Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products

Editors

Digvir S. Jayas

Fuji Jian

August 23-27, 2021

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Preface

The International Conferences on Controlled Atmosphere and Fumigation in Stored Products (CAF) are the major international forums for reporting advances in research and development of gaseous treatments for the preservation of stored commodities.

The conferences, which are held every four years, encompass the treatment of broad range of durable and other commodities held in storage utilizing controlled atmosphere or chemical fumigation for the control of pests. Over the years the conference objectives have been refined to report on advances in research and development and the current status of controlled atmosphere and fumigation for insect pests, microflora and quality control in stored products. Treatment of the products such as cereal grains, oilseeds, legumes, root crops, dried fruits, nuts and vegetables, and other agricultural products including bulbs and flowers are covered by these conferences. However, the CAF conferences do not cover controlled atmosphere treatments for the quality preservation of fresh agricultural products. The professional background for the CAF conference are: a) the need to ensure the continued use of phosphine against the pressures of increasing resistance to this valuable fumigant; b) the sustainable availability of novel MA technologies; and c) reports on new fumigants and among them are ethyl formate, ethane dinitrile and ozone.

The establishment of the International Steering Committee followed the first Symposium held in Rome in May 1980 organized by Assoreni and the first Coo-operative Bulk Handling Limited and the Australian Grain Institute Incorporated. The second one, which included fumigation, was held in Perth, Western Australia in 1983. This second symposium was given the title "International Symposium on Controlled Atmospheres and Fumigation" (CAF). Then the CAF International Permanent Committee emerged from the International Steering Committee in 1983 in Perth, West Australia. Only in CAF 1996 in Cyprus the title of “International Conference on Controlled Atmospheres and Fumigation in Stored Products” was adopted.

The Conferences after CAF Perth, Australia (1983) were in Singapore (1989); Winnipeg, Canada (1992); Nicosia, Cyprus (1996); Fresno, USA (2000); Gold Coast, Australia (2004); Chengdu, China (2008); Antalya, Turkey (2012); and New Delhi, India (2016).

This Conference is 11th in the series of the International Conferences on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), and originally planned to be held in Winnipeg, Canada, from August 23 to 28, 2020. But due to Covid-19 global pandemic, the CAF2020 was postponed to 2021; however, the name “CAF2020” was kept. Local Organizing Committee has hoped that the CAF2020 will be held in person but global pandemic prevented this. CAF2020 is being held virtually on August 23 to 27, 2021 organized by the Local Organizing Committee including members from the CAF International Permanent Committee under the leadership of Dr. Digvir S. Jayas. The Local Organizing Committee members are from the University of Manitoba, Agriculture of Agri-Food Canada, and Canadian Grain Commission, Canada. Since CAF2020 is being held virtually, a request was raised by the members of the Local CAF Conference Organizing Committee in Canada to hold in person meeting in Winnipeg in 2024. That means that the Local CAF Conference Organizing Committee in Canada will be responsible for the virtual conference in 2021 and in-person conference in 2024.

The CAF Conference Local Organizing Committee has done a great job in organizing the virtual conference and producing high quality refereed proceedings. The presentations of CAF2020 conference are organized in the following sessions: 1) Biological Response to Fumigants, 2) Engineering and Modeling of Fumigants, 3) Insect Resistance to Fumigants, 4) Hermetic Storage, 5) Controlled Atmospheres, 6) Quarantine and Alternatives to Methyl Bromide, 7) Alternate Fumigants, 8) Bio-fumigants, 9) Alternative Approaches, 10) Alternative Fumigants, and 11) Fumigation Overview. The proceedings include 41 full papers and 45 abstracts. The technical support provided by Ms. Judith Mate in preparation of the book of proceedings is greatly appreciated.

Prof. Dr. Shlomo Navarro
Chair, CAF International Conferences Permanent Committee

Prof. Dr. Digvir S. Jayas
Secretary, CAF International Conferences Permanent Committee and
Chair, CAF2020 Local Organizing Committee

Prof. Dr. Fuji Jian
Secretary, CAF2020 Local Organizing Committee

August 2021

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An appreciation of James (Jim) Gerald Leesch (1942–2020)

Dr. James (Jim) Leesch was born in Oakland, CA in 1942. He obtained his Associate's degree from Porterville College (CA) in 1962 and his Bachelor's degree from Occidental College (Los Angeles) in 1965; both in chemistry. He joined ARS in 1970 shortly after receiving his Ph.D. in Entomology from the University of California, Riverside.

Dr. Leesch worked at the Pest Control Technology Research Unit in Savannah, GA for 23 years. He developed the technology for assessing the suitability of fumigants in quarantine treatments. In addition, he developed the technology of in-transit ship fumigation. Over the years 1991 to 1993 served as Research Leader at the same laboratory.

In 1994 he was relocated to Crop Protection and Quality Research Unit (CPQRU), San Joaquin Valley Agricultural Sciences Center in Fresno CA, and later, to Parlier, CA. Dr. Leesch served as the CPQRU Research Leader from 2003 through his 2009 retirement.

Dr. Leesch served as the U.S. representative on the Permanent Committee of the International Conference on Controlled Atmosphere and Fumigation (CAF) in Stored Products. He has chaired the Local Organizing Committee of CAF 2000 conference in Fresno, CA. He also served on the Program Committee for the International Methyl Bromide Alternatives Outreach Research Conference from 2003 to 2017.

Dr. Leesch was world renowned in the field of postharvest fumigation, particularly as related to grain and other stored products, where he authored many book chapters and was the key contributor to the USDA Federal Grain Inspection Service (FGIS) Fumigation Handbook. His research conducted in the 1970's and 80's regarding shipboard fumigations continues to prove invaluable, as it is an integral component of the transoceanic, global marketing of wheat, corn, dried beans, logs, and wood chips.

He served as the key technical consultant for a USDA/U.S. Trade Representative team that won a World Trade Organization case against Japan concerning the value of varietal testing during phytosanitary treatments.

Dr. Leesch was known for his kindness, faith, humble nature and easy-going demeanor. He passed away near his home in Reedley, CA, on September 9, 2020. He is survived by his wife, Carolyn Leesch, and his sons Tucker and Marty Leesch. He will be dearly missed by his many friends.

Shlomo Navarro
Post-Harvest Research Entomologist, CEO of Green Storage Ltd.
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An appreciation of Zuxun Jin (1934–2021)

Mr. Zuxun Jin was born in Hebei Province of China in May, 1934, and died of illness in Beijing on July 19, 2021, at age of 88. From 1957 to 1965, Mr. Jin served as the Acting Head of Technology Office in the Scientific Institute of Food under the Ministry of Grain of China. From 1965 to 1989, he worked in Mianyang Scientific Institute of Food as the Deputy Director of the Reform Committee; Deputy Director and Director of Institute Academic Committee; and Editor-in-Chief of Food Storage magazine. From 1989 to 1996, he served as Deputy Party Secretary, Vice Dean, and Dean of Nanjing Institute of Food Economics (predecessor of Nanjing University of Finance and Economics). He also served on the Permanent Committee of the International Working Conference on the Stored-product Protection (IWCSPP) for 24 years (the first person from China ever to serve on this committee). His knowledge and wisdom were much appreciated by his peers and he contributed significantly to the development of food storage technologies within and outside China. He served the leader of the Expert Advisory Group of State Administration of Grains of China, and Vice Chair of the Chinese Cereals and Oils Association (CCOA), and Vice and Honorary Chair of the Storage Branch of CCOA. He played a critical role in promoting and recognizing Grain Storage as a scientific discipline in China, along with the establishment of the Storage Branch of CCOA. Mr. Jin devoted his life to the sciences and technologies of grain storage in China. He compiled and published more than 10 monographs and 50 papers, including Progress in Grain Storage Sciences and Technologies and Facilities and Technologies of Grain Storage in Ancient China. He was a senior engineer (Professor-level) and an Expert on Special Allowances for the State Council of China.

Mr. Zuxun Jin was one of proponents who led the founding of the Food Science Institute of Mianyang, which is the predecessor of Sinograin Chengdu Storage Research Institute. He was the lead person for several largescale National Science and Technology projects in grain storage, including the “Six-Five Plan” and “Seven-Five Plan” projects. His research and leadership

significantly contributed to and advanced the scientific knowledge and grain storage practices in China. A highlight of his contributions was a theoretical framework of grain storage ecosystems in China, along with a series of strategies of grain storage technology development, including the strategy of ”three lows three highs” (low losses, low pollution, and low costs; high quality, high nutrition, and high profits); strategies of “green grain storage”, “ecological grain storage” and “sustainable development of grain storage”; strategy of integrating field and post-harvest processes in managing stored grain; and the “four optimization strategy” (optimization of natural resources, ecological potential, economic profits, and human resource). He proposed a framework of safe grain storage in China and laid out a blueprint of China grain storage technology development model. His contributions laid a theoretical and philosophical foundation for developing safe food storage in China.

Mr. Zuxun Jin’s life was a life of trailblazing, a life of self-renewing, a life of devoting, and a life of accumulating knowledge. He may be gone from our sights, but he will never be gone from our hearts as a pioneer who opened the first page of modern grain storage research in China!

Sinograin Chengdu Storage Research Institute
Chengdu, China

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Athanassiou CG, Lampiri E, Agrafioti P (2021) Delayed mortality, resistance and the "sweet spot": the good the bad and the ugly in phosphine fumigations. Page 1. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Delayed mortality, resistance and the "sweet spot": the good the bad and the ugly in phosphine fumigations

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ABSTRACT

In a series of bioassays, adult mortality after specific exposure intervals to phosphine in selected stored product insect species was examined, as well as the appearance of the so called "sweet spot". The tested insects were: *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). For each species, we used populations that had different levels of phosphine resistance. In a first series of bioassays, we used the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK), with various exposures between 15 and 300 min at 3000 ppm, and after the termination of the exposure, the adults were transferred in phosphine-free environment. The majority of the adults of the susceptible populations of all species were instantly immobilized even in the shortest exposure period (15 min), in contrast with adults of the resistant populations that were active even after 300 min. At the post-exposure period, in most cases, most adults of the susceptible populations were dead, whereas adults of the resistant populations recovered regardless of the species and the exposure time. Another series of bioassays at 500, 1000, 2000 and 3000 ppm for 1, 3, 5, 20, 30 and 40 h clearly indicted the appearance of the "sweet spot", i.e., the decrease of mortality with the increase of the concentration, at certain exposure-concentration combinations. In fact, in most of the tested species the "sweet spot" appeared in 1000 and 2000 ppm and at a 5 h exposure interval. This observation is particularly important both in terms of the assessment of resistance and in the context of non-linearity of phosphine efficacy at elevated concentrations. Additional experimental work is needed to clarify if and how this non-linear response is related with the development of resistance to phosphine after exposure to high concentrations for short intervals.

Keywords: Phosphine, Fumigation, Resistance, Stored product insects, Mortality, Non-linear response

CAF2020 Abstract No. A-1-2-2

Nayak MK, Daglish GJ, Jagadeesan R, Pavic H, Burrill PR (2021) Phosphine fumigation strategies for effective control of bruchid pests. Page 2. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Phosphine fumigation strategies for effective control of bruchid pests

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ABSTRACT

The Australian pulse industry, especially mung bean and chickpeas, has been growing rapidly in recent years. Due to heavy focus on cereal industries, however, knowledge in post-harvest pest management in stored pulses is very limited. Bruchid pests, particularly the cosmopolitan cowpea weevil, *Callosobruchus maculatus* (F.) threatens industry's ability to maximise profits from this commodity. Although phosphine is being used predominantly to disinfest stored commodities in Australia, and fumigation protocols are available for major cereal pests, comprehensive research-based information on effectiveness of this key fumigant against bruchids is not available. The present study addressed this knowledge gap and attempted to establish detailed phosphine fumigation efficacy data particularly on the effect of concentration and exposure time against *C. maculatus*. Mixed-age populations (including all life stages) of a previously established laboratory population and a recently collected field population of *C. maculatus*, were raised in mung bean and chickpeas and fumigated at 25°C. Concentrations of 0.5 and 1.0 mg/L (360 and 720 ppm) were evaluated over multiple exposure periods up to 7 d. Apart from an adult mortality assessment at 7 d post-fumigation, the impact of the fumigation on immature stages (F1 progeny) was determined after 6 wk post-fumigation. Results suggest that irrespective of the concentrations used, a 7-d fumigation at 1 mg/L (720 ppm) was required to achieve complete control of bruchid populations. The field population of *C. maculatus* seemed to be more tolerant than the older laboratory population. These results were consistent across both mung bean and chickpeas. Our laboratory data were well supported by a silo-scale fumigation trial with mung beans. We concluded that the current Australian label for fumigation using aluminum phosphide formulations was adequate for controlling *C. maculatus* in stored pulses.

Keywords: Phosphine, Fumigation, Bruchids, Mung bean, Chickpea, Storage

CAF2020 Paper No. P-1-3-3

Alvarez E, Cardoso L, Bartosik R, Castellari C, de la Torre D, Erreguerena I, Bernadette Abadía B (2021) Evaluation of fungicidal action of four products on external fungal biota of grains. Pp. 3-9. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Evaluation of fungicidal action of four products on external fungal biota of grains

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Abstract

Corn, the main crop in Argentina, is normally harvested and stored in silo bags above the safe storage moisture content for 3-6 months, and faces high risk of quality losses. The evaluation of fungicidal/fungistatic products that could potentially be used to protect wet grain stored in silo bags, has not been widely studied. Thus, the objective of this study was to make a preliminary in-vitro comparative evaluation of four different fungicidal treatments in corn: 1) propionic acid (3 L/t); 2) a quaternary ammonium compound (3 L/t, diluted 1:25 with water); 3) sulfur dioxide (initial dose: 15% v/v); 4) phosphine (initial dose: 6.6 g/m^3) along with their respective controls (non-treated). In treatments 1 and 2 (liquids) corn was sprayed with the product and then stored in a sterilized glass jar. In treatment 3, sulfur dioxide was injected into a previously sealed glass jar. In treatment 4, glass jars were placed inside a plastic drum of 60 L capacity with two aluminum phosphide tablets (0.2 g phosphine each) and closed. Both liquids and the phosphine treatments had an exposure time of 15 d (at 25 ± 1°C), while in the sulfur dioxide treatment, three exposure times were evaluated (5 min, 5 h and 24 h, all at the same temperature). After the various treatments, the fungal biota (molds and yeasts) was evaluated. The results showed that the propionic acid treatment achieved a total control of fungal biota. Treatment with sulfur dioxide showed a reduction of 2-3 log10 (CFU/g DM) compared to non-treated samples. Finally, the quaternary ammonium compound and phosphine treatments only achieved a reduction in the number of colonies of 1 log10 (CFU/g DM). Based on the results of this study, propionic acid seems to be a good solution for improving the storability of wet corn in silo bags. Nevertheless, more information should be generated at laboratory and full scales.

Keywords: Propionic acid, Quaternary ammonium compound, Sulfur dioxide, Phosphine, Fungal colonies

Introduction

Molds have been designated as one of main causes of quality losses during grain storage (CAST, 1989). Fungal activity can cause rapid deterioration of grain, leading to dry matter loss, deterioration of nutritive value, seed viability reduction and production of mycotoxins. Storage molds are able to grow at relatively low water activities (a_w, 0.70-0.75) initiating grain spoilage (Magan and Lacey, 1988).

In the past few years, corn has become the most important crop in Argentina (50 Mt by 2019/20; Melo, 2021). Due to weather conditions in the fall, corn is usually harvested at 15-18% moisture content (m.c.) corresponding to a_w from 0.77 to 0.89, which is above the safe storage m.c. (<14%; a_w <0.7) and therefore requiring drying. However, grain drying is not always feasible at the farm level because most farms do not have grain dryers, and the price of fuel at the farms is too expensive. As a result, many farmers have to store wet corn in silo bags for 3-6 months and face a high risk of quality losses. The silo bag is a temporary storage system, is potentially hermetic, has an average holding capacity of 180-200 t of corn (2.74 m diameter, 60 m long, 235 µm polyethylene).

When wet grain is stored inside silo bags, the favourable modification of the atmosphere can assist in delaying the grain deterioration process. However, the airtightness of silo bags in the field is highly variable (depending on the sealing system implemented, perforations caused by animals, or misuse of equipment and tools around the bag) and normally decreases during storage (Cardoso et al., 2012). When stored in non-airtight silo bags, grain quality deteriorates after only a few months (Bartosik et al., 2008).

Several compounds had been proposed to reduce mold activity in corn and others grains, including organic acids (Chulze, 2010), phosphine (PH_3) (De Castro et al., 2001), and sulfur dioxide (SO_2) (Magan, 1992). There are also quaternary ammonium compounds (QAC) which are non-toxic products and have fungistatic or antifungal properties (normally used for disinfection of surfaces in the food industry) which were not previously evaluated in grain (Chauret, 2014).

Evaluation of fungicidal/fungistatic products that could potentially be used to protect wet grain stored in silo bags has not been widely studied. Liquids and gaseous candidate products must be applied under the particular conditions of storage in silo bags at the farm level. Liquid products can only be sprayed during the filling (or bagging) operation, usually at harvest time, while gaseous products can be applied during storage in silo bags with variable levels of airtightness. Thus, the objective of this study was to make a preliminary in-vitro comparative evaluation of four different fungicidal treatments in corn.

Materials and methods

The study was carried out at INTA Balcarce Research Station, Argentina. Four different products (two liquids and two gaseous) were evaluated: Test 1) Propionic acid (Lupro-Grain®, 3 L/t), Test 2) Quaternary ammonium compound/QAC (Virukill® = 3 L/t, diluted 1:25 with water), Test 3) Sulfur dioxide/SO_2 (initial dose: 15% v/v), Test 4) Phosphine/PH_3 (initial dose: 6.6 g/m^3), and their respective controls (non-treated).

A full-randomized design was proposed for each test. A factorial analysis was considered where each treatment was a combination of two factors: dose and storage time (Table 1).

The experimental unit was a glass jar with 1.8 kg of corn at 15% m.c., and three replicates were conducted for each treatment. In Tests 1 and 2 (liquids), half of the corn was sprayed with the product (and the grain mixed by hand), while the other half was used as a control. Both halves were divided into 4 portions of 1.8 kg each and stored in sterilized glass jars (non-hermetic). Test 3 consisted of different exposure times at 15% SO_2. The glass jar filled with corn was hermetically sealed and the SO_2 mixture (15%) was injected for 1 min through a rubber septum inserted in the metal lid of the jar (while purging was made through another needle inserted in the septum). The SO_2 concentration was measured with colorimetric tubes. In Test 4, the glass jars were placed inside a plastic container of 60 L capacity with two aluminum phosphide tablets (0.2 g phosphine each) and then closed with a partial sealing. Phosphine concentration was measured at different times employing colorimetric tubes inserted through a valve. All treatments were stored in a controlled temperature chamber (at 25 ± 1°C) during the exposure times indicated in Table 1.

Table 1. Dose and exposure time of different tests

Test number	Product	Dose	Exposure time
Test 1	Propionic acid	Non-treated	15 d
		3 L/t	
Test 2	Quaternary ammonium compound/QAC	Non-treated	15 d
		3 L/t (diluted 1:25)	
Test 3	Sulphur dioxide/SO_2	Non-treated	24 h
		15% v/v	5 min
			5 h
			24 h
Test 4	Phosphine/PH_3	Non-treated	15 d
		6.6 g/m^3	

After the prescribed exposure time, the jars were opened and the fungal biota (molds and yeasts) was evaluated using the method of counting in Petri dishes in potato dextrose agar (Britania®), with the addition of chloramphenicol (0.01% Anedra®). Plates were incubated in an oven at 28°C in darkness for 5 d (Pitt and Hocking, 2009). Counts were expressed as colony forming units per gram of dry matter (CFU/g DM).

Comparisons of treatments were performed with ANOVA, employing R software (version 3.6.3). Tukey's HSD (0.05) post hoc test was also used for mean comparison. Figures were created with Excel (Microsoft Office Professional Plus 2016).

Results and discussion

Table 2 shows that CFU counts in the non-treated samples had certain variability, from 3.42 log_{10}/g DM ($2.6x10^3$ CFU g/DM) for propionic acid to 4.12 log_{10}/g DM ($1.35x10^4$ CFU g/ DM) for SO_2. Despite the fact that the grain came from the same batch, this variability was expected because the tests were carried out at different times.

The four evaluated treatments had different results (Table 2). Test 1 with propionic acid completely eliminated the fungal load after 15 d (0 CFU/g DM). Test 2 with QAC only had a reduction of 1 log_{10} CFU/g DM in the fungal biota after 15 d of treatment ($p<0.05$). In Test 3 with SO_2 all of the exposure times resulted in a high reduction in the fungal biota ($p< 0.001$) of between 2-3 log_{10} CFU/g DM compared to the control. However, there were no significant differences among the various exposure times ($p>0.05$). The average final CFU count across all exposure times was 1.12 log_{10} CFU/g DM (13 CFU/g DM). In Test 4 with PH_3 there was a reduction of 1 log_{10} CFU/g DM, with the final count being 2.94 log_{10} CFU/g DM ($8.7x10^2$ CFU/g DM) ($p<0.05$), similar to that of Test 2.

Table 2. Average CFU counts (± SD) for different tests in wet corn. Different letters mean statistical differences within same product (p-value: 0.05)

Product	Dose	Exposure time	Log_{10} CFU/ g DM
Propionic Acid	Non-treated	15 d	3.42 ± 0.52 A
	3 L/t		0.00 ± 0.00 B
Quaternary ammonium compound/QAC	Non-treated	15 d	3.92 ± 0.62 a
	3 l/t (Diluted1:25)		2.94 ± 0.11 b
Sulfur dioxide/SO_2	Non-treated	24 h	4.12 ± 0.78 A
	15% v/v	5 min	1.80 ± 0.56 B
		5 h	1.08 ± 0.99 B
		24 h	0.50 ± 0.92 B
Phosphine/PH_3	Non-treated	15 d	3.93 ± 0.36 a
	6.6 g/m^3		2.94 ± 0.06 b

Gimeno and Martins (2011) proposed a threshold of $4x10^4$ CFU/g DM for a grain sample to be considered of good mycological quality. The CFU counts of all the control samples (non-treated) were below that threshold, indicating the grain used for this study was in good microbiological condition. Higher CFU counts (i.e., 10^5 CFU/g DM) would have indicated an early spoilage process (Magan, 1993).

Liquid products can only be applied during the bagging operation at the beginning of storage. In this sense, the desirable characteristics of the liquid products include an immediate reduction in fungal load and the persistence of this effect over time (residuality).

Propionic acid had the best performance of all the evaluated products as it completely eliminated the fungal load after 15 d. These results were in agreement with those reported by Fernandez Zambón (2018), who evaluated a dose of 4 L/t in wet corn (17% m.c.) stored for 6 months at pilot-scale (15 kg bags). He reported an immediate and significant reduction in CFU counts after the application, and that the CFU counts remained stable during storage (residual effect). Raeker et al. (1992) reported that lower doses than that evaluated in our study (0.5 and 1.0% (v/v)) allowed the preservation of wet corn (≥ 17.6% m.c.) for more than one year in laboratory tests. However, the application of low doses at real-scale represents a challenge in terms of achieving a proper distribution of the product throughout the entire grain mass (Chulze, 2010). As a negative effect of the use of propionic acid, a certain degree of grain discoloration was reported even in treatments with lower doses (Fernandez Zambón, 2018; Raeker et al., 1992), which could affect the commercial quality of the grain.
Quaternary ammonium compounds had certain fungicidal properties and had no negative effects on the grain (i.e., discoloration). However, a higher dose than what was explored in this study should be evaluated for achieving a suitable control. In addition, although this product showed a good residual effect on disinfected surfaces (Chauret, 2014), a more comprehensive study should be carried out for establishing its residual behavior in a grain matrix.

Gaseous treatments could be implemented at any point during storage, but they do not have residual effect. Additionally, a minimum airtightness is required to achieve the effective concentration-time in order to produce the expected fungicidal/fungistatic effect.

The theoretical concentration in the PH_3 treatment was 5000 ppm. However, after 120 h, the mean PH_3 concentration dropped to 3500 ppm, and to 800 ppm after 15 d, implying that the container was not hermetic. This PH_3 loss was about 270 ppm/day, equivalent to that achieved in a semi-hermetic system with a pressure decay test lower than 1 min (250-125 Pa) (Navarro and Zettler, 2000). This level of airtightness was similar to that reported by Carpaneto et al. (2016) in fumigated silo bags, implying that the results of the present study could be extrapolated to a real scale silo bag. The concentration-loss condition evaluated in the present study would reduce, but not eliminate, the fungal biota. A higher airtightness could yield higher fungicidal effect. De Castro et al. (2001) evaluated different PH_3 doses (0-4 g/m^3) and exposure times (1 to 15 d) in wet corn (0.85 and 0.98 a_w). They concluded that in the lower a_w condition, exposure time was more relevant than PH_3 concentration. Similarly, Lacey (1992) noted that PH_3 at 3.0-3.5 g/L over 90 d was fungicidal to 89% of storage fungi and fungistatic to another 7%. Lower PH_3 doses (i.e., 0.1 g/m^3) during longer exposure times could produce a slight decrease in fungal population or retard fungal growth (Hocking and Banks, 1993).

The evaluated dose of SO_2 during a short exposure time (1-24 h) produced a better result than PH_3 and QAC treatments. Magan and Aldred (2007) conducted a review about the fungicidal effect of SO_2, but their dosage recommendation was not perfectly elucidated. On the one hand they stated that 1.5% SO_2 (v/v) was required to reach long-term (5 m) grain conservation. However, studies suggested that much higher concentrations might be required because of the adsorption and binding of the SO_2 in the grain matrix. Furthermore, for wetter grain (0.92-0.96 a_w), 2 g/L (76% v/v) of SO_2 was suggested in order to reduce the fungal biota population from 1 to 2 log.

The shorter exposure time of SO_2 in comparison with the PH_3 treatment (1-24 h) implied that moderate/lower airtightness could be required and that a faster treatment could be made. However, at full-scale, a significantly higher volume of gas should be injected in the silo bag at the field, implying a greater operational complexity than the PH_3 treatment.

An additional challenge that must be considered for a full-scale application of SO_2 and propionic acid is their corrosive effect on metallic structures. This could disqualify the use of these products in metal bins, but not in silo bags since the polyethylene is not affected. Note, however, the application of propionic acid during the loading operation could also cause corrosion in the bagging machine. Based on the result of this study, propionic acid seems to be a good solution for improving the storability of wet corn in silo bags. Nevertheless, more information should be generated at laboratory and full-scale levels in order to make practical recommendations.

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Phosphine fumigation of stored turmeric in India

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Abstract

Among spices, turmeric (*Curcuma longa* L.) is significant for its role in culinary arts, cosmetics, medicine, and as a natural colouring agent (curcumin) for foodstuffs and fabrics. India is the major producer (0.93 million tonnes in 2020, global production 1.1 million tonnes), consumer (domestic consumption nearly 80%), and exporter (whole turmeric, turmeric powder and oil, oleoresins). Processed dried rhizomes of turmeric are stored as bulbs, fingers, and splits in gunny sacks in India. Around 20% is held by the farmers at their own premises, the balance stored by the entrepreneurs in private as well as government warehouses/market complexes and to a limited extent under cold storage. Storage period varies from a few months to five years.

Despite its claimed bio-pesticidal potency, during storage at farm and warehouse levels turmeric is still vulnerable to two major pests: the cigarette beetle, *Lasioderma serricorne* (F.) and the drug-store beetle, *Stegobium paniceum* (L.) along with threats from the red flour beetle, *Tribolium castaneum* (Herbst). Insect pest activity in stored turmeric affects its quality and marketability.

Fumigation with phosphine released from aluminum phosphide tablets and/or powder formulations (in sachets) is the common practice for the disinfestation of stored turmeric intended for the domestic market. Surveys in major production regions revealed that turmeric fumigations are carried out by farmers and unauthorized fumigators (routinely with improper phosphine dosages) and under poor quality gas-proof sheets and floor sealings. Due to these factors, control failures of repeated phosphine applications were noticed. In this context, awareness programs on Good Fumigation Practices in Stored Turmeric involving practical demonstrations were conducted by UPL Ltd., Mumbai, India, at selected centres in predominant turmeric storage areas for farmers as well as prominent turmeric traders. With supportive awareness programs being offered to farmers and other stakeholders, phosphine will continue to play a vital role in the protection of stored turmeric in India.

Keywords: Turmeric storage, India, Insect infestation, Phosphine treatment, Good fumigation practices in stored turmeric, Approaches and awareness programs

Introduction

Turmeric, *Curcuma longa*, is an herbaceous plant, belonging to the Zingiberaceae family. Turmeric crops take about 180-200 d to mature and need another 10-15 d for on-farm, post-harvest activities to make the rhizomes acceptable for consumption. As well as having medicinal applications as an antiseptic for skin abrasions and cuts, the rhizomes of the plant are used in a wide variety of foods of the cuisines of Southern Asia. Turmeric is consumed in powder form used in cooking, and as oleoresin in the medicinal industry (Table 1.). In India, turmeric is used whole or as a powder for its characteristic colour, and also as an ingredient for various spice mixes (Ragavan and Sujeetha, 2015).

Table 1. Turmeric and its value-added products

Category	Product
Whole turmeric	Bulb or rhizome
	Fingers
	Grits
Processed turmeric	Turmeric powder
	Ingredient in spice mixes
Extracts /Value added products	Curcumin
	Oleoresin

In 2019-20, the global production of turmeric was around 1.1 million tonnes. India dominates production by contributing 80% to the world supply, followed by China (8%) and Myanmar (4%). As well as being the largest producer and consumer of turmeric and its products in the world, India is also a leading exporter of this commodity (Table 2.). Major importers of India's turmeric supply include Bangladesh (15,889 tonnes), Iran (11,859 tonnes), Morocco (7,226 tonnes), USA (6,318 tonnes) and UAE (5,938 tonnes). There are other countries that import small quantities. Indian turmeric is considered to be the best in the world market because of its high curcumin content.

Table 2. Turmeric production and export from India (thousand tonnes)

Year	2017	2018	2019	2020
Production	952.97	862.77	972.97	926.91
Export-Quantity	116.50	107.30	133.60	136.00

The important turmeric growing states in India are Telangana (55,443 ha), Odisha (27,864 ha), Tamil Nadu (18,296 ha), West Bengal (17,711 ha), Karnataka (17,598 ha), Assam (16,550 ha), Maharashtra (14,511 ha), and Andhra Pradesh (13,223 ha) (PJTSAU 2020). The harvesting of

fresh turmeric in India starts from mid-January and ends in June. Stored turmeric is available for the market throughout the year. In light of the significance of this commodity, current turmeric storage and infestation control practices in India have been reviewed, and subsequently, the need and mode of good phosphine fumigation awareness programs for the farmer, trader and warehouse managers were elaborated.

Current storage practice

Harvested and cured turmeric rhizomes are stored at three stages: farmer, trader/*mandi*, and central storage (Fig. 1.). Turmeric is stored by farmers at their premises according to the requirement and as per the available space in their storerooms, and represents about 20% of total production. When there is inadequate space, farmers store the commodity in the Turmeric Market Complex/Private/Govt Market Committee warehouses, which accounts for the remaining 80% of the production. In certain areas (e.g., Nizamabad districts in Telangana) turmeric is stored by farmers and traders in cold storage units.

Fig. 1. Storage locations: farmer; trader/*mandi*; central storage.

Insect pest infestation

Turmeric, whether it be raw or processed/whole or ground, is vulnerable to insect pest attacks during storage. The quality, and hence the market value of stored turmeric, is decreased by insect infestation and microorganism infection (Gunasekaran et al., 2003). Besides consuming valuable product, these insect pests also contaminate the commodity with their excreta and body fragments, and disseminate microorganisms causing unacceptable levels of filth and mycotoxins. In India, stored turmeric is attacked predominantly by the Cigarette beetle, *Lasioderma serricorne* (F.) and the Drugstore beetle, *Stegobium paniceum* (L.). Turmeric fingers and bulbs are quite susceptible to *L. serricorne* (Jha and Yadav, 1991). Adult Cigarette beetles are active fliers and are short lived (2-6 wk) depending on the temperature and humidity. Both beetle species leave small round emergence holes in turmeric fingers/bulbs. The Red flour beetle, *Tribolium castaneum* (Herbst), the Coffee bean weevil, *Araecerus fasciculatus* (De Geer), the Lesser grain borer, *Rhyzopertha dominica* (F.), the Saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), the Almond moth, *Ephestia cautella* (Cautella), and the Rice moth, *Corcyra cephalonica* (Stainton) all occur as minor pests of stored turmeric and its products (Gunasekaran and Rajendran, 1999; Rajendran, 2003).

Current fumigation practice

Fumigation plays a vital role in the control of insect pests of stored turmeric. Phosphine released from metallic phosphide formulations is the only fumigant available for use on turmeric intended for the domestic market in India. Because of its high vapour pressure (29, 260 mm of Hg at 25°C), low molecular weight (34 g/mol), and gas density (1.17 kg/m^3), phosphine can distribute rapidly and evenly to act on all life stages of insect pests during fumigation. Furthermore, it is the least problematic fumigant in terms of residues in the treated commodity. At the central storage level, phosphine fumigation of commodities must be carried out by government storage agencies or accredited/approved commercial pest control agencies/operators as per NSPM-22 (Anon. 2017). Nevertheless, for the exclusive use of farmers, the Government of India has approved 10 g granular/powder formulation sachets or pouches containing 56% aluminum phosphide, as well as 12 g tablets in small flask packing containing 15% aluminum phosphide.

It has been observed that there is inappropriate selection of aluminum phosphide formulations which are not approved for use by farmers. These products are either approved for use by licensed operators only, or are counterfeit illegal packings sold to farmers through illegal channels.

The dosage applied is also on a per tonne basis, and the sealing integrity is not enforced either. Exposure periods vary from 15-30 d. The quality of the fumigation cover sheets is also not very good seeing as these sheets can rarely retain enough phosphine, thus leading to ineffective fumigation due to sub-lethal fumigant concentrations during the exposure period. Because of these failed attempts, fumigation has to be repeated every two months, despite which, the turmeric rhizomes are still damaged to a great extent.

After our interaction with turmeric growers and our subsequent assessment of storage locations, UPL Ltd. decided to organize training programs in the Erode District where farmers were suffering huge losses, not to mention being at high risk of phosphine exposure themselves.

Training/awareness programs

As one of the leading global manufacturers of aluminum phosphide formulations, UPL Ltd. felt/realized the need to organize training/awareness programs on Good Fumigation Practices in Stored Turmeric for farmers, traders and warehousing agencies. Accordingly, in the first instance, Good Fumigation Practices in Turmeric Storage was conducted at the selected centers viz., Turmeric Market Complex, Semmapalyam, near Erode Otthakadai, and Kodumudi in Tamil Nadu, India.

Fig. 2. Training activities, lectures and live demonstrations.

The objectives of the training/awareness programs were to:

1. Create awareness on safe usage of aluminum phosphide formulations for turmeric fumigation.
2. Display/demonstrate good fumigation practices to farmers for effective insect control.
3. Differentiate between genuine and spurious phosphine releasing aluminum phosphide products available in the market.

The training was imparted to about 1500 farmers and turmeric traders. The program was comprised of lectures followed by live demonstrations of the fumigation of turmeric bags with aluminum phosphide tablets and sachets (Fig. 2.). Farmers were made aware of approved aluminum phosphide formulations and brands in India which could be legally purchased for use by farmers. Certificates were distributed to the farmers/traders who participated in the training program. During these training sessions, there was open interaction with farmers and all queries were addressed for more clarity with regard to aluminum phosphide. Feedback was taken from the farmers regarding the relevance of the training and content, as well as any benefits they felt they had received from the sessions.

Way forward

Farmers and traders benefitted from the training on Good Fumigation Practices in Stored Turmeric and it created awareness on the safe and effective usage of approved aluminum phosphide formulations in turmeric storage. Learning these safe practices resulted in the wiser and more informed use of aluminum phosphide. Similar training sessions have been planned in other turmeric growing areas in the future.

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Research and application progress of controlling stored-grain insects by sulfuryl fluoride

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ABSTRACT

The paper highlighted the problems of insect resistance and shortage of aluminum phosphide for controlling stored-grain insects. Sulfuryl fluoride was identified as an alternative fumigant and its physical and chemical properties were analyzed. Also, insecticidal mechanism, control effect, residue and degradation, pesticide registration, fumigation technique, concentration detector, security, environmental protection, and mixture fumigation were discussed in the paper. Sulfuryl fluoride might become a common fumigant after elimination of methyl bromide, though there are some problems with its application, which was discussed in the paper.

Keywords: Sulfuryl fluoride, Fumigation, Stored-product insects

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Yujie Lu Y, Zhang C, Wang Z, Yan X, Emery RN (2021) Rapid detection of phosphine resistance in *Rhyzopertha dominica* (Coleoptera: Bostrichidae) from China using tetra-primer ARMS-PCR. Page 16. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Rapid detection of phosphine resistance in *Rhyzopertha dominica* (Coleoptera: Bostrichidae) from China using tetra-primer ARMS-PCR

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ABSTRACT

Strong resistance against the fumigant phosphine (PH_3) is becoming a serious problem in the control of *Rhyzopertha dominica* (F.) in grain storage system worldwide. The main molecular mechanism of strong resistance is caused by point genetic variants in the gene (*rph2*) encoding dihydrolipoamide dehydrogenase (DLD). The strong resistance population of R. dominica have been diagnosed mainly by the discrimination dose of phosphine (FAO recommended), which is always time consuming, inefficient and laborious.

To establish a suitable method for rapid identification of the P49S mutation in *R. dominica*, an efficient and simple molecular method was developed based on tetra-primer ARMS PCR. The method was involving in a set of four designed and optimized primers for distinguishing specially the different genotypes within one step. All the process of resistance detection only took 2 h. Using this method, 10 strong phosphine resistant populations of *R. dominica* field collected in China, were identified based on P49S mutation frequency of the populations.

This novel method was proven to be useful as an early warning system for resistance outbreaks of *R. dominica* to phosphine collected from field. This study highlighted the utility in accurate and rapid determination of strong phosphine resistant population of *R. dominica* in China for the first time. Application this method over larger scope would help in identifying rapidly resistance problems and making right pest management decisions.

Keywords: Phosphine, Resistance, *Rhyzopertha dominica*, Tetra-primer ARMS-PCR, Dihydrolipoamide dehydrogenase (DLD)

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Study on the control of DON-producing fusarium by fumigation in wheat

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ABSTRACT

Wheat is one of the three major food species in the world and it is also one of the most important food crops in China. Wheat suffers from many diseases during the production process, among which fusarium head blight (FHB) is one of the important diseases that plague the sustainable development of wheat production in China. With global warming and the changes in farming systems and methods, FHB is spreading. At present, FHB has become one of the important diseases worldwide. *Fusarium* is one of the dominant pathogens of FHB. The contamination of these pathogens will not only cause serious losses in the production, but also produce mycotoxins, such as DON and ZEN, which seriously affect the quality and safety of stored wheat and pose a potential threat to human and animal health. Therefore, the research on the *Fusarium* strains and its toxin production mechanism is of great significance for the prevention and control of FHB. In this study, we selected typical FHB wheat samples, and the main DON-producing *Fusarium* on the surface and inside of the head blighted grains was separated and purified. All isolates have been studied for their toxin production ability to verify their pathogenicity. Through ITS sequence analysis and phylogenetic analysis, the phylogenetic types were clarified, and the toxin-producing strains were genetically characterized. Then, the separated toxin-producing fungi were fumigated using ozone and chlorine dioxide gas. The results showed that the two different kinds of fumigants continuously inhibited *Fusarium*, and the ozone also showed a significant degradation effect on DON, which had a significant inhibitory effect on the production of DON by *Fusarium*.

Keywords: Grain storage, Fusarium head blight, Deoxynivalenol, *Fusarium* spp., Fumigation

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Exposure time prediction of N_2 treatment based on behavior and mortality of *Sitophilus oryzae* (L.) adults monitored by camera at different temperatures

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Abstract

The effect of controlled atmosphere (CA) on insects is usually evaluated by sampling and further incubating. This sampling and incubating method cannot monitor the control effect before the control is completed. Behaviour of insects in cages can be monitored by using video camera equipment. The knockdown time of *Sitophilus oryzae* (Linnaeus) adults monitored by a video camera located in an airtight chamber filled with 98% nitrogen was determined at 18, 23, and 28℃. The relationship between the exposure time, the knockdown percentage, and mortality was analyzed. It was 18, 16 and 10 d from the time of 50% knockdown to the time of 100% mortality at 18, 23, and 28℃, respectively. The equations describing the knockdown percentage (x) and the time difference from the knockdown to 100% mortality of adults (y) were $Y = -11.872x + 23.369$ ($R^2 = 0.9736$), $Y = -7.8218x + 18.678$（$R^2 = 0.856$), and $Y = -5.1382x + 12.402$ ($R^2 = 0.9659$) at 18, 23, and 28℃, respectively. The most tolerant life stage of *S. oryzae* was that of the pupa. The population extinction time could be predicted by using the developed regression equations associated with the most tolerant life stage under the tested conditions.

Keywords: High concentration nitrogen, *Sitophilus oryzae*, Knockdown percentage, Exposure time prediction, Controlled atmosphere storage

Introduction

The application of high concentration nitrogen to control stored grain insect pests has been used in many countries (Annis, 1986; Navarro, 2006; Carvalho, 2012; Cai, 2019; Moncini et al., 2020; Sule and Emekci, 2020; Lorenzo et al., 2020). Commercially available equipment called the ‘‘pressure-swing adsorption’’ system (PSA) uses the process of O_2 adsorption from compressed air passed through a molecular sieve bed. The system provides about 120 m^3/h of 98% N_2 (Navarro, 2012) and has been used for more than 15 million tonnes of stored grain in China (Yang et al.,

2016). The effect of this controlled atmosphere (CA) treatment is typically verified by inspecting the insect survival rate after this treatment process has been completed. Another method used to verify the killing effect of CA treatment in the field studies is to observe insect behaviour in cages or enclosures (Meenatchi et al., 2016). If some individuals survive in the enclosures, the CA treatment is considered a failure. The knockdown and death of insects in the CA enclosures are influenced by many factors including temperature, relative humidity, insect species, and life stages (Soderstrom et al., 1986; Donahaye,1996; Ofuya and Reichmuth, 2002; Gunasekaran and Rajendran, 2006; Hashem, 2012, Huang et al., 2020a). Temperature is the key factor, and low temperatures can extend the insect survival time (Huang et al., 2020a, b). Therefore, monitoring the insect behaviour continually in the enclosures is required due to the temperature fluctuations in storage structures. A convenient method is to use a high-resolution video camera to remotely monitor these small individual insects in the enclosures (Gavankar, 2013). In this study, the behaviour of *Sitophilus oryzae* at 18, 23, and 28°C and in 98% N_2 was monitored by using such a camera. The knockdown percentage of the adults was calculated. The relationships between the knockdown percentage and population extinction time were analyzed. This relationship was used to predict the exposure time needed in the field treatment.

Materials and methods

Insects and treatments

The *S. oryzae* was originally collected from a grain depot located in Nanning, Guangxi Zhuangzu Municipality, China and reared for five years at 28±1°C and 75±5% r.h. on wheat. More than 1,000 adults of mixed sex were transferred to a 1 L jar containing 800 g of wheat kernels for egg laying purposes. The adults were sieved out after 24 h. The wheat and eggs were kept at 28±1°C and 75±5% r.h. After another 24 h, the wheat kernels with the eggs were further divided into 5 g samples. This procedure was repeated for preparation of adults, pupae, and larvae after 42, 22, and 12 d, respectively. These time periods were calculated from the known *S. oryzae* development time at the rearing condition (Huang et al., 2020b).

Gas chamber and high N_2 management

The concentration of nitrogen with air mixture was maintained in a rectangular gas chamber with dimensions of 60×35×40 cm^3 (Fig. 1). The gas chamber was made of 2 mm armor plate, except for the top side, which was made of 2 mm transparent plexiglass. Saturated sodium chloride solution kept in a 250 mL beaker was used to maintain the 75±5% r.h. in the gas chamber. To keep the airtightness of the gas chamber, airproof sealing was used at the bolt and connection locations. The halftime of pressure drop of the chamber at the initial pressure of 500 Pa was 180 s. A cylinder containing compressed liquid of 99.999% N_2 was connected to the chamber by a rubber tube of 6 mm inner diameter (Fig. 1). The outlet from the gas chamber was connected to a recirculating pump (DC24V, Hailin Technology Co., Ltd., Chengdu, China) and an N_2 detector (MOT500—LM(N_2), Kenuoer Co., Shenzhen, P. R. China) using a rubber tube (Fig. 1). At the beginning of the operation, the valve was opened until the N_2 concentration inside the chamber reached 98%. The N_2 concentration in the chamber was then measured by the N_2 detector after the valve was closed. The N_2 detector can measure nitrogen concentrations in the range of 0 to 100%

with a 0.01% resolution. After the N_2 concentration inside the gas chamber was higher than 98%, the valve was closed. When the concentration of N_2 in chamber reached above 98%, the concentration of O_2 was 1.88 to 1.98% as measured by an O_2 detector (MOT500-O_2, Kenuoer Co., Shenzhen, China) capable of measuring O_2 concentrations from 0 to 100% with a 0.01% resolution (Fig. 1).

For video recording, the prepared 50 adults of mixed sex and the 2 g of wheat kernels were introduced into a 60 mm diameter petri dish. The petri dish was covered with nylon mesh (80 mesh) that was hot melted onto the petri dish rim. The petri dishes were installed at the top of the gas chamber and supported by a steel frame (Fig. 1). The nylon mesh was at the bottom so that the adult behaviour could be captured by the camera (HD1800, Sanhao Instrument Equipment Co., Ltd, Shenzhen, China) through the transparent plexiglass and transparent bottom of the petri dish.

Before the N_2 was introduced into the chamber, the petri dishes and insect cages holding 5 g wheat kernels with the adults (and hidden immature stages of insects) were located inside the chamber (Fig. 1). The cages were used to hold insects and grain, so that insects with different exposure times could be moved out of the gas chamber at different times. Each cage was 10 mm in diameter and 70 mm in length. There was one hole on one vertical side of the gas chamber that was sealed with a rubber glove. There was an N_2 gas cylinder on another vertical side that was sealed by two screw caps (80 mm in diameter and 200 mm in length). This hole, the airtight glove, and the cylinder were all used in assisting to remove the insect cages out of the gas chamber during the CA treatment without losing the airtightness of the chamber (Fig. 1). When a cage was removed out of the chamber, the cage was held by using the rubber glove and placed inside the cage removal cylinder. The cylinder was then sealed by turning the internal screw cap A. The cage was moved out of the cylinder once the outer screw cap B was removed. Insect cages were taken out of the CA chamber every 2 d during the 3 to 30 d period of the experiment.

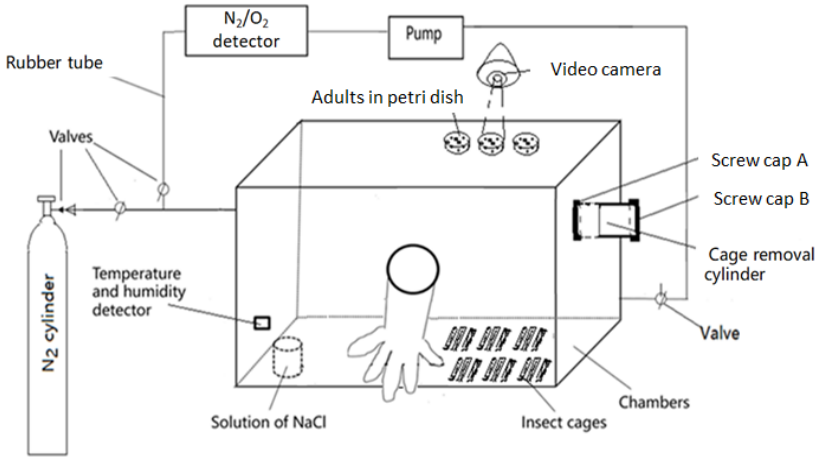

Fig. 1. Diagram of gas chamber and material arrangement

There were three replications for each observation. The control was conducted under the same conditions, except the air was used instead (Fig. 1). The gas chamber was kept inside the lab which was controlled by an air conditioning system at 18, 23, and 28℃ with ±1℃, respectively.

Insect behaviour monitoring and mortality evaluation

The recorded video was replayed on a computer and the knockdown time of each individual was counted. When an adult had no movement of body, antennae or legs, the adult was counted as a knockdown adult. The percentage of knockdown at different treatment times was calculated as: knockdown percentage = 100 × number of knockdown insects / total treated insects. The adult mortality in each cage was counted after 14 d. The mortality of the hidden immature stages of insects was evaluated by counting emerged adults over three successive days. After that, the survival of pupae, larvae and adults in wheat kernels was checked by cutting the kernels. All the tested insects and wheat kernels were kept at 30±1℃ and 75±5% r.h. after they were moved out of the chamber.

Statistical analysis

The time difference between 100% knockdown and 100% mortality was calculated for each stage and at each temperature. The percentages of knockdown and mortality at different times and different temperatures were compared by conducting Tukey's test. Linear regression between the percentage and time at each temperature was conducted. All data analysis was conducted by using IBM SPSS Statistics 20.

Results

Population extinction time of S. oryzae

At any treatment temperature, exposure time of 100% mortality from long to short was pupa > egg > larva > adult (Table 1.). The time of 100% mortality of pupa can be used as the population extinction exposure time. Compared with adults and at 23℃, the time of 100% mortality of pupa, egg, and larva was delayed for 8, 6, and 4 d, respectively. The time of 100% mortality of any stage of the *S. oryzae* increased with the decreasing temperature.

Table 1. Exposure time (d) of 100% mortality of *S. oryzae* in 98% N_2 at different temperatures

Temperature (℃)	Adult	Larva	Egg	Pupa
18	16.67±1.15a	19.00±1.73a	21.00±0.00a	25.00±1.63a
23	11.00±0.00b	15.33±0.81b	17.00±1.00b	19.00±1.00b
28	7.33±0.57c	11.00±1.00c	11.33±1.52c	12.67±0.57c

The different letters (a, b, c) in the same column are significantly different at $\alpha = 0.05$ level using Tukey's test.

Knockdown time and 100% mortality time

The knockdown time (TK) increased from 2.9 to 14.7 d at 18℃, from 2.1 to 9.9 d at 23℃, and from 1.5 to 6.3 d at 28℃, respectively, as the knockdown percentage increased from 10 to 100%. Therefore, knockdown percentage increased with the increase of the treatment time. The 100% knockdown time at 18℃ was more than double of that at 28℃ (Table 2.).

Table 2. Knockdown time (d) of *S. oryzae* adults in 98% N_2 at different temperatures

Knockdown percentage (%)	18℃	23℃	28℃
10	2.89±0.59aA	2.13±0.12bA	1.45±0.18cA
20	3.96±1.25aB	2.47±0.08bB	1.82±0.50cB
30	5.44±0.60aC	2.78±0.10bC	2.10±0.10cC
40	6.98±0.42aD	3.03±0.61bD	2.51±0.11cD
50	7.49±0.83aE	3.49±0.11bE	2.88±0.25cE
60	8.40±0.46aF	4.03±0.21bF	3.45±0.34cF
70	9.07±1.08aG	4.46±0.21bG	3.99±0.08cG
80	10.71±0.36aH	6.08±1.15bH	4.40±0.11cH
90	11.93±0.23aI	7.87±0.65bI	5.35±0.57cI
100	14.73±1.22aJ	9.90±0.61bJ	6.29±1.65cJ

The different letters (a, b, c) in the same row are significantly different. The different letters (A, B, C) in the same column are significantly different. All values are averages ± SE at α = 0.05 level using Tukey's test.

The time difference between knockdown time and 100% mortality decreased with the increase of temperature (Fig. 2). Even after the adults were in complete knockdown, it still required at least 10.3, 9.1, and 6.7 d to reach 100% mortality of the remaining population at 18, 23 and 28℃, respectively. If a knockdown percentage (x) was given, the time difference between TK and 100% mortality (Y) could be calculated as:

$$Y = -11.872x + 23.369,\ R^2 = 0.97,\ 18℃$$
$$Y = -7.8218x + 18.678,\ R^2 = 0.86,\ 23℃$$
$$Y = -5.1382x + 12.402,\ R^2 = 0.97,\ 28℃$$

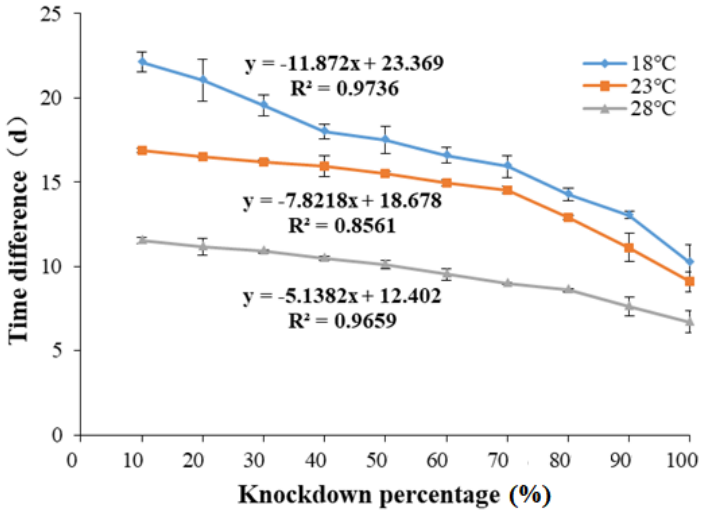

Fig. 2. Time difference between knockdown time and 100% mortality of *S. oryzae* pupae in 98% N_2 at different temperatures.

Conclusions

Temperature had significant influence on different stages of *S. oryzae* when they were treated in 98% N_2 mixed with air, not only for their knockdown time, but also for exposure time of 100% mortality or population extinction exposure time.

At any treatment temperature, exposure time of 100% mortality from long to short was pupa > egg > larva > adult. Even after the adults were in complete knockdown, it still required at least 10.3, 9.1, and 6.7 d to reach 100% mortality of the remaining population at 18, 23 and 28°C, respectively.

It is difficult to check the survival of immature life stages because they hide inside grain kernels. Knowing the response of adults during CA treatment by using a video camera and predicting the population extinction exposure times are important for the control of hidden insects. The equation between knockdown percentage (x) of adults and the time difference between the knockdown and 100% mortality (Y) were:

$Y = -11.872x + 23.369$ ($R^2 = 0.9736$) at 18°C
$Y = -7.8218x + 18.678$ ($R^2 = 0.8561$) at 23°C
$Y = -5.1382x + 12.402$ ($R^2 = 0.9659$) at 28°C

To predict the exposure time required for killing all of the stages, especially for the most tolerant stage of the pupa, using video recordings to monitor the adults' behaviour will be a useful tool to successfully conduct the control atmosphere storage.

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Phosphine fumigation: quo vadis?

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Abstract

Effective fumigation is required to control all life stages of stored product pests, achieve "pesticide-free" status, and avoid insect resistance. The current preferred global fumigant, phosphine (PH_3), initially patented in the 1930's as a solid metallic (aluminum) phosphide formulation, has evolved over the decades. The slow-release (2^+ d) solid formulations dominate the global fumigation market which has increasing contributions from solid quick release formulations and PH_3 gas / PH_3 mixtures in high-pressure industrial gas cylinders. Developments of PH_3 gas in cylinders include on-site mixing of 99% PH_3 with carbon dioxide or atmospheric air.

An advantage of the solid formulations is lower cost. Disadvantages of the solid formulations include inability to control/maintain optimum PH_3 concentration; operator safety with PH_3 exposure on handling; disposal of unreacted residues; flammability issues; and longer exposure times (2^+ d to generate and extended time to achieve uniform distribution). Advantages of gaseous PH_3 include rapid uniform gas distribution; reduced exposure times; ability to maintain/control optimum concentration; avoid operator exposure; and effective fumigation of non-gastight storage using flow-through PH_3 fumigation. A disadvantage of the gaseous PH_3 is higher cost.

The proven way to prevent resistance is to use PH_3 correctly in a gas-tight sealed storage by achieving the minimum Concentration x time (Ct) product to ensure effective fumigation. The majority of global grain storages are not "gastight". The Australian Standard, AS2628 (2010), states that sealable storage must perform a 5-min, half-life pressure test. Modern "bunker" storage can be sealed for fumigation. Specialist bulk grain storage sealing companies can achieve gastight status in many storages. Most "non-sealed" storage can be partially sealed and adapted to flow-through fumigation which uses low PH_3 levels (~150 ppm) with extended exposure times (3 wk). Flow-through PH_3 fumigation has been used in Australia for over 25 yr with tall vertical silos having the lowest treatment cost (~3 g/tonne).

Keywords: Phosphine fumigation, Aluminum phosphide fumigant, Cylindered phosphine fumigant, Sealed storages for fumigation, Flow-through fumigation, Pest control

Introduction

After 85 yr usage as a fumigant, PH_3 is the dominant global fumigant because of its low cost, efficacy, and environmental acceptance. Methyl bromide (MBr), first reported by Le Goupil (1932), is being phased out because of its ozone depleting effect. Actually, PH_3 replaced MBr long before its regulation by parties of the Montreal Protocol, wherever temperature and time were not constraints for PH_3 fumigation (Ducom, 2006). Because of its common use in global food production, the cereal grain industry is determined to maintain PH_3 as the priority grain fumigant. The major threat to the on-going use of PH_3 is the outbreaks of strong insect resistance. Fumigation needs to control insects, prevent food losses, and satisfy marketing requirements. Australia exports about 80% of the grains it produces, and in order to maintain market access, the grain must be free of live insects and pesticide residues. Insect control was initially achieved using grain protectants, but insect resistance and the requirement for insect and pesticide-free product led to increased use of phosphine (PH_3) fumigation. The solid aluminum phosphide (AlP) formulation developed in Germany (Freyberg, 1935) generates PH_3 gas on exposure to moisture in the atmosphere. This usage is relatively safe because the flammable PH_3 is released slowly over days by diffusion and dilution in the surrounding air. Phosphine is widely registered for disinfestation and is the only MBr alternative extensively used for cereals, legumes, dried fruits, nuts, beverages, herbs and spices. Widespread practical use began in the mid-1950s when alternatives were required for reasons of safety and portability to ethylene dibromide, ethylene dichloride, and carbon tetrachloride in the tropics, and methyl bromide in temperate areas (Munro, 1969). Phosphine is a naturally occurring gas, albeit short lived because it reacts with the atmosphere forming phosphoric acid (Fluck, 1973a), an acid used extensively as a food additive. In the mid-1980s, gaseous PH_3 was made available as a fumigant for agriculture in non-flammable gas mixtures in carbon dioxide (CO_2) and nitrogen (N_2). Over the past 20 years, new technology has enabled pure PH_3 to be supplied as a liquid under pressure in gas cylinders and mixed on-site with atmospheric air.

Flammability

Most historical and current fumigants are flammable including MBr (flammability ranges 10 to 16%); however, there is no reported fire incident associated with MBr while dust explosions in grain storage are well documented. All PH_3 products are manufactured from white phosphorus P_4 which is pyrophoric (any unreacted P_4 is a flammability hazard). The flammability issue of the pure PH_3 gas is resolved by formulating a non-flammable mixture in 2.6% CO_2, 2.0% N_2, or by rapid on-site dilution in atmospheric air to less than 1.8% flammability limit, prior to dispensing into the storage structure being fumigated.

Non flammability of PH_3 is important especially in the fumigation of large structures. It is critical to ensure that the PH_3 level being dosed into the storages is below the lower level of flammability to avoid fire or explosion. Explosions that can destroy the structure are relatively common in grain storage as there is always a “source of ignition” and high levels of dust which can lead to dust explosions. The reasoning behind the slow release of “solid” PH_3 formulations is to allow the flammable PH_3 to dissipate and dilute quickly into the surrounding air.

Phosphine products

The “solid” formulation refers to the solid tablets and plates which mainly consist of aluminum phosphide (e.g., AlP tablets), while the “liquid” is in high pressure industrial gas cylinders. The gaseous PH_3 is similar to gaseous CO_2 as both gases liquefy when compressed to high pressure (4MPa). The solid aluminum phosphide formulation developed in Germany and patented in the USA (Freyberg, 1935; 1938) generates PH_3 gas on exposure to moisture in the atmosphere. This usage is relatively safe because the flammable PH_3 is released slowly over days by diffusion and dilution in the surrounding air. The major advantages of the solid formulations are high portability, safety in use, low cost, and versatility of application under a variety of conditions. Negative issues include unreacted powder residues, disposal costs, and long exposure times.

The use of “liquid” PH_3 is made commercially available with the patented non-flammable PH_3 and CO_2 mixture (Ryan and Latif, 1989). This progresses to the onsite mixing patent of PH_3 and air (Ryan and Shore, 2005). The advantage of these gaseous PH_3 products is reduced exposure time as uniform distribution of the PH_3 is achieved in hours, not days. Other advantages of the gaseous PH_3 products include accurate control of PH_3 concentration, a more rapid delivery of PH_3 gas, better distribution in the grain mass without disturbing grain, and a controlled flow and dosage maintenance for long periods. Liquid PH_3 eliminates handling and disposal of the "spent" metallic phosphide tablets and requires less labour. The reaction of PH_3 with oxygen to form polymers is an issue which requires pre- and post- purging of PH_3 dispensing systems. Phosphine gas needs to be free of trace levels of diphosphine (P_2H_4) and higher phosphines to avoid being spontaneously flammable (Fluck, 1973b). The fumigation grade PH_3 has critical specifications for impurities such as P_2H_4 and P_4 which are pyrophoric (Ryan, 1997). While gaseous PH_3 has a longer history as a dopant in electronic silicon chip technology manufacture, it was initially investigated as a fumigant for the control of the fruit fly in 1976 (Ryan, 1997). A series of regular updates of the history of the commercial gaseous PH_3 products launched in the early 1980s have been published (Ryan, 1997). Phosphine has a wide flammable range in air, so various mixtures with CO_2 and/or N_2 have been patented to overcome this problem (Ryan, 1997; Ryan and Latif, 1989).

Significant timelines include: the original patented solid formulation (Detia, 1935); initial gaseous PH_3 (CIG, 1976); liquid PH_3/CO_2 mix (CIG, 1984); PH_3/CO_2 supported flow-through fumigation (CSIRO, 1986); PH_3/CO_2 (BOC, 1989); PH_3/N_2 (S&A, 1998); PH_3/Air mixing (GasApps, 1999); Solvay (CYTEC, 1999); PH_3/Air (Horn, 2001); PH_3/Air mixing (Solvay, 2005); UltraPhos 99% PH_3 (Specialty Gases, 2014).

Fumigation storage

Effective fumigations should be carried out in validated gastight storage. The Australian Standard, AS 2628 (2010), details the use of a decaying pressure test ($P_{0.5}$ > 5 min from 25 mm to 12 mm using a U-tube liquid manometer). Most grain storages fail this test; however, all can be fumigated using PH_3 flow-through fumigation. The liquid PH_3 formulations supported CSIRO’s flow-through fumigation process, SIROFLO (24/7 flow and 28 d exposure), used in non-gastight grain storages (Winks, 1987). The flow-through fumigation enables the fumigation of grain in “leaky” (non-gastight) storage; achieves pesticide residue-free and insect-free status for grain in “leaky” storage; makes old silos useful storage facilities; overcomes air ingress via small positive pressure and prevents fumigation failure; improves efficacy by achieving Ct product; uses low

concentrations for long exposure periods; increases workers' safety by using low PH_3 levels and constant low emissions levels. Any released PH_3 is short lived because it reacts with the atmosphere forming phosphoric acid. As flow-through fumigation equipment is used at unmanned rural sites, robust reliable design is required for rural "road" transport. The SIROFLO flow-through fumigation maintains a small positive pressure throughout the grain mass to ensure a uniform low concentration of PH_3 and can control PH_3 resistant insect strains in non-gastight storage (Winks and Ryan, 1990). The low PH_3 concentration (~100 ppm) if maintained up to 28 d will kill all stages of insects in non-gastight storages (these can be effectively "sealed" in critical areas).

Future

To predict the future, it is useful to review the past history of fumigants. The two significant issues with PH_3 are ineffective fumigation and insect tolerance / resistance. The expression "If you are not measuring, you are not fumigating" has been quoted for decades. This is still an issue and the major cause is non-gastight storage and/or failure to top-up the PH_3 concentrations. Effective fumigations should be carried out in validated gastight storage (AS 2628, 2010) using a decaying pressure test ($P_{0.5}$ > 5 min from 25mm to 12mm using a U-tube liquid manometer). Grain storages that fail this test should be candidates for PH_3 flow-through fumigation. The flow-through fumigation enables the fumigation of grain in "leaky" (non-gastight) storage and can achieve the required Ct product by maintaining low concentrations for long exposure periods.

Another major threat to the on-going use of PH_3 is outbreaks of strong insect resistance. Insect resistance to PH_3 fumigation is a critical issue for planning the future of this valued fumigant. Resistance issues detailed in a review by Ryan and DeLima (2014) include reported PH_3 resistance occurring in every insect species tested; variation in susceptibility of different life stages; improved efficacy by extending exposure periods; induced narcosis at high concentrations; widespread problems in most commercial storages; associated resistance with inadequate fumigation; and critical attainment of Ct product. The review also noted three levels of resistance ('weak' and strong' and 'very strong'). The development of very strong resistance (875x) in flat grain beetles *Cryptolestes ferrugineus* (Stephens) in large bulk storages in Australia poses a serious threat, however, an effective management of *this* strain through the use of sulfuryl fluoride as an alternative fumigant has been implemented. Also, PH_3 tolerant insects have been controlled using flow-through fumigation by extending the exposure period.

Of course, the last resort is to identify alternatives. The application of ethyl phosphine (Chaudhry et al., 1997) has the potential to counter PH_3 resistance in insects. Other alternatives may have potential in particular situations, but phosphine remains the most effective treatment at present. Among the alternatives, a broad-spectrum fumigant known as sulfuryl fluoride (SF) is the most promising. Although sulfuryl fluoride is being used in the effective management of strong resistant *C. ferrugineus* populations in bulk storage, SF does have an issue with efficacy against the egg stage of storage pests, particularly at lower temperatures. Carbon dioxide (CO_2) is well accepted as a treatment for organic grain and has excellent potential for rapid disinfestation at high pressure, but there are high costs associated with the construction and operation of high-pressure chambers. Carbonyl sulphide (COS) has not been commercialized although it has generally good efficacy, and ethyl formate (EF) can be effective against a range of insects when combined with CO_2. Hydrogen cyanide (HCN) has been used in a limited way on grain despite its high sorption, and

ethanedinitrile (C_2N_2) is a new broad-spectrum fumigant. Modified atmospheres involving elevated CO_2 or low O_2 have shown excellent effects, but issues of cost effectiveness and the need for long exposure times may be significant (Nayak et al., 2010).

Discussion

There have been significant changes from the high PH_3 doses of 10,000 ppm (14 g/m^3) used in the 1950s (Annis, 2001) to the current recommendations of 1-3 g PH_3/m^3 (718 - 2,154 ppm) or as low as ~100 ppm (0.14 g/m^3) in a continuous flow system (Anonymous, 1992). The critical requirement of a successful fumigation is to provide an adequate concentration (C) for a sufficient period of time (t). With most fumigants the Ct product is a constant (Miller et al., 2000), but the response of insects to PH_3 is far more effective if the exposure time is lengthened because PH_3 is a slow acting poison. High concentrations do not increase toxicity unless the exposure time is also increased (Bond et al., 1969; Howe, 1974; Hole et al., 1976; Winks, 1986; Winks and Hyne, 1994). Issues of PH_3 specific insect toxicity thresholds and of narcosis induced in insects at very high doses of PH_3 and the potential for inducing resistance in technically unfounded low dosages have been reported (Nakakita et al., 1974; Reichmuth, 1994; Winks, 1984, 1987). A unique characteristic of PH_3 is that in the absence of oxygen it is not absorbed and is therefore not toxic to insects (Bond et al., 1967, 1969; Cherfuka et al., 1976). Kashi and Bond (1975) showed that in the presence of 4% CO_2 there was a 20% increase in the uptake of oxygen and a 3-fold increase in the toxicity of PH_3 to insects. The action of phosphine is potentiated by carbon dioxide and the concentration and exposure time can be reduced when both CO_2 and O_2 are present. The optimum CO_2 concentration is in the range of 5-35%. At 5% CO_2, the PH_3 dose for LC_{90} efficacy can be reduced by ~50% (Kashi and Bond, 1975; Bond and Buckland, 1978).

In summary, the critical requirement of a successful fumigation is to provide an adequate Ct product. This needs a gastight storage or flow-through fumigation, especially in order to maintain the concentration, but the response of insects to PH_3 is also far more effective if the exposure time is lengthened. As a case history, in 1960, the Australian Government reacted to a crisis / customer revolt demanding a change to the "relaxed" attitude of regular shipments of infested grain being exported from Australia. The Export (Grain) Regulations at that time, prohibited the export of grain from Australia unless it was found to be free from insect pests. These days, the Australian $7 billion grain export revenue industry has ongoing independent government overview focusing on nil-insect and Maximum Residue Levels (MRL) requirements. In many global jurisdictions, changes are needed to improve the quality of fumigation, but this is unlikely to happen unless customers revolt or governments propagate and enforce regulations. The solutions are known: however, there is not a "burning platform" to initiate global change. The future of PH_3 as a fumigant may have a different outcome in individual countries. Reflecting on the long list of former insecticides, now discontinued due to insect pesticide resistance issues, should highlight the potential stark outcome.

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Potential of dosimeter tubes for monitoring phosphine fumigations

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ABSTRACT

Monitoring fumigation events is important for validating that a fumigation is adequate for controlling stored product insects. A variety of gas monitoring instruments are available. Some methods provide hourly data of the fumigation. Dosimeter tubes provide a single measurement representing the total dosage for the entire fumigation event. Dosimeter tubes are relatively inexpensive and easy to use. The total dosage is given as units of the measurement in ppm×h and represents the phosphine concentration × time. Two models of commercially available dosimeter tubes were evaluated (high range and low range). The high-range model, LPG-1, had a maximum range of 200,000 ppm×h. The low range model, LPG-2, had a maximum range of 20,000 ppm×h.

The dosimeter tube measurements were compared to a system that collected hourly phosphine concentration data using Wi-Fi sensors. The basic experimental factors included holding times of 24, 48, 96 h, and phosphine concentrations of 0, ~100, ~200, ~700, ~1400 ppm, and three replicates. The trials were conducted in sealed barrels. Wi-Fi phosphine sensors were placed in each barrel along with the dosimeter tubes to monitor the concentrations with time. Bioassays were included in all testing and contained adult lesser grain borer, *Rhizopertha dominica* (Fab.) and adult red flour beetle, *Tribolium castaneum* (Herbst). The bioassays of each species included two strains: one phosphine susceptible strain and one phosphine resistant strain.

Results of the experiment showed some of the limits of the dosimeters. The scale of the dosimeter tube was non-linear with wider spacing in the first decade of the scale. For the high range tube, they were more readable to 100,000 ppm×h. Also, model LPG-1, provided comparable C×T values for events less than 70,000 ppm×h. The LPG-1 dosimeter measurements were with ± 25% of the Wi-Fi system data. However, the low-range dosimeter tubes, LPG-2, tended to overestimate the C×T values by 50 to 100% for fumigation events less than 20,000 ppm×h. The insect bioassays provided some reference for level of control. The phosphine susceptible insects were controlled at C×T dosages of ~5000 ppm×h. But the phosphine resistance insects had varied control of 60 to100% at C×T levels of ~20,000 ppm×h.

Keywords: Concentration × time, Wi-Fi sensors, Insect resistance

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Evaluation of railcar fumigations during transportation

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ABSTRACT

Grain and grain-based products in railcars are often fumigated with phosphine during transportation but monitoring gas concentrations during transport can be challenging and typically no information is available on gas concentrations and exposure times obtained during transportation. The rationale for these treatments is typically as insurance to make sure that any insects in the cars or the product at the time of loading are eliminated or to prevent infestation during transportation. However, how effective these fumigations are can be impacted by many factors including the type of railcar, rail car condition, temperature, time spent moving, rate of movement, and trip duration. Rail transportation has restrictions that limit the use of conventional methods of gas monitoring, but new wireless phosphine monitoring devices that can be placed inside structures during treatments and that also have the ability to log and store gas concentration readings offer a new opportunity to understand fumigations during transport. We worked with collaborators to place Centaur PH_3 phosphine gas sensors into railcars after loading, but prior to fumigation and transportation. After railcars arrived at their destination the sensors were shipped back to the lab and the data were downloaded and analyzed. Results from monitoring multiple positions within a car, multiple cars and trips, and different car types will be presented. Implications for the use of this type of fumigation, ways to potentially improve the consistency and efficacy, and an evaluation of the use of these monitoring devices will be discussed.

Keywords: Phosphine, Fumigation, Railcar, Transportation, Monitoring

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Lampiri E, Athanassiou CG (2021) Insecticidal effect of phosphine on eggs of the khapra beetle. Page 34. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Insecticidal effect of phosphine on eggs of the khapra beetle

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ABSTRACT

Trogoderma granarium Everts (Coleoptera: Dermestidae) is one of the most important quarantine pests of stored grains. Control of this insect species can be achieved through a gaseous insecticide, phosphine. Many studies focus on the effect of phosphine on different developmental stages of insects, with most of them highlighting eggs as the most tolerant stage. Our data showed that 2 d-old eggs of *T. granarium* were more susceptible than 1 d-old eggs. Faster hatching was observed in eggs exposed to phosphine for 2 d compared to controls and the result was more pronounced for 1 d-old than 2 d-old eggs. In contrast to the 2 d exposure, hatching rates of eggs exposed to 4- and 6-d were notably reduced, while there was a delay in egg hatching compared to controls. Moreover, larval development from untreated eggs was faster than the larvae from treated eggs, regardless of the exposure time. These dissimilar patterns in larval growth may suggest certain delayed effects of phosphine fumigation. The results of the present work can be further utilized for the development of phosphine-based quarantine and pre-shipment treatments (QPS) for the control of *T. granarium*.

Keywords: *Trogoderma granarium*, Egg age, Phosphine, Egg hatching, Larval growth, Fumigation, Quarantine

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It all started with a kit - the Detia Degesch phosphine tolerance test and new data in the field of phosphine fumigation

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Abstract

Phosphine (PH_3) is still the most important and commonly used fumigant for the control of stored product insects globally. As there are many claims of decreasing susceptibility around the world in storage pest insects treated by phosphine fumigation, the need for additional knowledge and its consequences in adequate practice is increased. Responses towards unsuitable conditions, such as very low temperatures combined with minimum exposure times have shown unsuccessful treatments. In this account, low temperature fumigation trials were conducted at 5 and 10°C with dosages authorized in Europe (5 and 10 g PH_3/m^3). The results clearly suggested that, under these conditions, the efficacy of phosphine was different for various species and life stages.

Further research indicates the appearance of a so called "sweet spot" in adult beetles, the dependence of fumigation efficacy for phosphine regarding high concentrations, as well as a claim for minimum exposure periods to outrun those effects. They might also be involved in the process of selection of more tolerant insect strains. In this context, a tool named "Detia Degesch Phosphine Tolerance Test" was developed to assess the tolerance status of insect strains to phosphine in the field within a short time. The use of the test is based on the exposure of the insects to a high concentration of phosphine (3,000 ppm) for short exposure periods (from 8-15 min). In practice, the test should be used prior to a planned treatment, giving the user an option to prevent an unsuccessful treatment. Considering the option of ranges in dosage and exposure time has been already implemented in European registrations, the user is enabled to adapt the treatment according to the underlying conditions, by integrating a simple assessment tool into the procedure of a fumigation. Furthermore, spreading knowledge about efficacy related factors can support the improvement of fumigation practice.

Keywords: Stored product insects, Phosphine, Fumigation, Tolerance test

Introduction

Metal phosphide-based formulations for stored grain protection are still considered of high importance, when a) preventive measures fail and a threshold for damage is overcome and b) alternatives are not available or not permitted by authorities. Numerous publications already claim problems in successfully treating infested storages; not to mention the direct feedback by customers, complaining about active insect infestations. Resistant insect populations have been found around the globe, mostly using a standard FAO diagnostic to confirm their susceptibility status on a scientific basis.

The sector of stored-product protection has become a major interest in many parts of the world, judged by various outputs from scientific projects. The question is, how does the claim of resistance on the scientific basis relate to the real fumigations? Moreover, how do laboratory tests help a fumigator to decide how to conduct a real-world fumigation?

Lately, it has become a greater challenge to bring together the scientific world and the practical fumigation business. The fear of resistance against the highly important substance phosphine in various insect species, mostly having little to no alternative for many situations has driven the Detia Degesch Group to develop and continuously improve one simple, but highly expressive tool as useful addition to the procedure of a fumigation. The test is based on the narcotic effect during the exposure of storage pest insects to a high concentration of phosphine (3,000 ppm) within a short time. The principle is not new and has already been published before (Reichmuth, 1992; Steuerwald et al., 2006), as is the commercially available version: The updated "Detia Degesch Phosphine Tolerance Test" (Detia Degesch Group). This test allows every fumigator or persons involved in the decision for a treatment with phosphine to evaluate the underlying situation in the infested storage, even enabling multiple samples from the same location to be evaluated. The test has been validated recently by using laboratory and field strains from monitoring studies in Greece and other European countries (Agrafioti et al., 2019; Sakka et al., 2017) to optimize the realistic determination times for 13 common storage pest beetle species. Additionally, the test was correlated with the standard FAO method (FAO, 1975) with good results (Agrafioti et al., 2019).

One important factor to be considered prior to the treatment with phosphine is temperature. This is not solely due to the change in degassing behavior of metal-phosphide-based formulations, but most importantly due to the changes in metabolic activities of insects. In addition, exposure time is essential, especially in cases of low temperature treatments. To prove that even high dosages cannot replace a sufficient exposure time, GEP (Good Experimental Practice) trials were conducted in the laboratory of Detia Freyberg GmbH in Laudenbach, using breeding mixes containing all developmental stages of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), and the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). The major aim was to evaluate the effect of temperature at 5 and 10 g PH_3/m^3 in 5 d exposure time on insect mortalities, which is the minimum exposure period for an aluminum phosphide formulation in Europe.

One fairly recent, but highly discussed matter is the so called "sweet spot", which has been found in various stored-product insects species in response to elevated concentrations of phosphine (Walse et al., 2018; Lampiri et al., 2021). Basically, it describes the phenomenon of a change in the expected efficacy during exposure to phosphine at changing time intervals in correlation with

applied dosage. In accordance with the ongoing research, the delayed mortality aspect is investigated further as well, and seems to be a good indicator of susceptibility to phosphine (Athanassiou et al., 2019a, b). Based on the above-mentioned facts, the aim of this overview was to bring together the latest developments in the field of fumigation with phosphine generating formulations, but also to connect the scientific knowledge to the application in real-world fumigations.

Materials and methods

Phosphine efficacy test at suboptimal temperatures

To evaluate the efficacy of 5 and 10 g PH_3/m^3 dosages (the authorized European dosages of aluminum phosphide formulations by Detia Degesch GmbH under Reg. EC 1107/2009) under suboptimal temperatures, the tests were carried out in two identical gas-tight chambers (0.5 m^3 each) located next to each other in a climatized room at the laboratory of Detia Freyberg GmbH in Laudenbach, Germany. The trials were conducted consecutively as temperature had to be set at 5 or 10°C by using an air conditioning unit, climatizing the laboratory room to 5 or 10°C, respectively. The relative humidity (RH) in the fumigation chambers was adjusted to about 65% by placing a saturated ammonium nitrate solution (NH_4NO_3) in the chambers before the start of the experiment. The fumigation was conducted using PHOSTOXIN® Pellets (56% a.i.). The pellets for a target concentration of 5 g PH_3/m^3 and 10 g PH_3/m^3, respectively, were placed in petri dishes within the chamber.

Phosphine-susceptible strains of *S. granarius*, *T. castaneum,* and *P. interpunctella* were used, taken from the ongoing breeding strains at the biological laboratory of Detia Freyberg GmbH. For each test, 400 mL of breeding mixture containing all life stages was kept in a PVC vial (5 cm length, 4 cm in diameter) covered with gauze. The test conditions were monitored by an ebro EBI 20TH1 data logger. After the desired conditions reached, the insect mixtures were introduced into the chambers. The two chambers served as parallel tests, with five replicates per species. The insect mixture serving as controls were placed simultaneously in a separate box next to the fumigation chambers inside the climatized room. Additional controls were kept at 25 - 27°C and about 50% RH. After fumigants were introduced, the chambers were sealed.

During the 5-d exposure, phosphine concentrations in the chambers were measured after 1, 2, 4, 6, 8, 24, 32, 48, and 120 h, using Dräger-Tube system. After 5 d fumigation, the chambers were ventilated by extracting the gas via an exhaust air purification system. Before ventilation, the control samples were removed from the room and transferred to the biological laboratory. Immediately after ventilation, the treated insects were also transferred to the biological laboratory.

Efficacy was evaluated on the basis of adult survival compared to the survival of the adult stage in the control treatments. The efficacy of the fumigation on the development stages (egg, larva and pupa stage) was determined by incubating the treated insect mixtures under rearing conditions for 12 wk. The mixtures were checked weekly and freshly hatched adults were removed.

Phosphine Tolerance Test

To conduct the “Phosphine Tolerance Test”, the manual freely available on the Detia Degesch Group webpage was consulted. In brief, 20 adult beetles of each species were selected and placed inside a 100 mL syringe. To create the desired phosphine concentration, two test kit pellets (8% Mg_3P_2) were placed inside an unfolded 5 L plastic canister, and 50 mL of water were added. The

canister was closed and shaken thoroughly and carefully and then left for 5 min, until the pellets were completely decomposed. The phosphine concentration in the container was measured (Draeger tube, type 25/A, connected to pump Accuro, Draeger). The concentration might need to be diluted to 3,000 ppm by determining the amount of air and gas mixture from the plastic canister. After applying 3,000 ppm into the syringe, the beetles' activities were observed. Beetles not able to move or walk properly were considered to be narcotized. The manual includes determination/target times for 13 species, by which they can be categorized into normally susceptible or tolerant to phosphine.

The test can be used for a more scientific approach as well, by increasing the number of replicates and standardized age of the exposed individuals (pre-breeding of the strains). Additionally, investigation of delayed effects could be added to the set-up. This has been followed in various trials and exemplary descriptions can be found in Athanassiou et al. (2019a, b) and Aulicky et al. (2019).

"Sweet spot" and delayed mortality

The method to determine the relationship between exposure time and concentration, as well as the delayed effect reported by Lampiri et al. (2021) was used. In brief, insects were exposed for short periods (1-40 h) to concentrations of phosphine between 500 and 3,000 ppm.

Results and discussion

The efficacy of phosphine under suboptimal temperatures is illustrated in

Table **1**. Even at the highest dosage of 10 g PH_3/m^3, 5 d exposure at 10°C did not result in 100% mortality of *S. granarius*, while *T. castaneum* was successfully controlled under all tested conditions. The highest authorized dosage in Europe did not result in a successful treatment for all tested normally susceptible laboratory strains.

Table 1. Efficacy of phosphine exposure at different concentrations and low temperatures against three normally susceptible storage pest species.

Test conditions / Species	5°C / 5 days*		10°C / 5 days*	
	5 g PH_3/m^3	10 g PH_3/m^3	5 g PH_3/m^3	10 g PH_3/m^3
Sitophilus granarius				
Tribolium castaneum				
Plodia interpunctella				

*Green color: 100 % mortality of all developmental stages (no hatching), red: 100 % mortality of all adults, but also occurrence of new adults after 12 week breeding (post-exposure time).

High dosages were not able to outrun the exposure time under low temperatures. This might be caused by the low insect activity under low temperatures (<10°C inside the target commodity). The solution under suboptimal temperatures could only be the increase of exposure time, increased temperatures during treatment and ensuring of a proper sealing. Recent publications provided an insight into the reasons on why the claim of phosphine resistant insect population seems to have increased during the last years. The reasons seem to be: unsuitable fumigation conditions, poor sealing, and low dosages (e.g., Aulicky et al., 2019; Wang et al., 2020; Agrafioti et al., 2020). Agrafioti et al. (2020) used strains of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and showed decreased susceptibility towards phosphine exposure in real-world fumigations. This study found that the difference between laboratory and selected strains with prior phosphine contact did not differ much in reaction to a high-quality treatment in comparison to a poor-quality treatment. Hence, infestation with proven phosphine resistant insects does not necessarily exclude a treatment with phosphine in general. Additionally, the research on a mutation in the DLD (dihydrolipoamide dehydrogenase) gene of phosphine resistant insect strains has led to the conclusion that phosphine itself evokes this mutation (Nayak et al., 2020). Nevertheless, additional studies indicate that there are considerable variations within the same insect population with unknown biological and fitness cost (Malekpour et al., 2016, 2018; Agrafioti et al., 2021). Hence, results from field-collected strains in "real world" fumigations might not be directly comparable with data from laboratory strains that can be "homogenized" through periodical exposure to low concentrations of phosphine.

From a practical point of view, it is not uncommon to receive increasing numbers of requests indicating low efficacy of phosphine fumigations, without additional documentation regarding the dosage, exposure or the practice that was followed. In the vast majority of these cases, these failed applications are usually regarded as indications of resistance to phosphine, while, in reality, many of these failures are directly related with poor fumigation practices. In this context, we consider that there is an additional need to raise awareness, especially in the case of professional fumigations, that resistance is not an "on/off" phenomenon, and best management practices should always be the starting point.

A more recent research topic is the proof of a non-linear dose-response curve in storage pest insects to phosphine fumigation, named the "sweet spot" (Lampiri et al., 2021). It has caused major requests by costumers following the scientific update and has also led to the reoccurring demand for a Ct-product (concentration x time), which might not be always realistic in the case of treatments with phosphine. Apparently, this phenomenon has been highlighted and studied at the laboratory scale, with controlled environmental conditions and fixed concentrations. However, the sweet spot indicates that there is a hormetic response of stored product insects to phosphine, that is expressed at a certain concentration and exposure combination, that may, eventually, lead to increased survival at elevated phosphine concentrations. Even though Lampiri et al. (2021) highlighted certain first results, the possibility of a different sweet spot in developmental stages cannot be excluded yet, which is expected to constitute the calculation of the Ct product a very complicated procedure.

The authorization in Europe allows fumigators to choose from a range of dosages and exposure times for treatments with metal phosphide containing formulations. This offers a great deal of options, if biotic and abiotic conditions in the object appear critical, but of course does not prevent

good preparatory work, such as sealing and installment of concentration monitoring. Industry standards as implemented in the tobacco industry (CORESTA) give hope, that a cooperation and communication between fumigation, industry and science can work and lead to high quality treatments, adapting latest knowledge and take it into the practical world fumigation (Tobacco Asia, 2017). The ongoing research shows that even individuals or populations with a decreased phosphine susceptibility could still be treated successfully with the same substance, if good fumigation practices are followed, focusing on concentration and, especially the appropriate exposure interval. Nevertheless, the susceptibility status of storage pest insects against phosphine demands continuous attention and open conversation with all parties involved, aiming for awareness and communicating solutions for application issues.

