Review of research on the control of pine wood nematode (*Bursaphelenchus xylophilus*) using the fumigant sulfuryl fluoride and current status for inclusion in ISPM No.15

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Abstract

The pine wood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease, is endemic to North America. The unintentional introduction of the PWN into Asia and now Europe has caused considerable damage to forests in these regions. The PWN was first reported in Portugal in 1999 where it has now become established and it was also reported in Spain in 2008. This situation led the European Union (E.U.) Commission to take exceptional phytosanitary measures for wood trade within the E.U. Preliminary studies on the broad spectrum fumigant sulfuryl fluoride (SF) showed control of the nematode, but complete eradication was not achieved at the dosages tested. Further studies provided evidence that 100% control of PWN could be obtained by fumigating unseasoned wood with SF. A review of these research studies is presented together with a proposed fumigation schedule. This schedule and supporting efficacy data on PWN and quarantine insects has been submitted to the International Plant Protection Convention (IPPC) for inclusion of SF as an approved treatment in ISPM 15 (International Standard for Phytosanitary Measures) which regulates Wood Packaging Material in International Trade. The inclusion of SF in the standard would provide an alternative fumigant option to methyl bromide (MB) which will be banned for all uses in the E.U. in March of 2010.

Keywords: Sulfuryl fluoride, *Bursaphelenchus xylophilus,* ISPM No.15, Quarantine, Fumigation

1. Introduction

The use of wood pallets and packaging materials e.g. crates and boxes, widely used for agricultural commodities and industrial items, has increased significantly as global trade has developed over recent decades. This has resulted in an increased risk of the movement of non-native pest species on wood used in international transport. Some of these present a major threat to forestry and woodlands. This is the case of pine wood nematode (PWN); *Bursaphelenchus xylophilus* (Steiner and Buhrer), Nickle, 1970, which is native to North America and has spread to Asia and Europe with devastating effects on pine species not native to North America. This paper provides an overview on the research conducted on control of PWN with SF and the status of inclusion of sulfuryl fluoride (SF) in ISPM No.15.

1.1. Situation of PWN in E.U. and phytosanitary measures

The PWN is a nematode of about 1 mm length which is the agent responsible for Pine Wilt disease. It is vectored by pine sawyers of the genus *Monochamus* (Coleoptera, *Cerambycidae*). This nematode is native to North America (Canada, U.S.A. and Mexico). It first appeared in Japan at the beginning of 20th century on timber exports then spread to Korea, China and Taiwan. It was first detected in Portugal in 1999 in the Setubal region (Mota et al.,1999) where it appeared to be vectored by *Monochamus galloprovincialis* (Olivier) and other *Monochamus* spp. naturally present in that part of Europe.

In spite of E.U. measures to eradicate this pest since 2000, including the felling of 1 million pine trees, the spread could not be stopped and 70 outbreaks were recorded in 2008 outside the Setubal area, in North and central Portugal. (E.U. Commission, 2009) An isolated outbreak was notified to the E.U. Commission in Spain in November 2008 while several other countries reported presence of live PWN in wood packaging material (WPM) originated from Portugal. This resulted in strengthening emergency measures in an attempt to prevent the PWN spreading to other E.U. countries (E.U. Commission, 2006-2009). Beginning 1 January 2010, all WPM from Portugal to other E.U. Member Countries must be treated in accordance with ISPM 15 which allows two types of treatments: heat or fumigation with methyl bromide (MB).

The treatment choice, however, will be further restricted following an E.U. Commission Decision (2008/753/EC) of non-inclusion of MB in the Annex I of 91/414. This decision means the withdrawal of Plant Protection Authorization for this substance by 18 March 2009 with a transition period of one year. After 18 March 2010, MB treatment of WPM will be illegal in Portugal and throughout the E.U.; the only approved treatment for WPM will be heat i.e. 56°C at the core of the wood for 30 minutes. This requires large investment in fixed installations with powerful ovens to be able to effectively treat WPM. Heat can damage certain packaged goods. Fumigation offers a more flexible alternative e.g. loaded shipping containers with manufactured goods, and requires less investment.

1.2. Sulfuryl fluoride as a potential alternative to methyl bromide

SF has a long history of use being introduced by The Dow Chemical Company as the product Vikane® in 1961 in the USA for the eradication of termites, wood boring beetles, and other structure infesting pests. Since that time, many new registrations and commercial use patterns, e.g. in the food industry, have been established around the world (Thoms et al., 2008).

