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Bluefume (HCN) and EDN® as fumigation alternatives to methy bromide for control of primary stored product pests

Vaclav Stejskal[1)], Radek Aulicky[1)], Adam Jonas[2)], Jonas Hnatek[2)], Jarmila Malkova [2)]

1) Crop Research Institute, Prague, Drnovska 507, 161 06, Czech Republic, stejskal@vurv.cz
2) Lucebni zavody Draslovka Kolin a.s., Havlíčkova 605, 280 99 Kolín, Czech Republic adam.jonas@draslovka.cz
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Abstract

The presented paper provides preliminary results on the fumigation potential of two preparations: Bluefume (HCN - hydrogen cyanide) and EDN®. (Ethane-dinitrile). Their biological efficacy was tested on Granary weevil (*Sitophilus granarius*; Curculionidae; Coleoptera) as a primary stored product pest in the Czech Republic. In fumigation chamber, we tested temporal survival of various *S. granarius* strains following exposure of a dose of 9 $g.m^{-3}$ HCN (Bluefume). We compared differential sensitivity of one laboratory (i.e. sensitive) CRI-strain and 9 field strains collected from the Czech stores and mills. The HCN Ct products required to kill the tested *S. granarius* strains ranged from CTp= 30.5 $g.m^{-3}.h^{-1}$ to CTp= 51.7 g.m3.h^{-1}. The efficacy of EDN (30 $g.m^{-3}$) on various developmental stages *S. granarius* was tested in a fumigation chamber. No live individual of *S. granarius* belonging to any life stage was recorded following 18 hours of EDN exposure.

Keywords: gas, ethane dinitrile, hydrogen cyanide, Granary weevil, Sitophilus granarius,

Introduction

Fumigation of stored product pests has become a real challenge for both farmers and pest control professionals (PCOs) in the last two decades. The reason is that broad-spectrum pesticide methyl bromide is no longer available and pest resistance to the remaining major fumigant phosphine is on the rapid increase (Nayak, et al., 2017). Therefore, the alternatives to methyl bromide or "resistance phosphine breakers" (e.g., Nayak et al., 2016) are urgently needed. However, there are only few candidate active ingredients available even at the worldwide scale (Ducom, 2006). Currently two of them (EDN and HCN) are produced in the Czech Republic (Lucebni zavody Draslovka Kolin a.s.).

HCN (Bluefume)

Various formulations of hydrogen cyanide (HCN) has previously been used for pesticide/biocide fumigation in several countries, including USA, South Korea, France, Germany, Czech Republic, and Switzerland (Rambeau et al., 2001). HCN as an active ingredient shows quick and high efficacy on structural pests infesting mills (Bond 1984, Rambeau et al. 2001, Aulicky et al., 2015a) and ships (Monro, et al., 1952). Aulicky et al., (2015a) demonstrated a higher activity of HCN on *Tribolium confusum* eggs than the one documented for phoshine during the commercial mill fumigations in Czechia (Aulicky et al., 2015b). HCN has been historically used for the fumigation of many dry

foodstuffs, grains, tobacco and seeds (Bond 1984, Emekci, 2010, Stejskal, et. al., 2014b). HCN also shows promising level of biocidal activity on package and structural wood infesting pests such as *Hylotrupes bajulus*, *Anoplophora glabripennis* and pine wood nematode, *Bursaphelenchus xylophilus* (Stejskal et al., 2014a, Douda et al., 2015). Recent works Zouhar et al., (2016) reported high nematicide potential of hydrogen cyanide against *Ditylenchus dipsaci* nematode present inside garlic seedlings.

EDN®

Ethane-dinitrile (EDN) is an ozone-friendly alternative to methyl bromide. Its advantages are good penetration characteristics, high efficacy and short application time (Ryan et al., 2006). The EDN® main use is aimed at limiting the risks of pests and disease spreading, within the agricultural and timber industry. It can be used to sterilize soil and control insects, diseases, nematodes, weeds and other parasites, before planting. It can also be used to fumigate harvested timber and logs. Its excellent penetration characteristics and high efficacy make EDN® a great solution for eliminating wood-boring insects in timber as well as pathogens and nematodes which present a direct biosecurity risk to many importing countries.

As apparent from the previous paragraphs, most of the recently published studies on both fumigants dealt mainly with wooden, soil or structural pests. However, there are only limited (Hooper et al., 2003) and/or outdated (Monro, et al. 1952, Lindgren, et al. 1954, Lindgren, Vincent 1965) information documenting the efficacy of ECN or EDN on stored product pests.