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Affordable sealing of multiple grain storages – manifolded for CLF

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Abstract

In 1980, James Cook, a research director with Degesch America, patented phosphine (PH_3) recirculation fumigation, labeled the *J-System.* Cook documented phosphine's lower explosive limit (*LEL*) as 18,900 ppm. The *J-System* produced satisfactory results at gas flows from 0.001 to 0.01 cfm/bu (0.062 to 0.62 $m^3h^{-1}t^{-1}$). In 1988-89, I manifolded inlet and outlet gas piping of multiple grain storages of varied volumes to recirculation blowers (providing 0.208-0.104 $m^3h^{-1}t^{-1}$) to circulate gas through combined storages, which I called "Closed Loop Fumigation" - *CLF*. Closed Loop Fumigation established that storage units of variable sizes could be satisfactorily fumigated *as one storage volume*.

Sustainable Economic Sealing uses inexpensive high quality, off-the-shelf sealing materials. It requires strong attention to detail during application. Sealing methods include plugging visible light through walls and roofs, sealing structural joints with expandable adhesive foam, applying exterior grade caulking, using high quality duct tape and 6 mil polypropylene sheeting to seal to fan inlets and outlets, and sealing door facings using spray adhesives. Other leaks are from motor bearing shafts, conveyor covers and outlets, roof wall joints, roof and wall doors, slide gate adjustment rod openings and sleeves, roof vents, fill caps, down spouts, and other air leak points. Sealing grain structures to 'no leak' *pressure standards* to eliminate virtually all leaks may be ideal for *high-value* commodities. But, sealing commercial structures storing *low-value* commodities (wheat, corn, soybeans, field beans, rice) to *pressure standard* tightness is not economically affordable in most countries. Therefore, sealing major and minor leaks of steel bins, concrete silos, or warehouses (where you see points of daylight through walls and roofs) using economical sealing materials can provide affordable fumigations, especially when multiple adjacent storage structures are manifolded using *CLF*. Annual inspection, maintenance, and 'resealing' as needed are vitally important in *sustainable economic sealing.*

Keywords: Sustainable, Affordable, Sealing, Economic, CLF, Recirculation Fumigation, Low Pressure

Background

Phosphine gas was not recirculated until James Cook, a research director with Degesch America, obtained a U.S. Patent (Cook, 1980) on the *J-System* recirculation method. Cook documented that phosphine (PH_3) had a lower explosive limit (LEL) of 18,900 ppm. The *J-System* functioned well using gas flow rates from 0.062 to 0.62 $m^3h^{-1}t^{-1}$ (0.01 to 0.001 cfm/bu).

In 1987, I visited Fairfax Elevator, Kansas City, Kansas, and inspected two *J-Systems* used on a 10,000-t steel tank and a 550-t concrete silo. The steel tank used two 0.062 kW blowers each providing 0.0017 m^3/h gas flow. The concrete silo used one 0.062 kW blower (Noyes et al., 1989). After inspecting Fairfax Elevator's *J-Systems* in 1988, I developed a multiple bin recirculation system for an Oklahoma elevator, combining four 5,000-t steel bins as Oklahoma's first model PH_3 gas recirculation system (Fig. 1A), creating two side-by-side 10,000-t storage volumes, each with a 0.75 kW centrifugal blower (Fig. 1B) providing 0.0085 m^3/h, which I named "Closed Loop Fumigation" - *CLF* (Noyes, 1993).

An important steel bin piping design change from the first *CLF* model system (Fig. 1A) was connecting suction pipes through the bin wall just below the roof using two 90° Sched 40 PVC elbows (Fig. 1C). Suction pipes hang from bin walls, instead of laying on roof slopes. Hole saws cut smooth holes through bin walls, and pipes hang next to wall ladders and roof access doors. Suction pipes are clamped to bin walls or ladder siderails.

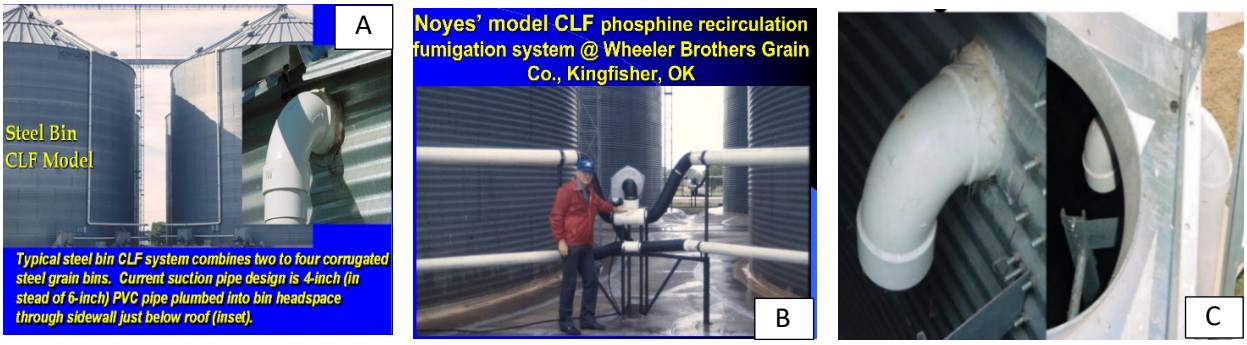

Fig. 1. First *CLF* model (A), caulked suction pipe elbows (B), *CLF* upper suction, lower pressure pipes (C).

Basic *CLF* Principles

1. Combined individual storage volumes vary widely with excellent efficacy; 2. Circulate PH_3 gas 1-2 days (3-5 volume changes) until at uniform peak; 3. Stop blower - continuous recirculation increases gas leakage; 4. Monitor headspace gas; when gas drops to marginal kill level (~ 200 ppm), operate blower 'a few minutes' to raise headspace gas to target level (~ 400-700 ppm); 5. Grain bulk in each storage provides PH_3 gas reserves.

In the 1990s many Oklahoma elevators implemented these CLF Principles manifolding all steel bins and concrete silos forming 10,000 to 50,000 t fumigation units. Design and economic data developed from these CLF systems were used in annual Oklahoma Elevator Management Workshops (Noyes et al. 1995).

In 2000, several grain companies requested *CLF* systems for flat storages. The best sealed warehouse was at Mid-Oklahoma Coop, in Kingfisher, Oklahoma. Two men worked 7 d to seal their 20,000-t warehouse and install 2 *CLF* blowers. In 2000, their peak gas concentration was 2050 ppm 24 h after dosage. Seven days later, their headspace gas level was 850 ppm. In 2001, Mid-OK Coop reduced dosage 50%. Their 24 h gas reading was 1050 ppm; after 7 d, their concentration was 450 ppm.

Closed loop fumigation systems typically use one recirculation blower for 2 to 10 steel tanks and 2 to 40 concrete silos as a unit. Thus, steel bin, concrete silo and warehouse storage facilities of 5,000-100,000 t are fumigated *as one unit* with *CLF*. Multiple fans are used on large warehouses. The largest *CLF* system in Oklahoma was a 90,000 t (45 m × 150 m) warehouse at Tulsa Port of Catoosa. This structure, with 18 aeration fans and ducts per side, used three pipe manifolds per side and had a 2.25 kW blower connected to six aeration fans / ducts per 50 m section.

After my *CLF* presentations at the 1998 *International Working Conference for Stored Product Protection* (*IWCSPP*) in Beijing, China (Noyes et al., 1998), the *China Oil and Seeds Committee* adopted *CLF* into the design of all concrete silos (*Squats*) and warehouses built in 19 new State Grain Depots across China between 1998-2000. In November 1999, Dr. Navarro and I lectured at two 2-day workshops in Nanjing and Beijing on the topic, *Aeration and CLF*, using photos of China's new *CLF* and aeration systems (Noyes and Navarro, 1999). We also presented a 5-h summary of *Aeration and CLF* to the 600 delegates of the *National Conference of Grain Depot Directors, Managers and Scientists* in Beijing.

Sealing storage structures for *CLF*

Sealing leaky bins, silos and warehouses is vital to successful *CLF* operation. Figure 2A illustrates available 'off-the-shelf' materials used for affordable grain storage sealing. These materials include expanding impervious adhesive foams, medium to strong tackiness adhesive sprays (red topped cans), elastomeric concrete foundation paint and nylon mesh (*Kool Seal*, Fig. 2A), heavy plastic contractor bags, high quality duct tape, exterior grade silicone caulk, and 6 mil polyethylene plastic sheeting. Please note that adhesive foam products like *Great Stuff* have an expansion ratio of 3 to 5 times their original volume. Practice using it slowly until you determine how fast and how much it expands before it becomes firm. Most expansion occurs in the first 10 to 15 min. Apply it in shallow layers; wait 10 to 15 min between layers to allow gradual filling of large spaces.

Primary Affordable Sealing Areas: (In priority sequence of major gas loss sealing importance)

Steel Bins: Roof-wall gaps; Roof Vents; Fill points; Discharge conveyors; Roof/Side Doors; Concrete base - sidewall seam.

Concrete Silos: Under-roof wall vents; Fill points; Unload conveyors; Roof deck - wall gap; Manhole doors.

Warehouses: Drive and walk-in doors; Windows; Roof vents; Fill points; Discharge conveyors; Roof leaks; Concrete Base - sidewall seam.

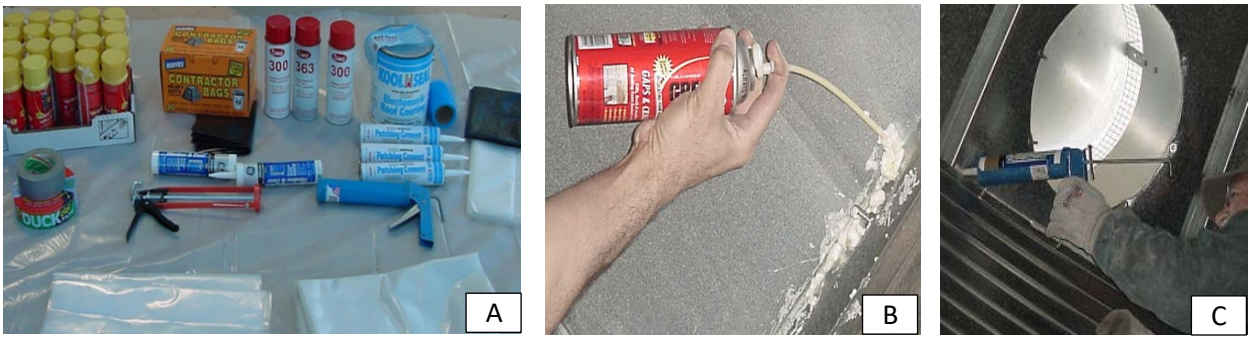

Fig. 2. Economical sealing materials (A), foam wide roof gaps (B), silicone caulk narrow roof gaps (C).

Sealing steel bin roof to sidewall air gaps

Roof sidewall air gaps are the largest gas leak areas in bolted steel bins which must be sealed (Figs. 2B and 2C). Roof panel ridge stiffeners at sidewalls (Fig. 3A) and fill cap flashing rings require critical sealing. Roof panel ridge ends under fill ring flashing collect dust, fines, and other airborne debris which must be cleaned out and sealed with expanding foam sealers like "Great Stuff" (Figs. 2A and 2B). Roof stiffener ridges at the sidewall need to be filled with adhesive spray foam (Fig. 2B). Steel (or wooden) plates that profile roof rib openings (Fig. 3A) can be caulked around edges for a tight seal, or openings can be foam sealed using a temporary blocking material until the foam hardens.

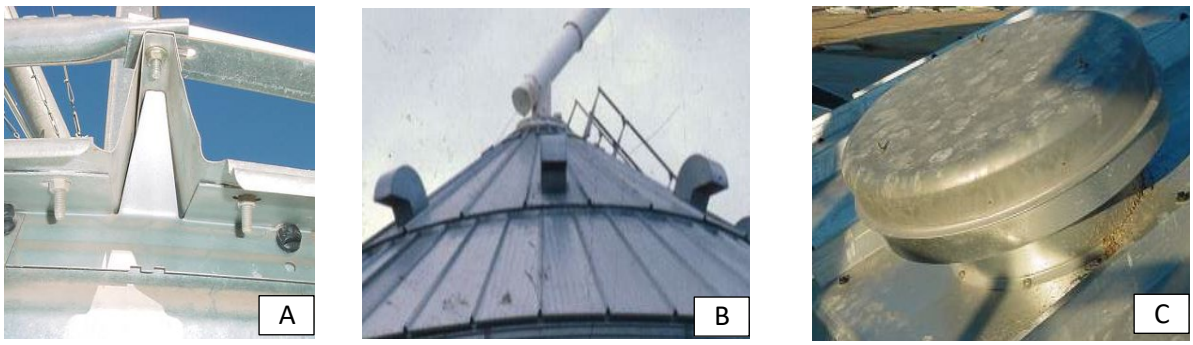

Fig. 3. Caulk edge of steel roof ridge plate (A), double bag roof vents (B), fill cap and down spout sealing (C).

Fill-cap openings and roof doors (Figs. 3B and 4A) are sealed using 6 mil plastic sheeting attached to raised flange openings and sides with adhesive spray. Plastic 6 mil sheets should be oversized by 20-30 cm so sheet edges can overlap below door and fill ring lips. Wrap excess plastic down sides and bind with duct tape, sealing plastic to steel surfaces. Seal roof vents (Figs. 3B and 3C) using doubled industrial plastic bags, duct tape around vent bases, and cross-tape tops of bags to provide wind resistance. Seal concrete foundation to steel wall base junction (Fig. 4B) with an exterior elastomeric paint for a durable flexible seal. Reseal concrete bin bases annually.

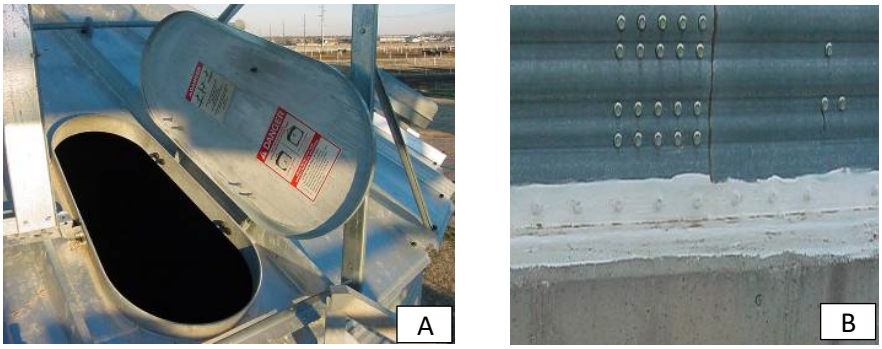

Fig. 4. Seal door flanges with adhesive spray and plastic sheeting (A), elastomeric paint base seal (B).

Many steel bins have fill spouts or auger discharge spouts installed through bin fill caps (Fig. 3B). Some downspouts have an extended "Y" with removable cleanout plates, so the spout can be 'stuffed' or 'foamed' to block gas flow up spouts but remove blockage after fumigation. Spouts with flange ring connectors above fill caps (which can be loosened) allow thin plates to be inserted between flange rings to block spout gas loss. If spouts cannot be sealed above the bin, spouts must be sealed inside bins using adhesive spray, 6 mil poly sheeting, or industrial plastic bags and duct tape.

Sealing roof and sidewall doors, and fan inlets

Bin roof doors and bin side doors are large openings where large volumes of gas can escape if not well sealed. These openings have flanges that can be sealed using adhesive spray, 6 mil plastic sheeting, and duct tape. Cut the plastic sheeting at least 20-30 cm wider than the outer dimensions of the roof and sidewall door flanges (Fig. 4A). Spray the flange surfaces with a medium to strong tackiness industrial adhesive (the 300 and 303 labelled cans in Fig. 2A), then lay the plastic sheet over the opening with the oversized plastic sheet centered on the door flanges. Lightly press the plastic sheet against flanges so the adhesive bonds. Read the label on how quickly tackiness sets up. As soon as the plastic sheet bonds, tuck excess plastic edges under the flange, and wrap duct tape tightly around the plastic so the plastic sheeting is held tightly across the flanges. Close and latch doors to protect the plastic sheeting from windblown debris that might penetrate the plastic, causing gas leaks.

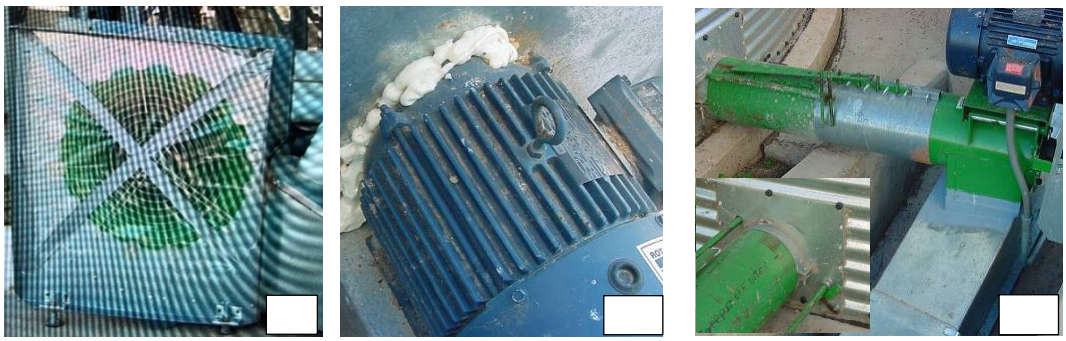

Fig. 5. Sealed fan inlet with plastic and X-braced (A), fan motor shaft foam sealed (B), caulk auger push rods and foam inside tube (C).

The primary sealing area of aeration fans is the fan inlet (Fig. 5A). Measure the diameter of the fan inlet orifice outer edges, then increase the diameter of the 6-mil polyethylene sheet material by 20-30 cm to allow material to wrap behind or under the orifice ring. Aeration fan guards are bolted near the outer edges of the fan inlet orifice. Silicon-caulk the bolts and holes before applying plastic sheeting over the inlet orifice. Apply duct tape in a cross (X) pattern across the fan guard plastic covering to keep it from flapping and being perforated during strong winds or fumigations.

Foam the motor shaft holes through fan housings to prevent gas leaks (Fig. 5B). Inspect aeration fan housings for holes, wiring conduit entry points, cracks, gaps at the mounting face to the bin or aeration ducts, or flange connections from fan outlet to aeration ducts, and then seal these leak points with caulk, adhesive foam, plastic sheeting and adhesive spray, or other suitable methods. Centrifugal fans mounted on concrete pads collect moisture and trash, and can rust through. Apply expanding foam under fan housings or caulk around housing base on concrete to seal fan housings.

Discharge conveyor sealing

Steel bin, concrete silo annex or warehouse discharge conveyors (Fig. 5C) are hard to seal. Remove U-trough conveyor cover close to wall, then add about 20-30 cm of expanding foam to the U-trough under the bin or warehouse wall. While the foam is still expanding, replace the cover panel so the foam pushes up against the cover. After fumigation, cut out most of the foam; remaining foam will be removed as grain discharges.

Most round tube auger conveyors have flange rings close to the foundation of storage structures. Disconnect flange rings and loosen shaft bearings to allow the auger tube to slide forward a 10-15 cm. Fill the auger tube cavity around the auger flighting at the flange ring opening toward the bin or warehouse foundation with a few centimeters of expanding foam, then reconnect the flange ring and tighten shaft bearing. The 10-15 cm of foam thickness will block gas flow, and the foam will shear away when the auger is started after the fumigation is completed. It may be possible to fill the end of the auger housing with foam to form an air-tight ‘plug’ of foam just ahead of the auger discharge if foam can be sprayed into the final 20-30 cm of the auger just ahead of the discharge outlet. The discharge auger (Fig. 5C) has a silver connecting band that can be loosened and slid forward enough to allow a short section of auger tube to be filled with expanding foam. If foam plugging of discharge conveyors is not feasible, the outlet of the conveyor including motor and drive must be ‘bagged’ with two to three industrial trash bags layered and sealed tightly with duct tape so there is plastic to steel tubing seal under the duct tape.

Sealing flat storage warehouses

Flat storage warehouses vary more in gas leakage than steel bins or silos. The best warehouses for sealing are those with long vertical wall sheets attached to horizontal steel stringers between sidewall and roof frames. Walking inside warehouses on sunny days will indicate leakiness from number and size of visible light spots. If there are a lot of light spots, the best option may be to coat and seal the inside walls with a thin tough exterior elastomeric spray or roller paint. Large roof eave, roof deck, conveyor inlet and discharge openings should be sealed like steel bins.

Sealing concrete silos

Most concrete silo leaks are at the top and bottom. Solid reinforced concrete sidewalls generally have few leaks, except for openings built for specific purposes, such as access doors, grain discharge chutes or spout outlets. Seal those with adhesive sprays, plastic sheeting or bags and duct tape.

Roof to sidewall air gaps are primary problems on concrete silos where roof decks were precast, then set on top of concrete silo walls without a filler material between roof and walls. Usually, these junctions are not caulked or sealed. Sealing the silo roof / wall cracks and external vent openings is best done when the silo is filled to within 1.5-2 m of the roof so boards or tarps on the grain surface make work platforms. If roof deck wall gaps are extremely close (0.5-1.0 cm), caulking may work. For larger gaps (1-2 cm), use foam spray to reduce material and labor.

Use expanding foam spray on exterior vents (Fig. 6A) building up shallow layers. An alternative is to cut plywood, cardboard (Fig. 6B) or steel plates anchored with "J" bolt hooks attached to vertical bars, and then silicone or adhesive foam spray around the edges, building up a thick layer of foam or caulk between concrete and plate materials. Another option is to cut a close-fitting plate and use a silicone bead or adhesive spray around the edges to push and secure it in place when the adhesive dries or the caulk sets. Discharge conveyors will be sealed like conveyors from steel bins or warehouses. Figures 6C and 7A illustrate methods of filling external vent openings. Figure 7B shows silo annex basement grain chute sealing points.

Fig. 6. Foam filling roof deck vents (A), roof vent sealing tools (B), roof vent foam sealing (C).

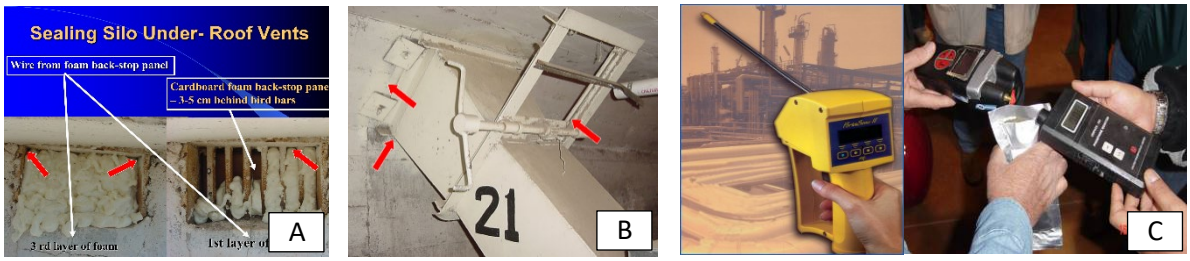

Fig. 7. Foam against cardboard (A), caulking silo spouts (B), PH_3 Gas meters are needed for sealing (C).

Storage leakage monitoring and inspections

Regardless of the type of storage, continued surveillance for leaks to improve storage seals improves fumigation. Finding gas leaks pays dividends in gas savings and higher fumigation efficacy. Your best sealing investment is a high quality PH_3 electronic tester (Fig. 7C) which allows for hundreds of quick samples. 'Sniffing' around discharge conveyors, aeration fans and ducts, the base of storages, around sidewall and roof openings after they are sealed, around fill and discharge conveyors, bin sidewall corrugated sheet junctions, fill rings and roof overhangs, bin base to concrete junctions during fumigations is vital to improving storage sealing. Enter empty storage units on sunny days and look for light points, and then seal them.

Closed loop fumigation systems - *CLF*

Combining multiple grain storages of various sizes into one larger fumigation volume provides a major improvement to James Cook's *J-System* recirculation fumigation model. Connecting storage units, regardless of size, as one recirculation fumigation volume is what I named "Closed Loop Fumigation" - *CLF*. Sealing grain storage structures permanently so they are ready to fumigate with minimal labor improves overall grain system management. Closed loop fumigation systems have greatly improved fumigation efficacy for controlling grain storage insects.

Closed loop fumigation system benefits

Although initial installation costs of *CLF* systems may be substantial, especially using outside contractors, financial payback can be relatively short while worker satisfaction and safety is improved because *CLF* creates: much faster response and purge timing than probe-tarp fumigation; (2) reduced fumigation labor; (3) improved worker safety; (4) reduced housekeeping; (5) reduced grain shrinkage and operating expense from no "turning" in concrete facilities; (6) lower fumigant dosages; (7) less gas release to environment; (8) high efficacy which minimizes insect PH_3 resistance.

Closed loop fumigation plumbing systems

In *CLF* systems, combined storages are connected by pressure and suction manifolds, like the upper and lower pipes connected to the *CLF* blower on steel bins (Figs. 1A and 1B). Pressure manifold pipes distributes gas from the blower (Figs. 8A and 8B) into the base of each storage through small lateral pipes (Figs. 8C and 8D) which tee off from the pressure manifold connecting to bottoms of silos. Lateral pipe shut-off valves (Figs. 8C and 8D) allow empty storage bins to be bypassed. In concrete silos, pressure pipes connect to grain discharge spouts through perforated boxes (Fig. 8C), so gas flows from the silo base up to the headspace. Blower suction pulls gas from all silo headspaces back to the *CLF* blower inlet (white pipe, Fig. 8A).

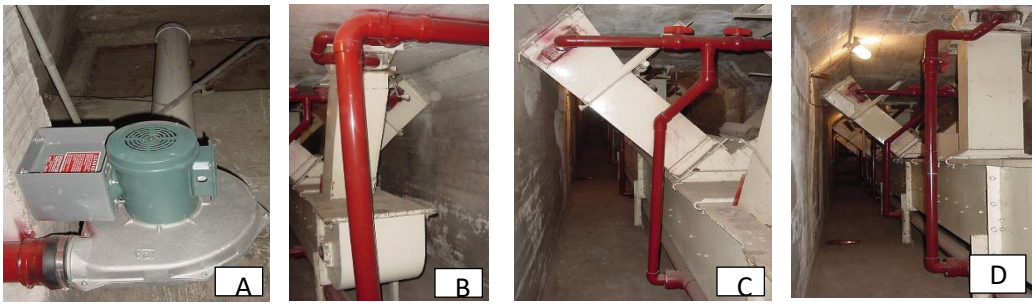

Fig. 8. CLF blower in silo annex (A), pressure manifold (B), lateral pipe to side silos (C), lateral pipe to center silos (D).

Closed loop fumigation plumbing materials

Schedule 40 UV resistant PVC pipe is the typical piping material used for *CLF* plumbing. Black flexible corrugated drainage tubing is practical and economical as it comes in long sections and follows the contour of storage bins and tanks, minimizing fittings. Gas velocity in *CLF* systems is quite low, so friction losses from flex tubing corrugations are negligible. Adapting between PVC pipes and flexible drainage hoses is relatively simple.

Closed loop fumigation blowers

Cast aluminum blowers work best for *CLF* systems. Cincinnati Fan Co. PB series 3450 RPM cast aluminum blowers (Fig. 9A) were used for *CLF* systems in all Oklahoma elevators. Figure 9B illustrates an aluminum blower circulating gas through six concrete silos. These blowers provide stable gas flows through *CLF* systems, are weather and spark resistant, and not subject to chemical reaction with phosphine gas. Closed loop fumigation blower performance data for the range of recirculation blowers are listed by Navarro and Noyes (2001). Degesch America and Chicago Blower Company market comparable cast aluminum blowers.

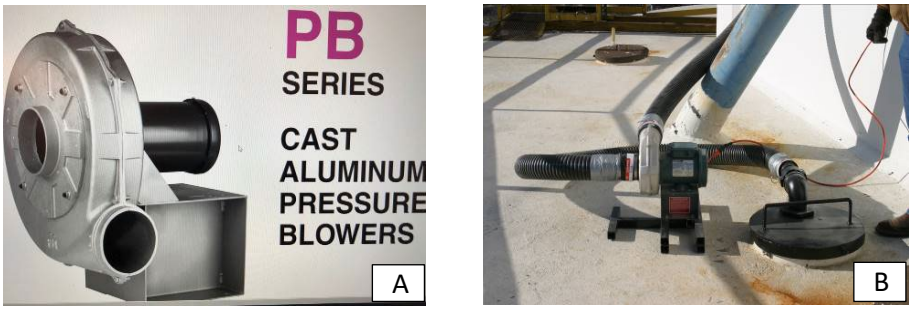

Fig. 9. Cast aluminum *CLF* blowers (A), *CLF* blower recirculating to 6 silos (B).

Venting fumigants with *CLF* fans

Once fumigation is complete, tanks or silos must be adequately vented before workers can re-enter storage units or grain can be shipped. For storages with aeration systems, unseal roof doors and vents, and operate the aeration fans for 3-6 h. Aeration fans should always be resealed immediately after the storage is vented to block insect reinfestation in the storage base.

When using *CLF* blowers to purge silos or tanks, disconnect blower suction piping then operate the blower to circulate ambient air to purge the storages. Open all roof vents or hatches so exhaust gas is immediately diluted with air moving across the tops of storage units. Storages without aeration fans can be purged much faster and to much lower gas levels using *CLF* blowers than by conventional gravity draft venting.

When *CLF* blowers are used for venting, 3-5 d of continuous blower operation should be used with open roof doors and vents. Storage bin, warehouse or silo base openings should remain sealed after purging fumigant gas to minimize insect reinfestation (Noyes et al., 1989).

Model *CLF* elevator

The *CLF* Elevator (Fig. 10) installed *CLF* in all three types of storage. They recirculated gas through steel tanks, concrete silos, and their flat storage warehouse using a 3.7 kW PB-14A centralized blower, delivering about 2900 m^3/h to their 36,000-t steel bin storage at 0.08 $m^3h^{-1}t^{-1}$, to their 23,000-t flat storage at 0.13 $m^3h^{-1}t^{-1}$, and to all 20 of their concrete silos (17,000-t) at 0.17 $m^3h^{-1}t^{-1}$. This *CLF* Elevator can fumigate all three types of storages in sequence by starting fumigation of each storage type 1-2 d apart. After each storage system has reached peak uniform concentration (in 1.5-2 d), the *CLF* blower can be switched to alternate manifolds between storages to maintain desired headspace gas concentrations in each storage (Noyes et al., 1998).

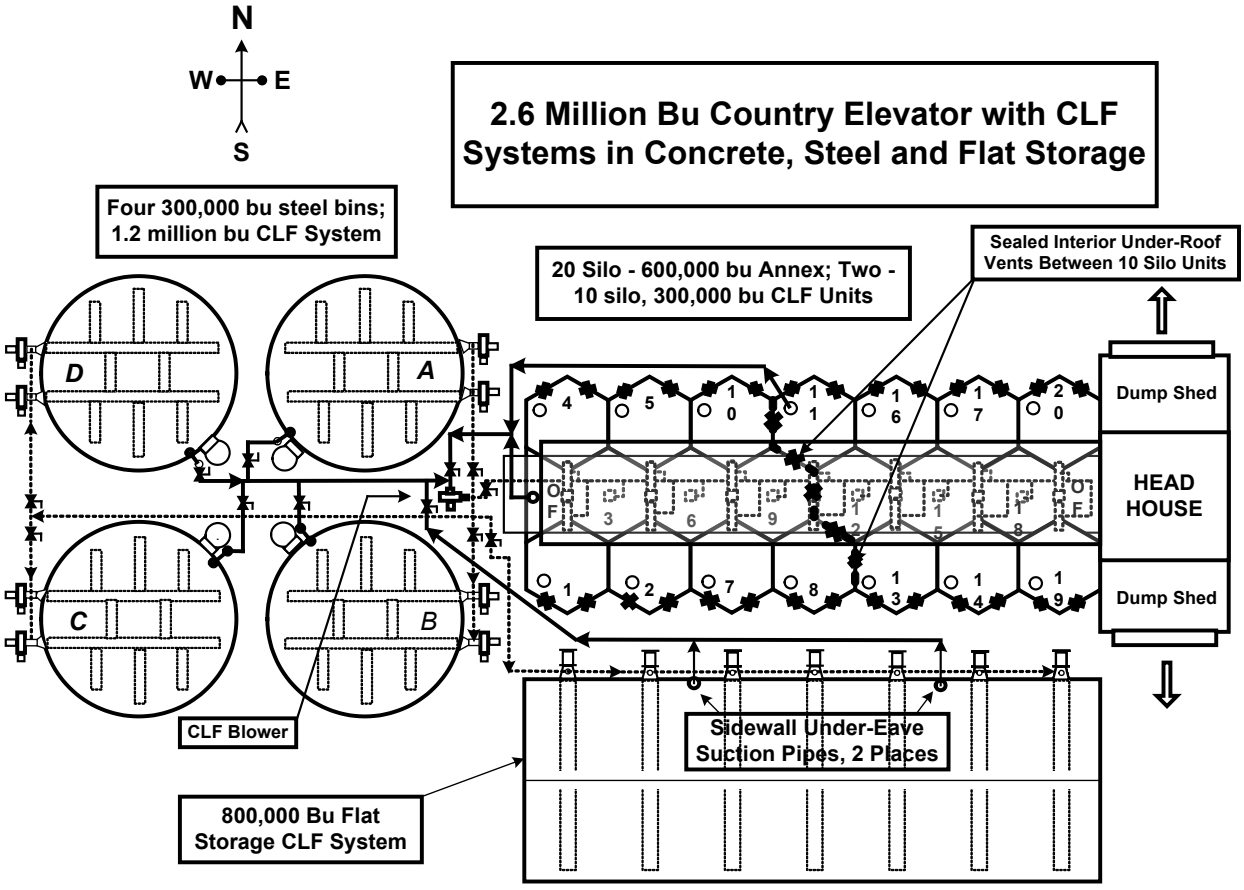

Fig. 10. Oklahoma elevator using central *CLF* blower for 4 steel bins, 20 concrete silos, and 1 flat Storage.

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CAF2020 Abstract No. A-2-2-15

Agrafioti P, Kaloudis E, Sotiroudas V, Bantas S, Athanassiou CG (2021) Predicting the concentration of phosphine and insect mortality with Computational Fluid Dynamics; Validation with field trials in cylindrical grain silos and shipping containers. Page 53. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Predicting the concentration of phosphine and insect mortality with Computational Fluid Dynamics; Validation with field trials in cylindrical grain silos and shipping containers

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ABSTRACT

In the present work, a Computational Fluid Dynamics (CFD) model was used to predict the distribution of phosphine gas in metal shipping containers and metal silos. The model results were compared with available data from phosphine sensors. The CFD model solves the equations for air velocity, temperature, gas transport, phosphine sorption, and their respective implementation in porous media. Weather conditions were used as boundary conditions and the phosphine gas release as a source term. Additionally, insect mortality was calculated as a function of their exposure to phosphine gas. For each fumigated facility, wireless sensors were placed to monitor the concentration of phosphine, along with vials with phosphine-susceptible and -resistant insect populations. The insect species used were *Rhyzopertha dominica* (F.) and *Oryzaephilus surinamensis* (L.), two of the most common species found in stored products. The first facility was a 12.2 m (40 ft) shipping container loaded with currants. Three Mg_3P_2 plates were used for the fumigation process. There was a time delay for phosphine to reach the sensors that were submerged inside the fumigated commodity, at the rear side of the container. The second facility tested was a metal silo (530 tonne capacity). Aluminum phosphide bags were placed to produce the phosphine gas and a recirculation system was used to improve the diffusion of phosphine throughout the grain bulk. The predictions of the computational model were in accordance with the phosphine concentration as recorded by the sensors. Concerning insect mortality data, in most of the cases, for both species, complete control was noted, regardless of the resistance level of the population tested. As results indicated that the CFD model correlated well with the phosphine concentration and insect mortality, a methodology for precision fumigation can be established.

Keywords: Shipping container, Metal silo, Fumigation, Phosphine, Wireless sensors, Stored-product insects, Monitoring, Computational fluid dynamics, Mathematical modeling

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Integrated CFD-based on-farm stored grain aeration model to predict the fan hours and investigate factors affecting the cooling potential

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Abstract

Physical velocity-based finite volume method was used to model the aeration cooling and re-wetting process in an on-farm silo (13.47 m high and 12.8 m diameter) filled with 1,000 tonnes of barley in Balaklava, South Australia. The 3-D transient model was validated with grain temperature (°C) and moisture content (% wb) data obtained from OPI Blue sensors (2018-19 storage season). Due to the Mediterranean climate, the initial grain conditions varied between 21.7-32°C temperature and 9.9-12.4% wet basis (wb) moisture content (mc). Thus, initial grain condition was sub-divided into 36 different sections (using User-Defined Functions) based on the number of cable sensors that eventually contributed to the accurate sorption (adsorption and desorption) process upon transient simulation. Heat of wetting (a primary component of heat of sorption), was added into the model to calculate the heat that would be released when water gets adsorbed into the grain capillaries. A grain domain mesh independency test and an appropriate time-step size analysis were conducted for solution optimization. The effect of solution methods such as pressure-velocity coupling schemes, spatial discretization schemes, and transient formulation schemes were investigated on the heat and moisture transfer in the stored grain ecosystem. The standard error of prediction (SEP) and percent mean relative deviation (MRD) for prediction of grain temperature and moisture content were 1.39°C and 0.15% wb; and 5.1% and 1.2%, respectively for the grain bulk which represents reasonable accuracy for the consideration of further facility optimization purposes. Heat of wetting showed a decreasing profile (magnitude wise) at a validated data point due to an increase in moisture value. ANSYS Fluent macros (DEFINE_EXECUTE_AT_END and DEFINE_PROFILE) were incorporated to formulate an air inlet control system, taking into consideration grain temperature and moisture from a certain height (inside grain bulk), that could work as a fan control for any allotted strategies.

Keywords: Physical velocity, Finite volume method, Aeration, Cooling and re-wetting, Heat of sorption, Fluent Macros, Inlet control

Introduction

Grain storage has undergone a major shift from bulk handlers to on-farm facilities in the past decade in Australia (GRDC, 2017). Farmers are keen on investing in their own storages for gaining higher return values, rather than directly selling to the bulk handlers. This has led stored-grain industries to expand the field of focus from a single type of storage unit to diverse structural facilities that can accommodate the increasing harvest in any given season.

Stored grain must be maintained at optimum conditions (defined by the grain temperature and moisture content) throughout the storage period in order to obtain a higher return value. This requisite is most-often achieved through implementing a common but crucial, non-chemical technique called aeration. Tropical and Subtropical regions prefer aeration (natural air) drying, while Temperate and Mediterranean regions prefer grain cooling to mitigate the insect habitat as quickly as possible via controlled moisture content (12-14% wb depending on grain type) (Panigrahi et al., 2019).

Although aeration remains the preferred technique over other chemical-based solutions, its efficiency is highly dependent on the ambient conditions. Knowing how the grain ecosystem is affected by any of these differences would assist in making more strategic use of any of the given prevailing conditions. To better understand and make management decisions, stored grain researchers have developed numerical models that have shown the intrinsic factors contributing to variations in grain temperature and moisture content during the aeration process. Different methods of modeling such as finite element (FE), finite volume (FV), and finite difference (FD) have been widely used for this purpose (Lawrence et al., 2013; Thorpe, 2008, 1997). However, the model's application directly depends on its level of accuracy. Accuracy level is further influenced by the method undertaken for modeling specific management processes. Panigrahi et al. (2019) have shown that the finite volume method (FVM) provides the best prediction during the aeration process. Ranjbaran et al. (2014) and Liu et al. (2016) have simulated drying and aeration cooling for paddy using FVM based Computational Fluid Dynamics (CFD) tool. Though they predicted outcomes with reasonable accuracy, the model was limited to the heat of vaporization that contributes to only the phase change process. However, in a large-scale storage silo, because of uneven initial grain conditions both adsorption and desorption take place during aeration cooling. Thus, there is a need to include the associated factors contributing to the above phenomena.

Model development is the first phase for creating a foundation for better management decisions other than relying on past experiences. As aeration efficiency depends on the strategic use of the local weather conditions, there is also a need to implement control measures to the model so that it can work as an integrated system for the desired aim. Lawrence and Maier (2011) used the 'if' logical scheme in Microsoft Excel to identify the desired ambient conditions and then used this information as an inlet to the developed 2D PHAST-FEM model. This was limited to the initial grain conditions and did not consider the changing grain condition during the aeration process. Thus, there is a need to integrate an automatic inlet control system to make the simulation more sophisticated and operate based on both changing grain and ambient conditions. Considering all the above issues, the study aimed to cool the grain more efficiently based on modelling approaches.

The objectives were as follows: a) to develop a 3D transient FVM model to predict grain temperature and moisture content during aeration cooling in a 1,000-t silo; and b) to develop and integrate an automatic inlet control system based on the changing grain conditions.

Materials and methods

On-farm storage silo

The on-farm grain storage structure was a flat-bottomed corrugated steel silo located at Balaklava, South Australia, Australia (34° 7' 26.22" S, 138° 27' 41.58" E) (Fig. 1). The silo was 12.8 m in diameter and 13.47 m high with an eave height of 10.06 m (Cyclone Model 4233CA, Newcastle, Australia). The silo floor had a V-shaped in-floor aeration duct of 10.4 m length and 0.9 m width. The grain had a peak height and diameter of 12.41 m and 12.8 m, respectively; with an angle of repose of 21º and eave height of 9.91 m. The silo was filled with barley.

The silo was equipped with one OPI Blue moisture sensing cable (M1/T1) having 10 RH and temperature sensors (near the central lid of the silo) and three temperature sensing cables (T2, T3 and T4) having 9 temperature sensors. Cable T2 was near the vents, and the other two were at the south (T3) and west (T4) corners (2 m in from the silo wall). The OPI system used registered equilibrium moisture content (EMC) curves to determine moisture content of the grain. Aeration cooling was operated for 24 h whenever the ambient temperature was lower than the grain temperature (lower bulk). A volumetric flow rate of 0.954 m^3/s and back pressure of 663 Pa was observed in the transition duct of a 3kW aeration fan during the aeration period.

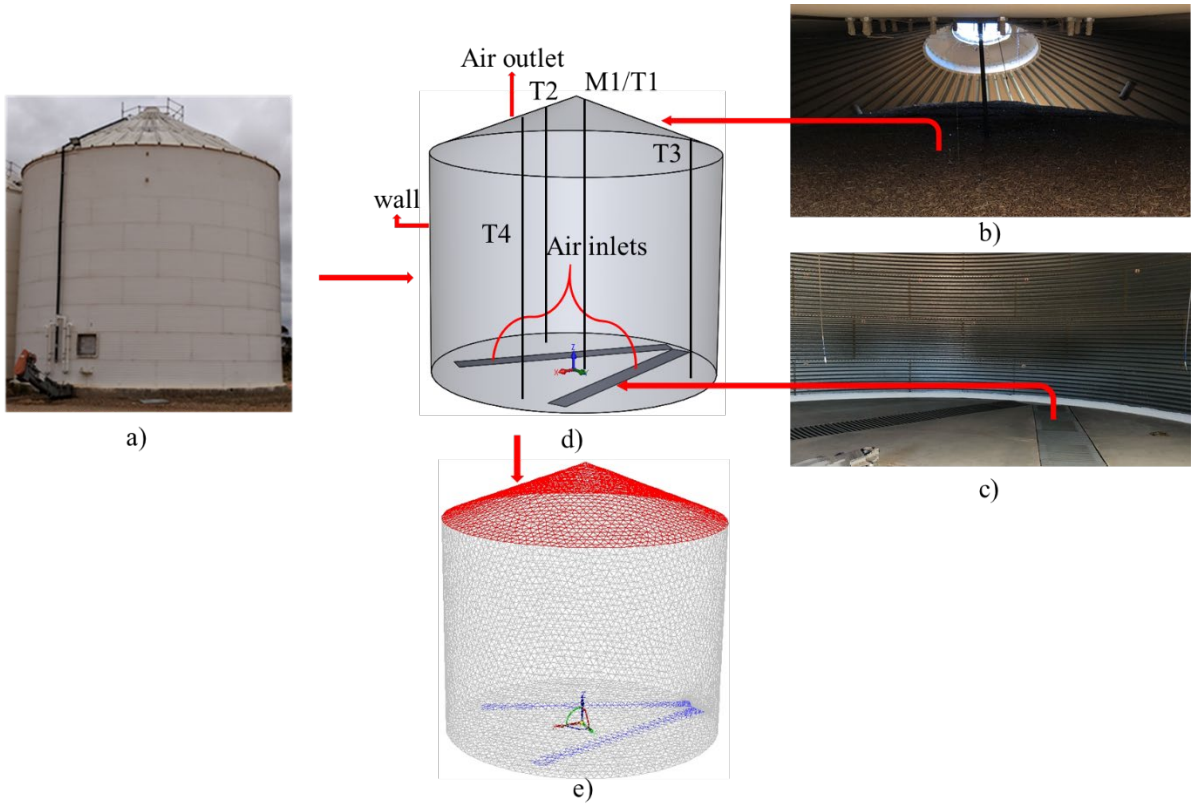

Fig. 1. On-farm grain storage silo: a) Experimental silo; b) Top grain surface; c) Aeration duct; d) 3D CAD model; e) Meshed (tetrahedral) geometry.

The flow rate and the duct surface area were used to determine the inlet face velocity (0.051 m/s). This system logged the ambient and grain temperature and moisture content with 1 h interval.

Model /control development

Local thermal equilibrium condition and laminar flow was assumed during the aeration process. Heat, moisture, and momentum conservation laws were incorporated with physical velocity formulations to account for the anisotropic porous medium across the radial direction. User Defined Scaler (UDS) was used to model the moisture transfer in the grain with absolute humidity of air as the scalar quantity. The moisture source term during the aeration cooling was based on Thorpe (2008) formulation with the inclusion of heat of sorption in the heat source term for the governing equation. The isosteric heat of sorption was derived from the combination of Clausius-Clapeyron equation and ideal gas law with the consideration of similar grain and free water temperature.

The following process was used to determine the differential heat of wetting:

$$h_w = h_v - h_s \quad (1)$$

Where h_w is differential heat of wetting (J/kg); h_v is latent heat of vaporization (J/kg) and h_s is heat of sorption (J/kg).

Respective constants associated with the source terms were used accordingly to the best fitted moisture adsorption or desorption process during aeration cooling.

Thermo-physical property equations such as bulk density, specific heat capacity and thermal conductivity for grain were formulated with temperature and moisture dependency functions (Alagusundaram et al., 1991; Otten and Samaan, 1978). For air, all property equations were a function of temperature and RH of the humid air (Basunia and Abe, 2005; Tsilingiris, 2008).

The on-farm silo was discretized into control volumes using tetrahedral elements to best represent the heaped surface of the peaked grain configuration (Fig. 1.e). The curvature and proximity mesh sizing and outlet face sizing criteria were opted to generate smaller size elements near the start of the V-shaped duct and at the domain outlet, respectively, in order to improve the mesh quality. Initial grain conditions were modeled as per 36 sensors in the silo. An assumption was made that the temperature is uniform within a zone (denoted by cable M1/T1, T2, T3 and T4, Fig. 1.d). Hourly ambient air temperature and absolute humidity data were used at the inlet and were linearly interpolated (to every second) during the simulation.

Mesh independency tests were conducted using 0.5, 0.35, 0.275 and 0.25 m element size. Time step size analysis was conducted using 1, 2, 4, 6, 8, 10 and 12 s for 24 h simulation. SIMPLE and COUPLED algorithms were opted as the pressure-velocity schemes to examine the difference in solution convergence and numerical solutions. Green-Gauss Node based and Least Squares Cell based interpolations were examined for Gradient discretization. Second order and PRESTO! methods were examined for pressure discretization. Second order scheme for momentum was employed, while first and second order for energy and UDS discretization were opted consecutively for examination. Both first and second order implicit transient formulations were examined to analyze the computational time and accuracy in the numerical solution. The absolute criteria for continuity, x, y and z-velocity, and for UDS were set at 0.0001; while 0.000001 was set for energy.

FLUENT macro DEFINE_EXECUTE_AT_END was used to determine the grain temperature at 1.5 m (coincident with first sensor of T1 cable) high from the silo floor. The above macro was also used to calculate the average inlet temperature and UDS value. These values were determined after every time-step, and based on the strategy "inlet temperature ≤ grain temperature". The inlet velocity was switched on with DEFINE_PROFILE macro.

FLUENT's parallel solver was used for computational processes. The UDF's developed aeration model (considering all functions and properties) was incorporated with #if PARALLEL… #endif arguments for calling out the sequence. All the simulations were run in parallel processing with 8 core dual Intel Xeon Gold 5215, 2.5 GHz, 192 GB memory with a bus speed of 2 ×10.4 GT/s. Model evaluation for all the simulations were based on the standard error of prediction (SEP) and mean relative deviation (MRD) for each of the sensor points. The analysis procedure was as follows: first SEP and MRD were calculated for a single sensor point (over the transient basis) followed by averaging with rest of the sensor points on a single cable. Then, the respective SEP and MRD for each of the cables were averaged (4 cables) and denoted as a single data.

Results and discussion

Simulation results showed that the inlet was operating with 0.051 m/s velocity throughout the 24 h cooling period. This was nearly coincident with the actual fan operation when the ambient temperature was less than the grain temperature (data not shown).

Considering the least values of computational time (2.25 h), SEP (1.39°C and 0.15% wb) and MRD (5.1% for temperature and 1.2% for moisture) among all the solution methods, the following were optimized: pressure discretization- PRESTO!; pressure-velocity scheme- SIMPLE; gradient- least square cell based; energy discretization- first order; UDS discretization- first order; and transient formulation- first order. These errors were significantly lower than the previous works, particularly for MRD values (Ranjbaran et al., 2014). It was also observed that second-order schemes took a significantly longer time than first order schemes; however, there was no significant difference in temperature and moisture error during aeration. This showed that opting for a scheme is "model-specific" and cannot be assumed instinctively. A 0.35 m element size (Panigrahi et al., 2020) and 4 s time-step size were observed to provide the least solution deviation for the 1,000-t storage silo configuration. These values should not be used in a general way when simulating a *different* storage structure. However, these values could be assumed the same for other types of grains stored in a *similar* silo structure. The transient temperature and moisture variation (M1/T1 cable) over the 24 h aeration period is shown in Fig. 2 for the best fitted validated model. Results for other cables are not shown.

The errors observed could be due to the assumption of uniform grain moisture across the thin layer. This could have altered the heat of sorption released during the adsorption process due to the tortuous flow path in anisotropic porous media. Moreover, the isotherm constants used in this model were different from the OPI's values which could result in a different value at the same validation point.

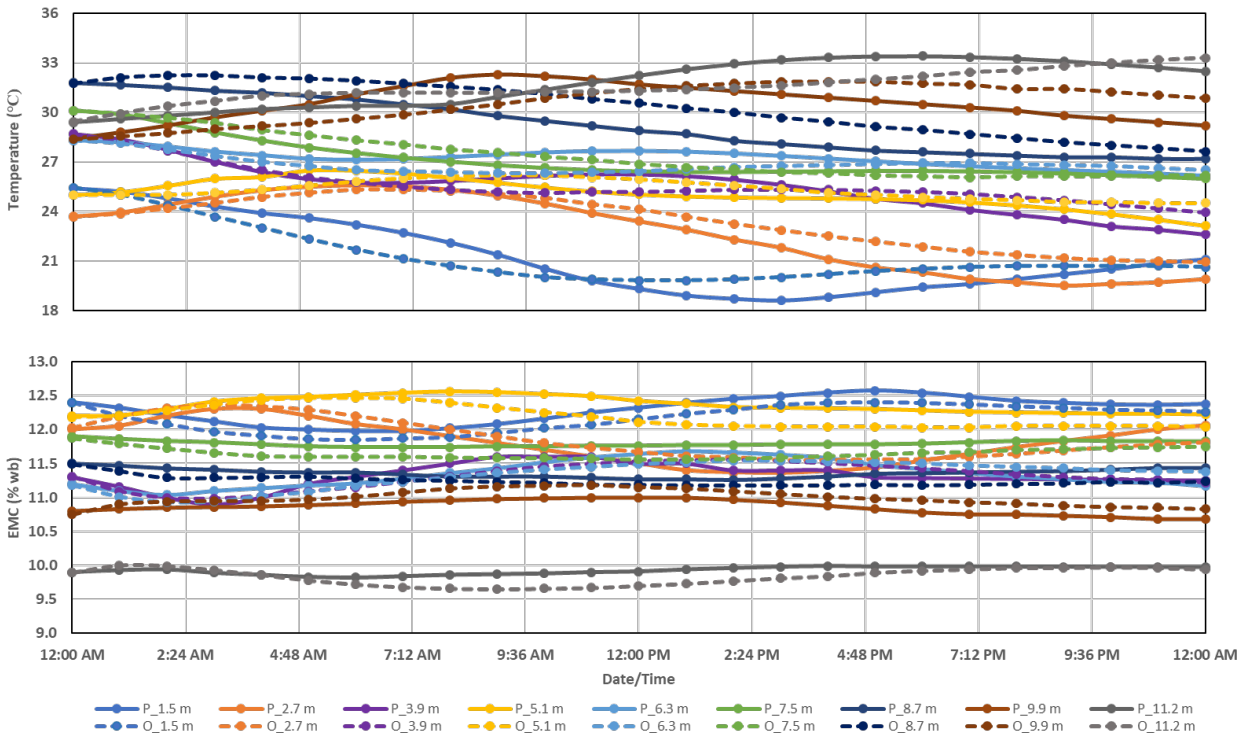

Fig. 2. Comparison of predicted and observed data during 24 h aeration cooling period.

Results showed that the cooling and moisture adsorption rate decreased along the bulk height. This was attributed to the loss in the release of the heat of wetting up the bulk (Fig. 3.). Negative values signified that h_v was less than h_s during the aeration cooling period due to extra heat taken to absorb the moisture into the grain.

However, negligible changes were observed above 8.5 m resulting in negligible moisture changes. During the cooling and re-wetting period, a linear decrease in h_w was observed at a particular grain depth (Fig. 4.), thus illustrating the decreasing cooling effect of the interstitial air along the height. The linear trend could be due to the assumption of constant moisture across the thin layer that might not be true as the grain moisture also depends on the temperature at every zone.

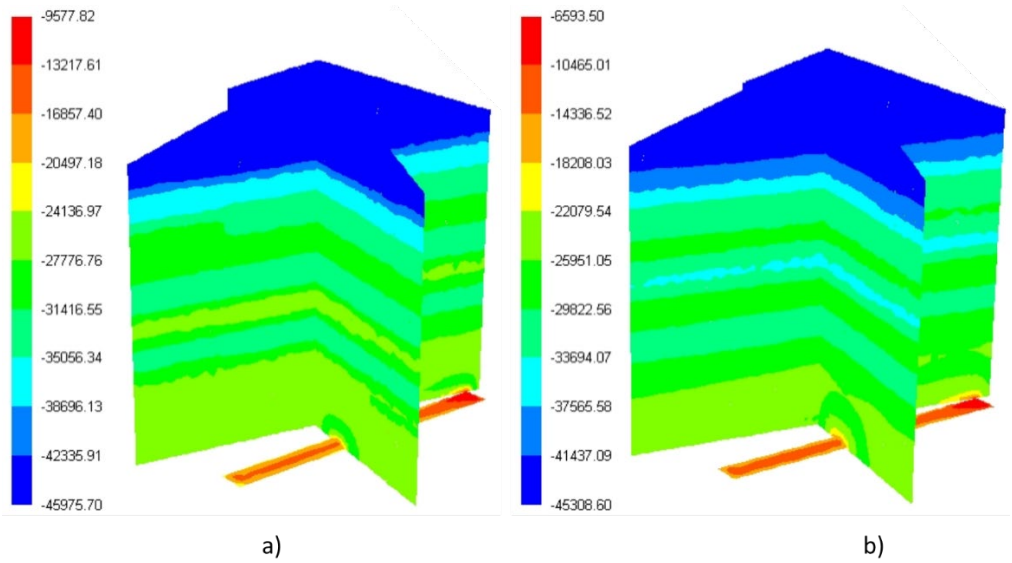

Fig. 3. Contour plots of heat of wetting (J/kg): a) Before start of the aeration; b) After 24 h aeration.

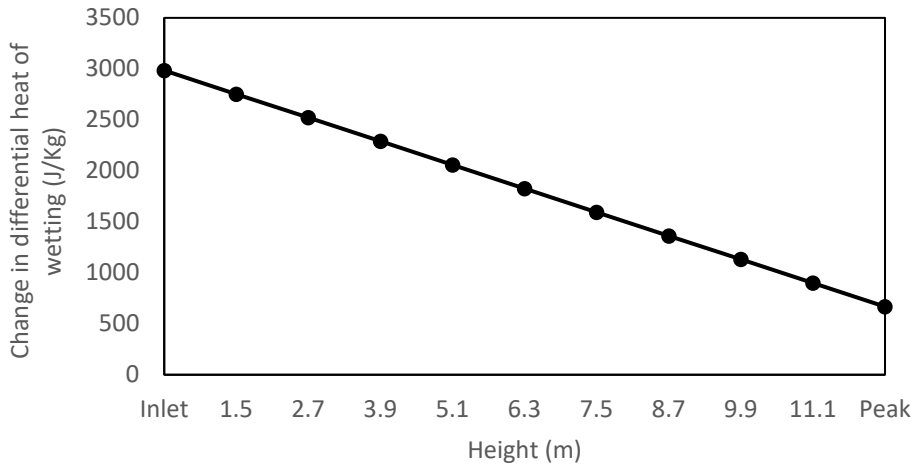

Fig. 4. Differential heat of wetting values between initial and final grain after 24 h aeration.

The above figure shows that to achieve efficient cooling along the height, the differential h_w should be maintained to a near-constant value. This can only be achieved by increasing the inlet velocity resulting in a higher temperature differential between incoming air and grain along the height.

Future work by the authors will include validating the above process on how the differential h_w varies with doubling the inlet velocity.

Conclusions

An integrated 3D CFD model was successfully developed with an inlet control system to predict the fan operation and the factors affecting the cooling potential in a 1,000-t storage silo. Mesh size, time-step size, and solutions methods were optimized for the above process. The optimized parameters resulted in a computational time of 2.25 h for simulating a 24 h real time aeration with SEP of 1.39°C and 0.15% wb and MRD of 5.1% and 1.2%, for temperature and grain moisture, respectively. Results showed that opting a scheme was model-specific and could not be assumed based on another model's output. A decreasing trend in differential h_w was observed during the cooling and rewetting process.

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CAF2020 Abstract No. A-2-4-17

Casada ME, Siliveru K, Arthur F, Brabec D, Campbell J, Maghirang R, Maier D, Conley T, Jones C (2021) Phosphine distribution patterns during fumigation in temporarily-sealed steel silos. Page 62. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Phosphine distribution patterns during fumigation in temporarily-sealed steel silos

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ABSTRACT

Phosphine is used extensively as a fumigant for controlling insects in stored grain. Effective application of the phosphine can be hampered by poor distribution of the gas. Leaks in the enclosure, foreign material in the grain, and improper placement can cause areas of insufficient phosphine concentration, allowing insects to survive and leading to phosphine-resistant insect populations. Phosphine concentrations were evaluated during field tests in temporarily sealed silos to compare distribution from application with conventional probing of aluminum phosphide tablets to the distribution from application with a closed-loop recirculation system. Constant pressure tests were performed on the silos prior to fumigation to assess leakage of the silo envelope. Large differences were observed between phosphine concentration patterns for the two application methods. Application with conventional probed tablets resulted in uneven distribution patterns as well as leakage over time, leading to many areas in the lower half of the grain mass remaining below the target phosphine concentration for the entire period of fumigation. Application with the closed-loop system using the same phosphine dosage yielded more uniform phosphine concentrations but there were equal or greater phosphine losses from leakage. These results highlight the need for even gas distribution from the phosphine application system and to exercise care to temporarily seal silos adequately to prevent excess leakage.

Keywords: Phosphine, AlP tablets, CLF, leakage, Constant pressure test

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Ducom V, Simioni F, Mercadal D, Busson D (2021) A comparative study of phosphine distribution using two application methods with 30,000 gas concentration measurements. Pp. 63-70. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

A comparative study of phosphine distribution using two application methods with 30,000 gas concentration measurements

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Abstract

Uniform phosphine distribution inside a silo is key to an effective fumigation. To eliminate all insect life stages, and avoid phosphine resistance, gas concentration needs to be held above 200 ppm throughout the storage space during the exposure period. The silos used in this study were four identical, 10,200 m^3 concrete cylinders each containing 8,000 tonnes (t) of durum wheat at a temperature between 27 and 30°C. The silos were partially sealed and did not have a recirculation system. Ten PhosCapt-MP phosphine monitors recorded and transmitted gas concentrations every 3 h from a total of 104 locations (26 points for each silo). The silos were divided into four 5 m vertical sections with 5 monitoring points located inside the grain of each section at East, West, North, South, and Center. Three additional points were located in the headspace and 3 others in the lower ventilation galleries. There were four treatments of two dosages (1.5 g and 3 g/m^3) generated from Aluminum phosphide (AlP) bag blankets. One of each dosage was placed at the top of two of the silos, and one of each dosage at the bottom of the other two silos. The fumigation monitoring was conducted over 37 d, recording a total of 30,784 measurements. Gas from the blanket introduced at the top quickly penetrated to a depth of 10 m from the top out of a total depth of 22 m of grain and reached 200 ppm in the first 12 h of fumigation, but not long enough to be effective. Then, its progression became very non-uniform for both gas dosages. In the bottom half of the silos, the concentrations never reached 200 ppm. From the blanket placed at the bottom, the gas propagation, regardless of the dosage, was slower and more uniform. It took 7 d for the gas to reach 200 ppm at 10 m from the silo bottoms and 10 d to obtain a complete admixture throughout the whole depth of the silo that was maintained for more than a week above 200 ppm. To conclude, introduction of fumigant at the bottom worked very well. The PH_3 convection inside the silo was also analyzed.

Keywords: Phosphine, PH_3, Fumigation, Gas distribution, Phosphine monitoring, Silo

Introduction

Uniform phosphine distribution inside a silo is key to an effective fumigation. To eliminate all insect life stages, and avoid phosphine resistance, gas concentration needs to be held above 200 ppm throughout the storage space during the exposure period. In large deep silos, it is difficult to attain uniform concentration. The goal of our study was to characterize the differences in phosphine penetration and distribution into a grain mass under real conditions using four silos each holding 8000 t of durum wheat, using two types of applications, one from the top of the silo and the other from the bottom. Two doses were tested.

Materials and methods

This study was conducted in Baziège, France in August 2019. The ambient air temperature was 22 – 29°C (26°C average) at the beginning and 18 – 25°C (22°C average) at the end. The tests were carried out at the Arterris cooperative site in four concrete silos (each silo was 24.5 m diameter, 19.8 m high and 5.5 m high cone-shaped metal roofs thus with a volume of 10,200 m^3 and held 7,600 to 8,000 t of recently harvested durum wheat). The grain conditions at the beginning were fairly homogeneous: temperatures 27 – 30°C, 11.5 – 13.7% moisture content, 810 – 820 kg/m^3 density, and 13 – 13.4% protein. The roofs were gas-proof, but not perfect. All four silos were equipped at the bottom with 24 ventilation pipes. The gassing was carried out using Aluminum phosphide generators Detia-Degesch Bag Blankets (BB) and mini-Bag Blankets (mBB). Each 3.4 kg BB released 1.1 kg of PH_3 and each 680 g mBB released 226 g of PH_3. The mBB were used for the bottom short-length aeration system. Silos A and C were fumigated with a dose of 1.5 g/m^3 PH_3, using 14 BB, and Silos B and D were fumigated with a dose of 3 g/m^3 using 17 BB and 50 mBB. Silos A and B had BBs placed at the top, and silos C and D had BBs and mBBs placed at the bottom.

The concentrations were measured every 3 h by 10 dual-sensor PhosCapt-MPs with email reporting (Fig. 1) from 26 locations in each silo (Fig. 2). Each device monitored twelve 4 mm ID PE lines up to 200 m length, with automatic sensor selection between high concentrations (up to 15,000 ppm, 1 ppm precision) and low concentrations (0.1 ppm to 20 ppm, 0.01 ppm precision). All the sensors were calibrated with the same gases at 940 ppm and 5 ppm.

Fig. 1. PhosCapt®-MP: 12 lines Phosphine monitor. CaptSystemes, France. (phoscapt.com)

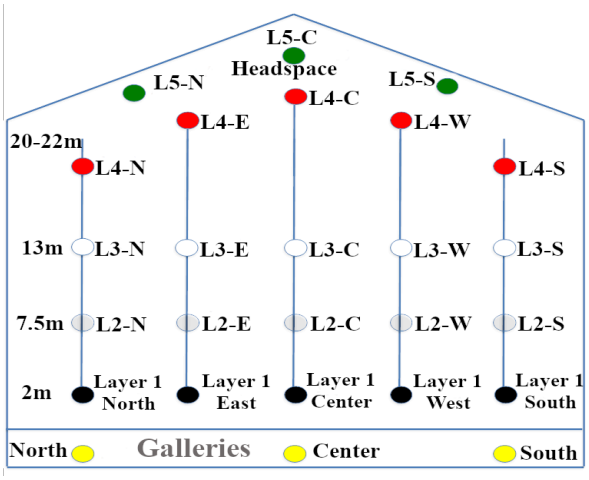

Fig. 2. Phosphine measurement locations in a silo.

The non-centered measurement lines were attached to the temperature sensor cables located halfway between the center and the wall (5.5 m from the wall). The four silos were fumigated in passive mode, meaning that no recirculation was used during the fumigation. The gassing operations were carried out simultaneously. One team gassed Silo A, while another team gassed Silo C. The same for Silos B and D. For silos gassed from the top: the closed BB were deposited in the center of the silo. Two operators opened the BB and arranged them in a star pattern on top of the grain. For silos gassed from the bottom: the closed BB were arranged in front of each ventilation pipe. Two operators began the gassing from the first pipe to be gassed, then went on to the next one. The BB were opened and were inserted into the pipes. The mini-BB were deposited at the pipe entrances. Efficacy was assessed by the gas measurements. The target was to maintain ≥ 200 ppm (Noyes and Philips, 2004) during the fumigation based on the temperature. In our case, temperature was at 27 – 30°C. Therefore, the minimum exposure time at 200 ppm was 144-168 h (Ducom, 2005).

Results and discussion

With 104 measurement locations in four silos and a 3 h measurement interval over 37 d, we had a total of 30,784 measurement data. Phosphine released from the top (Silos A and B) penetrated rapidly into the first few meters at the top of the silos, reaching 2,000 to 4,000 ppm (Fig. 3). We then observed the low phosphine concentration at lower layers of the grain mass which was below 100 ppm. The same trend was found in Silo B with the double dosage. These results differ from those found by Williams et al. (1996) for 2500-t silos gassed from the top with blankets. In their trials, the overall concentration was efficient at all levels, including the bottom, due to the very good sealing of the silos.

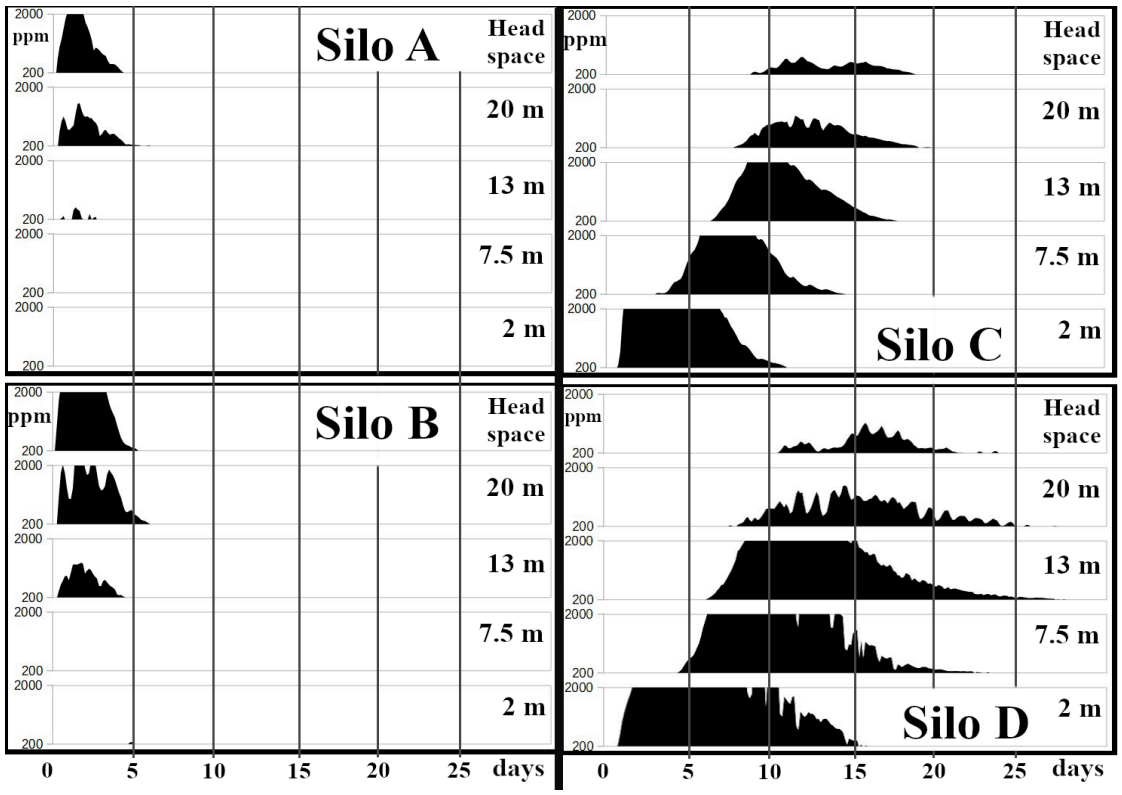

Fig. 3. Average concentration in the four silos (range: 200 to 2000 ppm)

The concentration time (Ct) products in each layer were calculated, not for the efficacy evaluation, but to calculate the quantity of gas. We noticed a huge difference in the Ct of PH_3 between gas releasing at the top and at the bottom (Table 1, Fig. 4). We also noted in Silo D (with a double dose at the bottom of the silo), a doubling in the Ct values for the bottom three layers, compared to Silo C (with a single dose). The differences in Ct products in the upper layers of Silos C and D were lower. This could be explained by a possible gas leakage at the top of the silos, even though the silo roofs were sealed.

Table 1. Concentration time product above 200 ppm in different layers (kppmh: kilo ppm × hour).

Layers	Silo A	Silo B	Silo C	Silo D
L5 Headspac	**122**	**269**	**112**	**156**
L4 20 m	**90**	**188**	**191**	**308**
L3 13 m	**15**	**77**	**343**	**694**
L2 7.5m	**0**	**0**	**410**	**812**
L1 2 m	**0**	**0**	**652**	**1174**

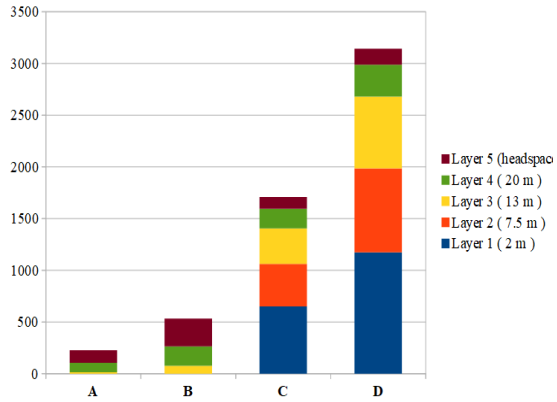

Fig. 4. Concentration time product above 200 ppm in different layers (kppmh).

During gas releasing, concentrations increase until the end of hydrolysis is at a peak and then start to decrease. The decrease is due to gas diffusion, sorption and leakage. In silos gassed from the top, the PH_3 peaks in the highest layers of the grain occurred between 30 and 51 h. In the lower layers, the peaks never reached 50 ppm (Fig. 5).

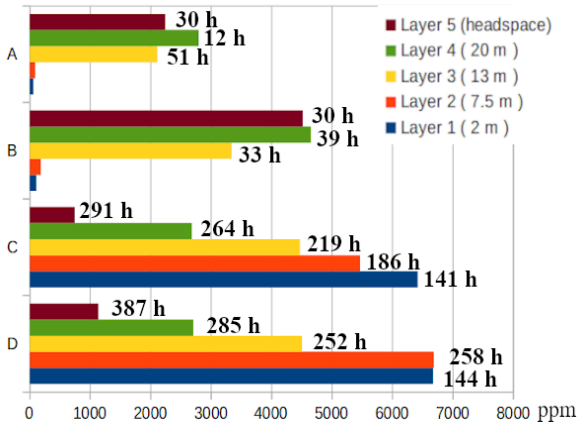

Fig. 5. Silo layer concentration peak times and values

Gassing from the bottom looked very different. In Silo C, where the PH_3 generators were placed in the ventilation ducts, the concentrations stayed above 11,000 ppm for about 100 h with a peak at 12,400 ppm. In Silo D (double dose), the ventilation duct concentration values were the same as for Silo C (single dose).

Gassing from the bottom showed a very slow gas penetration rate. Peaks at 1 m from the top of the surface (Layer 4) were obtained in 264-285 h (11 d). The peaks in the headspace were obtained in 12 to 16 d (Fig. 5). However, concentrations were high at all layers, including the highest layer where they reached nearly 400 ppm. Thus, there was a slow but remarkable rise in concentrations.

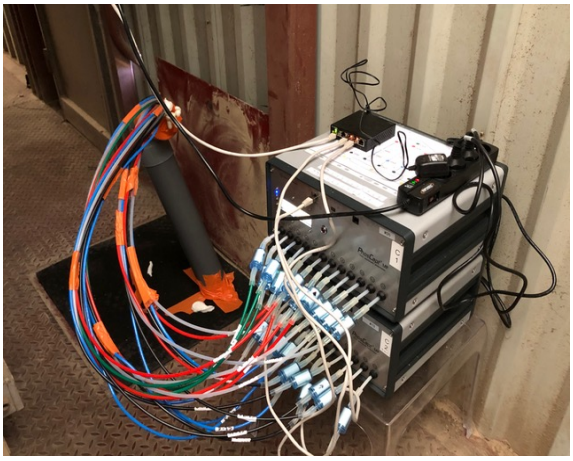

Fig. 6. Two PhosCapt-MPs placed in Silo C, monitoring 23 lines

The PhosCapt-MP (Fig. 6) is capable of measuring concentrations of up to 15,000 ppm (Fig. 5). Preliminary gassing tests in the bottoms of different silos showed that the concentrations never reached more than 12,000 ppm for application doses of 3 g/m^3 PH_3. This could have been due to a lack of water vapor that naturally limited the AlP hydrolysis speed and, as a result, the instantaneous quantity of PH_3 produced. We were thus well below the 17,900-ppm value, the flammability zone of phosphine (Green et al., 1983).

The fumigation insecticidal efficacy reference is the tandem '200 ppm for 144 h'. Thanks to the very large number of measurements taken, it was possible to precisely determine the ranges where the duo ≥ 200 ppm for > 144 h was obtained. In Silos A and B, (application from the top of the silos) did not allow us to obtain this tandem in all the layers. Fumigation was not effective. However, in Silos C and D (application from the bottom), efficacy was obtained in all the layers for the two application doses (1.5 and 3 g/m^3) (Table 2, Fig. 7).

Table 2: Exposure time (h), per layer, based on the concentrations above 200 ppm

Layers	Silo **A**	Silo **B**	Silo **C**	Silo **D**
L4 20 m	**135**	**135**	**294**	**444**
L3 13 m	**51**	**99**	**279**	**534**
L2 7.5m	**0**	**0**	**282**	**459**
L1 2 m	**0**	**0**	**252**	**369**

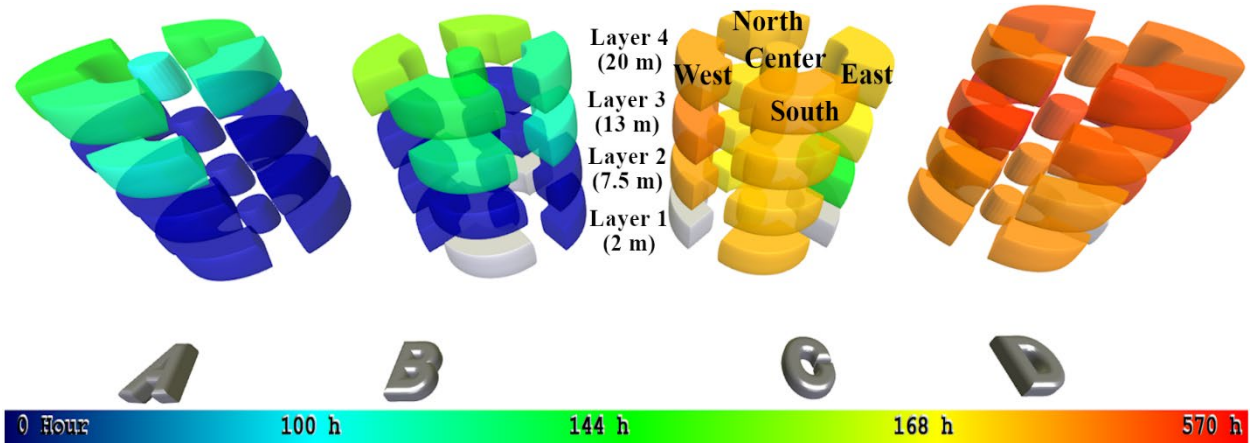

Fig. 7. 200ppm exposure time 3D cartography (Grey = data unavailable).

Phosphine concentrations in the grain were extremely variable in time and space. Constant gas movements were observed despite very stable general climatic conditions. The raw values given by the measuring devices showed very large fluctuations mainly in the center axis as shown in Figs. 8 to 11.

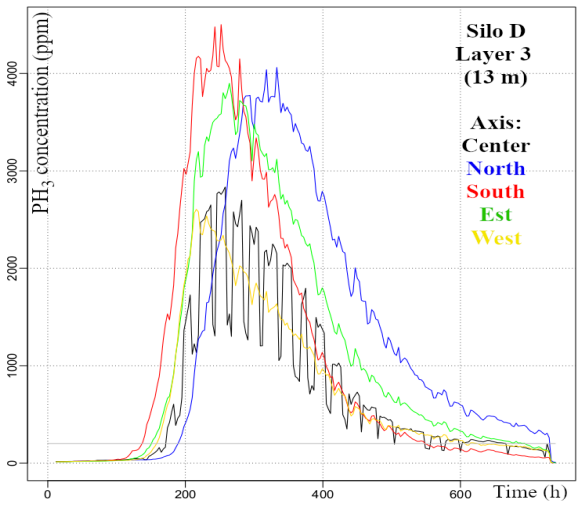

Fig. 8. Evolution of PH_3 concentration near the middle of Silo D, Layer 3 (13 m)

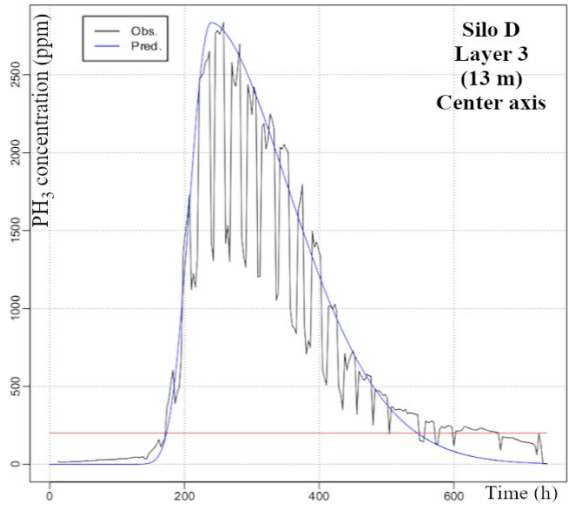

Fig. 9. Daily oscillations at the center axis

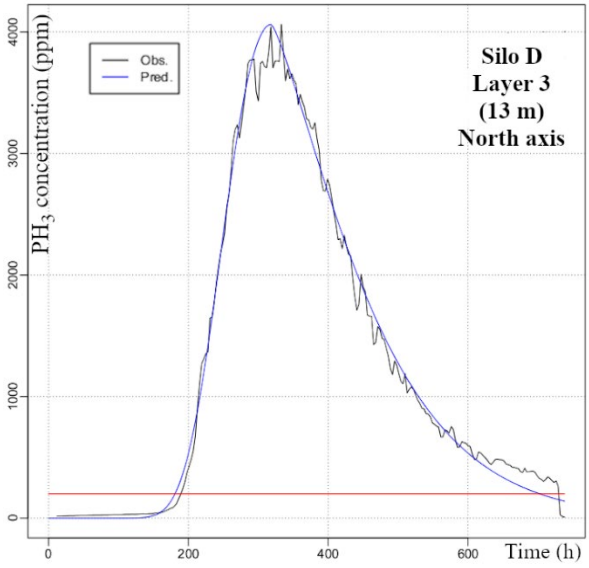

Fig. 10. Smaller oscillations in the north axis

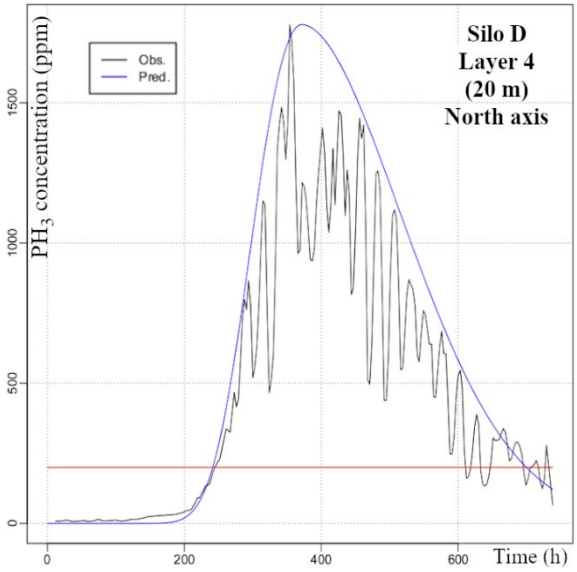

Fig. 11. Oscillations near the surface level

All the results for each line and each silo were subjected to a parametric smoothing to check the consistency of the measured values. This consisted in three steps: an apogee coordinate estimation, an ascending branch adjustment by a function inspired by the log-normal distribution probability density, and a descending branch adjustment by a function inspired by the Weibull distribution survival function. The curves were thus much more readable and showed the general trend (Figs. 9-11) despite the regular daily concentration oscillations.

The continuous measurements allowed us to observe remarkable daily oscillations for the first time in 8,000-t silos (Figs. 8-11). For the central lines, we noticed a regular daily evolution with a high amplitude: the concentration was the lowest in the morning, and the highest in the evening (Figs. 8-9). The Silo D center axis values showed a 1500 ppm variation for a 2800 ppm concentration. However, the oscillations of the non-centered lines (North, South, East and West) were much less accentuated. Their amplitude remained under 300 ppm as shown in Fig 10. Near the surface level (20 m), the PH_3 oscillations in the grain fluctuated in the morning and evening on all lines, but with a stronger and more irregular intensity. These oscillations were even accentuated on the central line. The "chimney effect" is clearly observed in Fig. 11.

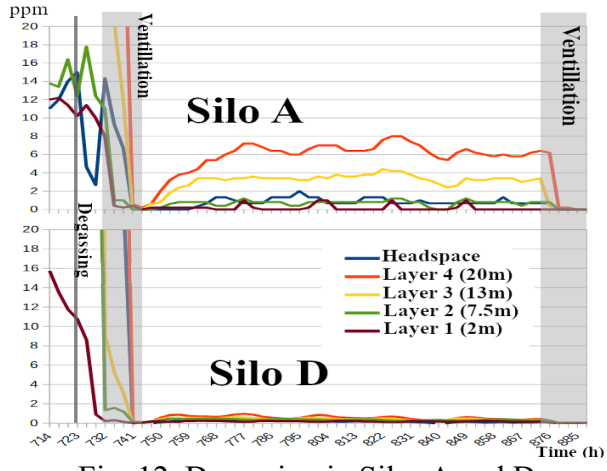

Fig. 12. Degassing in Silos A and D

The degassing started after 30 d (723 h) under gas. Ventilation ran for 12 h starting at hour 732. There was still between 30 and 50 ppm of gas in the silos gassed from the top. As we can see in Fig. 12, the fall in the concentrations was very rapid, reaching zero ppm in about 10 h.

When ventilation stopped, we then witnessed a slow rise in concentrations of 1 to 5 ppm in Layers 4 and 5 in Silo A, and less in the other layers. These values were quite stable for 5 d. The passive degassing was very slow. Ventilation was restarted 5 d later and the gas was completely evacuated in a few hours. For the silos gassed from the bottom, the concentrations measured in the grain were 50 to 160 ppm. After the first 12-hour ventilation, the concentrations dropped to zero ppm and rose again between 0.2 and 1 ppm. We noted that under the test conditions, the degassing was very rapid, thanks to the ventilation. After 7 d of degassing, the sorbed PH_3 was totally evacuated at hour 888 after the second ventilation cycle.

Conclusions

This full-scale trial was carried out in four 8,000-t silos of durum wheat. Measured concentrations from 104 locations during 37 days gave PH_3 concentration values at a 3-h interval. Our data showed that gassing from the bottom gave a total efficiency at all levels, estimated by the threshold of 200 ppm for 6-7 days. On the other hand, gassing from the top gave no efficacy throughout the silo, even at double the dose. This trial showed a large difference in gas distribution when gas was introduced from the bottom or from the top of a silo. Phosphine application is still in development in France, where silos were not built for fumigant use and are rarely gas-tight. The empirical data from our several million tonnes of treatment to date has taught us that everything is fumigable if we develop new fumigation techniques using multi-point monitoring. Our goal is to be efficient and not create PH_3 resistance, even when fumigating non-sealed silos.

For the first time, thanks to the 30,000+ measurements, we were able to visualize PH_3 distribution in all of its complexity. As our friend Jan Van Graver used to say, "If you are not monitoring, you are not fumigating." We can add today, "Monitor to better understand, monitor to innovate, monitor to succeed."

Acknowledgements

We would like to thank Patrick Ducom for his advice and always pertinent analysis; Thierry Ducom (CaptSystemes, France) for the data collection and Yves Le Gat (INRAE, France) for the statistical analysis. Thanks also to the Arterris cooperative for letting us use their installations with 32,000 t of durum wheat.

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Chaudhry MMA, Nadimi M, Hervet V, Paliwal J (2021) Understanding insect movement behavior in chickpea flour using X-ray micro-computed tomography. Page 71. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Understanding insect movement behavior in chickpea flour using X-ray micro-computed tomography

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ABSTRACT

Nutritional profiles of flours can be significantly altered, both chemically and physically, due to insect infestations. Chickpeas are a rich source of nutrition, especially protein, and their flours are used in different food formulations at varying fineness levels (particle sizes). This emphasizes the need for understanding and predicting insect movement in various streams of chickpea flours obtained from roller mills. Developing pest control strategies for stored chickpea flour require *a priori* knowledge of insect movement and behavior. The aim of the current study was to investigate the potential of 3D X-ray micro-computed tomography (micro-CT) for mapping the paths navigated by insects in chickpea flours at three different fineness levels obtained from a roller mill. Our preliminary results indicate that it was possible to identify the path length, pattern and direction of movement of *Tribolium madens* (Charpentier), black flour beetle, through flour bulks. This important behavior of insects can be utilized in developing appropriate pest management studies in flours.

