Stejskal V, Dlouhý M, Malkova J, Hampl J, Aulicky R (2016) Flour-mill fumigation using hydrogen cyanide insecticide gas. Pp. 173–177. In: Navarro S, Jayas DS, Alagusundaram K, (Eds.) Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Flour-mill fumigation using hydrogen cyanide insecticide gas

VACLAV STEJSKAL[1*], MILAN DLOUHÝ[2], JARMILA MALKOVA[2], JAN HAMPL[2], RADEK AULICKY[1]

[1]Crop Research Institute (CRI) – VURV vvi, Drnovska 507, 161 06 Prague 6, Czech Republic

ABSTRACT

Owing to their good penetration properties, fumigants are still one of the key components of integrated pest control programmes in flour mills in many countries. Hydrogen cyanide (HCN) is commercially available and registered in some EU countries as a biocide for mill and aeroplane fumigation. Although it is known (mostly from laboratory studies) that HCN has good biological activity on all pest stadia including eggs, currently there is insufficient information concerning biological HCN efficacy in relation to the CT-products reached during the real-world flour mill fumigations. The aim of this study was to (*i*) determine the extent of variation of HCN CT-products at various floors of flour mill building during a 'real world' commercial fumigation, (*ii*) determine HCN efficacy on 5 species of stored products pests, viz. *Tribolium castaneum* (Herbst), *Tribolium confusum* (L.), *Cryptolestes turcicus* (L.), *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (Fabricius) in the fumigated mill, and (*iii*) determine HCN biological efficacy on the confused flour beetle *Tribolium confusum* du val exposed to fumigant under 'naked' (i.e. pests enclosed in open empty vials and covered by mash lid) and 'obstructed' (i.e. pests enclosed in the same vials but containing various layers of wheat (*Triticum aestivum* L.) flour conditions. Although CT-products estimated for HCN were less than half of the labelled HCN rate (240 g h m^{-3}), 100% mortality was recorded for larvae and adults of *T. confusum* in all of the mill floors and locations tested. Regardless of the floor and CT variations, all adults and larvae of exposed *T. confusum* were dead. Contrary to the general belief that HCN is usually is poorly penetrative fumigant, we found 100% mortality of *T. confusum* enclosed in the vials containing wheat flour.

Key words: Dust, Flour, Fumigation, HCN, Mill, Storage pests

Owing to their good dispersion properties in treated structures and good penetration properties into treated materials, fumigants are one of the key components of the integrated pest control programmes in flour and produce mills in many countries. Unfortunately, nowadays there are only few registered insecticide active ingredients for stored product and structural (e.g. flour-mills) fumigation (e.g. PH_3 - Mueller, 1994; S_2F_3 - Chayaprasert et al., 2009). Widespread and repeated usage of limited number of active ingredients is connected with elevated risk of physiological resistance. For example, resistance to commonly used fumigant, phosphine (PH_3), has been recorded for several populations of storage pests from various parts of the world (Opit et al., 2012; Jagadeesan et al., 2012). In addition, reduced ovicidal activity was recorded for some of the currently used structural fumigants when low dosages were applied (Ciesla and Ducom 2010; Athanassiou et al., 2012) and/or if temperatures were not high (Aulicky et al., 2015). If fumigation of a particular mill is not fully effective - for any of the above mentioned reasons- what follows is a relatively quick pest population rebound (Campbell and Arbogast, 2004; Buckman et al., 2013).

Hydrogen cyanide (HCN) can be considered to be a one of few available alternatives to the major fumigants. The HCN is currently commercially available and registered in some EU countries as a biocide for structural fumigation (Aulicky et al., 2014). In the past, HCN was employed extensively as structural fumigant in tobacco stores and flour mills in the USA and Europe (Buttenberg et al., 1925). In

[2]Lucebni zavody Draslovka a.s. Kolin
[*]Corresponding author e-mail: *stejskal @vurv.cz*

the 1950's and 1960's, HCN was tested and used as a seed fumigant in the Czech Republic (Stejskal, 2014). Although it is not currently registered for seed fumigation, Ren et al. (1996) experimentally demonstrated that stored grain germination was not diminished and may have been slightly enhanced with hydrogen cyanide exposure. Recently, it has been documented that HCN has good activity on nematode *Ditylenchus dispaci* (Zouhar et al., 2016) infesting semi-dry garlic. Stejskal et al. (2012), reported good penetration of HCN through construction wood as well as its good biological efficacy on wood infesting pests.

It is known, mostly from laboratory studies, that HCN has excellent biological activity against various stored product and mill infesting pests and their developmental stadia (Lindgren and Vincent, 1965; Rambeau et al., 2001). However, currently there is only limited information (Aulicky et al., 2015) concerning biological HCN efficacy in relation to the CT-products reached under field conditions.

