have limited penetration into wood tissues where pathogens reside (Morrell 1995, Ren et al. 2011, Tubajika and Barak 2011). Shortcomings of SF and other fumigants are often attributed to slow or variable diffusion of the chemical into logs with high sapwood moisture content (Scheffrahn et al. 1992, Yang et al. 2019, Seabright et al. 2020). To date, EDN fumigation of softwood timbers (*Pseudotsuga menziesii* and *Pinus radiata*) demonstrates faster fumigant penetration and higher sorption than SF or MB (Ren et al. 2011) and appears to be largely unaffected by wood moisture content and end-grain sealing (Pranamornkith et al. 2014). In contrast, some studies that evaluated EDN fumigation of infested pine logs (*Pinus koraiensis* and *Pinus* spp.) indicate that the fumigant was less effective against insect pests in logs with high moisture content compared to dried wood, requiring higher fumigant concentrations or concentration-time (CT) products (Lee et al. 2017, Park et al. 2021, Hall and Adlam 2023). It remains unclear if the high moisture content of oak logs with bark intact will inhibit the ability of EDN to penetrate at a high enough concentration to kill *B. fagacearum*.

We conducted fumigation experiments in 2020 and 2021 to evaluate the efficacy of EDN fumigation as a phytosanitary treatment for logs harvested from oak wilt–affected trees. Our objective was to assess the rates of *B. fagacearum* colonization in small- and large-diameter logs from naturally infected (NI) and artificially inoculated (AI) red oak trees before and after treatment with 120 g/m^3 EDN for 24, 48, and/or 72 hours.

Materials and Methods

We conducted three separate fumigation trials on red oak (*Q. rubra* or *Q. ellipsoidalis*) logs to evaluate the potential for EDN to replace MB and identify a dosage that eradicated *B. fagacearum*. Fumigation treatments were conducted with increasing exposure times (24, 48, and/or 72 h) at the maximum recommended rate of 120 g/m^3 EDN. In this study, we refer to stem sections or bolts as ''logs'' for readability, though they were shorter in length than typical commercial veneer logs. In all the trials, we selected logs free of any exterior physical defects (e.g., ingrown bark, cracking, and knots) to mimic desired characteristics of veneer logs. In Trials 1 and 2, we used logs that were harvested from trees with naturally occurring oak wilt infections (NI) in Minnesota and Indiana. In Trial 3, we used logs that were harvested from trees following inoculation with *B. fagacearum* (AI) and development of disease symptoms in Wisconsin. The lengths and diameters of logs varied among the experiments (Table 1).

Selection of study trees

In August 2020 (Trial 1), we identified and harvested three *Q. ellipsoidalis* trees (diameter 9.1 to 14.2 cm at 1.5-m height; dbh) that displayed 60 to 90 percent crown wilt symptoms due to naturally occurring infections with *B. fagacearum* in Stacy, Minnesota. Logs (0.4 m length) were cut from the trees and transported to the study site at the University of Minnesota in St. Paul, Minnesota, for pretreatment pathogen assessment. Logs were then transported to Buzzards Bay, Massachusetts, for fumigation treatments in September 2020.

In August 2020 (Trial 2), we identified two *Q. rubra* trees (43 to 50 cm dbh) that were naturally infected with *B. fagacearum* at the Southeast Purdue Agricultural Center in Butlerville, Indiana. In February 2021, the trees (displaying 90% to 100% crown wilt symptoms) were harvested and cut into logs (0.7-m length). A section (0.3-m length) was cut from each log and transported to the University of Minnesota for pretreatment pathogen assessment. The shortened logs (0.4-m length) were transported to the University of Tennessee, Knoxville, for EDN fumigations in March 2021. The log sections and logs were sealed in plastic bags to prevent the spread of pathogen propagules during transport to Minnesota and Tennessee.

