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Validation of Hydrogen Cyanide Fumigation in Flourmills to Control the Confused Flour Beetle

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Abstract

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In the milling industry, the *Tribolium confusum* is hard to exterminate pest. We measured the concentration time product (Ct-P) achieved during the hydrogen cyanide (HCN) fumigation and examined whether the Ct-P levels in the explored Czech pilot mill were sufficient to control the field strain of *T. confusum*. Using an originally constructed, gastight fumigation chamber, it was estimated that the Ct-P required for the complete killing of the field strain of *T. confusum* was 4× higher (4.35 $g/h/m^3$) than the levels required to kill the laboratory strain (1.25 $g/h/m^3$). The Ct-P levels (ranging from 79 $g/h/m^3$ to 100 $g/h/m^3$) reached during the mill fumigations with HCN were less than half of the labelled HCN rate (240 $g/h/m^3$). Nevertheless, the current HCN dosage is still sufficient since the Ct-P reached during the mill fumigations was at least 99× higher than that required for the exterminating to the lab strain and 18× higher than that required for the field strain extermination. These results were confirmed in mill validation testing, where 100% mortality of *T. confusum* adults was achieved.

Keywords: mills; food safety; pest control; insecticides; fumigant

There is increasing evidence that pests may be frequent contaminators of flour and cereal products (Stejskal & Hubert 2008; Trematerra *et al.* 2011; Stejskal & Aulicky 2014, 2015; Stejskal *et al.* 2014a). Food safety is one of the most important political priorities in the EU. Flourmills are sensitive to the risk caused by many species of pests. Recently, *Tribolium* beetles (Figure 1) have emerged as a serious pest problem on a worldwide scale (Campbell & Arbogast 2004). In the Czech Republic, the confused flour beetle (*T. confusum*) is a persistent pest that is hard to monitor (Stejskal 1995). As noted by Ciesla and Ducom (2010), the pest control chemicals are becoming difficult to use in mills because many insecticides (i.e., methyl bromide) have been phased out as biocides in Europe. Promising alternatives, such as modified atmospheres and aerosols, are in minor use (Arthur 2012; Kucerova *et al.* 2013, 2014). However, fumigants are still key elements of the pest control in mills. The alternatives to methyl bromide may be corrosive and/or have a low ovicide efficacy at low dosages (Ducom 2012). Consequently, there is a renewed interest in hydrogen cyanide (HCN) for packaging (e.g., Stejskal *et al.* 2014b) and for mill fumigation. Furthermore, there is insufficient data from modern mills using HCN fumigation (Armstrong & Hill 1960). Rambeau *et al.* (2001) state that "knowledge on the efficacy of HCN as a fumigant is outdated, and existing data cannot be correlated with the present concept of the Ct product". Similarly, Reichmuth (1999) warns that there is a tremendous gap between the theoretical dosages and concentration–time–products (Ct-P) required for a given mortality and the recommendation of fumigation schedules. This disparity highlights the need for scientific studies to validate the use of HCN in modern mill fumigation. Validated Ct-Ps would enable re-evaluation of the

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(**A**) (**B**)

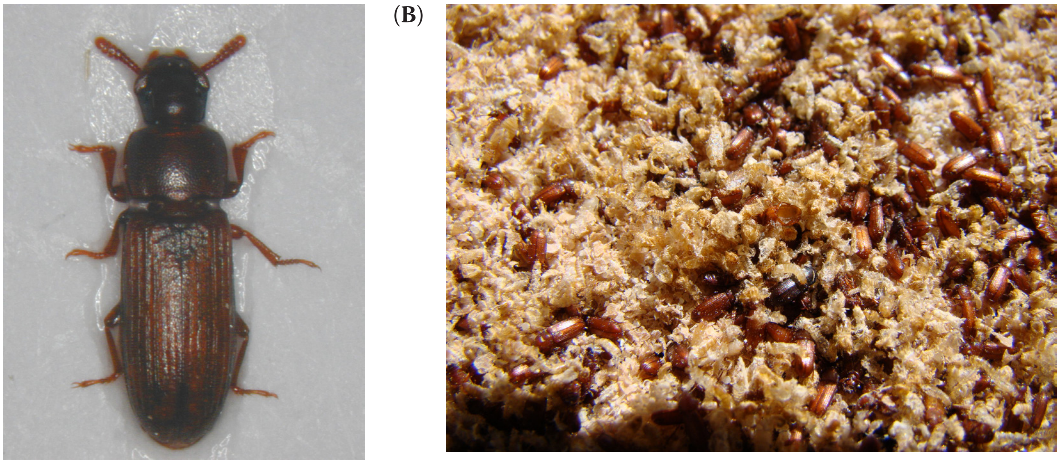

Figure 1. The confused flour beetle (*Tribolium confusum*) (**A**) is a major mill pest and (**B**) contaminated flour with physical fragments (i.e., faeces, carcasses) and excretions of carcinogenic chemicals (i.e., chinones)

