Review

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Efficacy of sulfuryl fluoride against the pinewood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchidae), in *Pinus pinaster* boards

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Abstract

BACKGROUND: The pinewood nematode (PWN) *Bursaphelenchus xylophilus* is an important conifer disease worldwide. It is the direct cause of the death of millions of pines in south-east Asia (mainly Japan, China and Korea) and has been established in Portugal since 1999. The phasing out of methyl bromide has created an urgent need for alternative treatment of wood packaging materials. The effect of sulfuryl fluoride (SF), a broad-spectrum fumigant used to control insects, was tested in *Pinus pinaster* boards naturally infested by PWN.

RESULTS: Boards were fumigated for 24 h at three different temperatures (15, 20 and 30 °C) with dosage ranges of 3169–4407, 1901–4051 and 1385–2141 gh m^{-3} respectively. Treated wood was sampled for nematode identification and counting, before treatment and after 24 h, 72 h and 21 days. No survival was found in the 15 °C and 30 °C treatments, while at 20 °C the mortality ranged from 94.06 to 100%. Some reasons for the survival at 20 °C are presented.

CONCLUSION: Results confirm SF to be an effective quarantine treatment for PWN at 15 and 30 °C. Further studies are needed to obtain the most effective dosage at 20 °C, and to determine the toxicity of SF fumigation on *B. xylophilus* at other temperatures, especially at 25 °C.

Keywords: pinewood nematode; quarantine; pesticide; control

1 INTRODUCTION

The pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, native to North America, is the causal agent of pine wilt disease, which has killed millions of pines in Japan[1] and China[2] since its introduction. It was first detected in Portugal in 1999 (this being the first record for Europe) in a dead maritime pine (*Pinus pinaster* Aiton) at Pegões, Setúbal Peninsula.[3] To infect a healthy pine tree, the nematode needs to be transported by an insect vector usually of the *Monochamus* genus.[4,5] In Portugal the only known vector is the native *Monochamus galloprovincialis*.[6]

Since the detection of the PWN in Portugal, significant efforts have been made to control the disease by cutting and destroying infested pine trees before the exit of the insect vector or by luring insect vectors with traps containing semiochemicals to capture them during their flight period.[7] *In situ* and real-time methods for early detection of the pine trees killed by PWN are also being developed and are important management tools to assess the effective impact of the nematode in the mortality of the pines, sorting *B. xylophilus*-induced mortality from trees killed by scolitids and other causes.[8,9]

Long-distance dispersal of the nematode and its vectors is related to the global commerce and transport of untreated round and sawn wood, and nematodes of the *Bursaphelenchus* genus are often detected in wood packaging materials worldwide.[10–16] To prevent such dispersal, International Standard for Phytosanitary Measures (ISPM) No. 15 for wood packaging material was first published in 2002 and later revised in 2009. In this last revision, emphasis was placed on 'phytosanitary measures that reduce the risk of introduction and spread of quarantine pests associated

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with the movement in international trade of wood packaging material made from raw wood'. Wood packaging material covered by this standard includes dunnage but excludes wood packaging (plywood) made from wood processed in such a way that it is free from pests.

Two control measures are currently included in the standard, namely fumigation with methyl bromide and heat treatment. Nevertheless, methyl bromide is currently being phased out of use globally and is no longer registered for use in the European Union (EU). Therefore, new alternatives are needed to eliminate nematodes from wood packaging materials. Among these, sulfuryl fluoride (SF) is a broad-spectrum fumigant used to control insects and tested on the pinewood nematode, and is known to have better timber penetration qualities than methyl bromide.[17] This gas is commercialised under the commercial brands Vikane®, ProFume® and Zythor®, which are registered in various countries for the control of stored-product insects and wood-destroying pests. In the EU, SF has been granted Annex 1 listing under EU Directive 98/8/EC (Biocides) for Product Type 8 (Wood Preservative) and Product Type 18 (Insecticides). Recently, Annex 1 listing under EU Directive 91/414 (Pesticides) was granted with effect from 1 November 2010, and the pest types include insects and nematodes.