A new HCN fumigation research program (including research institutions and industry partners) has been established in the Czech Republic, to generate sufficient data on HCN as a mill insecticide for registration purposes. This paper presents part of the results that have been obtained so far within the framework of this program.

The specific goals of this study were (i) to determine the extent of variation of HCN CT-products during a 'real world' commercial mill fumigation at various floors of the fumigated building, (ii) to determine HCN efficacy on 5 major pests species of stored product pests (Stejskal et al., 2015) in a fumigated mill, (iii) to estimate HCN biological efficacy on larvae and adults of the confused flour beetle, *Tribolium confusum* (L.) exposed to fumigant under 'naked' (pests enclosed in open empty vials and covered by mesh lid) and 'obstructed' (pests enclosed in the same vials but containing 2 different layers of wheat flour) conditions.

MATERIALS AND METHODS

Biological material and strains

In our experiment we used laboratory strain of 5 species stored product pests, viz. *Tribolium castaneum* (Herbst)*, Tribolium confusum, Cryptolestes turcicus, Sitophilus oryzae* (L.)*,* and *Rhyzopertha dominica* (Fabricius). The strains originated from cultures that were kept at the Crop Research Institute (CRI), Prague, Czech Republic, for >20 generations. Pests were reared in incubators set at 26^{o}C, 75% r.h., and continuous darkness, on a species-specific diet.

HCN formulation and application

For the fumigation of mills the commercially available HCN formulation Bluefume (formerly URAGAN D2^{TM}) - produced by LZ Draslovka Kolin (Czech Republic) - was used. The HCN was released from Bluefume saturated porous disks supplied in sealed cans. Bluefume was applied in the tested mill by an external certified Czech fumigation company. The dosage was 10 gm^{-3}(1 kg per 100 m^{-3}) that give for 24 exposure and ideal conditions (i.e., instant HCN release and even distribution in the mill; no leakage, no sorption), the theoretical maximal CT product of 240 g.h.m^{-3}.

Estimation of temperature and HCN concentrations during field fumigations

Temperature and relative humidity were recorded separately for the 1st, 3rd and 5th floors during the fumigation by using Gemini DATA LOGGERS (Tinytag Plus IS Dual, Gemini Data Loggers Ltd, United Kingdom). The data-loggers were located at the same locations where the bioassay samples had been placed. The 1st, 3rd and 5th floors locations were sampled for HCN concentration remotely via PVC-plastic tubing (diameter 6/9 mm), with sampling devices were connected to tubing from outside of the mill. Air samples were taken at a height of 50 cm above the locations on which the insects and the data loggers had been placed. Concentrations of the fumigant were monitored for two positions at each mill floor at regular intervals. HCN CT-products were calculated from measurements at all these points. HCN vapour samples were taken in Tedlar bags (from mills) and the HCN concentrations estimated using the GC technique (Shimadzu GC-17A, RT-QPLOT, 30 m, ID 0.53 mm, GC Software Clarity DataApex; Shimadzu Corp., Kyoto, Japan). The GC method was used to compare the detector response from a sample with the response from an external standard with a known concentration. The standard we used was 0.5% vol. HCN in nitrogen.

Biological efficacy

Bioassays (containers with pests) were placed in central parts of the 1st to 6th floors. After exposure, pests were transferred to the laboratory and checked for mortality 26 h after the spaces were ventilated. In each of the six locations (i.e. each central part of 1–6 mill floors) were two designs of bioassay. First design—a set of 30 bioassay containers were placed that included five replicates for each of the two life stages tested *Tribolium confusum* (adults, larvae) and three commodity combinations. The commodity

Table 1 Concentration time products at twelve measurement positions/check points (4 check points per 1st, 3rd and 5th floor) of a commercial flour mill during 24 h of commercial fumigation with HCN

Check point	1	2	3	4	5	6	7	8	9	10	11	12
Ct-product ghm^{-3}	75	78	60	92	95	94	54	100	88	84	67	64

Table 2 Mortality (% ± SE) of adults of five species stored-product pests at the six floors of the flour mill and control after the fumigation

Species	Floor	HCN mortality (%)
Sitophilus oryzae	1–6	100 ± 0.00
	Control	
Rhyzopertha dominica	1–6	100 ± 0.00
	Control	
Tribolium castaneum	1–6	100 ± 0.00
	Control	
Tribolium confusum	1–6	100 ± 0.00
	Control	
Cryptolestes turcicus	1–6	100 ± 0.00
	Control	

Table 3 Mortality (% ± SE) of adults and larvae of *Tribolium confusum* at the six floors of the flour mill and control after the fumigation period (Insects were bio-assayed in containers with four commodity types: 'no flour' and flour at two depths = 1 cm and 5 cm)

	Floor	No floor	1-cm floor	5-cm floor
Adults	1–6	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	Control	6.00 ± 2.96	8.00 ± 1.22	10.00 ± 4.18
Larvae	1–6	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	Control	5.00 ± 3.87	27.00 ± 5.38	1.00 ± 1.00

combinations were: (*i*) containers without flour, (*ii*) containers with 1 cm of flour, (*iii*) containers with 5 cm of flour. Within each container, 20 adults or larvae (third-fourth instar) were placed. Second design – a set of 50 bioassay plastic petri dishes that included 10 replicates for each of 5 species pests (adults of *T. confusum, T. castaneum, C. turcicus, R. dominica* and *S. oryzae*).