current dosage and exposure times with respect to the levels of absorption and the concentration losses due to structural leakage. The use of HCN is further complicated because it is not known whether the field strains of pests differ in HCN sensitivity from the laboratory strains.

Therefore, this study was designed to provide the first estimates of the Ct-P generated during HCN fumigations of current mill structures. In addition, we compared the laboratory and the field strains of *T. confusum* to determine whether the Ct-P reached in commercial mills is sufficient to control the field strains of pests that during their population history developed a tolerance to HCN.

MATERIAL AND METHODS

Tested strains of the flour-infesting pest. The laboratory strain of the confused flour beetle (*Tribolium confusum* Jacquelin du Val) originated from the cultures kept at the Crop Research Institute (Prague, Czech Republic). The field strains of *T. confusum* were sampled from a commercial mill in Moravia in 2012, where HCN fumigations had been regularly performed over the last 10 years.

Hydrogen cyanide formulations and estimated GC concentration in the air. Two HCN formulations were employed in this study. For the fumigation of mills, the commercially available HCN formulation URAGAN D2^{TM} (LZ Draslovka Kolin, Czech Republic) was used. In the laboratory (chamber experiments), we used an experimental liquid HCN formulation (cooled to $-5°$ C; stabilised with 0.1% phosphoric acid and 0.9–1.1% sulphur dioxide). The HCN vapour concentration in the fumigation chamber headspace or from sampling Tedlar bags from mills was 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 is based on comparing 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.

Validation of HCN under real-world mill fumigation conditions. HCN concentrations were measured in routine annual mill fumigation treatments performed by a professional, licensed pest control company. The dosage was 261 kg of HCN released into a mill structure with a volume of 26147 m^3 (10.02 g/m^3 of HCN). Biological checks (i.e. 10 Petri dishes per floor, each containing 10 *T. confusum* adults) were placed in central parts of the 1st, 3rd, and 5th floors, respectively. The pests were transferred to the laboratory and checked for mortality 26 h after the spaces were ventilated. HCN concentrations inside the mill were measured distantly via plastic tubing led to all of the floors. Each site (i.e. 1st, 3rd, and 5th floors) was sampled for HCN concentration by using sampling lines. Sampling bags made of Tedlar-polyvinyl fluoride were located outside the mill for safety reasons. The bags were filled with the HCN atmosphere from the inside of the mill via plastic sampling lines and a pumping device (KNF-N86Kt.16; KNF Neuberger GmbH, Freiburg, Germany). Temperature and humidity (Table 1) from the inside of the fumigated mill were recorded using Gemini DATA LOGGERS (Tinytag Plus).

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Table 1. Concentration time products (Ct-P) and estimated temperatures on three floors of a commercial mill during 24 h of fumigation with hydrogen cyanide HCN

Floor	Ct-P ($g/h/m^3$)	Mortality (%)	Temperature (°C)		
			average	min	max
1.	100.22	100^a	24.7^a	24.0	25.1
3.	95.11	100^a	26.5^b	25.9	27.1
5.	78.6	100^a	27.7^c	27.1	28.3

The efficacy of HCN was expressed as mortality of confused flour beetle adults (*Tribolium confusum*); $^{a–c}$means for the mortality of adults within a column followed by different lowercase letters are not significantly different ($P > 0.05$), whereas for the temperature means within a column followed by different lowercase letters are significantly different ($P = 0.01$)

Validation of HCN efficacy for two Tribolium confusum strains in a hermetically sealed chamber. All experiments were performed in a hermetically sealed, steel fumigation chamber (volume 650 l) with the forced air circulation and temperature regulation. The chamber allows for the continuous, non-invasive withdrawal of individual samples through an air-lock antechamber at the required time intervals. Using a syringe, liquid HCN was introduced into the fumigation chamber via a rubber septum. Strains of *T. confusum* having an evolutionary history from commercial mills and from the growth in a laboratory, respectively, were placed in Petri dishes (10/plate) and exposed to an atmosphere of HCN eight times (Figure 2A). For both strains, the exposure times were 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, and 420 minutes.