In June 2021 (Trial 3), we inoculated 11 *Q. ellipsoidalis* trees near Grantsburg, Wisconsin, with *B. fagacearum* following the protocol of Juzwik et al. (2019). Briefly, we inoculated trees by exposing three to four primary roots on the selected trees, drilling a small hole (2.0-cm depth, 0.64-cm diameter) into the uncovered roots, and dispensing an aqueous spore suspension (1 mL) of *B. fagacearum* endoconidia (10^6 spores/mL) into each hole. After uptake of the spore suspension by the tree, we sealed the holes with moldable epoxy putty and covered the roots with the

Table 1.—Size and diameter of logs from red oak trees that were naturally infected or artificially inoculated with Bretziella fagacearum *after removal of pretreatment log disks but prior to fumigation with ethanedinitrile.*

Fumigation trial no.	Species	Tree infection type	No. of logs	Mean log length ± SE (cm)	Mean log diameter ± SE (cm)
Trial 1	*Quercus ellipsoidalis*	Natural	20	15.2[a]	12.2 ± 0.4
Trial 2	*Quercus rubra*	Natural	16	41.3 ± 0.6	34.4 ± 2.0
Trial 3	*Q. ellipsoidalis*	Artificial	27	91.4 ± 0.5	34.7 ± 0.9

[a] Lengths of fumigated logs were not recorded for Trial 1. Trees were cut into logs (35.6-cm length) after tree harvest, and approximately 10.2 cm was removed from each log end for the pretreatment biological assay.

original soil. We monitored the inoculated trees throughout the growing season to document the progression of crown wilt. We selected eight AI trees (27 to 63 cm dbh) that displayed at least 60 percent crown wilt for EDN fumigation. In October 2021, the trees were felled, cut into logs (1.2-m length), and transported to the University of Minnesota for pretreatment pathogen assessment. Logs were transported in an enclosed vehicle to Buzzards Bay, Massachusetts, for fumigation treatments in November 2021.

Log preparation and sampling

For all trials, time between harvest and fumigation treatment was approximately 1 month, during which logs were stored at 40°C (Trial 1) or at ambient outdoor temperatures (Trials 2 and 3). The logs were acclimated to treatment temperature for at least 1 day prior to fumigation treatments. In the small log experiment (Trial 1), we removed sections (5-cm length) from the ends of each log and cut pretreatment sample disks (5-cm length) from the exposed end for biological evaluation. A sterile chisel was used to remove the bark to expose the outer sapwood (one to three outermost annual rings) at six equidistant locations around the disks. In the two large log trials, we removed sections (23-cm length) from one end of each log for Trial 2 and both ends of each log for Trial 3. Disks (7.6-cm length) were cut from the exposed end(s) of the logs for pretreatment pathogen assessment, and the remaining shortened logs were used for fumigation treatment. For biological assays, we removed bark at eight equidistant locations to expose the outer sapwood alternating with eight locations that exposed the inner sapwood (one to two annual rings before the heartwood).

In all trials, sapwood sample locations from each disk were assayed for viable *B. fagacearum*. We collected four small wood chips (0.6 mm^2) from discolored sapwood at the exposed locations that were characteristic of *B. fagacearum* colonization and placed them on Petri plates containing a glucose-phenylalanine growth medium that encourages rapid endocondia production and characteristic growth patterns for easy pathogen identification (Barnett 1953). The plates were incubated at room temperature (21°C to 23°C) with ambient lighting for a maximum of 21 days, during which we routinely monitored them for *B. fagacearum* growth. *B. fagacearum* identification in cultures was based on the occurrence of brown to olive-green colonies, characteristic fruity aroma, presence of endoconidia, and presence of wavy hyphae embedded in the medium (Barnett 1953). Sample locations were considered positive if at least one of the four wood chips yielded the fungus. Following EDN fumigation, we cut posttreatment disks and attempted isolation of *B. fagacearum* by repeating the same methods described above.

Experimental design and fumigation treatments

Prior to fumigation, we applied a thin layer of commercial end-grain sealant (Anchorseal 2; Seal-Once, Buffalo, NY) to the cut ends of the logs to simulate longer commercial logs in which fumigant penetration is expected to occur mostly through the bark. Fumigation parameters such as chamber volume, temperature, exposure time, and load factor differed among the trials (Table 2). For Trial 1, we randomly assigned logs ($n = 20$) to the following treatments: 120 or 0 g/m^3 EDN for 24 or 48 hours. The fumigations took place in glass chambers (10 liters) housed in a temperature-controlled unit held at 10°C with an average load factor of 17.2 percent (Table 2). Untreated logs were held at the same temperature for each of the designated durations. For Trial 2, we randomly assigned logs ($n = 16$) to treatments of either 120 or 0 g/m^3 EDN for 48 hours. For Trial 3, we randomly assigned logs ($n = 27$) to treatments of either 120 or 0 g/m^3 EDN for 72 hours. The treatments for Trial 3 were replicated two times. For Trials 2 and 3, fumigation took place in stainless-steel chambers (664 liters) with an average load factor of 23.4 and 31.0 percent for Trials 2 and 3, respectively, and untreated control logs were held at the same temperature for the duration of treatment (Table 2).

