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

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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


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 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 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 disinfest 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 (Fumiscope Model D, Key Chemical & Equipment Co., Clearwater, FL, U.S.A.) in order to calculate the concentration × time product (CT value) for the treatment over the 72 h fumigation. No subsequent addition of fumigant was required by the fact that the deeper sapwood isolation attempts for Pile 2) for the 4 logs with recoverable oak wilt fungus (by our isolation scheme) in the sapwood prior to fumigation, 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 x 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>
<thead>
<tr>
<th>Log #</th>
<th>Before Fumigation</th>
<th>After Fumigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pile 1</td>
<td>(CT 27,400 g h/m³)</td>
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<tr>
<td>1</td>
<td>37.5</td>
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</tr>
<tr>
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<td>0</td>
</tr>
<tr>
<td>Pile 2</td>
<td>(CT 35,010 g h/m³)</td>
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</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>5</td>
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</tr>
</tbody>
</table>

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

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 fumigation. 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.

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