RESULTS

HCN concentrations and CT-products during field fumigations

Table 1 shows that the CT-products obtained for the various positions and floors in the mill fumigation at an initial dosage of 10 g m^{-3} HCN. The maximum HCN CT-product obtained was 100 ghm^{-3} (4th position), while the lowest HCN CT product was 54 ghm^{-3} (7th position). The average CT-product was 91.31 ± 6.52 ghm^{-3}. Thus the observed average CT-product in the mill was less than half of the labelled HCN rate (240 ghm^{-3}) calculated on the basis of no loss from the initial dosage.

Biological efficacy of HCN on pests

The HCN caused 100% mortality of 5 major stored product Coleoptera species (*Tribolium castaneum, Tribolium confusum, Cryptolestes turcicus, Sitophilus oryzae* and *Rhizopertha dominica*) when fumigation was performed in a common type of commercial mill with a commercial applied dosage of 10 gm^{-3} achieving an average CT-product of 91.31 ± 6.52 ghm^{-3}.

At the HCN concentrations reached in this fumigation, after 24 h of exposure, all adults and larvae of *T. confusum* were dead (Table 3). Mortality was 100% regardless of the presence and the quantity of flour. It indicates that the hydrogen cyanide is able to penetrate flour that may cover the target insect individuals, at least at the flour quantity and height levels examined in this work (5 cm).

DISCUSSION AND CONCLUSIONS

The results of the present study illustrate CT-products, distribution, and efficacy of HCN on mills pests in a routine commercial flour mill in Central Europe. In the Czech Republic for mill fumigation, the recommended dosage of HCN is 10 gm^{-3} with 24 h exposure. This produces a theoretical maximum CT-product of 240 ghm^{-3} (i.e. under ideal conditions, with no leakage and/or absorption). Although CT-products estimated for HCN at six floors in this particular flour mill were less than half of the ideal HCN CT-product, still 100% mortality was recorded for all the bioassays containing adult pest species that may commonly occur in mills (e.g. *Tribolium castaneum, Tribolium confusum, Cryptolestes turcicus, Sitophilus oryzae* and *Rhizopertha dominica*). The maximum efficacy (100% mortality) was also observed for larvae of *T. confusum* in all the mill floors and locations tested.HCN is usually suspected to be a poorly penetrative fumigant through thick layers of organic materials. In the laboratory experiments, Rambeau et al. (2001) concluded that a Ct-P of 10 ghm^{-3} controlled the significant pests, *T. confusum*, *T. castaneum* and *Plodia interpunctella*, at all life stages in mills and food factories. However, according these authors, to ensure HCN penetration and to kill insects up to 10 cm deep in flour piles, the CT-product should be approximately 60 ghm^{-3}. In our mill experiment, we found 100% mortality of *T. confusum* enclosed in the vials containing wheat flour in layers 1 cm and 5 cm deep. Our findings are in accordance with the findings of Rambeau et al. (2001) since the average HCN CT-product (91 $g\ hm^{-3}$) reached in our experiment was higher than that reached (60 ghm^{-3}) in their experimental set up.

Sanitation may play an important role in fumigation efficacy (Williams et al., 2015). Layers of dust and flour found across a mill structure ranging from 1–5 cm represent moderately cleaned mill conditions. So, it would be a good idea to conduct future experiment that will cover situations with deeper dust/flour layers, e.g. ≥10 cm. Such level of unsanitary conditions can be usually found in old mills with complex structure and inaccessible places for cleaning or, more rarely, in modern flour mills with poor management.

We are convinced that the resulted presented in this work indicate good potential of HCN (Bluefume) for structural fumigation of flour mills.

ACKNOWLEDGMENT

This work was supported by the grant TAČR TA03020957 (Draslovka) and by the grant NAZV QJ1310057 and MZE RO0414 (VURV).

REFERENCES

Athanassiou CG, Phillips RW, Aikins MJ, Mahbub Hasan MM, Throne JE (2012) Effectiveness of sulfuryl fluoride for control of different life stages of stored-product Psocids (Psocoptera). Journal of Economic Entomology **105**: 282–287.