Fumigation treatments were conducted according to procedures detailed in previous SF and MB experiments (Yang et al. 2019, Seabright et al. 2019, 2020). Prior to fumigation, we pulled a slight vacuum on the chambers to allow room for EDN gas delivery. The pressure inside each chamber was reduced to ~70 mm Hg using a 1-liter gastight syringe (Hamilton Co., Reno, Nevada) in Trial 1 and a vacuum pump for Trials 2 and 3. The amount of EDN gas (99.9%; Draslovka, Kolín, Czech Republic) required to deliver a dose of 120 g/m^3 to the fumigation chambers was calculated volumetrically. For all the trials, EDN was delivered from a stainless-steel gas cylinder (Swagelok Co., Solon, OH) to tedlar bags (50 liters; SKC Inc., Eighty Four, PA) and then transferred to the fumigation chambers. Fans were used in the 664-liter chambers at the beginning of fumigation to promote gas circulation. We monitored EDN concentrations during treatments with an Agilent 490 micro gas chromatograph (GC; Agilent Technologies Inc., Santa Clara, CA). Gas was sampled through a stream selector valve (Valco Instruments Inc., Houston, TX) that pulled samples through PEEK tubing inserted into rubber septa inside stainless-steel ports (Swagelok) fitted on the fumigation chambers. The GC used a 10-m PoraPlot Q column held at 100°C with helium as the carrier gas set to 30 psi. Prior to fumigation, we calibrated the GC using EDN standards prepared in tedlar bags at varying concentrations. CT product and total EDN sorption for each fumigation replicate was calculated using gas concentration measure-

*Table 2.—Parameters for ethanedinitrile (EDN; 120 g/m^3) fumigation treatments of red oak (*Quercus rubra *or* Q. ellipsoidalis*) logs, including mean fumigant concentration-time (CT) products and percent sorption.*

Fumigation trial no.	Chamber volume (liters)	Temperature (°C)	Exposure time (h)	Mean CT product ± SE (h × mg/liter)	Mean EDN sorption ± SE (%)	Mean load factor ± SE (%)
Trial 1	10	10	24	2,019.9 ± 46.0	59.1 ± 1.8	15.8 ± 2.6
	10	10	48	2,358.2 ± 79.2	88.9 ± 0.7	18.6 ± 1.5
Trial 2	664	10	48	2,940.9 ± 113.9	87.7 ± 1.1	23.4 ± 1.5
Trial 3	664	5	72	2,157.9 ± 190.5	93.9 ± 1.0	31.0 ± 1.4

ments that were recorded every 4 to 6 hours throughout the treatments (Table 2).

Statistical analysis

We calculated the proportion of sapwood locations assayed where at least one of the four sapwood chips was positive for *B. fagacearum* before and after fumigation for all trials. Sapwood sample location was the experimental unit for all analyses. For the large log trials (Trials 2 and 3), generalized linear mixed effects models tested for differences in the frequency of pathogen isolation from the pretreatment samples. The models for both trials had the following form:

$$Y_{ijk} \sim \text{Bernouilli}(P_{ijk})$$

$$\text{Logit}\, P(Y_{ijk} = 1) = \mu + S_i + j + k_j$$

where Y_{ijk} is the assay result (positive or negative; 0 or 1) of a sapwood location and the probability of pathogen detection (P_{ijk}) follows a Bernoulli distribution. In the model, μ is the overall mean, S is the sapwood depth (outer sapwood or inner sapwood), and j and k are random effects for tree number and log number (nested within tree number), respectively. The models were run using the lme4 package in R and were tested with a Type II analysis of variance (Bates et al. 2015, R Core Team 2022). Estimated marginal means (i.e., predictions) of fungal detection from the inner and outer sapwood of NI and AI trees were calculated using the emmeans package (Lenth 2021). We used McNemar's test to determine whether the marginal probabilities of pathogen survival were the same between pre- and posttreatment samples (outer and inner sapwood samples combined). To better understand the relationship between applied fumigant concentration and pathogen survival, we also analyzed posttreatment data from Trial 3 using the same model as the pretreatment data with an added fixed effect for CT. Pretreatment isolation was initially included as a covariate in the posttreatment models, but it did not have a significant effect on posttreatment isolation and led to an increase in the Akaike and Bayesian information criteria. Values for CT were standardized to z-scores using the scale function in R prior to fitting the models because they were several orders of magnitude higher than the response variable.