Sulfuryl fluoride has been submitted and evaluated for inclusion in ISPM-15. Following its evaluations, the Technical Panel on Phytosanitary Treatments (TPPT) considered that published papers and historical use of SF fumigations have demonstrated that this treatment eliminates efficiently insect pests of concern in wood packaging material used in international trade. The present study was carried out to provide additional information to previous studies by Soma *et al.*,[18] Dwinell *et al.*[19,20] and Flack *et al.*[21] on the toxicity of sulfuryl fluoride against PWN, so that SF could be included in ISPM-15.

2 MATERIALS AND METHODS

2.1 Collection and preparation of wood material

A total of 29 dead maritime pine trees (*P. pinaster*) infested with the PWN were felled in February 2010 in Comporta, Portugal. The lower trunk was sawed into boards (5 cm thick × 10 cm wide × 45 cm long), while thinner wood sections and branches, with diameters between 10 and 20 cm, were cut into 40 cm logs to fill up the fumigation chambers. The wood was taken to the Instituto Nacional de Investigação Agrária e Veterinária (INIAV) laboratories in Oeiras and kept in climatic chambers at 25 °C and 70% relative humidity (RH) for 60 days to increase the number of propagative life stages of the nematodes to the target density of at least 100 000 nematodes per treatment, as required for Probit 9 statistical analysis. Subsequently, the temperature and RH were reduced to 12 °C and 50% for 7 days to promote the development of the dispersal juvenile stage (J_{III}), as this is considered to be the pinewood nematode's most resistant life stage.[22] This was done to obtain at least 60% of J_{III} nematodes, as required by the TPPT.

2.2 Fumigation chambers and fumigant

In the INIAV laboratories in Oeiras, Portugal, two 12.2 m long shipping containers were lined with 4 mm thick polystyrene insulation panels (Wallmate™; The Dow Chemical Company, Midland, MI) and placed at approximately 2.5 m above the floor to create an inner chamber. Thermostatically controlled air conditioners and coil-in-oil electric heaters were used to achieve the required temperatures.

Inside each shipping container, seven 1 m^3 polyvinyl chloride (PVC) chambers with galvanised steel frames were placed, representing six treatment chambers and one untreated (control) chamber. Each of the six chambers was connected to a 30 m long × 4 mm inner diameter SF introduction tube, a 20 m thermocouple line and two 30 m long × 2.5 mm monitoring tubes located in the top and bottom open air space of each chamber. The untreated chamber had the same set-up, except that no tube was introduced into the entry port, which was sealed. Temperature was measured using thermocouple lines and data loggers (Tinytag™; Gemini Data Loggers, Chichester, UK), which were placed inside the untreated chambers and set to record temperature and relative humidity at 15 min intervals.

Each fumigation chamber contained 36 boards, forming a stack of four piles, nine boards high, and another identical volume formed by at least nine logs. Nine replicate boards to be sampled were marked and placed in sets of three at the top, middle and bottom of the stack. The wood from each tree was dispersed among all treatments. The chambers, partially loaded with wood, were held within each shipping container set at the target temperature and allowed to acclimatise for approximately 48 h prior to introduction of SF.

A cylinder of commercial-grade ProFume® gas fumigant (99.9% sulfuryl fluoride; Dow AgroSciences, Indianapolis, IN) was placed on a Yellow Jacket® digital electronic weighing scale (Ritchie Engineering Company Inc., Bloomington, MN) and connected to the introduction tubes. The required amount of SF was dispensed via a needle valve to achieve target dosages into the polyvinyl chloride fumigation chambers.

Fumigations were conducted on 3, 8 and 13 May 2010 under three temperature regimes for the following target dosages: 3200 and 4000 gh m^{-3} at 15 °C; 2300, 2875 and 3450 gh m^{-3} at 20 °C; 1400 and 1750 gh m^{-3} at 30 °C. The concentration of SF was monitored using an infrared spectrometer (Spectros® Reporter IR; Spectros Instruments Inc., Hopedale, MA) at 0.5, 2, 4, 6, 12 and 24 h. There were three replicates for each dosage/temperature combination. Concentration readings were entered into Fumiguide® software (Dow AgroSciences, Indianapolis, IN) in order to calculate accumulated dosages.