Keywords: Chickpea, Flour, Roller mill, Black beetle, X-ray micro-CT

CAF2020 Abstract No. A-2-7-20

Buenavista RME, Casada ME, Siliveru K (2021) Determination of phosphine sorption isotherm in hard red winter wheat. Page 72. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Determination of phosphine sorption isotherm in hard red winter wheat

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ABSTRACT

With the growing population of phosphine-resistant stored grain insect pests, the sustainability of phosphine as an effective fumigant has been put at risk. Research studies are still insufficient to explain inconsistent phosphine concentrations in the grain bin during treatments. Limited data were gathered about sorption of phosphine into wheat kernels during fumigation. To fully account for this phenomenon in fumigation modeling studies, it is necessary to determine the sorption behavior of phosphine within the grain mass in response to varying environmental conditions and phosphine dose. The objective of this study was to characterize the phosphine sorption isotherm in wheat as affected by phosphine dose and temperature. Phosphine sorption isotherms in Hard Red Winter Wheat that has not been fumigated before were determined at 15°C, 25°C, and 35°C under a relative humidity level of 65%. Seven dosage levels (300, 600, 900, 1200, 1500, 1800, and 2100 ppm) were injected in separate airtight glass fumigation chambers with one-half wheat filling ratio. Phosphine headspace concentration was monitored at 2 h intervals for the first 8 h and once every 24 h until it approached equilibrium using a gas chromatograph equipped with a flame photometric detector set to phosphorus mode. The plot of sorbed phosphine concentration versus headspace phosphine concentration was fitted to various sorption isotherm models. Langmuir, Freundlich, and Redlich-Peterson sorption isotherm models were fitted to the sorption experimental data. Rate of phosphine sorption increased with an increase in temperature and decreased with time. Total sorbed phosphine at equilibrium is significant in determining the rate and maximum quantity of phosphine uptake in wheat. Phosphine sorption isotherm model for wheat kernels is useful in estimating the quantity of phosphine residue that needs to desorb from wheat kernels at given temperature and applied concentration.

Keywords: Phosphine sorption, Sorption isotherm, Sorption equilibrium, Wheat

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Jian F, Jayas DS (2021) Implications of dockage and foreign material distribution for fumigation and controlled atmosphere storage of grain in bins. Pp. 73-79. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Implications of dockage and foreign material distribution for fumigation and controlled atmosphere storage of grain in bins

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Abstract

In addition to sound grain kernels, stored grain contains many other materials such as chaff, straw, fine materials, dust, sand, animal droppings, dead insects, or insect fragments. Segregation of these materials is an unavoidable phenomenon during grain transportation, loading, and unloading. Center filling of grain into silos usually results in a concentrated accumulation of small and high-density particles (smaller than the sound grain kernels) at the center of the bin and particles with large sizes (larger than the sound grain kernels) and low densities (lower than the sound grain kernels) at the periphery. The accumulation of the dockage and foreign materials at the center of the silo attracts insects and favors insect and fungi growth and multiplication. Uneven distribution of these materials could result in slower movement of fumigants and applied gas in the center and faster movement at the periphery, possibly resulting in non-uniform distribution of fumigants and applied gas. This non-uniform distribution might result in low insect mortality in the stagnant and low concentration zones. Implications of this characterized distribution were discussed in the context of fumigation and controlled atmosphere. The dominant mechanisms resulting in this non-uniform distribution were discussed.

Keywords: Dockage, Foreign materials, Segregation, Fumigation, Controlled atmosphere

Introduction

Commercially stored bulk grain encompasses desired sound grain kernels and undesired materials including (but not limited to) straw, rachis, internode, chaff, awn, un-threshed spikelet, shrunken and broken kernels, stone pieces, other grains, weed seeds, ergot, insect excreta, dead insects, bird droppings, and animal filth. These undesired materials are usually referred to as dockage and foreign materials, which have different physical and chemical properties than sound grain kernels especially in size, shape, density, color and textural properties, and nutritional value. Dockage is usually referred to as any material that can be removed from the grain by using approved cleaning equipment such as mechanical dockage testers or sieves (Canadian Grain Commission, 2019), while "material other than grain of the same class that remains in the sample after the removal of dockage" is defined as foreign materials. Therefore, foreign materials are other grains or materials which have the same shape and size as the grain kernels. Grain may be handled and stored at

different stages, from harvesting to consumption. Segregation of these components during loading and unloading occurs, and dockage and foreign materials (DFM) in stored grain bulks do not have a uniform distribution due to their segregation. Uneven distribution of DFM, as well as grain kernels with different sizes, can cause many problems during storage, insect control (fumigation and controlled atmosphere storage), drying, and aeration because segregation of DFM in a grain bin can change the uniformity of small intergranular pores (Olatunde et al., 2016).

Fumigation is the process of using a gaseous fumigant or an aerosol in sufficient lethal concentration to control insect pests and/or microorganisms at the desired temperature, pressure, and relative humidity. In controlled atmospheres storage, intergranular gaseous composition is altered by modifying concentrations of carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2) to create lethal concentrations of high CO_2 or low oxygen (O_2). Fumigation or controlled atmosphere treatment of stored products is carried out in airtight enclosures and structures such as warehouses and silos. To successfully control insect pests, the processes of fumigation and controlled atmosphere ideally require uniform distribution of the applied gases inside the entire storage structure. This uniform distribution requires that the applied gas be uniformly forced through the pores among the grain kernels in the grain bulk. The uneven distribution of DFM will result in non-uniform distribution of the applied gases. In this study, we will analyze the implications of DFM material distribution for fumigation and controlled atmosphere in storage grain bins.

Segregation mechanisms

Segregation

Segregation is the tendency of particles with similar physical properties to collect in one zone during handling of free-flowing bulk materials (de Silva et al., 2000). In the grain industry, this segregation phenomenon usually occurs during heap formation when free-flowing bulk materials are filled into bags, silos, and hoppers (Fan et al., 2017; Jian et al., 2019). On the contrary, the behavior of cohesive or poorly flowing bulk materials (such as high moisture grain) is controlled by interparticle adhesion forces, which reduce the mobility of individual particles and therefore their tendency to segregate. The segregation process of different particles in bulk materials, particularly bulk grain mixtures, is very complicated because many factors and mechanisms are involved.

Mechanisms of particle segregation in bulk materials have been reviewed by several researchers and many different segregation mechanisms including rolling, sliding, embedding, sifting, avalanche, trajectory, fluidization, impact, displacement, percolation, air current, agglomeration, push-away, and bouncing have been identified (de Silva et al., 2000; Fan et al., 2017; Jian et al., 2019; Tang and Puri, 2004). Typically, more than one of these mechanisms occur simultaneously, and some of these mechanisms overlap each other or may be considered a special case for another mechanism (Jian et al., 2019; Tang and Puri, 2004). Furthermore, some mechanisms do not apply to bulk grain. For instance, agglomeration segregation can occur only during the mixing fine particles with a diameter smaller than 50 μm, or in cohesive fine particles like powders, due to interparticle forces (Tang and Puri, 2004). Narendran et al. (2019) observed the segregation effects of rolling, sliding, impact segregation, fluidization, trajectory, and avalanches during the loading of wheat mixtures with 3 or 6% in total of canola, kidney bean, and soybean into a bin. Jian et al. (2019) categorized the primary patterns of segregation into four main mechanisms occurring in bulk grain handling as: trajectory, fluidization, sifting, and impact segregation.

Trajectory segregation

Trajectory segregation is the combined effect of particle momentum and air drag, which changes the trajectory of moving particles. At the initial velocity of grain kernels being loaded into a bin, large particles have a higher momentum than small particles. In addition, different shapes of particles will have different air resistances (drag force) during moving or falling. This combined effect causes the larger particles to travel farther than the smaller particles (Fig. 1). Trajectory segregation is significant when bulk materials are being loaded in a horizontal or an inclined direction (such as loading into a bin using an inclined auger or pneumatic conveyor). It can also occur when the dropping height is high, or during relatively high rolling or sliding velocity scenarios. Hence, the rolling or sliding segregation effect on a heap is considered to be a special case of trajectory segregation (Tang and Puri, 2004). The result of trajectory segregation during central loading is that fines, dusts, broken grain kernels, and small stones are concentrated at the central dropping location, while larger particles such as chaff are deposited closer to the periphery of the bin (Jayas, 1987).

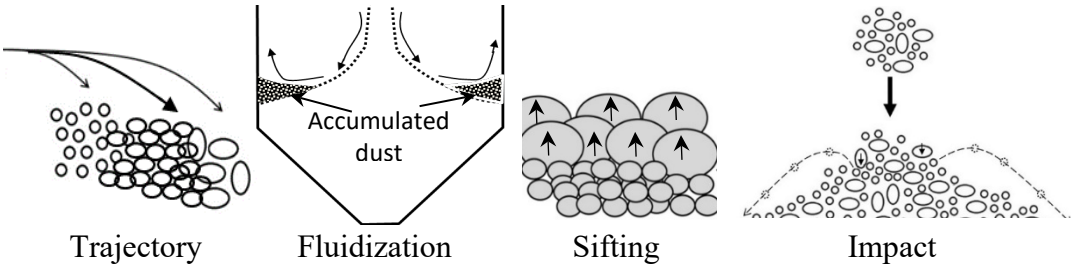

Fig. 1. Schematic of four primary segregation mechanisms in bulk materials suggested by Jian et al. (2019).

Fluidization segregation

Fluidization segregation can occur when a mixture containing fine particles and larger particles with low density (such as chaff) is loaded into a bin because the falling stream of mixture can induce air currents that carry the fine particles, or particles with low density to the periphery of the bin (Fig. 1). A greater drop height and loading rate can cause the air current effect to increase. If the grain bin is loaded several times periodically, the fine particles and less dense materials may be concentrated in layers near the bin walls. Fluidization segregation can also occur during grain loading and unloading when the mixture is moving.

Sifting segregation

Sifting segregation can occur when particles in the grain mixture roll or slide down on the surface of a grain pile (Fig. 1). The heap surface acts like a sieve because smaller particles are more likely to be embedded in the surface pores and gradually sink down to the bottom of the moving layer. Larger particles have a higher probability of sliding or rolling down further from the top of the heap and stay at the surface level of the heap (Tang and Puri, 2004). This embedding effect is a special case of the sifting phenomenon in grain segregation.

Impact segregation

Impact segregation is caused by the collision of moving particles during grain loading. When particles on surface of the heap are hit by the particles being loaded, the small particles due to their lower mass and lower momentum gain a higher velocity, and consequently tend to bounce farther

down the grain pile (Fig. 1). Impact segregation may result in a wider distribution of some small particles.

Distribution of DFM

Dominant mechanism during center loading

Grain silos are usually filled continually from the top center of the bins. Trajectory and fluidization segregations occur before particles are loaded on the grain heap, while impact segregation occurs when particles hit the heap, and sifting segregation occurs when particles are moving on the surface of the heap. The impact segregation acts in contrary to the sifting segregation (Jian et al., 2019). If the concentration of fine particles is low, the sifting segregation becomes more significant, while fluidization is the dominant mechanism of segregation at a high dropping height of the grain mixture with a high concentration of fine and less dense particles (Tang and Puri, 2004). Dusts and chaff might concentrate at the peripheries by the fluidization segregation but will not generate layers of dust if the grain is continually loaded. Avalanching might occur on the surface of a heap during bulk material loading when stationary layers are formed near the center of the heap. These layers gradually become unstable with increasing thickness of the layer and suddenly slide down the heap. Avalanching can intensify sifting segregation.

Distribution of DFM

When grain mixtures are center-loaded, the dominant segregations usually result in a concentrated distribution of small and high-density particles (smaller than the sound grain kernels) at the center of the bin, and particles with large sizes (larger than the sound grain kernels) and low densities (lower than the sound grain kernels) at the periphery. Even though different researchers had different conclusions on the effect of a single factor influencing segregation, researchers usually reported this similar distribution pattern (Bartosik and Maier, 2006; Jian et al., 2019; Narendran et al., 2019; Salarikia, 2020). In a 10-meter diameter bin, Salarikia (2020) found fine particles, dust, fragments, and foreign materials (corn and soybean kernels) mainly accumulated in the center, while shrunken and broken kernels accumulated mostly near the wall of the bin. Loading height did not influence true density and test weight of clean and unclean wheat. Thousand kernel weight, dimensions, and sphericity of wheat kernels were similar at different radial locations of the bin at different loading heights.

Detrimental effect of segregation on stored grain

Change in airflow resistance

The increase of fine particles (particles smaller than sound grains) increases the airflow resistance of bulk grains, while the increase of chaff (particles larger than sound grain kernels) has an inverse effect and decreases the airflow resistance. The accumulation of fine particles is of greater concern because it can create regions of high resistance, and fumigants or controlled atmosphere gases may not reach these areas at the same level as the rest of the bulk. Therefore, most of the papers demonstrating increase in airflow resistance due to fines are summarized in the following sentences. One of the most detrimental effects of segregation is the decreased pore size and porosity because fine particles fill the voids among grain kernels. After pores are filled by fine particles, the shape of the pores might also change. Decreasing pore size and porosity, along with any change of shape, result in an increase of airflow resistance (Górnicki and Kaleta, 2015 – Part I; Górnicki and Kaleta, 2015 – Part II). The airflow resistance of bulk corn increases with an

increase of fine particles smaller than 4.76 mm (Haque et al., 1978). Similarly, Grama et al. (1984) reported that with an increase in fine particles in shelled corn and with a decrease in size of these fine particles, airflow resistance increased. When these fine particles are removed from bulk corn either by screening or aspiration, airflow resistance is reduced significantly. Pressure drop in clean bulk wheat is lower than that of unclean wheat (Kumar and Muir, 1986). In oat seeds, the presence of fine materials has the same expected effect and increases the airflow resistance (Pagano et al., 2000). The increase of fine particles increases the airflow resistance of bulk flax seeds, while the increase of chaff has an inverse effect and decreases the airflow resistance (Pagano et al., 2000).

When grain is center-loaded into a bin with a partially perforated floor, a peak at the center of the bin rises at the repose angle of the grain, and this will further increase the airflow resistance which could then decrease the effectiveness of fumigation and controlled atmosphere. Aeration studies can further explain the effect of differential airflow resistances on fumigant distribution because such studies have not been done with fumigants. During aeration, Panigrahi et al. (2020) reported negligible airflow coming out from the grain peak with a quadratic increase in velocity from the peak to the walls. They found about 14.3% of the total grain volume, including a significant proportion of the top grain volume, exhibited a lower airflow rate than the recommended. Because 85% of the silo floor was non-perforated, 12.6% of the lower grain volume (up to a maximum height of 4.4 m above the floor) exhibited lower airflow than the recommended. Olatunde et al. (2016) reported the porosity at the core was lower than that near the walls. The solution for this uneven airflow resistance is to core the grain in order to reduce the height of the center grain. Bartosik and Maier (2006) found that the fines were 2.27% (from 0.77 to 3.55%) at the center, and 0.54% (from 0.11 to 0.93%) at the periphery in 14 farm bins. When the grain peak was not leveled in these 14 farm bins, the airflow distribution resulted in a non-uniformity factor of 89% versus 36% after coring.

Insect and fungi multiplication

Stored grain insects usually prefer grain with a high percentage of dockage, broken kernels, and foreign materials. Locations with a high percentage of dockage and broken grain kernels often have a higher moisture content. Because of this, grains stored at such locations are more likely to become highly infested by insects, and thus deteriorate more quickly than clean grain (Hagstrum and Flinn, 2012; Jian et al., 2005; McGregor, 1964). It has been reported that the moisture content of dockage is higher than that of the grain kernels (Hagstrum et al., 2012). Prasad et al. (1978) found the average moisture content of rapeseed dockage was significantly higher than the rapeseed kernel itself. Insects were found to be more active in the center of bin where the concentration of fine materials was higher (Athanassiou and Buchelos, 2020). McGregor (1964) reported that the adult red flour beetle, *Tribolium castaneum* (Herbst), preferred wheat with a high dockage content. Cracked and broken wheat kernels are more favorable than whole wheat kernels for the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). Sinha (1975) showed that the proportion of eggs that developed into adults in external infesters of grain like the rusty grain beetle, increased in the presence of dockage. Jian et al. (2005) found that rusty grain beetles preferred locations with higher than 10% dockage in wheat. Fine and broken kernels with a higher moisture content also provide suitable conditions for some stored grain fungi multiplication (Prasad et al., 1978). Fungi respiration produces heat and water which can encourage insect multiplication. Dust and moisture produced by insects provide even more suitable conditions for fungi growth. As a result, hotspots may develop at these locations. Thus, to effectively control insects and fungi, these locations should have enough concentration of fumigant. However, it might be difficult to achieve this goal

if the segregation of DFM and insect and fungi multiplication are not considered when fumigant and modified gas are applied.

Implications for fumigation and controlled atmosphere

To increase the effectiveness of fumigation and controlled atmosphere, fumigant or modified gas are usually applied by using an aeration system such as an aeration duct or a partially perforated floor at an aeration airflow rate. This system may result in uneven distribution of the applied fumigant or modified gas due to the uneven airflow resistance. Fumigation in grain bins with uneven distribution of dockage and foreign materials has an uneven distribution of fumigant which influences the fumigation result (Harein, 1961). The presence of dockage significantly reduces the effectiveness of fumigation in grain bins by increasing airflow resistance in some parts of the grain bin (Harein, 1961), and also reduces the mortality of insects when using diatomaceous earth (Kavallieratos et al., 2007). Navarro and Navarro (2020) reported the CO_2 treatment of a welded-steel silo filled with 6,881 t of winter wheat. After 39 h purging of CO_2 through the aeration duct at the bottom center of the silo, the moving-up speed of the CO_2 front at the center of the bin was slower than that at the periphery. The CO_2 concentration at the center peak was lower than 40%, while the other locations below the CO_2 front had higher than 80% CO_2. Therefore, CO_2 was added from the top of the grain bulk for an additional 106 h. After this adding, CO_2 was higher than 60% in the entire bin. During the maintenance period, CO_2 in the center bottom of the grain bulk was higher than the periphery because airflow resistance impeded the CO_2 diffusion in the area. Therefore, to overcome this uneven airflow resistance, the application system of fumigants or modified gas should be optimized for minimizing dead airflow zones.

Conclusions

Center-loading of grain into silos usually results in a concentrated distribution of both small and high-density particles at the center of the bin, and particles with large sizes and low densities at the periphery due to the dominant segregation mechanisms under this grain loading condition. This uneven distribution of dockage results in uneven distribution of airflow rate during aeration, natural air drying, and the application of fumigants and modified gas. The segregation of fines and broken kernels at the center of the silo also attracts insects and favors insect and fungi growth and multiplication. When fumigant or modified gas are applied, this uneven distribution of airflow rate also results in uneven distribution of the applied gas, and as a result, insects have a low mortality in these dead airflow zones.

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Carbon dioxide movement monitoring in a lab-scale grain bin

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ABSTRACT

Increased level of carbon dioxide (CO_2) in stored grain is one of the early indicators of spoilage. Carbon dioxide concentration of 600 to 1000 ppm in the bulk stored grain indicates early signs of spoilage due to increased microbial activity. A study aimed to simulate the CO_2 movement in a grain bin and to determine the rate of CO_2 movement from its point of origin (at the center of the study bin) to the headspace and the plenum was conducted. To introduce controlled amount of CO_2 into the small grain bin (2 m^3) a CO_2 gas cylinder was used. A regulated amount of approximately 0.075 L/s of CO_2 was introduced into the grain bin using a flexible tubing (3.2 mm opening) connected to the CO_2 cylinder. The flexible tube was inserted into a rigid metal pipe (12.7 mm diameter) which was then inserted together into the grain bin so that CO_2 was released approximately at the center of the bin. The equivalent ppm of CO_2 corresponding to the duration of injection was calculated using the Poiseuille Equation. The concentration of CO_2 in the headspace and near the bottom opening of hopper bottom bin was recorded using CO_2 sensors during and after the CO_2 injection. The concentration of CO_2 at the headspace was 0.1 to 0.2 times lower than at the plenum up to the injection time equivalent to 1500 ppm. Both plenum and headspace showed increased concentration within 3-5 min of release of CO_2. This preliminary study reflected the idea of monitoring CO_2 concentration in grain bin either in plenum or headspace that could be utilized as a potential tool for early detection of grain spoilage.

Keywords: Grain storage, Grain spoilage, CO_2 monitoring, Microbial activity

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Understanding pore structures in bulk grain for fumigation

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ABSTRACT

Quick diffusion and uniform distribution of the fumigant gas in bulk grain are critical for successful fumigation. Diffusion of fumigant gas in bulk grain is dependent on the pore structure of the grain bulk, including the total pore volume, individual pore size and shape, and connectivity of pores. The pore structure of a grain bulk is extremely complicated and difficult to quantify. This presentation summarizes imaging and numerical modelling methods to investigate pore structures in bulk grain. Molten wax was poured into the grain sample to "freeze" the pore structure of the grain bulk and the sample was then cut to expose the internal structures for image acquisition. The acquired images were then analyzed to quantify the pore structure, such as porosity and tortuosity. The discrete element method (DEM) was also used to simulate pore structures in bulk grain. In the DEM simulation model, a grain bulk was approximated as an assembly of spherical particles, which represented grain kernels. These particles interacted with each other through the forces at contacts, based on which the spatial arrangement of grain kernels in a grain bulk was predicted and the pore structure (pore volume, size and shape; channels connecting pores) was quantified. Based on the simulated pore structure, gas flow through connected pores in the grain bulk could be characterized. It was found that the pore structure generally varied from location to location in a grain bulk, which implied that local distribution of fumigant gas might not be uniform in a grain bulk.

Keywords: Grain bulk, Pore structure, Fumigation, Gas flow, Imaging processing, Discrete element modelling

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Characterizing engineering properties of hermetic storage bag technology for standard development

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ABSTRACT

Post-harvest handling operations, including storage, plays a vital role in keeping a commodity safe from deterioration. One of the growing innovative technologies which aim to improve food safety and security of smallholder farmers is hermetic storage. Hermetic storage can effectively control insect activity in stored grains, oilseeds and pulses without using pesticides; thus, preserving product quality. One type of this storage technology uses gas impermeable film as a liner inside a woven polypropylene (PP), or jute sack called hermetic bags. With the increasing adoption of these bags, various brands are becoming available in the market. Manufacturers and distributors make claims about the performance of their brand and their efficacy in controlling biological activity. This study aimed to identify fundamental engineering properties as a basis for establishing an international engineering standard for testing and rating hermeticity of plastic-lined bags for smallholder farmers. Six commercially available hermetic storage bag liners (AgroZbag, Elite, PICS bag, SuperGrainbag, Storezo, Zerofly bag) were tested for tensile, dart impact energy, tear force, and permeability (oxygen transmission rate and water-vapor transmission rate) following ASTM test methods. Results indicated substantial differences ($P < 0.05$) in material properties among types of storage bag liners. This provided a basis for evaluating how these properties were affected by usage and handling practices. The maximum allowable oxygen and water vapor transmission rates were recommended to ensure hermetic conditions can be achieved. Limits for tensile properties, tear strength, and failure strength of hermetic bag liners were identified to ensure bag liners were sufficiently strong to hold a crop. Analyzing these properties will not only help in identifying "real" from "fake" hermetic storage bags but also having a standard approach to rate hermetic crop storage bags which will ensure the continued successful adoption of this critically important storage technology to control biological activity.

Keywords: Hermetic storage bag, Engineering properties, Oxygen transmission rate, Water-vapor transmission rate, Standards, Impact failure, Tear force, Tensile strength

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A game changer: synthetic amorphous silica for protection from stored grain pests

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ABSTRACT

Keeping in mind high recommended dosages of diatomaceous earth (1,000 to 3,500 ppm) which can adversely affect physical and mechanical properties of grain and its wide-scale application as a stored-grain protectant, novel food grade synthetic amorphous silica (SAS) was tested for the first time in laboratory and field to assess its insecticidal properties against major stored grain pests including both phosphine susceptible and resistant strains in four major grain commodities. For comparison study, Dryacide®, Absorbacide® and Diafil 610® commercial diatomaceous earth formulation were selected. For preliminary selection of best performing SAS based MU powder, 19 powders were tested against *Lasioderma serricorne* (F.), *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) at 150 ppm. Based on their performance on all four stored grain insects, four best performing SAS powders were selected. These were than further subjected to laboratory trials with lower rates using above mentioned species, along with *T. castaneum and R. dominica resistant strains, Cryptolestes pusillus* (Schönherr), *C. ferrugineus* (Stephens) and *Oryzaephilus surinamensis* (L.). For *C. pusillus* and *C. ferrugineus,* 100% mortality can be seen from second day at 50 ppm. For *O. surinamensis* and *L. serricorne* 100% mortality was observed at 50 ppm within 7 d. For *T. castaneum, R. dominica* and *S. oryzae* 100% mortality was achieved at100-150 ppm within 7 d of treatment. Similar data were found to be for strong resistant *T. castaneum* and weak resistant *R. dominica.* For the effect of SAS on different grain commodities, the mortality of *T. castaneum*, *S. orzae* and *R. dominica*, were similar in barley when compared to wheat. In case of oats *S. orzae* and *R. dominica* were equally effective as in wheat but mortality of *T. castaneum* in oats showed reduction by 10%. Thus, we concluded that SAS based MU powder could be game changer for stored grain protection.

Keywords: Synthetic amorphous silica, Grain, Wheat, Diatomaceous earth, *Rhyzopertha dominica*, *Tribolium castaneum , Sitophilus oryzae,* Stored grain pests

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Design of an experimental setup to evaluate movement of adult insects in stored wheat

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Abstract

Stored grain losses, especially due to insect infestations, is one of the critical issues in preserving stored grains. In general, insects move inside stored grain bulks in search of suitable conditions for survival and multiplication. A complete understanding of the movement and distribution of insects inside stored grain bulks is of prime importance for developing proper management protocols. To analyze the movement of *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst) inside wheat bulk, 0.1 m sized cubes were made using metal rods of 1.6 mm diameter. Polylactic acid corners fabricated using a 3D printer were used to fasten the metal rods. These cubes were covered by a cloth screen with an opening of 1.4×1.4 mm^2. To evaluate the effects of the cube and screen on the movement of *C. ferrugineus*, a test was conducted by arranging 9 cubes to form a cuboid of size 0.3 m x 0.3 m x 0.1 m. The cuboid was filled with wheat at 16.5% moisture content and 100 adults were released at the center of the cuboid. The movement was stopped after 24 h. The same experiment was repeated without mesh cubes. Similarly, the effects of the cube and screen on the movement of *T. castaneum* was evaluated using the same experimental set up. The mesh cubes had no significant effect on the movement of *C. ferrugineus* and *T. castaneum.*

Keywords: Movement, Distribution, *Cryptolestes ferrugineus, Tribolium castaneum ,* Wheat, Three-dimensional, Stored grain ecosystem, Insects, Management protocols, Mesh cubes

Introduction

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) are the major economical granivorous pests associated with the Western Canadian stored grain ecosystem (Hulasare et al., 2003; Madrid et al., 1990). These insects move inside stored grain bins for various physical and biological reasons. One- and two-dimensional movements of insects inside grain columns and square boxes having grains with different temperatures and moisture contents, have been well established (Jian et al., 2003, 2004a, b, 2005a, b, c, 2007).

Surtees (1964) proposed a method to analyse the three-dimensional dispersion pattern of various insect species including *Sitophilus granarius* (L.), *Oryzaephilus surinamensis* (L.), *T. castaneum*, *C. ferrugineus,* and *Rhyzopertha dominica* (F.). The method used 3-inch cube bags made of 10 mesh net. The bags filled with wheat were arranged inside a Perspex cube and the insects were introduced at the top. The movement of insects, after a particular time, was studied by taking out the bags and placing them in prenumbered jars and counting the insects at each location, after sieving them. The major drawback of this method was the time associated in arranging the cubes (2 to 3 h to construct 4 layers of 16 cubes per layer), because of the considerable care required for proper arrangement. To address this drawback, our study used metal rods forming a cube and covered with a cloth screen. These metal rods maintained the rigidity of the bags even after filled with grains, thus reducing the time taken to construct the three-dimensional grain bulk. The objective of the present study was to determine whether the designed mesh cubes could be used to study the movement of *C. ferrugineus* and *T. castaneum* in three dimensions by comparing them to the insect movement in grain bulk without the use of mesh cubes.

Materials and methods

Wheat

Canada Western Red Spring Wheat (Grade no.1, cv. 'AC Barrie' certified) of moisture content 16.5 ± 0.1% was used in this study. A standard oven drying method was used to determine the moisture content of the wheat by drying 10 g samples at 130°C for 19 h in triplicates (ASABE, 2016).

Insects

The insect cultures of *C. ferrugineus* were reared in a media prepared with whole wheat kernels, cracked wheat, and wheat germ (90:5:5 w/w); whereas the cultures of *T. castaneum* were reared on wheat flour and brewer's yeast (95:5 w/w). Both the cultures were kept at 30 ± 1°C and 70 ± 5% RH in the dark. Mixed-sex adults with the age 1 d to 2 mo old, at the start of each experiment, were used for the study.

Experimental setup

Nine mesh cubes of 0.1 m each side, were prepared by fastening mild steel metal rods of 1.6 mm diameter using polylactic acid corners (Fig. 1). The polylactic acid corners were printed using a 3D printer (Cartesio W, Mauk CC, Maastricht, Netherlands). A cloth screen with an opening of 1.4 x 1.4 mm^2 was used to enclose the metal cube on all the sides, except the top so as to facilitate filling and emptying of the cube. The mesh size of the screen was chosen specifically to facilitate the movement of insects through it, while holding the wheat inside. The prepared mesh cubes were arranged in the form of a 3 x 3 cube, inside a wooden box with the inner dimension of 0.3 x 0.3 x 0.13 m^3 (Fig. 2). The wooden box was filled with wheat to the depth of 0.1 m. The top of the wooden box was covered with a cardboard sheet to prevent the influence of light, and then fastened with double-sided tape to prevent insects from escaping.

The location of the mesh cubes inside the wooden box were prenumbered as shown in Fig. 2. The same wooden box without mesh cubes was used as the control.

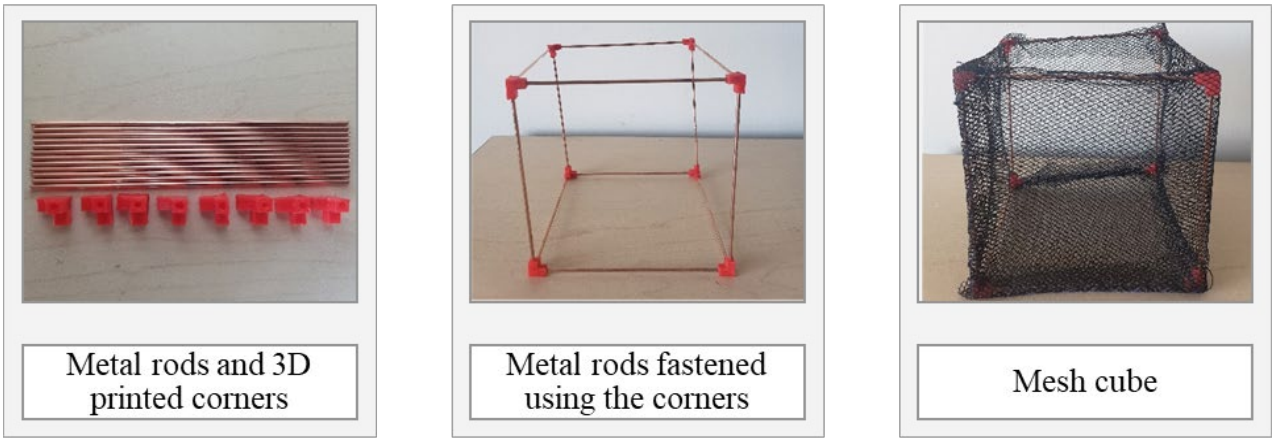

Fig. 1. Step by step preparation process of mesh cubes.

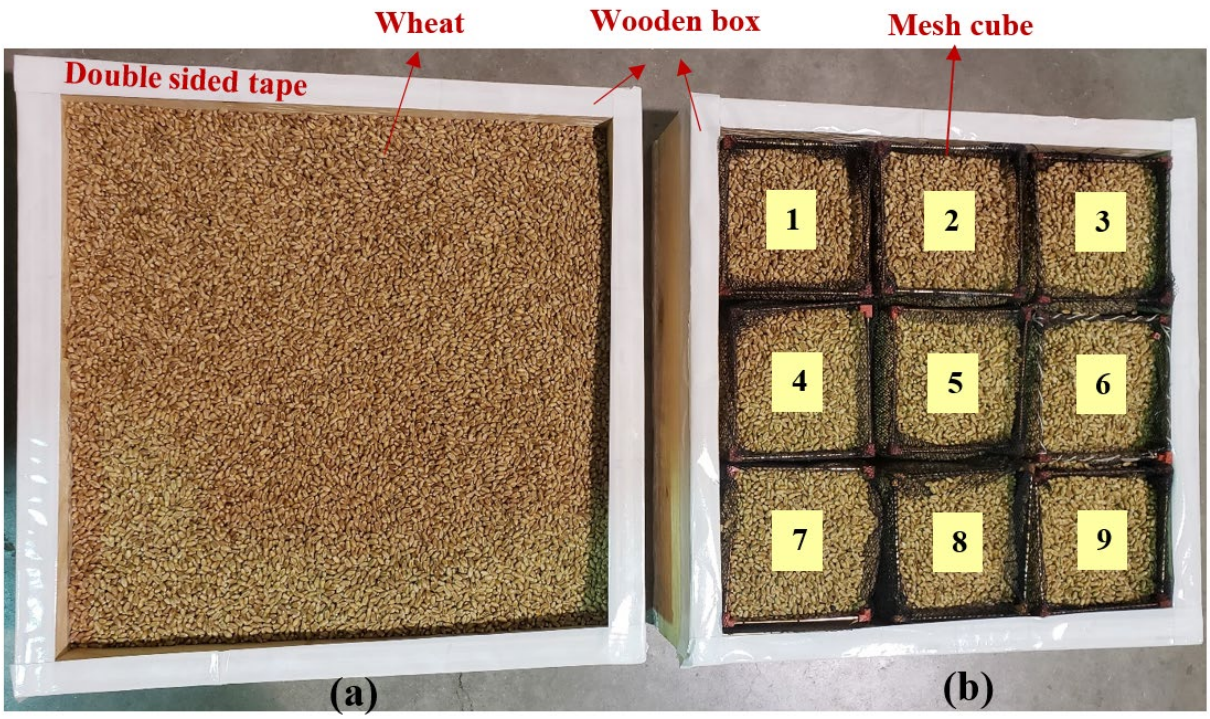

Fig. 2. Experimental set up: Wooden box without (a) and with (b) mesh cubes. The numbers are the marked locations of the mesh cube

Experimental procedure

To perform the experiments, 200 *C. ferrugineus* adults were sieved from the culture and placed in two different glass vials, each containing 100 insects. The insects in the vials were released at location 5 of the wooden box (center of the box) pre-filled with wheat, and with or without mesh cubes. After 24 h, the mesh cubes were carefully taken out of the wooden box and placed in the plastic bags that had prenumbered locations and secured tightly with a knot to prevent the insects from escaping. For the wooden box without mesh cubes, the wheat was segregated into 9 locations, similar to the ones with mesh cube, using the cardboard sheets. The cardboard sheets restricted the further movement of insects. The wheat in each section was vacuumed out along with the insects and then placed inside the plastic bags and secured tightly. A similar procedure was used to evaluate the effect of cubes and mesh screen on the movement of *T. castaneum.* Each experiment was repeated 3 times. The adults were sieved out from the wheat and the number of adults present at each location were counted. In addition, the wheat samples used for each experiment were kept at -15°C for 3 wk in order to eliminate any insect infestation before starting the experiment. The mean recovery rates were 92.7 ± 1.08% (n = 6) and 94.3 ± 0.94 % (n = 6) for *C. ferrugineus* and *T. castaneum*, respectively.

Statistical analysis

For the purpose of data analysis, the number of adults recovered from each box was adjusted to the initial number of adults introduced (i.e., 100) as follows (Jian et al., 2007).

$$Number\ of\ adults\ in\ each\ location = \frac{Number\ of\ adults\ recovered\ from\ each\ location \times 100}{Total\ number\ of\ adults\ recovered\ from\ that\ particular\ replication}$$

To determine if the developed mesh cube affected the movement and distribution of insects, the Two Sample Location Test and the Empirical Distribution Function (EDF) statistical tests (Jian et al., 2002) were conducted (SAS Institute, North Carolina, USA). These tests provided information on the difference in location, median location and the distribution of insects between the experiments with mesh cubes, and those without mesh cubes. The Wilcoxon, Median, and Kolmogorov-Smirnov options were used to perform the analysis.

Results and discussion

No significant differences in movement and distribution of *C. ferrugineus* and *T. castaneum* inside the wooden boxes (with or without mesh cubes) were observed (Table 1). These results indicated that the developed mesh cubes did not have significant effect on the movement of both *C. ferrugineus* and *T. castaneum* and can be used to study the three-dimensional movement of insects by locating the exact position of the insects after a required amount of time, with minimal external disturbances. Moreover, the rapid construction and dismantling of cubes not only reduce the time needed, but also improve the efficiency of the study.

Table 1. Results of the two sample location tests and EDF statistics to compare the movement of *Cryptolestes ferrugineus* and *Tribolium castaneum* in a box with and without the metal cubes covered with mesh screen.

Experiments		Wilcoxon		Median		Kolmogorov-Smirnov	
		Z	P > |Z|	Z	P > |Z|	KSa	P > KSa
With mesh cube vs without mesh cube	*Cryptolestes ferrugineus*	-0.2649	0.7911	-0.4581	0.6469	0.4714	0.9794
	Tribolium castaneum	-0.1766	0.8598	-0.4581	0.6469	0.4714	0.9794

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First report of strong phosphine resistance in stored grain insects in far northern region of Australia

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ABSTRACT

Although resistance to fumigant phosphine is being monitored in both farm and bulk grain storages across Australia over nearly three decades, the tropical far northern part of the continent has received little attention. To address this, a study is underway in the Townsville region, a central hub of Northern Queensland, for the first ever phosphine resistance survey in this region, consisting of both phenotypic and molecular screening of storage pests. Although not currently a major grain growing region, production is expected to increase in future. Sampling of several storages including silos, food processing facilities and feedlots was undertaken over two seasons, winter and summer, over a 12-mo period. The main pests being detected were *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), *S. zeamais* Motschulsky, *Oryzaephilus surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens), *C. pusillus* (Schönherr), bruchids, and psocids. A major finding of this study was the detection of strong phosphine resistance in samples of *T. castaneum*, *C. ferrugineus*, *R. dominica*, *S. oryzae,* and *O. surinamensis*. These insect samples are currently being subjected to high-througput molecular resistace screening that relys on detecting genetic variations within the phosphine resistance gene, *rph2*. This will allow accurate estimation of the resistance allele frequency and frequency of resistance allele carriers (i.e., those carrying either one or two copies of the allele). Moreover, the *rph2* screening data will augment the phenotypic resistance testing data, helping us to define the level of risk and for devising successful resistant management strategies of key pest species in this region.

Keywords: Stored grain, Phosphine, Strong resistance, Molecular screening

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Safety education – critical for grain fumigators

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Abstract

Fumigation with phosphine pellets or tablets is an important part of an integrated pest management strategy for keeping stored grain in good condition. The stored grain environment may cause concern for the safety of fumigant applicators who need to enter the structure because of unsafe atmospheric conditions, possible grain entrapment, and the use of a potentially dangerous insecticide. Instructions and equipment for testing the atmosphere, knowledge about how entrapment occurs and how to avoid it, and safety precautions for using a severely toxic insecticide are discussed. Atmosphere and phosphine monitors needed to test the air and keep applicators aware of the amount of fumigant in the area are needed for all fumigations. Being aware of the condition of the grain in storage and knowing whether any grain has been previously removed are important aspects to help prevent entrapment from occurring. Many steps must be taken and included in a fumigation management plan to keep applicators and workers safe from exposure to toxic insecticides. Education of applicators and employees is essential to keep everyone safe.

Keywords: Safety, Education, Fumigation, Entrapment, Gas monitors

Introduction

Integrated pest management (IPM) is an important strategy for maintaining the quality of stored products. Fumigation is an important component of stored product IPM. Proper care and procedures are needed when using a dangerous product and working in a dangerous environment to keep workers in the stored product industry safe.

Restricted use insecticide

Phosphine fumigants for stored grain protection are restricted-use pesticides due to the high acute inhalation toxicity of the gas. These insecticides are only available for retail sale to licensed dealers and certified applicators. Application is only permissible by certified applicators or personnel under their direct supervision, and only for uses covered by the certified applicator's certification and permitted in the applicator's manual.

First aid

Knowing the appropriate first aid is important for insecticide exposure. If phosphine gas is inhaled, move the person to fresh air. If the person is not breathing, call 911 or an ambulance and then give the person artificial respiration. If the phosphine product is swallowed, have the person sip a glass of water if the person can swallow. Do not induce vomiting unless told by a poison control center or doctor. In all cases, do not give anything by mouth to a person who is unconscious. If the phosphine product gets on clothing, take off the contaminated clothing immediately. If the product gets on skin, rinse immediately with plenty of water for 15-20 minutes. If the product enters eyes, hold the eye open and rinse slowly and gently with water for 15-20 min. Remove contact lenses, if present, after the first 5 min, then continue rinsing the eye. Contact a poison control center or doctor for treatment advice, and have the product container, label and applicator's manual available when calling or going for treatment.

Grain condition and the risks

There is a direct correlation between grain condition and safety hazards for workers in and around storage areas. Even small amounts of moldy grain, old grain, insect-infested grain, and trash can cause clumping to occur in the grain. Grain can stick to the walls of the structure. Bridges in the top layer of grain can form. During unloading, bridges and lumps will not go through the reclaim system. When employees enter the bin to break these clumps or bridges, entrapment and engulfment can occur when the bridge breaks through or the wall of grain on the bin walls breaks loose. The United States' Occupational Safety and Health Administration (OSHA) has strict laws about entering bins (OSHA, 1996). Full-body harnesses and lifelines are required anytime the grain is over the entrant's head or the grain is more than waist deep or providing a potential for the entrant to become entrapped or engulfed. For fumigators, this risk exists when checking for structure integrity from the inside or when treating with fumigant by entering the structure. These same precautions also apply to flat storage structures.

Air quality can be compromised by out of condition grain. Carbon dioxide and methane gas are products when grain spoils. This is motivation for monitoring air quality before entering the structure. Even when oxygen appears to be sufficient, other harmful gases may be present. Running aeration fans prior to entry can help remove these gases. However, testing the air is the best method of ensuring a safe environment.

Bin entry permits are required when entering a bin. If the plane between the inside of a bin and the outside environment is crossed, a permit is required. Bin entry permits are a safety checklist for the grain structure. Air monitoring, available safety equipment, grain levels and conditions, and entrant and attendant names with signatures are all part of the bin entry permit. Every time a bin is entered, a new bin entry permit is required. Grain moves and conditions can change between entries, making it essential that bin entry permit steps be performed and documented.

While fumigation is never performed alone, bin entry should never be performed alone either. An attendant trained in rescue and emergency procedures must remain at the entry point and in contact with the person entering and working in the bin. That attendant never leaves this position until the entry is complete, and the entrant is outside of the bin again.

Spoiled grain detection

Methods to detect grain spoilage and mold include temperature cables, CO_2 sensors, visual inspection, and detection of odors from the bin access points. Temperature will rise around areas in the grain that have biological activity such as insects or mold present. Each node on the temperature cable will sense about a meter around that node. Carbon dioxide sensors can detect biological activity when the fan starts. Any rise over the normal CO_2 range (about 400 – 600 ppm) of the bin indicates that someplace in the bin there are insects or mold is forming. During visual inspection, look for a whitish dull appearance in the grain. Also look for clumping and grain stuck to walls or in mounds. Out-of-condition grain also emits an odor. Take care when looking into a bin in case the odors are dangerous. Smell only from outside the bin at the bin entrance. It is important to train employees on these methods of spoilage detection. They may be the first ones to detect a change in the grain during their regular work activities. The earlier a problem is detected, the easier it is to remedy.

Sealing

It is critical to thoroughly seal the structure to be fumigated in order to contain the phosphine gas at the appropriate level for the designated time. Several products may be used to seal a structure: closed-foam sealant, duct tape, silicone sealant, heavy-weight (at least 6 mil) plastic, and elastomeric sealer (Fig. 1). Permanent areas that can be sealed include eaves and bases of structures, aeration fan motors and ducts, seams and missing hardware, and areas around unloading equipment. Areas that will be sealed temporarily include bin door entries, roof ventilation, manhole covers, unloading spouts, and aeration fan intakes.

Fig. 1. Sealing materials.

Closed loop fumigation

Controlling where phosphine fumigant travels in the bin and evacuation of the gas is a challenge and concern for fumigators. Closed loop systems take gas out of the headspace of the bin and return it to the bottom of the bin (Fig. 2). For systems to be successful, extensive sealing is critical. A small fan removes the gas from the headspace and forces the gas either directly into the bottom of the bin below the perforated flooring or into the aeration ductwork at the bottom of the bin. It is very important to seal the aeration fan and all vents to prevent the closed loop fan from forcing gas out of these areas. Closed loop systems have been shown to reduce the amount of fumigant required to maintain effective gas concentrations by as much as 75% while providing better distribution of gas when compared to conventional methods of fumigation.

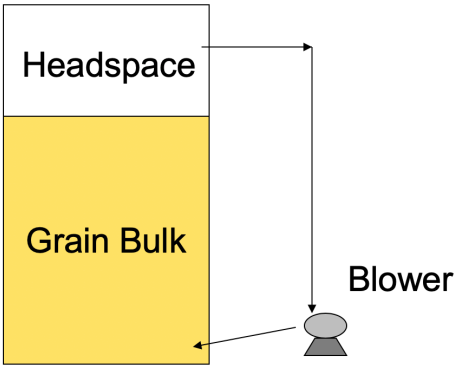

Fig. 2. Closed loop system.

Protective clothing

Protective clothing is necessary when using solid phosphine formulations. Wear dry cloves of cotton, preferably, or other material if contact with tablets, pellets or dust is likely. Gloves should remain dry during use. Always wash hands thoroughly after handling phosphine products. Aerate used gloves and other clothing that may be contaminated in a well-ventilated area prior to laundering.

Respiratory protection

Respiratory protection is required when concentration levels of phosphine are unknown or when concentrations exceed permissible exposure limits of 0.3 ppm. A NIOSH/MSHA approved full-face gas mask phosphine canister combination may be used at levels above 0.3 ppm up to 15 ppm. If the phosphine level is above 15 ppm or in situations where the phosphine concentration is unknown, a NIOSH/MSHA approved self-contained breathing apparatus (SCBA) must be worn. If phosphine is to be applied from within the structure to be fumigated, an approved full-face gas mask, phosphine canister combination or SCBA, must be available at the site of application in case it is needed. Respiratory protection must also be available for applications from outside the area to be fumigated such as the addition of tablets or pellets to automatic dispensing devices and outdoor applications.

Certified applicator

Whenever a fumigation is to be performed, a certified applicator must be physically present, responsible for, and maintain visual and/or voice contact with all fumigation workers during the opening of the product containers, during the application of the fumigant, and also during the initial opening of the fumigation structure for ventilation. During application of fumigant, at least two persons, a certified applicator and a trained person, or two trained persons under the direct supervision of the certified applicator, must be present. Monitoring must be conducted in order to characterize the application and determine the fumigator's exposure. Document the phosphine reading levels and retain documents for two years. Before re-entry into a structure, the facility must be ventilated until the phosphine level is 0.3 ppm or below.

Persons with documented training in the handling of phosphine products must be responsible for receiving, ventilating, and removal of placards from vehicles which have been fumigated in transit. The trained person must be trained by a certified applicator following the Environmental Protection Agency's (EPA) accepted product applicator's manual that must precede or be attached to the outside of the transport vehicle. When training has been completed and the employee demonstrates safety knowledge proficiency, the training date must be recorded and maintained in the employee's safety training record for a minimum of three years. Refresher training must be done annually and documented.

Gas detection equipment

Structures storing grains may develop hazardous atmospheres because of gases given off by decaying grain or from a previous fumigation that was not ventilated properly after the fumigation was complete. Before entering any storage structure, it is important to determine that there is sufficient oxygen present, and that the phosphine level is 0.3 ppm or lower. If a fumigant is detected or there is a lack of oxygen, the facility must be ventilated to ensure that the toxic gas levels are reduced to non-hazardous levels and that adequate oxygen levels are maintained.

Normal oxygen levels in the atmosphere range between 19-23%, but when they are lower than that range, workers can experience problems. Electronic oxygen sensors help prevent these dangers by accurately monitoring oxygen levels and triggering an alarm when the level drops below 19.5%, the current OSHA-mandated level (OSHA, 1996). Using an oxygen monitor helps determine that the working environment is safe to enter.

For phosphine gas detection, there are glass detection tubes or electronic gas monitors to determine the amount of phosphine gas present. Glass detection tubes (Fig. 3) with a sampling pump are portable, simple to use, only detect one gas, require little training to use, are relatively inexpensive, and are accurate. Glass tubes have an expiration date and must not be used if expired. Expired tubes may be retained and labeled “for training purposes only” to be used to train new employees but cannot be used to determine the amount of phosphine present during fumigation.

Fig. 3. Glass detection tube.

Fig. 4. Electronic gas monitor.

Electronic gas monitors (Fig. 4) are portable, may be complex to use and require extensive training, may be able to read multiple gases, are relatively rapid, are expensive, have a digital readout, must be calibrated according to the manufacturer instructions, and must be bump-tested before each use or at a minimum, every 30 d.

Electronic gas detection monitors must be calibrated against a known standard. Calibration can be done in-house with the required gas and testing equipment (regulators and adaptors) or the monitor can be sent back to the distributor or manufacturer for calibration. The calibration gas must be introduced at the proper pressure and flow rate. Over-pressurization can damage the sensor. Monitors should be calibrated at the altitude at which they will be used as changes in atmospheric pressure will influence the instrument’s response. For some instruments with an active sampling pump, the pump must be disconnected from the sensor and the gas flow rate set to match the sampling rate of the pump.

A bump test must be performed before every use of an electronic gas detection monitor. A bump test kit (Fig. 5) verifies calibration by exposing the instrument to a known concentration of test gas. Instruments should be zeroed before the bump test is performed in order to give a more accurate picture of the bump test results. When performing a bump test, the test gas concentration should be high enough to trigger the instrument's alarm. If the bump test does not trigger the alarm, then a full calibration must be performed.

Fig. 5. Bump test kit.

There has been some confusion regarding proper calibration procedures and frequency. To clarify the issue, the International Safety Equipment Association (ISEA) updated its position statement on instrument calibration in 2010 stating, "A bump test or full calibration check of direct-reading portable gas monitors should be conducted before each day's use in accordance with the manufacturer's instructions, using an appropriate test gas." If the instrument fails a bump test, it must be adjusted through a full calibration before it is used. The ISEA recommends more frequent testing if environmental conditions that could affect instrument performance are suspected, such as sensor poisons or excessively dusty conditions.

Placarding of fumigated areas

Placarding of structures and areas to be fumigated is essential to provide a safe environment for all personnel. All entrances to the fumigated area must be placarded. The placard (Fig. 6) must be made of substantial material that can be expected to withstand adverse weather conditions and must bear the wording as follows:

- The signal words DANGER/PELIGRO and the SKULL AND CROSSBONES symbol in red.
- The statement "Structure and/or commodity under fumigation, DO NOT ENTER/NO ENTRE".
- The statement, "This sign may only be removed by a certified applicator or a person with documented training after the structure and/or commodity is completely aerated (contains 0.3 ppm or less of phosphine gas). If incompletely aerated commodity is transferred to a new storage structure, the new structure must also be placarded if it contains more than 0.3 ppm phosphine gas. Worker exposure during this transfer must not exceed allowable limits.
- Date the fumigation begins.
- Name and EPA registration number of the fumigant.
- Name, address and telephone number of the Fumigation Company and/or applicator.
- A 24-h emergency response telephone number.

Fig. 6. Phosphine placard.

Where possible, placards should be placed in advance of the fumigation. For railroad hopper cars, placards must be placed on both sides of the car near the ladders and next to the top hatches into

which the fumigant is introduced. Do not remove placards until the commodity or area is ventilated down to 0.3 ppm phosphine gas or less.

Storage

Do not contaminate water, food, or feed by storing pesticides in the same areas used to store commodities. Store phosphine products in a dry, well-ventilated area away from heat, under lock and key. Make sure the door is posted as pesticide storage area. Do not store phosphine products in areas where temperature may exceed 54°C. Do not store phosphine in buildings where humans or domestic animals reside. As always, keep out of reach of children. For pellets and tablets supplied in gas-tight aluminum sealed flasks, once opened, the contents should be used completely. The shelf life of flask is virtually unlimited as long as the aluminum seal is not removed. At a minimum, the storage must be locked and marked with the following words and signs: Danger/Poison with skull and cross bones (Fig. 7.), phosphine NFPA chemical label (Fig. 8.), and Authorized Personnel Only.

Fig. 7. Danger/Poison skull and crossbones.

Fig. 8. Phosphine NFPA label.

Educating stored product workers

Grain elevator and fumigation workshops are critical in helping keep stored product managers and workers educated and up to date on safety requirements and insecticides used in controlling insects in stored products along with their proper usage. Certified applicators have a requirement to obtain CEUs or to re-test every few years to maintain their certification. Certified applicators and others working in this industry benefit from hearing regularly from university personnel and industry specialists working in the stored product area during these workshops. Fact sheets and news releases are important for the stored product industry to inform them of safety regulations, new insecticides, and good practices to utilize in this important agricultural endeavor.

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Gu C, Agarwal M, Ren Y (2021) Evaluation of ethyl formate for management of both resistant and susceptible strains of *Cryptolestes pusillus* and *Cryptolestes ferrugineus*. Page 98. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Evaluation of ethyl formate for management of both resistant and susceptible strains of *Cryptolestes pusillus* and *Cryptolestes ferrugineus*

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ABSTRACT

Insect pests are a major issue negatively affecting both quality and quantity of the grain during the post-harvest storage. Fumigation is a widely used method for control of a variety of insect pests due to its high degree of efficacy and cost effectiveness. Phosphine is the main fumigant deployed for the control of stored grain insect pests. However, insect resistance has been accorded to phosphine, this has led to ineffective control of many important stored grain insect species, particularly like *Cryptolestes pusillus* (Schönherr) and *Cryptolestes ferrugineus* (Stephens). As a result, there is an urgent need to identify and evaluate an alternative fumigant. Ethyl formate is proposed as a viable alternative to phosphine due to its status as a registered food additive, favourable environmental properties, kills insect rapidly and low toxicity to humans. This report evaluated ethyl formate as an alternative fumigant to manage phosphine susceptible and resistant strains of *C. pusillus* and *C. ferrugineus*. The results indicated that there were no significant LD_{50} and $LD_{99.5}$ with ethyl formate fumigation between the susceptible and resistant strains of *C. pusillus* and *C. ferrugineus*. That is, there are no cross resistance between ethyl formate and phosphine. The complete control of *C. pusillus* and *C. ferrugineus* can be achieved with in short period of exposure, such as 6 h with lower concentration of ethyl formate.

Keywords: Grain, Insect, *Cryptolestes pusillus, Cryptolestes ferrugineus,* Fumigation, Fumigant, Ethyl formate, Phosphine, Phosphine resistant

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Is phosphine or its derivatives responsible for mortality of stored product insects?

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ABSTRACT

Phosphine remains the single-most economic and recognised fumigant in global grain production systems. However, using a single fumigant and continued misuse of phosphine have resulted in development of chemical resistance in economically-important stored grain insects, leading to poor control and economic losses. Unlike methyl bromide and cyanide, that are directly toxic, phosphine creates multi derivatives of phosphine, that are toxic on different targets. As a result, phosphine fumigant shows the increased amount of toxin progressively during the fumigation. Moreover, the published mode of action reported the indirect and complex toxicity. By means of molecular biology, people believe that phosphine disrupts the energy metabolism inside each single cell. However, the pathways through the phosphine itself or/and its derivative molecules into the cells of insects are not discussed and reported. The interaction between phosphine molecules and its metabolites is also ignored. Therefore, we conducted systematic *in-vitro* experiments to explore the capability of phosphine itself to penetrate into reported insect targets and the possible interaction with energy metabolites. The results provided better understanding of phosphine toxicity for further understanding "phosphine" objective mode of action.

Keywords: Fumigant, Phosphine, Phosphine resistance, Toxicity, Metabolites, Mode of action

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Singaryan VT, Jagadeesan R, Schlipalius DI, Nayak MK, Ebert PR, Daglish GJ (2021) Does strong resistance to phosphine (PH_3) incur biological cost in *Cryptolestes ferrugineus* (Stephens): A two-way approach in dissecting the fitness cost? Page 100. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Does strong resistance to phosphine (PH_3) incur biological cost in *Cryptolestes ferrugineus* (Stephens): A two-way approach in dissecting the fitness cost?

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ABSTRACT

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), is a cosmopolitan pest of stored grains that has developed very high levels of resistance to fumigant, phosphine (PH_3) (up to 1200×). Despite new fumigation protocols to manage *C. ferrugineus*, information on the development and spread of resistance remains unexplored. In particular, it is not known whether there is a fitness cost associated with phosphine resistance in *C. ferrugineus*. In this study, we adopted two complementary approaches to detect fitness cost directly linked to phosphine resistance in *C. ferrugineus*. These included (i) investigating inherent differences in developmental and reproductive traits in strains having isogenic background except for phosphine resistance genes, and (ii) determining the change in resistance allele frequency in populations segregating for phosphine resistance using gene specific DNA markers for one of the two genes needed for strong resistance (*cf_rph2*) at discrete generations. In both of these approaches, there was no selection pressure (i.e., phosphine fumigation) applied to experimental insects because selection could favour resistance traits.

Developmental traits showed no significant difference between the strain in which the phosphine resistance genes were introgressed into a susceptible genetic background, and the phosphine susceptible strain itself. The introgressed resistant strain showed a marginal delay of 3.6% in time to 50% emergence but the two strains produced similar numbers of progeny indicating at most a small fitness cost. Genotyping randomly selected individuals from the progeny of a genetic cross that were segregating for the resistance allele *cf_rph2* in four discrete filial generations (F_5, F_{10}, F_{15} and F_{20}) indicated a significant change in the proportion of *cf_rph2* genotypes (*rr, rs, ss*), especially with increase in homozygous resistant genotypes (*rr*) from F_5 to F_{15}. However, this increase in resistant homozygotes (*rr*) was not significantly reflected in phenotype assays. Thus, both the approaches indicated that phosphine resistance caused at most a small fitness cost that could otherwise be exploited to minimise resistance development in the field.

Keywords: Phosphine, Resistance, Numbers of progeny, Allele frequency, Fitness

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Managing genetic resistance to phosphine in stored grain pests: an integrated strategy ensuring grain biosecurity and market access

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ABSTRACT

Pressure from insect infestation is so high that storage managers are forced to rely on chemical treatments, particularly the fumigant, phosphine (PH_3). The latter is a unique material and is favoured for several operational reasons and its acceptance as residue-free treatment in international markets. The development of resistance to phosphine, however, seriously jeopardises grain trade for Australia and food security in India. Two multi-faceted collaborative research projects on grain storage conducted in India and Australia over the last eight years (2012-2020) enhanced our scientific understanding, providing a basis for developing and implementing an integrated pest and resistance management approach (IPRM) in each country. The backbone of the Australian IPRM module is implementing improved pest and resistance monitoring programs (phenotypic and molecular), followed by adopting two major pest intervention strategies: (1) non-chemical (hygiene, aeration and grain cooling, and mass trapping) and (2) chemical (structural treatments, grain protectants, and fumigants). Successful adoption of selective combination these strategies facilitated managing Australian grain industry's most challenging pest, rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). Though, developing a similar IPRM program is difficult for India, our research identified the need for implementing the following: (i) improved fumigation methods for bag-stacks, (ii) new prophylactic treatments, (iii) transitioning into modern storages, and (iv) reducing losses in grain transports. The project also emphasized the need for outreach of the findings to the end-users in India, through a national alliance currently functioning in Australia for its perpetuity. Based on this, our team is developing an effective IPRM module for India, which can be selectively adopted in other countries, facilitating global food security and market access.

Keywords: Stored grains, Fumigants, Grain protectants, Resistance, Management

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Status of phosphine resistance of *Tribolium castaneum* in Indonesia

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Abstract

Storage period is the most vulnerable stage in post-harvest systems of all agricultural products. Fumigation using phosphine has long been an option in managing stored-product insect pests during the storage period. The long-term use of phosphine can lead to the development of resistant strains of stored-product insects. This study aimed to determine the distribution and resistance status of *Tribolium castaneum* in Indonesia. Samples of *T. castaneum* were obtained from various food and feed warehouses in 33 cities from 13 provinces in Indonesia which routinely conduct fumigation. The study was conducted using the FAO method for resistance test of stored-product insect pests. It was found that resistance cases had been spread out in various cities in Indonesia, and almost all *T. castaneum* samples collected had shown their resistance against phosphine. As many as 24 samples of *T. castaneum* collected from 24 cities in 11 different provinces (75% from total samples) had shown their resistance to phosphine with resistance factors (RF) ranging from 1.2 – 350.7 times. There were 8 cities from 6 provinces that had not experienced resistance to phosphine. The province with the lowest RF value was East Kalimantan at 0.6.

Keywords: Distribution, Phosphine, Resistance, *Tribolium castaneum*

Introduction

Storage period is the most vulnerable stage in post-harvest systems of all agricultural products. Insect pest attacks during the storage period can cause losses in quantity and quality of commodities. The magnitude of stored-product losses depends on the insect species involved, storage duration, storage facilities, and the pest control method implemented. Management of stored-product insects is most commonly carried out by fumigation.

Methyl bromide and phosphine are two fumigants that are commonly applied. Phosphine has been widely used as a fumigant for the control of stored-product insects for almost half a century (Price and Mills, 1988; Chaudhry, 2000). Since 1935, when phosphine gas was used for the first time as a fumigant, this gas has been used widely as a standard fumigant to manage insect pests in food storage facilities all over the world (Reichmuth, 1994; Chaudhry, 2000; Nath et al., 2011). Currently, phosphine is the main fumigant because methyl bromide has been banned due to its

ozone-depleting capacity (Waterford et al., 1994). The use of phosphine to fumigate cereal grain in Australia has reached 80% (Cao and Wang, 2001). Meanwhile in China, it has reached up to 90% (Ling et al., 2004).

In Indonesia, phosphine has also been used intensively. Phosphine has been chosen as the main alternative to methyl bromide because it has ideal characteristics as a fumigant, such as ease of application, no residues left in commodities, inexpensive, high penetration into any commodities and packaging materials, quick distribution throughout fumigation enclosures, and no impact on seed viability (Taylor, 1989; Liang, 1989, 1994; Afbh and Aciar, 1991; Chaudhry, 2000; Cao and Wang, 2001; Nath et al., 2011, Prijono, 2006; Nayak, 2012). These characteristics have allowed phosphine to play a critical role in stored-product pest management. Using phosphine as the main fumigant for warehouse treatment was supported by the Montreal Protocol which decided to phase out methyl bromide in 2015.

Repeated applications of phosphine in poorly sealed warehouses have been cited as the cause of the resistance development (Chaudry, 2000). Control failures by phosphine treatment have been reported in some countries (Taylor, 1989; Collins et al., 2002). Based on its survey in 1972-73, the Food and Agricultural Organization (FAO) reported the resistance of stored-product insects to phosphine in 33 out of 82 countries sampled (Champ and Dyte, 1976). In Indonesia, although studies on insect pests of stored products resistant to phosphine have been conducted since 2011 by Entomology Laboratory, SEAMEO BIOTROP, information about phosphine resistance of stored-product insect pests is still very limited. The insect samples for this study were collected from several provinces. This research aimed to monitor the resistance level of stored-product insects in Indonesia to phosphine, so that resistance management can be developed to minimize the problems.

Materials and methods

Insects

Between 2011 and 2018, a total of 33 samples containing *Tribolium castaneum* from 32 cities in 12 different provinces in Indonesia were collected by visiting food and feed storages in survey locations. In each visit, insect pests found in these facilities were collected manually using small paintbrushes and aspirators. Then, the insect samples were taken to the Entomology Laboratory in Bogor and used for resistance tests.

Resistance testing method

The test insects used were the first progeny (F1) of the collected insects, and the phosphine gas used was extracted from phosphine tablets (AlP) using H_2SO_4 10%. Extraction of phosphine gas was conducted using the apparatus for generating phosphine based on the FAO method (FAO 1975). Test insects of 50 adult beetles were introduced into a PVC insect cage (diameter and height of 2.5 cm) covered at both sides by a thin muslin cloth. Then, 2 such insect cages were placed on a wire mesh hung in the middle of a glass jar. Thus, each experimental unit was composed of one glass jar containing two insect cages. The lid of each glass jar had a hole for injecting phosphine gas, and this hole was plugged with a rubber stopper. The rubber stopper was then sealed with plasticine after phosphine gas was injected into the jar in order to prevent phosphine gas leakage.

Phosphine gas extracted from AlP (FAO 1975) was injected into the jars using a syringe at respective concentrations of 0.000, 0.005, 0.014, 0.023, 0.031, and 0.040 mg/L. A magnetic stirrer was used to stir the gas in the jars. Fumigation was conducted for 20 h exposure. After fumigation was completed, the test insects were taken out from the jars and put into new jars containing their appropriate feed. The test insects were kept in these respective jars for 14 d before their mortality was observed. If the test insects were still alive, there was indication of a resistance factor of more than 1. After that, a confirmation test was conducted by re-fumigation of those strains for 48 h. This test was intended to confirm that the insects were resistant to phosphine.

Data analysis

Mortality of the tested insects was observed 14 d after fumigation, and the data were analyzed using Probit Analysis (Polo PC) to obtain LC_{50} and $LC_{99.9}$ values from each tested insect sample. Those LC_{50} and $LC_{99.9}$ were then compared with Discriminating Concentration from FAO Manual No. 23 (FAO 1975) to obtain the resistance level. Resistance Factor (RF) was calculated using the formula:

$$RF = LC_{99.9} \text{ of test insect/Discriminating Concentration}$$

If the value of $LC_{99.9}$ was more than the discriminating concentration, the insects tested were considered resistant and were confirmed with a 48 h test. After the confirmation test, if $RF > 1$, then the insects tested were confirmed as resistant strains to phosphine. However, if $RF \leq 1$ after the confirmation test, then the resistance of these tested insects could not be determined and another round of tests was conducted.

Results and discussion

A total of 32 samples containing T. castaneum was collected from 12 provinces in Indonesia. The 12 provinces were: Bali, Banten, Central Java, East Borneo, East Java, Lampung, North Sulawesi, South Sulawesi, South Sumatera, West Java, West Nusa Tenggara, and West Sumatera. These provinces represent the big islands in Indonesia which include Java, Sulawesi, Sumatera, and Borneo, as well as the small islands including Bali and Nusa Tenggara. The test results from Borneo Island showed that T. castaneum samples from the East Borneo Province did not yet have resistance with an RF value of only 0.6-fold (Table 1). Overall, insect resistance had been distributed amongst almost all of the big islands in Indonesia including Java, Sulawesi, and Sumatera (Fig. 1).

More than 75% of the *T. castaneum* studied in this research showed their resistance against phosphine. The resistance levels varied with an RF value of 1.2 - 350.7 folds. A significant difference in resistance level from one city to another within the same province occurred in Banten Province with an RF value of 1 - 350.7 folds. This significant difference in resistance levels was thought to have occurred due to ineffective fumigation practices. Poor fumigation techniques (such as maintaining the fumigation chamber at a low air-tightness) could be one of the reasons resulting in fumigation failure and triggering the development of insect-resistant strains. According to Benhalima et al. (2004) and Collins et al. (2005), an insect population that has been under high selection pressure for many years may result in low mortality at the discriminating concentration.

Table 1. Probit analysis of 32 *Tribolium castaneum* assays using the FAO recommended method.

No.	City	Province	DC[a] (mg/L)	LC_{50}[a] (mg/L)	$LC_{99.9}$[a] (mg/L)	RF[b]	Confirmation
1	Balikpapan	East Borneo	0.040	0.010	0.020	0.600	Susceptible
2	Pare-pare	South Sulawesi	0.040	0.007	0.029	0.730	Susceptible
3	Wajo	South Sulawesi	0.040	0.008	0.032	0.800	Susceptible
4	East Lombok	West Nusa Tenggara	0.040	0.002	0.037	0.930	Susceptible
5	Lapadde	South Sulawesi	0.040	0.010	0.039	0.980	Susceptible
6	Surabaya	East Java	0.040	0.010	0.040	1.000	Susceptible
7	Jatake	Banten	0.040	0.020	0.040	1.000	Susceptible
8	Padang	West Sumatera	0.040	0.005	0.040	1.000	Susceptible
9	Tanette	South Sulawesi	0.040	0.006	0.048	1.200	Resistant
10	Bitung	North Sulawesi	0.040	0.004	0.049	1.220	Resistant
11	Makassar	South Sulawesi	0.040	0.013	0.050	1.250	Resistant
12	Mataram	West Nusa Tenggara	0.040	0.001	0.051	1.280	Resistant
13	East Lombok	West Nusa Tenggara	0.040	0.006	0.054	1.350	Resistant
14	South Padang	West Sumatera	0.040	0.009	0.062	1.550	Resistant
15	Sidrap	South Sulawesi	0.040	0.006	0.095	2.360	Resistant
16	Palembang	South Sumatera	0.040	0.006	0.106	2.650	Resistant
17	Painan	West Sumatera	0.040	0.011	0.112	2.800	Resistant
18	Tegal	Central Java	0.040	0.013	0.113	2.825	Resistant
19	Badung	Bali	0.040	0.009	0.113	2.830	Resistant
20	Central Lombok	West Nusa Tenggara	0.040	0.013	0.127	3.180	Resistant
21	Semarang	Central Java	0.040	0.004	0.131	3.275	Resistant
22	Panakkukang	South Sulawesi	0.040	0.010	0.142	3.550	Resistant
23	Lampung	Lampung	0.040	0.018	0.160	4.000	Resistant
24	Medan	North Sumatera	0.040	0.020	0.160	4.000	Resistant
25	Probolinggo	East Java	0.040	0.004	0.219	5.475	Resistant
26	Kotamobagu	North Sulawesi	0.040	0.013	0.302	7.560	Resistant
27	Indramayu	West Java	0.040	0.024	0.340	8.500	Resistant
28	Tabanan	Bali	0.040	0.030	0.566	14.150	Resistant
29	Serang 1	Banten	0.040	0.010	0.580	14.550	Resistant
30	Manado	North Sulawesi	0.040	0.014	0.932	23.300	Resistant
31	Bogor	West Java	0.040	0.070	1.020	25.450	Resistant
32	Serang 2	Banten	0.040	0.030	14.030	350.700	Resistant

DC[a] = Discriminating Concentration; LC[a] = Lethal Concentration; RF[b] = Resistance Factor (FAO 1980).

Resistant and non-resistant strains found within the same province also occurred in East Java, South Sulawesi, West Nusa Tenggara, and West Sumatera Province with an average RF value below 5 folds. Meanwhile, several areas had an RF value of more than 20 folds, namely Manado in North Sulawesi (23.3 folds), and Bogor in West Java (25.45 folds). All samples obtained from cities in these two provinces having resistance, had also shown to spread evenly. Apart from being triggered by inappropriate fumigation practices, the spread of insect-resistant strains could also be triggered by inter-city and provincial commodity shipping activities. The movement of resistant strains of insects carried in shipping commodities between cities and provinces is possible. The movement of insects due to the commodity trade is one of the factors that can influence the evolution of phosphine resistance (Benhalima et al., 2004).

Fig. 1. Distribution of insect strains resistant to phosphine in Indonesia: resistant strain (red); susceptible strain (blue).

Most of the insect samples used in this study were collected from rice, which is the main food commodity in Indonesia. This commodity has a high distribution potential between provinces as not all regions can independently meet their rice needs. Therefore, the development of *T. castaneum* resistance to phosphine in Indonesia might be caused by local selection and/or broad dispersal of the resistant population by the distribution of rice or the grain trade. Commodity distribution and poor fumigation application are most likely the major forces driving the development of resistant strains and phosphine resistance spread. Based on these results, it is necessary to evaluate commodity distribution activities, improve fumigation techniques, and look for alternative techniques or other fumigants to control resistant strains.

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Hermetic storage: under vacuum, under ambient pressure in bags, or in metal containers - what is most suitable?

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Abstract

Insect mortality in hermetic structures is a function of available oxygen, respiration, temperature, and gas tightness. A vacuum can be drawn, provided a gastight flexible storage containment is used, resulting in the following advantages: 1) the tension of the elastic film on the product shows the quality of the vacuum seal and is proof that no pest can enter; 2) the reduction of interstitial oxygen reduces the probability of insects hatching and penetrating through the film to the outside during the initial phase of storage; 3) the reduction of oxygen below 3% leads to mortality of insect pests and reduces the risk of grain quality degradation by molds. Laboratory tests at 20±1°C and 65±5% RH with samples of 1000 g wheat grain in commercially available vacuum bags and 100 infested grains of various developmental stages of *Sitophilus granarius* (L.) or 30 adults had no survivors at a vacuum of 0.5 bar (50 kPa) when the exposure time reached 5 wk. At ambient pressure and hermetic storage in similar bags, 8 wk were necessary for the same effect. A drawback is that liners for vacuum storage consist of multilayer plastics that require sophisticated production machinery and are not suitable for recycling or natural composting. Hermetic storage at ambient pressure using a PE or PP outer bag with gastight PA or HDPE inner bags does not show if the seal is gastight. Improperly sealed or punctured bags may allow influx of oxygen and thus the deterioration of stored products due to insect activity and microbials possibly causing mycotoxin contamination. Another option is the use of metal containers. Silo bins can be welded into suitable sizes, sealed from above and at the grain outlet, but need a shaded location to avoid condensation or the development of leaks from pressure changes. Underground hermetic storage used by our ancestors adds more safety from pests and cooler storage conditions to this type of storage. The question of which storage type is most suitable still needs to be answered.

Keywords: Hermetic, Vacuum, Moisture content, Pest, Mortality, Prevention, Grain quality

Introduction

As a side effect of global warming, granary weevils (*Sitophilus granarius* L.) hatching from wheat grains in the ear could be witnessed in Germany in the hot and dry summer of 2018. This means

that at an average monthly temperature of some 21°C, grain had become sufficiently dry and mature in the field for at least 7 wk. Furthermore, this implies that conditions in Central Europe more and more resemble those in tropical countries where a field infestation with stored product pests has to be expected on a regular basis (Adedire, 2001). Combined harvesting and threshing obviously are not sufficiently abrasive to prevent pests from being carried over into storage. At least in 2018, the observation of hatching weevils in the field coincided with a significant infestation with flour beetles (*Tribolium* spp.), rusty grain beetles (*Cryptolestes ferrugineus* Stephens), flat grain beetles (*Oryzaephilus surinamensis* L.), lesser grain borers (*Rhizopertha dominica* F.), and rice weevils (*Sitophilus oryzae* L.) in freshly harvested wheat grains in the silo bin of a project partner testing acoustic pest detection (Müller-Blenkle et al., 2018, 2020). The presence of a large proportion of rusty red flour beetles (*Tribolium castaneum* Herbst), rice weevils and lesser grain borers supports the notion that Central Europe may increasingly become inhabited by a more tropical stored product fauna.

As we search for improved grain storage methods, we should try to develop a system that controls an initial infestation and allows for sustainable long-term storage without toxic residues or hazards to storage operators. Given the remarkable capacity of stored product insects to find suitable stored products by smell (Adler and Ndomo-Moualeu, 2014), future storages should be insect-proof or more hermetic. Ideally, hermetic structures could prevent an insect attack from outside, and control an existing infestation by suffocation.

Hermetic storage has long been studied as a method suitable for stored product protection (Bailey, 1955; Navarro et al., 1994; Adler et al., 2000; Navarro, 2006). New developments in this area during the last five decades that made it into commercial application are perhaps the bunker storage in Australia, hermetic and vacuum storage in flexible cubes (Navarro and Donahaye, 1976), the Purdue Improved Crop Storage Bags (PICS Bags) (Baua et al., 2014), the development of hermetic silobag-storage (Bartosik, 2012), and the implementation of a gas-tightness standard for silo bins in Australia (AS 2628) using flexible paints for retro-sealing.

Flexible structures depend on the quality of the liners used to provide a gastight seal. Navarro et al. (1994) mentioned that PVC-liners with a thickness of 0.83 mm were permeable to oxygen from the ambient atmosphere by 87 mL $O_2 m^{-2} d^{-1}$. As PVC liners age under sunlight, plasticisers evaporate and some flexibility is lost, but permeability drops to some 50 mL $O_2 m^{-2} d^{-1}$ (Navarro et al., 1994). Oxygen permeability may counteract the insecticidal effect of oxygen depletion. Conversely, CO_2 accumulating inside due to respiration may be lost to the outside through permeable liners. However, a comparative practical on-farm study of Grain Pro Super Grain Bags, PICS Bags, and metal silos in Zimbabwe gave equally good results regarding insect mortality and grain quality (Mlambo et al., 2017).

Vacuum storage reduces the absolute amount of available oxygen at the start of storage, and the resulting anoxia can lead to a better protection of grains. Croft et al. (2012) reported that seeds of tropical plants like amaranth (*Amaranthus cruentus*), moringa (*Moringa oleifera*), pumpkin (*Cucurbita moschata*) or tomato (*Solanum lycopersicum*) could be kept in better quality regarding germination when stored under vacuum alone compared to freezing alone, while the best (but also most costly) results were achieved when both were combined. Already much earlier the insecticidal effect of vacuum against pest insects in tobacco had been described (Bare, 1948).

Navarro and Calderon (1979) studied the effects of low atmospheric pressures on the pupal stages of *Cadra* (*Ephestia*) *cautella*.

Finkelman et al. (2006) studied the efficacy of low pressure (50 mm Hg; 6.67 kPa) against the stored product beetles *Trogoderma granarium*, *Lasioderma serricorne* and *Oryzaephilus surinamensis* at 30°C and 50% RH. They found that eggs were the most tolerant stages, that times to achieve 99% mortality of this stage were between 32 h for *O. surinamensis* and 92 h for *L. serricorne*, and concluded that vacuum treatment might be an alternative to fumigation.

In a project on pest-proof storage of grain, horizontal warehouses were rendered insect-proof by sealing the buildings at the walls and doors (Adler et al., 2013; Adler and Ndomo-Moualeu, 2014, 2015). As an alternative to structural sealing, in a laboratory experiment 1.3 kg samples of wheat were stored under 0.5 bar (50 kPa) vacuum at 20±1°C for different periods of time. A moderate infestation had been simulated in some samples by placing 30 adult granary weevils onto the wheat 1 wk prior to drawing the vacuum. After the shortest exposure time tested (three months) granary weevils were found dead and the grain quality did not differ between infested and un-infested grain (Adler et al., 2016). This was the motivation to test shorter exposure times and investigate how developmental stages of grain insects may react to vacuum treatment. Individuals of the genus *Sitophilus* are known to be quite tolerant to hypoxic atmospheres; late larvae and pupae have been described as most tolerant developmental stages regarding anoxic conditions or modified atmospheres (Annis, 1987; Adler, 1993; Adler et al., 2000). The study described here aimed to identify the effect of vacuum storage of grain on the survival of different developmental stages of the granary weevil *S. granarius*.

Material and methods

Granary weevils and their developmental stages were taken from a weekly culture at 25±1°C. Insect culture: every week approximately 1900 young adult granary weevils (16 mL) were placed onto some 3,000 kernels of fresh uninfested wheat grain with a moisture content of 14±0.3%. After an oviposition period of 3 d the adults were removed by sieving, and the grain was kept in glass jars of 1L covered with cotton cloth held by two rubber bands. Through weekly repetition of this procedure, five developmental stages were produced, while within 6 wk the first weevils started to hatch.

Thirty young adult weevils (up to 2 wk old) were directly placed onto the grain in the vacuum bag. For developmental stages, 100 infested grains from a given weekly culture were filled into a small sachet made of nylon gauze with 0.5 mm mesh size. In order to keep oxygen consumption moderate, only two developmental stages were added to a given bag of grain filled with 1 kg of wheat (m.c. 14±0.3 %). A household vacuum machine combined with a welding station (Professional Vacuum Sealer V300, www.la-va.com) was used (Fig. 1). Vacuum bags were stored at 20°C for different time spans.

The vacuum bags (RS-Vac VL 1815) were commercially available bags (www.la-va.com, Germany) which consisted of four layers of plastic polymers, and were 160 µm thick, 250 mm wide and 400 mm in length.

Results and discussion

Results for the survival of granary weevils after different durations of storage at 20°C in vacuum bags are given in Table 1. Survival in vacuum bags was not found after exposure times of 5 wk or longer. In comparison, when similar bags were not vacuumized, but only sealed hermetically, it took 8 wk until no survivors were found. Results indicate that a vacuum environment causes a stress in granary weevils that may lead to death by lack of energy or water. In order to avoid high costs and excessive plastic waste, vacuum bags should be re-sealable.

Table 1. Comparison between total emergence of surviving developmental stages or surviving adult granary weevils after different durations of vacuum storage (0.5 bar; 50 kPa) or hermetic storage at 20±1°C

Exposure time (wk)	2	3	4	5	6	7	8
Vacuum Storage:							
Eggs	31.7	1.7	0	0	0	0	0
Young Larvae	79.3	49.7	0.3	0	0	0	0
Medium Larvae	32.3	8.3	6.7	0	0	0	0
Old Larvae	64	27.7	3.7	0	0	0	0
Pupae	63	23.3	3.3	0	0	0	0
Beetles	0.3	0	0	0	0	0	0
Hermetic Storage:							
Eggs	85.3	64.3	6.7	0	0	0	0
Young Larvae	91	86.7	61.7	12	0	0	0
Medium Larvae	53.3	25.3	17.7	0	0	0	0
Old Larvae	89	54	12	0	4.3	2	0
Pupae	52.3	31.7	5.3	5.3	0	0	0
Beetles	16.7	0	0	0	0	0	0

Mean values of three replicates

But which development is most promising? Under the focus of sustainability, one-way storage systems such as the Silobag storage and PICS Bags may be ruled out as they produce comparatively more waste and environmental costs including production, transportation and waste management in areas often remote from industrial production and treatment sites.

Fig. 1. Vacuum machine with welding station and vacuumized wheat bag with two nylon gauze cages containing 100 grains with immature stages.

A storage method for a basic food crop needs to be reliable, feasible, and sustainable. The simplest, most decentralized production and maintenance of hermetic storage structures may thus be possible with metal containers and silo bins.

Table 2 compares today's commercially available storage methods to underground hermetic storages found in archaeological sites since about the Iron Ages (800 B.C., Hill et al., 1983). Underground hermetic storages were constructed up to the 1850s when convicts built such cave-like structures in Cockatoo Island as part of the convict establishment in the harbour of Sidney (http://www.environment.gov.au/cgi-bin/ahdb/search.pl?mode=place_detail;place_id=105264). Some countries, e.g., in northern Africa and Asia still seem to use underground hermetic storage to a certain extent.

Table 2. Comparison of storage methods and environmental aspects

Method and resources required	Benefits	Risks and/or disadvantages	Environmental aspects
Bunker storage with low walls; Heavy tarpaulin; Drawn by caterpillars	Hermetic storage of large quantities; Semi-underground storage possible; Multiple use	Hermetic seal difficult; Permeability of liners; May be punctured (man, vertebrates)	Large space needed; Concrete surface/walls; Heavy machinery; Large plastic liners; Use more than once
Hermetic storage in Volcani cubes, Grain Pro cocoons etc., with/without vacuum	Hermetic storage in pre-designed cubes; Gastight seal with zipper; Multiple use	Shape flexible, but not volume; Permeability of liners may be punctured or become leaky	Multi-layer plastics; Full recycling not possible; Multiple use
Hermetic metal silos	Hermetic storage; Multiple use	Hermetic seals endangered by day-night temperatures and solar irradiation; Risk of condensation	Multiple use; Recycling possible
PICs Bag; Woven 60 kg or 100 kg bag with one or two gastight in-liner bags	Hermetic storage on a small farmer's level; Number of bags can adjust to harvest	Improper seal at top possible; Punctures or penetration from inside (insects) or outside (vertebrates, plants)	Plastic waste; Single use/unsustainable
Silo bags; Loading and unloading machinery	Hermetic storage of large quantities; Size flexible	Costly machinery; Protection against vertebrates needed; Single use/unsustainable	Large space needed; Plastic waste; Full recycling not possible
Gastight retro-sealing with white elastic paint; Gastight seal of grain inlet and outlet	Solar reflection; Gastight degree is high; Multiple use	Laborious; Hermetic seal difficult to reach	Paint covered metals; Not recyclable; Multiple use
Vacuum BigBag; Multi-layer liners and vacuum pump needed; May be suspended from rack	Vacuum pressure shows sealing quality; Hermetic storage; Faster pest control from reduced oxygen	May be punctured; Few uses	Plastic waste; Full recycling not possible
Underground hermetic storage	Hermetic storage; Natural cooling; No effect of diurnal temperature changes; No access for pests	Laborious construction; Unloading from above	Multiple use; Recycling possible depending on materials used

Pan et al. (2019) suggest the construction of an underground granary using a rigid steel skeleton and flexible polymers to seal the granary from the outside. The authors state that this method would be more ecologically sound and easier to disassemble compared to structures built from concrete. Thus, underground-hermetic storage still finds interest in modern research. Even today, however, the question of which storage type is most suitable for future grain storage still needs to be answered.

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Modified atmospheres in paddy rice hermetic storage are biogenerated according to different mechanisms in Portugal and Mozambique

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Abstract

Hermetic storage (HS) relies on modified atmospheres (MA) to deliver a safe and environmentally friendly extended storage life technology for agricultural commodities. Here, we discussed the implications of the initial levels of the moisture and temperature in determining which mechanism drives the increased storage capacity of paddy rice using results from experiments with HS under two distinct agronomic and environmental contexts, namely Portugal and Mozambique. Collectively, our results showed its importance as drivers for the modification of the atmosphere content of paddy rice hermetically stored. At low insect infestation conditions of Portugal, the atmosphere was modified only under high moisture content, despite the temperature. In Portugal, when the flux of the grain surpassed the drying capacity, delayed, incomplete or ineffective drying occurred (moistures >14%); therefore, HS could be successfully used. Additionally, we showed that under tropical conditions of Mozambique, even at low moisture (ca. 12%), insect infestation was common, and temperature was high (24 to 36ºC), thus increasing insect activity which changed the atmosphere conditions due to high respiration. MAs are among the best solutions for stored products protection. Our overall results suggested that HS with biogenerated atmospheres was a successful strategy for stored paddy rice under different contexts. In both conditions, between 4 to 12 mo storage, insect populations were controlled under HS when compared with control storage in Portugal (totally supressed at 24ºC) and traditional storage in Mozambique (96% reduction) but driven by specific underlying mechanisms.

