

Sulfuryl fluoride fumigation of red oak logs eradicates the oak wilt fungus

E. Schmidt, J. Juzwik, B. Schneider

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Preliminary field trials using red oak logs from trees dying from oak wilt disease were successful in eliminating the oak wilt fungus from sapwood after fumigation with sulfuryl fluoride for 72 h under tarp. These results support earlier laboratory data on the fungitoxicity of sulfuryl fluoride as a potential replacement for methyl bromide treatment of exported red oak veneer logs. However, not all other microorganisms were completely eradicated from oak sapwood at the treatment levels used in this trial.

Sulfurylfluorid-Begasung von Roteichenholz zur Bekämpfung des Eichenwelkepilzes

Erste Versuche zur Bekämpfung des Eichenwelkepilzes an befallenen Roteichenrundholz mit Sulfurylfluorid verliefen erfolgreich. Das Rundholz war 72 Stunden mit Sulfurylfluorid begast worden. Die Ergebnisse bestätigen frühere Laborbefunde über die fungizide Wirksamkeit von Sulfurylfluorid, das geeignet ist, das gebräuchliche Methylbromid zu ersetzen, womit exportiertes Furnierholz begast wird. Allerdings konnten unter den bisherigen Bedingungen nicht alle anderen Mikroorganismen völlig abgetötet werden.

1 Introduction

Concern over the accidental introduction of the oak wilt fungus (*Ceratocystis fagacearum* (T.W. Bretz) J. Hunt) into Europe prompted strong quarantine regulations in the 1970's. In order to prevent the import of the fungus from North America, oak wood is subject to specific treatment requirements under Council Directive 77/93/EC including

Elmer L. Schmidt
Dept. of Wood and Paper Sci.,
University of Minnesota, 2004 Folwell Ave,
St. Paul, MN USA 55108

Jennifer Juzwik
North Central Forest Experiment Station,
USDA Forest Service 1992 Folwell Ave,
St. Paul, MN, USA 55108

Brian Schneider
DowElanco Corp., 9330 Zionville Rd, Indianapolis,
IN, USA 46268

bark removal and drying or heat treatment (APHIS 1994). Oak logs intended for veneer production were granted exemption from these requirements due to concern that wood quality would drop using these measures, and the alternative treatment of log fumigation with methyl bromide (MB) was adapted as an accepted method for disinfecting red oak *Quercus rubra* L. (Liese et al. 1981; MacDonald et al. 1985; Schmidt et al. 1982; and Schmidt 1988). The pending restrictions of MB for phytosanitation use (McKenry et al. 1994) dictates that an alternative treatment must be found suitable for the the red oak veneer log export industry of North America (Kappenberg 1996).

Sulfuryl fluoride (Vikane, DowElanco, Indianapolis, IN, USA) is a fumigant which has been used to control wood-destroying insects in structures for over 35 years (DowElanco 1993; Kenaga 1961; Osbrink et al. 1987; and Stewart 1957). Sulfuryl fluoride (SF) has been shown to penetrate a variety of wood matrices more rapidly than MB (Scheffrahn et al. 1992). The first report of the fungitoxicity of SF (Woodward and Schmidt 1995) included its ability to kill the oak wilt fungus in short (15 cm), naturally-infected red oak log pieces of small diameter (15 cm). End-sealed pieces exposed to 280 g SF/m³ of space inside a sealed laboratory chamber for 72 h did not provide a single culture of viable fungus (present in over 50% of isolation attempts prior to fumigation).

The purpose of this research was to attempt eradication of the oak wilt fungus from commercial-sized logs of naturally-infected (wilted) red oak using SF as the fumigant under an outdoor tarpaulin fumigation. In addition, the frequency of isolation of other microorganisms from red oak sapwood was determined before and after fumigation of logs at two SF treatment levels.

2 Material and methods

Five red oak trees which had wilted from natural root-graft infection by the oak wilt fungus (60–100% of foliage wilted in late July) were felled in north-central Minnesota, U.S.A. during the first week of September. Two logs were cut from the bottom of each tree (2.4 m long) and one each assigned randomly to one of two treatment piles. Log diameters (inside bark) ranged from 28–58 cm with corresponding sapwood thickness of 2.5–5 cm. Logs were stacked on 10 cm high supports atop a nylon-vinylized tarp (5 for each pile). Disks (7 cm thick) were cut from each end of each log and sampled at five locations about the circumference according to the following isolation scheme: sapwood at each location was exposed using a chisel to split the disk at the sapwood-heartwood boundary; a sterile wood gouge was used to remove small (1.5 cm long) pieces of sapwood along the grain (four from outer sapwood within 1 cm of cambium and four from sapwood near the heartwood for each

media type providing a total of 80 isolation attempts for each disk); these samples were briefly flamed to surface disinfect and placed onto either a medium selective for the oak wilt fungus (Barnett 1953) or petri dishes containing 1.5% malt extract (Difco) and 2% agar agar (Difco). The malt extract agar (MEA) provided a non-selective medium to encourage growth of a wider variety of microorganisms found within the oak sapwood. All isolations of suspected oak wilt fungus on Barnett's media were subcultured onto potato dextrose agar and subsequently verified by microscopic confirmation of endoconidia. Other microorganisms developing on the MEA were grouped according to readily recognized form genera or otherwise noted as unknown filamentous fungi (melanized imperfects or ascomycetes or hyaline) or bacteria.