Following 24 h exposure, SF was aspirated from the chambers via extraction tubes and fans for approximately 2 h. Before handling the treated wood, complete exhaustion of SF was confirmed with an SF-ExplorIR device (Spectros Instruments Inc., Hopedale, MA).

2.3 Sampling for nematode extraction and counting

Boards (treated and untreated) were sampled for nematode presence on four occasions: before fumigation, after treatment, at 72 h and at 21 days after treatment. Separate wood bioassays were prepared for each sampling interval and treatment.

Wood samples for the nematode extraction consisted of 8 cm segments of each end of the wood piece, sawed to obtain a mixed sample of 100 g of wood cubes of approximately 1 cm^3, using a mechanical timber saw. After the sampling of each treated wood piece, the equipment was carefully brushed, vacuumed and washed with alcohol, to avoid cross-contamination.

Live nematodes were extracted using the modified tray method,[23] in which the wood sample was wrapped in paper and 'etamine' tissue and then placed on a plastic mesh overlaying a plastic tray. The tray was filled with tap water until the wood sample was completely immersed. After 48 h, the sample was removed and the water in the tray was passed through a 400 mesh

(38 μm) sieve. The remaining material was washed with distilled water in small plastic or glass containers, and stored at 4 °C until evaluation.

Samples obtained before fumigation were homogeneously mixed, and a 1 mL aliquot was placed inside a counting slide (Chalex Corporation, Grasonville, MD). The total number of PWN present (mixed stages) and the number of J_{III} were determined. If fewer than 100 individuals were present, a new aliquot was removed and the process was repeated until 100 individuals were counted or 5 × 1 mL accounted for. For the remaining sampling dates (day 1, day 3 and day 21 after fumigation), the entire sample volume was placed in a Doncaster petri dish (Doncaster, Kent, UK); the number of PWN present and the total number of J_{III} were determined. Each sample was evaluated separately by stereomicroscopy, and nematodes were identified by morphological characters and counted at the INIAV (Oeiras) and NemaLab/ICAAM (Évora).

2.4 Wood moisture content

After nematode extraction, each wood sample was oven dried (type U50; Memmert GmbH, Schwabach, Germany) for 48 h and weighed again. The percentage of initial moisture was calculated by subtracting the dry weight from the initial weight, and then dividing by the initial weight.

2.5 Statistical analysis

The data were subjected to statistical analysis to meet the requirements of a quarantine treatment.[24] A direct method of efficacy calculation (exposing more than 100 000 individuals to the treatment) followed that of Couey and Chew,[25] with the appropriate adjustment for control mortality.[26] This adjustment was necessary to account for natural mortality in the untreated controls. Other basic statistics such as ANOVA were performed using the Statistica® software (StatSoft, Inc., Tulsa, OK) package.[27]

3 RESULTS

3.1 Fumigation parameters

The fumigations were conducted using a chamber wood-loading factor of 16.2% (one pile of boards and one pile of logs with dimensions of 45 × 40 × 45 cm). The measured SF dosage ranges achieved were 3169–4407 gh m^{-3} at 15 °C, 1901–4051 gh m^{-3} at 20 °C and 1360–2141 gh m^{-3} at 30 °C. Because an unexpectedly low sorption of wood during the first set of fumigations at 20 °C resulted in a higher dosage than expected, during subsequent assays a correction to the amount of SF introduced was necessary. Fumigant leakage was measured from one of the fumigation chambers at 20 °C, and additional SF was immediately injected. However, the final obtained dosage of 1901 gh m^{-3} was below the minimum desired and therefore was not considered during subsequent analysis.

ANOVA adjusted to SF dosages measured in chambers revealed highly significant statistical differences for all tested temperatures and made it possible to determine three homogeneous groups for consideration in further analysis: $F(15\,°\text{C}) = 25.717$, df = 5, 30, $P < 0.0001$; $F(20\,°\text{C}) = 112.34$, df = 7, 40; $P < 0.0001$; $F(30\,°\text{C}) = 34.766$, df = 5, 30, $P < 0.0001$.