SF is a broad-spectrum fumigant which is a viable alternative to MB for fumigating a wide variety of structures and post-harvest commodities for pest control and eradication. The physical properties and inorganic nature of SF enable it to achieve deep penetration (diffusion) into matrices and it is more efficient in this respect compared to MB (Scheffrahn et al., 1992).

One area of active research and development of SF is fumigation of unseasoned wood, used in international trade, to control quarantine pests (Woodward and Schmidt, 1995; Mizobuti et al., 1996; Soma et al., 1996; 1997; 2001; Dwinell et al., 2003; 2005; Barak et al., 2006, 2010; Tubajika and Barak, 2006; Flack et al., 2008; Daojian et al., 2010). These data have been developed by leading international quarantine government scientists.

Quarantine specific use patterns already exist in a number of countries which include Finland, Germany, Norway, Sweden and the USA. China has also approved a specific treatment schedule for SF on logs for fumigation prior to export. In the E.U., SF has been granted Annex 1 listing under E.U. Directive 98/8/EC (Biocides) for Product Type 8 - wood preservative (E.U. Commission, 2006) and product Type 18 – insecticide (E.U. Commission, 2009). The submission of modern registration data packages and successful evaluations and registrations at E.U. and country level confirm that the fumigant will be available for use in the foreseeable future.

2. Trials on PWN from Soma et al. (2001) in Japan

2.1. Materials and methods

Red pine, *Pinus densiflora* Siebold et Zuccarini, naturally infested with PWN in Japan were sawn into boards (2 x 15 x 30 cm) and lumber (15 x 15 x 30 cm). Before fumigation, the pretreatment population of PWN was confirmed to exceed 10,000 PWN per 100 g of sample. Five pieces of boards and three pieces of lumber were tied up in a bundle by a plastic band and surrounded by boards and lumber of same size for achieving appropriate fumigation loading. Fumigations were carried out in 100 L fiberglass chambers, at 15°C and with 25% (v/v) load factor. Wood moisture content ranged from 25.8 to 27.3% in the boards and 20.1 to 33.4% in the lumber before fumigation. The mortality was assessed with Baermann funnel method 6-7 days and 20-21 days after fumigation.

2.2. Results and Conclusions

Nematode populations were high (20,400/100 g or greater) in all treatments before fumigation and in untreated controls after fumigation (Table 1). SF showed a clear dose response on the control of PWN with increased PWN mortality as the dosage increased. Nonetheless, the highest SF dosage tested (2932 g-h/m^3) was not sufficient to achieve 100% PWN mortality at 15°C in boards or lumber after fumigation. For the two highest SF dosages tested, PWN survivorship decreased at 20-21 days after fumigation compared to that of 6-7 days after fumigation. This might indicate a delayed mortality response of PWN to SF, which has been documented with insects (Osbrink et al., 1987). The best results were achieved at the highest dosage tested at 20-21 days after fumigation (0.34% survivors).

Table 1 Number of pine wood nematodes (PWN) before and after fumigation (percent survival) with sulfuryl fluoride at 15°C in boards and lumber (data extraction from Soma et al., 2001)

			Number PWN (survival %)				
			Board 2 x 15 x 30 cm		Lumber 15 x 15 x 30 cm		
SF Dose (g/m^3)	Exposure (h)	SF Dosage ($g\text{-}h/m^3$)	Days after fumigation		Days after fumigation		
			0[1]	6-7	0	6-7	20-21
Untreated			39000	33300 (85.4)	38600	39600 (102.6)	53100 (149.6)
30	24	765	-	-	20500	2183 (10.6)	3819 (18.6)
60	24	1539	20400	2704 (13.3)	22700	1906 (8.4)	454 (2.0)
60	48	2932	20400	1277 (6.0)	22700	426 (1.9)	78 (0.34)

[1]0 = prefumigation counts

3. Trials from Dwinell et al. (2003, 2005) in the U.S.A.

3.1. Materials and methods

For the April 2003 field trial, logs used were shortleaf pine, *Pinus echinata* Mill, that had been killed by the southern pine beetle (*Dendroctonus frontalis* Zimmerman) during the prior year were colonised by *Monochamus* spp. and the PWN (Dwinell et al., 2003) . The logs were salvaged ca. eight months after the pines had died, and debarked and sawn into timber *ca*. two weeks prior to each fumigation trial. For all subsequent lab and field fumigation trials, the wood was sawn from loblolly pine, *P. taeda* L.*,* which was also naturally colonised by *Monochamus* spp. and PWN. Sticks (2.5 x 2.5 x 25 cm) were sawn for laboratory chamber fumigations. Boards (2.5 or 5 x 2.5 to 15 cm), cants (12.7 x 12.7 or 10.2 x 10.2 cm), and slabs recovered from logs during the milling process were sawn in *ca*. 97 cm lengths for field fumigations.