Two logs in each pile were end-sealed with bitumen and aluminum foil at one end to prevent fumigant access to sapwood by adjacent endgrain. Fungitoxic activity beneath the foil would require fumigant entry through the intact bark or movement from the exposed log end (2.5 m distant).

Each pile was fitted with a wood frame to prevent tarp contact with log surfaces as well as two sample lines (high and low in the log pile) and a gas introduction tube. A recording thermograph was included in one pile to monitor temperature inside the tarp during the fumigation. Tarps were sealed by rolling and securing edges with metal clips. SF (Vikane) was added from a pressurized cylinder on a weight basis to pile 1 at a target loading of 280 g/m³ of space (pile enclosure approx. 3.1 m³) following the treatment dosage successful in earlier laboratory work (Woodward and Schmidt 1995). Pile 2 was given a target loading 50% higher than that for the first pile (420 g/m³) to potentially compensate for the reduced fumigant access to sapwood given the thicker bark and sapwood zones in these large logs. Fumigant concentrations at upper and lower zones in each pile were monitored three times daily using two thermal conductivity analyzers (Fumiscop Model D, Key Chemical & Equipment Co., Clearwater, FL, U.S.A.) in order to calculate the concentration \times time product (CT value) for the treatment over the 72 h fumigation. No subsequent addition of fumigant was required as initial concentrations dropped an average of only 10% over the course of the trial. Short sections of log were also stored under tarp (no fumigant) for isolation after 72 h to assure that microorganism viability was not reduced by heating beneath the tarp.

Logs were uncovered and allowed to degas for 24 h prior to repeating isolation attempts as was done prior to treatment. The disks for end-sealed logs were taken 6 cm from the foil wrap, whereas the remaining disks were taken at a distance of 1 m from non-sealed log ends. Moisture contents of sapwood of the logs was taken just prior to isolation attempts using an oven drying method.

3 Results and discussion

Moisture contents of the sapwood of the five trees ranged from 63–106% (dry wt. basis), suggesting a varied representation of disease progress in the wilted trees (sapwood moisture content drops after tree death and as secondary microbial invasion increases). Isolation data for the oak wilt fungus on Barnett's media before and after the fumigation treatment is noted in Table 1. No oak wilt fungus was isolated from any logs after either level of SF fumi-

Table 1. Percentage isolation of the oak wilt fungus from the outer sapwood of infected red oak logs before and after fumigation with sulfuryl fluoride for 72 h (Barnett's media)

Table 1. Anteil an isoliertem Eichenwelkepilz aus dem äußeren Splintbereich von Roteichenrundholz vor und nach Begasen mit Sulfurylfluorid

	Log #	Before Fumigation ¹	After Fumigation
Pile 1 (CT 27,400 g h/m ³)	1	37.5	0
	2	7.5	0
	3	2.5	0
	4	10.0	0
	5	0	0
Pile 2 (CT 35,010 g h/m ³)	1	12.5	0
	2	25.0	0
	3	10.0	0
	4	17.5	0
	5	0	0

¹ Each percentage is the number of positive cultures out of 40 sapwood isolation attempts (20 from each log end).

gation. Ignoring the tree which had no viable oak wilt fungus (by our isolation scheme) in the sapwood prior to fumigation (Log 5 in each pile), the average percentage of positive isolations from outer sapwood for pile 1 was 14.4 and 16.3 for pile 2. These positive recovery rates are far below the approx. 75% reported in lower bole sections of red oaks which had been inoculated with a conidial suspension at six locations about the bole circumference to induce disease in eradication trials with methyl bromide (MacDonald et al. 1985). Given that the trees in the SF trial were infected by root grafts from nearby infected trees, it is likely that development of the oak wilt fungus into the lower stems was restricted to longitudinal bands of xylem about the circumference making detection by random isolation more difficult. Competition from other fungi on the semiselective Barnett's media was not an obvious hinderance to isolation success. This is further suggested by the fact that the deeper sapwood isolation attempts were very low (4/160 attempts for Pile 1 and 6/160 attempts for Pile 2) for the 4 logs with recoverable oak wilt fungus. Even though only 3% of the deeper isolation attempts were successful prior to treatment, no fungus was recovered after fumigation suggesting that SF did have the ability to penetrate the sapwood thickness and kill the oak wilt organism in these logs. Four of 20 isolation attempts from outer sapwood of a tarped but not fumigated log were positive, confirming that the temperature range under the tarp (10–20 °C) did not decrease fungal viability.