The system was effective in achieving consistent temperatures following SF introduction in all fumigations. The temperatures recorded in the chambers were close to the target temperatures of 15, 20 and 30 °C in all assessment periods (Table 1).

The tested wood moisture content (WMC) varied from just 17% to 47% (average values per treatment in Table 1). Differences were found in WMC between boards where PWN was eliminated and boards tested at 20 °C where survival was recorded ($F_{(1,178)} = 30.29$, $P < 0.0001$). The relatively flat sorption curves of SF by *P. pinaster* wood in each fumigation chamber (Fig. 1) indicate that pinewood has poor SF sorption.

3.2 Fumigation effect on PWN populations

The PWN counts 48 h before fumigation of boards at 15 °C ranged from 166 000 to 883 000 (J_{III}) and from 21 000 to 253 000 (adults and all juveniles excluding the J_{III} stage) per dosage. At 30 °C, pretreatment counts ranged from 170 000 to 1 052 000 (J_{III}) and from 47 000 to 207 000 (adults and all juveniles excluding the J_{III} life stage) per dosage. The results of SF fumigations at the nine temperature–dose combinations are presented in Table 2. In boards fumigated at 15 and 30 °C, 100% mortality of PWN was achieved in all post-fumigation assessment periods (24 h, 72 h and 21 days) and for every dosage tested (3169–4407 gh m^{-3}). In boards at 20 °C, PWN survival was measured at all dosages, although for the 2328 gh m^{-3} SF dosage the nematodes were recovered from only one of the 27 analysed boards on all sampling occasions. The 20 °C PWN counts 48 h before fumigation revealed very high PWN populations, ranging from 279 000 to 1 011 000 (J_{III} life stage) and from 170 000 to 268 000 (adults and all juveniles excluding the J_{III} life stage). Nematode populations in the boards persisted during the 21 days of the assay.

Table 3 contains the corrected values for observed nematode mortality according to ISPM-15 requirements and Probit 9 calculations. At both 15 and 30 °C, the results provide Probit 9 efficacy and validate the SF dosage in the schedule to be proposed to ISPM-15 (Table 4). Values presented for the range between 20 and 30 °C should be considered as a basis for future studies.

4 DISCUSSION

Previous studies on sulfuryl fluoride toxicity mainly focused on various insects such as termites, wood-boring beetles and fruit flies.[28–32] The present study proves that SF is effective in eliminating high populations of *B. xylophilus* in infested wood boards. In fact, the nematode load in the fumigated boards was higher than the standard Probit 9 requirements (minimum of 100 000 individuals),[33,34] and, even with the highest survival treatment (a total of 3810 nematodes at 20 °C), almost 800 000 nematodes were eliminated with fumigation, confirming the toxicity and efficacy of the gas in eliminating the PWN.

The survival of the nematode in the 20 °C treatment without a dose–response relationship is difficult to explain, although it may be possible that nematodes survived fumigation at the egg stage, whereas under more unfavourable temperatures of 15 and 30 °C the eggs would have been much less abundant or even absent. It is known that insect eggs are less susceptible to SF fumigation than other life stages because the shell limits the passage of the gas; therefore, control of eggs requires an increased exposure time or increased concentrations of SF.[28,35–41] The same phenomenon applied to nematodes would explain the survival of some populations at 20 °C and the absence of *B. xylophilus* in almost all samples taken 24 h after fumigation (absent in 66 of 72 samples), as the egg is the only life stage not detected in the wood samples immersed in water, and after 21 days at favourable temperatures these same boards contained adult nematodes.