Keywords: Paddy rice, Modified atmospheres, Portugal, Mozambique, Insects

Introduction

Rice is important in the diet of populations of Mozambique and Portugal. In Mozambique, rice is mandatory for food security and one of the main cereals available, mainly in rural areas. This commodity is produced mainly by small farmers that use traditional unprofitable low-yield varieties. Moreover, additional losses are significant because of a deficient storage system (Guenha et al., 2014). For long-term storage, their facilities are not effective against the major pests of cereal

crops, particularly under high temperature and relative humidity (RH) that are typical conditions of tropical regions, which result in increasing the respiration rate of living organism and insect pests' populations, contributing to quality and quality losses (*Phophi et al., 2020*). Portugal, outshines as the largest rice consumer per capita [~15 kg/person (INE, 2021)] and because rice is a seasonal crop, it needs to be stored and available all over the year. Good storage means no infestation and enough low water activity, below 0.6-0.7 a_w, to prevent grain and mould respiration. One of the sustainable alternatives to the practice of insecticides application is the use of hermetic storage (HS) technology, which is effective in providing long-term, chemical free and sustainable grain storage. Under this technology, biogenerated atmospheres might be achieved naturally from the conditions created by the respiration of living aerobics organisms, changes gas content and, consequently, reduced biotic activity. This mechanism is reported to be as efficient as conventional fumigants to control insects (Navarro, 2010). The present work aimed at comparing the effectiveness of biogenerated modified atmospheres in two different real settings of paddy rice in Mozambique and in Portugal.

Material and methods

Independent trials were conducted in the Sado Valley, Portugal and in Boane district, Mozambique.

Trials in Mozambique (Mz)

Two experiments were conducted in Umbeluzi Agricultural Station, Institute for Agrarian Research of Mozambique (IIAM), Boane District (as detailed in Guenha et al., 2014; Carvalho et al., 2019; Covele et al., 2020). The first experiment (Mz1) was carried out for six months from January to July 2013 and the second experiment (Mz2) was conducted for one year, from February 2016 to February 2017. For both trials, the rice variety used was ITA 312, one of the most cultivated in Mozambique, mainly in the southern region. During the experiments, data loggers HOBO UX100-011 and HOBO UX120-006 M (Onset Hobo® Data Loggers, MA, USA) were used to monitor temperature and relative humidity outside (Mz1) and inside the storage containers (Mz2), located in the middle of the containers (E2) and in the warehouse environment (Mz1 and Mz2). Moisture content was determined using ISO-712 (2009) Norma (Guenha et al., 2014; Covele et al., 2020).

Mz1: To evaluate the efficacy of HS, super bags GrainPro (GrainPro Inc., Philippines) (SGB) were compared with traditional raffia bags, filled with 20 kg of paddy and replicated six times. Raffia bags were considered the control. At the end of 3 mo, three of the replications were opened, and the remaining three replications were opened at 6 mo. For each replication, samples were taken from several cardinal points at the top and bottom position as well as in the periphery and center of bags, thus mixed to obtain a composite sample of about 1 kg, using the methodology recommended by Webley (1985) and Mathur and Kongsdal (2003).

Mz2: Five storage containers were evaluated: 1) traditional Polypropylene bags (control - PP) filled with 50 kg of paddy grain; 2) Super Grain Bags (SGB) filled with 50 kg; 3) Polyethylene Drums (PD) filled with 210 kg; 4) Polyethylene Silo Tanks (PST) filled with 750 kg; and 5) Safe Grain Bags (GB), filled with 1000 kg; all with three replications per treatment. Grain samples and Storgard WB probe traps (Trece, Inc., Oklahoma, USA) were placed in the middle of all storage containers, in order to attract and collect insects. The probe traps were emptied in every sampling and replaced in the containers.

Samples were transported from the experimental site to the laboratory in airtight plastic packaging to preserve their integrity.

Trials in Portugal (PT)

Experiments were conducted in a paddy rice warehouse, in the Sado Valley region, Portugal. Three trials were carried out from December to November 2016: the 1st trial ran 4 mo from December to April (PT1); the 2nd trial ran 6 mo+ from December to July (PT2); and the 3rd trial ran 4 mo from July to November (PT3) (Carvalho et al., 2019). Experiments used two rice varieties: Ronaldo, *Oryza sativa sbsp. Japonica*, and Sírio, *Oryza sativa sbsp. Indica.* The relative humidity and temperature were monitored by Hobo® Data loggers, with the probe placed inside the bags. In all experiments, the two varieties were stored as paddy bulks and submitted to three different relative humidities: 67, 75 and 85% at three different average temperatures: 14 (PT1), 17 (PT2), and 24ºC (PT3). Moisture content was determined using ISO-712 (2009) Norma. At the end of the experiments, CheckpointII Portable O_2 and CO_2 Gas Analyzer was used to assess gas contents at the bottom and top of each bag, making for a total of six measurements per treatment, and the results registered. The gas content was expressed in terms of percentage by volume in air. For each treatment and variety, three replications were carried out.

GrainPro (GrainPro Inc., Philippines) (SGB) bags were used. The efficacy was measured using samples of 20 g infested with 1 wk old of ~20 unsexed *Sitophilus zeamais* Motschulsky, the dominant key-pest of stored rice in Portugal (Carvalho et al., 2013). These samples were placed inside paper bags and each bag was settled inside of each paddy bag. At the end of each trial, these samples were collected and brought to laboratory to evaluate mortality and population growth. *Sitophilus zeamais* was originally collected from Portuguese rice mills and reared in climatic chambers at 25±2ºC and 70± 2% RH.

Data analysis

Mozambique (Mz) - Microsoft Excel, Statistica 12 (*StatSoft, 2011*), and SPSS 20.0 software were used for data analysis: two-way factorial ANOVA analysis and Tukey's HSD mean separation test under 95% confidence limits and Pearson Product-Moment Correlation Coefficient (Guenha et al., 2013; Covele et al., 2020).

Portugal (PT) - R software (R Core Team, 2017) was used for data analysis: function *lm,* simple linear regressions, two-way ANOVA, interaction models, ANOVA models fitted with the R function *aov*, Tukey and Kruskal-Wallis with Fisher's least significant difference tests with functions HSD test and Kruskal from package 'agricolae' (Mendiburu, 2017).

Results

Temperature and relative humidity, moisture, and gas content

Mozambique (Mz) - Mz1: The average temperature was ~24°C [14 to 36ºC] and the mean RH was 67.0% (46 to 83%). The paddy was stored at ~12.0% moisture content (mc). After 3 and 6 mo of storage, mc increased slightly but with no significant differences and not limiting for insect development (Guenha et al., 2014).

Mz2: The variation of temperature was similar in all the containers and ranged between 19.0 to 29.0ºC with June the coolest period and February the hottest month. The relative humidity inside polypropylene bag showed fluctuations along the storage period which was not observed inside the HS. The paddy was stored at 11.0%. There was a significant difference in the mc of grain stored in polypropylene bag (PB), Super Grain Bag (SGB) and polyethylene silo tank (PST) after six months of storage. The Super Grain Bags recorded the lowest moisture content. No significant change was observed in the moisture content of the grain stored in the polyethylene drum (PD) over the storage period (Covele et al., 2020). Gas content was not analysed.

Portugal (PT) The relative humidity did not change inside the hermetic bags under all conditions. The temperature followed the environmental temperature (Fig. 1) and got an average of 14 (PT1), 17 (PT2), and 24ºC (PT3). Paddy mc varied 13% (under 67% RH), 14% (under 75% RH) and 15% (under 85% RH). Under 67% RH, there was no significant change in O_2 and CO_2 content, and no respiration was detected - a sign of no biological activity and good storage conditions. Depending on the increase in RH, more O_2 was consumed due to the increase in the respiration rate of the organisms, and consequently, an increase in CO_2 as well (Fig.2).

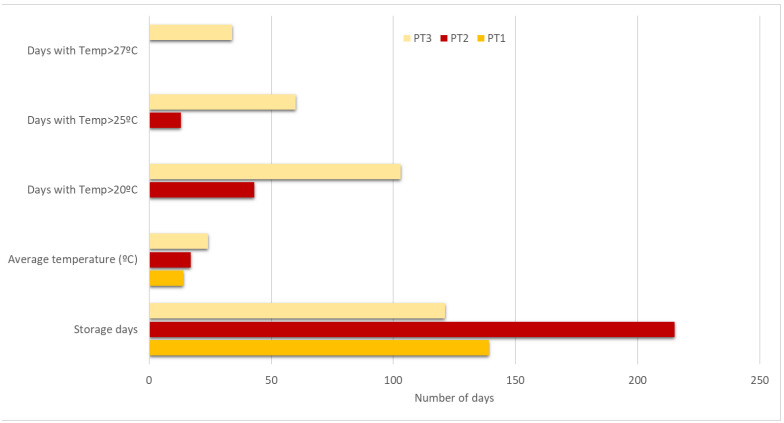


Fig. 1. PT: Average temperature and number of days with temperature greater than 20, 25 and 27ºC (data adapted from Carvalho et al., 2019).

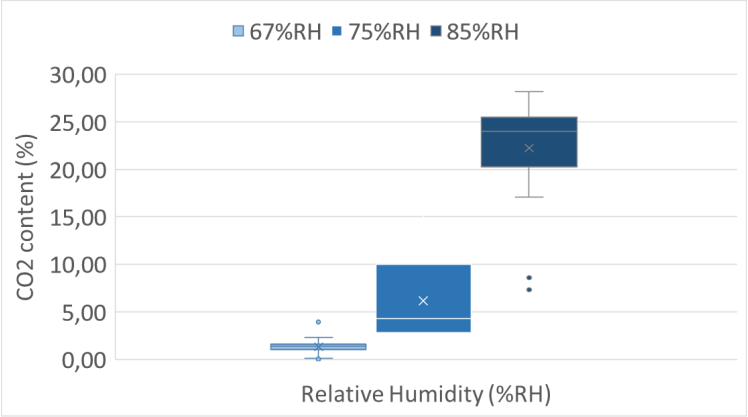
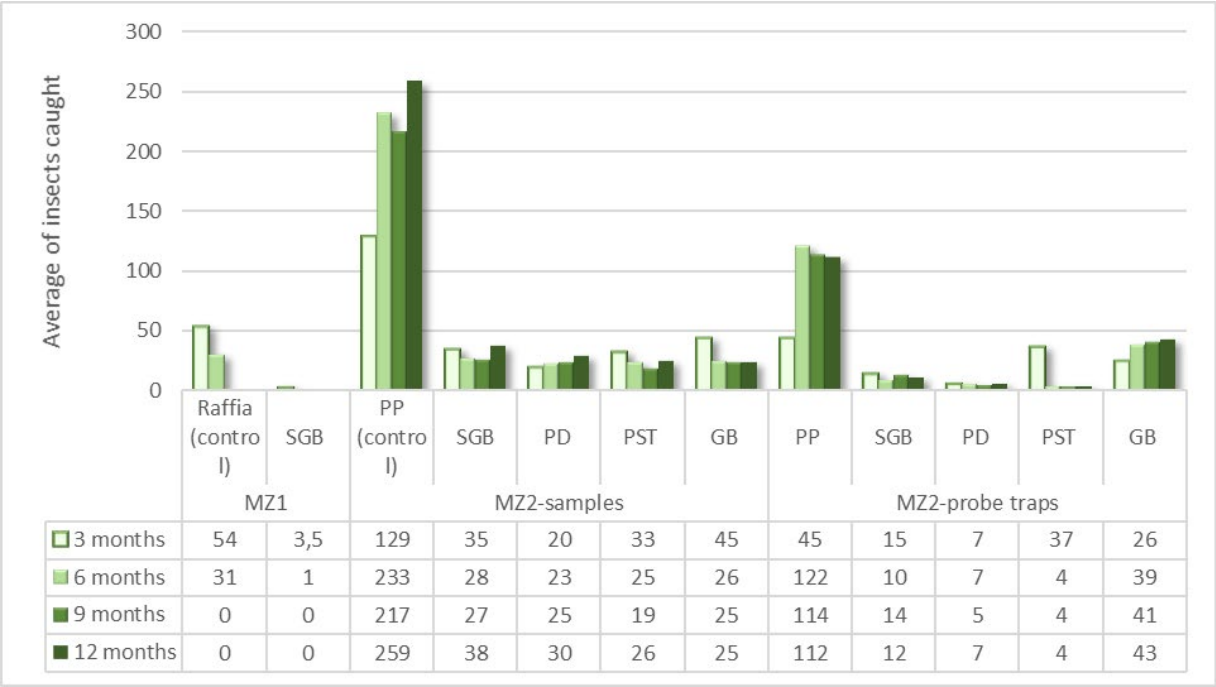


Fig. 2. PT: Relation of CO_2 (%) and RH (%) at the end of the experiment, for paddy rice under 67, 75 and 85% RH. The vertical bars indicate the standard deviation of the replications (data adapted from Carvalho et al., 2019).

Survival of stored product insect pests

Mz1-Samples: Four species were identified over the storage period: *Rhyzopertha dominica* Fab., *Tribolium castaneum* Hb., *Sitophilus* spp., and *Sitotroga cerealella* Oliv. The results showed that HS inhibited the increase of insect populations by over 99% (Fig. 3).

Mz2-Samples: The same four species plus the fifth species, *Cryptolestes ferrugineus* (Stephens), were identified. The lesser grain borer was the most predominant species (71% incidence) followed by the rice/maize weevil (incidence of 17%). *Sitotroga cerealella* were the least predominant species and were not even detected in any hermetic container from the 6 to 9 mo of trials. There were differences between polypropylene bags and HS in the number of insects caught over the storage period. Inside the polypropylene bags, infestation increased by 48%, after 3 mo of storage, and 80% after 6 mo of storage when compared with hermetic devices. During the first 3 mo, SGB showed a lower effect when compared with other HS, but at 6 mo, the SGB reduced the number of insects registered in samples by about two-fold. At 12 mo storage, the initial infestation level in HS was reduced by from 57 to 72%, with an average of 66% reduction in levels, compared with polypropylene bags. Using probe traps, there were no significant differences between any of the devices after three months of storage. After 6 mo of storage, SGB, PD and PST were significantly more effective at reducing insect populations (Fig. 3) (Covele et al., 2020).



	MZ1		MZ2-samples					MZ2-probe traps				
	Raffia (control)	SGB	PP (control)	SGB	PD	PST	GB	PP	SGB	PD	PST	GB
3 months	54	3,5	129	35	20	33	45	45	15	7	37	26
6 months	31	1	233	28	23	25	26	122	10	7	4	39
9 months	0	0	217	27	25	19	25	114	14	5	4	41
12 months	0	0	259	38	30	26	25	112	12	7	4	43

PP = Polypropylene bag; SGB = Super Grain Bag; PD = Polyethylene Drum; PST = Polyethylene Silo Tank; GB = Safe Grain Bag.

Fig. 3. **Mz1-Samples:** Average number of insects caught in raffia and HS before and after storage (adapted from Guenha et al, 2014); **Mz2-Samples:** Average number of insects caught at different storage containers; **Mz2-Probe Traps:** Average number of insects caught at different storage containers (data adapted from Covele et al., 2020).

PT: The average number of insects was considered, subtracting the 20 parental weevil adults used initially (Fig. 4) to better understand if there was progeny during the trials. Respiration rate increased with the increase of relative humidity, followed by average temperature of the trials. Since the number of insects was very low, the authors considered that the main organisms responsible for biogenerated atmospheres might be fungi and paddy rice. Under 85% RH the efficacy of the treatment was achieved for insect control (Carvalho et al., 2019).

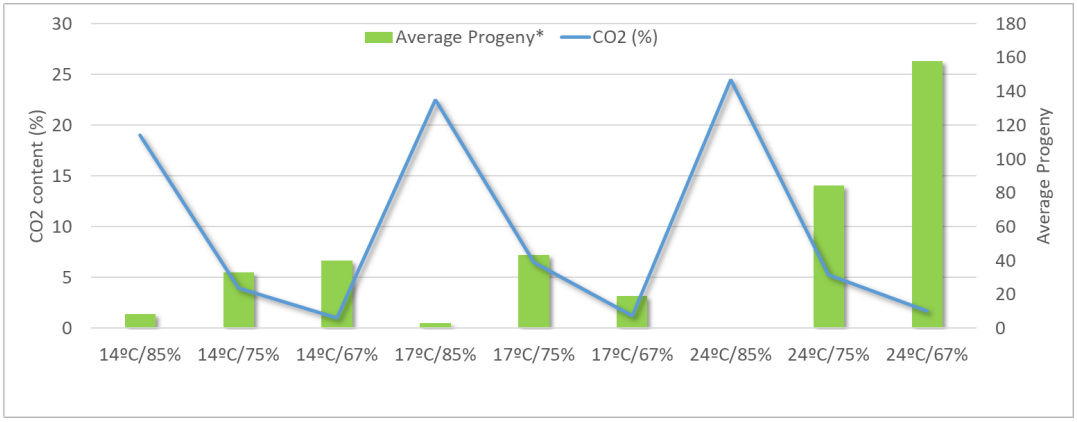


Fig. 4. PT: Average for the Progeny (*Progeny = alive adults + dead adults -20 parent adults) of *Sitophilus zeamais* under each temperature, relative humidity, and CO_2 condition (data adapted from Carvalho et al., 2019).

Discussion

Hermetic storage in temperate conditions is a successful practice for products with restricted infestations, in which modified atmospheres depend only on the relative humidity of the grain and time of storage, as we observed during the Portugal experiments. In contrast, in tropical environments, the grain to be stored typically has an initial high infestation. Under these conditions, the relative humidity and temperature are also high, so the respiration rates of insects, fungi and/or grain increase considerably during storage, reducing oxygen content to levels that make insect survival unsustainable and also reduce the activity of fungi and the activity of enzymes naturally present in the grains, thereby preserving the quality of the stored commodity (De Bruin et al., 2012), which matches the observations in the Mozambique studies. This suggests distinct underlying mechanisms are producing effective results. Therefore, our work emphasizes the implications of the levels of the moisture and temperature in determining the drivers of storage capacity of paddy rice using results from experiments with HS under two distinct agronomic and the contrasting environmental contexts of Portugal (temperate setting) and Mozambique (tropical setting). Collectively, we report that under the conditions of low insect infestation in Portugal, the atmosphere is modified only under high moisture content, despite the temperature and time of storage. In Portugal, when the harvested grain surpasses the drying capacity, then delayed, incomplete or ineffective drying occurs (moistures >14%); therefore, HS can be successfully used. It is mandatory for the grain to be free of insects so that MAs can act by maintaining the optimal conditions for product preservation. Under tropical conditions of Mozambique, even at low moisture (e.g., 12%), the common high insect infestation and high temperatures (24 to 36ºC) leads

to increasing insect activity, which then changes the atmosphere conditions to decreased O_2 and increased CO_2 levels (due to high respiration) and also results in restrictive conditions that control infestation. In both conditions, from 4 to 12 mo storage, insect populations were controlled under HS when compared with control in Portugal (totally supressed at 24ºC) and Mozambican traditional storage (96% reduction). In conclusion, we could highlight that biogenerated MAs were among the best solutions for stored paddy rice protection under different contexts but driven by specific underlying mechanisms.

Despite the fact that some synthetic pesticides are effective in controlling pests, their use is being challenged by environmental sustainability concerns and, more importantly, will suffer additional limitations due to the development of insect resistance, food contamination, and safety issues (Silva et al., 2019). The use of HS stands as a sustainable alternative to insecticide application. The MAs in this controlled environment depend on the respiration rate of insects, microorganisms, and the cereal itself (Villers et al., 2006). Any stored grain that is dry and free of insects can take weeks to reduce oxygen levels without the injection of supplemental carbon dioxide.

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Evaluation of storage of sunflower pellets in silo bags

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Abstract

Silo bags are a temporary and potentially hermetic storage system widely used for grains, oil seeds, legumes and other products. Oil extraction of sunflower seeds through the extrusion-expelling process generates a high protein sub product, which is pelletized for animal consumption. Storing sunflower pellets in silo bags could bring logistical advantages to animal production farms and the sunflower processing industry. Thus, the objectives of this work were to: 1) study the effect of silo bag storage on chemical composition and fungal biota evolution on sunflower pellets; and 2) quantify the mechanical damage due to the loading and unloading operation on pellet stability. The study was carried out in a facility near Crespo (Entre Rios Province, Argentina), between February and October of 2018. Samples were collected during the loading (initial) and unloading (final) of the pellets to/from the silo bag. Samples were analyzed for fungal biota, mycotoxins, and composition (moisture content, protein, fat, and fiber). Temperature, relative humidity and CO_2 concentration were measured during storage. Mechanical damage was evaluated by measuring pellet length and dust percentage. Storage moisture content was around 9% (dry basis) and did not change during storage, while relative humidity remained below 60%. The evolution of CO_2 concentration was related to the pellet temperature inside the bag (summer time 14.3% CO_2 and 30°C, winter time 6.0% CO_2 and 15.3°C). Fungal colony counts in the initial samples were low ($1.2x10^1$ CFU/g DM) and slightly increased during storage ($3.4x10^2$ CFU/g DM). Low concentrations of DON (12.5 µg/kg) and Zearalenone (5.5 µg/kg) were found in initial sampling, and no increase was detected during storage. Pellet length before bagging was 27.1 mm and decreased to 24.0 mm after storage, while dust percentage did not change (14.9%). These results indicated the feasibility of storing sunflower pellets in silo bags without quality deterioration.

Keywords: Sub product storage, Chemical composition, Carbone dioxide, Fungal colonies, Moisture content, Mechanical damage, Hermetic storage

Introduction

In the last decade (2009-2019), global sunflower oil production has increased 72% (13.4 to 23.1 million tonnes) and sunflower meal (main sub-product of oil extraction) also increased from 14.8 to 24.7 million tonnes. In this context, Argentina is the third world producer of sunflower seeds and sunflower by-products, and an important trader in the global market (USDA, 2020).

Protein and crude fiber are the main components in sunflower meal, ranging around 20-60% and 5-34%, respectively. The residual oil varies depending on the extraction process, starting from less than 3% when solvent extraction is applied (Pedroche, 2002). The sunflower meal is usually pelletized through a high pressure and temperature process (Arelovich, 2003). Pelletizing meal works to maximize bulk density and minimize dusting, which thereby reduces the storage and transportation cost of the sunflower meal. Pelletizing also increases the physical stability of the meal and increases the allowable storage time before consumption (Heuzé et al., 2020). Consequently, minimization of pellet damage during transportation, handling and conveying is highly desirable (Aarseth and Prestl, 2003). Additionally, it is important to preserve the chemical stability and prevent mycotoxins production during feed storage (Pereyra et al., 2019).

The silo bag is a temporary storage system widely employed for grain storage in Argentina and several countries (Bartosik, 2012). With the increase of sub products availability (WDGS, meals and pellets), silo bags began to be considered as an alternative storage system for animal feed (Alvarez, 2016). Thus, the objectives of this work were to: 1) study the effect of silo bag storage on chemical composition and fungal biota evolution on sunflower pellets; and 2) quantify the mechanical damage due to the loading and unloading operation on pellet stability.

Materials and methods

The study was carried out in a facility near Crespo city (Entre Rios Province, Argentina) between February and October of 2018. A standard silo bag of 2.7 m diameter and 60 m long (with recommended stretching) was filled with approximately 160 t of sunflower pellets. During the loading operation, 5 kg of pellet were collected directly in the flow from the wagon to the bagging machine (Mainero 2230, Bell Ville, Argentina) in four sites (every 10 linear meters approximately). Temperature and relative humidity (RH) were registered (every 3 h) with integrated data loggers (Ibutton DS 1923, Maxim, San Jose, USA) placed in two sites at 20 m from the ends of the bag, at one meter deep from the bag surface. Carbon dioxide concentration was measured every month along every 6 m of the bag, starting the first measuring point at 3 m from the end employing a portable gas analyzer (Silcheck, Junín, Argentina). After eight storage months the silo bag was unloaded and pellet samples were collected directly in the flow from the unloading machine (Richiger Ea 180, Sunchales, Argentina) to a truck. The degree of wear of the augers of the bagging and unloading machines was documented.

Fungal biota (filamentous fungi and yeasts) was evaluated using the method of counting in Petri dishes in agar, potato dextrose (Britania®), with the addition of chloramphenicol (0.1% Anedra®). Plates were incubated in an oven at 28°C for 5 d (Pitt and Hocking, 2009). Counts were expressed as colony forming units per gram of pellets dry matter (CFU/g DM). Mycotoxin concentrations (Aflatoxins, Fumonisins, Zearalenone, Ochratoxins, Deoxynivalenol (DON)) were analyzed using LC MS/MS method (UPLC Acquity H Class / detector MSMS: Xevo TQ-S micro, Waters, USA).

Crude fats, protein and fiber were analyzed by NIRS (FOSS DS 2500, USA). Moisture content (m.c.) was determined with the oven method: three sub-samples of 100 g were dried at 103°C during 24 h (ASAE, 2003). Chemical composition results were expressed in dry basis percentage (% d.b.). Mechanical damage was determined by measuring the length of 70 pellets (randomly selected) with a caliper (Hamilton C 10, China), and dust fraction was separated by employing a grain sieve (1,8 mm opening diameter). Then, dust fraction was collected and weighted (OHAUS, Pioneer PA 214, USA).

Comparisons of treatments were performed with ANOVA (R software version 3.6.3). Tukey's HSD ($\alpha = 0.05$) post hoc test was also used for mean comparison. Figures were created with Excel (Microsoft Office Professional Plus 2016).

Results and discussion

Figure 1 shows that the temperature at the beginning of storage (middle of the summer) was above 30°C while the mean ambient temperature was 25°C. During the two following months, the pellet temperature inside of the silo bags decreased until reaching 25°C, while the mean ambient temperature remained almost stable. Then, pellets and ambient temperature decreased towards the wintertime, reaching a minimum value of 11 and 14°C for ambient and pellets, respectively. In September (beginning of spring), both temperatures started to gradually increase, equilibrating to the same temperature (18°C) at the end of September. The RH at the beginning of storage was of 59%, trended to decrease towards winter (52%) and increased in early spring (54%). The maximum CO_2 concentration (15%) was registered after one month of storage (end of March), decreased in winter reaching a minimum of 6% by the end of July and then increased to 12% by September.

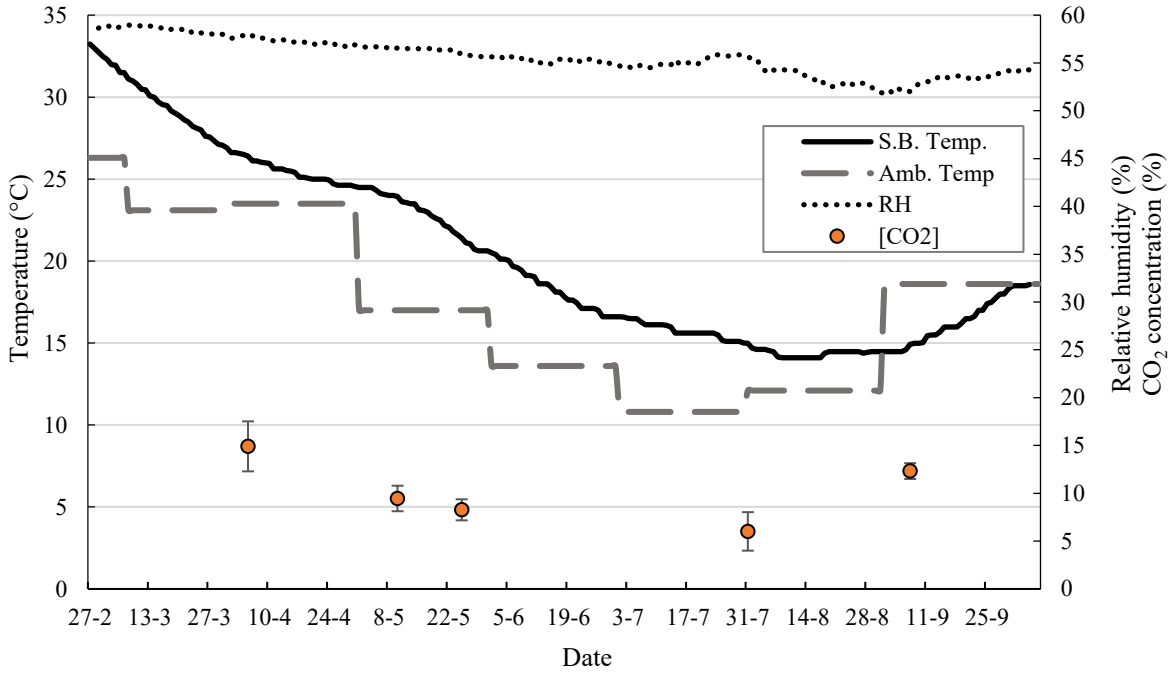

Fig. 1. Evolution of CO_2 concentration ([CO_2], %), relative humidity (RH, %), pellet temperature in silo bags (S.B. Temp., °C) and mean ambient temperature (Amb. Temp., °C), during silo bag storage of sunflower pellets. Vertical bars indicate SD.

Table 1 shows that pellets m.c. was near 9% (d.b.), the variability between sampling sites was low (SD less 0.5%) and did not change with storage time ($p>0.05$). Crude protein (mean= 36.9% d.b.), crude fiber (mean= 27.2% d.b.) and fat content (mean= 1.62% d.b.) also showed no variations with storage time ($p>0.05$).

Mean initial pellet length was approximately 27 mm (Table 1), and after pellet extraction the length decreased to 24.4 mm ($p= 0.0013$). Statistical analysis of dust fraction showed no variation between sampling time (mean= 14.8%; $p>0.05$). Variation between sampling sites at the beginning of storage (samples collected during the loading operation) was larger than at the end of storage (samples collected during the unloading operation) (SD= 7.7 vs 4.4 %).

Table 1. Chemical composition, pellet length and dust content (mean ± SD) during bagging (Initial sampling) and unloading operation (Final sampling)

Parameter	Initial sampling*	Final sampling
Moisture Content (% d.b.)	9.04 ± 0.44 **a**	8.60 ± 0.13 **a**
Fats (% d.b.)	1.81 ± 0.16 **a**	1.43 ± 0.52 **a**
Crude Fiber (% d.b.)	27.43 ± 1.26 **a**	27.04 ± 0.50 **a**
Crude Protein (% d.b.)	36.33 ± 2.87 **a**	37.37 ± 1.92 **a**
Pellet length (mm)	26.90 ± 0.61 **a**	24.40 ± 0.47 **b**
Dust (%)	13.55 ± 7.67 **a**	16.29 ± 4.39 **a**

*Different letters in same row indicate statistically significant differences between sampling times (p-value <0.05).

The initial count of fungal biota was 1.2 CFU/g DM (Table 2), and was mainly composed of *Alternaria sp.* and *A. flavus*. After storage, CFU count increased to 350 CFU/g DM ($p<0.0001$), while its fungal biota was composed of *A. flavus, A. niger,* and *Eurotium sp*. Table 2 also shows that the initial samples were contaminated with DON (12.5 µg/kg) and Zearalenone (5.5 µg/kg).

In both cases, the mean level of mycotoxin did not show variation with storage time ($p>0.05$). Moreover, the variation of contamination among sampling sites (SD) was larger than their mean values in both sampling times.

Table 2. Mold colony forming unit counts and mycotoxin contamination (mean ± SD) during bagging (initial sampling) and unloading operation (final sampling).

Parameter		Initial sampling*	Final sampling
Molds (CFU/g DM)		1.20 ± 1.40 **a**	347 ± 1.50 **b**
Mycotoxins (µg/kg)	DON	12.50 ± 16.20 **a**	10.25 ± 20.00 **a**
	Zearalenone	5.50 ± 6.47 **a**	3.80 ± 7.60 **a**
	Aflatoxin	**nd**	**nd**
	Fumonisin	**nd**	**nd**
	Ochratoxin	**nd**	**nd**

*Different letters in same row indicate statistically significant differences between sampling times (p-value <0.05); nd = mycotoxin non detected (detection limit: Ochratoxin: 10 µg/kg, others = 1 µg/kg).

The initial difference between ambient and pellet temperature could be explained by the high initial pellet temperature (due to the extrusion process). The temperature at which a product is bagged affects its temperature in the first weeks of storage (Cardoso et al., 2008). During the remaining storage time, the average pellet temperature followed the pattern of the average ambient air temperature. This influence of ambient temperature over the temperature of the product stored in silo bags had been previously documented (Bartosik et al., 2008; Bartosik 2012).

Temperature has a direct effect on microbial growth and also an indirect effect through the equilibrium RH (Magan et al., 2003). As temperature decreased toward winter, so did the equilibrium RH, even when the pellet m.c. did not change during storage.

The biological activity of the pellet, measured as CO_2 concentration, also followed the temperature pattern throughout the season (Fig. 1), this being a consequence of the effect of the temperature on the respiration rate of grains and byproducts (Ochandio et al., 2017). Rodríguez et al. (2008) found a similar pattern in the evolution of the CO_2 concentration through the seasons in wheat silo bags.

The CO_2 concentration in the first few storage months was similar to that reported by Bartosik et al. (2008) for sunflower seed stored in silo bags at a similar m.c. (16.5% CO_2 for 9.2% m.c. seed (d.b.)). However, since the oil content of the sunflower seed is substantially higher (around 50%) than that of the pellet (<2%), the equilibrium RH for 9% sunflower seed is also higher (close to 70% according to Maciel et al. (2018) than that measured for pellet in this study (52-58%). Therefore, it would be expected that for the same m.c., both the microbiological activity and the respiration of the sunflower pellets would be lower than that reported for sunflower seeds. The low initial CFU count could be due to the previous extrusion-expelling process and the subsequent pelletizing. The high temperature and pressure of those processes affect vegetative microorganisms to a great extent, and mold spores to a lesser extent (ICMSF, 2006). Therefore, when pelletizing is carried out correctly, the product results in low mold CFU counts ($<10^2$ CFU/g; IAG, 1993).

The initial biota was mainly composed of *Alternaria* and *Aspergillus* species, typical oilseed fungi (Stroka and Gonc, 2019). While *Alternaria* and *Aspergillus* species can colonize the grain in the field and produce mycotoxins, *Alternaria flavus*, in particular, is regarded as a storage mold (Magan and Lacey, 1988). However, the presence of this mold was not related to aflatoxin contamination of the initial samples. Zearalenona and DON are produced by the *Fusarium graminearum* and *F. culmorum* species (Miller, 1995) that typically colonize grain in the field (Fleurat-Lessard, 2017). The *Fusarium* species were not identified in initial samples; thus, it could be speculated that the contamination was produced before harvest.

Even though the equilibrium RH of the sunflower pellet was below the limit reported for filamentous fungi growth (70%) (Magan and Lacey, 1988), in the final samples some *Eurotium* sp. and *Aspergillus* sp. (xerotolerant storage mold (Fleurat-Lessard, 2017)) were found. During long term storage, the daily and seasonal changes in temperature often result in moisture migration in the product mass, creating favorable conditions for fungal growth in the silo bag periphery (Cardoso et al., 2007). In fact, during pellet extraction, a slight moisture condensation was observed in some areas where an air chamber between the plastic cover and the pellets was created. The equilibrium RH limit for fungal growth (70%) is lower than the limit for mycotoxins production (83-85%) (Fleurat-Lessard, 2017). Thus, it can be hypothesized that the xerophilic conditions during storage allowed the survival of *A. flavus* but prevented the development of Aflatoxin. Zearalenone and DON levels found in this study were lower than those reported by Boevre et al. (2012) in sunflower meal (81 µg/kg and 23-24 µg/kg, respectively). In general, the mycotoxin levels detected in the current study were below the maximum level recommended for feed by the European Commission (DON: 900 µg/kg; Zearalenone: 100 µg/kg, Aflatoxin: 5 µg/kg and Ochratoxin: 10 µg/kg), according to Stroka and Gonc (2019). On the other hand, the high variability in mycotoxin concentration among replicates could be due to a sampling method error or the heterogeneity of the matrix (FAO, 2003).

The moisture and fat contents of this study were lower than those reported by other authors for sunflower pellets (Arelovich, 2003) and sunflower meal (Heuzé et al., 2020), and this could be the reason for the high stability of the pellets during storage (Le Cleft and Kemper, 2015).

The reduction in the length of the pellets during the loading and unloading operation, although statistically significant, was only 2.5 mm (from 26.9 to 24.4 mm), remaining within what is considered to be a standard size (between 10 and 30 mm) according to Clef and Kemper (2013). This was probably due to the good condition of the bagger and extractor machine augers. Hidalgo et al. (2009) reported that auger characteristics, such as wear condition, length, and angle of operation, were the most important factors in grain breakage during rice loading and unloading operations. The amount of dust in the initial samples was high because the pellets were previously stored in a metal bin, so there was a previous handling of the product. It is also possible that the previous handling of the pellets caused a localized dust concentration in some sectors of the metal bin, which subsequently translated into dust concentration variability in the silo bag.

These results showed the feasibility of storing sunflower pellets in silo bags without quality deterioration.

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Predicting oxygen depletion during grain storage using hermetic bag technology

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ABSTRACT

The agriculture sector in low-income countries faces a significant challenge in solving problems with postharvest losses. Postharvest handling operations, including storage, plays a vital role in keeping the commodity safe from deterioration. One of the growing alternative innovative storage technologies that aim to improve smallholder farmers' food safety and security is the hermetic storage. Hermetic storage can effectively control insect activity in stored grain and thus preserve grain quality. Grain moisture content stabilizes while oxygen (O_2) level reduces, and carbon dioxide (CO_2) increases through respiration of the commodity, insects, and fungi. One kind of hermetic storage technology uses gas impermeable film as a liner inside a woven polypropylene (PP) or jute sack. Despite the increasing adoption of hermetic storage bag technology in Sub-Saharan Africa and Asia, there are still gaps needed to understand the factors that influence these hermetic liners' effectiveness. One of which is how these bags achieve and maintain a low-oxygen concentration and effectively preserve stored grains.

In this study, a spreadsheet was developed to calculate the predicted oxygen depletion in hermetic storage bags as a function of insect and grain respiration at different moisture contents and temperatures and the oxygen transmission rate. Results confirmed that insect respiration dominated oxygen depletion in maize stored at safe storage moisture contents of 12-14% while grain respiration was negligible. Results provided a basis for a better understanding of the hermetic storage technology. This will also assure the continued successful adoption of this critically important storage technology among smallholder farmers to control biological activity in stored grains.

Keywords: Grain respiration, Hermetic storage bag, Insects, Oxygen depletion, Oxygen transmission rate

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Obeng-Akrofi G, Maier DE, White WS, Akowuah JO, Bartosik R, Cardoso L (2021) Evaluation of hermetic bag technology to preserve shea nuts in rural Ghana. Page 133. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Evaluation of hermetic bag technology to preserve shea nuts in rural Ghana

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ABSTRACT

Shea nut is a key nutritional and economic food crop produced in rural shea growing communities in Africa. Storage losses are major constraints in the shea nut value chain, with substantial amount of shea nut lost prior to its processing and marketing. Hermetic storage bags have proven to be a viable option for the effective storage of grains in sub-Saharan Africa due to their simplicity, low cost, and efficacy. However, little is known on the effectiveness of hermetic storage bags in the storage of shea nuts. In this study, three different storage bags: hermetic bags, jute sacks, and polypropylene bags were used to store shea nuts with an initial moisture content of 7.3% (w.b.) over a six-month storage period. Each of the storage treatment was made up of 12 bags of 20 kg shea nuts of which samples were taken from 3 bags after every 6 wk. The quality of the stored shea nuts was assessed based on moisture content, insect infestation and damage, and mold presence and aflatoxin contamination. Temperature and relative humidity of the ambient condition was monitored versus the microclimatic conditions in the storage bags over the storage period. Carbon dioxide concentration in the hermetic bag was also monitored over the storage period. It was expected from the study that hermetic storage would provide a viable option for the quality preservation of shea nuts, and recommendation for their adoption.

Keywords: Shea nuts, Hermetic storage, Quality preservation, Technology adoption, Sub-Saharan Africa

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Assessing the viability of new deltamethrin treated hermetic storage bags in artificially infested wheat

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Abstract

Trogoderma granarium (Everts), the khapra beetle, has been named to the top 100 of the world's worst invasive exotic species and is one of the most destructive stored product insects. This species can feed on a wide variety of food products (>100) during storage and throughout the supply chain. The use of hermetic packaging is intended to maintain the quality and safety of the stored grain, while continuously protecting the grain from stored product insect contamination. Previous research on non-hermetic deltamethrin-treated packaging has shown to be effective in suppressing adult and larval survival of numerous species. The objective of this research was to determine the ability of a prototype deltamethrin all-in-one treated hermetic bag to maintain grain quality and inhibit *T. granarium*'s survival and growth in artificially infested wheat. The effectiveness of the deltamethrin on the outside of the bag was tested against *T. granarium* adults and larvae. There was a significant reduction in responsive adults after 5 d continuous exposure. More than 86% of larvae held on the outside of the packaging were unresponsive after 9 d exposure. Lots of 15 kg of wheat and 100 *T. granarium* larvae were placed inside treated and untreated storage bags, sealed, and stored in a semi-field warehouse and observed after 2, 6, and 8 wk for *T. granarium* survival and grain quality attributes. Live adult *T. granarium* were observed at 2 wk, but there were no live adults observed at 8 wk storage. The weight and number of insect damaged kernels was lower across all storage times for grain held in the treated bags, as compared with control bags (i.e., non-hermetic, without insecticide). The new deltamethrin hermetic bags maintained positive grain qualities; however, more information on the hermetic parameters is needed to understand how some larvae survived.

Keywords: Khapra beetle, Hermetic bags, Deltamethrin packaging, Grain quality

Introduction

Trogoderma granarium (Everts), the khapra beetle, has been named to the top 100 of the world's worst invasive alien species and is one of the most destructive stored product insects. This species can feed on a wide variety of food products (>100) throughout the supply chain and presents a major threat to global food security (Athanassiou et al., 2019). *Trogoderma granarium* is a major threat because it possesses the ability to survive for extended periods of time (months to years) under extreme conditions such as extreme temperatures, low relative humidity (RH) levels, and lack of food resources.

Detection and monitoring for signs of *T. granarium* presence is vital for controlling the spread of this species into countries without established *T. granarium* populations. However, when an infestation is established or detected there are several chemical and nonchemical control methods available for use. Methyl bromide is a powerful and effective fumigant to eradicate *T. granarium*. The use of contact insecticides can be applied to multiple surfaces in buildings and shipping containers to prevent the establishment or spread of *T. granarium* as quarantine and pre-shipment options at ports of entry, and do give residual control. However, the type of surface in which contact insecticides are applied play a significant factor into the efficacy of pyrethroid and insect growth regulator (IGR) insecticides (Arthur et al., 2018).

In many countries in the world where *T. granarium* is established, grain is stored in bulk bags. Recent investigations into the use of insecticide-treated packaging for control of stored product insect infestations have shown that both deltamethrin and methoprene-treated packaging material could be used to control populations of multiple stored product insect species (Kavallieratos et al., 2017; Scheff et al., 2017, 2018, 2019). Exposing adults of *Tribolium castaneum ,* the red flour beetle*,* for 48 h or longer on deltamethrin-treated packaging material results in no progeny production (Scheff et al., 2018). Kavallieratos et al. (2017) reported knockdown of five different adult stored-product beetles after 60 min exposure on deltamethrin-treated packaging material and increasing mortality as the beetles were continually exposed on the treated material.

In conjunction with the use of insecticide treated packaging material, the use of hermetic storage has been used successfully in many South American, Middle Eastern, and North African countries (Navarro et al., 2012). The use of hermetic packaging is intended to maintain the quality and safety of the stored grain, while continuously protecting the grain from stored product insect contamination. This method of storage takes advantage of the natural respiration of insects and the commodity by reducing oxygen (O_2) levels and increasing carbon dioxide (CO_2) concentrations to lethal amounts for the insects. Decreasing O_2 and increasing CO_2 is expected to cause the stored product insect to asphyxiate while maintaining the stored grain quality. The key to successful implementation of hermetic storage of grains is creating an airtight seal for a sufficient amount of time to create a hypoxic environment, and if this environment is not sustained long enough, the efficacy of the hermetic bag is lost.

Previous research has focused on the concepts of hermetic packaging and insecticide-treated packaging as fundamentally separate approaches for bagged storage of stored products. Their integration as one technology for storing grain has never been previously considered. But if we consider their combined effect as multiple modes of action against stored product insect infestation of stored grains, it could provide an advantageous technological improvement for small stakeholder farmers. Therefore, the objective of this research was to determine the ability of a

prototype deltamethrin all-in-one treated hermetic bag to maintain grain quality and inhibit *T. granarium*'s survival and growth in artificially infested wheat.

Materials and methods

This study was conducted at two different locations. The laboratory bioassays were conducted at the United States Department of Agriculture Animal and Plant Health Inspection Service, Center for Plant Health Science and Technology, Otis Laboratory, Buzzards Bay, MA, USA. *Trogoderma granarium* colonies were reared on a combination diet of ground dog food, wheat germ and rolled oats (4:1:1 ratio by volume). *Trogoderma granarium* colonies were maintained in an environmental growth chamber at 30°C and 50% RH in continuous darkness. All experiments were conducted in the Otis Laboratory containment facility under direct observation. The semi-field trials were conducted at the Agro-Farm facilities of the Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Nea Ionia, Greece. For these tests, *T. granarium* colonies were reared on wheat at 30°C and 65% RH in continuous darkness.

Prototype deltamethrin "all-in-one" treated hermetic bags used in this study were supplied by Vestergaard Frandsen Inc. The specifications of the prototypes tested were as follows: Base material: 45 GSM standard woven polypropylene bag; laminated with 15-micron deltamethrin/ PP coating, 5 GSM binding agent, 5 GSM Ethylene vinyl alcohol (EVOH – a formal copolymer of ethylene and vinyl alcohol). **A finalised product is now available with improved hermetic properties, available as ZeroFly® Hermetic Storage Bags.* Non-hermetic control bags were provided from a local wheat mill in Thessaly, Greece, and contained no pesticide.

Laboratory bioassay arenas were created as described by Scheff and Arthur (2018). Briefly, 9 cm diameter circles of the treated hermetic and untreated bag materials were cut and affixed to the bottom of a 100 x 20 mm plastic Petri dish. The edges of the material were secured with adhesive caulking material (DAP Kwik Seal, DAP Products Inc., Baltimore, MD, USA) and the sides of the Petri dish were coated with Fluon® (polytetrafluoroethylene, Sigma-Aldrich Co., St. Louis, MO, USA) to prevent insects from escaping the testing arena. Ten mixed-sex adult *T. granarium* adults or larvae (~4 mm in size) were placed on five untreated or five treated (outside surface) material, along with ~500 mg of diet. Adults were observed after 5 d post-insect addition for the number of responsive vs. non-responsive adults. Larvae were observed after 9 d post-insect addition for the number of responsive vs. non-responsive larvae. All bioassays were repeated three times for a total of 15 individual replicates. The percentage of responsive vs. non-responsive adult and larval *T. granarium* were transformed to angular values for statistical analysis (Zar, 2010) and analyzed for significant differences under the general linear model (GLM) procedure in SAS (SAS Institute, version 9.4, 2012) at $P = 0.05$.

The semi-field trials were conducted during the summer/fall of 2019 in sealed rooms (5.85 x 3.90 x 3 m high) in Velestino, Greece. Nine untreated and nine treated hermetic bags were filled with 15 kg of insect free organic wheat. Prior to sealing the bags, 100 *T. granarium* larvae, 4-5 mm in size, were introduced into each bag and then sealed using a three-step twist-tie method as follows: 1) air was squeezed out the bag of grain, 2) the bag material was gathered together and twisted five times, and 3) the twisted material was folded over and secured shut with a zip-tie/cable-tie. After the bags were closed, they were placed into experimental rooms and held at ambient conditions. Three hermetic bags, untreated and treated, were selected at random after 2, 6, and 8 wk storage.

After each storage duration, the stored wheat was assessed for grain quality parameters, effect on khapra beetle survival, and the presence of other stored product insect species.

Results and discussion

After 5 d, there was a significant reduction in mean percentage (± SE) of responsive *T. granarium* adults exposed on the treated hermetic packaging material (8.7 ± 12.9%) compared to the control (47.6 ± 16.6%) ($F = 50.13$, df = 1, 29; $P < 0.0001$). This correlates to >91% of adults that were unresponsive after 5 d of continual exposure on the treated hermetic packaging, which indicates that the treated material is highly effective on adult beetles. Similar to adults, *T. granarium* larvae were highly susceptible to the treated packaging material when continually exposed. After 9 d, >87% of larvae were unresponsive compared to 0% of larvae on the untreated packaging material ($F = 363.39$; df = 1, 29, $P < 0.0001$). All larvae on the untreated packaging material were healthy and responsive. It would be reasonable to postulate that continual exposure of adult or larvae of *T. granarium* on the treated packaging material would be highly effective in controlling *T. granarium* populations that encounter the treated bag material. Previous bioassays have shown that the use of pyrethroids on surfaces is generally considered effective for the control of this species (Arthur et al., 2018).

Regarding the semi-field bioassays, the number of insect damaged kernels (IDK) was greater in the untreated bags compared to the treated hermetic bags at 2, 6, and 8 wk storage. Additionally, the average amount of frass collected (mg) in untreated control bags was greater compared to the treated hermetic bags at all time points. At 8 wk storage, the control bags had 3733 mg of frass compared with 1838 mg in the treated bags. This indicates that there is more larval feeding in the control bags compared to the treatment bags, since the adult *T. granarium* does not feed (Athanassiou et al., 2019). Live adult *T. granarium* were observed at 2 wk in both untreated and treated bag materials, indicating that some of the larvae that were introduced at the start of the experiment made it to the adult stage. Perhaps the lower numbers of larvae in the treated bags did not create a sufficient hypoxic environment to cause larval mortality and thus some larvae could develop into adults. At 8 wk, *T. granarium* larvae were present in both the untreated and treated bags; however, very few adults were observed, and all adults were dead. The presence of larvae in the treated hermetic bag signifies there is ample supply of O_2 present inside the bag to sustain *T. granarium* development. One cause could be that the initial infestation level on the wheat was too low to create a low O_2 environment within the eight-week study. Secondly, the bags used in this study, 50 kg, were not filled to capacity and thus removing all the air prior to sealing would be difficult to achieve because of excess materials. It is likely that the way the bags were sealed, given that the wheat quantities were low, might have maintained some "oxygen nests" that allowed insect survival.

Conclusions

This was an initial test on a prototype all-in-one deltamethrin-treated hermetic bag material. Since this study is preliminary, it indicates that more information is needed to understand how this new technology can be improved. The new deltamethrin treated hermetic bags maintained positive grain quality throughout the testing period. Maintaining these grain quality parameters is imperative for farmers relying on bagged storage for protecting their stored grains. The deltamethrin component of the treated hermetic bag is highly effective on adults or larvae that would encounter the treated bag during storage and would provide an appropriate barrier to prevent

infestations from the outside. This new bag technology has the potential to be used as a preventive measure for the import/export of bagged grain products throughout the world. It could be one tool to help stop the spread of *T. granarium* into countries without an established *T. granarium* population, or from spreading within a country or geographic region. However, there is still the need for further studies on the all-in-one deltamethrin-treated hermetic bag material. Regardless, this is the first reported study on a new all-in-one deltamethrin treated hermetic bag and sets the groundwork for future studies on this new technology.

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Chigoverah AA, Mvumi BM (2021) Comparative survival of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) under large-scale hermetic grain storage conditions. Pp. 139-145. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Comparative survival of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) under large-scale hermetic grain storage conditions

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Abstract

We compared the responses of *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* Motschulsky to bio-generated modified atmospheres under hermetic field conditions. GrainPro Cocoons™ of 20-tonne capacity were loaded with bagged maize grain at three different sites. Twenty unsexed adult insects of each species were introduced into separate glass jars containing maize grain and the jars were closed using perforated lids to confine the insects in the jars during storage. The jars were placed at three elevations (Top, Middle and Bottom) inside each cocoon, with five position replicates at each level. Oxygen levels inside the cocoons were measured during the duration of the trials. Destructive sampling was carried out with one Cocoon being opened after 4 (Cocoon A), 8 (Cocoon B), and 12 mo (Cocoon C). The oxygen depletion in the cocoons was proportional to initial insect infestation levels in the stored grain. The lowest oxygen levels recorded were: 3.0% after 123 d in Cocoon A, 3.7% after 143 d in cocoon B and 1.5% after 14 d in Cocoon C. There was an increase in insect numbers in the glass jars with time. Fecundity at the experimental termination was higher for *S. zeamais* as compared to *P. truncatus* and there were significant differences ($P < 0.05$) between the two adult species for both insect numbers and grain powder except in Cocoon C. However, insect feeding-induced grain powder was high in glass jars containing *P. truncatus*. The highest live insect numbers and grain powder were recorded in Cocoon B for both species, and no live insects were recorded in Cocoon C at the end. The results showed that the efficacy of hermetic storage was hinged upon airtightness and rapid oxygen depletion to effectively suppress insect pest development and insect-induced damage, and to maintain grain quality.

Keywords: Bio-generated modified atmospheres, GrainPro Cocoon™, Oxygen depletion, Hermetic storage, Insect feeding-induced grain powder, Insect fecundity

Introduction

Storage insect pests control strategies in durable commodities have been centred on the use of synthetic pesticides. However, use of this curative method has faced challenges which include but are not limited to development of resistance among targeted species (Gautam et al., 2016), environmental pollution, and user and consumer health hazards (Damalas and Eleftherohorinos, 2011). Growing concerns and increased awareness on deleterious effects of synthetic pesticides on humans and the environment are influencing use of alternative control methods. This has seen an increase in research aligned to biorational control methods (Stevenson et al., 2012), and other non-chemical storage insect pest control options (Navarro, 2012).

Modifying atmospheric gaseous composition in grain storage structures or containers is another non-residual pesticide-free insect pest control option that is being promoted globally as an alternative to synthetic pesticides (Villers et al., 2006; Mvumi et al., 2013). Mode of action (Jay et al., 1971; Murdock et al., 2012; Ahn et al., 2013), performance and limitations (Jayas and Jeyamkondan, 2002; Chigoverah and Mvumi, 2016), types of modified atmospheres (MAs) and suitable facilities are well-documented (Navarro, 2012). Efficacy of MAs is hinged on low gas permeability of storage media which prevent replenishment of depleted oxygen and retention of produced carbon dioxide (Villers et al., 2006). GrainPro Cocoons are among the most common flexible structures being used for storing various dry agricultural commodities by applying the different types of MAs; bio-generated MAs, gas-hermetic fumigation, and vacuum-hermetic fumigation (Villers et al., 2006).

Insect species exhibit variable responses when exposed to MAs, hence it is important to understand the response of individual species to storage conditions to develop with efficient management strategies. Response of various storage insect pest species under hypoxic, hypercarbic and anoxic conditions are well-documented (Jay et al., 1971; Mitcham et al., 2006; Ahn et al., 2013). However, studies on the response of storage insect pest species of economic importance in sub-Saharan Africa like *Prostephanus truncatus* (Horn) (Coleoptera: Brostrichidae) are scarce, especially at commercial level. Moreover, knowledge gaps exist on the comparative responses of stored-maize pest species under bio-generated modified atmospheres. It is in this context that a comparative evaluation of the response of stored-maize insect pest species; *P. truncatus* and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) to bio-generated modified atmospheres was conducted under hermetic field conditions using GrainPro Cocoons.

Methodology

Trial sites and duration

The study was conducted at three Grain Marketing Board (GMB) depots in Zimbabwe; Marondera (mean annual temperature 16.7°C and rainfall 900 mm), Bindura (mean annual temperature 19.4°C and rainfall 847 mm) and Cleveland (mean annual temperature 18.4°C and rainfall 831 mm).

Maize grain and test insects

Freshly harvested untreated hybrid maize grain was supplied by GMB from deliveries made by farmers to the strategic grain reserve agency. The hybrid varieties used in the trial were Pioneer PHB 30G19 in Marondera, Seed Co SC727 in Bindura, and a mixture of varieties in Cleveland whose identity could not be ascertained.

Unsexed 7 to 14 d old adult *P. truncatus* and *S. zeamais* insect species were obtained from an existing culture in the Entomology Laboratory, Department of Crop Science at the University of Zimbabwe. The age of the insect species was determined during laboratory rearing as outlined by Tefera et al. (2010). The existing colony was monitored daily, and emerging insects were transferred over 10 consecutive days into separate jars containing clean disinfested grain until the required numbers were obtained. Insects which emerged on the same day were put in the same glass jar. The emerged insects were then kept under room conditions (27 ± 3°C) and insects which had emerged 7–14 d from the date of the first emergence were used in the trial.

Treatment layout

The trial evaluated the response of two storage insect pest species (*S. zeamais* and *P. truncatus*) to bio-generated atmospheres under hermetic field conditions. GrainPro Cocoons™ made from flexible polyvinyl chloride (PVC) with a capacity of 20 tonne were installed; one unit at each of the three GMB depots. A Cocoon consists of a top and bottom section which are brought together and sealed using a double-grooved gas-proof zipper. Each Cocoon was loaded to capacity with maize grain bagged in 50 kg polypropylene bags as described by Chigoverah et al., (2018). During loading, two 450 mL glass jars, each containing 200 g of sterilised maize grain, were artificially infested with 20 unsexed adult *S. zeamais* and the other jar with 20 unsexed adult *P. truncatus*. The jars were placed next to each other at each of the three levels: Top, Middle and Bottom layers. At each of the three levels, the pair of glass jars were placed at the four corners and at the centre. Thus, glass jars were placed at 15 positions in each Cocoon. The lids of the jars were perforated to allow free air circulation while restricting movement of insects in and out of the closed jars. Bags were stacked to recommended height and the dimensions of a fully loaded Cocoon were 4.4 x 3.4 x 2 m^3 (Length × Width × Height). A configured data logger (EL-USB-2, Lascar, Whiteparish, Wiltshire, UK) for measuring temperature and relative humidity was placed at each of the three levels before closing the Cocoons. Each Cocoon was closed by bringing the bottom and top sections together and sealing with the gas-tight zipper.

Sampling and sample analyses

An oxygen analyser (GPO_2-HH, GrainPro Inc, Subic Bay, Philippines) was used for monitoring oxygen levels in the cocoon from the day of setting up and subsequently; daily for the first two weeks after which readings were taken twice per week until the level reached less than 3%. Oxygen monitoring was also carried out at irregular intervals thereafter as a way of monitoring airtightness of the Cocoons.

The glass jars containing artificially infested maize grain were collected at termination of the trial after 4 mo in Marondera, 8 mo in Bindura and 12 mo at Cleveland, Harare. The grain in the collected jars was weighed and sieved (3 mm aperture) to separate whole grain and adult insects. The number of adult insects were counted (live and dead) and the grain powder from the jars was then weighed and recorded.

Data entry and analyses

Data were entered into Microsoft Excel and were subjected to normality tests using the Shapiro–Wilk test and transformed using log (X + 1) for insect count data and arcsine square root for percentage insect-induced grain powder following failure to meet assumptions of normality. GenStat 14 was used to subject the data to ANOVA and the LSD test ($P<0.05$) was used to separate treatment means to show statistically significant differences.

Results and discussion

Conditions inside installed Cocoons

There was a decrease in oxygen levels in all the Cocoons (Fig. 1). The rate of oxygen depletion was rapid at Cleveland where the lowest oxygen reading of 1.5% was recorded after 14 d. In Marondera, the lowest reading of 3.7% was recorded after 112 d storage and the lowest reading in Bindura was recorded after 143 d storage (Chigoverah et al., 2018*)*. The rate of oxygen depletion was directly proportional to level of insect infestation since insects were known to be the major consumers of oxygen in hermetically stored dry grain (Ahn et al., 2013). An increase in oxygen levels in Cocoons after attaining the lowest value was attributed to low biotic activity which creates a positive oxygen balance resulting in replenishment since rate of oxygen uptake would be less than the ingress oxygen. However, the rapid increase in oxygen readings after 92 d storage at Cleveland was due to an opening that was observed during routine inspections which are recommended given the importance of an airtight seal of the closed Cocoon.

The average conditions in the Cocoons were 19.0 ± 1.9°C and 56.3 ± 0.4% RH, 25.0 ± 1.2 °C and 53.9 ± 0.4 % RH, and 21.2 ± 0.6 °C and 53.9 ± 1.3% RH at Marondera, Bindura, and Cleveland; respectively. The sun-filtering shade pitched over the Cocoon regulated the effect of ambient conditions. The shade reduced the effect of diurnal temperature changes which led to relatively low temperatures during the duration of the trial.

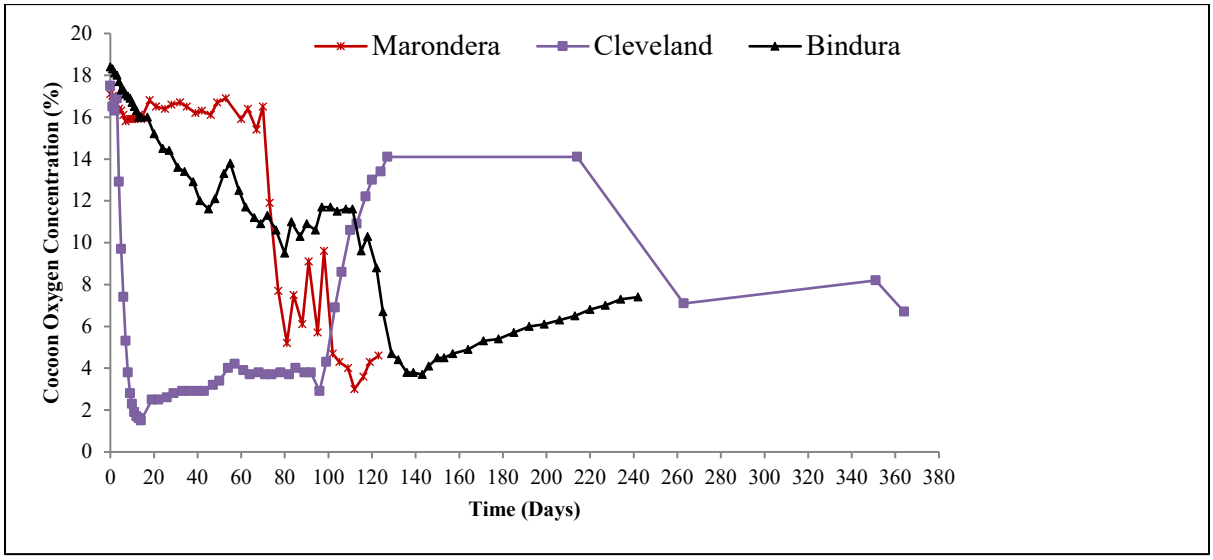

Fig. 1. Oxygen levels inside Cocoons A (Marondera), B (Bindura) and C (Cleveland) installed at three GMB depots (Chigoverah et al., 2018).

Insect Activity

There was an increase in insect numbers for both *P. Truncatus and S. zeamais* in Cocoon A in Marondera and Cocoon B in Bindura, while there were no changes in the total numbers in the Cocoon installed at Cleveland (Table 1). There were no live insects in Cocoon C. This could be attributed to rapid oxygen depletion in Cocoon C where readings of less than 3% were observed after 9 d storage. Previous studies have reported increased insect mortality under hypoxia and/or hypercarbia (Alvindia et al., 1994; Murdock et al., 2012).

The slow rate of oxygen depletion in Cocoon A and B supported insect development leading to an increase in insect numbers in the glass jars stored in the respective Cocoons. The glass jars in the two Cocoons (A and B) had considerable numbers of live *S. zeamais* with more than 130 live adults in glass jars in the Cocoon in Bindura after 8 mo storage. On the other hand, the rate of multiplication of *P. truncatus* in glass jars stored in Cocoon A and B was far less than that of *S. zeamais*. The results showed that *P. truncatus* was relatively more susceptible to modified atmospheres compared to *S. zeamais*. Previous studies have also reported the ability of *S. zeamais* to tolerate hypoxic conditions (Cao et al., 2010). However, literature also reported the lethal result of *P. truncatus* to specified modified atmospheres.

Table 1. Comparative mean insect numbers (±SEM) in glass jars placed in GrainPro Cocoons at three Grain Marketing Board depots (n = 15).

Insect Species	Cocoon A (4 Mo)		Cocoon B (8 Mo)		Cocoon C (12 Mo)	
	Total	Live	Total	Live	Total	Live
S. zeamais	50 ± 15.9^{b}	19 ± 11.2	380 ± 104.6^{b}	134 ± 52.7	20 ± 0.0^{a}	0.0
P. truncatus	24 ± 2.7^{a}	0.0	80 ± 17.0^{a}	2 ± 1.2	20 ± 0.0^{a}	0.0
ANOVA	$F_{1.58} < 0.01$		$F_{1.58} < 0.01$		$F_{1.58} > 0.05$	

*Means within a column for each site were compared and separated using LSD test ($P<0.05$) and different alphabetical letters indicate significant differences

Insect feeding-induced grain powder

Initially there was no grain powder in the glass jars. Insect feeding-induced grain powder was recorded in all the glass jars at termination. The glass jars in Cocoon B had the highest amount of grain powder followed by Cocoon A and Cocoon C which had the least values (Table 2). The trend could be attributed to the total insect numbers in the respective jars. Insect feeding-induced grain powder is highly correlated to insect population (Chigoverah and Mvumi, 2016). Even though there were significant numbers of insects in the glass jars, the amount of powder produced was low in relation to the numbers. This could be attributed to the antifeedant effect of MAs (Murdock et al., 2012). Glass jars containing *P. truncatus* had the most grain powder at all sites despite having lower insect numbers than those recorded in glass jars containing *S. zeamais*. While for other storage insect pests, feeding accounts for most grain losses, *P. truncatus* adult tunnelling accounts for as much as four times as the destruction due to both larval and adult feeding activities (Demianyk and Sinha, 1988). The results showed that under efficient bio-generated modified atmospheres, the severity of insect-induced damage could be suppressed thereby reducing storage losses.

Table 2. Comparative mean insect feeding-induced grain powder (± SEM) in glass jars containing artificially infested maize grain placed in GrainPro Cocoons at three Grain Marketing Board depots (n = 15).

	Marondera (4 Mo)	Bindura (8 Mo)	Cleveland (12 Mo)
Insect Species	Grain powder (g/kg)	Grain powder (g/kg)	Grain powder (g/kg)
S. zeamais	7.2 ± 3.22^{a}	88.5 ± 32.58^{a}	0.5 ± 0.12^{a}
P. truncatus	22.7 ± 4.11^{b}	96.2 ± 37.23^{a}	1.6 ± 0.20^{b}
ANOVA	$F_{1.58} < 0.01$	$F_{1.58} > 0.05$	$F_{1.58} < 0.01$

*Means within a column for each site were compared and separated using LSD test ($P<0.05$) and different alphabetical letters indicate significant differences.

Conclusions

The study showed that the two primary storage insect pests were relatively susceptible to bio-generated modified atmospheres under field commercial conditions. The results support the promotion of GrainPro Cocoons in African countries where *P. truncatus* and *S. zeamais* are pests of economic importance. However, there is need to generate more data on the response of *P. truncatus* under different types of MAs (bio-generated, gas-hermetic fumigation and vacuum-hermetic fumigation). There is also a need to ascertain durability of Cocoons under incoming *P. truncatus* infestation rather than just focussing on resident infestation.

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Testing of an inexpensive modified atmosphere chamber for small farmers using soaked grain as oxygen scavenger

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Abstract

Insects are one of the major causes of grain losses. Fumigation methods or controlled atmosphere (CA) treatments usually require sophisticated technologies that are not affordable in subsistence farming. This study focused in testing an inexpensive and effective modified atmosphere (MA) chamber using standard liners and soaked grain as O_2 scavenger. Freshly harvested wheat (14% m.c.) was bagged in 35 kg plastic raffia bags (non-hermetic). A stack of 13 bags was assembled inside a polyethylene liner of 70-micron thickness and dimensions of 1.0 m x 1.0 m x 1.8 m. The liner was closed by twisting, folding, and tying the opening with a rope. The airtightness was tested by a pressure decay test (PDT). Four treatments were evaluated, including a control (only dry grain) and three treatments with soaked grain as oxygen scavenger. Oxygen and CO_2 concentrations were measured with a portable gas analyzer. Results indicated that the respiration rate of soaked grain was 120 to 150 times greater that that of dry wheat. The incorporation of a small quantity of soaked grain as oxygen scavenger (1% dry matter) resulted in a reduction of the oxygen concentration to less than 2%, creating a lethal atmosphere for insects. The PDTs of the chambers were from 0.15 to more than 19 min. The variability in the final O_2 and CO_2 concentration achieved in all the treatments was quite low in spite of the variability of the PDT. This would suggest that the effect of incorporating oxygen scavengers was of a much larger magnitude than the differences in airtightness observed. This study demonstrated that an effective MA chamber can be created using standard 70-micron polyethylene liner, a simple sealing method and sacrificing only small portion of soaked grain as oxygen scavenger.

Keywords: Hermetic storage, Pest control, Subsistence farming, Plastic liner, Modified atmosphere, Wheat, Sealing method

Introduction

Insects are one of the major causes of grain losses in developing countries. Fumigation with phosphine is the most widely implemented insect control treatment in grains and non-perishable products (Agrafioti et al., 2020). However, it requires the manipulation of hazardous material (Bell, 2000), such as aluminum phosphide pellets, and there is a risk of development of resistance (Nayak et al., 2020). Controlled and modified atmospheres (CA/MA) under hermetic storage are suitable alternative treatments, with the benefit that there is no hazard for the user, it does not leave residue of pesticide in the grain and there is no development of resistance. However, to obtain full benefit of the hermetic storage it is required to achieve a low O_2 concentration (below 2%) or high CO_2 concentration (above 20%) (Navarro et al., 2012).

During MA treatment, the gas concentration inside the hermetic container is the result of the balance between the respiration rate of biotic agents (grains, fungi and insects) and the gas exchange rate with the outside (permeability and leakage) (Bartosik, 2012) and CO_2 sorption of the grain. Implementing MA storage in dry products, such as grains, implies an extra challenge. Non-perishable dry products typically have a low respiration rate. When the grain is dry and the initial insect population is low, the modification of the internal atmosphere by the respiration of the biotic agents inside the grain is insufficient (exchange rate higher than respiration rate) (Abalone et al., 2011a, 2011b). In this case, only when the insect population develops up to certain level, the O_2 consumption rate overpasses the O_2 entrance rate through the liner permeability, and the internal gas composition changes substantially. However, this process leads to grain quality losses before the critical atmosphere is achieved. The use of liners with gas barrier (i.e., EVOH) improves the effectiveness of hermetic storage systems, since gas leakage is reduced and a higher and faster modification of the internal atmosphere is achieved (Cardoso et al., 2016). However, liners with gas barrier are expensive and it might not be affordable for small farmers. Additionally, the use of liners with an O_2 barrier must be complemented with highly efficient sealing systems. One alternative is to incorporate O_2 scavengers to compensate the higher permeability of a standard polyethylene liner and the extra gas leakage of a simple sealing method. Taher and Bartosik (2018) proved that this concept could work in a MA system of 0.70 m^3 using a standard 70-micron polyethylene liner and soaked soybean as O_2 scavenger. However, in that study, the MA chamber was heat sealed. This sealing technology is quite expensive and might not be available in most rural areas. To successfully transfer this technology to small farmers an integral solution should be found. Thus, in the present study, a simple and inexpensive MA system made of a standard polyethylene liner, using soaked grain as an oxygen scavenger and sealed with a simple method, was evaluated for creating a lethal environment for insects.

Methodology

Respiration experiment

A sample of 45 kg of wheat (same as used in the MA treatment described below) was divided into three sets of 15 kg each to obtain three different wheat conditions: 1) whole wheat, dry; 2) whole wheat, soaked; and 3) grinded wheat, soaked. For the condition 1, the wheat was used as it came from the field. For the condition 2, the sample was divided in four sub-sets of 3.6 kg each, placed in plastic trays and independently soaked in 5.0 L of distilled water for 60 min. The soaked grain was then transferred to a perforated metal tray to drain the remaining water for 60 min.

For the condition 3, the wheat sample was also divided into four sub-sets of 3.6 kg each and grinded with an electric mill (Foss, Cyclotec, CT 293, Denmark). The grinded wheat was soaked following the same procedure as described for condition 2. All samples were stored in hermetic plastic containers for 24 h until the setup of the respiration experiment. The moisture content (m.c.) determined with the oven method (19 h at 130°C) by triplicate (ASAE, 2003) was 14.3, 28.0 and 56.0% for the dry, soaked, and grinded-soaked samples, respectively. The following day, for each grain condition, 12 samples of 300 g of product were placed in Erlenmeyer flasks of 560 mL capacity and hermetically sealed with a rubber cap. Previously, a septum was inserted in the center of the cap to measure the internal gas composition with a needle connected to the measuring device (Checkmate, Dansensor, Denmark). The volume of the void space of the Erlenmeyer flasks (interstitial air and headspace) containing wheat at different m.c. was determined by measuring the volume of distilled water that filled it (Weinberg et al., 2008), and was 366, 362 and 345 mL for dry wheat, soaked wheat and grinded-soaked wheat, respectively. Immediately after closing the Erlenmeyer, internal gas composition was measured and four replicates of each wheat condition were placed in temperature-controlled chambers at 20, 25 and 30°C. The internal gas composition was measured again after 90 min for the soaked and grinded-soaked samples, and after 24 h for the dry wheat samples. The respiration rate based on CO_2 generation and O_2 consumption was calculated as described by Ochandio et al. (2017).

Controlled atmosphere treatments

The wheat used in this study was harvested at 14% m.c. in December 2019 at INTA Balcarce Research Station (Balcarce, Buenos Aires, Argentina) and bagged in 30 kg polypropylene raffia bags (non-hermetic). Six MA chambers were made with a tubular polyethylene liner of 70-micron thickness and dimensions of 1.0 m x 1.0 m x 1.8 m, and heat-sealed at the bottom end. These plastic bags are widely available, inexpensive (about 3 USD), and are placed in the interior of the raffia big-bag for transportation of bulk construction material, such as sand. Thirteen raffia bags filled with dry wheat were placed inside each MA chamber, containing a total of 390 kg of grain per chamber (335 kg dry matter (DM)). The chamber was closed by twisting and folding the open end and tying it with a rope (Fig. 1). The dimension of the sealed MA chamber was 1.0 m x 1.0 m x 0.6 m. Each MA chamber was assembled on a wooden pallet covered with a foam sheet to protect the liner, and stored at the INTA Grain Postharvest Pilot Plant at Balcarce Research Station. Four treatments were evaluated considering three replicates (chambers) per treatment. The treatments were arranged in two consecutive trials of two treatments each. Treatment 1) only dry grain (control); Treatment 2) 1% DM of Grinded-Soaked wheat as oxygen scavenger; Treatment 3) 1% DM matter of Whole Soaked wheat as oxygen scavenger; and Treatment 4) 0.5% DM of Whole Soaked wheat as oxygen scavenger. The procedure for soaking the grain was similar to that described above.

Trial 1

The six MA chambers were divided in two groups of three. In Treatment 1 control, the chambers were closed to study the evolution of the internal gas concentration created by the dry wheat only. In Treatment 2, two plastic containers (0.35 m x 0.20 m x 0.12 m) filled with the equivalent of 3.35 kg DM of grinded-soaked wheat (or 1% of the grain dry mass in the stack) were incorporated as oxygen scavenger in each chamber. The airtightness of the sealed chamber was tested with the pressure decay test (PDT). Briefly, a vacuum pump (Dosivac, DV 95, Argentina) was connected to the sealed chamber through a previously incorporated valve in the liner.

After a negative pressure of 1200 Pa was generated, the valve was closed and the time that it takes for the internal pressure to drop from -1200 to -600 Pa was recorded (Navarro, 1998). Oxygen and CO_2 concentrations were measured once or twice per week with a portable gas analyzer (CheckPoint, Dansensor, Denmark) through a rubber septum previously inserted in the liner of the chamber.

Fig. 1. Sealing method of the modified atmosphere chamber. Left: opening of the bag twisted; Center: close-up of knot sealing the modified atmosphere chamber; Right: detail of a sealed chamber with the oxygen scavenger inside.

Trial 2

After finishing the treatments of Trial 1, the chambers were opened and ventilated. The six CA chambers were divided in two groups of three. In Treatment 3, two plastic containers filled with the equivalent of 3.35 kg DM of whole soaked wheat (or 1% of the grain dry mass in the stack) were incorporated as oxygen scavenger in each chamber, and in Treatment 4 one plastic container filled with the equivalent of 1.68 kg DM of whole soaked wheat (or 0.5% of the grain dry mass of the stack) was incorporated. The MA chambers were sealed, and the PDT was performed in the same way as explained for Trial 1. Oxygen and CO_2 concentrations were measured once or twice per week with a portable gas analyzer (CheckPoint, Dansensor, Denmark).

Results and discussion

Respiration Rate

In terms of CO_2 release, the soaked wheat respires from 115 to 150 folds more than the dry wheat, and the grinded-soaked wheat respires from 2.5 to 7.9 folds more than the soaked wheat. In terms of O_2 consumption, the soaked wheat respires from 120 to 150 folds more than the dry wheat, and the grinded-soaked wheat respires from 1 to 3.3 folds more than the soaked wheat. This implies that 1 kg DM of grinded-soaked wheat releases an amount of CO_2 equivalent from 400 to 1000 kg of dry wheat (depending on temperature), and consumes an amount of O_2 equivalent from 130 to 490 kg of dry wheat. Taher and Bartosik (2018) reported a higher respiration rate of soaked soybean (6950 mg CO_2/(kg DM day) and 7400 mg O_2/(kg DM day)) in comparison with the respiration of soaked wheat reported in this study.

Table 1. Carbon dioxide and oxygen respiration rates (mg/(kg DM day)) of wheat at different conditions and incubation temperatures. Values are average of four replicates (SD between brackets).

Grain condition	Temperature (°C)					
	20		25		30	
Dry (CO_2)	6	(3)	10	(6)	11.5	(9)
Soaked (CO_2)	755	(152)	1149	(112)	1682	(132)
Grinded-soaked (CO_2)	5960	(427)	4122	(60)	4236	(41)
Dry (O_2)	-6	(2)	-11	(4)	-13	(4)
Soaked (O_2)	-883	(193)	-1341	(69)	-1753	(152)
Grinded-soaked (O_2)	-2923	(180)	-1432	(26)	-2253	(26)

Pressure decay test

Table 2 shows the results of the PDT of the six grain MA chambers in trials 1 and 2. It can be observed that the PDT results were widely variable, from 15 s to more than 19 min. According to Navarro (1998), an hermetic structure 95% full should have a PDT of 3 min to be suitable for CA treatments and 5 min for MA storage. Based on this classification, in Trial 1 all chambers (except 3 and 4) accomplished the threshold of 5 min and could be suitable for MA treatments. After performing a failed PDT in chambers 3 and 4, the sealing procedure was repeated and a new PDT was conducted, but the results did not change. This would imply that most likely the liners of these two chambers had undetected leaking. In Trial 2 the results of the PDT were, in general, lower than in Trial 1. However, it was not possible to identify the causes of the lower PDT in the second trial. Based on these results, the twisting and folding sealing method could have the potential to achieve suitable airtightness levels for CA/MA treatments.

Table 2. Pressure decay test for the modified atmosphere chamber expressed as minutes to drop the internal pressure from -1200 Pa to -600 Pa.

Trial	Modified Atmosphere Chamber					
	1	2	3	4	5	6
1	> 5	> 5	0:30	2:30	> 5	> 5
2	1:15	> 3	0:15	2:40	> 5	> 5

Gas concentration inside the MA chambers

In Treatment 1 (Control) (Fig. 2), the CO_2 and O_2 concentrations after 20 d resulted in a small change of less than 1 percentage point (0.6% CO_2 and 19.9% CO_2). Clearly, this internal atmosphere would not be able to prevent pest development. In contrast, the treatments with the O_2 scavenger (Treatments 2 to 4) resulted in a substantial modification in the internal atmosphere. The treatment with 1% DM of grinded-soaked wheat quickly modified the internal atmosphere, tending to stabilize after 20 d of storage to about 5% of O_2 and CO_2 concentrations (Fig. 2).

Treatment 3 (Fig. 2) with 1% DM-soaked whole wheat had a lower rate of modification in the internal atmosphere in comparison with Treatment 2, grinded-soaked wheat, but in the long term stabilized in a lower O_2 concentration (1.7% after 50 d). Treatment 4, with 0.5% DM of whole and soaked wheat, also resulted with a substantial modification in the internal atmosphere. However, the O_2 concentration stabilized at about 4%, double as in Treatment 3, insufficient for achieving a lethal atmosphere.

The variability in the final O_2 and CO_2 concentration achieved in all the treatments was quite low (see error bars in Fig. 2) in spite of the different PDT of the different chambers. This would suggest that the effect of incorporating oxygen scavengers was of a much larger magnitude than the differences in airtightness observed. The amount of soaked grain that had to be sacrificed as oxygen scavenger is certainly a small portion of the total. In this study, 1% DM of the total grain mass was enough to compensate for the O_2 leakage. In a previous study Taher and Bartosik (2018) used soaked soybean seeds as oxygen scavenger and determined that a lethal environment could be achieved sacrificing about 0.5% DM.

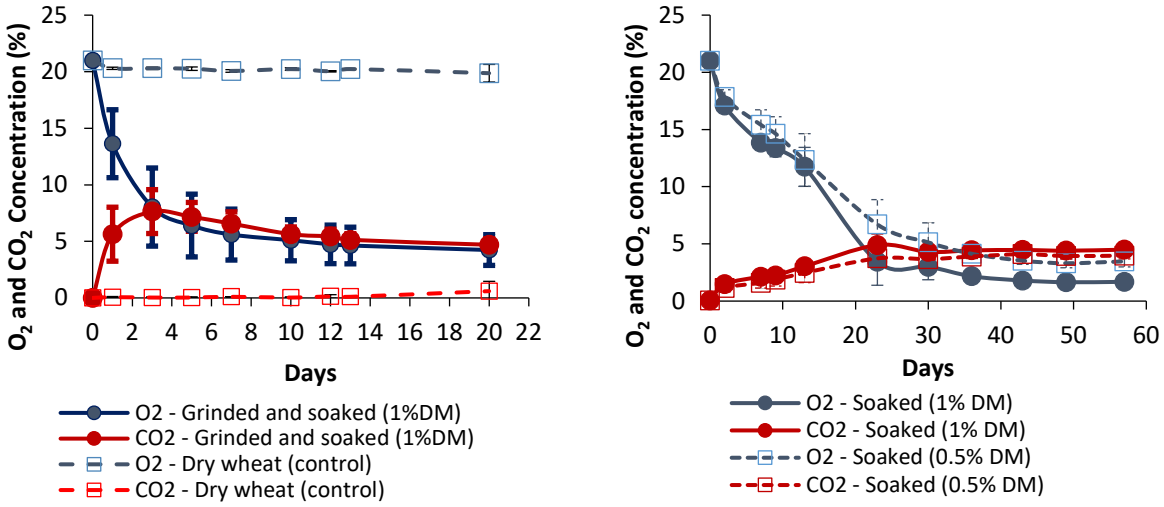

Figure 2. Average concentration of oxygen and carbon dioxide for Treatment 1 (control - only dry wheat) and Treatment 2 (1% DM of grinded-soaked wheat) (left); and for Treatment 3 (1% DM of soaked whole wheat) and Treatment 4 (0.5% DM of soaked whole wheat) (right). Error bars indicate the SD of the measured concentration.

Conclusions

The incorporation of a small quantity of soaked grain as oxygen scavenger (1% DM) was a suitable technical solution for creating a lethal atmosphere in the hermetic storage of dry products. The simple method of sealing the chamber by twisting, folding and tying the liner with a rope resulted in an appropriated MA atmosphere. This solution was not only technically sound, but also practical and economical.

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Efficacy of controlling two stored cashew nut insects in 1% O_2 and 99% N_2 at 43°C

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Abstract

The efficacy of controlling all life stages of storage insects *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.) were tested at 43°C and in 1% O_2 and 99% N_2 for 48 h of exposure time. For each species and each life stage, four replicates (each replicate consisted of 50 insects) were conducted. Two replicates for each species and each life stage served as control. Two control replicates were compared with treatment, one replicate served as control at 28°C and 60±5% RH and another served as control at 43°C and 60±5% RH. All tests were carried out by introducing insects to 100 g of cashew nuts in gastight 1 L containers. At the beginning and the end of each exposure time O_2 percent was measured. Insect counting was done immediately after exposure treatment and after 7-10 days of incubation at 28°C and 60±5% RH. Cashew nuts obtained were in equilibrium with 56.4% RH at 16.9°C. Increased mortality was observed due to exposure to 43°C in air (control) compared to exposure to 28°C. Adult stage of *O. surinamensis* was the most tolerant one with 99.5 % mortality after 48 h treatment. Larval and pupal stages could not survive the treatment and 100% mortality was recorded. Although in the egg stage in both species some larvae hatch was observed, they could not survive more than 24 h after the treatment. Average egg mortalities were counted as 97.5% for *T. castaneum* and 98% for *O. surinamensis*, the young hatched larvae were found dead immediately after treatment. The pupal and larval stages of both species were more susceptible to the treatment rather than the adult and egg stages. Treating both stored product insects, *T. castaneum* and *O. surinamensis,* at 43°C in 1% O_2 and 99% N_2 atmosphere for 48 h resulted in very effective control treatment. The survival rate was less than 0.5% of adult of *O. surinamensis*.

Keywords: Nitrogen atmosphere, Cashew nuts, *Tribolium castaneum*, *Oryzaephilus surinamensis*, High temperature

Introduction

Cashew (*Anacardium occidentale* L.) belongs to the family Anacardiaceae and is a native of Brazil. Around the 16th century, it reached the Far East where today, Vietnam serves as the largest supplier of cashew nuts to the international market. Cashew is a high economic value crop and is earning considerable foreign exchange for the country. In 2012, about 220,000 tonnes of cashew kernels were exported, with a turnover of over US$1.45 billion (Thai, 2012).

Since the harvest period is short, during storage, cashew nuts must be protected from insect infestation and weight loss. In most countries, food commodities fumigated with phosphine and subsequently aerated are considered to be phosphine free with no regulations attached. The US Food Quality Protection Act (FQPA), which became law in the US in 1996, has set a tolerance for phosphine of 0.01 ppm in processed food stuffs. This is well below the detection level available to most laboratories (Donahaye*,* 2000*).* As concerns about the safety of our food supply increases along with concerns about the impact of agricultural chemicals on our environment and resistant strains of stored product pests to phosphine increase, the development of nonchemical quarantine treatments to meet export requirements become increasingly necessary. Moreover, the increase in consumer demand for organic commodities in recent years has increased.

Therefore, the use of Modified or Controlled Atmospheres (MA/CA) offers a safe and environmentally benign alternative to the use of conventional residue-producing chemical fumigants for controlling insect pests that attack stored grains, oilseeds, processed commodities, and packaged foods (Navarro, 2006) and is increasing in general, and in particular for niche markets such as the treatment of organic commodities. The pre-condition for successful MA treatment is a tight hermetic seal. Sealing techniques (Andrews et al., 1994), and methods of verifying the seal (Navarro, 1999) are well developed, as are the application procedures (Navarro and Donahaye, 1990; Annis and van S. Graver 1991). Modified or Controlled Atmospheres to control insects can be obtained using nitrogen (N_2), provided that a hypoxic atmosphere of ≤ 1% O_2 can be maintained. Generally, the lower the oxygen level, the higher the mortality. For effective control, the O_2 level should be <3% and preferably <1% if a rapid kill is required (Navarro, 1978).