Aulicky R, Stejskal V, Dlouhy M, Liskova J (2014) Potential of hydrogen cyanide (HCN) fumigant for control of mill and wood infesting pests. International Pest Control **56**(4): 214–216.

Aulicky R, Stejskal V, Dlouhy M, Liskova J (2015) Validation of hydrogen cyanide fumigation in flourmills to control the confused flour beetle. Czech Journal of Food Science **33**: 174–179.

Buckman KA, Campbell JF, Subramanyam B (2013) *Tribolium castaneum* (Coleoptera: Tenebrionidae) associated with rice mills: Fumigation efficacy and population rebound. Journal of Economic Entomology **106**: 499–512.

Buttenberg P, Deckert W, Gahrtz G (1925) Weitere Erfahrungen be der Blausäuredurchgasung. Zeitschrift für Untersuchung der Nahr- und Genußmittel **50**: 92–103,

Campbell JF, Arbogast RT (2004) Stored-product insects in a flour mill: population dynamics and response to fumigation treatments. Entomologia Experimentalis et Applicata **112**: 217–225.

Chayaprasert W, Maier DE, Ileleji KE, Murthy JY (2009) Effects of weather conditions on sulfuryl fluoride and methyl bromide leakage during structural fumigation in a flourmill. Journal of Stored Products Research **45**: 1–9.

Ciesla Y, Ducom P (2010) Efficacy against eggs of *Tribolium confusum* and *Tribolium castaneum* after fumigations with sulfuryl fluoride in flour mills. Julius-Kühn- Archiv, Berlin, Nr. **429**: 48–51.

Jagadeesan R, Collins PJ, Daglish GJ, Ebert PR, Schlipalius DI (2012) Phosphine resistance in the rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): Inheritance, gene interactions and fitness costs. PLoS ONE **7**: e31582.

Lindgren DL, Vincent LE (1965) The susceptibility of laboratory-reared and field-collected cultures of *Tribolium confusum* and *T. castaneum* to ethylene dibromide, hydrocyanic acid and methyl bromide. Journal of Economic Entomology **58**: 551–555.

Mueller DK (1994) A new method of using low levels of phosphine in combination with heat and carbon dioxide, pp. 123–125. In Highley E, Wright EJ, Banks HJ, and Champ BR (Eds) Proceedings of the 6th International Working Conference on Stored-Product Protection. CAB International, Wallingford, Oxon.

Opit, GP, Phillips TW, Aikins MJ, Hasan MM (2012) Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. Journal of Economic Entomology **105**: 1,107–1,114.

Ren YL, O'Brien IG, Desmarchelier J (1996) Effect of hydrogen cyanide and carbonyl sulphide on the germination and plumule vigour of wheat. Pesticide Science **47**: 1–5.

Stejskal V, Simbera J, Aulicky R (2011) Uragan D2 jako fytokaranténní fumigant na dřevo: předběžné výsledky na modelové škůdce. Dezinfekce, Dezinsekce, Deratizace, **20**(4): 153–156.

Stejskal V, Liskova J, Ptacek P, Kucerova Z, Aulicky R (2012) Hydrogen cyanide for insecticide phytoquarantine treatment of package wood. In: Navarro S, Banks J, Jayas D, Bell C, Noyes R, Ferizli A, Emekci M, Isikber A and Alagusundaram K (Eds) Proceedings of 9th International Conference on Controlled Atmosphere and Fumigation in Stored Products. ARBER Professional Congress Services, Turkey. pp. 687-693.

Stejskal V, Douda O, Zouhar M, Manasova M, Dlouhy M, Simbera J, Aulicky R (2014a) Wood penetration ability of hydrogen cyanide and its efficacy for fumigation of *Anoplophora glabripennis, Hylotrupes bajulus* (Coleoptera) and *Bursaphelenchus xylophilus* (Nematoda). Int. Biodeterior. Biodegradation **86**: 189–195.

Stejskal V, Aulicky R, Kucerova Z (2014b) Pest control strategies and damage potential of seed-infesting pests in the Czech stores—a review. Plant Protection Science **50**(4): 165–173.

Stejskal V, Hubert J, Aulicky R, Kucerova Z (2015)

Overview of present and past and pest-associated risks in stored food and feed products: European perspective. Journal of Stored Products Research **64**: 122–132.

Strong RG, Lindgren DL (1959) Effect of methyl bromide and hydrocyanic acid fumigation on the germination of wheat. Journal of Economic Entomology **25**: 51–60.

Zouhar M, Douda O, Dlouhy M, Liskova J, Manasova M, Stejskal V (2016) Using of hydrogen cyanide against *Ditylenchus dipsaci* nematode present on garlic. Plant Soil and Environment **62**(4): 184–188.

Williams SB, Alexander CE, Mason LJ (2015) Sanitation's impact on the effectiveness of the pest management programs of food processing facilities. Journal of Stored Products Research **60**: 48–53.