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Wood penetration ability of hydrogen cyanide and its efficacy for fumigation of *Anoplophora glabripennis*, *Hylotrupes bajulus* (Coleoptera), and *Bursaphelenchus xylophilus* (Nematoda)

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ABSTRACT

Pinewood nematodes (*Bursaphelenchus xylophilus*) and Asian longhorned beetles (*Anoplophora glabripennis)* are the primary regulated pests for packaging wood and timber in the EU, while the house longhorned beetle (*Hylotrupes bajulus*) is the most important cosmopolitan pest of construction wood. Gaseous hydrogen cyanide (HCN) is one of the few fumigation alternatives to the banned ozone-depleting chemical methyl bromide (MBr). This study reports the results of HCN fumigation experiments in a hermetically sealed steel chamber regarding (1) the penetration and absorption rates of HCN in wooden blocks, and (2) the biological efficacy of HCN against the wood-infesting pests *B. xylophilus* (in sawdust), *A. glabripennis*, and *H. bajulus* (in wooden blocks). A concentration equilibrium for HCN (at 20 g m^{-3}) between the fumigation chamber headspace and the center of the treated spruce blocks (100 × 100 × 120 mm) was reached after 48 h in the saturated atmosphere. A dose of 10 g m^{-3} in the center of the spruce blocks was reached for both saturated and non-saturated atmospheres after 24 h of fumigation. The wood tested absorbed approximately 40–45% of the HCN, until equilibrium was reached. The highest tested HCN dose (20 g m^{-3}) led to 100% mortality of the *A. glabripennis* and *H. bajulus* larvae after less than 1 h of exposure. For 20 g m^{-3} and 1 h exposure the Ct product was <18.66 g*h/m^3 for *H. bajulus and* <17.67 g*h/m^3 for *A. glabripennis*. Hydrogen cyanide doses of 10 g m^{-3} and 20 g m^{-3} led to 100% *B. xylophilus* mortality in 40 and 18 h, respectively. For *B. xylophilus* the Ct product was <424.00 g*h/m^3 for 20 °C and 10 g m^{-3} and <349.51 g*h/m^3 for 25 °C and 20 g m^{-3}. The initial results are promising in terms of establishing an alternative technology and protocol to MBr for timber fumigation.

1. Introduction

Numerous pests deteriorate wooden construction, historical artifacts, and packaging wood. The house longhorn beetle, *Hylotrupes bajulus* L., is the most important cosmopolitan pest of construction wood and historical buildings. Leading EU authorities on plant health and pest risk assessment (EFSA and EPPO) have listed the Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky) and the pinewood nematode (*Bursaphelenchus xylophilus* s/ Steiner & Buhrer/Nickle) among the most serious regulated pests and as major threats to wood quarantine in Europe (OEPP/EPPO, 1986; Macleod et al., 2002; Cermak et al., 2013).

The regulated beetle *A. glabripennis* (EPPO Code: ANOLGL) is an EPPO 1 pest and is native to China and Korea. The regulated nematode *B. xylophilus* (EPPO Code: BURSXY) is also an EPPO A1 pest (list: No. 158) that was first described in the U.S. as *Aphelenchoides xylophilus* (Steiner and Buhrer, 1934). Eradication programs, based primarily on physical removal and the destruction of infested plants, have been established in several European countries, e.g., for *B. xylophilus* in Portugal and for *A. glabripennis* in Italy (Herard et al., 2009). Quarantine measures and techniques to slow the range expansion and exterminate these pests are limited. At present, heat treatment of wood originating in the areas of occurrence of *A. glabripennis* and *B. xylophilus* is the only available option, according to the International Standards for Phytosanitary Measures, Publication No. 15 (ISPM 15). However, this technique is time-consuming and presents the danger of changing the mechanical characteristics of the treated wood. In addition, recent scientific opinions from EFSA have questioned the reliability of some of the heat treatment methods. For example, EFSA (2010) concluded that the technical requirements (presented in the Portuguese dossier) did not adequately demonstrate the effectiveness

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and reliability of the proposed composting method as a treatment that would ensure that the treated pine bark was free from live *B. xylophilus*; the dossier did not provide evidence that all the bark particles within the lot had achieved a continuous core temperature of 56 °C for 30 min.

The EFSA Panel on Plant Health (EFSA, 2012) also expressed uncertainty when it was asked by the EC to provide an opinion on a technical file submitted by U.S. authorities in support of a request to list a new heat treatment (60 °C/60 min) among the EU import requirements for wood from beetle pest (*Agrilus planipennis*/ Fairmaire/) host plants. No alternative chemical treatment (including fumigants) has been available under ISPM 15 since the absolute ban on MBr by the EC (EC Regulation No. 2037/2000). Therefore, it behooves international commercial interests using wood packaging, as well as timber producers and processors, to extend the spectrum of active substances that can be applied against regulated wood-infesting pests.

Are there any alternative fumigants available for replacing MBr that have biological potential and industry support for industrial wood treatment and quarantine? According to Banks (2012), there are currently only four fumigants (sulfuryl fluoride, HCN, methyl iodide, and methyl isothiocyanate + sulfuryl fluoride/ = Ecotwin/) under consideration as alternatives to MBr for the ISPM-15-approved treatment of wood-packaging materials. Among the chemicals listed, HCN is the only prospective wood-protecting fumigant produced in central Europe. Hydrogen cyanide was identified as an existing active substance under Directive 98/8/EC for product types 08, 14, and 18 (wood preservatives, rodenticides, insecticides, acaricides, and products to control other arthropods). It was evaluated by experts and authorities of member states of EEA and approved as an active substance for the above mentioned PT with date of inclusion to the Annex I of Directive 98/8/EC set on 1 Oct. 2014. This means that biocidal product(s) (BP) containing HCN as an active substance can be subject to authorization of BP under EU Regulation 528/2012. An application for such authorization is now being prepared by Lucebni závody Draslovka a.s. Kolin, to be submitted to Czech authorities as soon as possible.