Laboratory experiments were conducted by Dow AgroSciences LLC at facilities at the Purdue University, West Lafayette, Indiana, USA using two, 28.3 L fibre glass chambers. The four field fumigation trials were set up between 2003 and 2004 by the USDA at the Whitehall Forest in Athens, Georgia, using 5 m^3 chambers made of timber frame and polyethylene sheeting cover. Wood was assayed for the PWN before and after fumigation, using the Baermann funnel procedure applied on a thin section of wood from the center of each stick (laboratory trials) or removing two wafer-thin sections by augering or sawing boards, cants, and slabs (field fumigations). The wood moisture content (WMC) was determined by drying a second set of samples at 105ºC for 24 h. Slabs, cants, logs and boards were examined periodically for evidence of *Monochamus* spp., activity and emergence after the fumigation.

3.2. Results and Conclusions

Table 2 Survival of pinewood nematode (PWN) in unseasoned pine sticks (2.5 x 2.5 x 25 cm), sawn from naturally-infested logs, following fumigation with sulfuryl fluoride (SF) for 24 h at varying dosages (g-h/m3) in temperature-controlled fumigation chambers, 2003-2004 (Dwinell et al., 2003, 2005).

Chamber ºC[1]	Target (Actual) SF Dose g/m^3	SF Dosage ($g\text{-}h/m^3$)	% Sticks Positive PWN[2]
15	90 (92)	2173	50[3]
15	125 (126)	2996	0[3]
20	30 (31)	694	70[4]
20	60 (61)	1393	10[4]
20	70 (71)	1671	50[4]
20	90 (89)	2099	0[5]
20	110 (109)	2566	0[5]
25	60 (60)	1420	0[4]
30	50 (51)	1204	0[4]
30	60 (60)	1426	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 *Pinus echinata* sticks per replicate, including non-fumigated controls. [4] 25 *Pinus taeda* sticks per replicate, including non-fumigated controls. [5] 25 *Pinus echinata* sticks per replicate, including non-fumigated controls

In laboratory fumigations (Table 2), all non-fumigated control sticks were 100% positive for PWN. The minimum dosage of SF tested resulting 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, and 1204 g-h/m^3 at 30ºC. The mean water moisture content (WMC) ranged from 37 to 92% on a dry weight basis.

Field fumigations were conducted, respectively, at a mean minimum temperature of 33°C, 32°C, 23°C and 10°C (Table 3). The WMC in fumigation conducted in April 2003 was 34% but was much higher in the other fumigations, between 52 and 87%. At 10°C, live PWN were extracted from timber at all dosages of SF tested (4203-5866 g-h/m^3). There was no presence of nematode on 100% of the boards sampled at a minimum dosage of 1533 g-h/m^3 at 23°C, 1402 g-h/m^3 at 32°C, and 997 g-h/m^3 at 33°C in field trials. There was no evidence of *Monochamus* spp. activity (i.e., fresh shavings) or emergence holes in the fumigated lumber from any field trial.

Table 3 Survival of pine wood nematodes (PWN) in pine boards (*Pinus echinata*, April 2003; *P. taeda*, all other fumigations) of varying dimensions sawn from naturally-infested logs following fumigation with SF, Whitehall Forest, USA, (Dwinell et al., 2003, 2005)