Table 1. Measured experimental parameters (temperature, wood moisture content and dosage; mean ± SEM) in sulfuryl fluoride (SF) fumigations[a] of *Pinus pinaster* boards infested with *Bursaphelenchus xylophilus* for 24 h in 1 m^3 chambers. Letters after sulfuryl fluoride achieved dosages are LSD homogeneous groups. Rows in bold represent mean values of replicates for each treatment (temperature/dosage). WMC: wood moisture content

Fumigation parameter				SF concentration (g m^{-3}) at time (h) after introduction					Mean dosage (gh m^{-3})
Temp. (°C)	WMC (%)	Dose (g m^{-3})	n	0.5 h	2 h	4 h	12 h	24 h	
14.6 ± 1.34	**25.3 ± 2.21**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**
14.6 ± 0.89	26.7 ± 1.53	140	9	145	123	137	133	132	3169 a
14.8 ± 1.10	29.6 ± 1.41	120	9	151	128	136	136	135	3217 a
14.7 ± 0.67	**28.2 ± 1.07**	**130.0 ± 10.00**	**18**	**148.0 ± 3.00**	**125.5 ± 2.50**	**136.5 ± 0.50**	**134.5 ± 1.50**	**133.5 ± 1.50**	**3193**
14.4 ± 0.55	30.0 ± 0.57	130	9	155	152	159	156	155	3691 b
15.4 ± 1.95	28.4 ± 1.23	140	9	169	146	164	160	158	3774 b
14.9 ± 1.02	**29.2 ± 0.69**	**135.0 ± 5.00**	**18**	**162.0 ± 7.00**	**149.0 ± 3.00**	**161.5 ± 2.50**	**158.0 ± 2.00**	**156.5 ± 1.50**	**3733**
15.2 ± 1.64	28.3 ± 1.12	170	9	205	184	191	183	184	4400 c
14.2 ± 0.84	30.6 ± 0.53	160	9	200	177	187	186	183	4407 c
14.7 ± 0.95	**29.5 ± 0.66**	**165.0 ± 5.00**	**18**	**202.5 ± 2.50**	**180.5 ± 3.50**	**189.0 ± 2.00**	**184.5 ± 1.50**	**183.5 ± 0.50**	**4404**
19.4 ± 1.52	**27.4 ± 0.67**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**
19.4 ± 1.14	29.4 ± 1.07	90	9	106	94	94	90	84	2145 a
19.2 ± 0.84	30.5 ± 0.48	90	9	114	101	99	99	96	2352 a
19.5 ± 0.58	28.6 ± 0.70	90	9	102	104	106	105	103	2488 a
19.4 ± 0.49	**29.5 ± 0.46**	**90.0 ± 0.00**	**27**	**107.3 ± 3.53**	**99.7 ± 2.96**	**99.7 ± 3.48**	**98.0 ± 4.36**	**94.3 ± 5.55**	**2328**
18.8 ± 1.30	**30.4 ± 1.09**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**
18.4 ± 0.55	31.5 ± 1.40	140	9	169	166	159	160	153	3768 b
18.3 ± 1.86	34.3 ± 3.20	140	9	170	167	164	163	158	3852 b
18.4 ± 0.91	**32.9 ± 1.73**	**140.0 ± 0.00**	**18**	**169.5 ± 0.50**	**166.5 ± 0.50**	**161.5 ± 2.50**	**161.5 ± 1.50**	**155.5 ± 2.50**	**3810**
20.0 ± 0.41	36.0 ± 2.70	170	9	181	178	178	176	172	4036 c
20.5 ± 0.71	29.8 ± 0.44	160	9	186	182	180	177	169	4045 c
19.2 ± 1.30	33.2 ± 0.99	150	9	176	172	172	171	166	4051 c
19.7 ± 0.71	**33.0 ± 1.06**	**160.0 ± 5.77**	**27**	**181.0 ± 2.89**	**177.3 ± 2.91**	**176.7 ± 2.40**	**174.7 ± 1.86**	**169.0 ± 1.73**	**4044**
31.4 ± 1.14	**27.7 ± 0.10**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**
30.8 ± 0.84	26.9 ± 0.48	60	9	54	59	59	58	57	1360 a
31.6 ± 1.14	26.5 ± 0.37	50	9	61	58	61	59	57	1385 a
30.4 ± 0.55	28.5 ± 0.40	60	9	40	68	64	64	63	1482 a
30.9 ± 0.55	**27.3 ± 0.29**	**56.7 ± 3.33**	**27**	**51.7 ± 6.17**	**61.7 ± 3.18**	**61.3 ± 1.45**	**60.3 ± 1.86**	**59.0 ± 2.00**	**1409**
31.2 ± 0.84	30.1 ± 0.92	70	9	86	78	76	76	75	1793 b
31.2 ± 0.84	29.0 ± 0.21	80	9	73	78	78	77	76	1804 b
31.2 ± 0.56	**29.5 ± 0.47**	**75.0 ± 5.00**	**18**	**79.5 ± 6.50**	**78.0 ± 0.00**	**77.0 ± 1.00**	**76.5 ± 0.50**	**75.5 ± 0.50**	**1799**
30.4 ± 0.55	27.0 ± 1.06	80	9	98	94	92	91	89	**2141** c