Insect mortality increases more rapidly as temperatures rise and their metabolism speeds up. Cool temperatures slow rates of mortality while lower relative humidity hastens toxic effects, notably in high CO_2 atmospheres because of desiccation of insects (Banks and Fields, 1995).

Sakka et al. (2020) investigated the use of MA on life stages of several storage pests at 40ºC for 2.5 d in commercial nitrogen chambers with phosphine susceptible and resistant populations of *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.). Complete mortality of all life stages of the tested pests was obtained with negligible progeny of *O. surinamensis* (0.3% ± 0.3) (Sakka et al., 2020). While Athanassiou et al. (2017) compared insect mortality when nitrogen (1% O_2) was applied at 25ºC, high insect mortality levels of *Tribolium confusum* Jacquelin du Val, *Ephestia elutella* (Hübner) and *O. surinamensis* were noted. However, in most cases there were a number of insects that survived the nitrogen treatment. In contrast, complete control was achieved at 38–43ºC for all insect species and life stages tested, with the exception of *T. confusum* larvae (Athanassiou et al., 2017). Therefore, the objective of the present study was to evaluate the effect of 99% N_2 nitrogen treatment to achieve 1% O_2 on two storage pests at 43ºC in a short exposure time on cashew nuts.

Materials and methods

Control of Temperature and O_2 level within atmosphere of 1% O_2

Insects were exposed to 1% O_2 in 1-L glass jars equipped with two 1/16" i.d. copper tubes soldered to the lid and seal was reassured by applying the half-time pressure decay test to hold a pressure of 6 to 3 mm H_2O for at least 5 min (Navarro, 1999). The temperature and RH in the gastight 1-L volume jars holding 200 g of cashew nuts were tested using data loggers (Elitech RC-4HC, Elitech,

London, UK). The outer ends of the tubes were connected to T-type valves. An electrolytic sensor type oxygen monitor (Oxycheck Bishop, UK) equipped with an internal pump delivering 500 mL/min gas sample was used for monitoring oxygen levels in the jars. To achieve the initial target oxygen concentration, the gastight jar containing 100 g cashew nuts and the insects was purged using N_2 at about 1000 mL/min until the O_2 concentration dropped to about 0.9% in the jar. For testing the gas concentration, the two T-type valves were kept at a position so that the gas flow would be from the O_2 monitor to the exhaust port of the jar. In that position the T-type valves were switched simultaneously to the ports to provide a closed loop gas flow between the gastight jar and the monitor. After 24 h and at the end of the exposure, readings were taken to ensure the gas concentration was maintained during the exposure period of 48 h. Oxygen concentration was measured three times; at the beginning, after 24 h and at the end of each treatment (48 h). Equilibrium relative humidity of 500 g cashew nuts was tested at the beginning of each trial using a Novasina RH monitor (Hygro Mate, Novasina, Switzerland).

Insects

Populations of storage insects *T. castaneum* and *O. surinamensis* were reared at the Green Storage (GS) Ltd. laboratories at 28ºC and 60±5% RH. The efficacy of controlling all life stages of these two species at 43ºC and 99% N_2 for 48 h of exposure time was tested (Fig. 1). There were four replicates for each species and each life stage (eggs, larvae, pupae and adults), and each replicate consisted of 50 insects. Two replicates from each species and each life stage served as control. One replicate served as control at 28ºC and 60±5% RH and the other replicate served as control at 43ºC and 60±5% RH. All tests were carried out by exposing insects to 100 g of cashew nuts in gastight 1 L containers (except the control which was not gastight).

Insect counting was done immediately after each treatment and after 7-10 d of incubation at 28ºC and 60±5%. The reason for the delayed counts was to make ensure there were no surviving insects. All hatched eggs were considered as alive.

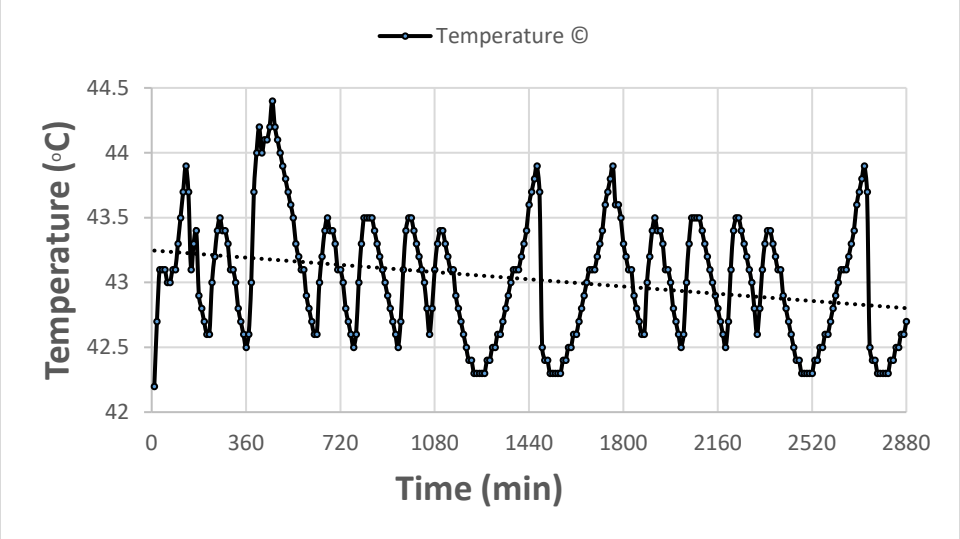

Fig. 1: Temperature obtained within the 1 L container containing 200 g of cashew nuts during a 48-h exposure time at a target temperature of 43ºC.

Results

Equilibrium relative humidity of 500 g cashew nuts was 56.4% at 16.9ºC. Figures 2-3 describe the temperature and RH maintained in the 1 L container during a 48-h exposure. Average temperature was 43.0ºC (Fig. 1) and the average ERH was 58.7 % (Fig. 2).

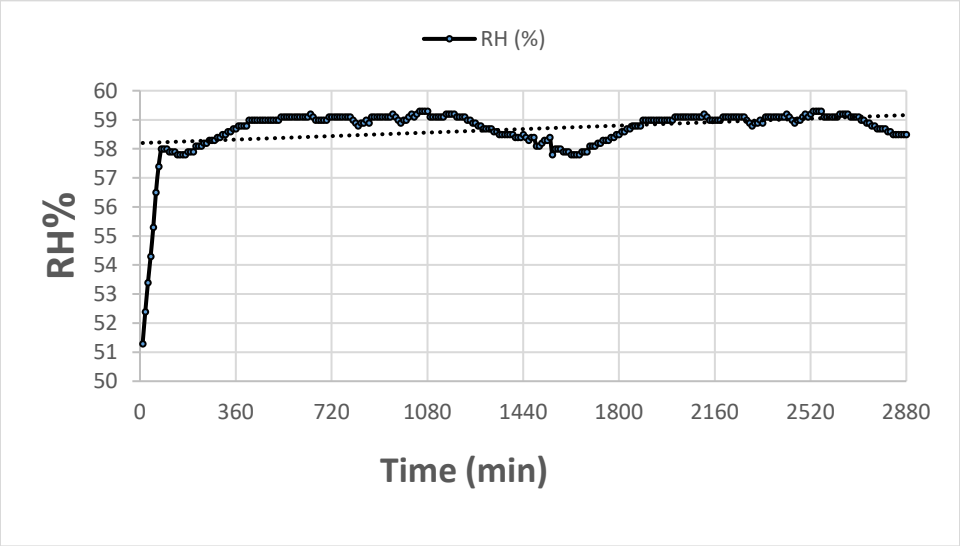

Fig. 2: Relative humidity (%) obtained in the 1 L container containing 200 g of cashew nuts during a 48-h exposure.

Table 1 shows the mortality of all life stages of *T. castaneum* and *O. surinamensis* after 48 h of exposure to 1% O_2 at 43ºC. All larvae were dead during counting (immediately after exposure time).

Table 1. Oxygen concentration (%) and mortality (%) of egg, larva, pupa and adult of *Tribolium castaneum* and *Oryzaephylus surinamensis* after 48 h of exposure to 1% O_2 at 43ºC and its control at 43ºC and 28 ºC.

Average temperature (ºC)	43		43		28	
	Average O_2 (%)	Average mortality (%)	O_2 (%)	Average mortality (%)	Average O_2 (%)	Average mortality (%)
Tribolium castaneum						
Adults	1.30	100.0	20.9	2.0	20.9	0.0
Pupae	1.05	100.0	20.9	42.0	20.9	0.0
Larvae	1.20	100.0	20.9	94.0	20.9	22.0
Eggs	2.35	100.0	20.9	98.0	20.9	76.0
Oryzaephilus surinamensis						
Adults	1.75	99.6	20.9	76.9	20.9	6.0
Pupae	1.25	100.0	20.9	90.9	20.9	62.5
Larvae	1.33	100.0	20.9	72.0	20.9	59.1
Eggs	1.45	100.0	20.9	93.0	20.9	48.0

As mentioned above, controls were carried out at both 43ºC and 28ºC to compare the effect of heat alone on insects. In Table 1, the effect of 43ºC alone in the control of both insects was apparent. Higher mortalities were observed in the controls at 43ºC compared to the controls at 28ºC. The adult stage of *O. surinamensis* was the most tolerant one with 99.5 % mortality on average. Larval and pupal stages could not survive the treatment and 100% mortality was recorded (Table 1).

Although some eggs of both species hatched, they could not survive more than 24 h after the treatment. Average egg mortalities were 97.5% for *T. castaneum* and 98% for *O. surinamensis.* Therefore, the mortalities for both species were considered as 100%.

Discussion

The target temperature of 43ºC, as is shown in Fig. 1, was reached in less than 30 min in the jars. In practice, when handling large commercial volumes, this process may take several hours or days to reach the target temperature at the core of the stack/bags, depending on the volume size (Donahaye et al., 1995).

According to Navarro (2012), to obtain rapid killing when using N_2, the O_2 concentration must be lower than 1%. Timlick et al. (2002) reported that in a sealed commercial storage that maintained less than 1% O_2, insect mortality was completed after 14 d at 17ºC. Therefore, N_2 has been considered unsuitable for bulk commodity treatment at export locations due to the length of time required for 100% mortality.

However, insect mortality increases more rapidly as temperatures rise (Navarro, 2012). In heat treatments carried out in commercial facilities, it was found that the young larvae of *T. castaneum* were the most heat tolerant at 50 to 60ºC within 24 to 36 h (Subramanyam et al., 2011). In our study, the effect of high temperature (43ºC) in the control is more pronounced especially for the egg, larva and pupa of *T. castaneum* which were much more susceptible than the adult (Table 1). In the control of *O. surinamensis,* all development stages were affected by the high temperature. Although eggs hatched to larvae, they were dead one day later.

After exposure to 43ºC in the control, adult *Tribolium castaneum* was the most tolerant stage to this high temperature (<2% mortality), followed by the pupal stage (42% mortality, Table 1). Navarro (1978) reported the significant differences of adult mortality of *T. castaneum* in N_2 which ranged between 2 d to 4.5 d at 0.1 to 1.0% O_2 at 26ºC. However, in these trials, adults of *T. castaneum* at 1% O_2 were much more susceptible due to the high temperatures. This is consistent with Sakka et al. (2020) that a complete mortality was achieved in 2.5 d at 40ºC and 1% O_2 and was emphasized at 28ºC where they obtained mean mortality of 96.7% after 9 d of exposure time.

The pupae and larvae of both species were more susceptible to the treatment than the adults and eggs in both species. Although it is reported that some percentage of eggs did hatch (Fig. 3), the hatched larvae were found all dead immediately after the treatment. The reason for the tolerance of eggs to the treatment might be due to the low respiration rate of the eggs, but the complete control was achieved due to the high temperatures on the hatched eggs which was lethal due to their susceptibility. Fields (2002) reports that at a temperature range of 45-50ºC, death is achieved in less than 24 h. However, at a range of 42-45ºC there is no data.

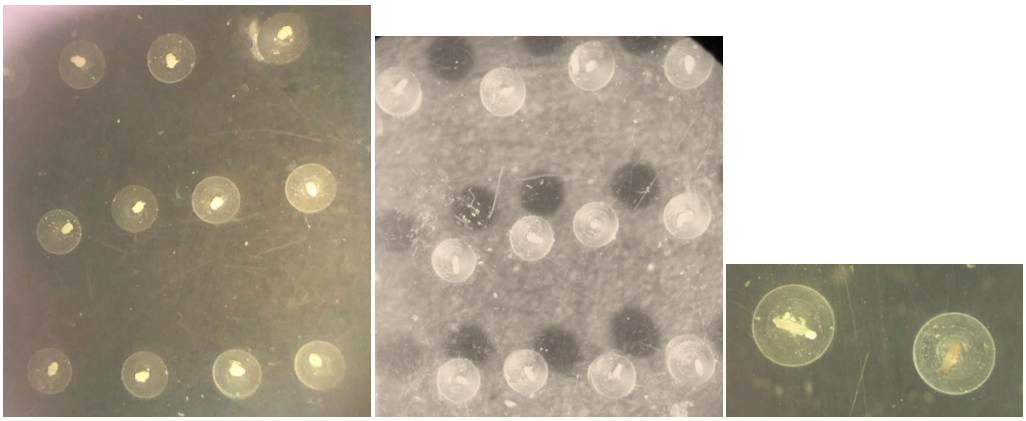

Fig. 3: *T. castaneum* eggs after treatment (left), *O. surinamensis* eggs after treatment (middle), and hatched eggs (right).

Protection of the beneficial qualities of cereals during storage depends on many factors. Among the detrimental factors that reduce the quality of cereals are insects and microflora (Navarro and Donahaye, 2005). Although the main objective of modified or controlled atmospheres is to control insect pests, the use of it enables quality preservation of the product as well, due to the sufficient sealed structure which maintains the vapor pressure (Navarro and Navarro, 2018). Even though in these trials, quality tests were not carried out, it is assumed that no quality deterioration occurred and the organoleptic characteristics were not affected.

Conclusions

Treating both stored product insects, *T. castaneum* and *O. surinamensis* at 43ºC in 1% O_2 using N_2 for 48 h resulted in very effective treatment. The survival rate was less than 0.5 % of adult of *O. surinamensis*. Increased mortality was observed at 43ºC compared to 28ºC. The combination of 43ºC with 1% O_2 in N_2 atmosphere was demonstrated as very effective in the control of all stages of both species. More data should be obtained to fill in the gaps of knowledge as for the tolerant stages of other insect species.

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YongLin Ren Y, Agarwal M, Newman J, Pan S, Li J, Xiao Y (2021) Delivery and adoption of nitrogen technology for the management of grain storage pests and grain quality. Page 160. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Delivery and adoption of nitrogen technology for the management of grain storage pests and grain quality

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ABSTRACT

To develop nitrogen technology as a complementary technology to the use of fumigants for control of key pests in Australian grain storages, market feedback from industry was that cost remained a barrier to uptake. The need for gas-tight storages was another barrier although this is reducing as insect resistance to the fumigant phosphine drives grain industry to uptake of sealed storages. However, recent advances in the cost of nitrogen generation (membrane technology) to assist industry overcome operational barriers to the uptake of the technology. A new generation membrane technology was sourced from Changshunanda, a large China-based company. The unit was deployed at the CBH grain port of Kwinana to compare performance against an older nitrogen generation technology (Pressure Swing Absorbance / PSA). To meet the strict operating conditions at the port the unit was upgraded by Changshunanda. A cost benchmark targeted at $0.50 per tonne of grain (the high-end cost of a phosphine fumigation). The research demonstrated an operational cost for the older PSA technology of $2.43 per tonne of grain. The technology also displayed operational limitations in its ability to generate and maintain the required level of nitrogen purity necessary to provide insect control (99%).

Keywords: Fumigant, Nitrogen, Fumigation, Grain, Stored Grain Insect, Grain Quality

CAF2020 Paper No. P-5-4-44

Song S, Du J, Hu B, Yang FY, Song JZ, Wang GY (2021) Low oxygen concentration hinders the occurrence of the cigarette beetle, *Lasioderma serricorne* under nitrogen-controlled atmosphere storage system. Pp. 161-166. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Low oxygen concentration hinders the occurrence of the cigarette beetle, *Lasioderma serricorne* under nitrogen-controlled atmosphere storage system

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Abstract

Cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) can infest a wide range of stored products such as food and cereal grains, and it is also particularly common on the infestation of tobacco. Fumigants, such as phosphine and sulfuryl fluoride have been widely used to control this insect pest, but some serious drawbacks, such as insect resistance to the fumigants and damage to the environment due to the excessive application, have limited their applications. Environmentally friendly alternatives to replace these chemical insecticides to control this pest are urgently required. Controlled atmosphere treatment such as using high concentration nitrogen (N_2) is an alternative to control this notorious pest. In this study, we investigated the mortality of different stages of cigarette beetles at different oxygen (O_2) concentrations (≤2 or 2 to 4%) by applying high concentration N_2 (≥99.95%) to a tobacco warehouse. The results revealed that the corrected mortality of eggs, larvae, pupae and adults was 100% under the low O_2 (≤2%) concentration after 27 d exposure. The corrected mortality of larvae exceeded 85% and hatching rate was less than 10% after 27 d when O_2 concentration was 2 to 4%. Thus, the result suggested that N_2 controlled atmosphere treatment was an environmentally friendly method to manage this stored tobacco pest.

Keywords: *Lasioderma serricorne*, Nitrogen treatment, Control atmosphere storage, Stored pest control

Introduction

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), is an economically important storage pest worldwide (Edde, 2019). The species is destructive and infests many stored products, such as tobacco, cereal grains and herbs (Cao et al., 2019). In particular, the infestation of cigarette beetle causes huge economic losses to the tobacco industry around the world. Several technologies have been developed to control *L. serricorne*, such as heat or cold treatments, biological control, fumigation, and radiation (Oliveira da Silva et al., 2018).

The common and effective control method is the phosphine fumigation (Oliveira da Silva et al., 2018; Edde, 2019). However, the excessive usage of phosphine has resulted in insect resistance to this fumigant and environmental pollution (Saglam et al., 2015; Li et al., 2018; Yang et al., 2019). Therefore, environmentally friendly alternatives for control of this pest are urgently needed.

Storage insect pests are animals requiring oxygen for their survival. Controlled atmosphere treatment (CA) offers an alternative that is safe and environmentally benign to the treated materials (Navarro, 2012). During the CA treatment, oxygen concentrations within a storage enclosure can be altered to the lethal O_2 concentration to the insect pests by adding N_2 or CO_2 (Navarro, 2012; Edde, 2019). The efficiency of CA depends on temperature, relative humidity (RH) and life stage of insects (Edde, 2019). Nitrogen controlled storage system has had a broad application after undergoing various optimizing processes and cost reductions in China (Zhang et al., 2015; Xiao et al., 2019). In recent years, several grain-storage companies have applied N_2 to control pest insects (Lin, 2019). Several studies have shown that high N_2 concentration in storage systems can effectively inhibit pest population increase (Lu et al., 2013; Liu, 2016; Xiao et al., 2019). In a study on the mortality of *L. serricorne* under the nitrogen treatment (O_2 concentration ≤2%), no live cigarette beetles were found on different linings of tobacco leaves after 45 d exposure (Xiao et al., 2019). Another report also showed that the mortality of the cigarette beetle adults and larvae could reach 100% under low O_2 (1% or 2%) and high temperatures (Liu, 2016). The effect of 98% nitrogen on the survival of other stored pests has also been studied (Lu et al., 2013).

In this study, we investigated the survival of different stages of cigarette beetle under low O_2 concentrations by applying high concentration N_2 (≥ 99.95%) into a tobacco warehouse. Our results could provide valuable information for developing an effective technique to control *L. serricorne*.

Materials and methods

Insects

The *L. serricorne* was provided by Zhengzhou Tobacco Research Institute of China National Tobacco Corporation. The culture was reared on whole wheat flour mixed with yeast (7:3, *w*/*w*) and maintained at 29 ± 1℃ and 75% ± 5% RH under dark conditions.

Tobacco warehouse

The re-roasted and processed tobacco leaves from Yunnan province were stored in a warehouse located at the Nanjing cigarette factory, China and kept at 18 to 26℃ and 55 to 65% RH. The tobacco stacks (14 m × 4.3 m × 3 m) were sealed with 0.12 mm nylon film on six sides of the stacks for air tightness.

Nitrogen treatment

The airtightness of the sealed stacks was tested before nitrogen application, and all the stacks reached the Chinese airtightness standard (half-life time of the pressure ≥ 5 min from 300 to 150 Pa). Highly purified nitrogen (≥99.95%) was produced from the air through zeolites by pressure swing adsorption in the nitrogen generation equipment (FD-PSA-100, Wuhan Dongchang Storage Technology Co. Ltd., China). The desired oxygen concentration (≤2% and 2 to 4%) was obtained by adding the produced N_2 (≥99.95%) to the stacks in the tobacco warehouse (26℃ and 65% RH) (Fig. 1).

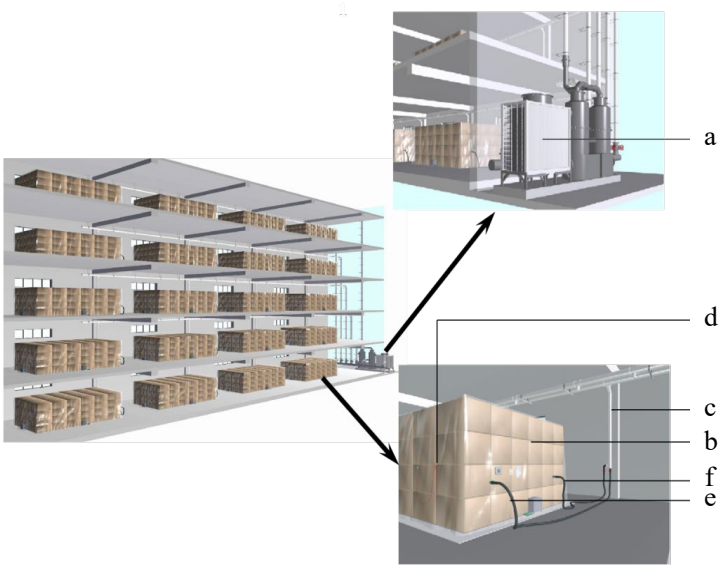

Fig. 1. Schematic diagram of nitrogen treatment of tobacco stacks: (a) Nitrogen generation device, (b) Tobacco stack, (c) Nitrogen filled/exhaust ducts, (d) Temperature/oxygen concentration probe, (e) Nitrogen filled ducts, and (f) Nitrogen exhaust ducts.

Treatment of insects

Different stages (eggs, larvae, pupae and adults) of *L. serricorne* were exposed to low oxygen (O_2) concentrations (≤2%, 2%-4%) under a controlled high N_2 (≥99.95%) atmosphere in the tobacco warehouse. The air (21% O_2) served as the control. The tested insects in plastic vials (8 cm × 12 cm) were located at 10-15 cm below the surface of tobacco stacks. The mortality of the treated insects (eggs, larvae, pupae and adults) was observed after 18, 21, 24, 27, 30 and 33 d exposure. The larvae or adults were considered dead if no movement was observed. The plastic vials which held the *L. serricorne* pupae were maintained in an incubator (29 ± 1°C and 75% ± 5% RH) after the low O_2 treatments, and the number of pupae which developed into adults was recorded daily for the following 7 d. The pupae that could not develop into adults were considered dead. The treated eggs were also maintained at the insect rearing condition after the treatment. The number of eggs which hatched into larvae was recorded daily for the following 7 d. The eggs that could not develop into larvae were considered dead. Three replicates were conducted for each treatment.

Results

Mortality of L. serricorne eggs, larvae, pupae and adults under normal O_2 concentration (21%)

We observed the survival of *L. serricorne* eggs, larvae, pupae and adults at normal O_2 concentration during the experiment (Fig. 2). The results showed that the mortality rate of eggs was the highest (34.4%) among the four stages. The larval mortality was 13.1% under the normal condition. For the pupae and adults, the mortalities were 3.1% and 6.3%, respectively.

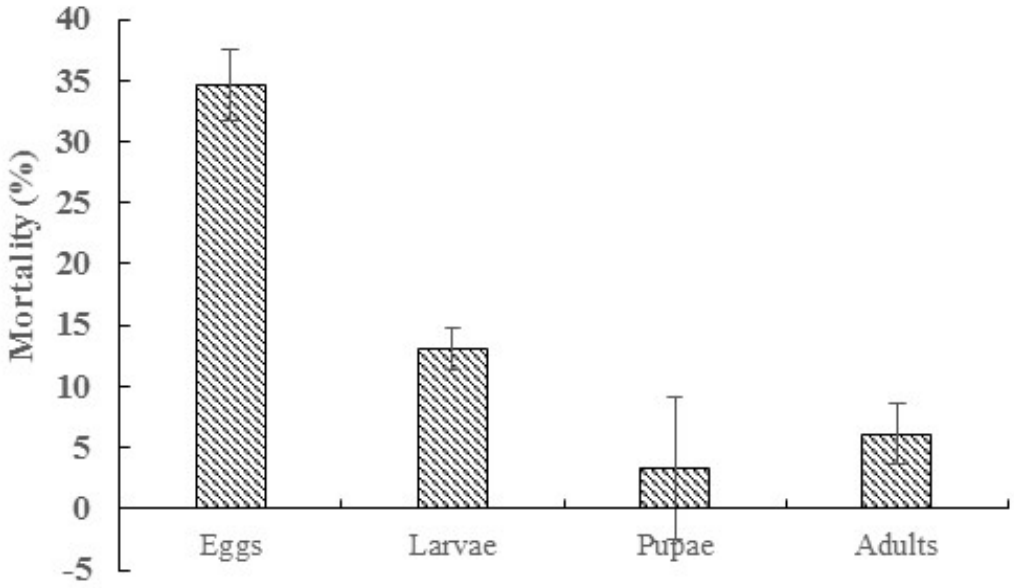

Fig. 2 Mortality of different stages (eggs, larvae, pupae and adults) *L. serricorne* at control condition (air with 21% O_2).

Effect of ≤2% O_2 on the survival of eggs, larvae, pupae and adults

When exposed to ≤2% O_2 for 18, 21 and 24 d, the corrected mortalities of eggs were 84.2, 92.7 and 99.0%, respectively, while larvae had mortalities of 89.6%, 95.3% and 98.6%, respectively (Fig. 3). The corrected mortality of eggs and larvae reached 100% under the low O_2 condition after 27 d. However, no live pupae and adults were found after 18 d under ≤2% O_2 (Fig. 3).

$$\text{Corrected mortality (\%)} = \frac{\text{Treatment mortality} - \text{Control mortality}}{1 - \text{Control mortality}} \times 100\%$$

Effect of 2 to 4% O_2 on the survival of eggs, larvae, pupae and adults

The highest corrected mortality of eggs and larvae were 93.5% and 87.6%, respectively, when exposed to 2 to 4% O_2 within 33 d (Fig. 4). For the pupae, the corrected mortalities were 97.1% and 99.5% when exposed to the low oxygen condition for 18 and 21 d, respectively. Live pupae were not observed after 24 d under any treatment (Fig. 4). The corrected mortality of adults was 99.4% under 2 to 4% O_2 treatment for 18 d, and reached 100% after 21 d (Fig. 4).

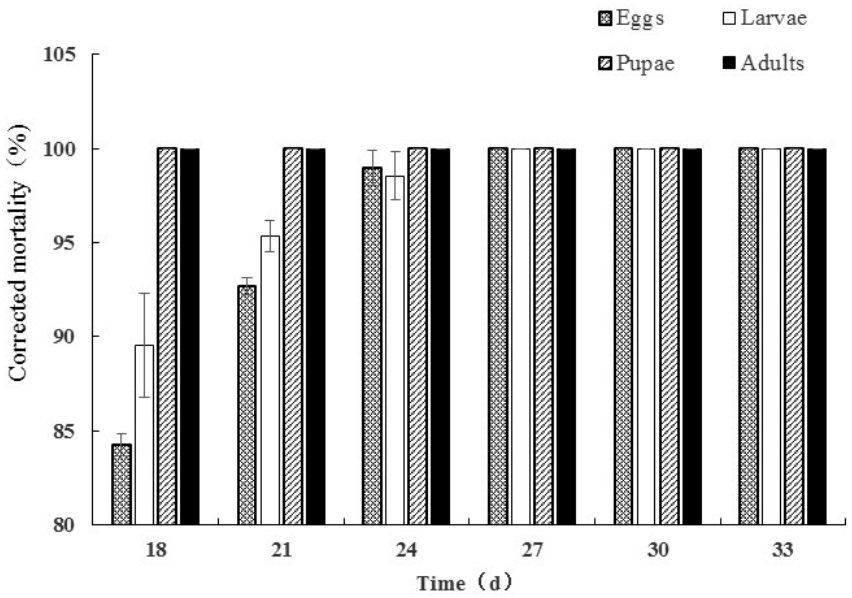

Fig. 3. Corrected mortality of different stages (eggs, larvae, pupae and adults) of *L. serricorne* under ≤2% O_2.

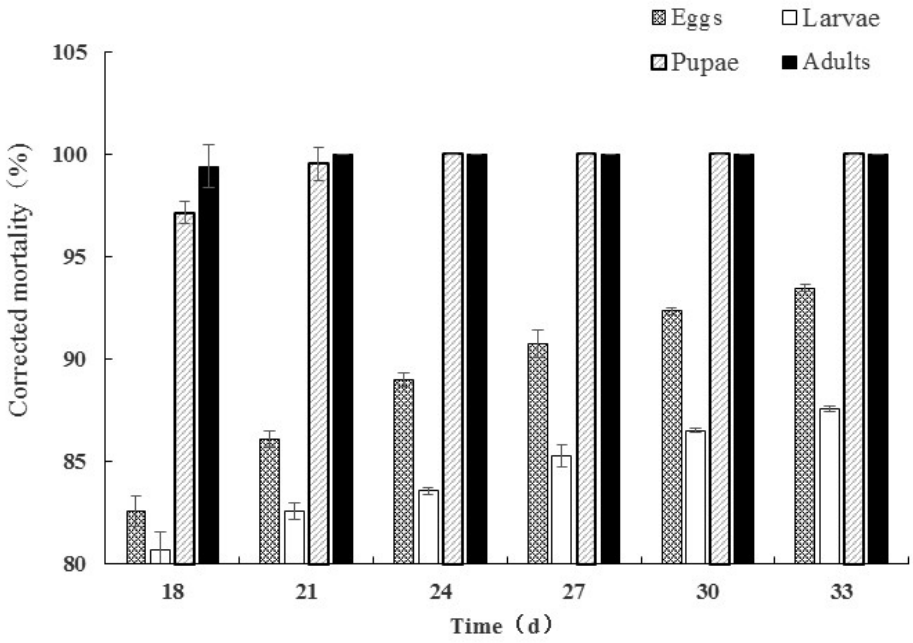

Fig. 4. Corrected mortality of different stages (eggs, larvae, pupae and adults) of *L. serricorne* under 2 to 4% O_2.

Discussion

In this study, eggs, larvae, pupae and adults of *L. serricorne* were exposed to low oxygen concentrations. The results indicated that low oxygen treatments (≤2%, 2%-4%) were effective in the control of different life stages of *L. serricorne*. The mortality of eggs, larvae, pupae, and adults was 100% under the low O_2 (≤2%) concentration after 27 d. No live pupae and adults were observed under 2 to 4% oxygen concentrations after 24 d; however, different stages of tobacco beetle had different tolerance to the low oxygen. When oxygen concentration was less than 2%, the mortality of eggs was 84.2% after 18 d, while pupae and adults had 100% mortality. Our data clearly showed that eggs were the most tolerant stage under the low oxygen concentration (≤2%). The tolerance of the four stages of *L. serricorne* to the low oxygen concentration (≤2%) in increasing order was adults, pupae, larvae, and eggs. When oxygen concentration was 2 to 4%, the larval stage had the highest tolerance because many larvae (12.4%) were still alive after 33 d. The tolerance of the four stages of *L. serricorne* to 2 to 4% oxygen concentration in increasing order was adults, pupae, eggs, and larvae. In conclusion, the low oxygen concentrations (≤2% or 2 to 4%) were effective to control *L. serricorne*.

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Efficacy of Bio-CO_2 as a non-chemical fumigant on pest control in wheat storage

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Abstract

The study evaluated the viability of Bio-CO_2 as a fumigant on mortality of adult *Rhyzopertha dominica,* seed germination, and seed quality. Bio-CO_2 is a by-product of the compressed biogas/ bio-methane production plant, which constitutes CO_2 as a major component and some of CH_4 and H_2S at ppm level. Wheat grain (Variety: H.D.-3086, Lot No. IARI/SPU/FA19286) was used in the present study. Fifteen grain plastic bottles of diameter 8.2 cm, height 19 cm, and total volume of one litre each were taken for the study. The purging of Bio-CO_2 fumigant was carried out at 0.5, 1.0, 1.5, and 2.0 L/min. The insect mortality of 100% was found in 1, 3, and 5 d using Bio-CO_2 as fumigant at 80, 60, and 40% concentration, respectively. Purging of the bottles was done at a gas flow rate of 1.5 L/min. There were no significant differences in seed weight observed after a storage period of 60 d under the elevated CO_2 storage atmosphere. However, seed weight loss of about 5.0% in controlled and 17.3% in grain stored with *R. dominica* without fumigation were found. The seed germination was reduced from 96 to 87.5%, 96 to 88.5%, and 96 to 90% at 80, 60, and 40% CO_2 concentration, respectively. However, the minimum seed germination certified by the SPU, ICAR-IARI, New Delhi was 85%. Thus, Bio-CO_2 as a fumigant resulted in better insect control than pure-CO_2 with no significant seed germination and seed quality change over a two-month storage period.

Keywords: Fumigant, Waste gas, Carbon dioxide, Insect mortality, Grain quality, Seed germination

Introduction

Stored food grain insects are the major threat to dried, stored, durable agricultural commodities and many value-added products globally (Arora et al., 2020). Post-harvest losses include losses during various unit operations, e.g., harvesting, threshing, cleaning, storage, processing, and transportation (Kumar and Kalita, 2017). These losses are estimated to be about 9% in developed and 20% in developing countries (Arora et al., 2020).

Altogether, the total post-harvest losses worldwide, the storage losses of rice, wheat, and maize have been around 2.7-15.1%, 0.9-56%, and 0.2-11.3%, respectively. The pest infestations are solely responsible for grain losses of about 5-10% during its storage in developed and 30-40% in developing countries (Kumar and Kalita, 2017; Cao et al., 2019). Meanwhile, the food demand is estimated to be increased by 70-100% by the end of the year 2050 (Godfray et al., 2010; Hodges et al., 2011). Therefore, reducing the losses of food grains by insect attack during storage is a major global concern.

So far, popular chemical fumigants like carbon tetrachloride, ethylene dibromide, aluminium phosphide, sodium cyanide, methyl bromide and phosphine have been used to a large extent to control pest infestation worldwide (Mohan and Gopalan, 1992; Ozkara et al., 2016, Cao et al., 2019). These fumigants effectively control insect pest growth in grain bulk; meanwhile, their excessive use leads to human health and environmental problems and thus banned in various countries (Proctor, 1994; Cheng et al., 2012). Whereas, due to the negative impact on ozone layer depletion (Sande et al., 2011), the use of methyl bromide had also been withdrawn in 2015 (Meyer and Newman, 2020). Phosphine also has a possible carcinogenic effect on humans and is still under review in the American and European countries (Bell, 2000). Another chemical fumigant that is ethanedinitrile (cyanogen), has recently been used in wheat storage to control pest infestation. The highly sorptive nature of ethanedinitrile in grains limits its use as an effective fumigant in storage pest control (Ramadan et al., 2020).

Reduced oxygen and raised carbon dioxide environment are the most suitable alternative to chemical fumigants. Presently, the storage of grains under an elevated carbon dioxide environment is getting more attention globally (Cao et al., 2019). The use of carbon dioxide as a fumigant is safe as it does not leave any harmful residues and is effective in controlling all life stages of a wide range of pests (Riudavets et al., 2014). This study evaluated wheat storage under fumigation by waste gas (mixture of carbon dioxide dominantly, some methane, and traces of hydrogen sulfide) as a fumigant. It is the first study of grain storage under fumigation by waste gas from compressed biogas (CBG) plant at Biogas Laboratory, Centre for Rural Development and Technology, Indian Institute of Technology Delhi, India; however, some studies of grains stored under raw biogas are available (Palaniswamy and Dakshinamurthy, 1986; Mohan and Gopalan, 1992; Chanakya et al., 2015). The use of raw biogas as fumigant is not appropriate due to its high calorific value with potential applications in cooking/ thermal energy, or as engine and vehicular fuel (Deng et al., 2020). In addition, the moisture content of raw biogas could wet the grain also. Therefore, this study was conducted to determine the efficacy of Bio-CO_2 as fumigant for insect control and effect of Bio-CO_2 on grain parameters like seed weight loss, and germination.

Material and methods

The mortality of adult *R. dominica* was determined at 40, 60, and 80% carbon dioxide in the waste gas from the CBG plant in five bottles for each treatment filled with grain to 25, 50, and 75% of its volume in a preliminary study. The bottle with grain at desired concentration of CO_2 was kept inside a temperature-controlled chamber at 28 ± 2°C.

Method of insect rearing

The newly emerged *R. dominica* adults were collected from the Division of Entomology, Indian Agricultural Research Institute, New Delhi, India. The adults were transferred into a box covered with a muslin cloth and fed with a mixture of 250 g coarse wheat and 50 g wheat flour (Noomhorm et al., 2009). The moisture content of the diet was 12.3%. The suggested moisture content of grain for *R. dominica* rearing is about 12-14% (Edde, 2012). The box was kept in a temperature-controlled room at 28±2°C with 70% RH and a light to dark hours of 16:8 as reported to be the optimum conditions for its growth (Edde, 2012; Wong-Corral et al., 2013).

Source of non-chemical fumigant

The waste gas was obtained from the off-stream channel of the CBG plant. The plant was based on water scrubbing technology. The waste gas obtained comprised around 73.8-74.2% CO_2, 12.7-14.2% CH_4, 0.5-1.6% O_2, 795-1509 ppm H_2S, and 9.8-12.1% as a balance. To obtain 90% of Bio-CO_2 concentration, the plant was operated at 0.6-0.7 MPa, 25 m^3/h biogas flow rate, and 5 m^3/h water flow rate.

Method of experiment

Fifteen tapered experimental plastic bottles of diameter 8.2 cm, height 19 cm, and total volume of one litre each were used. A glass tube of the inner diameter of 0.3 cm and 15 cm length was used as a gas inlet, and a similar tube was used as an outlet. The inlet and outlet glass tubes were connected with a silicon tube and two-way stopcock at the end, as shown in Fig. 1. The inlet tube was inserted up to 14-15 cm depth from the top of the bottle through a rubber cork. There were three replicates at each CO_2 concentration of 80, 60, and 40% with adult *R. dominica*, three replicates with *R. dominica* adults without fumigation, another three replicates without R. dominica and without fumigation as control were used for 60-d storage study.

Fig. 1. The experimental set-up for Bio-CO_2 purging.

A simple analytical method was used to quantify the carbon dioxide purging by knowing the bulk density and true density of wheat grain. The bulk density of the selected wheat grain was determined according to the methodology adopted by Chandra et al. (2012). The true density was determined using the standard Toluene displacement method (Singh et al., 2010). After that, the total void spaces available from which air to be removed to get a desired carbon dioxide concentration was calculated as:

$$\text{Total void spaces} = [\{\left(1 - \frac{\rho_b}{\rho_t}\right) \times V_g\} + V_h]$$

Where P_b, P_t, V_g, and V_h represents bulk density (kg/m^3), true density (kg/m^3), volume occupied by grain (L), and headspace volume of the bottle (L), respectively.

Quantity of CO_2 purging = Total void space in the bottle filled with grain (L) × desired concentration of CO_2 (decimal).

Optimization of CO_2 flow rate at different grain-fill volume

A rotameter (Flowstar FSC-100, Flow Star Engineering Pvt. Ltd., Faridabad, India), especially for carbon dioxide, was used to optimize the flow rate to achieve the best purging efficiency. The rotameter measured a flow rate of 0.5-10 L/min with the least count of 0.5 L/min. The trials with 0.5 to 2.0 L/min were carried out in the bottles filled with grains. A total of ten replications at each flow rate were carried out to get the best uniformity of carbon dioxide and purging flow rate. Best purging efficiency was achieved at 1.5 L/min (data not reported).

The purging efficiency of the grain bottle was determined by assuming the free mixing of carbon dioxide in the air (Mann et al., 1999).

Insect mortality test

The mortality was checked after 24, 48, 72, 96, and 120 h. Before the Bio-CO_2 fumigation, the bottle was filled up to one-fourth, half, and three-fourth with grains infested with 20, 50, and 70 adults of *R. dominica*. The insect mortality was checked according to the methodology reported by Noomhorm et al. (2009) and Riudavets et al. (2009).

Analysis of thousand-seed weight and germination

The Alberta seed testing standard (2016) was used to measure the thousand-seed weight and germination. The seed weight and germination were evaluated after a storage period of 60 d under Bio-CO_2 fumigation to determine any losses due to insect attack.

Results and discussion

Physical characteristics of grain and quantity of carbon dioxide purged

The bulk and true density of the wheat grain were 804.8 kg/m^3 and 1266.4 kg/m^3, respectively. The void space present in the grain was 36.5%. The quantity of carbon dioxide purged at different concentrations and grain-fill volumes is presented in Table 1.

Table 1. Quantity of carbon dioxide purged at 1.5 L/min flow rate to grain-fill volume

Grain-fill volume	Total void space[a] (L)	Quantity of CO_2 purged at different concentrations (L)		
		40%	60%	80%
25%	0.84	0.34	0.50	0.67
50%	0.68	0.27	0.41	0.54
75%	0.52	0.21	0.31	0.42

[a]Void space in grain + headspace volume.

The fumigant concentration was checked every 24 h using a portable biogas analyzer (Biogas 5000 Geotech, Netherland). The concentration of CO_2 in the storage bottles varied within ±2% from the desired concentrations. Loss of around 12.9-22.4% concentration occurred in 24 h, which was replenished regularly.

Insect mortality

To get 100% mortality of *R. dominica* 5, 3, and 1 d was sufficient at 40, 60, and 80% of CO_2. Riudavets et al. (2009) found 100% adult mortality of *R. dominica* at 8 and 4 d at 50 and 90% CO_2 at 25°C, respectively. Kaliyan et al. (2007) also reported the storage of grain under pure CO_2 at atmospheric pressure and storage temperature above 21°C; around 9, 11, and 17 d were enough to control pest infestation at 80, 60, and 40% CO_2, respectively. Our study showed that waste gas from the CBG plant gave better result than the previous studies.

Effect of bio-CO_2 fumigation on seed weight and germination

The grain storage bottles had no insects within 1-5 d at 40-80% CO_2. Therefore, no significant weight loss of wheat was observed after a 60-d storage period. The grain stored in the controls had a weight loss of about 5.0% due to pest multiplication. The highest loss of 17.3% was observed with the infestation of *R. dominica* without fumigation. Similar findings were reported by Mekali et al. (2013). After a 60-d storage under CO_2 fumigation using waste gas, the seed germination was reduced by 6, 7.5, and 8.5% with 40, 60, and 80% CO_2, respectively. The results were in agreement with the findings of Mitsuda and Yamamoto (1980). The effect might also be due to the presence of methane and traces of hydrogen sulfide in the waste gas used as a fumigant. Also, the germination of legume seed (pigeon pea) stored under biogas fumigation remained unaffected (Anonymous, 1992). It might be unchanged because of a short storage period.

Conclusions

The use of waste gas from a compressed biogas plant showed better efficacy in insect mortality than the previously reported studies based on pure CO_2. Some CH_4 and H_2S in waste gas might have a supplementary lethal effect on insects. No significant differences were found in the seed germination over a 60-d storage period. Furthermore, the viability of such fumigants needs to be evaluated in a larger scale storage system for long-term storage under actual field conditions. The future study should also assess the suitability of waste gas as a fumigant on other grain parameters

like nutritional quality, sensory evaluation. The efficacy of waste-gas as a fumigant could also be checked with other pests and grains.

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Jie Y, Shi T, Yang D, Jiang J, Li Q, Bi W (2021) Changes in quality of rice grains after storing in controlled nitrogen atmosphere. Page 174. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Changes in quality of rice grains after storing in controlled nitrogen atmosphere

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ABSTRACT

The use of controlled atmosphere with more than 98% N_2 is one of the most successful storage techniques, while the flavor change of grain after stored with N_2 is still unclear. High-quality rice grain was sealed in warehouses filled with 98% N_2 and stored at 20°C for 6 months; then unsealed and stored at 20°C for 6 months. The water loss, fatty acids value, appearance, pasting properties, texture properties and volatile compounds of rice grain after controlled nitrogen atmosphere were measured every month. The results showed that the controlled nitrogen atmosphere treatment reduced water loss and fatty acids value increase of rice grain, further reducing the production of off-odors in rice grain. This study provided a meaningful basis for large-scale application of nitrogen-controlled grain storage.

Keywords: Controlled atmosphere, Nitrogen, Volatile compounds, Shelf life

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Riudavets J, Iturralde-García RD, Castañé C, Bourne R, Wong-Corral FJ (2021) Effect of carbon dioxide sorption in packaged chickpeas on the susceptibility to modified atmospheres of *Rhyzopertha dominica* and *Callosobruchus chinensis*. Page 175. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Effect of carbon dioxide sorption in packaged chickpeas on the susceptibility to modified atmospheres of *Rhyzopertha dominica* and *Callosobruchus chinensis*

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ABSTRACT

Modified atmospheres (MAs) with 50 or 90% CO_2 were tested in containers filled with chickpeas at different filling ratios (24, 48 and 96%) to assess the amount of CO_2 sorbed by the pulse. The maximum sorption (1.28 gCO_2/kg of chickpea) was obtained with the lowest filling ratio tested (24%) and with an initial concentration of 90% CO_2. Time needed to reach equilibrium sorption varied between 27 and 141 h, depending on the initial CO_2 concentration and filling ratio. The negative pressure produced by sorption inside the containers incremented with the increase of the filling ratio and the initial CO_2 concentration.

Mortality of the internal feeders *Rhyzopertha dominica* (F.) and *Callosobruchus chinensis* (L.) was assessed in packages filled with two extreme filling ratios (4 and 96% of chickpeas) and with 50 or 90% CO_2. For both pest species, the exposure time to reach 50% mortality ranged from 7 h (larvae with 90% CO_2) to 2 d (pupae with 50% CO_2) at the lower filling ratio tested (4%). When increasing the filling ratio to 96% of chickpeas, mortality of *R. dominica* eggs and adults decreased significantly while did not vary for the internal developmental stages. A similar effect was observed (a decrease in mortality of external developmental stages) in *C. chinensis* at 96% filling ratio with 50% CO_2. However, mortality remained the same for the eggs and pupae at 90% CO_2.

The decline in mortality of external developmental stages of both weevils was probably due to the sorption of CO_2 by the chickpeas, which caused a loss of intergranular levels of CO_2. In conclusion, when chickpeas were packaged with high CO_2 MAs, a decrease in the mortality of the external stages of the pests could be expected due to sorption, whereas for internal stages effectiveness could be anticipated to be the same.

Keywords: Modified atmospheres, Filling ratio, Sorption, Negative pressure, Weevils, Chickpeas, Insect pests, Control, Vacuum, Legumes

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Hulasare Heat treatment: an effective, viable alternative to methyl bromide for controlling stored product insects in food processing facilities. Page 176. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Heat treatment: an effective, viable alternative to methyl bromide for controlling stored product insects in food processing facilities

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ABSTRACT

Environmental concerns, consumer preference and increased insect resistance are the drivers in search for effective alternatives to chemical fumigants such as methyl bromide. Heat treatment involves raising and maintaining temperatures of grain storage structures, warehouses, and food-processing facilities between 50 to 60°C to manage stored-product insect species. The duration of heat treatment is application-specific and may vary from 6 h for an empty storage structure (bin/silo) or portion of a food-processing facility to 24 h for an entire facility.

Using direct-fired, make-up air heaters, 'positive pressurization' is applied forcing 100% heated fresh air into the facility. The process is engineered to generate specific number of air changes per hour based on structural parameters of the facility. The process uses a combination of direct-fired make-up heaters, fans, and ductwork for airflow management and maintains a relatively constant lethal temperature profile throughout the treatment area.

Laboratory and commercial trials with high temperatures during the last decade, especially with forced air gas heaters, have resulted in a wealth of information on (1) understanding responses of insect species and life stages to heat, (2) heat distribution within a treated area, and (3) techniques necessary for gauging effectiveness of commercial heat treatments. Insect responses vary with the temperature, among species, and within a species among life stages.

Insect bioassays and monitoring insect populations before and after a heat treatment are important to understand the degree and duration of insect suppression obtained in commercial facilities. Heat treatments are safe, effective, and a viable tool for the organic and non-organic sector. High temperature treatment of whole or part of the facility by maintaining temperatures between 50 to 60°C has been shown to be safe, effective and a viable alternative to manage stored product insects.

Keywords: Heat treatment, Forced Air, Flour mills, MB alternative, Stored product insects

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Arora S, Stanley J, Patil N, Adak T, Srivastava C, Singh JP, Jena M, Ramya RS, Navik O, Asher PP, Patel F, Patel M, Gupta JP (2021) Phosphine as methyl bromide alternative for QPS treatment of food grains in India. Pp. 177-183. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Phosphine as methyl bromide alternative for QPS treatment of food grains in India

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Abstract

Currently, phosphine and methyl bromide are the only registered fumigants in India. The latter is used exclusively for Quarantine and Pre-Shipment (QPS) treatments. However, in view of its ozone depleting potential a necessity has arisen to seek an alternative. In this context, phosphine was evaluated as an alternative for methyl bromide for QPS treatment of food grains. The efficacy of phosphine was assessed based on multi-location field trials with different climatic conditions in India. Fumigation trials were conducted on bagged wheat, milled raw rice, green gram, chickpea and yellow pea stacks (5 tonnes per replicate, 3 replicates per dose/exposure period) at locations with different climates. The temperature and humidity conditions ranged from 20-39°C and 38-78%, respectively. The stacks were fumigated using an on-site phosphine generator with QuickPH1o -R 77.5% granular aluminum phosphide (AlP) formulation at 1.0 and 1.5 g phosphine/m^3, and by conventional application with 56% AlP tablet formulation at 1.5 (2 Tab/tonne) and 2.3 g phosphine/m^3 (3 Tab/tonne), each in triplicate along with untreated controls. The experiments were carried out for 7 and 10 d exposure periods against life stages of *Tribolium castaneum* , *Sitophilus oryzae*, *Rhyzopertha dominica,* and *Callosobruchus maculatus* from laboratory cultures as well as field populations. Phosphine concentrations in each stack were monitored at 24 h intervals during the exposure period. On termination of the treatment, grain samples were drawn from fumigated and untreated control stacks for phosphine residue analysis and infestation monitoring.

The results revealed the minimum effective phosphine dosage to be 1.5 g phosphine/m^3 with 7 d exposure, as 100% mortality of test insects was achieved. The average terminal phosphine concentration at the end of 7 d was 500 ppm (300-725 ppm) for cereals and pulses grains. Estimated residues of phosphine in the fumigated grain samples were below the Maximum Residue Limit (MRL) of 0.1 ppm of Codex Alimentarius Commission (CAC) for all dosages.

Keywords: Methyl bromide alternative, QPS treatments, Phosphine, Food grains, Efficacy, Residues

Introduction

The post-harvest losses in durable agricultural commodities including food grains and many value-added food products and non-food derivatives of agricultural products are reported worldwide due to infestation of stored product by insect pests. The FAO in 2011 report estimated about 1.3 billion tonnes of annual global loss or wastage of food (Gustavasson et al., 2011). However, around 14% of the world's food loss is from post-harvest losses (FAO, 2019). The estimated post-harvest losses of pulses during storage are 5-10% in India (Lal and Verma, 2007). The annual quantitative food losses and wastes for cereals are around 30% globally (Sawicka, 2019). Out of total 10% post-harvest losses of grains, a significant quantity of 6% are damaged during their storage. The World Bank report has estimated 7–10% of grain loss (approximate 12-16 million tonnes) in post-harvest operations in the field and 4–5% of losses on the market and distribution in India during 1999 (Kumar and Kalita, 2017; Shah 2013; Ognakossan et al., 2013). Out of these post-harvest losses in India, storage insects alone account for 2.0 to 4.2%. The annual storage losses have been estimated at 14 million tonnes worth of Rs. 7,000 crores (US$ 1 Billion), where insects alone account for nearly Rs. 1,300 crores (US$ 185 million) (Kumar and Kalita, 2017).

During storage, cereal grains are attacked by a number of storage insect pests, such as *Tribolium castaneum* (Herbst) (rust-red flour beetle), *Sitophilus oryzae* (Lin) (rice weevil), and *Rhyzopertha dominica* (Fab) (lesser grain borer); and pulses are attacked by the pulse beetle, *Callosobruchus maculatus* (Fab), responsible for post-harvest losses accounting for the nearly Rs. 1,300 crores noted above (IGMRI, 2019). Due to phasing out the ozone-depleting methyl bromide (MBr) fumigant, phosphine is widely used as an effective fumigant in the world due to its desirable properties across many types of storage structure and its acceptance as a residue-free treatment by international markets (Nayak et al., 2020).

In this context laboratory experiments were conducted to study the effectiveness of phosphine against life stages of stored grain insect pests. Following the laboratory trials, the test insects were used to carry out field trials. Phosphine is registered in India as aluminum phosphide in two formulations, 56% aluminum phosphide (AlP) tablet and 77.5% aluminum phosphide granules for domestic storage, and needs testing for long term food grain storage and for quarantine and pre-shipment treatments. This study investigated the efficacy of phosphine against insect pests of cereals (wheat and rice) and pulses (green gram, chickpea and yellow pea) in storage under varied climates using both formulations.

Materials and methods

The on-site gas generation (through QuickPHlo-R^{TM} gas generator, m/s UPL Limited, Vapi, India) using 77.5% AlP granular formulation at 1.0 and 1.5 g phosphine/m^3 dosages were used for fumigation of food grains at all the locations. The same was compared with the application of conventional 56% AlP tablet at 2 Tab/tonne (~1.5 g phosphine/m^3) and 3 Tab/tonne (~2.3 g phosphine/m^3). The experiments were conducted on custom-built 5 tonne stacks for 7 and 10 d exposure periods in triplicate including control during 2017-19. There were 100 bags each of 50 kg grains in every stack. Gas monitoring lines were fixed at top, middle and bottom levels in each stack including untreated control stacks to keep track of the phosphine gas concentration during the experiments. The temperature and relative humidity were monitored every 24 h during the course of field fumigation trials at each location (Table 1). Also grain moisture was checked for each commodity before initiation of the experiments.

Nucleus culture of *T. castaneum, S. oryzae* and *R. dominica* were procured from Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, ICAR-National Rice Research Institute, Cuttack, Odisha, and ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttrakhand, India. The cultures were maintained by rearing the insects at 28-30°C and 65-70% relative humidity (RH) on whole wheat flour: yeast (19:1) or broken wheat as media (Arora et al., 2021). A culture of *C. maculatus* was reared separately for green gram, chickpea and yellow pea in an incubator set at 27±3°C and 70–75% RH. A photoperiod of 16 L: 8 D was maintained for rearing all the test insects (Arora and Srivastava, 2021).

The tests insects enclosed in twelve test containers were taken for different life stage of *T. castaneum* (adults and larvae), *S. oryzae* (adults), and *R. dominica* (adults), *C. maculatus* (adults and eggs) separately, and placed uniformly at three levels (top, middle and bottom) across four sides of each stack. The field population of these insects in individual grain stacks was also monitored. For fumigating each stack two methods of fumigation were applied in triplicate including untreated control at all the locations for each commodity. The experiments were conducted each for 7 and 10 d exposure periods separately for both the formulations.

For granular 77.5% formulation, stacks were covered with 200 GSM gas proof sheets (HDPE) followed by floor sealing using sand bags prior to delivery of phosphine through on-site gas generation; while, for tablet formulation, stacks were sealed after placing the tablets. Phosphine gas concentration was monitored at 3 levels (bottom, middle and top) every 24 h throughout the experimental period using UNIPHOS FumiSense Pro (Uniphos Envirotronic P. Ltd., Nahuli, INDIA measuring range 1-2000 ppm) through gas monitoring lines for all commodities. It was initiated on zero day itself for on-site gas generation, and one day after placement of tablets.

On termination of the treatment, the stacks were aerated for 5 d and after a withholding period of 2 d, representative grain samples (~1 kg) were drawn from fumigated and untreated control stacks before initiation and on termination of fumigation trials, for phosphine residue analysis using GC method (Nowicki, 1978). These samples were also used for infestation monitoring in field population as well as test containers.

Results and discussion

A significant variation was observed in phosphine gas concentrations and dissipation in food grains in trials conducted at different locations depending on grain moisture and weather conditions (Table 1).

Table 1. Details of phosphine fumigation trials conducted on grain stacks at different locations by conventional tablet and on-site-generation applications and achieved terminal concentrations

Grain	Location	Temperature (°C)	Humidity (%)	Moisture (%)	Exposure period (d)	Two applications of PH_3*	Average gas loss (%)	Terminal concentration (ppm) in 7 d
Wheat	Delhi	25-29	43-80	10.8± 0.1	7, 10	Tablet	17.3	500-1100
						On-site gas	13.4	250-650
	Pithoragarh	21-25	43-58	12.5± 0	7, 10	Tablet	13.2	400-1000
						On-site gas	11.5	200-500
Milled raw rice	Sonepat	29-36	52-56	13.6±0.1	7	Tablet	14.4	795
						On-site gas	17.6	443
	Delhi	29-34	67-82	13.1±0.1	10	Tablet	16.0	575
						On-site gas	14.9	290
	Cuttack	29-34	54-78	12.88±0.1	7, 10	Tablet	15.9	300-900
						On-site gas	16.5	200-600
	Jagatpur	20-30	45-61	11.3±0.2	7, 10	Tablet	16.1	600-1300
						On-site gas	13.9	200-500
Green gram	Jagatpur	20-29	50-83	9.63±0.1	7, 10	Tablet	21.1	250-700
						On-site gas	16.8	150-250
Chick pea	Jaipur	27-35	38-72	9.3±0.1	7, 10	Tablet	19.4	250-700
						On-site gas	18.7	150-300
Yellow pea	Mumbai	28-39	60-76	11.85±0.1	7, 10	Tablet	12.2	200-400
						On-site gas	22.4	300-500

*Tablet application at 2 or 3 Tab/tonne (~ 1.5 and 2.25 g phosphine/m^3); on-site gas application using gas generator at 1.0 and 1.5 g phosphine/m^3.

The grain moisture was significantly different ($Pr>F;\ p < 0.001$) for the food grains fumigated under field experiments at various locations. However, no significant difference was observed in the moisture of chickpea and green gram treated at two different locations. Phosphine gas loss was significantly different with respect to commodities ($Pr>F;\ p < 0.0026$), but not with dosage or exposure periods. Moreover, no significant difference was observed between any of the pulses but was with cereals. The t-test revealed significant difference for chickpea with rice and wheat grains at Delhi, wheat at Pithoragarh and rice at Jagatpur. Similar was the case for green gram with wheat at Delhi and Pithoragarh, and rice at Jagatpur. Gas loss was noted to be significantly higher in pulses when compared with those of cereals, i.e., wheat and rice at different locations. Moisture content of the pulses were less than that of cereal grains fumigated under different climatic conditions. Rice (paddy and milled) and other high moisture grains are known to absorb phosphine very rapidly (Banks, 1989). Reddy et al. (2007) has also reported that among cereals paddy rice was more sorptive (~60%), increased doses of 3-6 g/m^3 were required to attain the target concentration of 1000 ppm. The amount of phosphine sorbed has been observed to be mainly affected by the type of substrate, temperature, moisture content, and exposure of reactive surfaces by course grinding of the cereal seeds (Ben, 1968).

Phosphine gas loss was observed to higher extent in pulses with minimum grain moisture when compared with those of cereal grains. The results under present study are in accordance with the reports available in literature. Although rice grains have been reported to have higher sorption of phosphine gas followed by pulses and wheat. However, pulses were observed with higher sorption in present study, which might be due to variation in temperature and humidity conditions and moisture content of the grains at respective centers.

Absorption by the treated commodities affects the terminal gas concentration during fumigation. Pulses with higher sorption resulted in lower terminal concentrations of phosphine when compared with those of wheat and rice at different locations. Furthermore, the terminal gas concentrations using on-site gas generator were significantly lower than that of using conventional tablet formulation. Reason being the peak concentration achieved at zero day itself using on-site gas; while, it takes 24-72 h to reach peak value depending on the temperature and humidity conditions when tablets are used. In the present study 100% mortality of test insects as well as field population was observed during grain fumigations conducted at all locations. No emergence of insects was found when treated grain samples were monitored under laboratory conditions for 60 days, indicating mortality of all developmental stages of insects.

Varied climatic conditions at different centers affected the grain moisture, phosphine gas dissipation and terminal concentrations. However, fumigation trials proved the effectiveness of phosphine fumigant for managing stored grain insect pests. Moreover, phosphine provided insect control up to 60 d of treatment until cross contaminated.

Residues of phosphine in grain samples collected from the experimental stacks were noted. However, the levels of residues were in the range of 0.005-0.095 ppm, below MRL (0.1 ppm), fixed by CAC of FAO/WHO. Hence, phosphine can be an effective substitute for methyl bromide fumigant in quarantine pre-shipment treatment of food grains.

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New diagnostic tool for *Trogoderma* species: visible near infrared hyperspectral (VNIH) technique coupled with machine learning

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ABSTRACT

Trogoderma sp., khapra beetle (*Trogoderma granarium* Everts) is one of the major stored grain pests in the world. In most of the developed countries it is among the top quarantine pests. The economic consequences of an incursion of this species would be very serious as the pest is difficult to be controlled by existing methods and there will be loss of premium (khapra-free) market reputation for the country. Most of the times accurate and reliable identification becomes very difficult as it morphologically resembles other native *Trogoderma* species. Until now taxonomic method by trained personnel is the only available method which could be labour intensive, time consuming and often suspected *Trogoderma* sp. found in grain products is the body fractions such as larval skins or fragmented adult which is impossible to diagnose morphologically. Hence under this study a new diagnostic system for khapra beetle using visible near-infrared hyperspectral (VNIH) imaging method to address biosecurity surveillance and identification gaps for khapra beetle is developed. Hyperspectral imaging is the technique of acquiring a 2D image where every pixel in the image contains a continuous spectrum. A regular camera records three spectral channels in every pixel (red, green, and blue), while hyperspectral imagers can record hundreds of spectral channels to form a hypercube. This technique is useful because different materials have unique reflectance spectra, and this difference in reflectance spectra can thus be used to identify various constituents in an image. About 2000 hyperspectral images were acquired, comprising of *T. granarium* and *T. variabile* (Ballion), adult, larvae, larvae skin, fragments of adult and larvae images, which were subjected to two deep learning models; Convolutional Neural Networks (CNN) and Capsule Network for analysis. For adult whole body and adult fragments, the accuracy achieved was 96 and 92% respectively. For whole larvae, larvae skin and larvae fragment, an accuracy of 93, 92 and 90% was achieved. Thus, the technology offered new concept and possibility of an effective identification of *Trogoderma* sp. from its body fragments and larvae skins which are otherwise impossible to diagnose taxonomically.

Keywords: *Trogoderma* diagnostic, Khapra beetle, Deep learning, Capsule network, Convolutional neural networks, Visible near infrared hyper spectroscopy

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Insecticidal effect of phosphine in different life stages of the khapra beetle, *Trogoderma granarium*

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ABSTRACT

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is an important pest of stored products worldwide. It is considered as one of the most serious quarantine pests in many parts of the world, and often cannot be controlled by insecticides that are usually effective against other stored product insects. The use of phosphine gas has been proven to be effective against a wide range of stored-product insect species, but there is still inadequate information in the case of *T. granarium*. In the present study, we evaluated the effectiveness of phosphine in different life stages of this species, including its diapausing larvae. The evaluation protocols used were: a) the Food and Agricultural Organization (FAO) protocol, i.e., exposure for 20 h at 30 ppm, b) the dose response protocol, i.e., exposure for 3 d at different concentrations (50, 100, 200, 500 and 1000 ppm), and c) the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK, Detia Degesch GmbH), which is based on short exposures (usually minutes) at 3000 ppm. The adults, pupae and larvae of *T. granarium* were susceptible to phosphine, as mortality was 100 % after 3 d of exposure, even at 50 ppm. The same holds for diapausing larvae, which had similar susceptibility to phosphine with non-diapausing ones. In contrast, after 3 d exposure, there was some egg survival at 500 ppm, while mortality was 100 % only at 1000 ppm. The data of the present study can be further utilized for the control of this species, especially in the case of quarantine and pre-shipment treatments.

Keywords: *Trogoderma granarium*, Phosphine, Life stages, Fumigation, Quarantine species

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Early-warning detection protocol of khapra beetle (*Trogoderma granarium* Everts) and other insect pests associated with stored grains in Portugal - preliminary results

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Abstract

The contamination of stored grains by insects and fungi is one of the most prevalent problems within food storage. *Trogoderma granarium* Everts is an important quarantine species in several countries with high activity in the international trade of cereals, such USA, Canada, Brazil, and Australia. It has been considered as one of the most destructive stored product insects worldwide. The aims of this work were to detect the presence of *T. granarium* in Portugal and to identify other insects captured, through a sampling protocol developed by the consortia of countries (Portugal, Spain, Italy, and Greece). Seven warehouses were surveyed for *T. granarium* and other stored products insect pests in 2019, for three months, in different regions from Portugal. Two types of traps were used: Storgard Probe traps, and Storgard Dome traps. Molecular tools were used to identify *Trogoderma* spp. *Trogoderma granarium* was not identified in Portugal, but *Trogoderma inclusum* LeConte, was registered; however, it does not have a concerning pest status. This sampling program allowed the identification of other stored grain pests, with a total of 17055 insects captured. Nine species of Coleopteran pests were identified, as well as three Lepidopteran pest species. Among the captured insects, three species/order were present in all facilities: *Sitophilus zeamais* (Motschulsky), *Cryptolestes ferrugineus* Stephens, and Psocoptera. The most abundant species were: *S. zeamais*, *Oryzaephilus surinamensis* (L.), and *C. ferrugineus*. Asymmetries on stored grains associated insect monitoring along Portugal may be linked not only to different stored products, and the origin of those products, but also with different management strategies held by the storage facilities. Further investigation on this should be pursued and a continuous monitoring program shall be held in order to monitor for the entry of new stored product pest species, including *T. granarium*.

Keywords: Stored grains and derivatives, Insect pests, *Trogoderma granarium*, Monitoring

Introduction

Stored products insect pests may infest all cereal grains and may be responsible for postharvest losses in the order of 9% and up to 20%, in developed and developing countries, respectively (Pimentel, 1991; Phillips and Throne, 2010). The contamination of the food products with insect materials (dejects, body fragments, dead bodies, chemical excretions) may also affect the product quality and decrease its value or the possibility of its consumption. New commodities, which are entering consumers' diets (for example chia, quinoa, or millet), are also susceptible to stored products insect pests, and studies within this area are required to establish the risk of infestation (Cominelli et al., 2020). Although some insect species may have some nutritional value and are not referenced as allergenic to humans (Singh and Sinha, 1977), Dermestidae species have abundant and often barbed setae on the larvae that have been documented as a possible source of allergens for humans (Okumura, 1967; Hirao, 2000; Mullen and Durden, 2009; *Gorgojo et al., 2015*; *MacArthur et al., 2016*). Regarding *T. granarium*, besides contamination by insect activity as chemical secretions and cast skins or urticating hairs released by the larvae, that may cause severe health hazards when consumed, causing quality losses, is also classified as highly destructive of cereal grains, even if in good storage conditions, the damages done by larval feeding (Athanassiou et al., 2019).

Trogoderma granarium stands out as one of the 100 worst invasive species worldwide (Lowe et al., 2000; Luque et al., 2014). This species is native to India (Paini and Yemshanov, 2012); however, is believed to be established in 34 countries in Asia, Africa, and Europe, and registered as introductions already eradicated or no longer occurring in an additional 32 countries in Europe, North and South America (Athanassiou et al., 2019). It is a highly polyphagous species, as more than 100 commodities have registered attacks by *T. granarium* (Athanassiou et al., 2019). In Portugal, this species has been registered in the last century, but no further efforts were made since then to know the extent of *T. granarium* in the country, and the current status of this species is absent, with interceptions (EPPO, 2021). A recent study from Spain, using monitoring and molecular tools, stated that *T. granarium* was no longer present in the country, changing the status of this pest situation to absent by the EPPO (European and Mediterranean Plant Protection Organization) (Castañé et al., 2020; EPPO, 2021). This illustrates the need of effective and continuous monitoring programs, including inspections and quarantine measures, regarding this pest, as it may imply some restrictions on the commercial trade of some commodities. The difficulties arisen in the detection, control and/or eradication of *T. granarium* may be explained by several factors, for example: 1) the cryptic habits of these insects, which are not easily detected, and therefore are easily transported; 2) the larval diapause capacity, which enables the larvae to remain in the same instar for years and the tolerance of these diapausing larvae to adverse conditions and to pesticides is enhanced (Banks, 1977; Athanassiou et al., 2019); and 3) the identification of adults and larvae is difficult, requiring taxonomists and molecular tools, both resources that are not easily available within phytosanitary authorities in most countries (Athanassiou et al., 2019). Additionally, the increases in the international trade of raw materials and global warming favour the *T. granarium* spreading (Athanassiou et al.,2019). The aim of this work was to survey the presence (or state the absence) of *T. granarium* in Portuguese territory. Additionally, this survey allowed the data gathering of several other insect species associated with stored products.

Material and methods

Nine warehouses were surveyed for *T. granarium* and other stored-product insect pests, located in different regions from Portugal: two warehouses each in Azores islands (2019) and in Aveiro ports (2017 and 2019); one in a pasta mill (2017) and in two ports (2017 and 2019) in Lisbon; one in a flour mill in Castelo Branco (2019); one in a port in Porto (2019); and a rice mill in Santiago do Cacém (2017). In 2017, a preliminary test was carried out using XLure R.T.U. MST Beetle Floor Trap. These devices were used to monitor five different warehouses with two traps for each site. All lures were replaced every month for three months (June to August). In 2019, warehouses were sampled monthly for three months between June and August, except for Azores, which was from September to October. This choice was related to a higher activity of insects associated with stored products during spring and summer, already registered for the Iberian Peninsula (e.g., Castañé et al., 2020). In 2019, five floor traps (STORGARD® Standard DOME™ Trap, Trécé, Inc., Adair, Oklahoma, United States of America) were placed in each location: Aveiro (AV1 and AV2), Castelo Branco (CB), Lisbon (LB) and Porto (PT), except in the Azores, where three Dome traps were placed in each location (AZ1 and AZ2). These traps were baited with a commercially available gel combination including sex and aggregation pheromones, and food attractants (PantryPatrol™, Insects Limited Inc., Westfield, Indiana, United States of America). This gel combining sex and aggregation pheromones and food attractants is not specific to *T. granarium*, therefor, also attracts other stored products insect pests. This kairomone was impregnated into a paper filter disk placed inside the Dome. All lures were replaced every month. Three Probe traps (STORGARD®, Trécé, Inc.) were placed in the warehouses which allowed access to the stored products (AZ1 and AV1); however, in the end of the survey, only one trap was available on each site, precluding the use of the data related to the missing traps. Insects were collected and separated into different orders, suborders, families, genus, and/or species, based on morphological features, and counted.

Molecular identification of *T. granarium* was done by IRTA, Spain in 2017; and in 2019 it was done at a laboratory of the Instituto Superior of Agronomy, University of Lisbon, Portugal. In Portugal, the molecular identification was carried out using a previously described method, with slight adaptations (Olson et al., 2014). Extraction of DNA was done with a DNeasy blood and tissue kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, and a Real-Time PCR was performed. A pair of *T. granarium* specific primers was used (der16SF4 and der16SR1; Olson et al., 2014). Also, an additional set of primers considered to be more generalist was used (Castañé et al., 2020). *Trogoderma granarium* specimens obtained from Spain were used to a positive control, and the only suspected specimen captured within this work was submitted to this analysis.

Results and discussion

Regarding the preliminary detection campaign in 2017, khapra beetle was not found. In Lisbon warehouses, 9 Dermestidae larvae, 2 *Sitophilus* sp., 2 *Ptinus* sp., 1 *Gnatocerus cornutus*. 1 *Lasioderma serricorne*, and 1 *Cephalonomia* sp. were found. At the pasta mill, only Psocoptera insects were found and in the rice mill, 8 *Sitophilus zeamais* and 3 *T. castaneum* were registered. In the sampling program of 2019, a total of 17055 arthropods were captured, 14965 in Dome traps, and 2090 in probe traps. In the dome traps survey, in the Azorean warehouses (AZ1 and AZ2), *S. zeamais* and *O. surinamensis* prevailed (Fig. 1).

In Aveiro region (AV1 and AV2), Psocoptera and *S. zeamais* were the most abundant insects, as well as in Porto (PT), although *C. ferrugineus* was also numerous. In Castelo Branco (CB) warehouses, *R. dominica* and Collembola were the most prevalent insects. In Lisbon (LB) region, *S. cereallela* and *L. serricorne* were the most abundant insects captured.

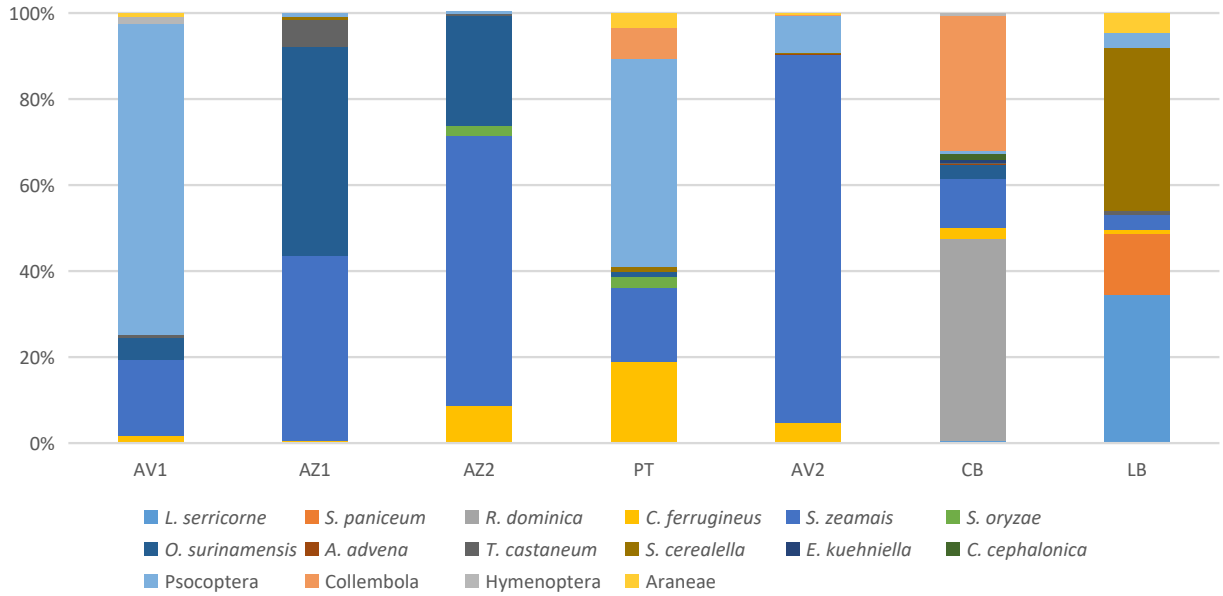

Fig. 1. Proportion of arthropods captured in dome traps in each of the Portuguese warehouses surveyed, located in Azores region (AZ1 and AZ2), Aveiro region (AV1 and AV2), Castelo Branco Region (CB), Porto region (PT) and Lisbon region (LB).

In the probe traps survey, Psocoptera were very abundant in the Aveiro region warehouse (AV1). In the Azorean warehouse (AZ1), *A. diaperinus* and *T. castaneum* were the prevailing insects (Fig. 2). *Sitophilus zeamais* stands out as the most abundant pest captured, present in all the warehouses surveyed except AZ1 probe trap. *Oryzaephilus surinamensis* was the second most abundant species, although these numbers are due to high captures in the Azorean warehouses. *Cryptolestes ferrugineus* was the third species in terms of abundance, and these insects were captured in all the warehouses surveyed, as well as Psocoptera.

Dome and probe traps are more adapted to capture crawling insects; therefore, the majority of captures are from insects belonging to the Coleoptera order, although this order bears the majority of stored products insect pest species. Nine species of Coleopteran pests were identified, as well as three Lepidopteran pest species. The survey demonstrated a rich stored products insect pest community within most Portuguese warehouses, regardless of the kind of product stored. *Sitophilus zeamais* seems to be the dominant species regarding its distribution and abundance on northern warehouses and in the Azores. This species is regarded as one of the most destructive primary pests worldwide, besides the direct damage done to grains, it also facilitates the establishment of secondary pests and pathogens (Trematerra et al., 2007). From the southern warehouses, Castelo Branco and Lisboa, the dominant species were, respectively, *R. dominica* and *S. paniceum*. Among the captured insects, three species/order were present in all monitored facilities: *S. zeamais*, *C. ferrugineus*, and Psocoptera; and the most abundant species were: *S.*

zeamais, O. surinamensis, and *C. ferrugineus*. High numbers of some species are related to the Azorean region, for example, *A. diaperinus, O. surinamensis, T. castaneum,* and to some extent *S. zeamais*. Psocids (order Psocoptera) were considered minor pests for a long time, although concern was raised after observations of their high tolerance to pesticides, high associated costs, and potentially negative effects on health and safety (Nayak et al., 2014). In this survey, psocids showed a wide distribution, and this fact should be further investigated, namely their identification to species, as infestations may comprise more than one species and different species may interact and have different responses to treatments, influencing the pest management strategies success (Athanassiou et al., 2014; Nayak et al., 2014).

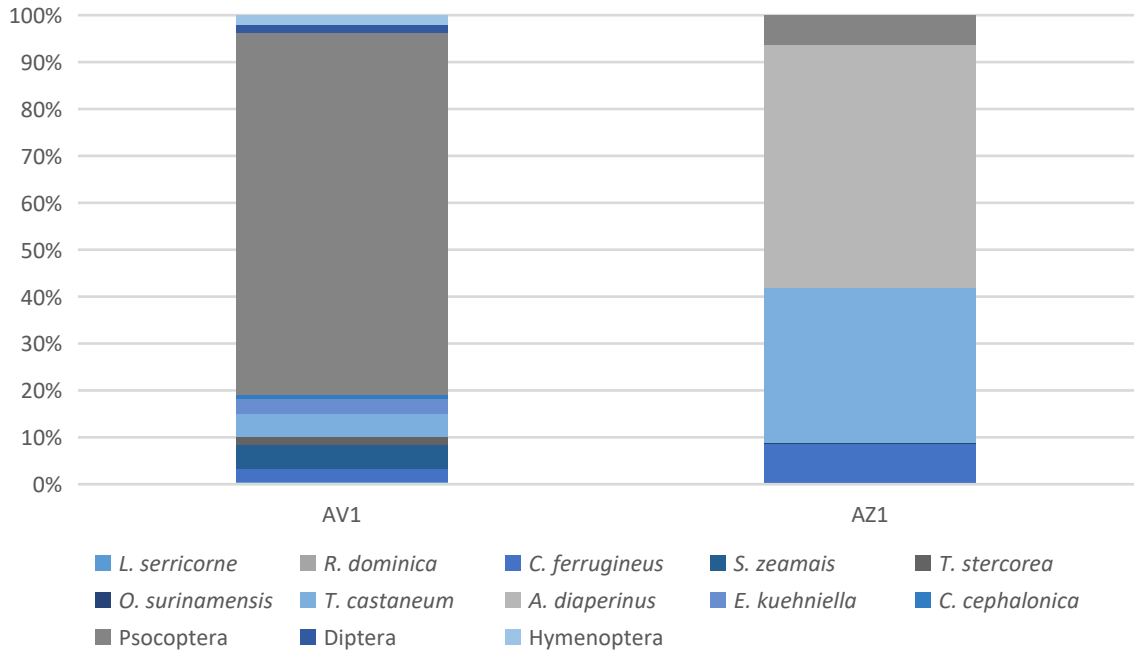

Fig. 2. Proportion of arthropods captured in probe traps in each of the Portuguese warehouses surveyed, located in Aveiro region (AV1) and Azores region (AZ1).

Regarding the identification of *T. granarium* specimens, it was caught one adult specimen, in Castelo Branco, that raised suspicions on its identification, so it was processed into molecular identification and the identification was *T. inclusum*, which is not a species of concern. Castañé et al. (2020) performed a survey in Spain, including one Portuguese warehouse, and the results were similar: no *T. granarium* was identified, this resulted in the classification regarding *T. granarium* in Spain being defined as "Absent, confirmed by survey" (EPPO, 2021). In Spain, the species belonging to *Trogoderma* genus prevailing is clearly *T. inclusum* (Castañé et al., 2020). *Trogoderma granarium* has been intercepted in Europe multiple times, but there are no data indicating that it is established in European countries, except for Cyprus and Turkey, where it has restricted distribution (Stejskal et al., 2015; EPPO, 2021). The maintenance of a detection survey directed to this species would be important regarding the aggressive features of this invasive species (Banks, 1977; Athanassiou et al., 2019). The use of molecular identification techniques already developed for the identification of *T. granarium*, as described by Olson et al. (2014) and Castañé et al. (2020) are valuable tools to allow a proper survey and identification of this species.

Data presented here is preliminary, as the survey will continue in the following years. Monitoring programs at the national level should be accomplished by governmental and/or industrial task forces, to allow correct identification of stored products insect pests, facilitating the proper management of pests by the owners, and enabling the establishment of previously defined early warning methods regarding invasive species.

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Efficacy of propylene oxide as a postharvest fumigant for fresh citrus pest disinfestation and its impact on citrus fruit quality

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Abstract

Arthropods found on citrus at the time of harvest but not present in countries where the fruit is exported must be controlled to prevent their accidental introduction. Regulatory and technical logistics related to methyl bromide, a postharvest fumigant long relied upon by the California citrus industry, is upon the discretion of importing countries. We evaluated the toxicity of fumigant propylene oxide (PPO) in combination with CO_2 on California red scale (*Aonidiella aurantii* Maskell), Fuller rose beetle [(*Naupacts godmanni* (Crotch)], bean thrips [*Caliothrips fasciatus* (Pergande)]; and three mite species, namely, *Brevipalpus californicus* (Banks), *Brevipalpus lewisi* McGregor, and *Lorryia formosa* Cooreman Caged. Arthropod specimens were exposed to different concentrations of PPO during a 2 h fumigation at 15.6°C to generate dose-response mortality. Results showed that propylene oxide was toxic to all species tested but response to treatments varied greatly among species. Fuller rose beetle egg control was not achieved during the 2 h fumigation schedule; therefore, 24 h fumigation exposure was tested. Among the species tested during 2 h exposure, bean thrips adults and *B. californicus* adults were the most susceptible and the most tolerant species, respectively, and required 27.8 and 151.1 mg/hL to achieve 99% mortality. Propylene oxide at 40 mg/L did not cause phytotoxic effects on mandarins and navel oranges, but exposure to 112 mg/L resulted in higher level of decay of navel oranges. These data showed that PPO was toxic to arthropods of export concern to the citrus industry. Future research will aim at studying the time-concentration relationship, effects of fumigation schedule on fruit quality, and residues. Potential of PPO as promising fumigant alternative to methyl bromide for the California citrus industry and future studies were discussed.

Keywords: Postharvest fumigation, Export, Citrus

Introduction

California is the leading producer of fresh citrus in the U.S. with annual production exceeding 3.7 million tonnes with a farmgate value of $3.4 bn (NASS 2019). Export markets contribute nearly 1/3rd of the total revenue (CCQC 2020). Postharvest control of the arthropods that may be present

on citrus at the time of harvest but not present in importing countries is one of the challenges the citrus industry must overcome for retention and expansion of the export markets. As such, one fumigant methyl bromide (CH_3Br abbreviated as MeBr) had dominated the industry as a nearly ideal postharvest fumigant choice. However, regulatory concerns regarding the ozone depleting nature of this chemical have led to its limited use for the Quarantine and Pre-Shipment (QPS) treatments. Even QPS MeBr is on borrowed time and at the discretion of importing countries and there are concerns about future availability and cost of this fumigant (Walse, 2017). Subsequently, postharvest treatments to replace MeBr must be developed to provide biological safeguard against pests of export concern.

Current method of mitigation of export pests is system's approach or MeBr fumigation is used for all citrus arriving South Korea (Pupin et al., 2013). Many fumigants have been studied as a MeBr replacement for a phytosanitary treatment (Pupin et al., 2013; Walse, 2017; Bikoba et al., 2019). Phosphine is the only other fumigant alternative to MeBr but requires ≥ 6-fold longer exposure to control pests compared to 2 h MeBr treatments. In this study we evaluated the toxicity of propylene oxide in combination of carbon dioxide on export concern species.

Propylene oxide (PPO) is an FDA approved sterilant to kill bacteria, mold, and yeast contamination on processed spices, cocoa, and processed nutmeats except peanuts (Griffith, 1999). It is a favored treatment method for pasteurizing almonds. The major disadvantage, its flammability, can be overcome by fumigating under vacuum or in combination with CO_2 (Navarro et al., 2004). Propoxide, 8:92 (wt: wt) combination of PPO and CO_2 is a registered fumigant for controlling stored-product insect pests in the United States.

The overarching goal of this study was to determine the potential of PPO as a postharvest treatment for fresh fruits. First, we established efficacy data for citrus pests of export concern. Second, we determined PPO sorption by citrus at different chamber capacities. Next, we also evaluated the phytotoxic effects of PPO on navels and mandarins. The potential of PPO as a postharvest fumigant for fresh produce as well as the need for future studies were discussed.

Materials and methods

Insects and rearing

California red scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), used for the experiment, was initiated from insects collected from an insecticide free backyard lemon tree, *Citrus limon* L., in Porterville, CA in 1991 and maintained on green lemons at 24 ± 3°C and 12:12 (L:D). Fuller rose beetle, *Naupactus godmanni* (Crotch) (Coleoptera: Curculionidae), eggs were obtained from adults collected from citrus orchards near Parlier, California. Bean thrips, *Caliothrips fasciatus* (Pergande) (Thysanoptera: Thripidae), adults for the experiments were collected from an organic alfalfa at KARE. *Brevipalpus californicus* (Banks) (Arachnida: Acari: Tenuipalpidae) and *Brevipalpus lewisi* McGregor (Arachnida: Acari: Tenuipalpidae) were obtained from laboratory colonies maintained on Valencia oranges at 26 ± 1°C and 60 ± 5% RH. *Lorryia formosa* for the experiment was obtained from laboratory colony maintained on rough lemon plants. Experimental setups for fumigation were prepared as shown in Fig. 1.

