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# Complementary studies to validate the proposed fumigation schedules of sulfuryl fluoride for inclusion in ISPM No. 15 for the eradication of *Bursaphelenchus xylophilus* from wood packaging material

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## INTRODUCTION

Sulfuryl fluoride (SF), a broad-spectrum fumigant, is under evaluation for inclusion in standard ISPM No. 15 - Guidelines for Regulating Wood Packaging Material in International Trade. The Technical Panel on Phytosanitary Treatments (TPPT) has evaluated the efficacy data submitted on SF against a range of insect species and pine wood nematode (*Bursaphelenchus xylophilus*) [PWN] to support the fumigant inclusion in the Standard. Following their evaluation, the TPPT considered that there were no further requirements for insects; however, additional information on PWN was requested. Several efficacy studies of SF on PWN were conducted and submitted: (Dwinell *et al.* 2005); (Flack *et al.* 2008) and (Bonifácio *et al.* 2013) but additional information on efficacy within 18 -29.9°C was requested. A new study was completed on PWN in Portugal in 2013 to validate a proposed treatment schedule for that temperature range.

## MATERIALS AND METHODS

Boards of 45cm x 10cm x 5cm were prepared from PWN-contaminated pine trees (*Pinus pinaster*) felled in Portugal, where this pest is now naturalized. The boards were maintained in incubation chamber at 25°C 75% rh to provide optimal conditions for reproduction of PWN. Boards were then placed in 1 m<sup>3</sup> chamber and fumigated with

commercial grade ProFume<sup>®</sup> gas fumigant (99.8% sulfuryl fluoride, Dow AgroSciences, Indianapolis, IN USA). A range of SF dosages and two exposure times were tested at 20°C as follows: 2,514-

4,263 g-h/m<sup>3</sup> at 24 h exposure and 2,459-3,216 g-h/m<sup>3</sup> at 48 h exposure. Each fumigation was monitored for temperature with a thermocouple and for SF concentration with an IR-specific monitoring device. Once target exposure time was achieved (24 or 48 h), chambers were aerated and wood boards placed in incubation chamber before counting PWN. Representative samples from both ends of boards were prepared by cutting them into wood cubes of ca. 1 cm<sup>3</sup>. Live PWN were immediately extracted by total immersion of the wood cubes in water for 48 h, then sieving the water through a 38µm sieve to identify and count the nematodes under a microscope (Penas *et al.* 2002). Complementary genomic DNA identification of extracted nematodes was performed when no adult nematodes were found.

## RESULTS AND CONCLUSION

The study conditions created a demanding, worst case scenario for PWN infestation. Initial populations of PWN ranged from 237 to 331 individuals per gram of wood, exceeding 3 million individuals per treatment, and further increased in the controls after fumigation of the treated samples. Infested wood contained a high proportion (53-90%) of the J<sub>III</sub> juvenile dispersal stage. Wood moisture content was 43-61.4% before fumigation and decreased to 15.7-19.4% 21 days after fumigation. At 3 days after application, 100% control of the J<sub>III</sub> juvenile dispersal stage was achieved with all SF dosages and exposure times. All dosages tested at 48 h exposure, compared to similar dosages at 24 h exposure, achieved better nematode control; 99.971-100% versus 99.617-99.998%, respectively. Survivors for 24 h exposure were mainly young larvae (J<sub>2</sub>, J<sub>3</sub> stages), which would support the assumption that only eggs survived the SF treatment at this exposure. At 21 days after application, nematode control was 99.852-99.999% at 24 h exposure and 99.991-100% at 48 h exposure.

The dosage of 3,000 g-h/m<sup>3</sup> in 48 h achieved 99.999% to 100% control at T<sub>3</sub> and T<sub>21</sub> and was selected for fumigation at 20°C-29.9°C. As a general rule, SF fumigant dosage decreases as temperature increases. A previous study verified this observation on PWN at 20 and 25°C (Flack *et al.* 2008). New proposed SF fumigation schedules, using the SF schedules developed in 2010 (Bonifácio *et al.* 2013) for temperatures below 20°C (3,200 g-h/m<sup>3</sup> with 24 h exposure) and above 30°C (1,400 g-h/m<sup>3</sup> with 24 h exposure) and adding 3,000 g-h/m<sup>3</sup> with 48 h exposure for 20°C-29.9°C, were submitted to TPPT.

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