The question that is of prime importance in this preliminary study is the estimate of the probability that the oak wilt fungus was, in fact, eradicated in the infected logs treated with SF. Using the approach noted by MacDonald et al. (1985) for methyl bromide treatment of red oak (which followed a similar sampling scheme to that used in the SF fumigation), the odds of finding at least one infected sample if n samples are taken and $p \times 100\%$ of the sapwood is infected could be estimated using a binomial approximation to the hyper-geometric distribution. The probability of detecting an infected log with approx. 15% of the sapwood infected would be .9925 using the 40 attempts/log scheme. Given the fact that few organisms were cultured even on the MEA after fumigation, the MEA attempts from outer sapwood can be legitimately considered

Table 2. Summary of microorganisms (other than the oak wilt fungus) isolated from red oak inner and outer sapwood before and after fumigation with sulfuryl fluoride for 72 h

Tabelle 2. Anzahl an Mikroorganismen (außer Eichenwelkepilz), die aus dem inneren und äußeren Splintbereich von Eichenrundholz isoliert wurden. Werte vor und nach dem 72 stündigen Begasen mit Sulfurylfluorid

Pile Designation (CT)	Microbe Type	Pre-fumigation		Post fumigation	
		Outer	Inner	Outer	Inner
1 (27,400 g h/m ³)	melanized fungi	12	5	0	1
	<i>Trichoderma</i> spp.	7	0	0	0
	<i>Penicillium</i> spp.	2	1	1	1
	other fungi	28	24	6	0
	bacteria	5	3	4	3
Total Positive Cultures (200 attempts on MEA)		54	33	11	5
2 (35,010 g hr/m ³)	melanized fungi	28	3	0	2
	<i>Trichoderma</i> spp.	16	9	0	1
	<i>Penicillium</i> spp.	4	2	1	0
	other fungi	50	33	1	2
	bacteria	6	0	3	3
Total Positive Cultures (200 attempts on MEA)		104	47	5	8

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as attempts to find the oak wilt fungus which would provide 80 total isolation attempts/log.

If the presumption is made that each log in a treated pile retained at least 1% of the circumference with viable fungus after the SF treatments ($n = 5$ logs/pile), and that the disk sampling method from two locations/log covered approximately one-half of the sapwood circumference, a Monte Carlo simulation program can be used to estimate the probability of identifying an infected log. Results indicate that, presuming vertical strip infection bands in the sapwood, the chance that an infected log would not be detected would be only 1%. More powerful levels of confidence that no fungus remains in treated logs would require further trials with more intensive isolation efforts and a more thorough level of sapwood colonization by the oak wilt fungus.

The fumigation of logs in the treatment piles also greatly reduced but did not eradicate other microorganisms from the sapwood regions (Table. 2). These field data are in contrast to the findings in the laboratory (Woodward and Schmidt 1995) where a CT value of 18,530 g h/m³ not only eradicated the oak wilt fungus, but all other organisms originally present in the small red oak log pieces as well. Despite the increased severity of the treatments (48% and 89% higher CT for piles 1 and 2 respectively) measured in the field on larger logs, complete eradication of all microbial types cultured from sapwood was not achieved. The reductions in positive isolates were quite dramatic (even for inner sapwood areas), however, suggesting that longer treatment times might well achieve the goal of complete eradication desired for phytosanitation purposes. The greater efficacy of the fumigant against the oak wilt fungus as compared to all other microorganisms was noted in earlier work with methyl bromide (MacDonald et al. 1985), and may reflect the fact that the location of the oak wilt fungus and propagules within the wood matrix (vessels and ray tissue) makes it more accessible to the SF accumulating in the wood. The great reductions in viable organisms deep within the sapwood (at the heartwood boundary) also confirms the fact that complete sapwood penetration can be achieved even in such large logs.

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Conclusions

The oak wilt fungus can be killed throughout the sapwood depth in large red oak logs from naturally-infected trees

after fumigation with SF at treatment levels equal to or greater than 27,400 g h/m³. The fumigant effectively eradicated the pathogen in sapwood adjacent to ends of 2.4 m long logs sealed to prevent longitudinal entry at the proximal endgrain, and would, therefore, be expected to also be similarly effective in logs of 5 m length as are common in commercial trade. Sulfuryl fluoride fumigation of red oak veneer logs to prevent export of the oak wilt fungus is supported by these data. Not all microorganisms, however, were eliminated at the fumigant levels tested and use of SF as a sterilant for red oak sapwood requires further experimentation.

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