		% boards positive for PWN by board dimension (No. of boards fumigated)					
SF Dosage (g-h/m^3)	**Actual Total h Exposure**	**2.5 x 2.5 cm**	**2.5 x 5 cm**	**2.5 x 12.7[1] or 2.5 x 10 cm**	**5 x 12.7[1] or 5 x 10.2 cm**	**12.7 x 12.7[1] or 10.2 x 10.2 cm**	**Slabs**
Fumigation April 2003, mean temperature 35 °C, WMC[2] 34%							
997	6.5	-	-	0 (13)	0 (13)	0 (13)	-
1039	6.5	-	-	0 (13)	0 (13)	0 (13)	-
1192	6.5	-	-	0 (13)	0 (13)	0 (13)	-
1506	6.5	-	-	0 (13)	0 (13)	0 (13)	-
1538	6.5	-	-	0 (13)	0 (13)	0 (13)	-
1751	6.5	-	-	0 (13)	0 (13)	0 (13)	-
Control		-	-	89 (39)	83 (39)	74 (39)	-
Fumigation August 2003, mean temperature 33°C, WMC 52% to 87%							
1151	20	54 (11)	27 (11)	44 (25)	71 (7)	41 (22)	88 (8)
1402	22.5	0 (12)	0 (12)	0 (23)	0 (7)	0 (22)	0 (8)
1916	20	0 (12)	0 (12)	0 (23)	0 (7)	0 (22)	0 (8)
1937	22.5	0 (12)	0 (12)	0 (23)	0 (7)	0 (22)	0 (7)
1942	19.5	0 (12)	0 (11)	0 (23)	0 (7)	0 (21)	0 (7)
2092	21.5	0 (12)	0 (13)	0 (23)	0 (7)	0 (22)	0 (7)
Control		87 (24)	100 (24)	94 (46)	100 (14)	100 (44)	100 (14)
Fumigation February 2004, mean temperature 10°C, WMC 68%							
4203	16.5	-	-	20 (10)	27 (11)	15 (13)	8 (12)
4381	16.5	-	-	30 (10)	64 (11)	62 (13)	33 (12)
5066	16.5	-	-	10 (10)	36 (11)	0 (13)	0 (13)
5515	17	-	-	30 (10)	18 (11)	15 (13)	0 (12)
5866	17	-	-	0 (10)	18 (11)	15 (13)	0 (12)
Control		-	-	80 (20)	73 (22)	100 (26)	85 (22)
Fumigation April 2004: mean temperature 24°C, WMC 72%							
1160	22	-	-	0 (13)	17 (12)	42 (7)	0 (12)
1207	22	-	-	0 (13)	25 (12)	57 (7)	18 (12)
1533	23	-	-	0 (13)	0 (12)	0 (7)	0 (12)
1597	23	-	-	0 (13)	0 (12)	0 (7)	0 (12)
2171	22	-	-	0 (12)	0 (13)	0 (7)	0 (12)
2357	24	-	-	0 (13)	0 (12)	0 (7)	0 (12)
Control		-	-	85 (26)	100 (24)	100 (14)	82 (24)

[1] Only in April 2003 fumigation [2]Wood moisture content

These trials showed evidence that total control of PWN nematode and of its vector *Monochamus* spp. could be achieved with appropriate dosage of SF when temperature was 15°C or higher, even with wood

of high WMC exceeding the saturation point. This research resulted in a fumigation schedule with SF that was submitted to the TPPT by German Federal Ministry of Agriculture for inclusion in the ISPM 15, suggesting the target dosage for PWN applied for all quarantine pests since the other pests required less dosage. The dosages submitted and their associated concentrations in 24 h exposures were as follows: 3000 g-h/m^3 at 15 to 19.9°C, 2100 g-h/m^3 at 20 to 24.9°C, 1500 g-h/m^3 at 25 to 29.9°C, 1400 g-h/m^3 at 30 to 34.9°C, and 1000 g-h/m^3 for 35°C or greater.

4. Trials from Flack et al. (2008) in the USA

4.1. Materials and methods

The TPPT identified four potential issues concerning trials previously submitted (Dwinell et al., 2003; 2005) evaluating SF fumigation for control of the PWN: 1) the absence of number of pests for each treatment and the control; 2) the lack of information on which life stage was present and was the most resistant to SF; 3) requirement to have mortality data using the Baermann funnel after 6 and 21 days to allow for incubation of PWN; 4) requirement to extract nematodes from larger samples than wood slivers used in previous studies. Therefore, additional trials were conducted by Flack et al. in 2008 to provide additional data to validate proposed SF quarantine treatment schedule for 20ºC and 25ºC.