Therefore in the present work we evaluated the potential of two fumigantion preparations (Bluefume -HCN- hydrogen cyanide and EDN® -Ethane-dinitrile) regarding their biological efficacy on the primary stored product pest granary weevil (*Sitophilus granarius*).

Materials and Methods

Pest species

Granary weevil (*Sitophilus granarius*, Curculionidae; Coleoptera) was selected as a model species, since it is a major primary pests in the Czech Republic stores (Stejskal et al., 2014b; Stejskal et al. 2015). Both field (for HCN testing) and laboratory (for EDN or HCN testing) strains of *S. granarius* were included in the study.

HCN (Bluefume)

The efficacy of HCN on various strains of *S. granarius* was tested. In the tests, the efficacy of BLUEFUME (with the active substance HCN) at the initial dose of 9 g. m^{-3} (0.75%) of HCN on *S. granarius* adults was estimated. Testing was carried out in a small fumigation chamber of 650 litres (for detailed description of methods see Stejskal et al., 2014a). In total, 10 strains (one laboratory and 9 field strains) were included in the experiments. The following HCN exposure times were used: 15; 45; 60; 90; 180; 240; 300; 360; 420; 480; 540; 600 and 660 minutes. The reason for using short exposure times and a reduced dose of HCN was to divide the mortality of the tested individuals over a wider time span.

EDN®

The efficacy of EDN on various stages of *S. granarius* was tested. The tested EDN dose was 30 $g.m^{-3}$ and the exposure time was 18 hours. The tests were carried out in a 650-liter fumigation chamber (Stejskal et al., 2014a). The individual stages of development of the *S. granarius* were separately placed (in grain mass of winter wheat) in plastic containers with a diameter of 50 mm and a height of 70 mm. A hole of 40 mm diameter was formed in the lid and bottom of the plastic container, which was overlaid with a breathable fabric (miralon). Biological samples with grain containing

different stages of *S. granarius* were exposed in separate plastic containers. Adults aged 7-14 days were inserted into grain mass in the test vial one day before trial.

Eggs of *S. granarius* were ovtained by exposing adult females to grain mass for 3-4 days before trial. For the testing of pupae, the infested grains were used 42-48 days after exposure to *S. granarius* females. The biological efficacy of EDN on various stages was evaluated after removal and ventilation of samples in the laboratory. In adults, the efficacy was assesed visually, directly on the basis of their knockdown and mortality. In pupae, larvae, and eggs, however, the EDN efficacy was evaluated indirectly: according to adult emergence from the exposed grain-infested samples.

Results and Discussion

HCN (Bluefume)

Figure 1 shows comparison of the HCN sensitivity of one laboratory strain with 9 field strains of *S. granarius* (collected from the Czech stores and mills). The maximum time required to kill all of the tested 10 *S. granarius* strains was 660 minutes. The Ct products - required to kill the tested 10 *S. granarius* strains - ranged from CTp= 30.5 $g.m^{-3}. h^{-1}$ to CTp= 51,7 $g.m^{-3}. h^{-1}$.

Monro, et al. (1952), were the first who published a comparison of the efficacy of HCN and methyl bromide as fumigants for the treatment of stored product pests in empty ships. Later Lindgren, et al. (1954) compared the laboratory efficiency of 10 fumigants to selected types of food and warehouse pests. Their work showed the high efficiency of HCN in 8 tested species. Grain weevil (*S.granarius*) and Rice weevil (*S. oryzae*) were less tolerant to HCN than other pests including *Tribolium confusum, Acathoscelides obtectus, Oryzaephilus surinamensis* and *Rhizopertha dominica*. However, Ct products were not established in their work. The first study, which documented the HCN Ct products required to kill *S. granarius*, was the work of the French researchers Rambeau et. al. (2001). They claimed that *S. granarius* was more tolerant to HCN than to methylbromide (CH_3Br). The Ct product and mortality of *S. granarius* for HCN was 36 $g.m^{-3}$ for LD_{90}, and 15.7 $g.m^{-3}$ for CH_3Br. Rambeau et al. (2001) did not use HCN formulations in the form of liquid or liquid soaked in porous matter - a formulation that uses BLUEFUME but worked with HCN released from cyanide salts.

EDN®

Table 1 shows that the exposure of *S. granarius* by EDN fumigant at a dose of 30 $g.m^{-3}$ led to 100% mortality in all of the tested pest stages. According to Ducom (2006) EDN was much more toxic than methyl bromide and killed most of the pests very quickly. However, S*itophilus* sp. was an exeception, since a very long exposure of 5 days was required to achieve complete mortality of the egg stage. However, our laboratory experiments suggested that 100% mortality can be reached after 18 hours in all stages for the EDN dose of 30 $g.m^{-3}$. This preliminary result show good potential of EDN to be used to combat infestations from storage pests and it is therefore, desirable to continue with further validation tests against other pest species.