Results

Pathogen presence in logs before fumigation

In all the trials, *B. fagacearum* was isolated from at least one of the outer sapwood locations before fumigation in nearly all logs (Fig. 1). In general, the frequency of *B. fagacearum* isolation was higher in the trial using logs from AI trees than the trials using logs from NI trees. In Trials 2 and 3, the frequency of pathogen isolation was greater ($P < 0.001$) in the outer sapwood (57% in logs from NI trees and 82% from AI trees; Supplemental Table S1) compared to samples taken from the innermost sapwood (23% in logs from NI trees and 60% from AI trees).

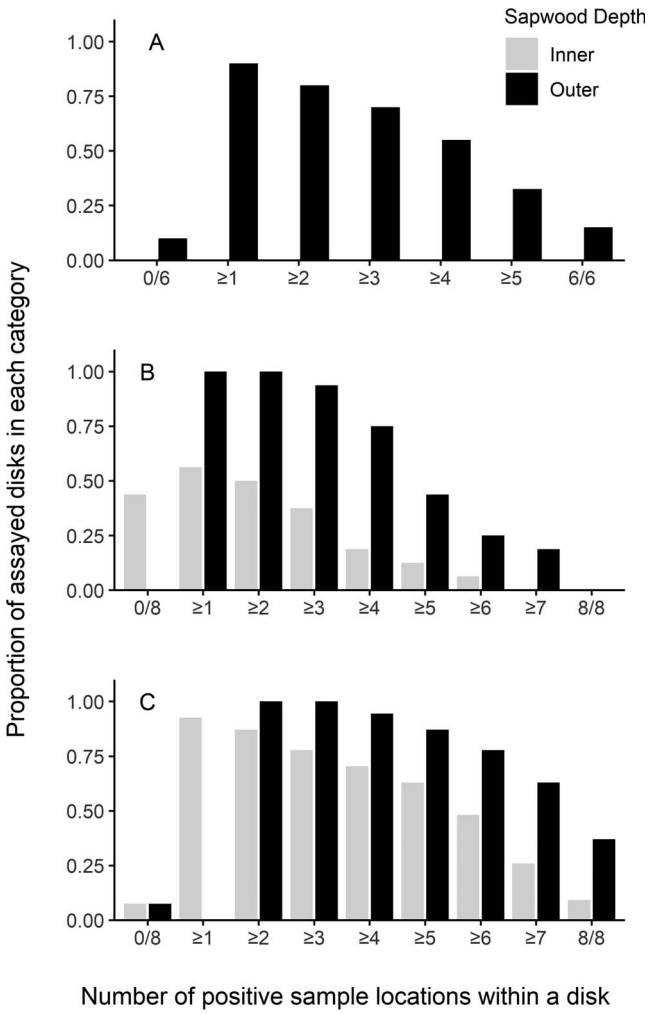

Figure 1.—Cumulative proportion of pretreatment sapwood samples yielding Bretziella fagacearum *per log disk based on the total number of disks (*n *= 40 disks for Trial 1;* n *= 16 disks for Trial 2;* n *= 54 disks for Trial 3). Log disks were assayed from naturally infected (A and B; Trials 1 and 2, respectively) and artificially inoculated (C; Trial 3) trees prior to fumigation with ethanedinitrile (EDN) in 2020 and 2021. Samples were taken from the outer and inner sapwood during Trials 2 and 3 and taken only from the outer sapwood during Trial 1.*