Logs of dead pine (*Pinus* spp.) subsequently colonised with PWN were salvaged, debarked and sawn into sticks one week before fumigation trial. A natural infestation of PWN was augmented with laboratory cultured PWN 24-48 h before fumigation. Fumigations were conducted in 10 l glass chambers at 20 and 25°C for 24 h. Three target concentration x time (CT) dosages (g-h/m^3) were evaluated per temperature. Entire wood sticks were assayed for PWN after 7 and 21 days after fumigation using the Baermann funnel method and the numbers of juvenile and adult nematodes occurring in each extraction sample were determined. The wood moisture content was also measured by drying at 105°C for 24 h.

4.2. Results and conclusions

The mean WMC of wood sticks was 59.1 ± 4.3% (mean ± SD), indicating wood was water-saturated (Table 4). SF dosages ranged from 1947-2287 g-h/m^3 at 20°C and 1342-1586 g-h/m^3 at 25°C which represented dosages less than, approximately equal to, and above the proposed quarantine dosages of 2100 g-h/m^3 at 20°C and 1500 g-h/m^3 at 25°C. Extractions at 7 d indicated high numbers of PWN (3000 at 25°C and 1300 at 20°C) from untreated controls. At 21 d, the number of extracted nematodes from untreated control dropped to 846 at 20°C and 78 at 25°C.

Table 4 Pine wood nematode (PWN) extracted from unseasoned pine 7 and 21 d following 24 h fumigation with sulfuryl fluoride (SF) at 20° and 25°C (data extraction from Flack et al., 2008).

			PWN extracted 7 days post fumigation			**PWN extracted 21 days post fumigation**		
Temp. (°C)	**Chamber #**	**Cumulative SF Dosage (g-h/m^3)**	**No. Juv. PWN**	**No. Adult PWN**	**Total No. PWN**	**No. Juv. PWN**	**No. Adult PWN**	**Total No. PWN**
20	1	1962	2	0	2	8	1	9
	2	2046	46	3	49	30	6	36
	3	2143	334	41	375	220	42	262
	4	1947	2	0	2	0	0	2
	5	2183	0	0	0	0	0	0
	6	2287	0	0	0	0	0	0
	7	0	2850	150	3000	690	156	846
25	1	1342	0	0	0	2	0	2
	2	1487	0	0	0	0	0	0
	3	1586	0	0	0	0	0	0
	4	1334	0	0	0	0	0	0
	5	1495	0	0	0	0	0	0
	6	1568	0	0	0	0	0	0
	7	0	1040	260	1300	54	24	78

At 25°C, no PWN were extracted from sticks in five of the six fumigation treatments. Two PWN juveniles were extracted from one treatment (1342 g-h/m^3) which was less than the proposed quarantine SF dosage of 1500 g-h/m^3. These trials confirm that the proposed quarantine dosages at 25°C are effective.

Results were more variable at 20°C, with some survivors in four out of six fumigations. This was attributed by the authors to the high WMC which may have delayed penetration of the gas. Therefore, the proposed quarantine dosages for 20°C and 15°C were increased by 200 g-h/m^3 to account for variability in WMC and an updated treatment schedule (Table 5) was submitted to the TPPT in 2008.

Table 5 Updated 24 h treatment schedule sulfuryl fluoride (SF) fumigation of unseasoned pine for control of pine wood nematode (PWN).

	Min. Target SF Dosage (g-h/m^3)	SF Dose (g/m^3)	Minimum SF Concentration (g/m^3) at hour:				
Mean ºC			0.5	2	4	12	24
15-19.9	3200	183	188	176	163	131	93
20-24.9	2300	131	136	128	118	95	67
25-29.9	1500	88	94	83	78	62	44
30-34.9	1400	82	87	78	73	58	41
35 or above	1000	60	63	57	53	42	30

5. Current status and perspective of inclusion of sulfuryl fluoride in ISPM 15

Studies by leading nematologists and quarantine experts have shown effective control of PWN by SF. However, further guidance of IPCC on efficacy data requirements for PWN were produced in 2009 (Magnusson and Schröder, 2009) to applicants for proposed treatments in ISPM 15. The requirements included the need for more efficacy data on the dispersal life stage (J3 larvae) and a greater number of individuals for Probit 9 analysis. An additional study on PWN is planned with SF in Portugal in 2010 to meet these requirements. Following inclusion in ISPM 15, the use of SF would contribute to eradication and confinement of PWN alongside other methods of control, leading to enhanced trade within and outside the E.U.

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