[a] Fumigations were conducted during April 2010 in Oeiras, Portugal. The *CT* (concentration × time) values were calculated from the concentrations below. The loading factor of the fumigation chambers was 16.2% (vol:vol).

Another possible explanation for survival at 20 °C is that variations in board moisture content (with higher content where survival occurred) could have influenced SF penetration, as water-saturated pinewood is known to reduce the penetration of SF considerably.[42] However, the level of moisture in all boards was generally low, with the highest mean value of just 33%, which is distant from water saturation and would hardly be a barrier to SF entry. Indeed, pre-tests at Fera UK using freshly cut pine logs confirmed that effective penetration of SF at WMC above 60% occurred in a range of pine and hardwood species.[43]

The results at 15 and 30 °C assured Probit 9 efficacy and validated the toxicity of the SF dosage in the proposed ISPM-15 schedule (Table 4), significantly reducing the risk of introduction and/or spread of quarantine pests, as stated by ISPM-15 norms. On the other hand, the Probit 9 standard for quarantine treatment efficacy (99.9968% mortality), which was originally recommended for tropical fruit flies, may be too stringent for quarantine pests of insects on poor hosts.[44] Similarly, living pine trees should be considered as the host plant to the PWN and its insect vector, rather than debarked wood packaging material, which is exposed to changing environmental conditions and is a very poor host that poorly supports nematode development. Furthermore logs with bark would be expected to absorb less SF than processed boards, as SF penetration in logs occurs mainly at the cut ends, whereas in boards all sides are permeable.

Mortality rates resulting from quarantine fumigations show widely varying risks and do not necessarily give foolproof levels of security.[45] The stated goal of ISPM-15 is to 'reduce the risk of introduction and/or spread of quarantine pests' and to describe measures that 'significantly reduce the risk of pest spread'. Such a reduction was achieved in the present study, given the size of the eradicated nematode populations.

Table 2. Nematode numbers before and after sulfuryl fluoride fumigation of *Pinus pinaster* boards infested with *Bursaphelenchus xylophilus* (PWN) at three temperatures (15, 20 and 30 °C) for 24 h in 1 m^3 chambers. The numbers of *B. xylophilus* (total nematodes and percentage at life stage J_{III}) in boards were counted before fumigation and in samples taken after 24 h, 72 h and 21 days

Fumigation parameters		Total number of nematodes (mean/g wood × weight of infected board[a])											
		48 h before fumigation			After fumigation								
					24 h			72 h			21 days		
Temp. (°C)	Dose (gh m^{-3})	N[b]	Total PWN	J_{III} life stage (%)	N[b]	Total PWN	J_{III} life stage (%)	N[b]	Total PWN	J_{III} life stage (%)	*N*[b]	Total PWN	J_{III} life stage (%)
15	0	9	424 000	72.81	9	546 000	77.27	9	395 000	83.65	9	177 000	70.18
15	3193	18	405 000	85.96	0	0	0	0	0	0	0	0	0
15	3733	18	1 630 000	81.82	0	0	0	0	0	0	0	0	0
15	4404	18	1 190 000	74.74	NR[c]	NR	NR	0	0	0	0	0	0
20	0	9	1 067 000	81.38	9	461 000	87.02	9	200 000	72.18	9	93 000	84.99
20	2328	27	2 780 000	75.10	1	180	100	1	30	0	1	15	0
20	0	9	551 000	74.23	9	402 000	82.63	9	301 000	79.46	9	426 000	92.70
20	3810	18	814 000	56.35	1	105	28.57	5	2830	20.10	5	24 760	19.11
20	4044	27	3 055 000	63.10	4	329	72.69	10	4520	13.59	10	36 980	19.94
30	0	9	1 182 000	88.99	9	684 000	80.78	9	609 000	93.94	9	112 000	87.45
30	1409	27	1 952 000	82.94	0	0	0	0	0	0	0	0	0
30	1799	18	1 454 000	87.10	NR	NR	NR	0	0	0	0	0	0
30	2141	9	800 000	75.39	NR	NR	NR	0	0	0	0	0	0