Pathogen presence in logs after fumigation

The pathogen was not isolated from any of the logs in the small log experiment following fumigation with 120 g/m^3 EDN for 24 or 48 hours (Table 3). In comparison, *B. fagacearum* was isolated from at least one of the four wood chips in 65 percent of the outer sapwood locations sampled from four control logs. In Trials 2 and 3, the pathogen was isolated from three out of 12 NI logs and 11 out of 19 AI logs following fumigation with 120 g/m^3 EDN for 48 and 72 hours, respectively. The treatments reduced the overall probability of pathogen survival (McNemar's $\chi^2 = 94.9$, $P < 0.001$, and McNemar's $\chi^2 = 54.3$, $P < 0.001$, for Trials 2 and 3, respectively). Pathogen survival in logs from AI trees was negatively correlated with the achieved CT product, but there was no difference in the likelihood of survival between sapwood depths after fumigation (Supplemental Table S2). Although not statistically tested, the frequency of pathogen isolation in the untreated logs did not appear to differ between pre- and posttreatment samples, suggesting that pathogen viability in the logs did not change over the time course of the experiments.

Discussion

In this study, EDN fumigation eradicated all viable *B. fagacearum* in small-diameter oak logs (average 12.2-cm diameter) using 120 g/m^3 EDN for 24 or 48 hours. However, treatments were less effective against the pathogen in large-diameter logs (average 34.4- and 34.7-cm diameter for Trials 2 and 3, respectively) using 120 g/m^3 EDN for 48 or 72 hours, particularly in the experiment with logs from trees artificially inoculated with the pathogen. Although the pathogen was not isolated from any of the fumigated logs in the trial with the smallest-diameter logs, the relatively thin bark and sapwood layer of these trees presumably did not mimic the magnitude of physical barriers (e.g., thicker bark and wider sapwood) that fumigants may encounter in commercially sized logs. Characteristics of the larger logs in Trials 2 and 3 serve as a closer approximation to commercially fumigated logs. In the larger-log trials, the total reduction of viable *B. fagacearum* (pretreatment vs. posttreatment; outer and inner sapwood samples combined) in the treated logs was 94.0 percent for NI logs and 86.2 percent for AI logs. Treatment time differed between the trials with NI and AI logs, making it difficult to infer whether increasing the fumigation time of NI logs to 72 hours would have resulted in full pathogen eradication. However, the low rate of pathogen recovery in NI logs treated for 48 hours warrants further evaluation in otherwise merchantable logs harvested from red oak trees with naturally occurring oak wilt infections.

One concern regarding EDN as a replacement to MB for QPS treatment is that its high sorption and water solubility may affect its penetration into green wood and thus complicate appropriate fumigant dosing (CSIRO et al. 1996, Armstrong et al. 2014, Hall et al. 2018, Park et al. 2021, Hall and Adlam 2023). Although we did not measure sapwood moisture content in our experiments, similar phytosanitary trials have reported relatively high sapwood moisture content (83% to 88%) in logs from recently harvested oak trees (Juzwik et al. 2019, Yang et al. 2019). Our fumigation treatments achieved high sorption across all treatments, which is congruent with several studies that reported rapid and high sorption of EDN into wet and end-sealed wood products (Pranamornkith et al. 2014, Najar-Rodriguez et al. 2020). Even with high sorption in the larger-log trials, *B. fagacearum* was not completely eradicated, suggesting that adequate fumigant penetration did not occur during our fumigation treatments. Research on the penetration and fate of EDN in wet wood is limited, and it remains unclear whether EDN persists in a gaseous state after it penetrates the wood, whether it dissolves in the water in the wood, or whether it decomposes into other products (CISRO 1996, Hall et al. 2018, Hall and Adlam 2023).

The minimum concentration of EDN (50 g/m^3 for 3 h at 10°C) that is lethal to *B. fagacearum* on inoculated barley was documented by Uzunovic et al. (2022) and theoretically represents the threshold dose needed for pathogen eradication in wood. However, changes in fumigant efficacy can occur when scaling phytosanitary trials from in vitro conditions to commercial-sized logs and lethal CT combinations for *B. fagacearum* colonized wood remain unknown (Yang et al. 2019, Seabright et al. 2020, Uzunovic et al. 2022). Results from Trial 3 using logs harvested from AI trees indicate that as CT products increased, the likelihood of pathogen survival decreased. The efficacy of EDN could potentially be increased through achieving higher CT products by increasing either fumigant concentration or treatment time. However, CT products achieved during log fumigations are also influenced by interrelated factors, such as treatment temperature, load factor, wood moisture content, and bark characteristics (Hall and Adlam 2023).