Tab. 1 The efficacy of EDN on various stages of *Sitophilus granarius* (dose: 30 g/m3, exposure time: 18 hours) in a fumigation chamber.

	Mortality (%)		Larval emergence (ks)	
Developmental stage	Treated	Control	Treated	Control
Adults	100	3,6	n	n
Larvae	n	n	0	483,4
Pupae	n	n	0	651,6
Eggs	n	n	0	2,8

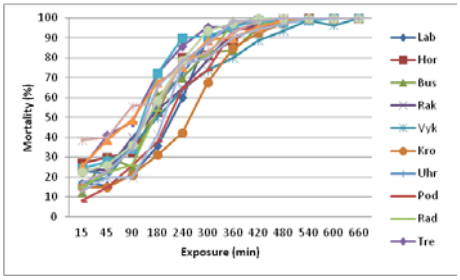

Fig. 1 Mortality of 10 strains (9 field and 1 laboratory) of *Sitophilus granarius* after HCN exposure in a dose of 9 $g.m^{-3}$ in a fumigation chamber.

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Improved Analysis of Propylene Oxide, Propylene Chlorohydrin and Propylene Bromohydrin

Wiley A. Hall[1], Spencer S. Walse[2], Leonel Jimenez[3]

[1]Safe Food Alliance, 2037 Morgan Drive, Kingsburg CA 93631, wileyh@safefoodalliance.com
[2]USDA-ARS-SJVASC, 9611 Riverbend Drive, Parlier, CA 93648 spencer.walse@ars.usda.gov
[3]Horn Technologies & Services Inc, 2020 S Golden State Blvd Ste 103, Fowler, CA 93625, ljimenez@horn-technologies.com
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Abstract

The benefits and deficiencies of several methods of analysis for PPO and PXH, including the aqueous extraction used in ASTA method 23.1 and the MTBE extraction method previously reported by the authors, will be discussed. Novel methods utilizing dynamic headspace extraction and solid phase microextraction (SPME) will also be reported with particular emphasis on preventing artefactual effects. Preliminary experiments have found that dynamic headspace sampling can lower detection limits by up to 3 orders of magnitude.

Keywords: Propylene Oxide, Fumigation, Sterilent, Headspace-SPME, Pesticide Degradants.

Introduction

The importance of propylene oxide (PPO) treatments for stored product protection has only increased in recent years, especially as the implementation of FSMA in the US puts pressure on tree nut producers to pasteurize their product. In the search for post-harvest methyl bromide replacements; PPO/SF blends, with PPO overcoming the ovicidal deficiencies of sulfuryl fluoride, have been shown to be effective against several stored product pests.

With the increasing variety in PPO treatments across commodity types and a "deharmonized" global MRLs comes an increasing need for the quick and accurate quantification of PPO residues. Analysis is complicated by the ease with which PPO will undergo nucleophilic reaction with water to form propylene glycol, or with chloride and bromide to form propylene chloro and bromo- hydrin, which can artificially lower the detected PPO residue. Avoiding the formation of these halohadrins (PXH) is of particular importance as they face regulatory scrutiny as carcinogens.

Materials and Methods

Jimenez et. al. Method:

Almonds or walnuts are added to an explosion proof blender along with chilled, deionized water and MTBE and homogenized. 45mL of the homogenate is centrifuged and a 1 mL aliquot of the MTBE supernatant is transferred to a 2 mL amber glass vial for analysis. A 10x concentration (10 mL to 1 mL) of the MTBE supernatant could be performed to increase detection of PBH-1 and PBH-2. Analysis was performed via cool on-column injections in an Agilent 6890 gas chromatograph (GC) equipped with a 5973N mass spectrometer (MS).

Dynamic Headspace Extraction Method:

Three almonds or walnuts are chopped roughly, transferred into a 20 mL headspace vial and sealed. The vial is then incubated at 80C for 42 min in a Perkin-Elmer Turbomatrix dynamic headspace autosampler, and three cycles of pressurizing the vial to 15 psi and allowing it to vent through an adsorbent trap are performed prior to ballistically heating the trap and directing the sample flow into a Perkin-Elmer Clarus SQ8 GCMS.

SPME-Headspace Method:

An approx. 50g sample of almond or walnuts is cryogenically milled under liquid N2 and a 2g subsample is transferred to a 20 mL headspace vial and sealed. SPME extraction is performed with