SULFURYL FLUORIDE AS A QUARANTINE TREATMENT FOR THE PINEWOOD NEMATODE IN UNSEASONED PINE

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The pinewood nematode (PWN) (*Bursaphelenchus xylophilus* Steiner and Buhrer, 1934 Nickle, 1970), the causal agent of pine wilt disease, has been intercepted in pine chips, unseasoned pine lumber, and wood packing material (WPM). Several countries now regulate the import of all coniferous chips, logs, sawn wood, and WPM to protect their forest from the PWN and other exotic pests.

Although methyl bromide can be used for quarantine purposes under the Montreal Protocol, there is interest internationally in finding an alternative fumigant for wood packing material (Dwinell 2001, 2004). Sulfuryl fluoride (SF) may serve as an alternative for methyl bromide for managing the PWN and its insect vectors in unseasoned pine wood. A preliminary report of research evaluating the efficacy of SF to control PWN in pine wood was published in 2003 (Dwinell et al. 2003). This study evaluated the efficacy of SF to kill the PWN and its insect vectors (*Monochamus* spp) in unseasoned pine lumber fumigated in laboratory chambers and in field chambers. Quarantine fumigation schedules are suggested based on the results of this study and Dwinell et al. 2003.

Materials and Methods

Wood preparation: Wood was sawn from loblolly pine (*Pinus taeda*) and shortleaf pine (*P. echinata*) logs that had been attacked by *Monochamus* spp. and colonized by PWN in June 2003. Sticks (2.5 x 2.5 x 25 cm) were sawn for laboratory fumigations. Boards, cants, and slabs were sawn in ca. 97 cm lengths for field fumigations.

Laboratory Chamber Fumigations: Experiments were conducted at Purdue University, West Lafayette, IN using two 28.3L fiberglass chambers equipped with a 25 cfm fan for air circulation and placed in a temperature-controlled environmental chamber. Each fumigation chamber was loaded with 20 or 25 sticks with the same number of sticks placed in the environmental chamber as untreated controls. Dosages ranging from 50-125 g/l were evaluated at 15, 20, or 30ºC for 24 h exposure period. Each chamber treatment was replicated twice. The appropriate amount of SF was introduced in gaseous form using a calibrated glass syringe. SF concentrations in the fumigation chambers were confirmed 30 min after gas introduction and 30 min before aeration using a Model 7500 portable gas chromatograph (Carle Instruments, Inc. Fullerton, CA). A standard curve was prepared using various SF gas concentrations measured by the GC and recorded on an Esterline Angus Port-A-Graph recorder (Indianapolis, IN).

Field Chamber Fumigations: Three field fumigation trials were conducted at the Whitehall Forest, Athens, GA; in Aug 2003 and Feb and April 2004. Fumigation chambers (5 m^3) were constructed of lumber frames and covered with 4-mil polyethylene for the Aug 2003 fumigation and 6-mil polyethylene for the subsequent fumigations. Six fumigated chambers were built on one concrete pad located 104 m from the concrete pad on which the two non-fumigated, control chambers were built. Duct tape and spray adhesive was used for sealing tarps together and to the concrete pad. Each chamber load consisted of 3-6 dimensions of lumber replicated 7-22 times, based on fumigation trial. Wood loads were approximately equal per chamber in each fumigation trial. Wood volume load factor averaged 10-15% for this study. Layers of wood were loaded and separated by common wood spacers. Wood equilibrated in sealed chambers overnight before SF was introduced. One battery-operated 4" desk fan was placed in each chamber to aid SF dispersion after introduction.

The introduction of SF (99.8%, Dow AgroSciences, Indianapolis, IN) was measured and controlled using a programmable charging scale (Yellow Jacket, Ritchie Engineering Co., Bloomington, MN). The SF concentrations in each chamber, fumigated and non-fumigated, were measured using a Fumiscope (Key Chemical and Equipment Co., Clearwater, FL) and an infrared (IR) analyzer (Spectros, Walpole, MA). The calibration of the TC and IR instruments were checked daily using SF diluted to a known concentration.

The ambient air temperature in each chamber was recorded using Hobo Model H8 Temperature dataloggers (Onset Computer Co., Bourne, MA). Following fumigation, chambers were aerated until SF concentrations were non-detectable, as measured using an Interscan gas analyzer (Model GF1900, Interscan Co., Chatsworth, CA).

PWN Bioassays: Wood was assayed for the PWN before and after fumigation. Following laboratory fumigations, a thin section of wood was sawn from the center of each stick to extract nematodes using the Baermann funnel procedure (Southey 1985). The occurrence of PWN in each dimension of lumber was recorded and expressed as percent of lumber positive for PWN. Boards were examined periodically for evidence of *Monochamus* spp. activity and emergence after the fumigation. The wood moisture content, expressed on a dry weight basis, was determined by drying a second set of borings or wafers at 105^{o}C for 24 h. All bioassays and wood moisture content measurements were conducted in Athens, GA.