Table 3.—Posttreatment proportion of inner and outer sapwood samples yielding Bretziella fagacearum *of logs taken from naturally infected and artificially inoculated red oak trees after fumigation with ethanedinitrile (EDN).*

Fumigation trial no.	Tree infection type	No. of logs	EDN treatment (g/m^3)	Treatment time (h)	No. of positive logs	Proportion of positive sapwood locations	
						Outer	Inner
Trial 1[a]	Natural	2	0 (control)	24	2	0.54	—[b]
		2	0 (control)	48	2	0.75	—
		8	120	24	0	0.00	—
		8	120	48	0	0.00	—
Trial 2	Natural	4	0 (control)	48	4	0.59	0.40
		12	120	48	3	0.02	0.03
Trial 3	Artificial	8	0 (control)	72	8	0.73	0.63
		19	120	72	11	0.09	0.12

[a] Data represent the proportion of positive sapwood locations based on $n = 12$ attempts per log for Trial 1, $n = 8$ attempts per log at each depth for Trial 2, and $n = 16$ attempts per log at each depth for Trial 3.
[b] Inner sapwood locations were not sampled in Trial 1.

Maintaining wood quality for veneer processing is a priority for exported logs; thus, measures to increase fumigant penetration (e.g., removing bark or drying wood) may interfere with veneer quality (Morrell 1995).

Although this study did not allow for a direct comparison of NI and AI trees for EDN efficacy against *B. fagacearum*, this work highlights the challenges and benefits of using both tree infection types for phytosanitary evaluations. In the larger-log trials, overall rates of pathogen isolation before treatment were higher in logs obtained from AI trees compared to logs from NI trees (71.2% and 39.8%, respectively). These outcomes align with previous tests of SF and MB efficacy on logs from NI and AI trees in which AI trees provided a more rigorous standard for phytosanitary evaluations (Yang et al. 2019). The common concern that tests on logs from AI trees pose an unrealistic challenge for fumigant evaluation due to higher rates of pathogen colonization is mitigated at least in part by observations that *B. fagacearum* colonization in NI trees is both spatially and temporally variable (MacDonald et al. 1985, Schmidt et al. 1997). The inconsistency of pathogen colonization of NI trees introduces substantial uncertainty as to whether fumigation treatments fully eradicate the fungus. Thus, fumigation trials using logs from both AI and NI trees may offer the greatest opportunity for accurate inference.

The increasing restrictions on the use of MB as a phytosanitary treatment of exported logs creates an urgent need to identify suitable alternatives to maintain log exports. There are significant advantages to replace the current MB schedule with EDN; however, our study reveals that similar to SF, EDN may not be a suitable stand-alone treatment to eradicate *B. fagacearum* in logs with intact bark from diseased trees. Compared to other wood products that may be debarked or dried, commercial-sized veneer logs present a unique challenge to fumigant efficacy, as the large diameter, presence of bark, and high sapwood moisture affect fumigant penetration. Future work that focuses on understanding obstacles and limitations of EDN penetration may help to determine how to deliver lethal dosages to *B. fagacearum* in colonized logs. Very high levels of predicted efficacy (>99% mortality) have often been the standard to evaluate stand-alone phytosanitary measures such as MB fumigation. This is challenging to achieve in phytosanitary experiments using large-diameter logs due to the potential cost and labor required to produce an adequate sample size to verify treatment outcomes (Haack et al. 2011, Shortemeyer et al. 2011). Further research, including quantitative wood pathways analyses, is still needed to assess new solutions for oak log exports and potential regulatory measures.

Conclusions

Fumigation with 120 g/m^3 EDN for 48 or 72 hours can eradicate the majority of viable *B. fagacearum* in logs harvested from large-diameter diseased red oak trees, but it may not be suitable as a stand-alone phytosanitary treatment. In this study, survival of *B. fagacearum* was higher following fumigation with EDN (120 g/m^3 for 72 h) than previously published survival after MB (240 g/m^3 for 72 h) fumigation of logs harvested from inoculated trees (10% and 2% pathogen viability for EDN and MB treatments, respectively; Yang et al. 2019). Our experiments highlight the complex nature of fumigant treatment evaluation in logs and justifies the need for further evaluation at a commercial scale.

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