Statistical methods and the calculation of the concentration time products (Ct-P). The results were analysed by a logistic regression mortality model (χ^2-test) for LT_{50} and LT_{99} (LT – Lethal Time) using the statistics program XLSTAT (Addinsoft, Paris, France). Non-parametric Mann-Whitney U tests were used for the statistical comparison of the mortality of both strains and Non-parametric Kruskall-Wallis tests were used for the statistical comparison of the temperature of three floors (Statistica, v. 10; StatSoft CR s.r.o, Prague, Czech Republic). The Z score is a test of statistical significance that helps to decide whether or not to reject the null hypothesis. The *P*-value is the probability the null hypothesis has been falsely rejected. The Ct-P was estimated based on Bond (1984) and FAO methods (http://www.fao.org/inpho_archive/content/documents/vlibrary/new_fao/x5417e/x5417e0u.htm).

RESULTS

In mill fumigations with an initial dosage of 10 g/m^3 HCN, the average Ct-P was 91.31 ± 6.52 $g/h/m^3$. Table 1 shows the differences in the field measurements of Ct-P. The Ct-P, determined for each of the three mill floors ranged from 79 to 100 $g/h/m^3$. Table 1 also shows the biological efficacy of HCN on *T. confusum* adults (enclosed in mills in open dishes) for the respective floors. The Ct-P levels on each of the floors resulted in 100% mortality of the exposed *T. confusum*.

The mortality rates for two strains of adult *T. confusum*, tested for multiple durations in a fumigation chamber, are shown in Figure 2B. The relevant

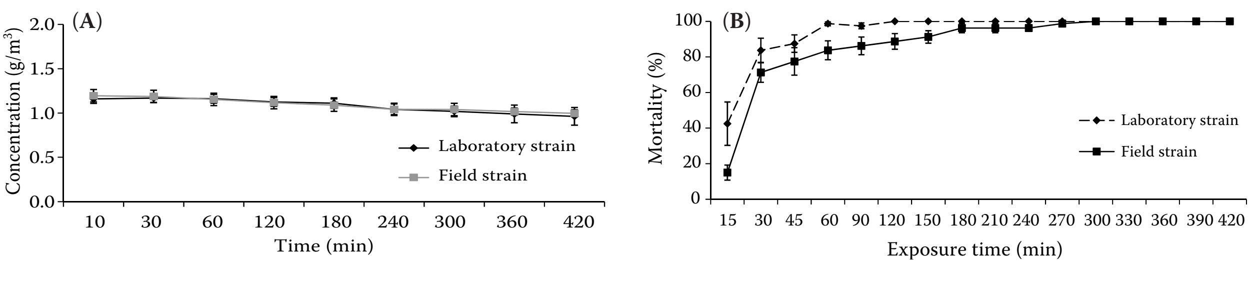

Figure 2. (**A**) Fumigation chamber headspace hydrogen cyanide (HCN) concentrations during fumigation and (**B**) mortality of two strains of adult *Tribolium confusum* during the course of HCN chamber fumigation. The concentration time product (Ct-P) for the laboratory strain WGS 1.25 $g/h/m^3$ and for the field (mill) strain 4.35 $g/h/m^3$

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concentrations of HCN in the headspace, estimated chromatographically, are shown in Figure 2A. For the dose used in experiments (1.12 ± 0.12 g HCN/m^3), complete mortality occurred after 120 min in the laboratory strain and after 300 min in the field strain. These results are significantly different (Z = 3.14; *P* = 0.002). The relevant values of the lethal times (LT_{50}, LT_{99}) and Ct-P were calculated for both strains. In the laboratory strain, LT_{50} = 15.53 (10.03–19.56) min; LT_{99} = 76.81 (66.90–92.59) min; and Ct-P = 1.25 $g/h/m^3$. In the field (mill) strain, LT_{50} = 14.80 (1.40–25.43) min; LT_{99} = 243.37 (216.41–280.65) min; Ct-P = 4,35 $g/h/m^3$). These data show, for the first time, that the sensitivity to HCN is decreased in the strain of *T. confusum* from mills that have been repeatedly treated with HCN. The Ct-P required for the complete kill of the field strain of *T. confusum* was 4× higher than that required for the laboratory strain. This decreased sensitivity cannot be called resistance because the Ct product of the field strain was still far below those of the actual Ct-P achieved in the mill (Table 1).