Results and Discussion

Laboratory Chamber Fumigations: PWN survivorship in non-fumigated controls was high (100%), indicating that the handling procedures in shipping sticks to and from IN did not adversely affect PWN. The mean WMC of samples for the laboratory fumigations ranged from 37 to 92% on a dry weight basis. The WMC of the samples were well above the fiber saturation point of wood, generally taken as 28%. Laboratory fumigations demonstrated that increasing temperature decreased the CT dosage of SF required to kill PWN (Table 1). The minimum CT dosage tested that resulted in no PWN

detected in wood sticks was 2996 g-h/m^3 at 15ºC, 2099 g-h/m^3 at 20ºC, 1420 g-h/m^3 at 25ºC (Dwinell et al. 2003), and 1204 g-h/m^3 at 30ºC.

Field Chamber Fumigations: Fumigations had exposure times of at least 16.5 h. For the Aug 2003 fumigation, the HLT to 13.3 ± 4.4 h. Additional SF was added to two fumigation chambers. For the Feb. and April 2004 fumigations, the polyethylene sheeting was 6-mil, rather than 4-mil used in the previous fumigation, which improved the HLT to 67 ± 43 h and 129 ± 29 h, respectively. Only one chamber needed additional SF added during the April 2004 trial.

Infestation rate of PWN in untreated controls was high, with 82-100% of the controls positive for PWN following the fumigation (Tables 2-4). As with the laboratory trials, increasing temperature decreased the CT dosage of SF required to kill PWN in field trials. The mean chamber temperature for the Aug 2003 (Table 2) was 33ºC. Wood moisture content was 52% *P. echinata* and 87% *P. taeda.* During these trials, only the lowest CT dosage tested, 1151 gm-h/m^3, had live PWN extracted from lumber of all dimensions. All CT dosages 1402 gm-h/m^3 or greater resulted in no PWN extracted.

The mean chamber temperature for the April 2004 trial (Table 3) was 24ºC. The highest mean wood moisture content, 72%, was tested during these trials. CT dosages at or below 1207 gm-h/m^3 had live PWN extracted from lumber. All CT dosages 1533 gm-h/m^3 or greater resulted in no PWN extracted.

The coldest temperature evaluated in the field was a mean 10ºC during the Feb 2004 field trial (Table 4). Mean wood moisture content was 68%. Rainfall occurred during the night of the fumigation and accumulated water broke the seal on one chamber. Therefore, CT dosage and HLT could not be determined for this chamber; results of the remaining five fumigated chambers are presented. During these trials, live PWN were extracted from lumber at all CT dosages tested (4203-5866 gm-h/m^3).

There was no evidence of *Monochamus* spp. activity (i.e., fresh shavings) or emergence holes in the fumigated lumber from any field trial, in spite of extraction of PWN from fumigation treatments tested during three of the four field trials. Cerambycid beetles appear to be more susceptible to SF than PWN, based on these observations and recent research completed by Barak et al. (2005) on dosages required to kill the another cerambycid beetle, the Asian longhorned beetle *Anoplophora glabripennis.*

Based on laboratory and field trials, recommended minimum CT dosages by temperature and quarantine treatment schedule are proposed in Table 5. Fumigation of PWN at mean temperatures below 15ºC will not be recommended at this time. The dosages in the treatment schedule should also kill the insect vector, *Monochamus* spp., based on field observations in these trials. In conclusion, sulfuryl fluoride could be a viable alternative to methyl bromide for eradicating the PWN and its *Monochamus* spp. vectors in pine lumber and WPM.

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Table 1. Survival of pinewood nematode in unseasoned shortleaf and loblolly pine sticks (2.5 x 2.5 x 25 cm), sawn from naturally-infested logs, following fumigation with sulfuryl fluoride for 24 h at varying CT dosages (g-h/m^3) in temperature-controlled fumigation chambers, 2003-2004.

Chamber ºC[1]	Target (Actual) Initial g/m^3	SF CT (g-h/m^3)	% Sticks Positive PWN[2]
15	90 (92)	2173	50[3]
15	125 (126)	2996	0[3]
20	70 (71)	1671	50[4]
20	90 (89)	2099	0[5]
20	110 (109)	2566	0[5]
30	50 (51)	1204	0[4]

[1] Two replicated chamber fumigations per temperature and target initial g/m^3
[2] All non-fumigated control sticks were 100% positive for PWN; nematodes extracted using Baermann funnel method
[3] 20 shortleaf pine sticks per replicate, including non-fumigated controls
[4] 25 loblolly pine sticks per replicate, including non-fumigated controls
[5] 25 shortleaf pine sticks per replicate, including non-fumigated controls

Table 2. Survival of pinewood nematode in unseasoned loblolly and shortleaf pine . *echinata*) boards of varying dimensions sawn from naturally-infested logs following fumigation with sulfuryl fluoride (SF) at mean 33ºC, Whitehall Forest, Athens, GA, August 2003.