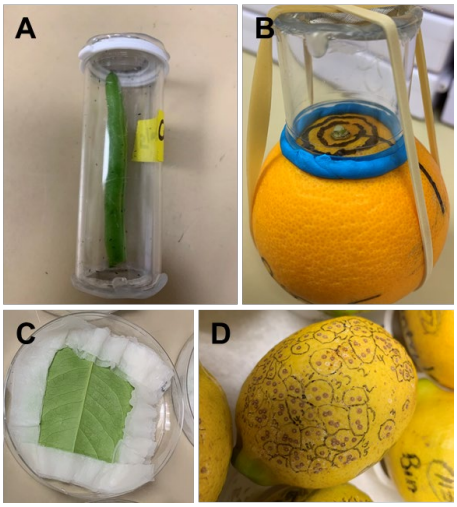

Fig. 1. Experimental setup for fumigation to control bean thrips (A), *B. californicus* (B), *L. formosa* (C), and California red scale (D).

Fumigation

Laboratory-scale exploratory fumigations were conducted in 28.3-L Labconco® vacuum desiccators (Labconco® # 5530000) (referred to as chambers hereafter) housed in a 17 m^3 environmental room with programmable temperature and humidity control at the SJVASC, USDA-ARS, Parlier, CA.

Pure liquid propylene oxide (≥ 99.5%; #82320 Aldrich, Sigma-Aldrich Co. St. Louis, MO) was drawn from a 50-mL conical flask with a glass stopper under a certified fume hood using a 0.1 mL gas syringe (Hamilton, Foxboro/Analabs, North Haven, CT) or 1-, 2-, or 5- mL gas syringes (Precision syringe, Dynatech Precision Sampling, Baton Rouge, LA) befitting the applied dosages and injected through a modified septum onto a petri dish lined with filter paper. Carbon dioxide was drawn from a cylinder (13.4 x 45.7 cm) containing compressed CO_2 using 500-, 1,000-, or 1,500-mL gas syringes or weighted out using a scale and injected through stopcock before injecting PPO.

The range of concentrations of propylene oxide tested for California red scale, fuller rose beetle, bean thrips, *B. californicus*, *B. lewisi*, and *L. formosa* were 4-32, 16-80, 4-32, 4-96, 2-96, and 4-96 mg/L, respectively. For each species, at least seven different concentrations within the range were tested. Each treatment was replicated at least 3-5 times. Prior to injecting liquid PPO into the chambers, a pressure of 150 mmHg was created to ensure space for CO_2 and PPO volatilization while preventing the development of positive pressure in the chamber. To simulate 8:92 mixture of PPO:CO2, volume of CO_2 equivalent to that which would have been administered into each chamber in a scenario where the gas introduced was premixed in the same ratio. After the PPO volatilized (within 2-5 min), the pressure inside the chamber was normalized by permitting air in from the stopcock. This marked the start of a 2 h (or 24 h in case of Fuller rose beetle) fumigation exposure period.

Concentrations of PPO inside each chamber were determined at the start (after the pressure was normalized) and at end of the experiment. Gas samples were taken using a 100 mL gas syringe (Becton, Dickinson and Co., Franklin Lakes, NJ) to withdraw 40 mL of gas through the stopcock. Concentrations of PPO in fumigation chambers were quantitatively monitored and analyzed using a gas chromatograph (GC) (Model 3800, Varian Inc., Walnut Creek, CA). Doses of PPO expressed as concentration × time (CT) product (mg h/L) were calculated by the method of Bond (1984). The temperature set point for the exploratory fumigations was 15.6°C (60°F).

For all experiments, PPO concentrations in the chamber headspace were measured using a gas chromatograph (GC) (Model 3800, Varian Inc., Walnut Creek, CA) equipped with a 1 cc gas sampling loop and a flame ionization detector (FID).

Mortality evaluation and effects of PPO on navel oranges and mandarins

After fumigation, adult mites, thrips, California red scale, and Fuller rose beetle eggs were incubated and mortality evaluations were conducted 3, 3, 14, and 28 d, respectively. All mortality assessments were conducted using a stereoscope and survivors were diagnosed based on their ability to move one body length when prodded (adults), except CRS gravid females which were determined visually (discoloration). Eggs were evaluated as hatched or unhatched.

For determining the effects of PPO on fruit quality, commercially packed, export grade navel oranges and mandarins sourced from local packinghouses were fumigated. Two different concentrations of PPO, 40 and 112 mg/L during a 2 h fumigation at 15.6°C were tested. Fruit was repacked into modified cardboard boxes (to fit inside the fumigation chamber) for fumigation. Fumigation was conducted in 9 ft^3 stainless steel chambers and the fumigation procedure was the same as that described above. Following fumigation, fruit was stored for 3 wk at 2.8°C (37°F) to simulate sea shipment plus 1 wk at 20°C (68°F) to simulate handling and shelf life. Evaluations were conducted 4 wk after storage. Fruit was checked weekly during 4 wk storage (3 wk at 2.8°C and 1 wk at 20°C) and those showing symptoms of decay were removed. Final evaluations were conducted at the end of the 4 wk storage period for peel color, overall external appearance, decay, stem end rot, firmness, and juice content.

Data Analysis

Dose response data for each species tested were subjected to probit analysis using PoloPlus (LeOra Software, Petaluma, CA; LeOra Software, 2005) to estimate lethal concentrations to kill 50, 95, and 99% of the individuals and their 95% confidence intervals and to predict probit 9 values. For determining the phytotoxic effects of PPO on navel oranges and mandarins, data were analyzed separately for percentage decay, firmness, fruit color, and juice content using one-way ANOVA analysis (Statgraphics, 2018).

Results and discussion

Propylene Oxide Toxicity

Propylene oxide was toxic to all species tested at 15.6°C for 2 h; however, the tested species responded differently to propylene oxide (Table 1).

The number of specimens tested, slope of regression estimate, estimated CT exposures to cause 50, 95, 99, and 99.9968% mortalities and the upper (UL) and lower limits (LL) of the 95% confidence limits, chi-square, degrees of freedom, and regression heterogeneity (H) are presented in Table 1.

Among the species tested, bean thrips were the most susceptible species and required 27.3 mgh/L (13.6 mg/L) to kill 99% of the individuals. Fuller rose beetle was the most difficult to kill, requiring 1,694.5 mgh/L for 99% mortality. California red scale required 51.4 mgh/L.

Table 1. Probit analyses parameters of citrus export concern species following exposure to propylene oxide at 15.6°C. Lethal exposure values (LEs) are expressed as mgh/L

Species	Number tested	Slope ± SE	LE_{50} (95% CI)	LE_{99} (95% CI)	P_9 (95% CI)	X^2 (df) [H*]
California red scale	1,939	12.8 ± 0.81	33.8 (32.3-35.2)	51.4 (47.9-57.4)	69.5 (61.3-84.9)	53.1(17) [3.1]
Bean thrips	1,033	12.7 ± 0.95	18.3 (17.6-18.8)	27.8 (26.3-30.3)	37.7 (33.6-43.9)	60.5(38) [1.6]
Fuller rose beetle eggs*	6,542	4.02 ± 0.13	447.2 (374.0-513.3)	1,694.5 (1,322.5-2,529.8)	4,418.8 (2,869.29,116.2)	591.6(40) [14.8]
<u>*Mites*</u>						
B. californicus	2,632	2.8 ± 0.11	22.7 (19.1-26.3)	151.1 (113.6-225.9)	590.3 (360.1-1,206.0)	131.7(21) [6.3]
B. lewisi	2,513	9.3 ± 0.43	22.1 (20.9-23.1)	39.2 (36.3-43.6)	62.6 (51.1-92.7)	77.6(21) [3.7]
L. formosa	800	3.4 ± 0.27	15.7 (13.6-17.7)	77.1 (63.9-98.5)	242.4 (175.0-375.9)	21.1(30) [0.7]

*Heterogeneity factor

**Exposure period for Fuller rose beetle was 24 h.

For the mites, 99% mortality was achieved in the range of 31.7 to 151.1 mgh/L. The most tolerant species was *B. californicus*. Efficacy of PPO for controlling insect pests during short exposures such as 2 or 4 h, has been reported for many stored-product species (Isikber et al., 2006; Gautam, 2013). Prior to our study, the effects of PPO on arthropod pests infesting fresh fruit produce had not been explored. Among other postharvest fumigants being studied as a MeBr alternatives, phosphine and ethyl formate are effective (Bikoba et al., 2019; Walse et al., 2016). However, phosphine, the only other fumigant registered for use in citrus, requires longer exposures, ca. ≥ 48 h to control mites.

Tolerance of PPO by navel oranges and mandarins

Two concentrations of PPO, 40 mg/L (effective on California red scale) and 112 mg/L (effective on mites), were well tolerated by navel oranges and mandarins (Figs. 2 and 3). Exposure to 40 mg/L did not affect firmness, brix, acidity, and percentage decay in both varieties compared to control. At higher concentration, 112 mg/L, percentage decay in navels was significantly higher, but not on mandarins. At 112 mg/L, the button end mandarin was dry and darker in color compared to control. There were no significant differences between brix and acidity between treated and the control fruit suggesting no likely effect on fruit flavor as a result of exposure to PPO. Effects of PPO on fruit quality parameters have not been explored prior to this report. Effects of other fumigants such as ethyl formate, methyl bromide and phosphine on citrus fruit quality have been reported by other researchers (Pupin et al., 2013; Arpaia et al., 2021). Fruit tolerance likely varies with commodity, dose, and time after fumigation (Pupin et al., 2013). Our results on fruit sourced from three different packinghouses suggests that recommended 40 mg/L doses of PPO have minimal effects on citrus.

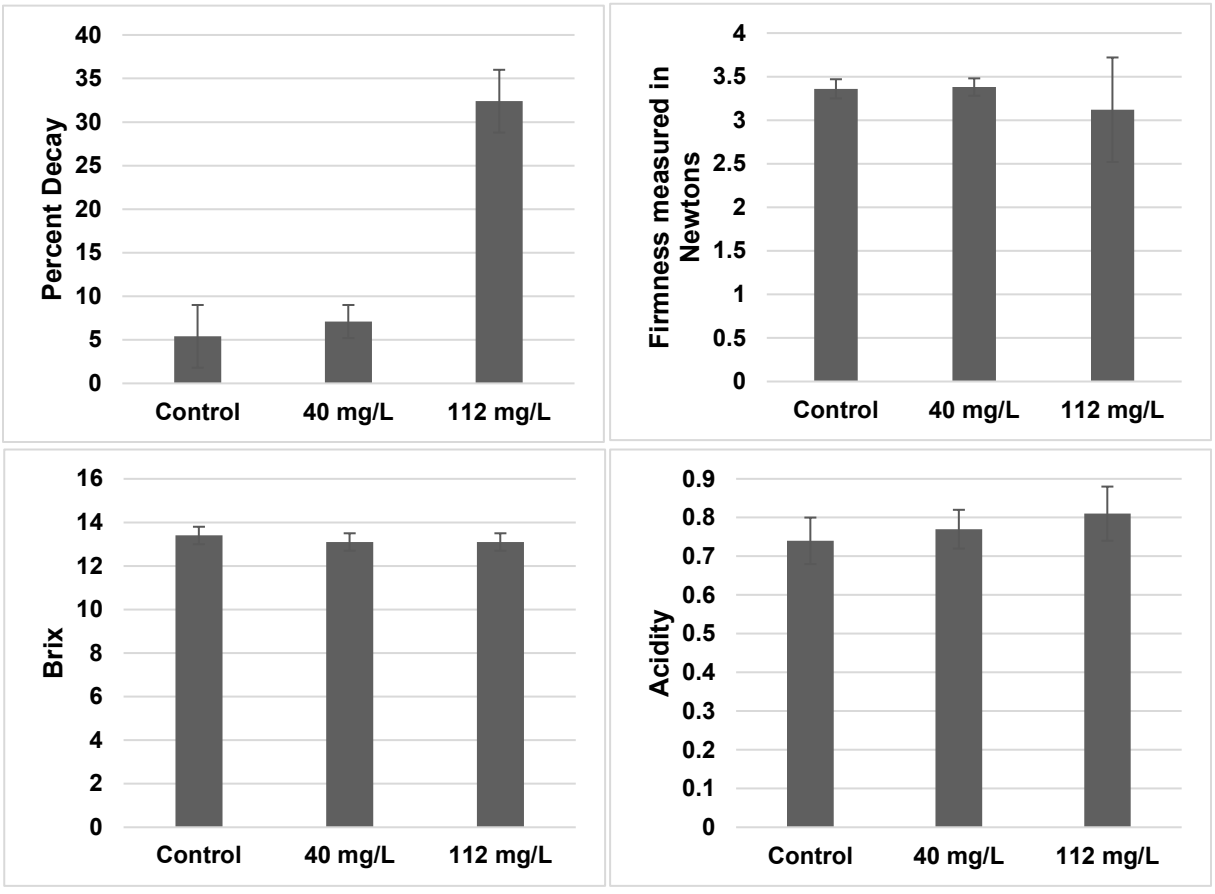

Fig. 2. General fruit quality parameters of navel oranges: percent decay (A), firmness (B), Brix (C), and acidity (D) of navels oranges exposed to propylene oxide fumigation.

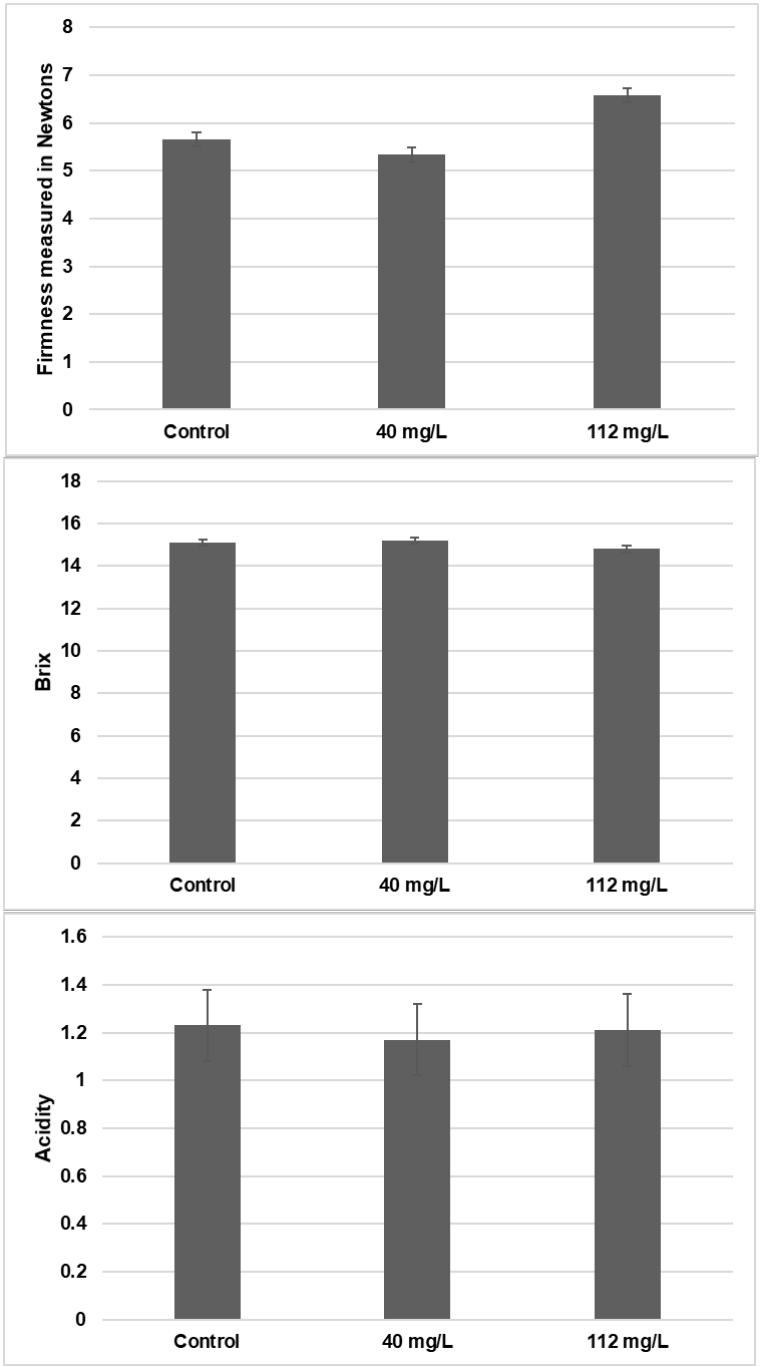

Fig. 3. General fruit quality parameters: firmness (A), Brix (B) and acidity (C) of mandarins exposed to propylene oxide fumigation. Fruit decay was not observed in mandarins.

Conclusions

Results showed evidence that propylene oxide was toxic to all pests of postharvest concern in citrus. Recommended concentrations to achieve probit-9 mortalities, 40 mg/L of two important pests, bean thrips and California red scale, did not have any negative effects on fruit quality. However, residues of PPO on different citrus varieties might also need to be considered to develop a treatment schedule for export of citrus fruit. Future studies will focus on developing time-temperature relationship, sorption, residues, and validating results during commercial scale trials.

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Recent advances in methyl bromide alternatives for fresh fruit at USDA-ARS

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Abstract

This research generally serves technical interaction between industry, ARS Office of Pest Management Policy (OPMP), the US State Department, USEPA, and the UN Environmental Program, Methyl Bromide Technical Options Committee to support postharvest compliance with the Montreal Protocol. Research on off-gassing potential and labeling of postharvest fumigants: methyl bromide (MB), phosphine, propylene oxide, and sulfur dioxide, which critically supported mandatory reviews by California and USEPA, was detailed specific to fresh fruit. Moreover, recent research findings for fresh fruit treatments were presented and discussed, including: the application of ethyl formate to fruit in field bins, the use of a sulfuryl fluoride-propylene oxide blend to disinfect and disinfest tree nuts and dried fruit, and as detailed in this article for drosophila control in fresh citrus, a Quarantine Pre-Shipment (QPS) application of phosphine.

Keywords: Postharvest, Methyl bromide alternatives, Phosphine, Ethyl formate, Sulfuryl fluoride

Introduction

Other than methyl bromide, only a single postharvest fumigant, phosphine, is currently approved to treat citrus in the USA. Owing to the pioneering work of Dr. Fransiskus Horn (Horn and Horn, 2004) in the late 1990s, cylinderized phosphine is now used across the globe to treat numerous types of horticultural crops, typically at the optimal cold-storage temperature, and maximum residue limit (MRL) of 10 µg/kg (ppb) is established in key markets, including those relevant to citrus exports from California, USA. A 12 h postharvest fumigation with cylinderized phosphine is now used in a quarantine and pre-shipment (QPS) capacity to control bean thrips *Caliothrips fasciatus* (Pergande) in "Navel" orange, *Citrus sinensis* (L.), exports from California, USA to key international markets (Walse and Jimenez, 2021). Spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is now a pest of concern to certain countries and below results are detailed to support an efficacious phosphine schedule with, and without, considering the additionally toxicity of *in transit* cold storage.

Materials and methods

Experimental details were as reported in Walse and Corbett (2019). Briefly, the methodologies used to develop phytosanitary treatments for control SWD, including general rearing and fruit infestation methods, are as originally described in Walse et al. (2012), and more recently outlined in Walse et al. (2021). Fruit was first transferred into a cage with ~2,000 adult SWD for a predetermined amount of time. Infestations were synchronized so that a treatment could be conducted on fruit infested with distinct age groupings, spanning from the egg through pupal life stages. Fruit respective to the age groupings were treated simultaneously, and the relative survivorship was used to identify the most treatment-tolerant age grouping. The variance associated with SWD development on fruit hosts (Bellamy et al. 2013) resulted in age groupings that are comprised of different proportions of life stages, whereby the most treatment-tolerant life stage was most prevalent tin the most treatment-tolerant age group. The most treatment tolerant life stage was used in all exploratory laboratory-scale fumigations to identify efficacious treatment parameters, facilitated by mortality-response models (e.g., probit). The evaluation of SWD mortality following a treatment, which required counting the numbers of adults that emerged from treated fruit, was as detailed in Walse et al. (2016). Results of exploratory treatments were verified in confirmatory treatments at the pilot- or commercial-scale. The overall number of SWD treated, or alternatively the number of SWD survivors, across a series of trials was calculated by summing the results and propagating the error (Walse et al. 2016). The evaluation of overall treatment efficacy following fumigation, cold, or sequential combinations thereof, were as described in Walse and Bellamy (2012). Procedural aspects of phosphine fumigation, as well as the sourcing of citrus fruit, were as described in Walse and Jimenez (2021) and were consistent with previous work to control stored product insects.

Results and discussion

Exploratory fumigations

The average air temperature, 4.8ºC, was calculated across all trials. Deviation in temperature was assumed to follow a normal distribution with the estimated margin of error reported as ± 2*s*, 0.2ºC, the 95% confidence interval (Quinn, 1983). For each of the four isolated age groupings, duration-mortality regressions for (applied doses) PH_3 steady-state concentration ($[PH_3]_{ss}$) of 0.4 (250), 0.8 (500), and 1.5 g/m^3 (1000 ppmv($\mu L/L^1$)) were modeled using Polo Plus (LeOra Software, 2002-2007). The number of specimens treated, the regression heterogeneity (H), the projected durations to cause 50, 95, and 99% mortality in the treated population (respectively LT_{50}, LT_{90}, LT_{99}, LT_{P9}), and the corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95% confidence level (CL) are shown in Table 1. Across the age groupings, for each of the three $[PH_3]_{ss}$, likelihood ratio-based hypothesis testing of equality was rejected (0.4 g/m^3: $P = 0.000$, $\chi^2 = 122$, $df = 6$; 0.8 g/m^3: $P = 0.000$, $\chi^2 = 103$, $df = 6$; 1.5 g/m^3: $P = 0.000$, $\chi^2 = 77$, $df = 6$), indicating that the slopes as well as the intercepts of the regressions respective to $[PH_3]_{ss}$ were significantly different. Likelihood ratio-based hypothesis testing of parallelism was also rejected (0.4 g/m^3: $P = 0.000$, $\chi^2 = 23$, $df = 3$; 0.8 g/m^3: $P = 0.000$, $\chi^2 = 19.2$, $df = 3$) indicating that the slopes of the regressions respective to the age grouping were significantly different for the two lowest levels of $[PH_3]_{ss}$. However, at 1.5 g/m^3 parallelism was not rejected ($P = 0.5$, $\chi^2 = 2.2$, $df = 3$), indicating that the slopes of the regressions respective to the age groupings were not significantly different.

Table 1 shows the probit regression parameters (^) for mortality of spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), following fumigation with 1.6% (v/v) phosphine balanced with nitrogen at air temperature 4.8 ± 0.2 ºC ($\bar{x} \pm 2s$) and probit regression analyses of the duration mortality response respective to applied doses and steady state headspace concentrations, $[PH_3]_{ss}$ of 0.4 (250), 0.8 (500), and 1.5 g/m^3 (1000 ppmv (µL/L)).

Table 1. Probit regression parameters for mortality of spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura)

Probit Regression Parameters

	12- to 60-h old (eggs)			36- to 84-h old (small larvae)			108- to 156-h old (large larvae)			300- to 348-h old (pupae)		
		95% CL			95% CL			95% CL			95% CL	
250 ppmv $[PH3]_{ss}$	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL
LT_{50}	4.9	4.2	5.6	2.7	1.8	3.5	3.9	3.0	4.7	4.3	3.4	5.2
LT_{95}	13.3	11.9	15.3	10.7	9.5	12.4	10.6	9.4	12.5	12.9	11.3	15.4
LT_{99}	19.9	17.1	24.0	19.0	15.8	25.0	15.9	13.2	22.2	20.2	16.6	27.2
Slope	3.9 (+/-) 0.2			2.8 (+/-) 0.2			3.9 (+/-) 0.2			3.5 (+/-) 0.2		
Heterogeneity	4.35			3.4			4.9			5.6		
Treated	6420			6616			6708			6312		
500 ppmv $[PH3]_{ss}$		95% CL			95% CL			95% CL			95% CL	
	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL
LT_{50}	5.7	5.3	6.1	4.6	4.0	5.0	5.5	4.9	5.5	4.6	4.3	4.9
LT_{95}	13.1	12.2	14.4	10.4	9.6	11.4	10.2	9.6	11.0	11.7	11.0	12.4
LT_{99}	18.6	16.7	21.2	14.5	12.9	17.0	13.5	12.3	15.1	17.1	15.8	18.9
Slope	4.5 (+/-) 0.2			4.6 (+/-) 0.3			5.7 (+/-) 0.3			4.1 (+/-) 0.2		
Heterogeneity	2.28			2.0			1.5			0.7		
Treated	6420			6616			6708			6312		
1000 ppmv $[PH3]_{ss}$		95% CL			95% CL			95% CL			95% CL	
	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL	"duration"$_x$	LL	UL
LT_{50}	5.7	5.2	6.3	4.8	4.0	5.4	4.8	3.8	5.5	5.1	4.8	5.5
LT_{95}	14.1	12.9	15.9	11.8	10.6	13.8	11.1	9.7	13.7	13.2	12.4	14.2
LT_{99}	20.5	17.9	24.6	17.2	14.6	22.0	15.8	13.0	21.9	19.5	17.7	21.9
Slope	4.2 (+/-) 0.6			4.2 (+/-) 0.2			4.5 (+/-) 0.3			3.5 (+/-) 0.2		
Heterogeneity	3.78			4.9			1.4			5.6		
Treated	6420			6616			6708			6312		

^Number of specimens treated, the regression heterogeneity (H), the projected durations to cause 50, 95, and 99% mortality in the age groupings (respectively LT_{50}, LT_{90}, and LT_{99}), and the corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95 % confidence level (CL).

To identify the most phosphine-tolerant age grouping at each of the three $[PH_3]_{ss}$, lethal time ratios (LTRs), where the response to 12 to 60 h old specimens was normalized to that of older groupings, were calculated with (±) 95% confidence intervals across the durations projected to cause 10 to 99% mortality in the treated population. The LTRs were used to identify, irrespective of $[PH_3]_{ss}$, that the older age groupings were less phosphine-tolerant than 12 to 60 h old specimens, as LTRs respective to durations > LT_{85} all overlapped or superseded a value of 1 (unity).

Lethal time ratios (LTRs), where the response to $[PH_3]_{ss}$ of 0.4 g/m^3 (250 ppmv) was normalized to that of 0.8 (500) or 1.5 g/m^3 (1000 ppmv), were calculated with (±) 95% confidence intervals across the durations projected to cause 10 to 99% mortality in the treated population of 12 to 60 h old specimens and provide potential insight into the "narcosis threshold", or "sweet spot", associated with this pest (Winks and Waterford, 1986). The tabulated regression data as well as Fig. 1 shows the projected durations to cause 99% mortality in the treated population (LT_{99}) did not vary as a function of $[PH_3]_{ss}$, indicating that variability in $[PH_3]$ between 0.4 and 1.5 g/m^3 (250 and 1000 ppmv) did not change the efficacy. The LTRs are consistent with the above finding, and identify that $[PH_3]_{ss}$ of 0.8 (500), or 1.5 g/m^3 (1000 ppmv) were no more efficacious toward the most phosphine-tolerant age of SWD, 12 to 60 h old specimens, than a $[PH_3]_{ss}$ of 0.4 g/m^3 (250 ppmv), as LTRs respective to durations > LT_{30} all overlapped or superseded a value of 1 (unity). However, both LT ratios decrease as mortality in the treated population increases, suggesting that if $[PH_3] < 0.4$ g/m^3 (250 ppmv and > 1.5 g/m^3 (1000 ppmv), longer treatment times may be required to achieve the same level of control as observed when $[PH_3] \geq 0.4$ g/m^3 (250 ppmv) and ≤ 1.5 g/m^3 (1000 ppmv), or at least $\cong 0.8$ g/m^3 (500 ppmv (μL/L)). In this case of treating fresh fruit with phosphine, or any other product in which minimizing the duration required for efficacy is desired, an "optimal" treatment maintains the $[PH_3]$ level within the upper and lower limits of the narcosis threshold.

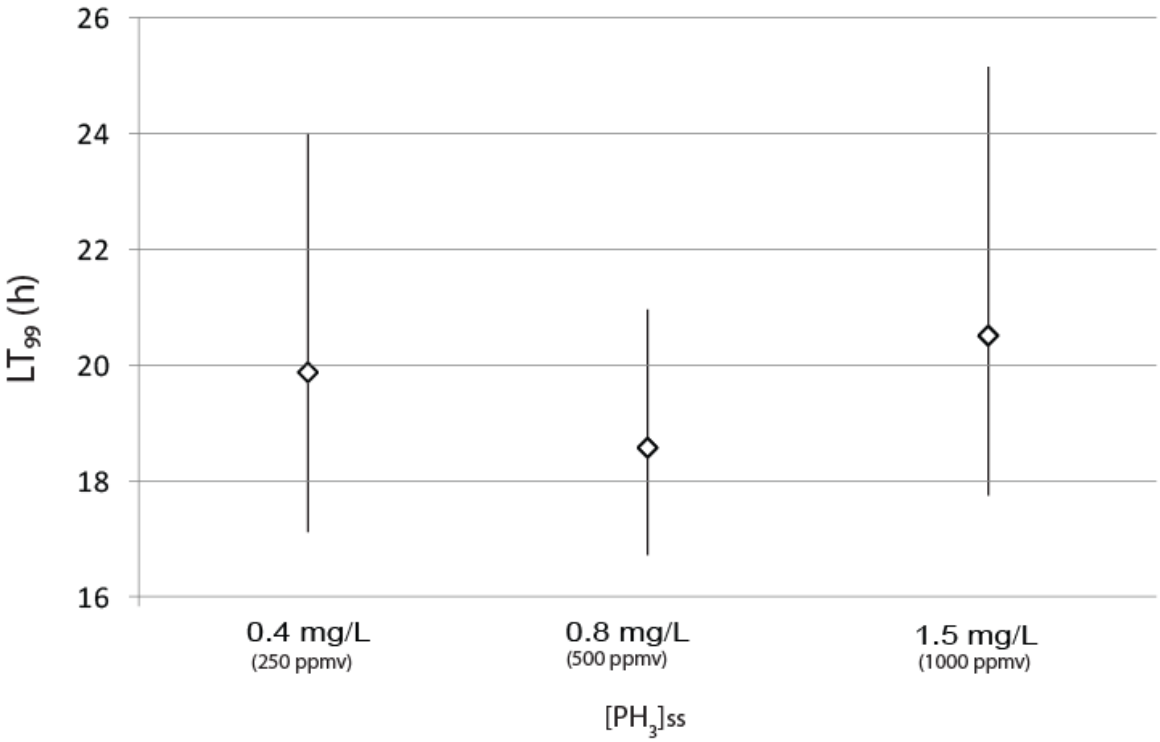

Fig. 1. Projected durations to cause 99% mortality in the treated population (LT_{99}) of population of 12 to 60 h old SWD (the most phosphine-tolerant age).

The projected durations did not significantly vary as a function of steady-state headspace concentrations, $[PH_3]_{ss}$, over the range 0.4 to 1.5 g/m^3 (250 to 1000 ppmv (μL/L)), suggesting that variability in phosphine levels within that range will not change the efficacy of fumigation.

Error bars are the estimates of the bounds (upper (UL) and lower (LL) limits) at the 95% confidence level (CL). These results indicate that the narcosis threshold for adult BT spans [PH_3] ≥ 0.4 and ≤ 1.5 g/m^3 (250 ≥ and ≤ 1000 ppmv (µL/L)). If headspace concentrations of phosphine, [PH_3], are recorded outside this "optimal" range, a longer treatment duration could potentially be required to achieve 99% mortality.

Collectively, these results suggest that the narcosis threshold for 12 to 60 h old specimens, the most phosphine-tolerant SWD age, was bounded by [PH_3] ≥ 0.4 and ≤ 1.5 g/m^3 (250 ≥ and ≤ 1000 ppmv). Future work will more clearly outline the upper and lower limits of [PH_3] for an "optimal" treatment, as well as define the how much longer (than 36 h) is required when [PH_3] is "sub-optimal", or at least ≤ 0.4 (250 ppmv) and ≥ 1.5 g/m^3 (1000 ppmv). Moreover, it should be noted that loads of fresh fruits that vary by amount (load factor) and type (variety, size, etc.) are known to only minimally influence [PH_3] levels, as equilibrium between headspace in the enclosure and the load is typically reached within 30 min of application. Accordingly, the efficacy of phosphine toward SWD at *T* of ca. 5°C is nearly equivalent across all fruit types, let alone citrus types.

Confirmatory fumigations: 36 h Phosphine fumigation

Exploratory results provided evidence that phosphine fumigation of fresh citrus at *T* ≥ 5.0ºC will control SWD infestations if [PH_3] is maintained ≥ 0.4 g/m^3 (250 ppmv) and ≤ 1.5 g/ m^3 (1000 ppmv (µL/L)) for a duration ≥ 36 h. To test this prediction, a series of confirmatory trials were conducted to verify efficacy toward 12- to 60-h old SWD, the most phosphine-tolerant age, infesting the navel of sweet oranges following application of ca. 1.5 (1000 ppmv) or 0.8 g/m^3 (500 ppmv (µL/L)) phosphine for 36 h at *T* ≤ 5.0ºC. The average temperature was calculated over the course of each trial as described above. Demonstrating mortality of quarantine insect pests as a function of probit analyses and associated confidence levels is often requested to qualify phytosanitary treatment efficacy, particularly when commodity is moved internationally (Couey and Chew, 1986; Follet and Nevin, 2006). Toward this end, confirmatory testing resulted in 0 survivors from 103910 ± 2627 (*n* ± *s*) (probit 9, 96.4% CL) and 103850 ± 2271 (*n* ± *s*) (probit 9, 96.4% CL) SWD treated, respectively, with an applied dose of ca. 1.5 (1000 ppmv) and 0.8 g/m^3 (500 ppmv).

It is critical to note efficacy was consistent across the three different phosphine formulations (1.6% (v/v) balanced in nitrogen, VAPORPH3OS®, and ECOFUME®), with each formulation resulting in 0 survivors from ca. 30,000 total treated specimens. From a technical perspective, this result supports the decision to use 1.6% (v/v) phosphine balanced with nitrogen in exploratory fumigations to outline efficacious fumigation parameters, at least with respect to this pest. Moreover, it provides evidence that shows increasing carbon dioxide levels in chamber headspace, over the range ca. 0.4 to ca. 63 g/m^3 (365 to 49,000 ppmv; 0.036 to 5%) did not impact treatment efficacy. Important from an operational perspective, this later result indicates that both commercially available formulations of cylinderized phosphine, VAPORPH3OS® or ECOFUME® are equivalently efficacious toward SWD and either could be used for the proposed schedule.

Confirmatory fumigations: 12 h PH_3 fumigation followed by 10 d refrigeration at 5°C

Exploratory results provided evidence that phosphine fumigation of fresh citrus at *T* ≥ 5.0ºC will control ca. 95% of 12- to 60-h old SWD, the most phosphine-tolerant age, if [PH_3] is maintained ≥ 0.4 g/m^3 (250 ppmv) and ≤ 1.5 g/m^3 (1000 ppmv) for a duration of 12 h. To test this prediction, a series of confirmatory trials were conducted to verify efficacy following application of ca. 1.5

(1000 ppmv) or 0.5 g/m^3 (300 ppmv) phosphine for 12 h at $T \leq 5.0$ ºC. Confirmatory testing resulted in 18 survivors from 50560 ± 1313 ($n \pm s$) (probit 8.20, 95% confidence level) and 15 survivors from 51210 ± 1167 ($n \pm s$) (probit 8.24, 95% CL) treated SWD, respectively, with an applied dose of ca. 1.5 (1000 ppmv) and 0.5 g/m^3 (300 ppmv).

Previous work on SWD suggested that (the few) survivors of a 12 h fumigation were not likely to also survive a subsequent refrigeration. To test this hypothesis, half of the infested fruit was transferred to refrigeration at ~5ºC for 10-d following the 12-h fumigation. Testing resulted in 0 survivors from 50560 ± 1313 ($n \pm s$) treated (probit 8.68, 95% CL; probit 9, 80.2% CL) when refrigeration at $T = (4.9 \pm 1.2)$ ($\overline{x}_{GM} \pm 2\ s_{pooled}$) followed an applied dose of ca. 1.5 (1000 ppmv). An applied dose of 0.5 g/m^3 (300 ppmv) followed by $T = 4.9 \pm 1.2$ ($\overline{x}_{GM} \pm 2\ s_{pooled}$), resulted in 0 survivors from 51210 ± 1167 ($n \pm s$) (probit 8.69, 95% CL; probit 9, 80.5% CL) treated SWD. It is critical to note that citrus exported to key export markets is refrigerated during the 14 to 21 d required to reach port, with pulp temperature (T) rarely outside the range of 0.5 to 5ºC.

Discussion

Results indicate that SWD can be controlled in citrus. For the 36 h fumigations, across all formulations, an applied dose of ca. 1.5 (1000 ppmv) resulted in 0 survivors from 103,910 ± 2,627 ($n \pm s$) (probit 9, 96.4% confidence level (CL)) treated SWD, while an applied dose of 0.8 g/m^3 (500 ppmv) resulted in 0 survivors from 103,850 ± 2,271 ($n \pm s$) (probit 9, 96.4% CL) treated. The 12 h fumigations at $T \leq 5$ °C, across all formulations, resulted in ca. 95% control as expected. Importantly, however, when the 12 h fumigation was followed by 10 d of refrigeration at $T \cong 5$°C, an applied dose of ca. 1.5 (1000 ppmv) resulted in 0 survivors from 50,560 ± 1,313 ($n \pm s$) treated (probit 8.68, 95% CL; probit 9, 80.2% CL), while an applied dose of 0.5 g/m^3 (300 ppmv) resulted in 0 survivors from 51,210 ± 1,167 ($n \pm s$) treated (probit 8.69, 95% CL; probit 9, 80.5% CL).

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Potential of monitoring carbon dioxide to detect insect infestation in a wheat bulk

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Abstract

Monitoring carbon dioxide (CO_2) concentrations in a bulk of grain has been considered for detecting insect infestation. To evaluate the potential of monitoring CO_2 concentrations to detect insect presence at different bulk depths, in relation to changing temperature under aeration, a trial was carried out in Israel in a concrete bin of 180 tonnes containing wheat of 11.6% moisture content. Samples of wheat were taken from depths of 1, 3, 5, 7 and 9 m, thermocouples and gas sampling tubes were inserted to the same depths. Examination of wheat samples indicated presence of initial infestation in mid-June from average of 0.15 live insects per kg wheat to 2.05 live insects per kg wheat in March. In April live insects per kg wheat dropped to 0.8, probably due to the low temperatures achieved in the grain bulk. The observations lasted ten months during which aeration was performed intermittently to reduce the temperature of the grain bulk from initial 28.3°-34.8°C in June to 16.5°-20.0°C in March. Carbon dioxide concentrations were measured at all sampled depths and they varied from close to undetectable levels at initial samples to up to 9% when wheat temperature dropped to 20°C in March. Carbon dioxide concentrations progressively increased with the increase in insect populations. Although the bin was not sealed and no sealing operations were performed, CO_2 concentrations were detectable. A peculiar observation indicated that increase in CO_2 concentrations occurred in gas samples after aeration was cessed. The authors have no reasonable explanation for this observation. Insect populations consisted of *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (Linnaeus), *Rhyzopertha dominica* (Fabricius) and *Oryzaephilus surinamensis* (Linnaeus). The initial low insect populations correlated well with low CO_2 concentrations. Whereas, the increased CO_2 concentration was affected by additional factors like level of gas tightness of the bin, temperature gradients and forced aeration.

Keywords: CO_2 monitoring, Insect infestation, Detection, Wheat, Grain bulk, Aeration

Introduction

To determine the presence of insects in bulk stored grain, manual samples, traps, and probes have been used. Manual inspection has been based on sieving, cracking-floatation and Berlese funnels to detect insects in grain bulks. Since these methods are time consuming, alternative detection methods like acoustic detection (Mankin and Hagstrum, 2012), CO_2 measurement, uric acid

measurement, near-infrared spectroscopy, and soft X-ray methods have been proposed to the industry. The advantages and limitations of these insect detection methods have been evaluated by Neethirajan et al. (2007).

Several scientists have used CO_2 as an indicator of insect infestation (Howe and Oxley, 1952; Calderon and Shayya, 1961) or microbial infection (Steele et al., 1969; White et al., 1982) or both (Muir et al., 1980). Sinha and Wallace (1977) measured CO_2 concentrations in and around a small column (0.6% of total bulk) of spoiling rapeseed at 11 to 13.5% moisture content in a 46 tonnes bulk of dry rapeseed at 8.5 to 9.4% moisture content. Concentrations of CO_2 in the spoilage pocket were up to 2%. Measurements of CO_2 in a 27-tonnes cone-shaped pile of wheat placed directly on the ground without a cover indicated that CO_2 did not diffuse rapidly out of the grain bulk (Muir et al., 1980). Singh et al. (1983) mathematically modelled dispersion of CO_2 from three possible spoilage areas in a grain bin (6 m in diameter filled with wheat to 4.6 m height) and concluded that a CO_2 sensor with a resolution of 2 g/m^3 (0.1%) located near the center of the bin could detect grain spoilage when spoilage location is not known.

Ileleji et al. (2006) employed commercial CO_2 sensors near the vents and exhaust air stream of fans in the grain bin for measuring CO_2. They concluded that hot spots and early spoilage of grain can be detected inside grain bin using CO_2 sensors.

Maier et al. (2006) studied elevated CO_2 levels in localized pockets of a grain mass caused by insects, fungi, and grain metabolism. The primary objective of the study conducted by Maier et al. (2006) was to monitor CO_2 levels for early detection of spoilage. The measured CO_2 levels ranged from 0.05% to 0.5% and higher levels of up to 2.5 % were recorded with a portable CO_2 monitor. Maier et al. (2010) in their work on monitoring carbon dioxide concentration for early detection of spoilage in stored grain reported the presence of heavy stored-product insect population of up to 27 live insects/kg of grain.

Bartosik et al. (2008) carried out periodic CO_2 monitoring of silo-bags holding about 200 tonnes of wheat for the early detection of biological activity and spoiled grain. A distinctive value of CO_2 for different MC grains was established, which represents the typical atmospheric composition for a silo-bag with and without spoilage.

Gonzales et al. (2009) examined use of relative humidity (RH), temperature, and carbon dioxide (CO_2) sensors for their suitability to determine adverse storage conditions of wheat. Wheat at approximately 11% MC was aerated with the air that passed through high-moisture grain conditioned to nominal MCs of 14%, 16%, and 18% (wet basis). Sensors monitored air conditions during the entire storage period. Aeration was provided over 3-h periods at rates of 5 m^3h^{-1} $tonne^{-1}$ and 10 m^3h^{-1} $tonne^{-1}$. Carbon dioxide sensors were effective in indirectly detecting moist grain conditions due to the large amount of CO_2 generated from the wet grain. Carbon dioxide levels monitored at the exhaust of the aeration duct were generally adequate in determining adverse storage conditions. A detailed overview of the types of CO_2 sensors, their sensing mechanisms and characteristics covering different aspects of the CO_2 sensor technology has been synthesized by Neethirajan et al. (2009). Also, Neethirajan et al. (2010) developed a carbon dioxide sensor using polyaniline boronic acid conducting polymer as the electrically conductive region. The sensor was demonstrated for use in detecting spoilage in stored grain.

Jian et al. (2014) studied the interstitial concentrations of carbon dioxide and oxygen in stored canola, soybean, and wheat seeds under various conditions. Sum of CO_2 and O_2 concentrations were close to 21%-22% at most airtight storage times and within any crop.

Muir et al. (1985) tried CO_2 as an early indicator of stored cereal and oilseed spoilage. Concentrations of CO_2 were measured in 39 farm-stored bulks of wheat, rapeseed, barley and corn in Canada and U.S.A. Spoilage was confirmed by analyses of grain samples in 97% of the 34 bins having CO_2 concentrations greater than 0.03% of ambient air.

Taher et al. (2019) attempted to predict soybean losses using CO_2 monitoring during storage in silo bags. Based on their results, a correlation to predict grain losses was developed, which considered grain moisture and a predictor related to the CO_2 concentration at the silo bag closing end as independent variables.

Zhang et al. (2014) studied a site-directed CO_2 detection method for monitoring the spoilage of stored grains by insects and fungi in Chinese horizontal warehouses. The CO_2 produced by grain respiration was relatively low, and the activity of insect and mould significantly affected the CO_2 concentration in the bulk.

So far, the reports were concentrated on detection methods mostly on spoilage of grain and very few were only for detecting insect activity using CO_2. Grain respiration, fungal activity, and insect activity on CO_2 detection were extensively investigated. None of the studies focused to correlate insect infestation in relation to CO_2 detection inside the grain bulks. In addition, the changes in temperature that affect the convection currents that take place in large bulks and the influence of mechanical aeration on CO_2 retention has not been reported. Therefore, the present study was carried out to test the CO_2 concentrations that can be developed as a result of natural insect infestation in a vertical aerated silo containing dry wheat.

Materials and methods

Storage bin

The observations were carried out during 10 mo of storage in a concrete bin containing 180 tonnes of wheat. The bin was square 4x4 m and 14.5 m high with a hopper of 2 m height from the discharge hatch. The base of the hopper was equipped with an aeration tube of 20.5 cm diameter. The bin had a loading opening of 60x60 cm through which thermocouple sampling tubes were inserted and grain samples were taken. The bin was equipped with an aeration system capable to deliver 2.8 m^3/(h/tonne). The aeration was operated to deliver ambient air from the bottom of the bin and the fan was activated by a thermostat that selected the suitable ambient temperature for aeration. Number of hours of fan operation was registered by a time recorder located adjacent to the fan.

Wheat grain

The wheat stored in the bin was a local cultivar "Nanasit" having moisture content (wet basis) in the range of 10.5 – 12.6%. Wheat samples were taken using a Prob-A-Vac (Minneapolis, MN U.S.A.) grain sampler, a probe that used vacuum to sample stored grain. Grain samples of 1 kg were taken periodically from the depths of the grain bulk at 1, 3, 5, 7, and 9 m deep from the

surface. Moisture content was electronically measured using Motomco Model 919 grain moisture meter (Paterson, New Jersey, U.S.A.) calibrated for the local grain cultivar. For identification of insect infestation, the grain samples were sieved through a standard USA mesh #10. The separated live and dead insects were identified and counted.

Measurement of temperature and gas

Thermocouples and gas sampling tubes were installed using the external tube of the Prob-A-Vac at the same depths as grain samples were taken (at 1, 3, 5, 7, and 9 m deep from the bulk surface). Grain temperature was measured on a weekly to bi-weekly basis. Ambient air temperature and relative humidity was recorded using a thermo-hygrograph that recorded data from inside a meteorological station located adjacent to the bin aeration fan.

Gas samples were taken from the indicated depths using gas sampling PVC tubes of 3 mm internal diameter. Gas samples were analysed using thermal conductivity meter (Gow Mac, Bethlehem, PA, U.S.A.). Gas samples were taken and analysed on the same days when temperature and grain samples were taken. The gas analyser was tested and calibrated against known CO_2 concentrations taken from cylindered CO_2 gas before each sampling date.

Results

Insect infestation

Insect population consisted of *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Oryzaephilus surinamensis* (L.) and *Tribolium castaneum* (Herbst). Their total counted numbers served to assess the relationship of their presence in the wheat samples and the associated measured CO_2 concentrations detected at the various depths of the wheat bulk. Although all samples and measurements were taken from 1, 3, 5, 7, and 9 m deep from the grain bulk surface, in Fig. 1 and 2, only the depths of 1, 5 and 9 m were presented. Figure 1 shows that insects were present throughout the observation period from June to mid April. Examination of wheat samples indicated presence of initial infestation in mid-June from average of 0.15 live insects per kg wheat to 2.05 live insects per kg wheat in March. This insect population was greatest at depths of 3 m (not shown in Fig. 1) and 5 m from the grain bulk surface. Examining the insect populations revealed that towards December there was a slight increase in the total population size, then a gradual and significant decrease towards March. The initial insect population was of 1 to 4 live insects/kg of wheat. This population grew up to 25 insects/kg of wheat in October-November. At the end of the observations in mid April the insect population decreased to a range of 1 up to 5 live adults/kg under the influence of aeration during the cold months of the year.

Changes in temperature of the grain bulk and the ambient

Wheat temperature was initially high at about 30ºC. By the operation of aeration system there was a gradual decrease in temperature at all tested depths in accordance with the cooling of ambient air, particularly after October and during the cold months prevailing in Israel (October-March). At depths of 3 (not shown in Fig. 1) and 5 m there was a slight increase of temperature by 1ºC and up to 5.5ºC, respectively. The aeration was performed intermittently to reduce the temperature of the grain bulk from initial 28.3º-34.8ºC in June to 16.5º-20.0ºC in March.

Changes in carbon dioxide concentrations

Carbon dioxide concentrations were measured at all sampled depths and they varied from close to undetectable levels at initial samples to up to 9% when wheat temperature dropped to 20ºC in March. Carbon dioxide concentrations were detected at all tested depths and throughout the observations period from June to mid April. There was a fluctuation in the gas concentrations that increased consistently after each operation of the aeration system. Those concentrations gradually increased with the increase in insect populations. However, the CO_2 concentrations were at their minimal level just before operating the aeration system and increased immediately after the cessation of operating the aeration system and then gradually dropped until the next fan operation. Such increase in CO_2 concentration was observed after each aeration period.

Wheat moisture content

The initial moisture content (wet basis) of the wheat samples at the start of observations in June was in the range of 10.5% to 12.6%, while at the end of the observations in April was of 10.4% to 10.9%. Examination of the samples taken from different depths of the grain bulk showed no increase in moisture content.

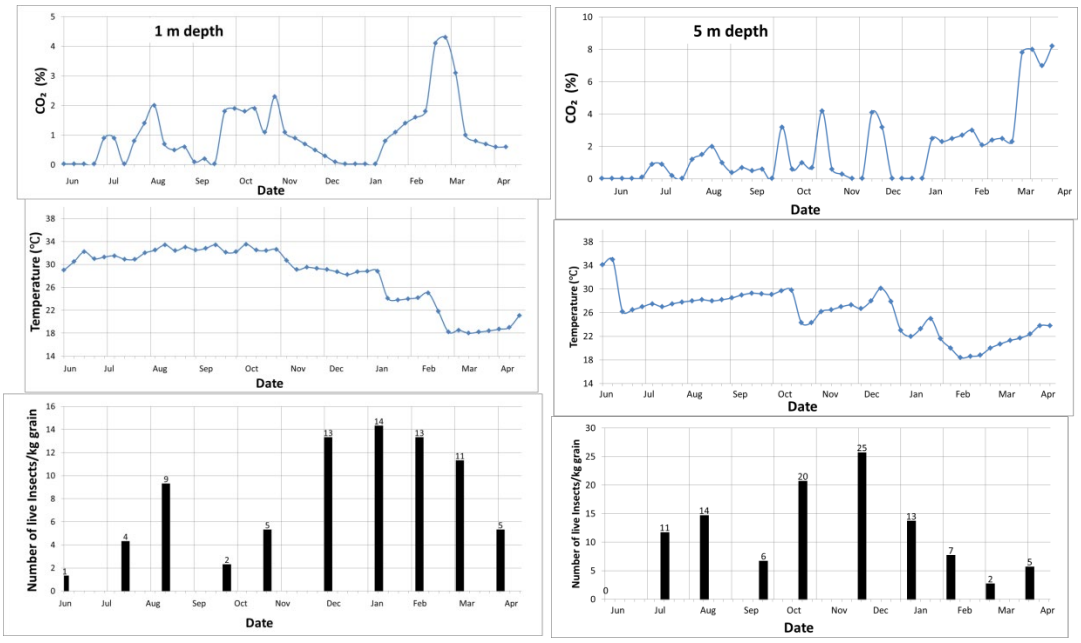

Fig. 1. Monitored CO_2 concentrations, temperatures and number of live insects/kg wheat found in grain samples taken from depths of 1 m (left) and 5 m (right) during 10 months observation of a wheat bulk of 180 tonnes stored in a vertical bin of 14.5 m height.

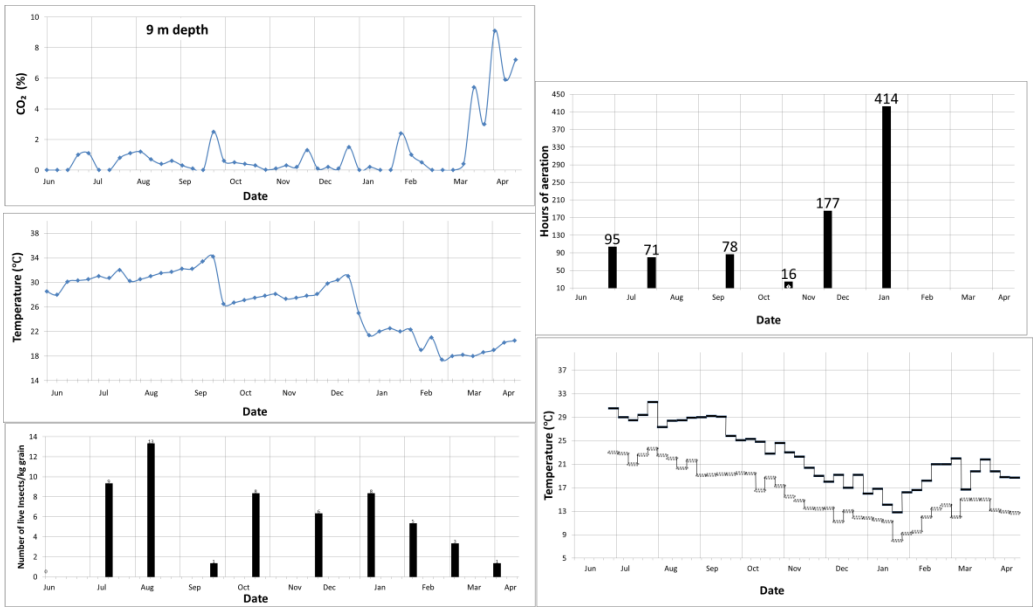

Fig. 2. Monitored CO_2 concentrations, temperatures and number of live insects/kg wheat found in grain samples taken from depth of 9 m (left), number of hours of aeration fan operation, ambient average maximum and minimum weekly temperatures during 10 months of observation of a wheat bulk of 180 tonnes stored in a vertical bin of 14.5 m height.

Discussion

Early works carried out by several researchers have already indicated the advantages of detecting insects by their CO_2 production (Calderon and Shayya, 1961; Howe and Oxley, 1952; Muir et al., 1980; Muir et al., 1985; Neethirajan et al., 2007) and more recently Zhang et al. (2014) has reported on a site-directed CO_2 detection method for monitoring the spoilage of stored grains by insects. Although all those early works led to promising results, the tested CO_2 production by insects, was not disturbed by air currents. Furthermore, some of the works were carried out under laboratory conditions and very little information was made available to correlate the effect of convection currents or the effect of aeration systems in detecting CO_2 concentrations in relation to insect population. Therefore, those works have not considered how quickly the CO_2 produced by insects can be easily detected and correlated when there is air movement inside the grain bulk. In addition, some of those works have considered particularly detecting only the spoilage of grain mainly under the influence of microbial activity developed at moisture contents above their critical level for safe storage (Bartosik et al., 2008; Maier et al., 2006; Maier et al., 2010; Jian et al., 2014) and not due to insect activities.

The present study indicates that the CO_2 produced can be attributed only to insect activity because the tested wheat bulk had a very low moisture content (10.4% to 10.9%). This moisture, in terms of equilibrium relative humidity in air is very close to 53%. That moisture eliminates the possibility of any CO_2 production due to microflora activity.

An interesting unexpected peculiar development was the increase in CO_2 concentration immediately after aeration. When we compare the CO_2 concentrations immediately after aeration, there was always an increase of CO_2 concentration, almost at all tested depths of the grain bulk. In the present study, to condense the report, only three depths were reported (1 m, 5 m and 9 m depth). However, in reality, we tested two more depths (3 m and 7 m depth) that exhibited the same pattern as in the reported depths. Authors have no logical explanation to this remarkable and peculiar development to the increase in CO_2 concentration.

Carbon dioxide concentrations progressively increased with the increase in insect populations. At 5 m depth it was 8% and at the depth of 9 m it was 9%. Although the bin was not sealed and no gas tightness operations were performed, CO_2 concentrations were detectable even after operating the aeration system that practically should have removed all CO_2. Although these results indicate the possibility of detecting insect infestation deep in the grain bulk, at the end of the observations, at 1 m depth CO_2 concentration was only 0.5%. These results indicate the difficulty to correlate the gas concentration to the size of insect population. We postulate because the CO_2 gas is constantly under the influence of convection currents created due to the changing temperature in the grain bulk, mainly in the tested particular case, due to the effect of ambient aeration. This study revealed the potential and the limitations of the application the CO_2 as an indicator to detect insect infestation.

Conclusions

This study revealed the possibility that even in aerated dry wheat bulks, insect presence could be detected by their metabolic activity that generates significantly detectable CO_2 levels as high as 9% in the interstitial air. There was a progressive increase in the size of insect population and the detected CO_2 concentrations. There was also an unexpected peculiar development of CO_2 concentration immediately after each intermittent aeration. It was clear that CO_2 readings were strongly affected, due to the influence of convection currents, that prevented the possibility to correlate with the existing insect populations.

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CAF2020 Abstract No. A-7-2-56

Du X, Ren Y (2021) Study on using synthetic amorphous silica (SAS) as phosphine resistance breaker. Page 216. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Study on using synthetic amorphous silica (SAS) as phosphine resistance breaker

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ABSTRACT

Phosphine resistance has occurred in most grain production countries that rely on the phosphine fumigation for management of stored grain insect pests. Inert dusts, such as diatomaceous earth (DE) dust is a kind of physical treatment, has long been used as a non-chemical method for pest control purpose. Particularly, food grade synthetic amorphous silica (SAS), one of the most effective powders, was evaluated for control of phosphine resistant stored grain insects. The mortalities of primary and secondary stored product insects of phosphine susceptible and resistant lesser grain borer, *Rhyzopertha dominica* (F.), and red flour beetle, *Tribolium castaneum* (Herbst) were compared under the SAS treatment. The complete control was achieved after 3 d treatment with 50 g SAS dust per tonne of grain. The dead adult insects were analysed. The results showed that sugars and amino acids were completely exhausted, which indicated the SAS could induce further energy exhaustion by deteriorating energy metabolic chain. Therefore, the SAS offered equally fatal to both phosphine susceptible and resistant stored grain insects. This study showed that SAS product could be an alternative to provide a solution to solve phosphine resistant issues and offer industry a non-chemical alternative for management of phosphine resistant insects in stored grain.

Keywords: Synthetic amorphous silica, Phosphine resistance, Metabolites, Stored grain insects, Lesser grain borer (*Rhyzopertha dominica*), Red flour beetle (*Tribolium castaneum*)

CAF2020 Abstract No. A-7-3-57

Sun W, Xia L, Cui M, Yu Q, Cao Y, Wu Y (2021) Age-stage, two-sex life tables and predation analyses of the predatory mite *Cheyletus malaccensis* (Oudemans) at different temperatures. Page 217. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Age-stage, two-sex life tables and predation analyses of the predatory mite *Cheyletus malaccensis* Oudemans at different temperatures

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ABSTRACT

Cheyletus malaccensis Oudemans is a predatory mite inhabiting grain depots. The relationship between temperature and the population growth rate of *C. malaccensis* will be useful for predicting its population dynamics. Age-stage, two-sex life tables of the predator, *C. malaccensis*, reared on *Acarus siro* were constructed under laboratory conditions at 22, 24, 28, 30 and 32°C, and 75% relative humidity in dark. Based on predation analysis, predation capacity of *C. malaccensis* on *A. siro* was evaluated for the treatment temperatures of 22, 30 and 32°C. Results showed that increasing temperature led to a shorter development time of immature stages. The life history of *C. malaccensis* varied from 11.10 d to 27.50 d. Life table parameters showed that 28°C was the optimum temperature for the growth and development of *C. malaccensis*. The highest net reproductive rate (R_0 = 290.25) and highest fecundity (544.52) occurred at 28°C. Temperature significantly affected the intrinsic rate of increase (r), fecundity, and finite rate of increase (λ). The peak value of the age-specific fecundity curve (m_x) was 28°C>24°C>30°C>32°C>22°C, while the peak value of the age-stage-specific fecundity curve (f_{x4}) showed the same trend. The age-specific predation rates increased inconsistently with age and the order was 32°C>30°C>22°C. The order for cumulative net predation rate was 22°C>30°C>32°C and for the transformation rate was 30°C>22°C>32°C. The predation rate at 32°C was the highest while the cumulative net predation was the lowest, and the predation rate at 22°C was the lowest while the cumulative net predation was the highest. The adults of *C. malaccensis* with the highest predatory ability had a short developmental duration and a low cumulative net predatory rate at high temperatures. Supplementary introduction of *C. malaccensis* might be a better biological control strategy.

Keywords: *Cheyletus malaccensis* Oudemans, Biological control, Age-stage two-sex life table, Predation rate

CAF2020 Abstract No. A-7-4-58

Cui M, Sun W, Xia L, Wang Z, Cao Y, Wu Y (2021) Killing stored grain pests by radio frequency and its effect on grain quality. Page 218. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Killing stored grain pests by radio frequency and its effect on grain quality

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ABSTRACT

In this study, four radio frequencies (RFs) were studied for insecticidal effect. Eexperiments were carried out on different insect species, and the insecticidal effect in paddy with different moisture content was studied. The results showed that when heated to 58°C by RF without heat preservation, the mortality of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) adults reached 100%. It took at least 8 min to reach the lethal temperature of *R. dominica* at 40.68 MHz. The main influencing factor of insecticidal rate in different grain varieties was the density of the grain. The higher density, faster was the insecticidal effect. Under the condition of 27.12 MHz, the time needed to increase mortality was not linear with the increase of moisture content of paddy. Furthermore, paddy after RF treatment had no significant effect on processing quality, storage quality and seed quality. These studies provided alternative physical methods for killing stored grain pests to replace chemical fumigation.

Keywords: Radio frequency, Stored grain pests, Insecticidal effect, Quality

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Kawagoe ZA, Walse SS (2021) Characterization and measurement of semiochemicals for management of stored product pests. Pp. 219-222. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Characterization and measurement of semiochemicals for management of stored product pests

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Abstract

This work describes research conducted as part of a cooperative agreement between ARS and Sensor Development Corporation (SDC). ARS and SDC have teamed to identify key natural products used by stored product insects for signaling, as well as chemical signals that are diagnostic of infestation. SDC specializes in the engineering and commercial application of gas sensor technologies, particularly the detection of volatile organic compounds in commercial as well as quarantine settings across the globe. SDC has developed a prototype detector capable of detecting several natural products produced by a suite of stored product pests. Recent research findings will be presented for proof-of-concept testing for detection of indianmeal moth and navel orangeworm in simplified laboratory-scale experiments.

Keywords: Semiochemical, Stored product pest, Indianmeal moth, Navel orangeworm

Introduction

Insect pests are a key contributor to the estimated postharvest food loss of 9-20% worldwide (Pimentel, 1991) and 20-40% in African countries (Kumar and Kalita, 2017). Current post-harvest technologies for detecting early stages of infestation are either cost-prohibitive or ineffective, at least for key pests such as indianmeal moth (IMM), *plodia interpunctella* (Hübner), and navel orangeworm (NOW), *Amyelois transitella* (Walker). Immunoassay-based approaches to monitoring stored products are effective but are destructive and require sample preparation, staff training, and analysis. A novel nanocrystalline tin-oxide sensor developed by Sensor Development Corporation (Elyria, OH) has shown promise in proof-of-concept testing at the laboratory-scale as a rapid, inexpensive, and easy-to-operate real-time (or near real-time) detector for a host of such early infestations. The aim of the work described herein is to demonstrate effective detection of IMM and NOW in simplified conditions and set a detection baseline for future testing and development endeavors.

Materials and methods

All life stages of IMM and NOW were sourced from the USDA-ARS-SJVASC insectary (Parlier, CA, USA). Flour was purchased at a local grocery store, and bins of almonds were provided by a local packing house. Headspace sampling and analysis were performed using a custom thin-film sensor unit produced by SDC (Sensor Development Corporation, Elyria, Ohio). Two 37.9 L galvanized steel pails with loose-fitting lids were modified with 3/8 in stainless steel tubing and stainless-steel Swagelok (Solon, OH) fittings. A Swagelok fitting was installed 12 cm from the top of each pail which connected to an 11 cm length of tubing connected to a ball valve. Both ball valves were connected to opposite arms of a tee union with a 3.5 cm length of stainless-steel tubing, thereby linking the pails. The prototype gas sensor was attached to a length of tubing that connected to the stem of the tee union. Each pail was loaded with 2.3 kg of store-bought all-purpose flour. One-pint Ball (Ball Corporation, Broomfield, CO) glass canning jars fitted with metal mesh lids were loaded with a single life stage of either IMM or NOW: egg, larva, pupa or adult.

Galvanized pail testing

The galvanized pails were used to assess the relative sensitivity of the SDC sensor to various numbers of each IMM life stage. One metal galvanized pail was loaded with a glass jar containing one life stage of IMM and the other contained only the store-bought flour. The headspace of the flour-only pail was sampled until the sensor had established a steady baseline and then the flow for the flour pail was toggled off and the pail with the IMM jar was toggled on. The pail with the IMM in it was sampled for 15 min and then the gas flow was toggled back to sampling the flour-only pail while the IMM jar was changed out.

The SDC sensor was also tested with all life stages of NOW in amounts of 50 pupae, larvae, or adults; 100 eggs, pupae, larvae, or adults; and 200 eggs. Test conditions were the same as those previously described for the IMM galvanized pail testing.

Blind testing

A set of single-blind-type tests were performed with the sensor in which a glass jar containing 5, 25, 100, 150, or 225 IMM pupae were placed in the test pail without the sensor operator knowing the contents of the pail. The sensor operator then used the data collected in the previously described galvanized pail testing to estimate the number of pupae present for each group. The accuracy of the sensor for each group of IMM pupae was calculated by the formula

$$Accuracy = \left(1 - \frac{|A - C|}{A}\right) * 100\%$$

Where; A is the actual count of pupae and C is the calculated number of pupae from the linear regression model. Pupae were used for the test because they elicited the strongest response per insect, and each jar was tested a single time in the blind testing.

Results and discussion

The SDC sensor was able to detect all life stages of IMM. However, the detection response varied for each life stage. IMM pupae gave the highest detection response (Fig. 1), and the response from IMM eggs, larvae and adults was lower. The data collected so far indicate that the sensor could be used to detect IMM before they develop to adults and have the opportunity to lay eggs. This would pre-empt trapping and visual detection of adults for cases in which eggs are brought into a storage situation or the first-generation adults are undetected. In this early sensor development stage, there were some possible concerns with baseline drift as seen in the flour only sections of Fig. 1.

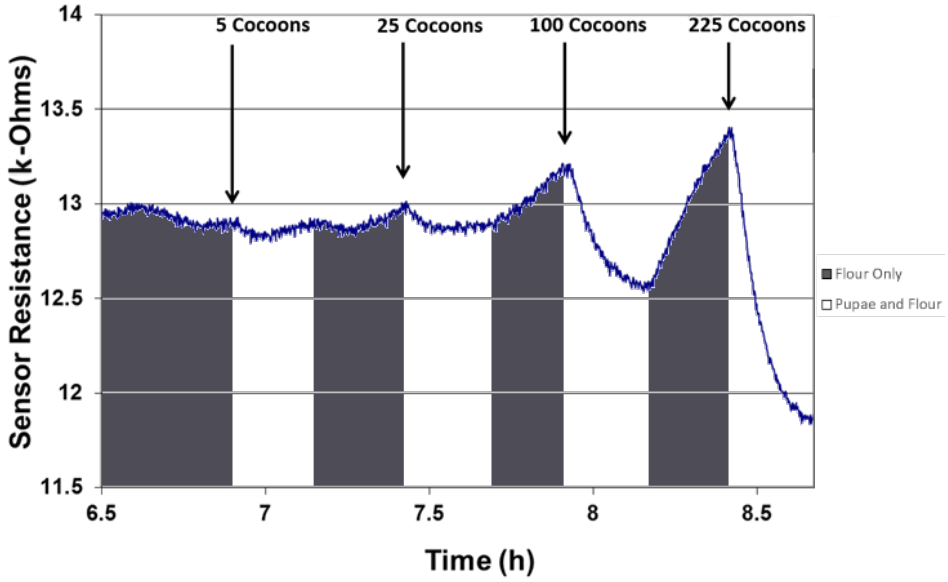

Fig. 1. Flour pail test results with cocooned IMM pupae. Sampling alternated between the flour only pail and the IMM/flour pail as indicated by the shading below the sensor readout.

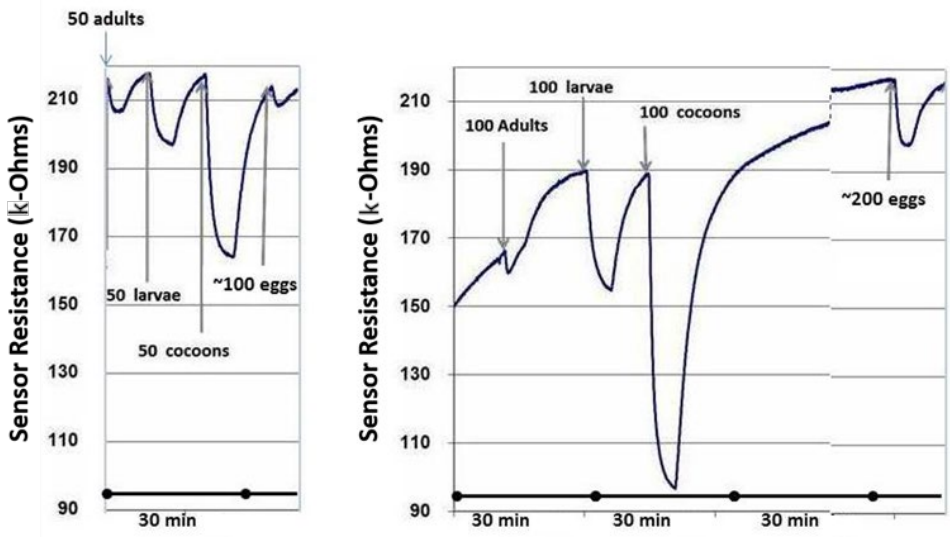

Fig. 2. Sensor readout data from navel orangeworm life stage detection. Although all life stages were detected, cocooned pupae elicited the strongest response.

The SDC sensor was able to detect all life stages of NOW, and the sensor had greater sensitivity for NOW than IMM (Fig. 2). Cocooned pupae elicit the strongest response, followed by larvae, adults, and eggs, in decreasing order of response strength.

Sensor response (net conductivity) data from the IMM pail testing was fitted with a linear least-squares regression model and used to estimate the number of specimens in a series of single-blind tests. Estimations based on the regression model were 76-92% accurate over a range of values from 5 to 225 IMM pupae (Table 1). The linear response of the sensors shows promise for determining the presence of pests as well as the severity of the infestation, thereby guiding disinfestation and IPM strategies.

Table 1. Single-blind testing results for SDC sensor with IMM pupae; each quantity of pupae was tested once

Actual Pupae	Calculated Pupae	Accuracy
5	4	80%
25	23	92%
100	124	76%
150	135	90%
225	253	88%

If proven as a robust and accurate pest detector, the novel nanocrystalline tin oxide sensor developed by SDC presents a viable electronic monitoring option that would be price-accessible, simple to use, and easy to maintain for quality control and storehouse monitoring of IMM, NOW, and other stored product pests. So far, the SDC sensor has demonstrated the ability to detect IMM and NOW in simple proof of concept testing, and the range of detection is encouraging for possible use as an early detector of pest infestation. The SDC sensor, which is non-intrusive toward commodities, has thus shown promise for use in QC sampling.

Future studies will explore the sensor's ability to differentiate between pest species, but in most expected applications any pest presence is problematic and provides valuable information for the user. Improving baseline stability will also be a focus of future investigation. Characterization and optimization of the sensor using insect semiochemicals via gas chromatography mass spectrometry is planned and will be conducted once a beta test unit is available.

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Effect of *Callosobruchus maculatus* infestation on nutritional parameters of *urad (Vigna mungo)* beans

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Abstract

Urad *(Vigna mungo)* belongs to family *Leguminosae* and is an excellent source of protein in common Indian diet. In view of specific crop cycle of legumes, proper storage is an important aspect of postharvest management. The cowpea weevil, *Callosobruchus maculatus* (F.), is one of the most important storage insect pests of grain legumes. Infestation by *C. maculatus* may cause the qualitative and quantitative loss to stored legumes. Hence present investigation aimed to study the effect of infestation on nutritional parameters of stored *Urad* beans. Infested (50% damaged grains) and un-infested *Urad* samples were analysed for various nutritional parameters such as carbohydrate, fat, protein, ash, and amino acid content. Results revealed that carbohydrate and crude fat, decreased by 4.04% and 40.38%, respectively; while on other hand there was an increase in ash content (9.21%) and crude protein (9.01%) due to pest infestation. Also, amino acid profile of infested *Urad* beans changed. Significant decrease (ranging from 6.81 to 61.39%) in level of several Amino acids such as Serine, Lysine, Methionine, Isoleucine, Valine, Arginine and Cysteine was noted. However, significant enhancement (ranging from 1.10 to 79.87%) was seen in level of Histidine, Glutamic acid, Aspartic acid, and Alanine. Insect infestation therefore, in addition to quantitative loss had consequences for human nutrition.

Keywords: Urad, *Vigna mungo*, Insect infestation, *Callosobruchus maculatus,* Amino acid profile

Introduction

Food legumes belong to the family *Leguminosae,* also known as *Fabaceae*. Grain legumes are used as pulse (whole/split dal) and are an excellent source of protein (22-24%), carbohydrate, and a fairly good source of thiamine, niacin, calcium, and iron. Pulses are the leading supplement of protein in a typical Indian diet. Among all legumes, *V. mungo* (local name Urad*)* is India's important pulse crop as it contains higher protein content than other pulse crops. The crude protein of raw seeds of *V. mungo* is 23.6% (Bravo et al., 1999). India is the largest producer and consumer of Urad in the world. Legumes are less remunerative and high-risk crops than cereals due to their susceptibility towards insect pest infestation during crop growth as well as storage. India is the largest producer (25% of global production), the consumer (27% of global consumption), and the importer (14% of global) of pulses. Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, and

Karnataka are the top five legume-producing states in India (Mohanthy et al., 2015). Despite being top pulse-producing country, India still has to depend on other countries to meet domestic demands (Mangaraj et al., 2013). Before consumption, crops undergo different stages of postharvest operations. Good postharvest management is the key to reducing the postharvest losses thus contributing to food and nutritional security (Stathers et al., 2013; Sheahan and Barrett, 2017). It has been noted that significant losses occur during storage and milling. The major organisms infesting storage grains are insects, fungi, bacteria, and rodents. Among all, insect damage in stored grains alone may amount to 10-15% which is controlled by using several chemical pesticides such as malathion, chlorpyrifos and aluminum phosphide. These chemical pesticides are hazardous for the environment and human health (Satya et al., 2016).

Coleopteran insects of the family Bruchidae (also commonly known as pulse weevils/beetles) are the main insects associated with legumes (Sales et al*.,* 2000). Some pulse bruchids are *Callosobruchus maculatus* (F.), *C. chinensis* (L.), *C. analis* (F.), *C. rhodesianus* (Pic)*, C. phaseoli* (G.), and *C. dolichosi* (G.). These insects multiply at a rapid rate under suitable environmental conditions such as high humidity and temperature (Ahmed et al., 2003). Among these *C. maculatus* is ubiquitous and reported very harmful for stored pulses. Literature survey reveals that a lot of the research work has focused on quantitative losses, expressed as weight loss (De Lima, 1979; Boxall, 1986), whereas very little work on measuring the effect of insect-mediated postharvest losses on quality has been done (Hodges, 2013; Affognon et al., 2015). Losses in the quality of stored legumes not only negatively affect the market value but also impact household nutrition. In view of the above, present investigation was focused on effect of *C. maculatus* infestation on nutritive value of stored *V. mungo.*

Materials and methods

Insects rearing

Callosobruchus maculatus culture was obtained from Division of Entomology, IARI (Indian Agriculture Research Institute, New Delhi) and maintained in the Laboratory. It was done in a Biological Oxygen Demand (BOD) incubator at a temperature of 27±2°C (12:12 h day:night light cycle) and 65±5% RH. Initially, 20 pairs of freshly emerged adults were placed in a Polyethylene Terephthalate (PET) jar containing *V. mungo* grains (500 g). The jars were covered with muslin cloth and remained sealed to allow mating and oviposition. Then, initial stock culture was removed, and each jar's remaining content (grains and freshly laid eggs) was kept for further multiplication. The newly emerged beetles were used for experimental tests.

Setting up grain storage bioassay jars and sampling

Polyethylene Terephthalate (PET) jars (1000 mL) were washed and air dried. These jars were filled with 500 g organic urad (from 'Navdhanya Organic' Hauzkhas in Delhi). Two treatments (in triplicate) were used: 1) control (with no insects); 2) *C. maculatus* infested with 20 unsexed 0–3 d old adults. Attempting experiments with a single female was deemed to be an unacceptable risk of early mortality, so minimum two females were required to ensure infestation occurred. After the addition of insects (if any), jars were sealed with a muslin cloth and tighten with a rubber band. All these jars were placed in the BOD incubator at a temperature of 27±2°C (12:12 h day:night light cycle) and 65±5% RH.

The relevant subset of jars (three replicates for each type) was removed from the chamber, opened, and deteriorated sample (~50% damage) was taken out and analysed for the various parameters (proximate analysis and amino acid content).

Analysis of samples

Total nitrogen was determined by using a CHNS analyser (Vario ELIII, Elementar, Germany), and protein content was calculated as (Khalil & Manan, 1990):

Total Protein (%) = 100 × 6.25 × Total nitrogen
Where; 6.25 is the conversion factor.

The other constituents, the ash, moisture, and crude fat, were determined by standard methods (AOAC, 2000). The total carbohydrate content was obtained by the difference method:

Total Carbohydrate (%) = 100 – (Crude Protein + Crude Fat + Ash + Moisture)
Where; the crude protein, crude fat, ash, and moisture are in percentage.

The amino acid contents of the samples were determined using the HPLC method (ITC Labs, Panchkula, Haryana).

Data analysis

All experiments were performed in triplicates, and values were represented as mean. Data were analysed using Microsoft excel-2010.

Results and discussion

Proximate Analysis

The chemical analysis results (Table 1) revealed that carbohydrate and crude fat decreased by 4.04% and 40.38%, respectively. On the other hand, there was a slight increase in ash content (9.21%) and crude protein (9.01%) of the samples with infestation. The increase and decrease in values of the nutrients were found to be statistically significant. Figure 1 shows the graphical representation of change in nutritional composition of infested samples. Our findings agree with the result obtained by Etokakpan et al. (1982) which showed an increase in ash, crude protein, and crude fibre content of cowpea with insect infestation. Results of Bamaiyi et al. (2006) also showed the decrease in fat and carbohydrate content with infestation in stored cowpeas.

A general increase was seen in the percentage of total protein with the infestation. It might be due to the accumulation of insect frass and exuviae inside the grains, which increases the total nitrogen content of the grains. The insects' feeding decreased the actual protein content of grains.

An increase in total nitrogen, nonprotein nitrogen, total protein, and uric acid with increased levels of pest infestation, mainly 10%, 25%, and 50%, affect the nutritional quality of stored wheat at different levels (Jood et al., 1993, 1996; Soris et al., 2010). *Callosobruchus maculatus* feeds inside the grains, leaving the outer covering, which is mostly fibre, and raising the ash content (Bamaiyi et al., 2006). This result agrees with FAO (1984), which shows a decrease in the nutritional value of grains due to an increase in dietary fibre levels in infested grains. Increased dietary fibre intake lowers intestinal transit time, significantly reducing the rate of digested food absorption.

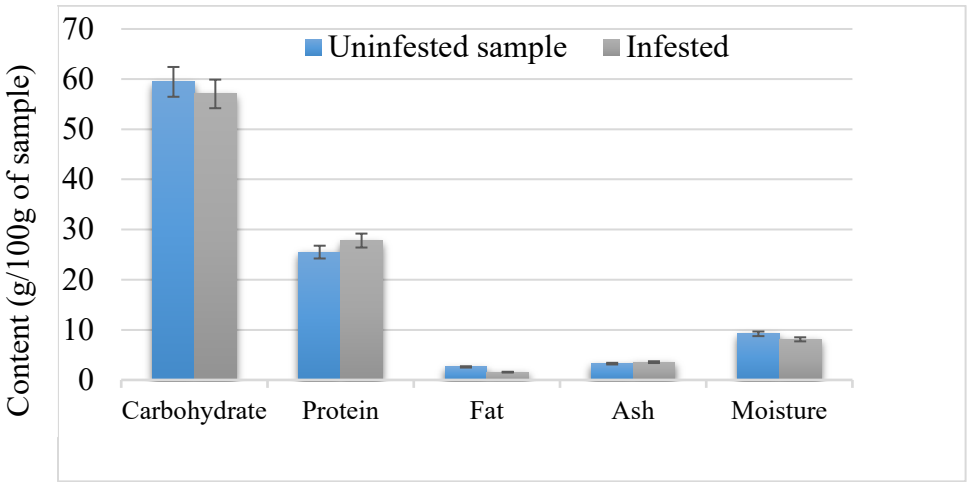

Fig.1 Comparison of nutritional composition of infested and uninfested Urad grain

Table 1. Proximate composition of infested and uninfested Urad (*V. mungo)* (mean ± SD)

Parameters	Uninfested Urad (g/100g)	Infested Urad *(g/100g)*	Percentage Change (%)
Carbohydrate	59.45±0.20	57.05±0.24	-4.04%
Protein	25.50±0.60	27.80±0.60	9.01%
Fat	2.60±0.03	1.55±0.05	-40.38%
Ash	3.25±0.03	3.55±0.04	9.21%
Moisture	9.20±0.07	8.10±0.07	-11.95%

Amino acid profile

Amino acids are the main nutritional constituents of legumes and act as essential components in the human body's specific metabolic processes. Their deficiency can adversely affect various functions of the human body.

Amino acid composition of infested and uninfested samples is presented in Table 2. It clearly shows the change in amino acid pattern. There was a significant decrease (ranging from 6.81 to 61.39%) in serine, lysine, methionine, isoleucine, valine, arginine, and cysteine. Sulphur containing amino acids (i.e., methionine and cysteine) are very limiting in Urad, and infestation caused a decrease in the level of these limiting amino acids. Our findings agree with research work of Etokakpan et al. (1982), which showed decrease in lysine, histidine, glutamic acid, valine, leucine, and methionine in infested cowpea. A decrease in amino acid content could be due to pests using them for physiological needs or due to change in physical conditions. However, significant enhancement (ranging from 1.10 to 79.87%) was seen in the level of histidine, glutamic acid, aspartic acid, and alanine.

Table 2. Amino acid profile of infested and uninfested Urad (V. mungo) samples

Amino Acid	Uninfested Sample(mg/100g)	Infested Sample(mg/100g)	Percentage change (%)
Arginine	876.32	715.48	-18.35
Cysteine	105.67	99.15	-6.17
Isoleucine	131.722	98.84	-24.96
Leucine	171.46	159.78	-6.81
Lysine	595.81	343.78	-42.3
Methionine	95.85	65.7	-31.45
Histidine	212.07	214.47	1.1
Tryptophan	206.19	160.88	-21.97
Serine	1988.90	767.88	-61.39
Valine	1964.66	1557.1	-20.74
Alanine	103.22	140.37	35.99
Glutamic acid	3518.41	3639.34	3.43
Glycine	5167.34	4573.37	-11.49
Aspartic Acid	11413.67	20532.82	79.87

Overall, present preliminary investigation clearly revealed that insect infestation reduced the nutritional and aesthetic value of stored Urad *(V. mungo)*, further decreasing its market value. This was mainly due to a change in amino acid profile and a change in certain nutritional parameters of stored legume due to infestation. So, there is need to develop effective methodology, based on design of storage bins along with eco-friendly pest control measures for storage of legumes, to reduce the nutritional and storage losses.

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Changes in RH and wet-bulb temperature of an Indica paddy bulk in a large warehouse in southeast China

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Abstract

Based on the isotherm of indica paddy, the relative humidity and wet-bulb temperature in the intergranular air of a paddy bulk were calculated using the Newton-Raphson method. The dry-bulb temperature, relative humidity, and wet-bulb temperature in 3500 tonnes of the paddy bulk were measured. Fumigation was conducted from 29 April to 28 June, 2019 and air-conditioned cooling was carried on from 17 July to 15 October, which kept Layer 1 of the paddy bulk below 26°C. The average dry-bulb temperature in Layers 2, 3 and 4 from August to September was below 24.5°C, 22.5°C and 22.5°C, respectively. The relative humidity in Layers 1, 2, 3 and 4 was below 58%, which indicated that the paddy was dry. The wet-bulb temperatures in Layers 1, 2, 3, and 4 were below 20°C, 18.5°C, 17°C, and 17°C, respectively, which suggested a possible insect infestation in Layer 1 and 2. In 2020, the nitrogen treatment was conducted from 23 March to 25 September, and a cooling from 11 May to 25 June kept Layer 1 of the paddy bulk below 25°C. The average dry-bulb temperature in Layers 2 and 3 from August to September was below 23°C. The relative humidity in Layers 1, 2, and 3 was below 60%. The wet-bulb temperatures in Layers 1, 2, and 3 were below 20°C, 18°C, and 17°C, respectively. The fees for fumigation and cooling in 2019 were 3.05 RMB per tonne of paddy. In 2020, the fees for fumigation and nitrogen treatment were 6.31RMB per tonne.

Keywords: Indica paddy, Storage cost, Air-condition, Fumigation, Nitrogen treatment, Wet-bulb temperature, Relative humidity

Introduction

Rice is amongst the oldest domesticated cereal grains in the world, and it is estimated that ten billion people will consume rice as main food and that the demand will be around 880 million tonnes by the year of 2025 (Rehal et al., 2017). The total rice production was 472.3 million tonnes with China leading in rice production of 144 million tonnes and India following with a production of 106 million tonnes in 2014 (FAO, 2014). Rice is a difficult to handle cereal in that head rice

and grain breakage can seriously affect market value (Iguaz and Vírseda, 2007). The evaluation parameters for the efficiency of paddy processes are head rice percentage and whiteness (Yadav and Jindal, 2007), and the moisture content (m.c.) of paddy is an important factor for these two parameters. A test in vertical mill suggested the optimal m.c. for paddy processing is 15% wet basis (Yan et al., 2005). But in practice, the long-term safe storage m.c. of indica paddy is usually lower than 13.5%. In south China, the main storage technology for dry paddy is to control the damage caused by pests. Thus, air-conditioners and nitrogen-filling techniques are being extensively applied. Few studies deal with the intergranular air properties in a large grain bulk. Changle Depot of Fujian Provincial Grain Reserve Administration Co. Ltd. is located at the Fuzhou Plain, China. This region belongs to a climate of subtropical marine monsoon. The present study analyzed the changes in dry-bulb temperature, relative humidity (RH), wet-bulb temperature in different layers of a paddy bulk in a large warehouse during the first-year storage with fumigation and cooling, and the second-year storage with cooling and nitrogen treatment. The overall aim was to find the variation trends of the above parameters in a paddy bulk under subtropical marine monsoon climate.