Hydrogen cyanide is not a novel insecticide molecule; however, it offers several significant advantages. It occurs naturally in the environment, it features very high penetration properties, and it is highly reactive and therefore easily and quickly degradable, while leaving minimal undesirable residues. The risks associated with high toxicity can be decreased by proper cylinder formulation, use in a fumigation chamber, and application by professionally trained staff. Hydrogen cyanide is commercially available and is registered in some EU countries as a biocide for mill and airplane fumigation (Ducom, 2012). Outside Europe, HCN has been registered in India, New Zealand (Navarro, 2006), and South Korea. Misumi (2011), in his review of quarantine methods, documented that HCN has been used in Japan for the quarantine of apples (dose: 1.8 g m^{-3}; exposure: 30 min).

Lindgren et al. (1954) and Aulicky et al. (2012) demonstrated significant HCN efficacy against beetle pests infesting important products stored at mills. Rambeau et al. (2001) reported that all of the life stages of significant pests in mills and food factories, including *Tribolium confusum* Jacquelin du Val, *Tribolium castaneum* (Herbst), and *Plodia interpunctella* (Hübner) could be controlled at a Ct product of 10 ghm^{-3}, although to ensure HCN penetration and kill insects at a depth of approximately 10 cm in heaps of flour, the prevailing Ct product should be approximately 60 ghm^{-3}. In the presence of minor leakage, initial HCN concentrations were proposed at 5 g m^{-3}.

Although there are no current plans for HCN fumigation in the USDA-APHIS Treatment Manual (Neven, 2010), it has previously been employed to fumigate fresh fruits in the U.S. and Europe (Bond, 1984). Hydrogen cyanide may be used for the fumigation of many dried foodstuffs, grains, and seeds (Emekci, 2010). Although HCN is strongly absorbed by many materials, this action is usually reversible when they are dry, and, given enough time, all of the fumigant vapors are desorbed (Navarro, 2006). Recent experiments performed in the Czech Republic (Manasova et al., 2012) demonstrated the nematicidal properties of HCN for the free-living nematode *Caenorhabditis elegans* (Maupas). There have been long-term practical experiences with the structural treatment of wood (Rambeau et al., 2001) because HCN has been used as a biocide for controlling pests that are infesting wood in churches and museums in Europe. Hydrogen cyanide fumigation methods have been developed for treating parts of galleries as well as structural woodwork that had been infested by wood pests (Grosser and Roßmann, 1974; Emmerling, 1995). There are data published on the efficacy of HCN for controlling wood-infesting pests of the genera *Anobium* sp. and *Lyctus* sp. under various temperatures, concentrations, and exposures (Parkin and Busvine, 1937; Bletchly, 1953). Soma et al. (2001a), Soma et al. (2001b) compared the efficacy of several fumigants in controlling nematodes infesting wood. However, experimental data are lacking on the efficacy of HCN at controlling not only newly emerging wood-packaging pests (*A. glabripennis* and *B. xylophilus*) but also, surprisingly, some "old" wood-infesting pests, such as *H. bajulus*.

Because of the lack of existing fumigation treatments against significant EPPO 1 wood-packaging infesting pests, we explored the potential of gaseous HCN for their control. This study reports results on (1) the penetration ability of HCN into wooden blocks and its rate of absorption, and (2) the biological efficacy of two doses of HCN (10 g m^{-3}, 20 g m^{-3}) for both of the regulated pests *A. glabripennis* and *B. xylophilus* and for the significant cosmopolitan non-regulated structural wood-infesting pest *H. bajulus*.

2. Materials and methods

2.1. Fumigation chamber, HCN formulation, and concentration estimation

All of the experiments were performed in a hermetically sealed steel fumigation chamber (volume, 650 L) with forced air circulation and temperature regulation that is located at the Lucebni zavody Draslovka a.s., Kolin (Czech Republic) (Fig. 1A). If there is an experimental need, the chamber allows for the continuous, non-invasive withdrawal of individual samples through an air-lock antechamber (Fig. 1B) during the required time intervals. The experimental HCN formulation was used in a cooled (5 °C) liquid form. It was stabilized with 0.1% phosphoric acid and with 0.9–1.1% sulfur dioxide. The HCN was introduced into the fumigation chamber using a syringe via a rubber septum (Figs. 1A and 2). Inside the fumigation chamber, the HCN vapor concentration (inside and outside/ = headspace/wooden spruce block) was estimated using the GC technique (Shimadzu GC-17A, RT-QPLOT, 30 m, ID 0.53 mm, GC Software Clarity DataApex). The GC method is based on comparing the detector response from the sample with an external standard with a known concentration. We used 0.5 vol.% HCN in nitrogen as the standard (Linde Gas).

2.2. HCN wood penetration

Spruce wood (*Picea alba* (L.) *H. Karst.*) was selected for the HCN penetration experiments because it is the most common material used for wooden pallet construction. Wooden Euro pallets are composed of top and bottom duckboards (1–3 cm in width) connected by stringers or blocks (100 × 100 × 10 mm). For the HCN penetration test, five wooden blocks (moisture 18.5% ± 0.44%;