DISCUSSION

Historically, few authors (e.g. Lindgren & Vincent 1965; Rambeau *et al.* 2001; Stejskal *et al.* 2014b) have experimentally demonstrated the significant HCN efficacy against pests in stored products and wooden packaging. Although HCN is not a new fumigant, the Ct-P has not been established, even though these values are cornerstones of modern fumigation practice in the milling industry. The first attempt to establish Ct products for HCN was the work of Rambeau *et al.* (2001). Their laboratory experiments concluded that a Ct-P of 10 $g/h/m^3$ controlled the significant pests (*T. confusum*, *T. castaneum* (Herbst), and *Plodia interpunctella* (Hübner)) at all life stages, in mills and food factories. Under the same conditions, methyl bromide (MB) fumigation requires a Ct-P of 25.7 $g/h/m^3$ to control adult pests, while HCN needs a Ct-P of only 0.8 $g/h/m^3$. However, to ensure HCN penetration and to kill insects up to 10 cm deep in flour piles, the Ct-P should be approximately 60 $g/h/m^3$ (Rambeau *et al.* 2001). For mill fumigation in Europe, the dose of HCN has been historically labelled as 10 g/m^3 at 24-h of exposure. Under ideal conditions, with no leakage and/or absorption, the application of the label dosage produces a Ct-P of 240 $g/h/m^3$. This is 4× higher than the Ct-P recommended by Rambeau *et al.* (2001) in laboratory studies. Ideal conditions in mills are not realistic. Because they are not hermetically sealed, fumigants will be lost by both leakage and absorption. Therefore, the label dosage is higher to compensate for these losses. However, how high should the compensation be? The compensation must be based on the data from the field studies that determine the dynamics of gas and the temporal decline of concentration in multiple conditions. MacDonald and Reichmuth (1996) demonstrated that 50–90% of the initial dose of MB is lost when applied in a flourmill. Data such as this is only available for sulfuryl fluoride and MB in the USA (Chayaprasert *et al.* 2012). With the exception of an old study by Armstrong and Hill (1960), there have been no adequate measurements of HCN coming from modern mills. Our measurements of HCN dynamics during routine mill fumigation showed that the average Ct-P (91.31 ± 6.52 $g/h/m^3$) was less than half of the labelled HCN rate (240 $g/h/m^3$); Ct-P ranged from 79 to 100 $g/h/m^3$ depending on the mill floor. The Ct-P obtained in our mill measurements was above the 60 $g/h/m^3$ recommended by Rambeau *et al.* (2001), based on their laboratory experiments.

When establishing an effective dose of fumigant for the use in the field, the "pest-strain-factor" must be accounted for in addition to conditions such as the mill structure, pest developmental stage, and pest age (Rambeau *et al.* 2001). Laboratory data are typically generated using pests coming from sensitive strains or from strains of unknown origin and pesticide exposure history. However, repeated exposure to HCN may lead to strains that develop tolerance or even resistance. Therefore, there may be a risk of HCN resistance resulting from an evolutionary response to exposure experienced by some phytophagous insects. In support of this idea, there are reports of HCN resistance in phytophagous, scaled-insects that infest fruits in the USA (Quayle 1916, 1922; Lindgren & Dickson 1945; Gerhard & Lindgren 1951). Lindgren and Dickson (1945) demonstrated that fruit infesting, scaled-insects might develop HCN resistance after only 6 to 9 fumigations. A different situation was documented for pests infesting mills and food. A report by Gough (1939) showed that the laboratory strains of mill pests have a low potential (F7 – 1.7×) to develop HCN resistance. In mill-collected strains of storage pests, the resistance was almost negligible (up to 1.4×) in the USA (1.6×; Lindgren & Vincent 1965) and Australia (1.4×;

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Champ 1979) a few decades ago. However, there have been no recent publications on sensitivity or resistance towards HCN. Our work represents the only current study comparing the sensitivity of a laboratory strain to that of a field strain, which was collected from a mill that had been repeatedly fumigated with HCN. The Ct-P required for a complete killing of the field strain of *T. confusum* was estimated to be 4× higher (4.35 g/h/m^3) than that required for the laboratory strain (1.25 g/h/m^3). However, the current HCN dosage is sufficient to control both strains. The Ct-P reached during the tested mill fumigation was at least 99× higher than that required for complete killing of the sensitive lab strain and 18× higher than that necessary for the less sensitive field strain. These data were validated by HCN testing in mills, where 100% mortality of *T. confusum* adults was achieved when all the technological and hermetical sealing procedures required by the HCN biocide label had been implemented.

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