Materials and methods

Condition and management of the paddy warehouse

The experiment was carried out at the Changle Depot, Fujian Provincial Grain Reserve Administration Co. Ltd., Fuzhou, Fujian province, China. This depot is located at the plain with 25.9°N, 119.5°E, and 63 meters of average altitude, which has a tropical monsoon climate. The average annual temperature in this plain is 19.3°C, and average temperature in January and July is 10.3 and 28.3°C, respectively. The warehouse used in this study had an inner dimension of 42 m length and 24 m width, and was in the east-west direction. The warehouse had three pairs of U-shaped ground cage air channels along the width direction and three ground vents in the bottom of the north gable wall. Two pairs of 0.55 kW axial fans (0.55 kW power, YBF280M1-4, Huasheng Machinery Co., Ltd., Leqing City, China) were installed in two windows located at the top of north and east gables. Each fan supplied air to1.5 pairs of U-shaped air channels. The ratio of longest to shortest pathway of the supplied air was 1.35.

The late maturation indica paddy, 3500 tonnes harvested at Nanping, China was loaded into the warehouse on 24 March 2019, with a 6.1 m of grain height. The grain had 11.4% m.c.,15 mg KOH/100 g free fatty acid content, and 0.5% foreign materials. After levelling the grain bulk surface, aeration was conducted by running the four axial fans from 24 to 25 March, 2019. Three centrifugal fans (7.5 kW, Type 4-72-6C, Huasheng Machinery Co., Ltd., Leqing City, China), which provided 16576 m^3/h and1116 mmHg (148.8 kPa) static pressure, were continually run for 48 h (9:00, 31 December 2019 to the next day). The supplied air entered warehouse through the three vents and then passed upward through the grain bulk, finally was exhausted by the two axial fans. The axial fans provided a negative pressure. On 13, 15, 20, and 22 January, and 17 and 21 February, the axial fans were continually run for 16 h (from 17:00 to 11:00 on next day).

During the aeration, the warehouse doors were closed, the three vents were opened, and air duct to the four axial fans was opened. The four fans provided 320 to 220 Pa of static pressure and 9090 m^3 air per hour. Thus, the ventilation rate was 10.4 $m^3h^{-1}t^{-1}$.

Before conducting the fumigation, the bulk surface was covered with plastic films. On 29 April, 36.7 kg aluminum phosphide tablets (56%effective content, Jining Yongfeng Chemical Plant, Shandong province, China) were loaded into a phosphine generator located outside the warehouse, and the generator (LM-KF3608-VI controllable phosphine generator, Beijing Liangmao Science and Technology Co. Ltd., China) produced 350 mL/m^3phosphine. The whole warehouse was fumigated with a circulating current system till 18 June. On 19 to 27 June, the phosphine generator was stopped, but the fans were continually run to remove the phosphine from the warehouse, until no residue was detected (on 28 June).

Two air conditioners (TS-LS051S, Henan Grain Storage Equipment Technology Co., Ltd., China) were used to control the headspace temperature during summer months. The two air conditioners, which had a cooling capacity of 5880 kW, were installed on the eastern and western walls of the warehouse and located at 1.8 m above the paddy bulk surface. The supplied cooling air was evenly distributed within the suspended ceiling through eight outlets from the air conditioners. In 2019, the air-conditioners were set at 24°C, and run from 17 July to 15 October. In 2020, the air-conditioners were set at 23°C, and run from 11 May to 27 October. Nitrogen with 83.7% to 97.8% concentration was applied to the warehouse on 23 March, 6 and 13 April, 15 and 18 May, 22 June, and 15 and 24 July. The nitrogen application time at each day was 24 or 26 h. The nitrogen concentration was monitored at six locations inside the paddy bulk. The nitrogen analyzer included an air compressor (type GA75, Wuxi Atlas Copco Compressor Co. Ltd., Wuxi, China), two filters (type CTA-030), one oil remover (type CY-15), one freeze dryer (type WCD-150GF, 17 Nm^3/min capacity, Cold medium R22, Guangzhou Weiton Industry Gas Co. LTD., Guangzhou, China), and a nitrogen generator (type NP995-310B, Guangzhou Weiton Industry Gas Co. Ltd.). The nitrogen generator had a capacity of 310 Nm^3/h with 99.5% of nitrogen purity at 0.75-0.8 MPa.

Measured parameters

Grain temperature

A temperature and RH sensor located at1.5 m above the bulk surface measured the headspace temperature and RH (Fig.1). The grain bulk temperature was detected every three to four days, and 240 data of temperature were recorded in the software of Grain Status Monitoring System. Twelve cables along the width direction about 3.7 m apart were parallelly connected to a trunk which was 41 m long along the length direction. Each branch cable had five lines and was about 5.8 m apart along the width direction. Four temperature sensors were located in each line, and had distance of 0.2, 2.1, 4.0, and 5.8 m in proper order from the bulk surface, assigned as layer 1, layer 2, layer 3 and layer 4, respectively. The data for paddy bulk temperature, bin headspace RH and temperature, and ambient air RH and temperature were collected from 6 March 2019 to 27 October, 2020.

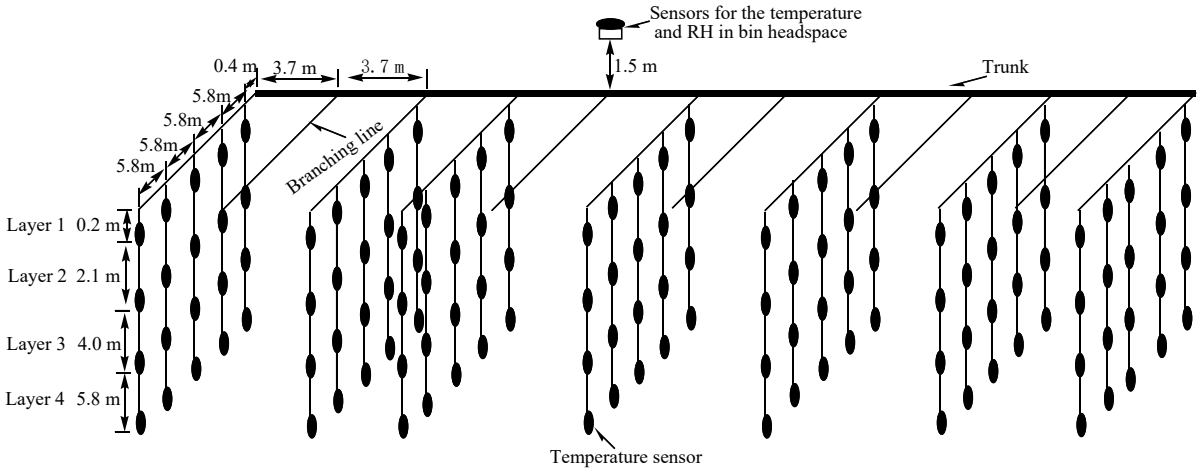

Fig. 1. Distribution of temperature sensors in the paddy bulk and a RH/Temperature sensor in the warehouse headspace.

Grain moisture content

The warehouse P27# was in west-east orientation, and had four sampling locations in the bulk surface which was about 13.7 meters apart along the north or south wall and had 0.5 m distance from the wall. Three sampling locations passed through the centre line with a location in the central point, and two other locations had 10 meters distance from the centre site. The depths of sampling were 0.3, 3.0, and 5.8 m from the bulk surface, assigned as layer L1, L2 and L3, respectively. The sampling dates in 2019 were 1March, 1 April, 2 October, and 1 November. The sampling dates in 2020 were 17 January, 18 March, and 26 October. About 0.5 kg grain was sampled at each of thirty-three locations at each sampling time. The sampled grain was kept in plastic bags (20 cm length, 14 cm width) and transferred to lab. Grain moisture content of the sample was measured using a capacitance moisture meter (LDS-1G, Taizhou Grain Instrument Factory, Taizhou, China).

Intergranular air relative humidity in grain sample

The modified Chung-Pfost equation was used to calculate the RH inside the sample:

$$RH = 100 \exp\left[-\frac{a}{T+b}\exp\left(-c \cdot M\right)\right] \quad (1)$$

Where; RH is the intergranular air relative humidity (%); M is the grain moisture content (%, wet basis); T is grain temperature(°C); and the values of a, b, c are 564.019, 63.041, and 0.219, respectively (Li et al., 2014).

The following equations were used to calculate the humidity ratio of the paddy bulk:

$$w = 0.622 \cdot \frac{RH \cdot p_s}{p_{atm} - RH \cdot p_s} \quad (2)$$

$$p_s = \frac{6 \times 10^{25}}{(T+273.15)^5} \exp\left(-\frac{6800}{T+273.15}\right) \quad (3)$$

Where; w is the intergranular humidity ratio of grain bulk (kg of water/kg of dry air); p_{atm} is the atmosphere pressure (101,325 Pa); and p_s is the saturation vapor pressure of intergranular air at grain temperature (Pa).

The wet-bulb temperature (T_w) was calculated as:

$$f(T_w) = w - w_w(T_w) + [4.042 \times 10^{-4} + 5.816 \times 10^{-7} w_w(T_w)](T - T_w) \quad (4)$$

Where;T_w is intergranular air wet-bulb temperature (°C); w_w is the humidity ratio of the saturation vapor pressure at the wet-bulb temperature (kg/kg).

The right-hand side of the Eq. (4) depends only on the wet-bulb temperature when $f(T_w) = 0$.

The Newton-Raphson method was used to search for the T_w (Thorpe, 2002):

$$T_w^{p+1} = T_w^p - \frac{f(T_w^p)}{df(T_w^p)/dT_w^p} \quad (5)$$

Where; T_w^p is the past estimated wet-bulb temperature (°C).

$$\frac{df(T_w)}{dT_w} = -\frac{dw_w}{dT_w} + (4.042 \times 10^{-4} + 5.816 \times 10^{-7}) \left[T \frac{dw_w}{dT_w} - \frac{d(w_w T_w)}{dT_w}\right] \quad (6)$$

When the absolute difference between successive values of the wet-bulb temperature was less than 10^{-6}, the iteration was stopped.

Results

Dry and wet-bulb temperatures and RH in headspace

Figure 2 shows the dry and wet-bulb temperatures and RH in headspace of the warehouse for two years. The highest and lowest temperature of ambient air at the depot was in the early August and January. The local air RH was in the range of 60 to 95%. The average headspace temperatures were below 25°C and 23°C from May to October 2019 and 2020, respectively. The average headspace RH was 65% and 54% in 2019 and 2020, respectively. From May to October, the average wet-bulb temperatures of the ambient air were 23.8°C and 24.2°C in 2019 and 2000, respectively, while the headspace had average wet-bulb temperatures of 18°C and 15.7°C in 2019 and 2000, respectively.

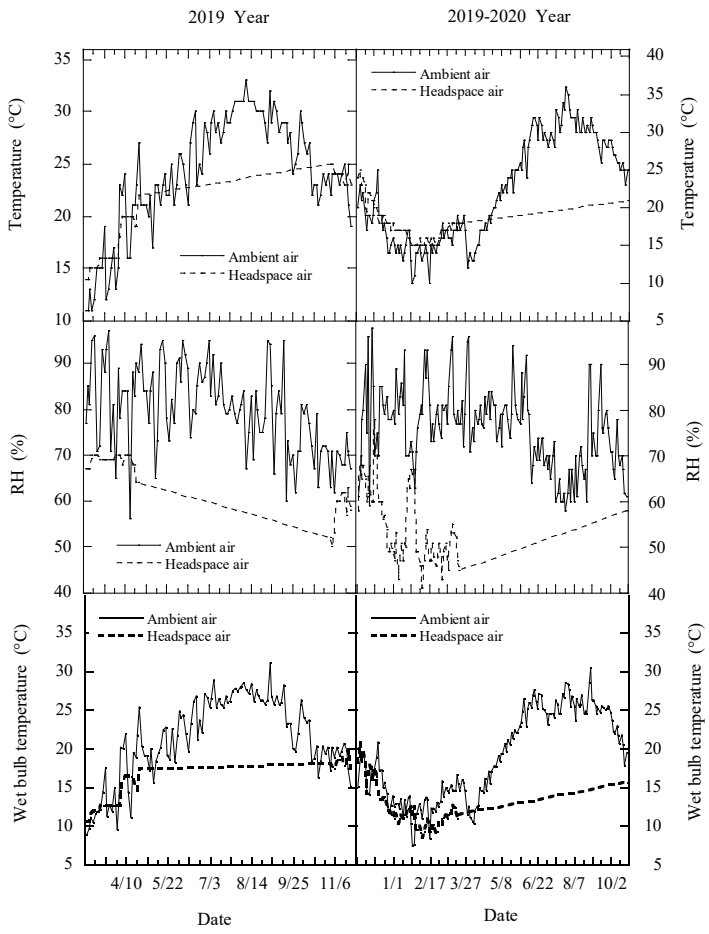

Fig. 2. Dry and wet-bulb temperatures and RH in headspace of the warehouse.

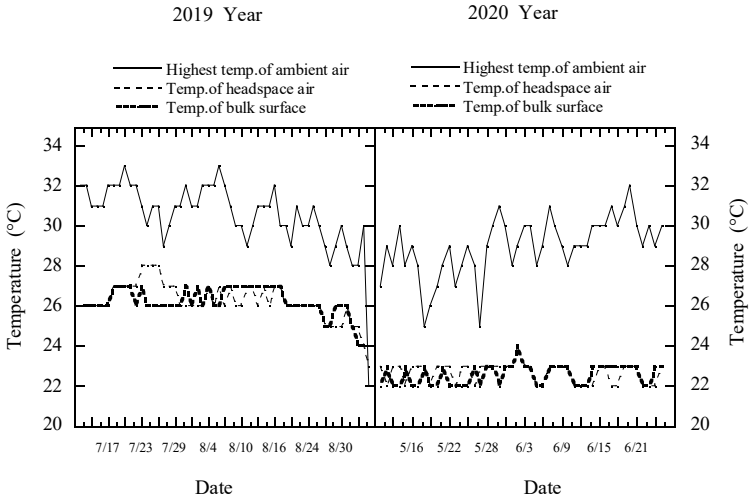

Fig. 3. Changes in the highest temperature of ambient air, temperature of headspace air and bulk surface of P27# warehouse when air conditioning was conducted.

Figure 3 shows the changes in the highest temperature of ambient air, temperature of headspace air and bulk surface of P27# warehouse for two years. These values during 12 July to 5 September, 2019 were 28-32°C, 24-28°C, and 24-27°C, respectively, while these were 25-32°C, 22-23°C, and 22-23°C during 11 May to 27 October, 2020, respectively.

The grain moisture content, air RH, and wet-bulb temperature in the grain bulk

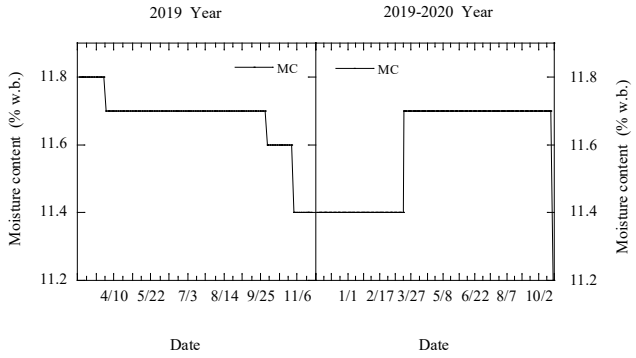

Fig. 4. Changes in moisture content of paddy bulk in the P27# warehouse.

The average moisture content of the paddy bulk was from 11.8% to 11.4% in 2019, and 11.7% to 11.2% in 2020 (Fig.4). The changes in dry and wet-bulb temperature and RH in the paddy bulk had the similar trends in 2019 (Fig.5).

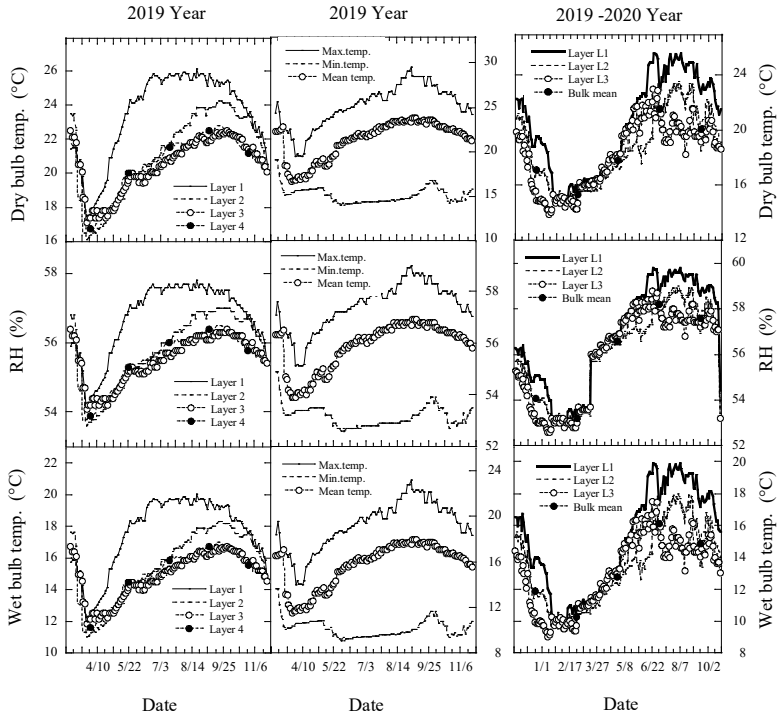

Fig. 5. The grain moisture content, air RH, and wet-bulb temperature in the grain bulk in 2019 and 2020.

From 1 April to 22 November, the dry and wet-bulb temperature and RH in Layer 1 of the paddy bulk were significantly higher than those of Layers 2, 3 and 4. Both Layers 3 and 4 had a similar dry and wet-bulb temperature and RH. The maximum, minimum, and mean dry and wet-bulb temperature and RH in the paddy bulk had the similar trends.

Changes in dry and wet-bulb temperature and RH in the paddy bulk in 2020 had the similar variation trends as 2019. During May to October, the average dry-bulb temperature, RH, and wet-bulb temperature in Layer 1 were 23.4°C, 58.9%, and 17.8°C, while in Layer 2 were 20.6°C, 57.7%, and 15.3°C, respectively. These average values in Layer3 and the whole bulk were 20.3°C, 57.6%, and 15.0°C, and 21.4°C, 58.1%, and 16.1°C, respectively.

Discussion

The depot in this study was located at Fuzhou, China, which has an average ambient air temperature higher than 28°C from May to October. The main technology to safely store indica paddy with 11.5% m.c. is to control insect pests. In 2019, fumigation was conducted from 29 April to 28 June, and air-condition cooling from 17 July to 15 October kept the temperature in Layer 1 of the paddy bulk below 26°C. Due to the poor thermal conductivity of the grain, the average dry-bulb temperature in Layers 2, 3 and 4 from August to September was below 24.5°C, 22.5°C and 22.5°C, respectively. The air relative humidity in Layers 1, 2, 3 and 4 was below 58%, which indicated that the paddy was dry. The wet-bulb temperatures in Layers 1, 2, 3, and 4 were below 20, 18.5, 17, and 17°C, respectively, which suggested a possible infestation of insect pests in Layers 1 and 2.

In 2020, the nitrogen treatment was conducted from 23 March to 25 September, and air-condition cooling from 11 May to 27 October kept the temperature in Layer 1 of the paddy bulk below 25°C. The average dry-bulb temperature in Layers 2, 3 and the whole bulk from August to September was below 23°C. The air relative humidity in the whole bulk was below 60%, which indicated a safe storage condition of the paddy bulk. The wet-bulb temperatures in Layers 1, 2, and 3 were below 20°C, 18.0°C, and 17°C, respectively. The fees for the fumigation and cooling in 2019 were 3.05 RMB per tonne of paddy. In 2020, the fees for the nitrogen treatment and cooling were 6.31 RMB per tonne.

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Ren Y, Coetzee EM, Du X, Thomas M, van der Merwe J, McKirdy SJ (2021) In-transit fumigation of shipping containers with ethyl formate + nitrogen during full travel on road and on sea. Page 238. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

In-transit fumigation of shipping containers with ethyl formate + nitrogen during full travel on road and on sea

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ABSTRACT

The use of shipping containers for cargo transportation has the potential to transport insect pests from infested to non-infested areas. Therefore, fumigation is required as an appropriate biosecurity measure to exterminate these pests. In-transit fumigation trials were conducted in four 6.1 m (20 ft) shipping containers during a two-day journey. Ethyl formate (EF) (90 g/m^3) was purged with nitrogen (EF+N2) into the containers. Ethyl formate concentration inside containers and the surrounding environment were monitored at timed intervals throughout the journey on road and sea. Fumigation achieved sufficient concentration × time (Ct) products in the containers during the journey, which exterminated all stages of most common insect pests. Levels of EF in the environment between 1-15 m downwind from the containers and driver's cabin were less than 0.5 ppm at each of the timed intervals below 100 ppm of EF Threshold Limit Value (TLV). Ethyl formate concentrations inside containers and the surrounding environment on the barge were monitored at timed intervals throughout the overnight voyage. This research had also demonstrated that there was no detectable risk to the public, crew members on the barge or workers on discharging area throughout the journey. In addition, all tested containers were ready to be opened and unloaded after 5-10 min aeration or without aeration upon arrival at discharging area.

Keywords: In-transit fumigation, Insect pest control, Fumigant, Ethyl formate, Worker safety, Environmental safety

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Zhao Y, Xi J, Li Y, Dong H, Song J (2021) Attractiveness of *Matthiola incana* (L.) R. Br. powder and its volatile compounds to *Lasioderma serricorne*. Pp. 239-245. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Attractiveness of *Matthiola incana* (L.) R. Br. powder and its volatile compounds to *Lasioderma serricorne*

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Abstract

As a worldwide insect pest in stored tobacco, *Lasioderma serricorne* is harmful during the entire tobacco storage and processing. To effectively monitor the occurrence and damage of *L. serricorne*, 10 plant materials with different characteristic odours were used to test the attractiveness to *L. serricorne* adults. The results showed that the attractiveness of *Matthiola incana* (L.) R. Br. powder was the highest, and the selection index was more than 75%. The static headspace gas chromatography was used to analyze the volatile compounds from *M. incana* powder, and 46 volatile compounds were tested on *L. serricorne* adults. The results indicated that octanal exhibited the highest attractiveness to *L. serricorne*, with a 70.8% selection index at 11.32 nL/cm^2. Both *β*-Ionone and 2, 3-hexadione also had good attractiveness, and their selection indices were more than 60% at 11.32 nL/cm^2. Thus, *M. incana* and its volatile compounds could be used in tobacco storage to monitor the occurrence and damage of *L. serricorne*.

Keywords: *Matthiola incana* (L.) R. Br., Volatile compounds, *Lasioderma serricorne*, Selection index

Introduction

Lasioderma serricorne (Fabricius) (Coleoptera: Anobiidae) is a significant storage insect pest worldwide, which not only damages tobacco, but also infests other stored products, such as herbs, spices, and cereal grains (Mahroof et al., 2007; Ebadollahi et al., 2010; Yu et al., 2019). At present, the control of *L. serricorne* mainly relies on phosphine fumigation (Weizheng et al., 2014). However, the excessive use of this chemical fumigant causes many negative effects, such as high insecticide residue and insect resistance to the insecticide (Kim et al., 2003). Therefore, development of environmentally friendly pest control methods is critically important (Isman et al., 2006; Junyu et al., 2019).

Plant volatiles play important roles in insect host searching and oviposition site selection, and it has been reported that some plant volatiles are attractive to insects (Landolt et al., 1997). More and more studies focused on using plant attractants have been reported. For example, the attractiveness of mulberry leaf tea and chili to *L. serricorne* have been reported (Phoonan et al., 2014). In addition, to monitor the occurrence of pests, plant volatiles can be applied with sex pheromones to trap pests, which can enhance the trapping efficiency of the sex pheromone (Cox et al., 2004; Phoonan et al., 2014; Xintian, 2010). There is a need to identify more attractants for enhancing insect control strategies, and there is a lack of attractive effect analysis of plant volatiles.

In this study, we investigated the olfactory responses of *L. serricorne* to 10 plant materials and analyzed the volatile compounds extracted from the *M. incana.* Our results could pave a foundation for developing plant-based attractants to *L. serricorne*.

Materials and methods

Insect

The cigarette beetles collected from the tobacco storage laboratory, Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China, and reared for several generations. The laboratory colony was reared at 28 ± 2°C and 70% ± 5% RH under dark conditions on whole oatmeal containing 30% wheat flour by weight. The adults used in the experiment were collected one week after emergence. The collected adults were starved for 24 h before the olfactory behavior test.

Plant materials

The leaves of following 10 plant materials were used for this study: *M. incana*, *Osmanthus fragrans* (Thunb.) Lour., *Nicotiana tabacum* L. (flue-cured tobacco), *Rubus corchorifolius* L. f., Camellia sinensis var. Assamica (Pu-erh tea), *Rosa rugosa* Thunb., *Rhododendron simsii* Planch (yellow), *Rubus idaeus* L.*, Rosa multiflora* Thunb., *Aspidistra elatior* Blume and *Indocalamus* leaves. The leaves of these materials were crushed by using a high-speed grinder (FW100, Tianjin Tester Instrument Co. Ltd., Tianjin, China). The powder collected after this grinding was used for the attractant test.

Chemicals

The following chemicals were used in this study: 2-Methylbutyraldehyde (98%), 5-Methyl furfural (98%), 2-Methyltetrahydrofuran-3-one (98%), Furfuryl alcohol (98%), 2-Methylpyrazine (98%), 2,5-Dimethylpyrazine (98%), 2-Ethylfuran (98%), 2-Pentylfuran (98%), Benzaldehyde (98%), γ-Butyrolactone (98%), 2-Decanone (98%), 2(5H)-Furanone (98%), Neophytadiene (98%), Phenylacetaldehyde (98%), Palmitic acid (98%), Pyrrole-2-carboxaldehyde (98%), Octanal (99%), Nonanoic acid (98%), Furfuryl Acetate (98%), Methyl palmitate (98%), Maltol (99%), Methyl eugenol (98%), Nonadecane (98%), Nonanal (98%), β-Damascenone (98%), 2,3-Dimethylpyrazine (99%), Decanal (97%), Pentadecane (98%), Terpinyl Acetate (98%), Hexadecane (99%), Dodecane (99%), Acetoxyacetone (98%), 2,3-Heptanedione (98%), 1-Hexadecene (98%), Methyl glycolate (98%), DL-Pantolactone (98%), 2-Acetylpyrrole (98%), Methyl furan-2-carboxylate (98%), 4-Ethylguaiacol (98%), Nonane (98%), p-Tolualdehyde (98%), 2,3-Hexanedione (98%), 1,4-Dimethylpyrazole (98%), Propioin (98%), and β-Ionone (98%). Acetophenone (99%) was purchased from Innochem or J&K Co. Ltd., Beijing, China as model attractant and n-Hexane (98%) was used as a solvent.

Olfactory response of L. serricorne to plant powders

The area preference method (Shanshan et al., 2016) was used to test the attractiveness of the material powders to the cigarette beetle. Petri dishes (15 cm in diameter) were used to confine cigarette beetles during the experiment. Filter paper (15 cm in diameter) was cut in half, and one half (marked as A) was treated with 1.0 g of powder while the other half (marked as B) was blank as control. Sixteen mixed sex adults were released at the center of the petri dish. The petri dishes were then covered and transferred to a chamber set at constant temperature and humidity (28 ± 2°C, 70% ± 5% RH). The number of insects presenting on both halves were counted after 30 and 60 min. For each sample, three replicates were done.

The selection index (SI) was calculated using the following formula:

$$SI(\%) = [(A - B)/(A + B)] \times 100 \qquad (1)$$

Where; A and B are the insect numbers on the side A and B, respectively and SI is the selection index.

If SI is positive, the material has an attractive effect, otherwise has a repellent effect. The competitive olfactory response of *L. serricorne* to the plant materials and the tobacco was tested using the same procedure mentioned above, except that one half of the filter paper was treated with 0.5 g plant powder (marked as A) and the other half (control) was treated with 0.5 g tobacco powder (marked as B).

Analysis of volatile compounds in M. incana

Static headspace sampler (7697A, Agilent) was used to collect volatile compounds. Volatile components of the *M. incana* were analyzed by using a GC-MS (7890A/5977B, Agilent). The GC-MS was equipped with a DB-5ms (50 m × 0.25 mm i.d. × 0.25 μm film thickness) capillary column. The oven temperature started at 40°C for 1 min, then increased to 150°C at 2°C/min, increased to 250°C at 8°C/min and held for 10 min, and then increased at 20°C/min until the final temperature of 280°C. Helium (1.0 mL/min) was used as the carrier gas. Mass spectra were recorded from 50 to 550 amu with electronic impact ionization at 70 eV. The retention indices were determined by comparing their mass spectra with those stored in NIST 17.

Olfactory response of L. serricorne to volatile compounds

The area preference method was used to test the attractiveness of volatile compounds. Three solutions (56.62, 11.32 and 2.26 nL/cm^2) were prepared by diluting the model attractants (listed in *Chemicals*) with n-hexane. Each solution was evenly applied to the half of a filter paper using a micropipette. The other half was treated with 1000 μL n-hexane as the control. Both halves were air dried to completely evaporate the solvent. The two halves were attached with solid glue, so a full disk was kept during the operation. Other procedure was the same as that mentioned in 1.4. There were three replications for each concentration.

Statistics

One-Way ANOVA and Duncan's new multiple range test were conducted using SPSS 22.0.

Results and discussion

Olfactory response of L. serricorne to plant powders

All plant powders exhibited attractiveness to *L. serricorne* adults (Fig. 1). After 30 min, more than 75.0% adults of *L. serricorne* oriented to powders of *M. incana* and *R. simsii*, and 72.9% to flue-cured tobacco. These three materials showed a better attractancy than other tested materials. After 60 min, the SI associated with powders of *M. incana*, *R. idaeus,* and *Rosa rugosa* was 79.2%, 77.1%, 75.0%, respectively. Therefore, powder of *M. incana* had the highest attractiveness to *L. serricorne* adults.

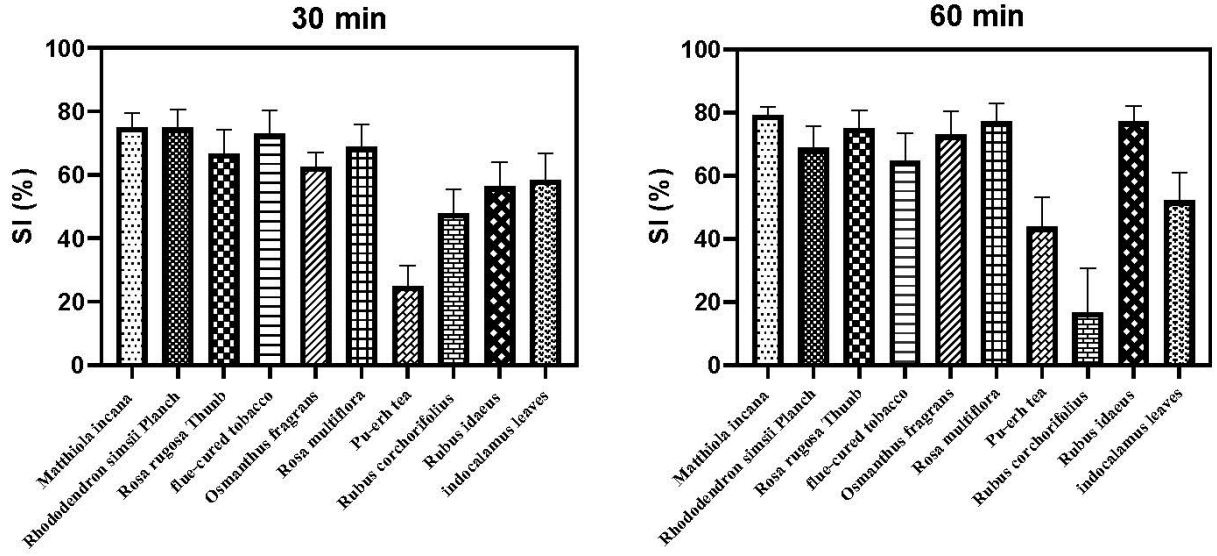

Fig.1. Olfactory response of *L. serricorne* adults to different plant powders.

Olfactory responses of L. serricorne to six plant powders when tobacco powder was the control

The six plant powders (*M. incana*, *R. rugosa*, *O. fragrans*, *R. simsii*, *R. idaeus, R. multiflora*) had a significant higher attractiveness than that of flue cured tobacco powder (Table 1). After 30 min, the SI associated with *M. incana* and *R. simsii* were 16.7% and 10.4%, respectively, which indicated that the attractiveness of these two plant powders was stronger than the flue cured tobacco. In the 60 min test, *M. incana* and *O. fragrans* powders were also more attractive than the flue cured tobacco. Thus, the attractiveness of *M. incana*, *R. simsii*, and *O. fragrans* powders was higher than that of flue cured tobacco.

Table 1. Olfactory responses of *L. serricorne* adults to six plant powders when tobacco powder was the control

Plant	30 min [(a)]			60 min [(a)]		
	A-adult	B-adult	SI (%)	A-adult	B-adult	SI (%)
Matthiola incana	9.33±1.75a	6.67±1.75b	16.67	9.00±2.00a	7.00±2.00a	12.50
Rhododendron simsii (yellow)	8.83±2.04a	7.17±2.04b	10.42	8.17±2.48a	7.83±2.48a	2.08
Rosa multiflora	7.83±1.94a	8.17±1.94b	-2.08	7.83±2.48a	8.17±2.48a	-2.08
Osmanthus fragrans	8.17±1.72a	7.83±1.72b	2.08	8.50±1.87a	7.5±1.87a	6.25
Rosa rugosa	7.67±3.01a	8.33±3.01b	-4.17	8.33±1.21a	7.67±1.21a	4.17
Rubus idaeus	4.83±1.72b	11.17±1.72a	-39.58	6.83±1.72a	9.17±1.72a	-14.58

[(a)]Different letter in the same row indicates a significant difference at $P < 0.05$. A, B were the number of *L. serricorne* adults in A and B side, respectively.

The identified volatile compounds from Matthiola incana (L.) R. Br.

One hundred and two volatile compounds were identified from the powder of *M. incana*. The total ion flow diagram is showed in Fig. 2. The components identified accounted for 89.3% of the total volatile compounds, including aldehydes (59.5%), ketones (6.5%), esters (2.9%), alcohols (2.3%), phenols (6.1%), organic acids (0.5%), hydrocarbon compounds such as alkanes and alkenes (2.4%), and heterocyclic compounds containing nitrogen and oxygen (9.1%).

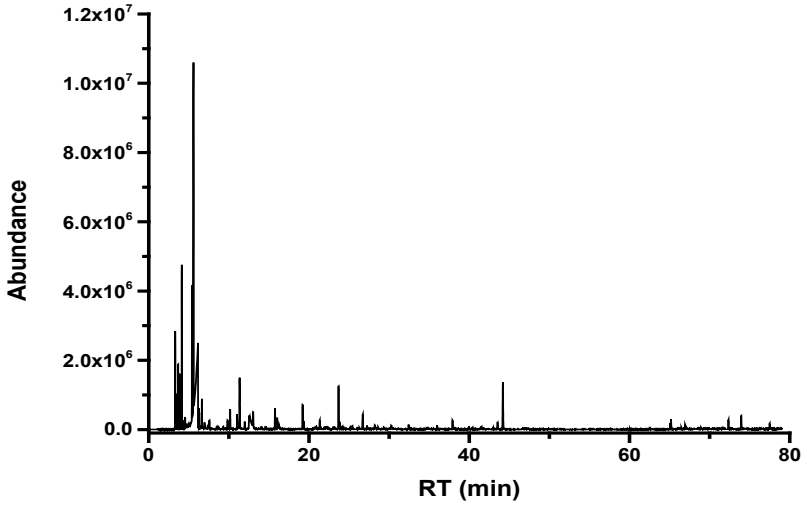

Fig. 2. The total ion flow diagram of *Matthiola incana* (L.) R. Br.

Attractiveness of volatile compounds to L. serricorne

Based on their safety, cost, and repellency, 46 kinds of volatile compounds extracted from *M. incana* were selected for the test (Table 2). The results revealed that the selection indices of Octanal, *β*-Ionone, and 2,3-Hexanedione were 70.8%, 66.7%, 62.5%, respectively, at 11.32

nL/cm^2, which showed higher attractiveness to *L. serricorne*. The selection indexes of Propioin and Neophytadiene were over 50% at 2.26 nL/cm^2, while 4-Ethylguaiacol and Methyl glycolate also reached 58.3% at 11.32 nL/cm^2 and 56.62 nL/cm^2. Therefore, these four materials also had stronger attractiveness.

Compared with other tested compounds, Octanal exhibited the highest attractiveness to *L. serricorne* at 11.32 nL/cm^2. Octanal might be the key volatile component that attracts adults of *L. serricorne* to *M. incana*, which has not been reported. However, the relative content of 2-Methylbutyraldehyde was the highest in the volatile compounds, so it should be noted that the active attractants were often not the major compounds. The attractiveness of volatile compounds was generally less than powders, therefore, synergistic activity of components extracted from *M. incana* might exist, which should be further studied.

Table 2. Attractiveness of *L. serricorne* to volatile components extracted from *M. incana* (SI (%))

Chemicals	Concentrations			Chemicals	Concentrations		
	2.26 nL/cm^2	11.32 nL/cm^2	56.62 nL/cm^2		2.26 nL/cm^2	11.32 nL/cm^2	56.62 nL/cm^2
2-Methylbutyraldehyde	4.17	-4.17	4.17	Methyl glycolate	16.67	12.50	58.33
5-Methyl furfural	8.33	16.67	8.33	4-Ethylguaiacol	37.50	58.33	-8.33
2-Methyltetrahydrofuran-3-one	4.17	16.67	12.50	2,3-Hexanedione	33.33	62.50	12.50
Furfuryl alcohol	4.17	-4.17	-25.00	Nonanoic acid	29.17	8.33	-8.33
2-Methylpyrazine	0.00	4.17	-12.50	Methyl furan-2-carboxylate	-16.67	-25.00	-25.00
2-Pentylfuran	-20.83	-25.00	-45.83	1,4-Dimethylpyrazole	-29.17	-25.00	-33.33
Benzaldehyde	-25.00	-41.67	-33.33	Propioin	54.17	-12.50	2.08
γ-Butyrolactone	-8.33	20.83	-16.67	p-Tolualdehyde	-8.33	4.17	-29.17
2-Decanone	-25.00	-20.83	-54.17	Acetophenone	4.17	-16.67	4.17
2(5H)-Furanone	4.17	4.17	0.00	DL-Pantolactone	4.17	-12.50	-29.17
Phenylacetaldehyde	4.17	8.33	-45.83	Methyl palmitate	25.00	12.50	4.17
Octanal	41.67	70.83	33.33	Nonadecane	8.33	4.17	-8.33
Furfuryl Acetate	8.33	12.50	4.17	Hexadecane	-8.33	12.50	4.17
Terpinyl Acetate	-37.50	-45.83	-54.17	Pentadecane	8.33	12.50	-8.33
Acetoxyacetone	20.83	8.33	12.50	Dodecane	4.17	8.33	-8.33
2,5-Dimethylpyrazine	16.67	25.00	4.17	Nonane	12.50	4.17	0.00
β-Ionone	33.33	66.67	41.67	2-Acetylpyrrole	20.83	16.67	8.33
2,3-Dimethylpyrazine	-54.17	0.00	-12.50	Maltol	20.83	25.00	8.33
Nonanal	20.83	4.17	20.83	Palmitic acid	-8.33	-4.17	-29.17
Decanal	12.50	-12.50	-8.33	Neophytadiene	50.00	37.50	12.50
2,3-Heptanedione	37.50	-4.17	-8.33	β-Damascenone	16.67	12.50	41.67
1-Hexadecene	25.00	-41.67	4.17	Methyl eugenol	8.33	4.17	-4.17
2-Ethylfuran	12.50	20.83	12.50	Pyrrole-2-carboxaldehyde	8.33	16.67	-12.50

Conclusions

Compared with other materials, *Matthiola incana* (L.) R. Br. had the highest attractiveness to *L. serricorne*, while *R. simsii* and *R. rugosa* were the next highest with SI > 65%. The *R. corchorifolius* and Pu-erh tea had relatively poor attractive effect, and their attractiveness fluctuated with the extension of time of the test time. *M. incana*, *Osmanthus fragrans*, *R. simsii*, *Rubus idaeus, Rosa multiflora*, and *Rosa rugosa* were tested when the flue cured tobacco was used as the control, and the results showed that *M. incana* had the highest attractiveness to the tobacco beetle. Furthermore, 46 volatile compounds extracted from *M. incana* were selected for attractive test, and the results indicated that the SI of Octanal, *β*-Ionone and 2,3-Hexadione reached more than 60% when the concentration was 11.32 nL/cm^2, while Octanal exhibited the highest attractiveness.

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Acute toxicity of two bio-fumigants against larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)

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Abstract

The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), is a polyphagous pest of stored products such as dried cassava chips, maize and timbers. It causes a reduction of nutritional value and of product weight, and consequently constitutes a major threat to food security and income generation, if left uncontrolled. Synthetic fumigants, despite their age-long application, have some demerits such as the tendency of a pest to develop resistance against them, the high-cost implications and the concerns for ecological safety. Therefore, it is crucial to search for eco-friendly alternatives. In this research, essential oils (EOs) of *Cymbopogon citratus* and *Thuja plicata* were obtained via distillation for four hours using Clevenger type apparatus. Each EO was separately assayed against *P. truncatus* for fumigant toxicity at 6.66, 13.33, 26.66, and 53.33 µL/L air; and latter subjected to Gas Chromatography Mass Spectrometry. The percentage oil yields from *C. citratus*, *and T. plicata* were 0.263% and 0.393% (v/w), respectively. When each EO was applied at 55.33 µL/L air, *C. citratus* caused significantly higher mortality of 100% against *P. truncatus* at 6 h after treatment (HAT), while *T. plicata* caused 100% mortality at 48 HAT. The predominant compounds identified in *C. citratus* EO were Citral (44.92%), Verbenol (34.87%), and Geraniol (6.51); while *T. plicata* EO was predominated by 3-Carene (30.68%), α-Pinene (24.85%), Caryophyllene (8.60%), Humulene (5.94%) and Cedrol (4.32%). The results indicated the superior bioactivity of *C. citratus* over *T. plicata* as bio-fumigant against *P. truncatus*.

Keywords: *Prostephanus truncatus,* Essential oil, Acute toxicity, *Cymbopogon citratus, Thuja plicata,* GC-MS

Introduction

Prostephanus truncatus (Horn) known as the larger grain borer is a significant pest of dried cassava, maize, and woody plants in the tropics. The insect represents the main destructive storage pest of maize and cassava stock over a short period of time. The adult bores into cassava or maize cobs or grains making neat holes and tunnels by producing a large quantity of dust (Nang'ayo et

al., 2002; Nansen et al., 2004). Losses to *P. truncatus* on field and storage has constituted a major constraint to food security and income generation in sub-Saharan Africa (Abebe et al., 2009; Osipitan et al., 2011).

The use of botanicals to control *P. truncatus* has been in existence in developing countries where they are cheaply available. Several scientists have reported the bio-efficacy of different plants formulation against *P. truncatus* and other insects. *Thuja plicata* is commonly called western cedar, giant cedar. It is a species of *Thuja* an evergreen coniferous tree in the cypress family Cupressaceae native to Western North America. *Cymbopogom citratus* is a perennial plant with long thin leaves, is one of the largely cultivated medicinal plant for its essential oil in part of tropical and subtropical areas of Asia, Africa and America (Chanthal et al., 2012). The leaves usually produced yellow or amber colour aromatic essential oil when squeezed (Adejuwon and Ester, 2007).

Synthetic insecticides have been used against this pest; however, due to several problems associated with the use of synthetic insecticides, it is necessary to consider alternatives to synthetic insecticides that cause no harm to human health and the environment. Since the geographical location of plant has tendency to affect the chemical composition of its essential oil (EO), conducting an empirical study to establish the bioactivity of the EO would not be out of place, even when such studies had been carried out in other geographical locations. To the best of the authors' knowledge, the toxicity of Nigeria-grown *C. citratus* and *T. plicata* against *P. truncatus* has not been studied. Therefore, the research was designed to evaluate the toxicity of the bio-fumigants against *P. truncatus.*

Materials and methods

Insect culture

Prostephanus truncatus adults were obtained from a heavily infested dried cassava chips kept in the Entomology Laboratory of Crop and Environmental Protection, Department, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. They were introduced into clean transparent jars that contained uninfested dried cassava chips (cultivar Oko Iyawo), obtained from Araada Market, Ogbomoso and reared according to established methods (Osipitan, 2011). The jars were covered with a muslin cloth and kept in the laboratory, and monitored under a fluctuating temperature (28±2°C) and relative humidity (65±5%).

Preparation of plant materials for extraction of essential oil

The bio-fumigants were obtained from fresh leaves of *C. citratus* and air-dried leaves of *T. plicata* via hydro-distillation method for 4 h using Clevenger apparatus. (Shiva Scientific Glass Private Ltd, New Delhi, India). The plants were collected from June to August, 2017. The leaves of *C. citratus* were collected from LAUTECH Teaching and Research Farm, Ogbomoso; while the leaves of *T. plicata* were collected from Ilorin, Kwara State, Nigeria.

Gas chromatography-mass spectrometry analysis

The conditions and the procedures for the Gas Chromatography Mass Spectrometry analysis of the bio-fumigant followed Babarinde et al. *(*2019), using AGILENT (19091S–33HP–5MS) GC (Agilent Technologies, Palo Alto, CA, USA) interfaced with a double focusing mass spectrometer VG Analytical 70–250S) (VG Analytical Ltd, Manchester, UK).

Fumigant toxicity of the bio-fumigants on P. truncatus

To assess the fumigant toxicity of the bio-fumigants on *P. truncatus*, the different doses (5, 10, 20 and 40 µL) of the essential oils corresponding to 6.66, 13.33, 26.66, 53.33µL/L air were separately dissolved in 0.2 mL of acetone and applied to filter papers (Whatman N° 1, ~8 cm²), that was pasted under the bottle cover of 75 mL plastic bottle. The bottle cover was dried in air for 20 min to allow for escape of acetone, and treatment was replicated three times. In the control bottles, 0.2 mL acetone was applied on the filter papers and also replicated three times. Twenty mixed sexed *P. truncatus* adults were placed in the covered plastic bottles. Data were collected at 6, 12, 24, 36, and 48 HAT on the number of dead and alive insects. Percentage of dead adult (PDA) was computed as:

$$PDA = \frac{Number\ of\ dead\ insects}{Total\ number\ of\ assayed\ insects} \times \frac{100}{1}$$

Statistical analysis

The data were subjected to analysis of variance. Significant means were separated using SNK at 5% probability level.

Results and discussion

Yield and chemical composition of studied bio-fumigants

The oil yields from *C. citratus* and *T. plicata* were 0.263 and 0.393% (v/w), respectively. *Cymbopogon citratus* EO was dominated by oxygenated monoterpene with citral (44.92%) and verbenol (34.97%) as the predominant compounds (Table 1); while *T. plicata* was dominated by hydrogenated monoterpene with 3-carene (30.68%) and alpha pinene (24.85%) being the predominant compounds (Table 2).

Table 1. Chemical composition of *Cymbopogon citratus* leaf essential oil

Retention Index [a]	Compound name [b]	Composition (%) [c]	Chemical class
958	Myrcene	0.47	Monoterpene
948	3-Carene	0.06	Monoterpene
1074	p-Menth-8-ene, cis	0.04	Monoterpene
1082	Linalool	1.03	Oxygenated monoterpene
1175	alpha-Cyclocitral	0.09	Oxygenated monoterpene
1131	trans-3(10)-caren-2-ol	0.20	Oxygenated monoterpene
948	Carene, 4,5-epoxy, trans	0.15	Oxygenated monoterpene
1136	cis-Verbenol	0.26	Oxygenated monoterpene
928	5-Isopropyl-1,4-dimethylcyclopentene	0.61	Monoterpene
1206	Carveol	1.13	Oxygenated monoterpene
1196	Isopregol	0.38	Oxygenated monoterpene

Retention Index [a]	Compound name [b]	Composition (%) [c]	Chemical class
1143	alpha-Terpineol	0.17	Oxygenated monoterpene
949	Cyclobutaneethanol, beta-methylene	0.09	Hydrocarbon
1228	Geraniol	7.74	Oxygenated monoterpene
1269	Oxiranemethanol, 3-methyl-3-(4-methyl-3-pentenyl)-	0.13	Oxygenated monoterpene
1136	Verbenol	34.97	Oxygenated monoterpene
1174	Citral	44.92	Oxygenated monoterpene
1224	Epoxy-linalool oxide	1.00	Hydrocarbon
1342	Neric acid	1.23	Acid
1352	Geraniol acetate	0.78	Monoterpene
1030	2,3-Dimethylcyclohexanol	0.70	Alcohol
1430	trans-alpha-Bergamotene	0.08	Sesquiterpene
1449	Tridecanone	1.07	Sesquiterpene
1507	Caryophyllene oxide	0.41	Oxygenated Sesquiterpene
1593	Selina-6-en 4-ol	0.20	Oxygenated Sesquiterpene
1324	(IS,4R)-p-Mentha-2,8-diene, 1-hydroperoxide	0.08	Oxygenated monoterpene
2192	trans-Geranylgeraniol	0.45	Oxygenated diterpene
1211	alpha-Santalene	0.91	Sesquiterpene
1107	Rhodinal	0.55	Oxygenated monoterpene

[a] Kovats retention indices relative to n alkanes on fused silica capillary column Optima® 5MS.
[b] The components are listed in ascending order of retention time.
[c] Percent composition is peak area relative to total peak area obtained from total ion chromatogram peak report.

Table 2. Chemical composition of *Thuja plicata* leaf essential oil

Retention Index [a]	Compound name [b]	Composition (%) [c]	Chemical class
932	2-Bornene	0.08	Monoterpene
903	alpha-Thujene	1.27	Monoterpene
948	alpha-Pinene	24.85	Monoterpene
943	Camphene	1.93	Monoterpene
897	Thujene, 4(10).	1.36`	Monoterpene
943	L-beta-Pinene	1.01	Monoterpene
958	Myrcene	1.41	Monoterpene
948	3-Carene	30.68	Monoterpene
948	2-Carene	0.16	Monoterpene
1042	m-Cymene	0.08	Monoterpene
948	alpha-Pinene	0.59	Monoterpene
1018	d-Limonene	1.96	Monoterpene
976	Ocimene	0.14	Monoterpene

Retention Index [a]	Compound name [b]	Composition (%) [c]	Chemical class
998	Moslene	0.27	Monoterpene
1052	Terpinolene	4.65	Monoterpene
787	Beta-biisocrotyl	0.05	Hydrocarbon
1136	(S)-cis-verbenol	0.02	Oxygenated monoterpene
1125	alpha-Phellandren-8-ol	0.23	Oxygenated monoterpene
1137	1-Terpinen-4-ol	0.31	Oxygenated monoterpene
1197	p-Cymen-8-ol	0.04	Oxygenated monoterpene
1143	alpha-Terpineol	0.02	Oxygenated monoterpene
1117	1-Ethyl-2-methylenecycloheptanol	0.04	Alcohol
1168	4-Isopropylidene-cyclohexanol	0.18	Alcohol
1277	Bornyl acetate	0.16	Oxygenated monoterpene
1032	Terpinolene	0.06	Monoterpene
1152	Bicyclo[3.3.1]non-6-en-3-ol	0.06	Alcohol
1333	alpha-Terpinyl acetate	0.80	Monoterpene
1221	Copane	0,08	Sesquiterpene
1398	beta-Elemene	0.47	Sesquiterpene
1451	Zingiberene	0.05	Sesquiterpene
1458	alpha-Farnesene	0.06	Sesquiterpene
1494	Caryophyllene	8.60	Sesquiterpene
1398	beta-Cedrene	0.70	Sesquiterpene
1416	Thujopsene	0.20	Sesquiterpene
1579	Humulene	5.94	Sesquiterpene
1435	gamma-Muurolene	0.62	Sesquiterpene
1474	alpha-Selinene	0.82	Sesquiterpene
1507	Beta-Chamigrene	0.44	Sesquiterpene
1469	delta-Cadinene	1.14	Sesquiterpene
15.22	alpha-Elemol	0.07	Oxygenated sesquiterpene
1507	Caryophyllene oxide	0.80	Oxygenated sesquiterpene
1539	Ledol	0.45	Oxygenated sesquiterpene
1529	Humulene epoxide II	0.52	Oxygenated sesquiterpene
1543	Cedrol	4.32	Oxygenated sesquiterpene
1580	Cubenol	0.34	Oxygenated sesquiterpene
1580	alpha-Cadinol	0.05	Oxygenated sesquiterpene
1593	Eudesm-4(14)-en-11-ol	0.14	Oxygenated sesquiterpene
1469	Eudesm-4(14)-en-11-diene	0.03	Oxygenated sesquiterpene
1531	Cis-Z-alpha-Bisabolene-epoxide	0.04	Oxygenated sesquiterpene

[a] Kovats retention indices relative to n alkanes on fused silica capillary column Optima® 5MS.
[b] The components are listed in ascending order of retention time.
[c] Percent composition is peak area relative to total peak area obtained from total ion chromatogram peak report.

Fumigant toxicity of two bio-fumigants on Prostephanus truncatus

Of the two bio-fumigants that were assayed against *P. truncatus*, the toxicity of *C. citratus* was superior to that of *T. plicata;* and the toxicity was generally dose and exposure duration dependent. Application of *C. citratus* at 13.33 and 26.66 µL/L air evoked significant mortality at 36 and 48 h after treatment (HAT), whereas in *T. plicata*, significant mortality was observed when 26.66 µL/L air at 48 HAT. Application of *C. citratus* EO at 53.33 µL/L air evoked 100.0% mortality at 6 HAT, whereas 100.0% mortality was observed in *T. plicata* at 48 HAT (Tables 3 and 4).

Table 3. Fumigant toxicity of *Cymbopogon citratus* essential oil against *Prostephanus truncatus*

Treatment (µL/L air)	Percentage mortality at Hours After Treatment				
	6	12	24	36	48
Acetone	0.00±0.0a	0.00±0.0a	1.67±1.7a	1.67±1.7a	10.00±2.9a
6.66	0.00±0.0a	0.00±0.0a	6.67±3.3a,b	13.33±1.7a	21.67±1.7a
13.33	0.00±0.0a	0.00±0.0a	15.00±2.9b	15.00±2.9b	23.33±6.0a
26.66	0.00±0.0a	0.00±0.0a	25.00±5.0c	25.00±5.0b	58.33±10.1b
53.33	100.00±0.0b	100.00±0.0b	100.00±0.0d	100.00±0c.0	100.00±0.0c
ANOVA	df=4,14	df=4,14	df=4,14	df=4,14	df=4,14
Result	F=2931	F=2931	F=264.538	F=200.107	F=45.120
	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001

Means (±SE) followed by the same letter of alphabet within the column are not significantly different using SNK 5% significance level.

Table 4. Fumigant toxicity of *Thuja plicata* essential oil against *Prostephanus truncates*

Treatment (µL/L air)	Percentage mortality at Hours After Treatment				
	6	12	24	36	48
Acetone	0.00±0.0a	0.00±0.0a	1.67±1.7a	1.67±1.7a	10.00±2.9a
6.66	0.00±0.0a	0.00±0.0a	5.00±2.9a	5.00±2.9a	20.00±2.9a
13.33	0.00±0.0a	0.00±0.0a	5.00±2.9a	10.00±5.8a	25.00±5.0a
26.66	0.00±0.0a	0.00±0.0a	13.33±3.3a	28.33±6.0b	46.67±7.3b
53.33	16.67±1.7b	26.67±1.7b	50.00±2.9b	83.33±7.3c	100.00±0.0c
ANOVA	df=4,14	df=4,14	df=4,14	df=4,14	df=4,14
Result	F=100.00	F=256.000	F=51.607	F=42.948	F=68.412
	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001

Means (±SE) followed by the same letter of alphabet within the column are not significantly different using SNK 5% significance level.

The toxicity of the bio-fumigants agrees with previous authors (Buxton et al., 2014; Gariba et al., 2021) who showed various levels of bioactivity of plant products against adult *P. truncatus.* The principles of fumigation include inhalation of the EOs by insects via the spiracles. Consequently,

specific compounds had different mechanisms of action which include neurotoxicity and modulation of some enzymatic reactions. Essential oils are preferred to other botanical formulations because they are effective at low doses without detrimental effects on non-target organisms (Babarinde et al., 2015). Although, the scope of the present study did not include evaluation of the specific EO compounds, the major compounds identified in the bio-fumigants have been reported to be insecticidal. In conclusion, the study showed the potentials of the two bio-fumigants for use in the control of *P. truncatus* under simulated hermetic storage devices.

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Chemical constituents and insecticidal activity of essential oils against three stored product beetles in stored wheat

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Abstract

Laboratory experiments were conducted to determine the chemical constituents and insecticidal activity of *Chenopodium botrys*, *Citrus reticulata*, and *Callistimone citrinus* essential oils against *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), and *Tribolium castaneum* (Herbst) in stored wheat. In the *Chenopodium botrys* essential oil, Ascaridol (39.89%), Terpinyl acetate (17.85%) and Para cymene (11.78 %) were major compounds. The *Citrus reticulata* essential oil contained Limonene (69.32%), Linolool (7.83%) and Myrcen (5.38%) as major compounds. The essential oil of *Callistimon citrinus* contained 1-8 Cineole (42.03%), Alpha-terpineol (15.65%), Limonene (11.23%) as major compounds. The toxicity test found that selected essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* completely suppressed feeding and breeding as they caused full inhibition in progeny development of *S. oryzae*, *R. dominica* and *T. castaneum* after one year of treatment at 0.2 and 0.4% concentration. The essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* resulted in 100% mortality of *S. oryzae*, *R. dominica* and *T. castaneum* after 24 h treatment. The essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* had highly repellent activity against *S. oryzae*, *R. dominica* and *T. castaneum*. All tested essential oils were highly effective in terms of fumigant toxicity and mortality against *S. oryzae*, *R. dominica* and *T. castaneum* with strong repellent activity, as they contained potent insecticidal constituents.

Keywords: *Chenopodium botrys, Citrus reticulata, Callistimon citrinus,* Chemical constituents*, Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum ,* Fumigant toxicity, Mortality, Repellent activity

Introduction

The *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) are notorious

insects of stored wheat in India. Each year, enormous amounts of globally-stored human food resources are spoiled due to the infestation of several insect pests. Human food security depends not only on primary agricultural production, but also on sufficient post-harvest storage and distribution of agricultural commodities and food products. In order to search for alternatives to traditional chemical fumigants, several essential oils from the plant kingdom were evaluated for their fumigant toxicity, contact toxicity, ovicidal activity, mortality and repellent activity against insect pests of stored commodities (Kumar, 2017; Kumar et al., 2020; Rajendran et al., 2008; Tripathi et al., 2002). The objective of the present experiment was to determine the chemical constituents and insecticidal activity of essential oils against three stored product beetles in stored wheat.

Material and methods

Culture of insects

The experiments were conducted in the Postharvest Entomology Laboratory, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Pure cultures of *S. oryzae*, *R. dominica* and *T. castaneum* were reared in an incubator maintained at 27 ±1°C and 70±5% relative humidity (RH). Plastic jars of 1.0 kg capacity were used for rearing purposes. At the center of the lid a hole of 1.8 cm diameter was made and covered with 30 mesh copper wire net to facilitate air exchange in the jar. *Sitophilus oryzae* and *R. dominica* were reared on wheat kernels (variety DBW 14), while *T. castaneum* was reared on wheat flour (95%) and yeast powder (5%). The appropriate feed was filled in plastic jars and 100 adults (mixed sex) were released in each jar and maintained in the incubator. First generation adults (0-7 d old) were used for all tests.

Extraction and analysis of essential oil

Fresh leaves of *C. botrys, C. citrinus* and peels of *C. reticulata* were collected from fields during winter, and then their essential oils were extracted by steam distillation using a Clevenger apparatus (model-475/4, JSGW, Ambala Cant, India).

The analysis of chemical constituents in these essential oils was carried out using a gas chromatograph (HP-5890 series II, Rtx-1-MS-889068, USA), equipped with a flam ionization detector (FID) and an electronic pressure control (EPC) injector. A polar HP-innowax (PEG) column (30 m × 0.25 mm × 0.25 µm film thickness) and a polar HP-5 column (30 m × 0.25 mm coated with 5% phenyl methyl silicone and 95% di methyl polysiloxane, 0.25µm film thickness) from Agilent were used. Carrier gas (N_2) flow was 30.0 mL/min and the split ratio was 80:20. Analysis was performed using the following temperature program: oven kept isothermally at 70°C for 2 min, increased from 70 to 180°C at the rate of 4°C/min for 8 min, and from 180 to 230°C at the rate of 6°C/min for 12 min. Injector and detector temperature were held at 270 and 280°C, respectively. The GC-MS analysis was made using the HP-5972 Mass Spectrometer with electron impact ionization (70 eV) coupled with the HP-5890 series 2^{nd} Gas Chromatograph. Helium was used as the carrier gas with a flow rate of 1.21 mL/min and split ratio of 80:20. Scan time and mass range were 0.50 s and 40-850 m/z, respectively. The essential oil volatile compounds were identified by calculating their retention time relative to (C9-C18) n-alkenes, from data for authentic compounds available in the literature, and also by matching their mass spectrum fragmentation patterns with corresponding data stored in the mass spectra library of the GC-MS data system (NIST) and other published mass spectra. The percentage compositions of these essential oils were calculated according to the area of chromatographic peaks.

Fumigant toxicity test

The fumigant toxicity of these essential oils against *S. oryzae*, *R. dominica,* and *T. castaneum,* were evaluated on untreated and graded wheat seed, variety DBW-14. The experiment was conducted in plastic jars with 1 kg capacity in which 500 g of wheat with a moisture content of 13.5% (wb) were filled. Twenty adults of each test insect were released into each jar. One week after the insect introduction, the filter paper soaked with essential oil was inserted into the jars and jars were sealed for 12 mo. There were three replicates for each treatment. After 12 mo, the grains were analyzed to record percent inhibition and the number of adults that had emerged. The data were analyzed by STPR 3 software with log (X+1) transformation.

Mortality test

The experiment was conducted on *S. oryzae*, *R. dominica,* and *T. castaneum* to find out the actual time required for the essential oils to cause 100% insect mortality. The experiment was performed under controlled conditions at 27±1°C and 70±5% RH. Fifty grams of wheat grain with 13.5% moisture content was filled in a 100 mL plastic vial. Four sets of these vials were prepared for test insects to record their mortality after 6, 12, 18 and 24 h. Ten adult insects (0-7 d old) of *S. oryzae*, *R. dominica*, and *T. castaneum* were released in each vial. After 24 h of releasing the adults, the required quantity of oil soaked on absorbent mats was inserted in each vial, after which each one was closed and sealed with paraffin wax. These treatments were replicated three times. Observation was recorded after every 6 h of treatment up to 24 h.

Repellency test

A repellency test was conducted as per the method of Talukdar and Howse (1993). Petri dishes, 9 cm in diameter, were used to confine insects during the experiment. The essential oils were diluted in ethanol to 1.0, 2.0, 3.0 and 4.0% concentrations and pure ethanol was used as a control. Filter paper of 9 cm diameter was cut into two halves and 1 mL of each concentration was applied separately to one half with a micropipette. The other half was treated with 1mL of pure ethanol. Both the treated halves were then air dried to evaporate the solvent completely. A full disc was carefully recreated by attaching each treated half with transparent tape. Care was taken so that the attachment did not prevent free movement of insects from one half to the other, and then this filter paper was placed in a petri dish. Twenty insects were released in the centre of each filter paper disc, and then covered with the petri dish lid. Three replications were used and the experiment was repeated twice for each test insect. The calculation of % repellency was done as per Abbott (1925) and the repellent class was categorized as per the scale of Roy et al. (2005).

Result and discussion

Chemical constituents of C. botrys essential oil

There were 31 compounds identified in *C. botrys* essential oil (Fig.1). These included Ascaridole (33.89%), Terpinyl acetate (17.85%), Para cymene (11.78%), Kemitracin (5.42%), Cyclo octanone (4.31%), Linalool acetate (2.92%), 1-Octadecene (2.58%), Doecenol (2.21%), Phytol acetate (2.12%), Thymol (2.06%), 1-Propanol (1.90%), p Cumic aldehyde (1.41%), Heptadecane (1.35%), Phthalic acid (1.30%), Precocene (1.16%), Hexonic acid (1.14%), Cyclobutane ethanol (1.02%), and other remaining compounds in trace amounts.

The pinene major compound in essential oil of *M. koenigii*, has been reported to be toxic to several stored grain insects (Kordali, 2006). These oxygenated monoterpenes have strong fumigant action against *S. oryzae* (Lee et al., 2001).

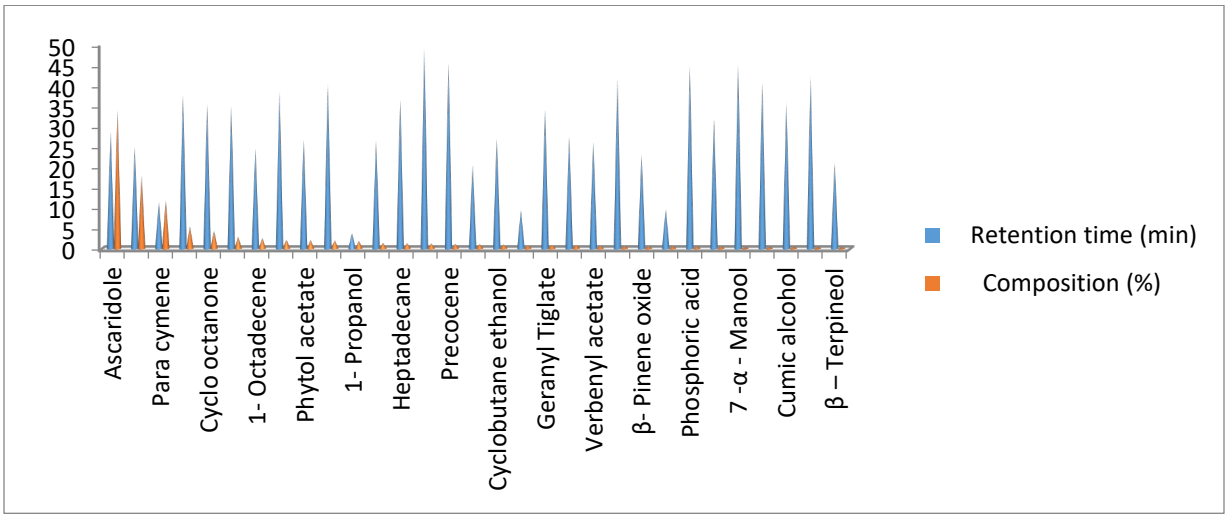

Fig. 1. Chemical composition of *C. botrys* essential oil.

***Chemical constituents of C. reticulata* essential oil**

There were 18 compounds identified in *C. reticulata* essential oil (Fig. 2). These included Limonene (69.32%), Linalool (7.83%), Myrcene (5.38%), α-Terpineol (3.35%), Sabinene (2.16%), α-Pinene (2.11%), Octanol (1.74%), Geranial (1.13%), Carene (1.05%), and other remaining compounds in trace quantities. Tripathi et al. (2002) reported the insecticidal activity of Limonene (a major constituent of citrus oil) against *T. castaneum* to be effective in inhibiting progeny production.

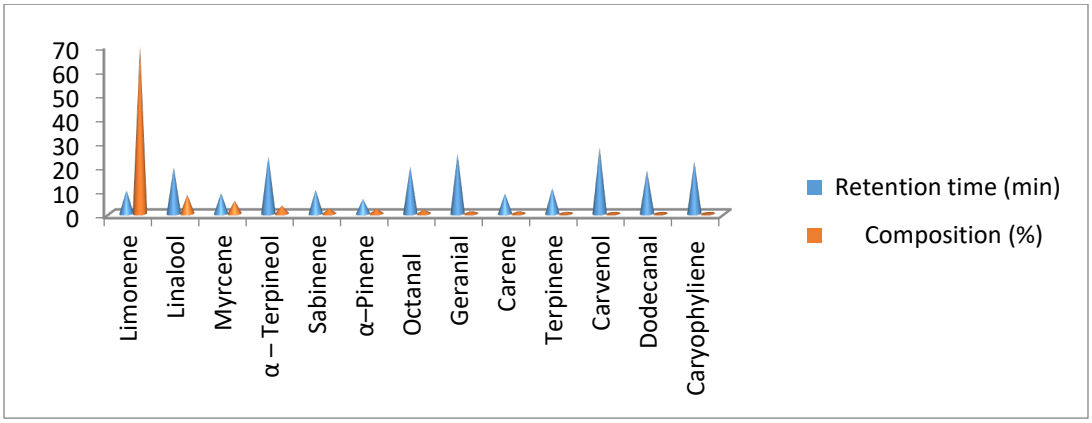

Fig. 2. Chemical composition of *C. reticulata* essential oil.

Chemical constituents of C. citrinus essential oil

There were 15 compounds identified in the essential oil of *C. citrinus* (Fig. 3). These included 1,8-Cineol (42.03%), α-Terpineol (15.65%) and Limonene (11.23%) as major compounds, followed by α-Pinene (10.47%), Cymene (5.93%), α-Phellandrene (3.21%), Linalool (3.05%), Myrcene (1.5%), and other remaining compounds in trace quantities.

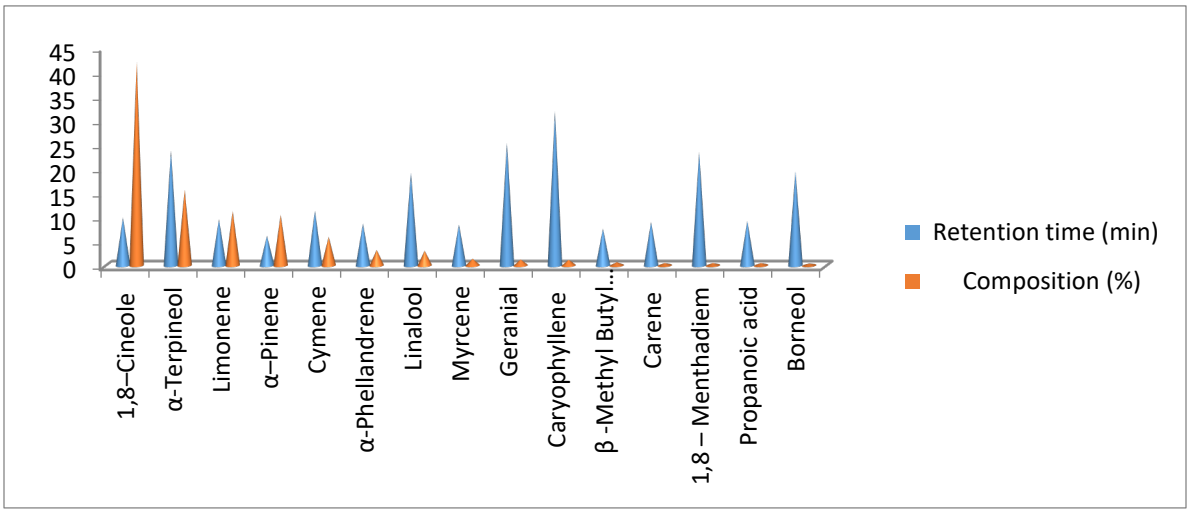

Fig. 3. Chemical composition of *C. citrinus* essential oil.

Fumigant toxicity of essential oils against test insects

The essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* caused full inhibition (zero emergence) in progeny development of *S. oryzae*, *R. dominica*, and *T. castaneum* after one year of treatment at 0.2 and 0.4% concentration as compared to untreated controls with emergence of 14.5, 65.0 and 43.0%, respectively. The essential oils of *M. koenigii*, *C. reticulata*, *C. citrinus* either alone at 0.2% or two component combinations were highly effective against *S. oryzae* and *R. dominica* (Kumar et al., 2018). The essential oils of *C. botrys, C. reticulata, L. camara, P. roxburghii* at 0.1, 0.2, 0.3, and 0.4% were highly effective against *T. castaneum* seeing as full inhibitions were caused compared to the untreated control (Kumar et al., 2021).

Mortality test against test insects

The average mortality caused by the essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* against *S. oryzae*, *R. dominica*, and *T. castaneum* after 24 h of treatment was 100% as compared to the untreated control. Tunc et al. (2000), observed fumigant toxicity with 100% mortality caused by the essential oil of Cumin (*C. cyminum*) against the eggs of *T. confusum* and *Ephestia kuehniella* (Z.).

Repellency test against test insects

The essential oils of *C. botrys*, *C. reticulata* and *C. citrinus* at 1.0, 2.0, 3.0, and 4.0% showed strong repellency against *S. Oryzae*, *R. dominica*, and *T. castaneum* (Table 1.) because of their potent insecticidal constituents. Joshi and Tiwari (2019) reported that *C. limetta, M. koenigii, C. citrinus, C. longa* and *P. roxburghii* at 3% concentration exhibited 92.51, 83.48, 92.75, 98.20 and 89.78% mean repellency against *R. dominica*, respectively.

Conclusions

All tested essential oils were highly effective in terms of fumigant toxicity and mortality against *S. oryzae*, *R. dominica* and *T. castaneum* with strong repellent activity, as they contained potent insecticidal constituents.

Table 1. Mean repellency of essential oils against tested insects

Essential oil	Conc. %	Mean repellency after 24 h of treatment		
		S. oryzae	*R. dominica*	*T. castaneum*
C. botrys	1.0	96.01	95.32	98.39
	2.0	97.10	96.19	100.00
	3.0	98.66	97.83	100.00
	4.0	99.53	99.10	100.00
C. reticulata	1.0	94.82	93.16	97.84
	2.0	97.31	94.50	100.00
	3.0	98.56	95.87	100.00
	4.0	99.95	97.54	100.00
C. citrinus	1.0	96.02	94.47	98.65
	2.0	96.53	95.31	100.00
	3.0	97.81	96.99	100.00
	4.0	98.89	98.64	100.00

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Evaluation of effect of ozone on two species of stored grain insects *Tribolium castaneum* and *Rhyzopertha dominica* and grain quality

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ABSTRACT

Ozone is a highly oxidative gas. It is generally recognized as safe (GRAS) by the United States Food and Drug Administration, and it is approved for use as an antimicrobial agent on processed food, including meat and operation theatre. The use of ozone to manage stored-grain insets has been explored more than a decade ago. This study reported the results on effects of evaluation of ozone on two species of stored grain insects *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) with and without wheat and grain quality. All four stages of two species of insects were easily killed without wheat, 100% mortality achieved using 700 ppm ozone for 1.8-2.3, 2.5-3, 3-3.5 and >4 h exposure for adults, larvae, eggs and pupae, respectively. However, unlike without grain, with grain 100% mortality needed to extend the exposure time 8-10 times longer, depending on the location and distance of ozone penetration. There was no effect of ozone on wheat quality, such as starch, protein, moisture content and bulk density.

Keywords: Fumigant, Ozone, *Tribolium castaneum* , *Rhyzopertha dominica,* Mortality, Wheat, Quality

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Fumigant toxicity of garlic essential oil with the combined effect of ozone gas and determination of phytochemical residues from the treated rice weevil *Sitophilus oryzae* and wheat grains

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Abstract

Ozone gas (OEG) is a strong oxidizing agent and it has been recognised as potential fumigant to manage stored product insect pests. The present study was carried out to understand the ozonation effect on garlic essential oil (GEO) fumigation and residual characteristics on the fumigated food grain and insect pests. Fumigant toxicity of GEO and OEG against *Sitophilus oryzae* adults were evaluated using 1 kg of wheat grain at 30 to 120 µL/kg and 0.2 to 2.4 g/h concentrations, respectively. In order to saturate GEO in stored wheat grain, OEG was passed at 1 g/h flow rate along with GEO at 225 µL/kg concentration. Mortality of *S. oryzae* adults was observed and phytochemical residues were collected from the treated grains and beetles at different exposure durations. After 72 h of exposure, 64.10 µL/kg of LC_{50} value was obtained for GEO fumigation against *S. oryzae* adults. Fumigant toxicity of GEO was increased with the combined treatment of OEG at specific flow rate of 1 g/h and 100% mortality was achieved ($P \leq 0.05$) within 72 h exposure. In gas chromatography analysis, diallyl sulfide, diallyl disulfide and diallyl trisulfide compounds were detected as major fumigant residues from the treated *S. oryzae* adults and wheat grain. The residual level of phytochemical fumigants was remarkably reduced in GEO+OEG treatment as compared to GEO alone treatment. The study results suggests that GEO fumigation with the combination of OEG at mild dose could be a promising method in order to prevent *S. oryzae* infestation in stored wheat grain.

Keywords: Ozone, Garlic essential oil, *Sitophilus oryzae*, Phytochemical residue, Fumigation, Eco-friendly insecticide

Introduction

Plant essential oils and phytochemical volatiles have been widely recognized as promising alternative natural sources to conventional gaseous fumigants against stored product insects (SPI). However, the vapour pressure and diffusion properties of plant volatile molecules are less (≤1 mmHg at 20°C) as compared to gaseous fumigants with respect to the practical applications (Dambolena et al., 2016). Garlic (*Allium sativa*) essential oil (GEO) recorded as a one of the potential bio-fumigant against several SPI (Demeter et al., 2021). In-order to enhance the fumigant action, GEO was investigated in combination of carbon dioxide (CO_2) and 4.9-fold increased fumigant toxicity was recorded against *Tribolium confusum* (Herbst) as compared to GEO alone treatment (Işikber, 2010). Ozone (O_3) gas (OEG) is also a potential fumigant and its insecticidal activities have been reported against a number of SPI species (Kells et al., 2001; Iskiber and Oztekin, 2009; Mishra et al., 2019). According to Graham (1997), OEG had been recognized as safe for application in food processing sector under GRAS status. Rajendran and Sriranjini (2008) suggested that phytochemical fumigant studies with carrier gases are needed to validate the potentiality of essential oil based bio-fumigants for SPI control. The *Sitophilus oryzae* (Linnaeus), commonly known as rice weevil is a major SPI that causes severe damages in a wide range of cereals including rice, wheat, maize. During the past few decades, a wide range of plant essential oils and phytochemical volatiles have been extensively studied for the control of *S. oryzae*. In our recent study, we have investigated the fumigant toxicity of selected essential oils against *S. oryzae* adults and analyzed the phytochemical residue profiles on the fumigated rice grain (Vendan et al., 2017). In another study, we have evaluated the persistence and ingestion characteristics of five different phytochemical volatiles in *S. oryzae* adults (Sahu et al., 2021). The present study was aimed to investigate the fumigant toxicity of combination of GEO with OEG and to determine the phytochemical residues on the treated wheat grain and *S. oryzae* adults.

Materials and methods

Essential oil and chemicals

The garlic essential oil, HPLC-grade n-hexane solvent and other chemicals were procured from Sigma-Aldrich Chemicals Pvt. Ltd., India.

Insect culture

Different species of SPI were regularly maintained in the insectary with controlled temperature (30 ± 2°C), relative humidity (75 ± 5%) and photoperiod conditions (13:11; light: dark). From the stock culture, a sub-culture of *S. oryzae* was prepared and maintained with wheat grain. Newly emerged (one-week-old) *S. oryzae* adults in the sub-culture were used for fumigation bioassays.

Fumigation bioassays

Fumigant toxicity of GEO was evaluated against *S. oryzae* adults at 30, 60 and 120 µL/kg concentrations with 1 kg of wheat grain in 1.5 L container under airtight condition. Thirty individuals of *S. oryzae* adults were released into each container filled with wheat grain. Garlic essential oil was loaded on the Whatman No 1 filter paper strips (15 x 1 cm) and inserted at the center of wheat grains inside the container. Control sample was maintained without GEO treatment. Three replicates were maintained for each concentration treatment. Mortality of *S. oryzae* adult was recorded at 24, 48 and 72 h of treatment durations.

Fumigant toxicity of OEG was evaluated against *S. oryzae* adults at 0.2, 1.0 and 2.4 g/h flow rates with 1 kg of wheat grain in 1.5 L container under airtight condition. OEG was generated using atmospheric oxygen with the help of an ozone generator (Model A5G, Faraday Ozone Products Private Limited, Coimbatore, India) and passed into the fumigation container containing wheat grains and inoculated 30 individuals of *S. oryzae* adults. The OEG was passed into experimental containers through the inlet (bottom of the container) and the remaining OEG was liberated through the outlet of the container (top of the container). Ozone gas fumigation was carried out for 1.0 h and the fumigated grain was stored up to 72 h for assessing the beetle mortalities. Control sample was maintained without OEG treatment. For each treatment condition, three replicates were maintained with control samples. After 24, 48 and 72 h of treatment, *S. oryzae* adults were separated from the wheat grain using sieves (Test Sieves, Jayant Scientific India) and the beetle mortalities were recorded.

Fumigant toxicity of GEO+OEG combination (225 µL/kg of GEO and 1.0 g/h flow rate of OEG) was evaluated against *S. oryzae* adults at 0.25, 0.50 and 1.00 h exposures with 1 kg of wheat grain in 1.5 L container. Concentration of GEO and flow rate of OEG were determined based on the obtained LC_{90} values and flow rate option in the OEG generator. Similar to the above method (OEG fumigation bioassay), GEO+OEG fumigation experimental set up was prepared and GEO was loaded on the Whatman No 1 filter paper strip (15 x 1 cm) and placed at the end of the OEG inlet connecting to the 1.5 L container containing wheat grains and inoculated 30 *S. oryzae* adults. Garlic essential oil exposure without OEG treatment vice versa was maintained for comparative analysis and control sample was maintained without any fumigant treatment. Three replicates were maintained for each concentration and treatment. Treated beetles were separated and mortality was recorded at 24, 48 and 72 h of treatment duration, similar to the above bioassays.

Fumigant residue analysis

Fumigation experiments were carried out similar to the above method (GEO+OEG fumigation bioassay) and the fumigated grain and beetles were used for fumigant residues analysis. After 72 h of GEO and GEO+OEG fumigation treatments, three replicates of each treatment were pooled into single samples. Randomly, 50 g of wheat grain and 30 individuals of *S. oryzae* adults were separated from the pooled samples. Both wheat grain and beetle samples were soaked for 1 h in 100 and 5 ml of HPLC-grade hexane, respectively. Then the hexane extract was collected by filtration using Whatman No 1 filter paper and all the extracts were concentrated up to 1 mL by air-drying. Final concentrates were filtered using PTFE syringe filter (0.2 µm pore size) and all the hexane extract samples were stored at -20°C till further use.

Gas Chromatography analysis

Phytochemical fumigant residues were analyzed using a gas chromatograph coupled flame photometric detector (GC-FPD) (Agilent 7890B, version 2019) system. HP-5 capillary column (30 m x 0.32 mm x 0.25 µm film thickness) was used and split less mode of injection was followed for analyses of all the samples. Nitrogen was used as a carrier gas in a constant flow mode (25 mL/min). About 4 µL of hexane extract samples were injected into the column and the following specifications were employed in the GC-FPD system for analysis; initial temperature at 150°C for 1 min, ramped to 210°C at the rate of 20°C/min, and ramped again at the rate of 4°C/min to 300°C for the last 10 min. The compounds detected in the tested samples were identified by comparing the respective retention times of the standard and control samples.

Data analysis

Percentage corrected mortality of *S. oryzae* adults was calculated using Abbott's correction formula (Abbott, 1925). The significance of results was analysed by one-way ANOVA and the effective treatments were separated by Tukey's multiple range test. Differences between means were considered significant when $P \leq 0.05$. The mortality data were further used for Probit analysis to estimate the LC_{50}, LC_{90}, LT_{50} and LT_{90} values of treatments (Finney, 1971). ANOVA and Probit analysis were performed using the SPSS (16.0 version) software program.

Results and discussion

In the present study, combined treatment of GEO with OEG was investigated for evaluating synergistic fumigant toxicities against *S. oryzae* adults. In the first phase of the study, fumigant toxicity of GEO and OEG was evaluated against *S. oryzae* adults with 1 kg of wheat grain at three different concentrations (30, 60 and 120 µL/kg of GEO and 0.2, 1.0 and 2.4 g/h of OEG). After 72 h of treatment, 24.3 and 41.8% of mortality were recorded for 30 and 60 µL/kg of GEO concentrations, respectively, whereas at 24 and 48 h treatments mortality was nil (Fig. 1a). Remarkably, 76.5% of mortality was observed for 120 µL/kg of GEO concentration with 1.0 kg of wheat grain. About 64.10 and 224.24 µL/kg of LC_{50} and LC_{90} values were recorded respectively, at 72 h of fumigation treatment in the present study. Previously, Chaubey (2016) recorded 0.24 µL/cm^3 of LC_{50} value for GEO against *S. oryzae* adults for 48 h of fumigation exposure without food. Most recently, Demeter et al. (2021) studied the fumigant toxicity of twenty-five different essential oils with 8 g of wheat grain against *S. granarius* (Linnaeus), and they highlighted that GEO was most toxic with 0.64% for LC_{50} value at 24 h exposure. In the present study in OEG fumigation, no mortality was observed for 0.2 g/h exposure during 24 to 72 h of treatment durations (Fig. 1b). Remarkably, 100% mortality of *S. oryzae* adult was observed for 1.0 and 2.4 g/h of OEG exposures at 72 h treatment duration. The Probit analysis results revealed 0.45 g/h for LC_{50} and 0.60 g/h for LC_{90} of OEG against *S. oryzae* adults at 72 h of treatment duration (Fig. 1b). Previously, Kells et al. (2001) reported 100% mortality of *S. zeamais* Motschulsky adult for 50 ppm of OEG fumigation on 8.9 tonnes of maize at 72 h of treatment.

In the second phase of the study, fumigant toxicity of combination of GEO+OEG (225 µL/kg of GEO and 1.0 g/h flow rate of OEG) was evaluated against *S. oryzae* adults with 1 kg of wheat grain at 0.25, 0.50 and 1.00 h of fumigation exposures. In GEO and OEG alone treatments, mortalities were not observed for 0.25 and 0.50 h fumigation exposures up to 72 h of treatment duration (Fig. 1c). For 1 h fumigation exposure, 30 and 100% mortality rates were recorded for GEO and OEG alone treatments at 72 h treatment duration. Fumigant toxicity of combination of GEO+OEG was directly proportional to the fumigation exposures and treatment durations (Fig. 1c). Remarkably, 100% mortality of *S. oryzae* adult was recorded for 1 h of fumigation of GEO+OEG combination and OEG alone at 72 h of treatment. Interestingly, fumigant toxicity of GEO (225 µL/kg) was increased with the combination of OEG (1.0 g/h flow rate) treatment and 96.66% of mortality of *S. oryzae* adult was recorded within 48 h of treatment period compared to GEO and OEG alone treatments with the significance of $P \leq 0.05$. About 0.28 and 0.74 h of least LT_{50} & LT_{90} values were recorded for GEO+OEG combination at 72 h of treatment duration, respectively (Fig. 1c).

Previously, Işikber (2010) studied the combined effect of GEO with CO_2 treatment and 98.3% of mortality of *Tribolium confusum* adult with 0.38 µL/L of LC_{50} value was recorded within 24 h of exposure period without food. The present study results suggested that GEO+OEG combination treatment was better than GEO and OEG alone treatments against *S. oryzae* adults in with food condition.

