COMPLETE SCHEDULE FOR SULFURYL FLUORIDE APPLICATION ON WOOD PACKAGING AGAINST THE PINEWOOD NEMATODE AND ITS INSECT VECTORS

Bonifácio*, L., M., L. Inácio, P. Naves and E. Sousa; INIAV, Oeiras, Portugal; S. Buckley, Dow AgroSciences SAS., Valbonne, France and E. M. Thoms, Douglas Products, Gainesville, FL, USA.

INTRODUCTION

Several efficacy studies of sulfuryl fluoride (SF) on Pine Wood Nematode (*Bursaphelenchus xylophilus*) (PWN) were conducted (Dwinell *et al.* 2005; Flack *et al.* 2008; Bonifácio *et al.* 2014) and submitted to the IPPC for inclusion in the Standard ISPM No. 15 - Guidelines for Regulating Wood Packaging Material in International Trade, and also the ISPM No. 28 – Phytosanitary treatment for regulated pests. The complete treatment schedule proposed for inclusion is presented along with description of the factors conditioning SF efficiency and PWN survival. Comparative analysis of SF efficiency and other PWN control methods available or under development (i.e. other fumigants, heat treatment) will be discussed.

MATERIALS AND METHODS

PWN was first detected in Portugal in 1999. Since then, Portugal is the only affected European country, apart from several successive contaminations detected and eradicated in Spain. Boards (45 cm x 10 cm x 5 cm) prepared from felled pine trees (*Pinus pinaster*) naturally-infested with PWN were maintained in an incubation chamber at 25°C 75% RH to provide optimal conditions for reproduction of PWN. Population levels and instar composition of PWN were regularly evaluated to achieve the required 100,000 nematodes per treatment, with 60% in the resistant stage JIII (Magnusson and Schröder 2009).Boards were then placed in hermetic chambers with controlled temperature and fumigated with ProFume® gas fumigant (99.8% SF, Douglas Products, Liberty, MO, USA). Each fumigation was monitored for temperature with a thermocouple and for SF concentration with an IR-specific monitoring device.

The measured SF dosage ranges, achieved in 24 h exposure, were 3,169–4,407 g-h/m^3 at 15°C, 1,901–4,263 g-h/m^3 at 20°C and 1,360–2,141 g-h/m^3 at 30°C. Additional assays were done at 20°C with 48 h exposure with the SF dosage range of 2,459-3,216 g-h/m^3.
Once target exposure time was achieved (24 or 48 h), chambers were aerated and wood boards returned to the incubation chamber. Representative samples were prepared by cutting wood cubes of ca. 1 cm^3 from both ends of each board. Live PWN were immediately extracted by total immersion of the wood cubes in water for 48 h, followed by straining water through a 38 µm sieve. Extracted nematodes were identified and counted using a microscope. Complementary genomic DNA identification of extracted nematodes was performed when no adult nematodes were found.

RESULTS AND CONCLUSION

The pre-fumigation populations of PWN ranged from 405,000 to 3 million individuals per treatment and contained a high proportion (53-90%) of the JIII resistant stage. These populations surpassed requested worst case scenario for PWN infestation and represented extreme infestations not previously found in nature (Sousa et al. 2011a).The wide range of wood moisture content (25-61%) before fumigation decreased sharply to 15.7-19.4% by

final sampling 21 days after fumigation. Complete eradication of PWN was achieved in all dosages at 15°C and 30°C, and for 20°C with 48h exposure PROBIT 9 mortality was also obtained 99.991-100%. All *Monochamus galloprovincialis* (only insect vector in Portugal) larvae recovered from the fumigated wood were killed, confirming SF insecticide effect. Surviving PWN observed immediately (1 h and 24 h) after fumigation for 24 h at 20°C were only young juveniles (J2 stage), indicating only eggs survived the SF treatment and JIII stage is not tolerant to SF. These surviving PWN populations gradually increased at 10, 15 and 21 days post-fumigation if wood moisture content was over 20%. This situation can also occur in heat-treated wood that becomes reinfested after being placed in direct contact with PWN-infested wood (Sousa et al. 2011b).

On the other hand, some boards after 24 h exposure at 20°C revealed PWN survivors at day 3, but none at day 21, because wood moisture content decreased under 20%. Therefore, results obtained in studies carried out using wood chips can't be extrapolated to wood packaging, since chips dry fast and nematodes may die due to the low moisture content rather than chemical application.

As a general rule, SF fumigant dosage for a given pest decreases as temperature increases. A previous study verified this observation on PWN at 20 and 25°C (Flack et al. 2008). Therefore, the proposed SF fumigation schedule is 3,200 g-h/m^3 for 24 h for temperatures below 20°C; 3,000 g-h/$m^3$for 48 h at 20°C-29.9°C; and 1,400 g-h/m^3 for 24 h at and above 30°C). The TPPT recommended two SF treatment schedules, one for insects and one for PWN and insects, as annexes to ISPM nos. 15 and 28. These SF schedules were approved by Standards Committee and are currently under review by IPPC members. This review is to be completed Nov. 30, 2015.

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