[a] Mean weight of infested boards = 1500 g.
[b] *N*: number of infested boards.
[c] NR: not recorded.

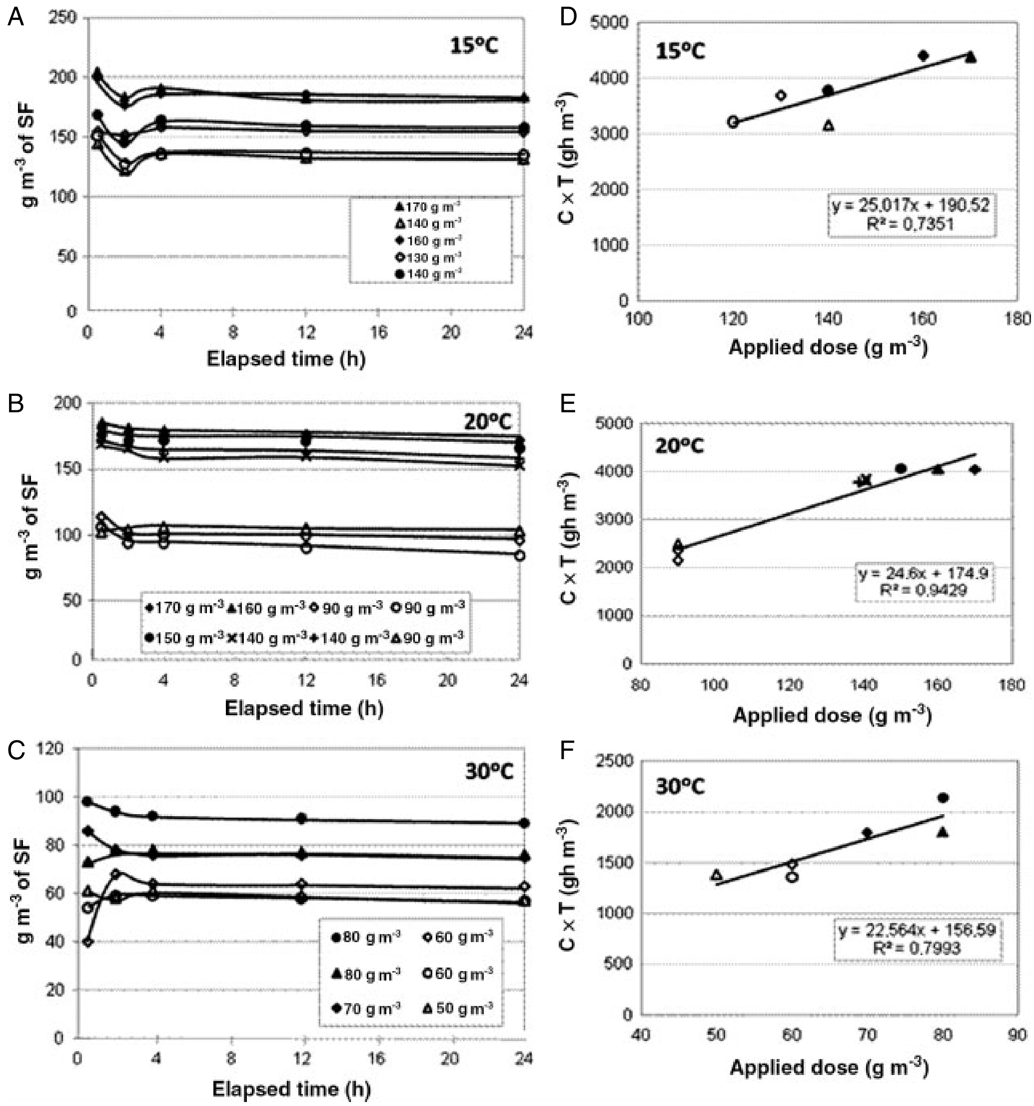

Figure 1. Parameters of the fumigation with sulfuryl fluoride (SF) of *Pinus pinaster* boards naturally infected with pinewood nematode *Bursaphelenchus xylophilus* at three temperatures. Sorption curves registered in fumigation chambers at 15 °C (A), 20 °C (B) and 30 °C (C). Relation of final *CT* product (concentration × time) in fumigation chambers at 15 °C (D), 20 °C (E) and 30 °C (F).

The infested wood had been obtained from dead pines in locations where various species of bark and wood-boring beetles were present, such as scolitids (*Ips sexdentatus* and *Orthotomicus erosus*) and the cerambycids *Arhopalus syriacus* and *M. galloprovincialis*, normal insect fauna associated with dead *P. pinaster* in Portugal.[7,46] Although not quantified, all larvae, pupae and adult insects present in the wood were killed with SF fumigation at the three tested temperatures, thereby confirming previous results on the efficacy of SF as an insecticide.[29,32,47,48] The limited survival of the pinewood nematode in low-moisture-content wood[49] and the combined eradication of both the nematode and its insect vector from SF-fumigated boards effectively remove the risk of dispersing pine wilt disease, even if low levels of *B. xylophilus* survive, because the nematode depends on a living vector in the wood for dissemination to a healthy host tree.

Overall, the present results confirm that fumigation with SF is a suitable quarantine treatment for wood and wood packaging materials infested with *B. xylophilus*, and that SF may be an alternative chemical fumigant needed for commodity quarantine treatments for pests in the absence of methyl bromide. Additional studies are needed, however, to determine the effective dosage and/or exposure time at 20 °C to obtain 100% mortality, and to determine the toxicity of SF fumigation on *B. xylophilus* at other temperatures, especially at 25 °C.