SF CT (g-h/m^3)[1]	Mean (Max.) Chamber ºC	Percent boards positive for PWN by board dimension in cm (No. of boards fumigated)[2]					
		2.5 x 2.5	2.5 x 5	2.5 x 10	5 x 5	5 x 10.2	10.2 x 10.2
1151	34 (49)	54 (11)	27 (11)	44 (25)	71 (7)	41 (22)	88 (8)
1402	33 (45)	0 (12)	0 (12)	0 (23)	0 (7)	0 (22)	0 (8)
1916	32 (42)	0 (12)	0 (12)	0 (23)	0 (7)	0 (22)	0 (8)
1937	34 (47)	0 (12)	0 (12)	0 (23)	0 (7)	0 (22)	0 (7)
1942	33 (45)	0 (12)	0 (11)	0 (23)	0 (7)	0 (21)	0 (7)
2092	33 (44)	0 (12)	0 (13)	0 (23)	0 (7)	0 (22)	0 (7)
Control	33 (44)	87 (24)	100 (24)	94 (46)	100 (14)	100 (44)	100 (14)

[1] One chamber per SF CT evaluated; 2 control chambers
[2] 2.5 x 2.5 lumber was *P. echinata*; remaining lumber was *P. taeda.*

Table 3. Survival of pinewood nematode in unseasoned loblolly pine boards of varying dimensions sawn from naturally-infested logs following fumigation with sulfuryl fluoride (SF) at mean 24ºC, Whitehall Forest, Athens, GA, April 2004.

SF CT (g-h/m^3)[1]	Mean (Max.) Chamber ºC	Percent boards positive for PWN by board dimension in cm (No. of boards fumigated)			
		2.5 x 10	5 x 10.2	10.2 x 10.2	Slabs
1160	24 (41)	0 (13)	17 (12)	42 (7)	0 (12)
1207	23 (38)	0 (13)	25 (12)	57 (7)	18 (12)
1533	23 (35)	0 (13)	0 (12)	0 (7)	0 (12)
1597	24 (44)	0 (13)	0 (12)	0 (7)	0 (12)
2171	23 (37)	0 (12)	0 (13)	0 (7)	0 (12)
2357	25 (43)	0 (13)	0 (12)	0 (7)	0 (12)
Control	24 (37)	85 (26)	100 (24)	100 (14)	82 (24)

[1] One chamber per SF CT evaluated; 2 control chambers

Table 4. Survival of pinewood nematode in unseasoned loblolly pine boards of varying dimensions sawn from naturally-infested logs following fumigation with sulfuryl fluoride (SF) at mean 10ºC, Whitehall Forest, Athens, GA, February 2004.

SF CT (g-h/m^3)[1]	Mean (Max.) Chamber ºC	Percent boards positive for PWN by board dimension in cm (No. of boards fumigated)			
		2.5 x 10	5 x 10.2	10.2 x 10.2	Slabs
4203	10 (15)	20 (10)	27 (11)	15 (13)	8 (12)
4381	11 (17)	30 (10)	64 (11)	62 (13)	33 (12)
5066	10 (15)	10 (10)	36 (11)	0 (13)	0 (13)
5515	10 (15)	30 (10)	18 (11)	15 (13)	0 (12)
5866	10 (12)	0 (10)	18 (11)	15 (13)	0 (12)
Control	10 (16)	80 (20)	73 (22)	100 (26)	85 (22)

[1] One chamber per SF CT evaluated; 2 control chambers. Results of one CT dosage chamber excluded because chamber seal broke after water accumulation following rainfall; CT dosage and HLT could not be determined for this chamber

Table 5. Proposed treatment schedule for 24 h sulfuryl fluoride (SF) fumigation of unseasoned pine for control of pinewood nematode.

Mean ºC	Min. Target CT Dosage (g-h/m^3)	SF Dose (g/m^3)	Minimum Concentration (g/m^3) at hour:				
			0.5	2	4	12	24
15 to 19.9	3000	175	179	165	155	123	87
20 to 24.9	2100	124	130	118	111	88	62
25 to 29.9	1500	88	94	83	78	62	44
30 to 34.9	1400	82	87	78	73	58	41
35 or greater	1000	60	63	57	53	42	30