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Table 3. Percentage mortality of *Bursaphelenchus xylophilus* in boards of *Pinus pinaster* after sulfuryl fluoride fumigation. Percentages are relative to nematode populations in untreated control boards at each sampling date

Fumigation parameters		24 h after fumigation		72 h after fumigation		21 days after fumigation	
Temp.(°C)	Dose (gh m^{-3})	% Mortality[a]	% Mortality[b]	% Mortality[a]	% Mortality[b]	% Mortality[a]	% Mortality[b]
15	0	—		6.7456		58.3097	
15	3193	100	100	100	100	100	100
15	3733	100	100	100	100	100	100
15	4404	100	100	100	100	100	100
20	0	56.7905		81.2446		88.2027	
20	2328	99.9935	99.9935	99.9850	99.9989	99.9995	99.9954
20	0	26.9409		45.3743		22.6292	
20	3810	99.9871	99.9823	99.6528	99.3643	96.9587	96.0691
20	4044	99.9892	99.9852	99.8521	99.7293	98.7898	98.4358
30	0	42.1557		48.4793		89.3823	
30	1409	100	100	100	100	100	100
30	1799	100	100	100	100	100	100
30	2141	100	100	100	100	100	100

[a] Not adjusted for untreated mortality.
[b] Adjusted by the Henderson–Hilton formula, accounting for natural mortality observed in the respective control.

Table 4. Proposed concentration–time dosages and effective doses of sulfuryl fluoride (SF) to eliminate *Bursaphelenchus xylophilus* in *Pinus pinaster* boards

			Minimum concentration (g m^{-3})				
Mean temperature (°C)	Minimum target *CT* dosage (gh m^{-3})	SF dose (g m^{-3})	at 0.5 h	at 2 h	at 4 h	at 12 h	at 24 h
15–19.9	3200	183	188	176	163	131	93
20–24.9	4400	250	255	240	228	183	130
25–29.9	3200	183	188	176	163	131	93
30 and above	1400	82	87	78	73	58	41

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