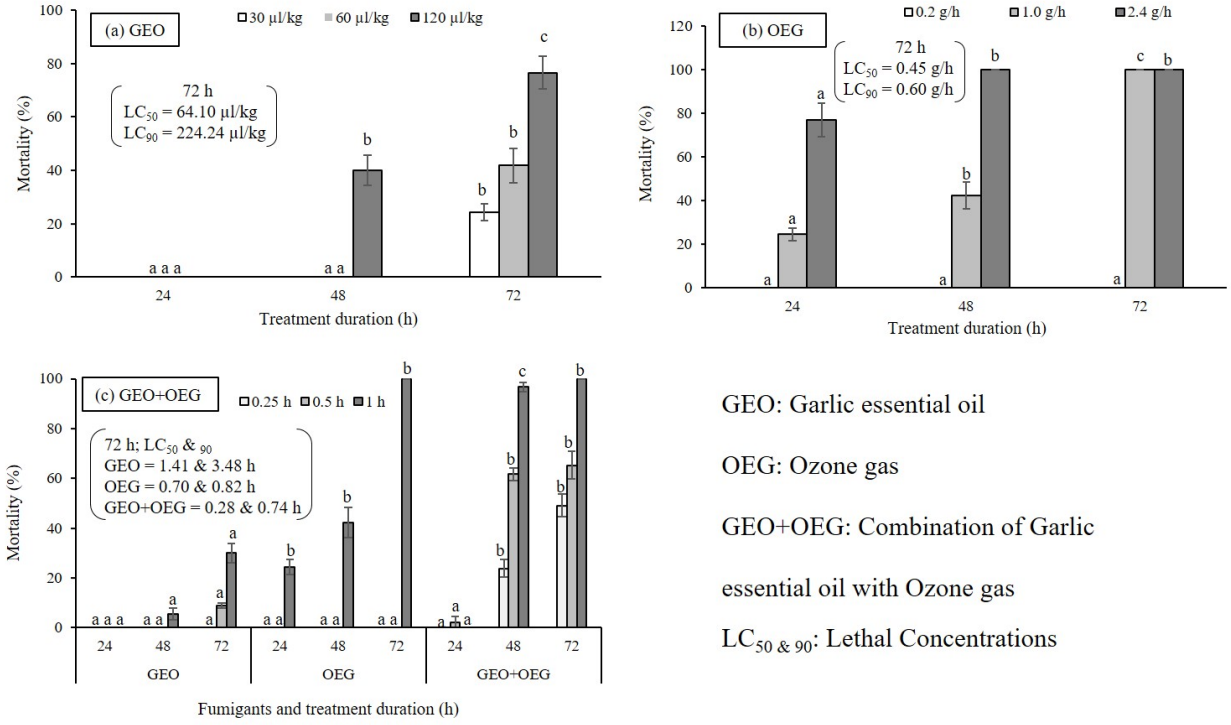

Fig. 1. Mortality of *Sitophilus oryzae* adults due to the fumigation effect of garlic essential oil, ozone gas and combination of garlic essential oil with ozone gas.

Each vertical bar is a mean of three replicates with standard error (% Mean ± SE). (a & b). Means within a concentration (60, 120 and 240 µL/kg) and between treatment duration (24, 48 and 72 h), different letters are significantly (P≤0.05) different from each other as determined by Tukey's test. (c). Means within a fumigant exposure (0.25, 0.50 and 1.00 h) and between different treatment duration (24, 48 and 72 h), different letters are significantly (P≤ 0.05) different from each other as determined by Tukey's test.

In this present study, phytochemical fumigant residues were examined and the diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS) were detected as major residues in the GEO alone and GEO+OEG combination treated wheat grain and *S. oryzae* adults (Fig. 2 and Table 1). Remarkably, residual level of phytochemicals was decreased (99.97 and 99.98% of DADS and

DATS residues, respectively) in GEO+OEG treated wheat grains compared to GEO alone treated wheat grain. According to Law and Kiss (1991), OEG may not sediment as fumigant residue on the surface of fumigated samples and hence fumigant residues were not examined for OEG treatment in the present study.

The residual property of phytochemical fumigants may be linked to the polar surface area of fumigant molecules (Vendan et al., 2017). Interestingly, in the present study phytochemical residual level was decreased (99.98 and 99.97% of DADS and DATS residues, respectively) in GEO+OEG treated wheat grains when compared to GEO alone treated wheat grains. In *S. oryzae* adults, DADS level was increased from 39.98 peak area (GEO treatment) to 585.27 peak area (GEO+OEG treatment), which might be attributed to the enhanced mortality of *S. oryzae* adults. More recently, it was reported that phytochemical fumigant persistence on the body surface of *S. oryzae* adults was positively correlated with the fumigant toxicity and fumigation exposure (Sahu et al., 2021).

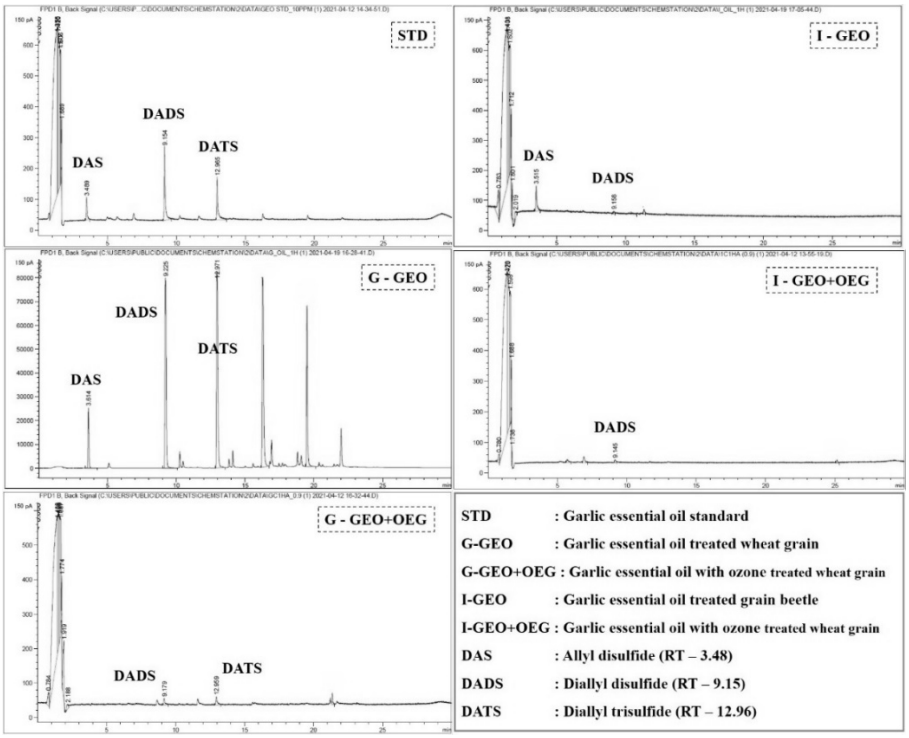

Fig. 2. GC-FPD chromatograms of phytochemical residues detected from the treated wheat grain and *Sitophilus oryzae* adults.

Table 1. Phytochemical residual level on treated wheat and *Sitophilus oryzae* adults.

RT (min)	Compound	Residue Level (Peak area[#])					
		GEO		OEG		GEO + OEG	
		Grain	Insect	Grain	Insect	Grain	Insect
3.48	Diallyl sulfide	532.44	46.90	nt	nt	-	-
9.15	Diallyl disulfide	2572.54	39.98	nt	nt	0.61	585.27
12.96	Diallyl trisulfide	2812.55	-	nt	nt	0.78	-

RT= Retention Time, GEO= Garlic essential oil, OEG= Ozone gas, GEO+OEG= Combination of Garlic essential oil with Ozone gas. [#]Peak area (pA*s) per gram of wheat grain and beetle, nt= not tested.

Conclusion

The current study revealed that the fumigant toxicity of GEO against *S. oryzae* adults had significantly increased during the combination treatment of GEO+OEG. Additionally, the residual level of phytochemical fumigants was reduced in GEO+OEG treatment as compared to GEO alone treatment. It was also noted that the trace levels of DAS, DADS and DATS residues detected in the treated wheat grain were safe for consumption with reference to GRAS status. This study results could be used for the further studies for the development of environment-friendly bio-fumigant process for the safe storage of food grains from insect pests.

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Ethyl formate: review of a rapid-acting fumigant

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Abstract

Ethyl formate (EF) is a historical flammable liquid fumigant to disinfest dry fruits, with uses extended to horticulture and cereal grains. Enhanced efficacy is achieved when EF is applied in a non-flammable vaporized carbon dioxide (CO_2) mixture. Ethyl formate is an effective bulk grain fumigant (complete control at 70 g/m^3 in 24 h) with sorption issues being accommodated by rapid dispensing ensuring uniform distribution. Recent review found 78 insect species controlled by EF, albeit at different rates or exposure times. Ethyl formate is registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA) to control 41 species of these pests. Current APVMA Permit allows for in-transit EF fumigation. Registration of EF has not kept pace with recent research. The brown marmorated stink bug (EF probit 9 efficacy reported as 10.5 g/m^3, 10°C , 4 h), khapra beetle, tomato potato psyllid, and tramp ants are candidates for EF fumigation.

The EF usually requires relatively high dose (70 g/m^3); however, its predominant attribute, like methyl bromide (MBr), is short exposure times (i.e., hours not days). Ethyl formate can be used at lower temperatures than other fumigants. The volatile and flammable EF is a proven fumigant and a candidate for replacing ozone depleting MBr. Unlike other MBr alternatives, EF kills insects rapidly and has advantages for worker and environment safety. Ethyl formate (Threshold Limit Value, TLV = 100 ppm) is an effective and less toxic fumigant for horticulture and stored product pests, including during transit on road and sea. Recent research identified EF as a candidate alternative fumigant for MBr (TLV=5 ppm) in the elimination of exotic quarantine pests. An effective low dose of EF allows for non-flammable on-site EF mixing to be competitive with the existing MBr quarantine fumigation. In addition, other benefits include environmental release (unlike MBr, EF is not an ozone depletor and has limited life in the atmosphere).

Keywords: Quarantine fumigation, Non-flammable fumigants, On-site mixing fumigant, Methyl bromide, Alternative fumigants, Food grade fumigant

Introduction

Ethyl formate (EF) is a historical liquid fumigant (1929) to disinfest dry fruits and its use has been extended to horticulture and cereal grains. Ethyl formate also has a history of safe use as a food additive. Interest in EF as a fumigant declined following the introduction of carbon disulphide and

subsequently of methyl bromide (MBr) and phosphine (PH_3) in the 1950's (Ren and Mahon, 2006). However, in 2002, carbon disulphide was deregistered for use as a fumigant in Australia (Ren and Mahon, 2006). Methyl bromide is the fumigant with the widest range of applications (Bell, 2000) but was due to be phased out for stored commodities after 2005 (Ren and Mahon, 2006). There are restrictions on the use of MBr as mandated by the Montreal Protocol on substances that deplete the ozone layer (TEAP, 2000). The use patterns of fumigants continue to change because there are continuing pressures on fumigants due to registration requirements, atmospheric emissions controls, fears on safety or human health, the incidence of resistance. These changes are occurring as the world expects increasingly high standards of pest control in international trade (Bell, 2000). The registrations of EF have not kept pace with recent research due to the existing preference for other fumigants.

Ethyl formate background

Ethyl formate is also known as ethyl methanoate, formic acid ethyl ester, ethyl formic ester, and formic ether (Merck Index, 1989; Ryan and De Lima, 2012). Ethyl formate is present naturally in soil, water, vegetation, and in a range of plant and animal products. These products include food grains, fruits, vegetables, beer, wine and spirits, tuna, meat, mussels, milk, cheese and bread (Desmarchelier et al., 1999; Ren and Mahon, 2006; Ryan and De Lima, 2012). Ethyl formate is a central nervous system depressant (Ryan and De Lima, 2012). Ethyl formate can irritate eyes, skin, mucous membranes and the respiratory system, particularly above 100 ppm (Ryan and De Lima, 2012; Safe Work Australia, 2019). The gas is weakly pungent at 100 ppm and annoyingly pungent at 1,000 ppm (Safe Work Australia, 2019). Agarwal et al. (2015) found that EF had a pleasant aromatic odour. Ethyl formate has the characteristic smell of rum and is partly responsible for the flavour of raspberries (Ryan and De Lima, 2014). Commercially, EF is used in the manufacture of artificial rum, as a flavour for lemonade and essences, as a fungicide, larvicide and as an organic solvent (Merck Index, 1989; Safe Work Australia, 2019). In industry, EF is used as a solvent for cellulose nitrate, cellulose acetate, oils and greases (Ryan and De Lima, 2012).

The oral LD_{50} for rats and rabbits is >1,800 mg/kg and TLV 100 ppm (Safe Work Australia, 2019). Ethyl formate is not classified as a carcinogen (Safe Work Australia, 2019) and holds "generally regarded as safe" (GRAS) status with the US Food and Drug Administration (FDA) for its use as a food additive (Ducom, 2006; Haritos et al., 2006). EF has the advantage of a very short fumigation period, low toxicity to mammals and the environment, and a rapid breakdown with minimum or no residues (Coetzee et al., 2019; Haritos et al., 2006). Some pests are controlled after one hour of fumigation and one hour of venting (Bikoba et al., 2019).

Ethyl formate uses

The low toxicity EF can require relatively high dose (70 g/m^3); however, its predominant attribute, like MBr, is short exposure times (i.e., hours not days). Mixing with an inert gas is required to achieve a non-flammable mixture. Unlike PH_3, EF kills insects rapidly and its residues break down to naturally occurring products such as formic acid and ethanol (Desmarchelier et al., 1999; Ren and Mahon, 2006). In Australia, there are no Maximum Residue Level required for EF when used for baled hay, as a fumigant for cereals, pulses and canola and associated storage structures and machinery, as a fumigant for cocoa, and as a post-harvest fumigant of fruit and vegetables (Reuss et al., 2001; Ren and Mahon, 2006). Ethyl formate is rapidly sorbed and degraded by most

commodities where they have high moisture or are warm (Ren and Mahon, 2006). Itis effective on many horticulture insect pests. Additionally, EF is efficacious on stored product insects and has synergist effects when applying non-flammable EF/CO_2 vapour on stored grain insects (Haritos et al., 2006). Ethyl formate was an effective bulk grain fumigant with sorption issues being mitigated by rapid dispensing (Dojchinov et al., 2010). Ethyl formate can be removed from rice products through unforced ventilation (Reuss et al., 2001).

Ethyl formate is registered in Indonesia, Israel, Malaysia, New Zealand, Philippines and South Korea (Wolmarans et al., 2017; Simpson et al., 2007). There are three EF products registrations with the Australian Pesticides and Veterinary Medicines Authority (APVMA), one as a 98% liquid product and two with EF/CO_2 liquefied gas mixtures (Ryan and De Lima, 2014). Ethyl formate is registered by APVMA to control 41 pest species. Additionally, the APVMA has issued permits (Permit 87993) for the use of EF for the movement of foodstuffs and general goods to the environmentally sensitive Barrow Island in Western Australia. The application rate must be sufficient to ensure that the concentration × time (Ct) is greater than 270 g.h/m^3. Permit 86953 allows in-transit fumigation with EF at 90 g/m^3 for 6 h.

To minimize flammability, an EF/CO_2 in a 1:5 non-flammable mixture in high pressure industrial gas cylinders was patented (Ryan and Bishop, 2003; Haritos et al., 2003; Damcevski et al., 2003). Addition of carbon dioxide to the EF significantly enhanced efficacy of the fumigant (Haritos et al., 2006). Also, CO_2 accelerates the penetration of insecticides into insects' spiracles (Ryan and De Lima, 2014). Since about 2000, EF was effective in controlling a range of insects in citrus, grapes, strawberries, bananas, sweet corn, stored cereals, pulses, dates and fodder crops (Ryan and De Lima, 2014).

Treatment periods are frequently 1-2 h (Simpson et al., 2007; Agarwal et al., 2015). Ethyl formate is efficacious at low fumigation temperatures (e.g., 9.2°C); these temperatures are not recommended for fumigation with MBr or some other fumigants (Tarri et al., 2007). Cold (5°C) Navel oranges did not need to be warmed prior to treatment with EF and CO_2 to treat bean thrips (Bikoba et al., 2019) hence prolonging fruit shelf life and minimizing handling costs and time. Chhagan et al. (2013) also treated apricots at 5°C without adverse effect on fruit. De Lima (2011) tested EF successfully in temperatures ranging from 10°C to 20°C.

On farm fumigation

The Australian Commonwealth Scientific and Industrial Research Organization (CSIRO) were early adopters of EF fumigation with Allen and Desmarchelier (2000) initiating the treatment of grain sampling equipment at grain export terminals. Another significant input was CSIRO Entomology (2013) report on the GRDC project (#CSE009) which detailed EF/CO2 as a fast insecticide fumigant for small grain storages (50-200 t). The data generated in this project was used to extend the APVMA pesticide registration approval. This report concluded EF could be used as a PH_3 resistance management tool. The availability of effective alternative treatments is a method of supporting PH_3 use in the industry. Ethyl formate is completely effective against PH_3 resistant insects. Ethyl formate acts rapidly to kill insects. Application takes less than 15 min and fumigation takes as little as 3 h. Ethyl formate sorption is minimized by rapid dispensing, one gas exchange of EF in 12 min. Venting of the gas at the end of fumigation takes less than 2 h. Grain can then be safely out loaded without a withholding period. This means growers who want to sell

grain quickly but find it is infested can treat and outload in less than a day. The grain can be immediately out loaded for sale and use for human and animal purposes after venting of excess fumigant. Silo requirements include an aeration fan and some level of sealing. This CSIRO project developed an application technology that is specifically designed for small scale silos (50-200 t) thereby directly benefiting growers who choose to store on-farm. Mixed age cultures of three stored grain insects were chosen for the major efficacy studies based on the frequency these insects are found in storages, the economic damage they cause to stored grains and their known tolerance of insecticidal treatments. These included a highly PH_3 resistant field strain of the lesser grain borer, and laboratory strains of the flour beetle and the rice weevil.

A Draeger X-AM 7000 multi gas detector (www.draeger.com) measures EF and CO_2 fumigation levels, and the Miran® SapphIRe (Thermo Environmental Instruments; www.thermofisher.com /Miran) programmable infra-red gas analyzer measures EF at occupational exposure levels (100 ppm) and below.

The major outcome of project CSE00009 has been the successful delivery of a new grain fumigant for the Australian grains industry and in particular, farm-scale storers of grain.

Treatment of exotic quarantine pests

Brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is highly polyphagous and is found on at least 211 plants across 88 plant taxa. Currently, there are three approved treatment options for BMSB detections at the Australian border in international cargo (DAWE, 2020). Heat treatments require that consignments be treated at 56°C or higher at the coldest surface of the goods, for a minimum of 30 min or 60°C or higher at the coldest surface of the goods, for a minimum of 10 min.

Alternatively, MBr is an option with a dose of 24 g/m³ or above, at 10°C or above, for a minimum of 12 h (but less than 24 h), with all start time concentration readings above 24 g/m³ and a minimum end point reading of 12 g/m³. Another fumigant option in Australia is sulfuryl fluoride. The treatment dose is 24 g/m³ or above, at 10°C or above, for a minimum of 12 h (but less than 24 h), with all start time concentration readings above 24 g/m³ and a minimum end point reading of 12 g/m³. The treatment has failed if the concentration of fumigant falls below the minimum end point reading at any point during the treatment (DAWE, 2020).

Ethyl formate is a potential BMSB quarantine fumigant. Kawagoe et al. (2017) presented data requiring low EF doses (median 10 g/m^3, 4 h) to eliminate BMSB. The Lethal Exposure, LE_{99} (Ct) varied from 20.52 (10.26 mg/L) for 2 h exposure to 29.29 (2.44 mg/L) for 12 h exposure. Probit Curve data gave the LE_{99} (Ct) of 33.02 (16.5 mg/L) for 2 h exposure, 41.9 (10.5 mg/L) for 4 h and 58.77 (4.9 mg/L) for 12 h exposure. Also, these results were achieved at 10°C (below the recommended temperature limit for many fumigants). The majority of current MBr fumigation for BMSB are carried out in low density packed containers (e.g., motor cars and associated non-food shipments) which avoids issues of sorption and uniform distribution related to densely packed grain storage. In the consumables required to eliminate BMSB at the USDA, median 10 g/m^3, 4 h exposure, fumigation would be cost competitive with the current MBr treatment.

Ethyl formate can be used in-transit shipping containers and offers savings in labour cost, elimination of the time for a container to remain stationary in a fumigation facility and a significant decrease in time spent between dispatch and receival (Coetzee et al., 2019). There were nil detections of EF in the immediate surroundings, up to 15 m downwind or inside and outside of the truck cabin (Coetzee et al., 2019). Similarly, EF (90 g/m^3) and nitrogen fumigation of 20 ft shipping containers were monitored during an overnight voyage (Coetzee et al., 2020).

Conclusions

Recent review (Ryan and Dominiak, 2020) found 78 insect species that could be controlled by EF, albeit at different rates or exposure times. These insects include five weevils, six aphids, six thrips, seven moths, 18 scale and mealy bugs, and ten beetles. Of these, EF is registered to control 41 of these pests. There is an opportunity to add more pests to the registered uses based on available science. Also, there is opportunity to evaluate more pests from the more established EF control groups such as thrips, moths and beetles to assist interstate trade.

Unlike some alternatives, EF kills insects rapidly (Ren and Mahon, 2006). Ethyl formate has advantages for worker and environment safety (Ren and Mahon, 2006; Coetzee et al., 2019). EF is much safer for human use compared to MBr (Ryan and De Lima, 2014; Park et al., 2020). Ethyl formate is an effective and less toxic fumigant for horticulture and stored product pests, including during transit on road and sea. Research identified EF as a candidate alternative fumigant for MBr in the elimination of exotic quarantine pests. The effective low dose of EF allows for non-flammable on-site EF mixing to be competitive with the existing MBr quarantine fumigation. In addition, other benefits include environmental release (unlike MBr, EF is not an ozone depletor and has limited life in the atmosphere), occupational (TLV's: EF = 100 ppm and MBr = 5 ppm) and the reduced aeration time should reduce facilities costs. Ethyl formate has less onerous requirements for PPE and no recapture technology is required. Ethyl formate is an attractive alternative fumigant compared with many industry standards.

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Low temperature as an alternative to fumigation: An example using *Acanthoscelides obtectus*

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ABSTRACT

Insects are affected by temperature in all aspects of their biology: ecology, reproduction, behaviour, physiology and biochemistry. Stored-product insects reproduce between 15 and 35°C. Above and below these temperatures insects can move, but cannot complete their development. Temperatures below 5°C and above 40°C insects cannot walk, and will eventually die. Between -7 and -25°C insects freeze and die instantaneously. There are significant changes to these general patterns depending upon species, life stage, acclimation and diapause. For example, insects can become 10 times more resistant to cold if acclimated at cool temperatures (5 to15°C) before being exposed to sub-zero temperatures.

Acanthoscelides obtectus (Say), the bean weevil, is capable of causing severe damage to stored whole pulses by boring through the seed coat to consume the cotyledons and contaminating sound grain with dead bodies and excreta. It is originally from South America, but is now found across North America, Europe and Africa. It is a quarantine pest for India and China. More natural control measures are being evaluated worldwide as replacements for chemical control. Here, we tested the survival time of different life stages of *A. obtectus* at -5°C. The life stages studied were: eggs and adults (external stages) and young larvae, old larvae and pupae (internal stages). Since *A. obtectus* larvae feed within seeds, 2D soft X-rays were used to confirm if a seed was infested with a single larva and to determine the internal life stage of it. The insects were exposed to -5°C for 0, 1, 2, 3, 4, 7, 10 and 14 d. Treated insects were monitored at 25°C and 65% RH to assess survival. Eggs were considered as alive if they emerged as larvae; young larvae, old larvae and pupae were considered alive if they emerged as an adult; adults were considered as alive if they were able to walk. The eggs had a lethal time to 50% mortality at -5°C (LT_{50}) of 2.3 d, young larvae, old larvae and adults had a LT_{50} of approximately 4 d. Data for pupae is not available at this time. The supercooling point (freezing point) of eggs was -26.8°C and for adults it was -14.0°C. Preliminary encouraging results establish that cold treatment of bean weevils could be used as an effective alternative to fumigation.

Keywords: Quarantine; X-ray, Pulses, Alternative to methyl bromide

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Effectiveness of Cryonite system in treatment of stored product insects

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Abstract

The Cryonite system turns liquid carbon dioxide to snow at an extremely low temperature (-79°C). The snow is sprayed into cracks and crevices to attempt to lower the temperature of insects below their supercooling point (SCP), the temperature at which they freeze and instantly die. To determine the effectiveness of this alternative treatment in a flour mill setting, Cryonite was applied to cracks in concrete blocks filled with adult insects, *Tribolium castaneum* (Herbst), and flour using two different nozzle types (standard and jet) and at various application durations. Cryonite was used in a grain storage facility to test the efficacy of the system in a real-life scenario. It was found that a 5 s application using the jet nozzle gave 100% mortality within the top 10 cm of the 5 mm wide crack with no flour in crack. The 30, 45, and 60 s applications of the standard nozzle gave mortality rates within the top 10 cm of 79, 97, and 99%, respectively. With cracks deeper than 10 cm, neither the jet nor standard nozzles were effective. Cryonite was ineffective in cracks with flour. For applications in the grain storage facility, the results varied significantly depending on materials and nature of the voids. We recommend that Cryonite not be used as a stand-alone treatment in storage facilities, but rather as a complementary treatment to fumigation or heat treatment.

Keywords: Carbon dioxide, Flour mill, Storage facility, Cryonite, Spray, Freezing

Introduction

After harvest, commodities are stored and processed in a variety of facilities including grain bins, elevators, mills, and warehouses. While food products are being processed for sale, they are susceptible to be attacked by stored-product insects. These insects specialize on feeding on processed plant and animal materials. Historic control of these insects required the use of chemical insecticides, however, through repeated use and misuse of these chemicals, some negative consequences have been discovered (Fields, 1992; Abd El-Aziz, 2011). First, these chemicals are broad spectrum and pose threats to non-target organisms (Fields, 1992). Second, some chemicals, such as methyl bromide, have been proven to be harmful to the environment and are being phased out (Fields and White, 2002; Abd El-Aziz, 2011). Third, insect resistance from over-use or improper use of chemicals has occurred globally (Fields and White, 2002).

Thus, there is interest in finding alternative, non-chemical control methods for stored-product insects. Carbon dioxide can be used to control stored-product insects in two main ways. It can be used as a fumigant either alone or in combination with other fumigants (Boyer et al., 2012). When carbon dioxide gas is pumped into a storage bin or facility, the atmospheric composition changes, reducing the amount of oxygen available, turning the bin into an anoxic environment and inhibiting glycolysis within the insect (Alder, 1994; Boyer et al., 2012; Husain et al., 2017). However, in facilities that are not sealed tightly, it can take a long time to kill pests due to lower concentrations of carbon dioxide. Another way carbon dioxide can be used as a control method is the Cryonite system which produces carbon dioxide snow that freezes insects (CTS Technologies AG, 2008). The system was designed to be used on pests in close contact with humans such as cockroaches, bedbugs, termites, and stored-product insects. The system converts liquid carbon dioxide into a -79°C snow as it is released. This snow is safe to use on a variety of surfaces such as clothing, plastics, wood, metal, and electronics, as it has no residues.

The use of cold temperatures is effective in controlling stored-product insects in a variety of settings (Fields, 1992). The optimal temperature range for most stored-product insects is between 25-33°C (Fields, 1992; Abd El-Aziz, 2011). Temperatures below 20°C cause a halt in development of stored-product insects, and insects eventually die. Lower temperatures cause insects to die faster (Fields, 1992). If temperatures are cold enough, insects will freeze and die instantaneously. This temperature referred to as the supercooling point (SCP), varies from -5 to -30°C depending on species, life stage and acclimation (Fields, 1992). This can be a very effective and an environmentally friendly control method. However, most field studies to date focus on freezing temperature in grain bins over the winter months or in mills that are undergoing freeze-outs. The Cryonite system introduces a new method of using freezing temperatures to control insects in spot/ local treatments within facilities.

For this experiment, we tested the efficacy of the Cryonite system on the red flour beetle, *Tribolium castaneum* (Herbst), using simulated concrete cracks, and in a real grain storage facility. *Tribolium castaneum* has a supercooling point of -12.3°C (Fields, 1992). This is the point at which the body water freezes and becomes lethal (Andreadis and Athanassiou, 2016). Initially, we tested nozzle type, duration of application and width of crack on the temperatures at different depths in the crack. Once this was determined, *T. castaneum* was introduced into the system to determine the effectiveness of the Cryonite system at reducing populations of this stored-product insect. The system was then used in a storage facility to measure temperatures produced by the Cryonite system in voids of varying sizes and composed of various materials.

Materials and methods

Standard nozzle tests without insects

To create a crack that could vary in size, two solid concrete blocks were made, and thermocouples were embedded throughout (Fig. 1). The ten thermocouples could be selectively monitored based on the requirements of the experiment. The temperatures were measured every 1 s. The simulated concrete cracks tests had two main variables: crack size and application duration. The crack widths were of 1, 3, and 5 mm, and the application durations were 30, 45, and 60 s, which resulted in 9 combinations that were tested. The spray handle was held at 50% capacity for each duration.

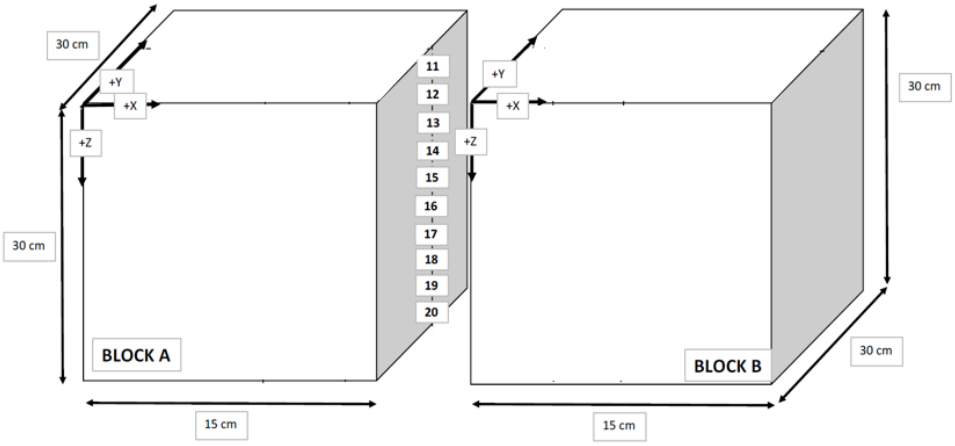

Fig. 1. Locations of thermocouples within the concrete block.

Standard nozzle with insects

This experiment used the same methods as described above with the addition of insects (*T. castaneum*). To keep the *T. castaneum* at a specific location within the crack, 25 adult insects were placed into a small fine mesh bag. Three of these bags were tied to a string at 2, 10 and 20 cm positions. The string was then tied to the top of the block and the insects were placed into the 5 mm crack being held at specific locations along the string. Three replicates were performed for each spray duration. This experiment was completed with insects in the crack and also with insects and flour in the crack.

Jet nozzle with insects

This experiment followed the guidelines recommended in the documentation from CTS Technologies. The jet nozzle was sprayed for a 5 s duration. As in the previous experiment, the crack size was held constant at 5 mm, and the string method with 3 bags of 25 adult *T. castaneum* and no flour.

Application in a storage facility

This experiment followed the same guidelines as the jet nozzle experiment. The Cryonite system, using the jet nozzle was sprayed for a 5 s duration into cracks and crevices. Temperature was monitored in each location. Locations were scouted prior to application so the test would include a variety of crack sizes and materials. Ten locations were selected for Cryonite spot treatments that included bin footings, electrical boxes, wooden beams, vinyl baseboards and other various obstacles that would be hard to access for other control methods. Additionally, five locations in a grain cleaner were selected as this would be a prime location for pests due to the food residues that are often left behind after machine use (Fig. 2). These locations were all sprayed twice to determine minimum temperature reached by the Cryonite jet nozzle.

Results

Standard nozzle without insects

When using the standard nozzle, the lowest temperatures were found in the largest crack sizes. Temperatures increased with depth. In the larger crack sizes, lower temperatures were observed deeper into the concrete block (Table 1). Using the supercooling point for *T. castaneum* of -12.3°C as a point at which the insects would die, the results indicate that the standard nozzle was ineffective for crack sizes of 1 mm. For the 3- and 5-mm cracks, a 45 s application is sufficient to achieve the minimum desired temperature of -12.3°C, and death would be expected under these conditions.

Table 1. Temperatures for locations 11, 12 and 13 (1, 4 and 7 cm depth in crack, respectively) in three crack widths (1, 3 and 5 mm) after three durations of spraying Cryonite (30, 45 and 60 s) with no flour.

Depth in Crack (cm)	Temperature (°C)								
	30 s			45 s			60 s		
	1 mm	3 mm	5 mm	1 mm	3 mm	5 mm	1 mm	3 mm	5 mm
1	-4.8	-33.6*	-31.8*	-13.2*	-23.3*	-37.4*	-9.9	-30.2*	-40.5*
4	13.6	-16.4*	-14.6*	12.5	1.8	-19.8*	11.3	-10.1	-22.3*
7	17.8	-14.2*	-11.6	13.8	3.6	-18.2*	13.9	-9.3	-18.9*

*Temperatures below the supercooling point of *T. castaneum* (-12.3°C), where death should occur (Fields, 1992)

Standard nozzle with insects

When insects were added to the crack, 100% mortality of *T. castaneum* was seen in the top of the crack when Cryonite was sprayed for 45 and 60 s using the standard nozzle. For the 30 s spray duration, mortality was also high at 96% death. Mortality remained high at a depth of 10 cm in the 45 s and 60 s time treatments but mortality was low at depths of 20 cm. Mortality increased with increased spray time ($F=5.52$, $p=0.01$) and decreased within increased depth ($F=20.12$, $p< 0.001$, Table 2).

When flour was added to the crack, no mortality was observed for *T. castaneum* because temperatures below 0°C were not reached at 2 cm below the surface and lower. Mortality was not significantly affected by spray time ($F=1.0$, $p=0.38$) or depth ($F=1.0$, $p=0.38$).

There was a significant discrepancy between the temperature data and the insect mortality. For each replicate, only the thermocouple 2 cm below the surface showed negative minimum temperatures (Table 3), which is inconsistent with the high mortality rates throughout the top 10 cm. This may be due to the method of application, where the snow is getting in contact with the bags of insects, but not the thermocouples directly.

Table 2. Average mortality of *Tribolium castaneum* at three depths (2, 10 and 20 cm deep in crack), three durations of spraying Cryonite (30, 45 and 60 s) into a 5 mm crack with no flour.

Depth in Crack	30 s	45 s	60 s
(cm)	Mortality (%)	Mortality (%)	Mortality (%)
2	96	100	100
10	68	93	97
20	24	53	39

Table 3. Average mortality of *Tribolium castaneum* at three depths (2, 10, and 20 cm) after three spray durations into a 5 mm crack filled with flour.

	30 s		45 s		60 s	
Depth in Crack (cm)	Mortality (%)	Temperature (°C)	Mortality (%)	Temp. (°C)	Mortality (%)	Temperature (°C)
2	0	3.79	1	-7.61	0	-17.45
10	0	22.61	0	20.98	0	20.69
20	0	21.11	0	21.18	0	20.79

Jet nozzle with insects

When using the jet nozzle for only 5 s, 100% mortality of *T. castaneum* was seen up to 10 cm deep in the concrete block. Mortality decreased with increased depth ($F=6.23x10^{30}$, $p< 0.001$, Table 4). Temperatures recorded by the data loggers were, however, inconsistent with temperatures that would cause death for *T. castaneum.* Temperatures at 20 cm deep in the concrete block were too warm to kill *T. castaneum*.

Table 4. Average mortality of *Tribolium castaneum* at three depths (2, 10 , and 20 cm) after a 5 s spray duration into a 5 mm crack.

Depth in Crack (cm)	Mortality after 5 s (%)	Temperature (˚C)
2	100	-21.8
10	100	7.7
20	0	18.9

Applications in a storage facility

For applications in the grain storage facility, the results varied depending on materials, nature of the voids and between applications. Two applications were completed in each location. Temperatures detected varied up to 30°C between the two applications. This suggests that error in aiming of the nozzle likely plays an important role in the efficacy of this system. Interestingly, applications into cracks within wood and within metal provided some of the coldest temperatures and may be more effective in voids of certain materials.

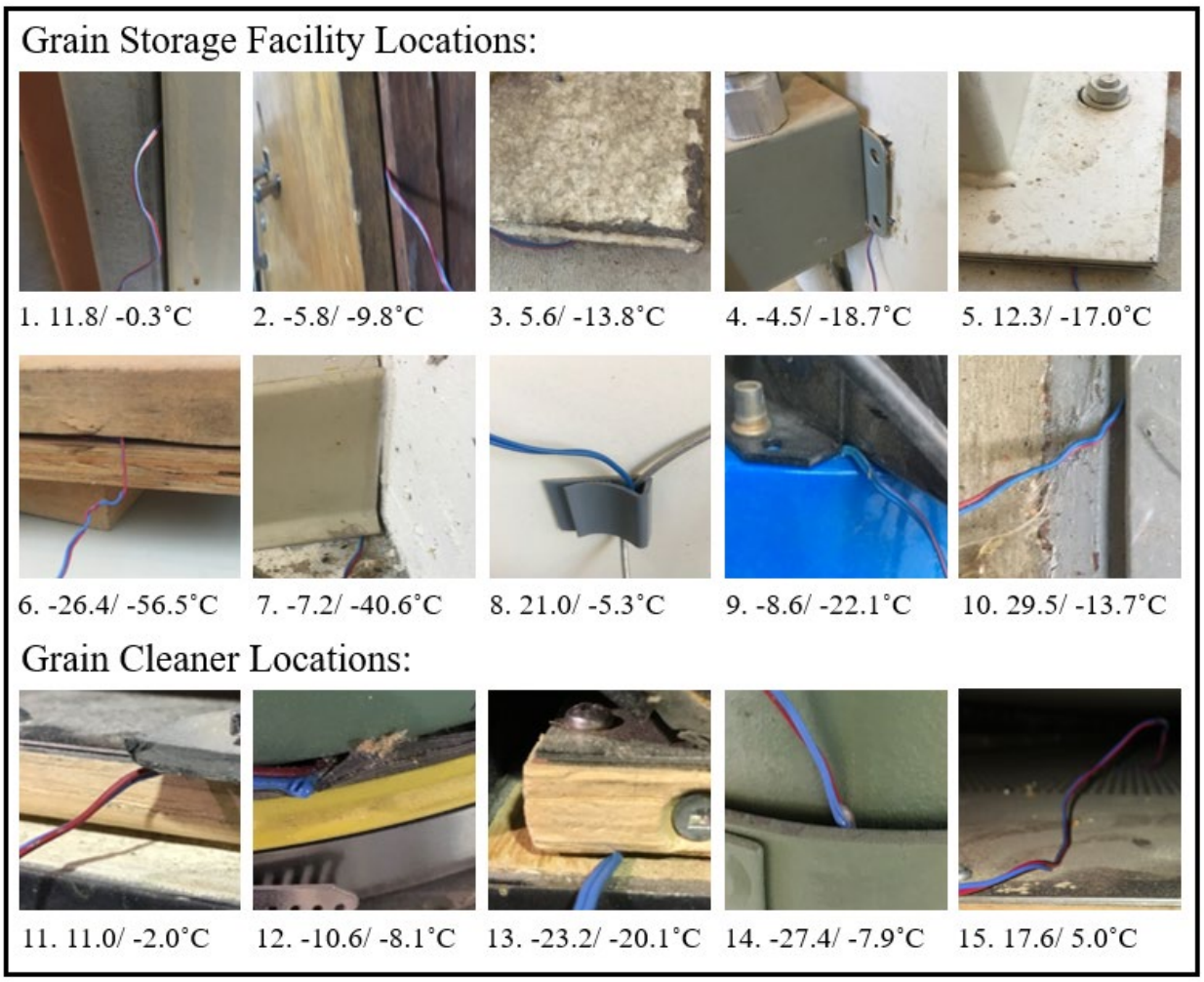

Fig. 2. Locations in Storage Facility with the minimum temperature reached on the two trials: 1. Column with two metal plates bolted together; 2. Inside of drawer on wood cart; 3. Between concrete floor and metal bin footing; 4. Between metal electrical box and drywall; 5. Between concrete floor and metal bin footing; 6. Between a piece of plywood and a wooden block; 7. Behind vinyl baseboard in corner of concrete post and drywall; 8. Inside of plastic clip used to secure small wires; 9. Corner of metal machine where 3 pieces of sheet metal are secured via bolts; and 10. Diamond plate floor access hatch. Locations in Grain Cleaner: 11. Between rubber flap and metal; 12. Between ribbed plastic and metal; 13. Between two pieces of plywood; 14. Along edge of small access hatch to metal chute; and 15. Between layers of sieves, approximately 30 cm away from spray edge.

Discussion

The Cryonite system was able to, under certain conditions, drop temperatures in cracks to below the supercooling point of *T. castaneum* and was able to kill *T. castaneum* adults placed in cracks. The conditions where it worked were in shallow cracks, 10 cm or less, and cracks 5 mm wide or larger. The conditions that it did not work in were cracks of any nature filled with debris or flour, and in thin cracks (less than 5 mm wide) if they were deeper than 4 cm. Therefore, for applications

within cracks, the jet nozzle is the preferred method over the standard nozzle. However, this method is only ideal for shallow cracks. While the storage guideline document notes that the jet nozzle should only be used in voids and crack due to the reduced quality of snow produced by this nozzle, the jet nozzle provides the highest mortality rate in the shortest amount of time with the least amount of carbon dioxide.

Stored-product insects all die if frozen, so if SCP temperatures are obtained insects will be controlled. These range from -8 to -20°C (Fields, 1992). Thus, knowing the correct identity of the insect being controlled for is crucial when using this product. The use of ice nucleation bacteria (Fields, 1992) could raise the SCP, making insects more susceptible to this treatment. Additionally, we would recommend that the Cryonite system be used in conjunction with another treatment method, such as a heat or fumigation, where Cryonite be used as an additional treatment in hard-to-reach places. The Cryonite system will not work against insects in cracks filled with flour or dust, so cleaning must take place prior to the use of this control method.

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Identification and functional analysis of cytochrome P450 CYP346 family genes associated with phosphine resistance in *Tribolium castaneum*

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ABSTRACT

Resistance to phosphine fumigation has been frequently reported in insect pests of stored products and remains one of the obstacles in controlling these pests, including *Tribolium castaneum* (Herbst). In this study, six field populations of *T. castaneum* were collected from different localities in China. Bioassay data showed that SZ population was strongly resistant to phosphine, followed by moderate-resistance populations WL and SF, and three susceptible populations JX, YN, and ML. In addition, synergism assays showed that PBO significantly increased the toxicity of phosphine in resistant population SZ. Furthermore, CYP346B subfamily genes: CYP346B1, CYP346B2, and CYP346B3, were significantly overexpressed in resistant populations. Expression of CYP346B1, CYP346B2, and CYP346B3 were significantly upregulated following exposure to phosphine. RNAi assays showed that depletions on the expression levels of CYP346B1, CYP346B2, and CYP346B3 resulted in an increase of susceptibility to phosphine in *T. castaneum*. Our data demonstrated that CYP346B subfamily genes in *T. castaneum* were associated with the resistance of phosphine. Moreover, the study also advanced our understanding of phosphine resistance at the molecular level in stored pest insects.

Keywords: *Tribolium castaneum* , Phosphine resistance, P450, Overexpression, RNAi

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Comprehensive evaluation of direct aeration combined with closed internal circular ventilation in squat silos

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ABSTRACT

Ventilation is widely used in granaries. A well-designed aeration system improves ventilation efficiency and reduces the cost by lowering energy consumption. We investigated two common aeration systems: direct mechanical ventilation and direct ventilation combined with closed internal circular ventilation in 6789 tonne and 7500 tonne squat silos, respectively. Compared to direct mechanical ventilation system, the direct ventilation combined with closed internal circular ventilation system decreased operating time by 13.13% and energy consumption by 2381 kWh. When the direct ventilation combined with closed internal circular ventilation was conducted, the grain lost 0.5% less water, and grain moisture content also had a more homogenous distribution within the grain bulk. The two systems had a 0.5% difference of moisture content. Mild condensation was detected in the silo with direct mechanical ventilation system. These results could provide a guideline for choosing an effective ventilation system in squat silos.

Keywords: Squat silo, Direct mechanical ventilation; Direct ventilation combined with closed internal circular ventilation, Operating time, Grain moisture homogeneity

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Zhao H, Zhang H, Cao Y, Li Y Efficacy of food grade synthetic amorphous silica in small farms. Page 286. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Efficacy of food grade synthetic amorphous silica in small farms

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ABSTRACT

There are few available techniques to control stored products insects in small farms, which account for about 30% volume of stored grain in China. This research evaluated application of synthetic amorphous silica dust on small farms at the dosage of 100 mg/kg. Populations of *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens), *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst), *and Sitotroga cerealella* Olivier reduced 90.4%,78.3%, 100%, 100%, and 76.9% after 50 d of application, respectively. After 70 d of application, populations of *S. zeamais*, *C. ferrugineus*, *R. dominica*, *T. castaneum, and S. cerealella* reduced 81.9%, 100%, 100%, 100% and 50%, respectively. The losses caused by insects and molds were 1.67±0.16% and 0.46±0.12% in the treatment groups, whereas 3.55±0.33% and 1.29±0.33% in the control groups. This study provided the information of a new non-chemical technology for pest management in small farm grain storage.

Keywords: Food grade, Synthetic amorphous silica, Stored grain insect pest, Small farm storage

CAF2020 Abstract No. A-9-6-74

Wu G, Zhao Y, Zheng J, Xi J (2021) Study on fumigation of tobacco lamina with sulfuryl fluoride at low temperatures. Page 287. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Study on fumigation of tobacco lamina with sulfuryl fluoride at low temperatures

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ABSTRACT

Temperature has a strong relationship with lethal effect of sulfuryl fluoride. The effect of sulfuryl fluoride with different concentrations on *Lasioderma serricorne* (F.) in different types of tobacco storages from 0 to 10°C was studied. The results showed that 100% of four stages of tobacco beetles were killed by sulfuryl fluoride under 30 g/m^3 in 7 d. When the concentration of sulfuryl fluoride was lower than 20 g/m^3, a small number of eggs still hatched. In addition, when sulfuryl fluoride was injected into the sealing fume stack through guide pipes, the phenomenon of volatile frostiness could be observed in the cylinder valve, guide pipe, and outlet during application. Not only should the injection rate not be higher than 10 kg/h, but also the outlet of the guide pipe should not be in direct contact with the tobacco box, otherwise, dew and even frost could occur on the tobacco box. The results of this study provided technical information for the application of sulfuryl fluoride fumigation in the cold region of northern China.

Keywords: Sulfuryl fluoride, Low temperatures, *Lasioderma serricorne,* northern China

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Nitric oxide fumigation for postharvest control of pests and pathogens

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Abstract

Nitric oxide (NO) is a recently discovered fumigant and NO fumigation has been demonstrated to be effective against all insects and mites tested to date, including external and internal pests, fresh and stored product pests. However, as NO reacts with O_2 spontaneously to form nitrogen dioxide (NO_2), NO fumigation must be conducted under ultralow oxygen conditions. As it is impractical to remove all oxygen, NO fumigation always has NO_2 as a result of interaction of NO with residual oxygen in fumigation chamber. Recently, NO_2 was demonstrated to be effective against microbes. Effective control of *Aspergillus flavus* spores was achieved in NO and NO_2 fumigations. Complete control of bacteria and fungi on stored almonds and peanuts were also achieved in NO_2 fumigations. Therefore, NO fumigation has potential to control both pest and pathogens. When conducted properly, NO fumigation is also safe for use on fresh products and helps to maintain storage/shelf-life of fresh fruit. Nitric oxide fumigation does not leave toxic residues on fresh or stored products. These studies indicate that NO fumigation is feasible to control both insects and microbes in a single fumigation treatment. This is important in expanding potential applications of NO fumigation and making NO fumigation more cost effective for potential commercial applications.

Keywords: Nitric oxide, Nitrogen dioxide, Fumigation, Pest control, Microbial control, Almond, Peanuts, Stored products, *Aspergillus flavus*

Introduction

Nitric oxide (NO) is a chemical produced naturally in fossil fuel combustion and lightning and commercially as an intermediate in fertilizer production. Nitric oxide was discovered in 1980s to be a ubiquitous cell messenger molecule and has since been found to play diverse functions in physiological and biochemical processes in organisms (Lamattina et al., 2003). Nitric oxide is also found to be an inhibitor of ethylene biosynthesis in plants and can be used to enhance postharvest quality and prolongs shelf-life of fresh fruit and vegetables (Soegiarto and Wills, 2004; Manjunatha et al., 2012). Recently, NO is found to be a potent fumigant for postharvest pest control and is effective against all insects and mites tested to date (Liu, 2013, 2015; Liu and Yang, 2016, 2018).

Nitric oxide fumigation is also safe to postharvest quality of fresh products when conducted properly (Liu, 2016, 2017; Liu and Yang, 2018; Yang and Liu, 2018a). Nitric oxide fumigation does not leave significant nitrate and nitrite as residues on both fresh and stored products when conducted properly (Yang and Liu, 2017, 2019). In the past few years, NO_2 fumigations were found to be effective against microbes (Liu et al., 2019; Oh and Liu, 2020; Oh et al., 2020). Therefore, it is possible that a single NO fumigation treatment can effectively control pests and microbes on stored products and, thereby, make NO fumigation more useful and cost effective. In this paper, NO fumigation research was reviewed and discussed with an emphasis on the most recent studies on the efficacy of NO fumigation against insects and microbes.

Nitric oxide fumigation

Nitric oxide reacts with oxygen spontaneously to form nitrogen dioxide (NO_2) (Beckman and Koppenol, 1996). Therefore, NO fumigation must be conducted under ultralow oxygen (ULO) conditions to preserve NO. Procedures for NO fumigation have been thoroughly described and demonstrated (Liu and Yang, 2016; Liu et al., 2017). The key points are airtight seal of fumigation chamber and flushing with nitrogen gas at the beginning of the process to establish ULO and at the end to dilute NO in the fumigation chamber to prevent NO_2 formation.

Nitric oxide fumigation has more stringent requirements on fumigation apparatus and procedures due to the requirement of ULO environment and the need to keep the fumigation chamber airtight. For commercial scale NO fumigation, N_2 supply expenses include costs of air compressor and N_2 generator, and operating costs for N_2 generation including energy and maintenance costs. This adds extra expense to NO fumigation and makes NO fumigation more expensive. Costs of fumigation equipment and energy to operate N_2 generation equipment for NO fumigation was previously analyzed (Liu, 2015). Based on the numbers of air exchanges with the N_2 flush, volumes of N_2 and energy cost can be estimated for establishing ULO conditions for NO fumigation. It is estimated that energy cost for initial establishment of ULO conditions for NO fumigation and N_2 flush at the end of NO fumigation is moderate. The added expenses related to N_2 generation may be compensated by the benefits of NO fumigation as compared with fumigations with more toxic fumigants that are less effective and leave toxic residues in fumigated food products (Liu, 2015).

As it is impractical to remove all oxygen in a fumigation chamber especially in commercial scale fumigations, NO fumigation always contains certain levels of NO_2. Nitrogen dioxide; however, has strong antimicrobial property. Nitrogen dioxide fumigation was demonstrated to kill *Aspergillus flavus* spores and control microbes on stored products (Liu et al., 2019; Oh and Liu, 2020; Oh et al., 2020). In fact, NO_2 levels in NO fumigation can be controlled by controlling ULO levels. Therefore, NO fumigation can be established with desired NO and NO_2 levels and may have potential to control both pests and pathogens on stored products.

Efficacy of NO fumigation against postharvest pests

Nitric oxide fumigation has been demonstrated to be effective against over 14 pest species tested to date at different life stages (Liu, 2013, 2015; Liu and Yang, 2016; Liu et al., 2018; Yang et al., 2020) (Table 1). Insect species and their life stages differ in susceptibility to NO fumigation.

Table 1. Fumigation treatments with NO at different concentrations for different durations at different temperatures that resulted in 100% mortality of insects and mites at different life stages.

Species	Life stage	NO (%)	Time (h)	Temp (°C)
Western flower thrips	larva, adult	0.2	8	2
		2.0	2	2
Lettuce aphid	nymph, adult	0.2	12	2
		0.5	9	2
		1.0	3	2
Long-tailed mealybug	nymph, adult	2.0	2	2
Light brown apple moth	egg	3.0	12	2
	larva, pupa	2.0	8	2
Spotted wing drosophila	egg, larva (in cherries)	3.0	8	2
Codling moth	egg, larva, pupa	2.0	48	2
	large larva (in apples)	5.0	24	2
Indianmeal moth	egg	1.0	24	20
Naval orangeworm	egg	2.0	16	25
	larva (in walnuts)	1.0	8	25
Confused flour beetle	egg	2.0	24	10
	larva, pupa	0.5	24	20
	adult	0.5	8	20
Rice weevil	egg	1.0	48	25
	adult	1.0	24	25
Granary weevil	adult	1.0	24	25
False spider mites	larva, adult	0.5	6	2
Bulb mites	larva, adult	2.0	24	20
Ham mites	egg	2.0	8	25
	larva, adult	2.0	4	25

Small external soft body insects on fresh products are more susceptible to NO fumigation than insects on stored product and insects that feed internally on fruits and vegetables. The treatment time is shorter for mobile stages than for pupa and egg stages (Liu, 2013, 2015; Liu and Yang, 2016). It takes a few hours to control external feeding insects including western flower thrips (*Frankliniella occidentalis* (Pergande)), lettuce aphid (*Nasonovia ribisnigri* (Mosley, 1841)), and longtailed mealybug (*Pseudococcus longispinus* (Targioni Tozzetti)) with NO fumigations at ≤1.0% at a low temperature of 2°C (Liu, 2013). Internal feeding larvae of spotted wing drosophila (*Drosophila suzukii* (Matsumura)) in infested cherries takes 8 h to control with 2.5% NO

fumigation (Liu and Yang, 2016). Codling moth (*Cydia pomonella* L.) larvae in infested apples take 24 h NO fumigation at 5.0% concentration at 2°C to achieve complete control (Liu et al., 2016). Nitric oxide fumigation at 1-2% concentrations takes 24 to 72 h at 15-25°C to control stored product insects including Indian meal moth (*Plodia interpunctella* (Hübner)), confused flour beetle (*Tribolium confusum* Jacquelin du Val), and rice weevil (*Sitophilus oryzae* (Linnaeus)) (Liu, 2013, 2015; Liu and Yang, 2016). Nitric oxide fumigation under ULO established with CO_2 flush is also effective against stored product insects (Liu, 2020). Nitric dioxide fumigation was also tested against Navel orangeworm (*Amyelois transitella* (Walker)) on an artificial diet and in infested walnuts. Navel orangeworm eggs are more tolerant to NO fumigation than larvae and pupae and complete control of eggs was achieved in 8 and 16 h fumigation with 3.0 and 2.0% NO, respectively (Yang et al., 2020). Nitric dioxide fumigation is also effective against mites. Bulb mites (*Rhizoglyphus spp*.) on infested peanuts were controlled with 2.0% NO in 24 h at 20°C (Liu, 2017). Complete control of false spider mites (*Brevipalpus phoenicis* (Geijskes)) and ham mites (*Tyrophagus putrescentiae* (Schrank)) was also achieved (Table 1). All of these results show that NO fumigation has good efficacy against all pests at any life stages.

Effects of NO and NO_2 fumigation on microorganisms

Both NO and NO_2 can kill microbes (Table 2). However, NO_2 is far more effective in controlling microbes than NO. In 3 h fumigation tests, 0.1% NO_2 had complete control of *A. flavus* spores (Liu et al., 2019). Nitrogen dioxide fumigation is also effective in controlling both bacteria and fungi on almonds and unshelled peanuts (Oh and Liu, 2020; Oh et al., 2020). Unpasteurized almonds were fumigated with NO_2 at 0.1, 0.3, or 1.0% concentrations by inject NO under ambient O_2 for 1 and 3 d at 25°C. A rapid enumeration test was used to determine microbial loads in diluted wash-off samples from NO_2 fumigated almonds and controls. Nitrogen dioxide fumigation treatments showed either greatly reduced microbial loads or complete control of microorganisms, depending on NO_2 concentration and treatment duration. Nitrogen dioxide fumigation was more effective against fungi than against bacteria. Effective control of microbes was also achieved on unshelled peanuts. Bacteria and fungi on outer surfaces and inside unshelled peanuts were effectively controlled with 3 d NO_2 fumigations and complete control of both bacteria and fungi was achieved with 1.0% NO_2 fumigations (Oh et al., 2020) (Table 2). These results suggest that postharvest NO fumigation with proper levels of NO and NO_2 can be used for insect and microorganism control on stored almonds and peanuts.

Safety of NO fumigation

The safety of fumigation treatment for pest and disease management includes preserving product quality and enhancing consumer safety by reducing toxic residues on fumigated products. When conducted properly, NO fumigation is safe to fresh product quality and also does not leave unacceptable residues on fumigated products. In small scale tests, NO fumigation was demonstrated to be safe to all fresh products tested to date including lettuce, broccoli, cucumber, pepper, tomato, strawberries, apple, pear, orange, and lemon when terminated with N_2 flush as there are no significant differences between the treatment and the control (Liu, 2016; Yang and Liu, 2018a, 2018b). When NO fumigation is terminated by directly opening the fumigation

chamber to ambient air without flushing with N_2 gas, NO reacts with O_2 to produce NO_2 in the fumigation chamber and results in stains on delicate fresh products including leafy vegetables, broccoli, squash, and peach. Stains also occur to some apples when NO fumigation was terminated without N_2 flush (Liu, 2016). The key aspect to ensure safety of NO fumigation to fresh products is to prevent or reduce exposure of products to NO_2 at a high level that causes damage to fresh products.

Table 2. Effects of NO and NO_2 fumigation on relative colony forming unit (CFU) of microorganisms on artificial media and stored products at 25°C.

Source of microorganism	Treatment	Relative CFU (%)
Aspergillus flavus spores on cellulose filter discs	Control	100
	0.1% NO, 3h	79.2
	1.0% NO, 3h	0
	0.1% NO_2, 3h	0
Bacteria and fungi on almonds	Control	100
	0.1% NO_2, 24h	68.5
	0.3% NO_2, 24h	27.5
	1.0% NO_2, 24h	0
Fungi on almonds	Control	100
	0.1% NO_2, 24h	5.5
	0.3% NO_2, 24h	0
	1.0% NO_2, 24h	0
Bacteria and fungi on outer surfaces of intact unshelled peanuts	Control	100
	0.3% NO_2, 72h	8.3
	1.0% NO_2, 72h	0
	3.0% NO_2, 72h	0
Bacteria and fungi on inside and outer surfaces of cracked unshelled peanuts	Control	100
	0.3% NO_2, 72h	6.2
	1.0% NO_2, 72h	0
	3.0% NO_2, 72h	0
Fungi on outer surfaces of intact unshelled peanuts	Control	100
	0.3% NO_2, 72h	2.0
	1.0% NO_2, 72h	0
	3.0% NO_2, 72h	0
Fungi on inside and outer surfaces of cracked unshelled peanuts	Control	100
	0.3% NO_2, 72h	16.9
	1.0% NO_2, 72h	0.2
	3.0% NO_2, 72h	0

Nitric oxide fumigation can extend postharvest storage and shelf life of fresh products due to its antagonistic effects on ethylene biosynthesis (Soegiarto and Wills, 2004; Manjunatha et al., 2010, 2012). Nitric dioxide fumigations for control of western flower thrips and codling moth also result in better postharvest quality of strawberries and apples respectively (Liu, 2016; Liu et al., 2016). These results are consistent with other studies and suggest that NO fumigation for postharvest pest control may also provide additional benefits of extended storage/shelf-life to some fresh products.

Nitrogen dioxide, nitrate, and nitrite are expected residues of NO fumigation and N_2 flush is critical to prevent significant accumulations of these residues. When NO fumigation is terminated with a N_2 flush, most NO will be flushed out to prevent its reaction with O_2 to form NO_2. As NO_2 has a high boiling point of about 21°C and readily reacts with water, NO_2 is expected to be adsorbed onto fumigated products and may be converted to nitrate (NO_3^-) and nitrite (NO_2^-) as residues.

Twenty fresh products and 10 stored products were studied for residues after NO fumigation treatments (Yang and Liu, 2017, 2019). For most fresh products, when NO fumigation is terminated properly with N_2 flush, it does not result in significantly higher nitrate or nitrite levels as compared with controls (Yang and Liu, 2017). For the 10 stored products, NO fumigation also does not significantly increase nitrate or nitrite levels in fumigated stored products as compared with controls (Yang and Liu, 2019). When NO fumigation is terminated without N_2 flush, there are significant increases in nitrate and sometime also nitrite levels in fumigated fresh and stored products (Yang and Liu, 2017, 2019). Nitrate and nitrite naturally exist in food products and have nutritional values as they may contribute to the blood pressure–lowering effects and nitrate is an important part of our bodies' defenses against gastroenteritis (Santamaria, 2006; Hord et al., 2009). Nitric oxide fumigation, therefore, does not leave toxic residues in fumigated products and is safe to food quality and human health.

Prospects of NO fumigation

Efficacy of NO fumigation has been well demonstrated against different life stages of 14 pest species representing diverse pest groups including external and internal feeders, fresh and stored product pests. It is, therefore, reasonably to expect that NO fumigation will be effective against most other pests. Because it is technically feasible to establish NO fumigation with desired levels of NO and NO_2, and NO_2 fumigation was demonstrated to be effective against microbes on stored almonds and peanuts as well as *Aspergillus flavus* spores, NO fumigation has the potential to control pests and pathogens in a single fumigation treatment. This is expected to expand usage of NO fumigation, make NO fumigation more cost effective, and promote its commercial applications. However, approval of NO fumigation for pest and microbial control by regulatory agencies must occur before any prospect for commercial application of NO fumigation can be realized. Active involvement of industry is critical in the regulatory approval process and development of specialized fumigation equipment for NO fumigation. The anticipated expanded use of NO fumigation for control of both pests and microbes is likely to make NO fumigation more attractive to industry to increase efforts to register NO fumigation for commercial applications.

Nitric oxide has advantages in efficacy in comparison with the commercial alternatives: phosphine, sulfuryl fluoride, and ethyl formate. Phosphine, as a major methyl bromide alternative fumigant for postharvest pest control, is not effective against some pests due to tolerance or resistance and phosphine fumigation and, in general, also has long treatment times which may extend over 10 d to achieve effective control of some pests (Hole et al., 1976). Although phosphine fumigation in an oxygen enriched atmosphere (oxygenated phosphine fumigation) has significantly increased the efficacy of phosphine fumigation against phosphine tolerant insects (Liu, 2011), the prospect for commercial application of oxygenated phosphine fumigation remains unclear. Sulfuryl fluoride has the disadvantage of being ineffective against insect eggs (Bell et al., 1998) and having phytotoxicity to fresh products (Aung et al., 2001). The absorbing rate of ethyl formate in fresh products and its phytotoxicity on fresh products are also concerns for wide applications of ethyl

formate fumigation (Zoffoli et al., 2013). In contrast, NO fumigation is not only effective against all pests and all life stages but also controls microbes that often occur with pest infestation and need effective control.

For delicate fresh fruits and vegetables, NO fumigation may have additional benefits of extending storage/shelf-life. Some harvested fresh products are treated with chemical agents to maintain proper storage life. Nitric dioxide; however, is an inhibitor of ethylene biosynthesis (Manjunatha et al., 2010) and can also help to maintain postharvest storage life (Soegiarto and Wills, 2004; Manjunatha et al., 2012; Liu et al., 2016; Yang and Liu, 2018a). It is possible that NO fumigation for postharvest pest control can also reduce or replace the usage of chemical agents for postharvest storage of fresh fruit. This suggests NO fumigation may provide additional benefits and enhance food safety.

There have been extensive efforts with limited progresses to find alternative treatments for postharvest control of pests and microbes to replace methyl bromide. Therefore, there is a severe lack of safe and effective alternative fumigants to meet the demand for postharvest pest and disease management. As a recently discovered new fumigant, NO fumigation has high efficacy against a wide variety of pests and associated NO_2 control of microbes. Furthermore, the lack of toxic residues and extended storage life of fresh products treated with NO fumigation should far offset the disadvantages of the complex and strict fumigation procedures and associated costs on acquisition and operation of N_2 generation equipment. Therefore, more efforts are warranted to speed up the commercial applications of NO fumigation including developing effective and safe treatments for specific pests on a variety of products, developing and demonstrating commercial scale treatment protocols, and registration efforts from industries for commercial applications.

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Asher PP, Patel FR (2021) Results of fumigation of fresh flowers – QuickPHlo – R™ phosphine generator. Page 296. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Results of fumigation of fresh flowers – QuickPHlo – R™ phosphine generator

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ABSTRACT

There is a demand for phosphine as fumigation with methyl bromide being banned in many countries for quarantine fumigation. Methyl bromide application is complex and does not disperse easily inside the enclosures. It leaves residue in the commodity. Phosphine is the most preferred fumigant. However, aluminum phosphide tablets cannot be used for fresh flower fumigation because they are phytotoxic.

UPL Ltd. has developed a technology to generate pure phosphine gas in an hour and is ammonia free. QuickPHlo – R phosphine generator is used with QuickPHlo – R aluminum phosphide 77.5% granules for the trials.

We conducted experiments on roses at various locations and on tulip bulbs to assess the mortality on insect pests – *Tetranychus* spp (Mites), *Frankliniella* spp (Thrips) and *Thaumatotibia leucotreta* (FCM) and to determine for change in colour or any other detrimental effects. Different behaviours in different insect pests were observed at various locations.

Keywords: Fumigation, Phosphine, QuickPHlo – R phosphine generator, QuickPHlo – R aluminum phosphide 77.5 % granules, Insect pests, Cut flowers

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Xi J, Zhao Y, Li Y, Dong H, Wang J, Gu X, Song J (2021) Mixed fumigation of sulfuryl fluoride and carbon dioxide to tobacco lamina. Pp. 297-300. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Mixed fumigation of sulfuryl fluoride and carbon dioxide to tobacco lamina

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Abstract

Tobacco lamina is often damaged by stored product insect pests during storage and processing. Currently, phosphine fumigation is widely used to control the pests, however, its application has been restricted due to its high toxic, flammable, explosive, and corrosive to metals. Sulfuryl fluoride is favored by tobacco factories because of its wide lethal effect to different species of insect pests, high diffusivity, permeability, low toxicity, low residue, and no corrosive to metals. To improve the safety and lethal effect of sulfuryl fluoride, the mixed fumigation of sulfuryl fluoride and carbon dioxide (weight ratio 7:3) was studied. Results showed that all of the eggs, larvae, pupae and adults of *Lasioderma serricorne* at 30 g/m^3 of the mixture were dead after being treated at 28±2℃, 60±5% RH for 4 d（Ct value 2880 g.h/m^3). The mixture treatment with different concentrations had no significant effect on the chemical composition of tobacco lamina, and only the total plant alkaloid was slightly decreased. The sensory quality of tobacco lamina did not change, and the residue was not significantly increased. The mixed fumigation can reduce the use of sulfuryl fluoride and improve the safety during tobacco storage, which is significant for the ecological control of stored tobacco pests and the reduction of environmental pollution.

Keywords: Sulfuryl fluoride, Carbon dioxide, Mixed fumigation, Tobacco lamina, *Lasioderma serricorne*

Introduction

Lasioderma serricorne is one of the most destructive insect pests to stored tobacco leaves and cigarettes (Edde, 2019). Phosphine fumigation is the most widely used chemical control method by various tobacco processing factories during tobacco storage (Zhu et al., 1995). However, its application is restricted to certain predetermined conditions due to its high toxicity, flammability, explosiveness, and corrosivity to metals. Sulfuryl fluoride is favored by tobacco factories and has

a broad application due to its high diffusivity, permeability, low toxicity, low residue, and non-corrosivity to metals. Research has shown that carbon dioxide can enhance the penetrability of phosphine, which makes phosphine uniformly distributed in the enclosure. In addition, carbon dioxide can stimulate the insect respiration, hence enhancing the lethal effect on insects (Jian et al., 1995). To improve the fumigation effectiveness, shorten fumigation time, and reduce sulfuryl fluoride deflagration risk, mixture of sulfuryl fluoride and carbon dioxide was used to control tobacco insects during tobacco storage. Relationships among exposure time, concentration of sulfuryl fluoride and carbon dioxide, and mortality of Lasioderma serricorne were determined in this study which provided the basic information for the determination of dose of sulfuryl fluoride and carbon dioxide, and exposure time for the mixed fumigation.

Materials and methods

Insects

Lasioderma serricorne was collected from a tobacco factory located at Zhengzhou, Henan, China, and reared on a diet of 45% flour, 5% yeast and 50% wheat kernels in weight. Pupae of *Lasioderma serricorne* were obtained from the culture. The 2 d old eggs of *L. serricorne* were provided by the Henan University of Technology, Henan, China.

Treatments

Sulfuryl fluoride and carbon dioxide were provided by Shanxi Xinjinye Tobacco Technology Co., China. The weight ratio of sulfuryl fluoride and carbon dioxide was 7:3 and the concentration of the mixture was 30 g/m^3. The exposure time was 48, 72, 96, and 120 h. There were three replicates for each treatment.

Each pile with 12 tobacco stacks were sealed with polyethylene film and adhesive tape. The volume of a pile was about 60 m^3. During treatment, temperature and relative humidity were maintained at 28±2°C and 60±5%, respectively. One hundred each stage (egg, larva, pupa, and adult) of *L. serricorne* were introduced into a glass vial filled with tobacco leaves, and the glass vial was covered by 200 mesh screen.

Placement of air sampling tube

To install the air sampling tube, one cigarette box at the center of each stack was selected. One plastic tube was inserted into the center of the cigarette box through a premade hole, and the tube was fixed by adhesive tape. The gas sampling tube was also fixed at the outside of the tobacco pile. The concentration of sulfuryl fluoride in the cigarette box was measured every 0.5 h up to 12 h at beginning of the treatment, and then every 2 h up to the end of the treatment.

Determination of insect mortality

After the polyethylene film which sealed the tobacco pile was removed, the glass vials holding the treated insects and tobacco leaves were removed and the insects were transferred to petri dishes. The survival of the insects (larvae, pupae, and adults) in the petri dishes was observed. The eggs in the petri dishes were incubated at 30°C and 70% RH for 14 d.

Results and discussion

Effect of the mixture on insect mortality

As shown in Table 1, the longer the treatment time, the higher the mortality for any stage of the insect. Eggs, larvae, pupae, and adults were dead at 96 h.

Table 1. Effect of the mixture on insect mortality

Exposure time (h)	Mortality (mean ± standard deviation)			
	Adult	Larva	Pupa	Egg
48	100±0	90±2.5	100±0	85±4.5
72	100±0	98±1.5	100±0	96±3.5
96	100±0	100±0	100±0	100±0
120	100±0	100±0	100±0	100±0
Control	15±3	0±0	0±0	37±6.5

Effect of the mixture on chemical composition of tobacco lamina

The effect of the mixture on the chemical composition of tobacco lamina is shown in Table 2. The mixture had no obvious effect on the chemical composition of the tobacco lamina. Compared with the control, the concentrations of total nitrogen, reducing sugar, potassium, chlorine, total volatile acid, and total volatile base did not change, while the total alkaloid decreased slightly.

Table 2. Effect of the mixture on chemical composition of tobacco lamina

Exposure time (h)	Total alkaloid (%)	Total nitrogen (%)	Reducing sugar (%)	Potassium (%)	Chlorine (%)	Volatile acid (%)	Volatile base (%)
48	3.03	2.01	21.96	1.70	0.32	0.175	0.191
72	3.05	2.03	22.17	1.74	0.31	0.173	0.188
96	3.10	2.02	22.38	1.69	0.33	0.176	0.182
120	3.24	2.00	23.68	1.65	0.30	0.175	0.190
Control	3.23	2.01	22.55	1.69	0.31	0.174	0.190

Effect of the mixture on sensory quality of tobacco lamina

The sensory quality after the 120-h treatment was better than the control, while the sucking quality after 48, 72 and 96 h treatments were equivalent to that of the control. After the mixture treatment, the sensory quality of tobacco lamina had no adverse effect.

Table 3. Effect of the mixture on sensory quality of tobacco lamina

Exposure time (h)	Aromatic quality	Amount of aroma	Con[a]	Mis[a]	Ene[a]	Irr[a]	Aft[a]	Quality Grade
48	6.5	6.5	6.0	6.0	6.5	6.0	6.5	6.5
72	6.0	6.5	6.0	6.0	6.5	6.0	6.5	6.5
96	6.0	6.5	6.0	6.0	6.5	6.0	6.5	6.5
120	6.0	6.5	6.0	6.0	6.5	6.0	6.0	6.0
Control	6.0	6.5	6.0	6.0	6.5	6.0	6.5	6.5

[a]Con = Concentration, Mis = Miscellaneous gas, Ene = Energy, Irr = Irritability, Aft = Aftertaste

Effect of the mixture on fluorine residue

With the increase of the dosage of sulfuryl fluoride or the exposure time, the residue of fluoride in the tobacco lamina increased. Compared with the control, the fluoride residue increased by 3.1 mg/kg at 30 g/m^3 of the mixture concentration and 4 d treatment (Table 4).

Table 4. Effect of the mixture on fluorine residue

Exposure time (h)	48	72	96	120	Control
Residue (mg/kg)	26.8	27.7	31.0	31.0	27.9

Discussion and conclusions

At 28 ± 2°C and 60 ± 5% RH, all insect stages (eggs, larvae, pupae, and adults) were dead under the mixture concentration of 30 g/m^3 and 4 d treatment, which had a Ct product of 2880 g·h/cm^3. The mixture treatment with different concentrations did not significantly influence the chemical composition of tobacco lamina, and only the total alkaloid was slightly decreased. After the fumigation, the sensory quality of tobacco lamina did not change, and the residue was not increased significantly.

The mixed fumigation of sulfuryl fluoride and carbon dioxide requires a higher airtightness level of the treated enclosures. In practice, when mixed fumigation is applied, the airtightness of the enclosure must be inspected carefully. If the airtightness of enclosure cannot meet the requirement, the amount of sulfuryl fluoride must be increased to compromise the less sealing level. Otherwise, the fumigation time should be increased.

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McKirdy HL, McKirdy SJ, Ren Y (2021) The application of ethyl formate for the treatment of stored grain insect pests. Page 301. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

The application of ethyl formate for the treatment of stored grain insect pests

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ABSTRACT

Fumigation treatment of stored grain products is an important component of maintaining commodity integrity. Here, we reported laboratory preliminary trials to evaluate the efficacy of liquid ethyl formate (EF) as a potential fumigant for controlling stored grain insects. Ethyl formate was applied twice with a four-hour interval between applications within a simulated mini grain silo containing 52 kg of wheat and three common durable commodity insect pests (*Sitophilus oryzae* (Linnaeus), *Rhyzopertha dominica* (Fabricius) and *Tribolium castaneum* (Herbst)) in the different locations. Ethyl formate was highly effective in causing acute mortality in the adult life stage of three insect species assessed, with adult mortality greater than 99% across all species 24 h post-fumigation. Variation in the pattern of emergence of adults in the fumigation treatment compared to the control indicate EF had a chronic effect on internal stages (eggs, larvae and pupae) within the grain. Immature life stages of *R. dominica* exhibited the greatest susceptibility to EF, with *T. castaneum* displaying the most tolerance. Additional applications of the fumigant would be necessary to fully control all life stages. Given the acute success of EF in treating the adult life stages of the three species investigated and the chronic effect on immature life stages within the grain, the compound remains a viable grain fumigant for the control of durable commodity pests. Further trials will be required to determine the efficacy of the fumigant to other common insect pest species and the various life stages of these insects.

Keywords: Fumigant, Methyl bromide alternative, Ethyl formate, Fumigation, Stored grain insect pests, Insect mortality

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Thalavaisundaram S (2021) Ethanedinitrile (EDN) – a new broad-spectrum fumigant for biosecurity applications. Page 302. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Ethanedinitrile (EDN) – a new broad-spectrum fumigant for biosecurity applications

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ABSTRACT

EDN (active ingredient - Ethanedinitrile C_2N_2) is a new broad-spectrum fumigant, highly toxic to insects, nematodes and fungal pathogens of timber and logs. EDN is neither an ozone-depleting substance nor it is a green-house gas. It is currently registered in Australia, South Korea and in the Czech Republic under permit. It is in the process of approval in a number of countries. ISPM-28 for treatment of wood for insect pests is currently in the approval process.

EDN has a number of advantages for post-harvest application. The boiling point of EDN is -21°C and can be applied as a gas, and it is efficacious at low temperature. EDN is a smaller molecule and has a high vapour pressure; hence it can penetrate quickly along and across the grain of the timber and achieve equilibrium quickly in a fumigation environment resulting in higher efficacy. Hence, EDN has potential as a phytosanitary alternative to methyl bromide for treatment of pallets, sawn timber and logs.

Lab and field studies were conducted by the New Zealand PFR on three timber pests – burnt pine longhorn beetle, *Arhopalus ferus;* Black pine bark beetle, *Hylastes ater;* and Golden-haired pine bark beetle, *Hylurgus ligniperda.* Results shown 100 g/m^3 for 20 h was efficacious to all timber pests.

Efficacy studies conducted at FPInnovation Canada, on Pinewood nematode (*Bursaphelenchus xylophilus)* and four fungal pathogens (*Heterobasidion annosum, Geosmithia morbida, Phytophthora ramorum,* and *Ceratocystis fagacearum)* at two dose rates and temperatures between 1 and 24 h exposure shown that EDN was efficacious to all the target nematodes and fungal pathogen.

USDA and the University of Tennessee study on Pinewood nematode in artificially infested wood showed that EDN was highly effective and provided complete mortality at 40 g/m^3 for 24 h treatment than other alternative fumigants available.

Keywords: EDN, Pinewood nematode, Timber pests, Timber pathogens, Quarantine treatment

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Mahroof RM, Paudel S (2021) Dose-response of selected stored product insects to ozone treated on various surface materials. Pp. 303-308. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Dose-response of selected stored product insects to ozone treated on various surface materials

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Abstract

Since the phase out of methyl bromide, researchers are looking for alternatives to control stored insect pests and ozone has shown some promises as an effective control agent for stored insect pests. However, there exist many discrepancies on the effects of ozone on stored insect pests, which might be due to the differences on dose, exposure time, storage surface material, and method of application. The goal of this study was to determine the effects of ozone toxicity for different stored insect pests that could occur in different storage structures. The materials used commonly in storage structures included aluminum, cement, wood, glass, and vinyl. Preliminary experiments were done with eggs, larvae, pupae and adults of two stored insect pests, Indianmeal moth, *Plodia interpunctella* (Hubner), and red flour beetle, *Tribolium castaneum* (Herbst). Insect life stages were exposed to ozone gas on glass, aluminum, concrete, vinyl, and wood surfaces at 900 ppm. This study explored the time-concentration requirements of ozone gas to attain LT_{99} for the most tolerant life stage of *P. interpunctella* and *T. castaneum*.

Keywords: Fumigations, Grain storage, Ozone, Indianmeal moth, Insect management, Red flour beetle, Surface materials, Stored products

Introduction

The consequences of insect infestation in storage, milling, processing and warehouse facilities are far greater than the dollar costs of products (Arthur and Phillips, 2003). In general, stored product insects in bulk grain have been managed by fumigation using phosphine. Phosphine fumigation is an effective method to control stored-product insects, but its continuous and indiscriminate use has resulted in the evolution of resistant populations and control failures (Zettler and Keever, 1994; Shi et al., 2012).

More research on viable alternatives that can effectively control stored product insects in bulk storages deem necessary. Ozone is one such alternative, it can control insects and moulds associated with grain (Mahroof et al., 2017; 2018 a, b). Ozone is a toxic gas that can kill insects effectively in relatively short period. The most desirable aspect of ozone is that it decomposes rapidly to molecular oxygen without leaving a residue in treated food. Stored product insects such

as Indianmeal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) and red flour beetle, Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) are known to successfully feed, develop, and breed on a variety of durable commodities, causing significant losses to stored grains, pulses, dried fruits and nuts, and processed foods (Howe, 1957). Losses due to these insect infestations and damage can account for millions of dollars in the food industry. These insects are cosmopolitan in distribution and the most common indication of infestation is adult activity on the surface of the food. Plodia interpunctella commonly forms webbing or silken cocoons. Larval and adult tunnels are common in infested commodities as well as on dusty surfaces in food-processing facilities and grain elevators (Hagstrum and Subramanayam, 2009). In heavy infestations, the food material may be discolored, kernel structure may be disintegrated and dust and particles may be prevalent.

Ozone kills stored-grain insects such as Sitophilus oryzae (L.), Oryzaephilus surinamensis (L.), O. *mercator* (Fauvel), *Lasioderma serricorne* (F.), and *Ephestia elutella* (Hübner) and various fruit flies (Endman, 1979; Endman, 1980; Mahroof et al., 2017; 2018 a; Amoah and Mahroof, 2018). Endman (1980) who pioneered the effort of ozonation for killing stored product insects used low concentration gas with extended exposure time, up to 7 h. He reported that the younger larval and pupal stages of *T. confusum* were more ozone sensitive than similar older stages; 28-d-old pupae were the most ozone resistant of the stages tested. Other studies, including those by Mahroof et al. (2018 a, b) and Amoah and Mahroof (2018) have suggested that toxicity caused by ozone is usually delayed but dependent on the species, life stages, dose and exposure time.

Lack of data on dose-mortality response for various life stages of stored product insects alongside flow and penetration characteristic of ozone through various building materials or floor surfaces render uncertainly in application of this technique to various types of storage bins, food processing buildings or packaging. Therefore, there is a need to close the data gap that exist in the literature, in order to better popularize and utilize this pest control technique. The objective of this study was to evaluate the efficacy of ozone on the most tolerant life stages of *P. interpunctella* and *T. castaneum* and using the most tolerant stage to expose on surfaces, resembling flooring or construction of food processing plants or grain storage structures.

Methods

Ozone equipment, ozone generation, and application

A bench-top model of ozone generating equipment that produces ozone in the range of 0-8000 ppm with a continuous flow rate of 1-2 L/min using the corona discharge method, was custom built and obtained from Ozone Solutions Inc., Hull, IA. The equipment was made of five major components: the oxygen concentrator, a control box with ozone generator, the analyser, the ozone chamber, and the ozone destruction unit. The ambient air was taken up by the oxygen concentrator; O_2 in the air was concentrated and delivered between high voltage plates to simulate corona discharge. Oxygen was broken apart and recombined into ozone. The concentration of ozone was regulated by adjusting the push button selector switch on a touch screen panel. The accuracy of the concentration was further verified by the analyzer. The analyser was calibrated yearly according to NIST standards to maintain accuracy. Accuracy of the set concentrations varied by ± 5 ppm. The ozone chamber, where test specimens were placed, maintained user-defined ozone concentrations. Ozone was delivered to the chamber through three outlets. A circulation fan located inside the ozone chamber evenly distributed ozone throughout the chamber.

Establishing optimal lethal concentration

As a preliminary study, eggs, larvae, pupae, and adults of *P. interpunctella* and *T. castaneum* life stages were tested at 400, 500 and 600 ppm for 6 h exposure to select the most tolerant life stages. We did not report the results of the preliminary data herein. Based on the study, we selected eggs of *P. interpunctella* and *T. castaneum* for further studies. Twenty-four-hour old eggs transferred to Petri dishes either with 20 g of diet or without diet were exposed at 600 ppm concentration for 9 h. Lab artificial medium or 15 (whole wheat flour):1 (yeast) was used as the diet for *P. interpunctella* and *T. castaneum*, respectively. The control experiment was not to expose eggs to ozone gas, but rather to apply every other condition given to experimental unit including control treatments with or without diet. Post treatment, eggs were transferred to an environmental growth chamber at 28ºC and 60-65% RH. Egg hatch was observed daily until 10 d. The number of eggs that hatched both in treated and control experiments were recorded. If mortality exceeded >15% in control units, then treatment mortality was corrected based on the Abbott's Formula.

The experiment was a Randomized Complete Block Design (RCBD) with replication over time (block based on time). Experiments for *P. interpunctella* eggs were replicated six times and for *T. castaneum*, it was replicated nine times. At a given time for a give species, a total of 100 eggs were treated as 10 eggs/dish (10 × 10 = 100). These 10 dishes were considered as pseudo replicates. Egg that did not hatch were expressed as percentage mortality and subjected to two-way ANOVA using PROC MIXED program. Means were separated using LS means procedure at $\alpha = 0.05$.

Evaluating the efficacy of ozone when exposed to various surfaces

The toxicity of ozone was evaluated for eggs of *P. interpunctella* by exposing them on various surfaces that resemble flooring or construction materials of food processing plants or grain storage structures. Glass, aluminum, concrete, vinyl, and wood were tested. Twenty-four-hour old eggs with or without diet were placed on the laboratory-fabricated arena. Eggs were exposed to ozone at 600 ppm for 9 h. A control experiment was maintained by introducing eggs to various surfaces with or without diet but not exposing them to ozone. Post treatment, eggs were transferred to an environmental growth chamber at 28ºC and 60-65% RH. Egg hatch was observed daily until 10 d. Number of eggs that hatched both in treated and control experiments were recorded. If mortality exceeded >15% in control units, then treatment mortality was corrected based on Abbott's Formula. The experiment was replicated over six times (block based on time). At a given time, a total of 100 eggs were treated as 50 eggs/dish (50 × 2 = 100). These 2 dishes were considered as pseudo replicates. Mean percentage mortality in treated arenas and corresponding control arenas, whether with diet or without diet were estimated.

Results and discussion

Establishing optimal lethal concentration

Susceptibility of *P. interpunctella* and *T. castaneum* eggs was found when exposed to ozone at 600 ppm for 9 h. Egg mortality was not dependent upon whether presence or absence of diet in the treatment arena (Table 1). Ozone treated eggs with or without diet showed significantly lower hatch, resulting high egg mortality when compared to corresponding controls, for both species (*P. interpunctella:* $F = 337.41$, $df = 3,116$ $P < 0.0001$; *T. castaneum*: $F = 116.31$, $df = 3,176$ $P < 0.0001$). Egg mortality was consistently high for *P. interpunctella* eggs in control treatments. We presume this could be due to mechanical and physical damages caused during handling.

Comparing the two species, *P. interpunctella* eggs were more susceptible to chosen Ct (Concentration X time) resulting in closer to 100% mortality. However, *T. castaneum* eggs required a higher Ct value to attain 100% mortality. Moth eggs, in general, had more aerophyles and micropyles when compared to beetle eggs, allowing them to intake a relatively higher amount of gas during exposure, making ozone relatively more toxic to moth eggs. In *T. castaneum*, a higher percentage of egg mortality was encountered when eggs were treated without diet. Ozone is adsorbed by physical surfaces including diet and the gas had to penetrate through food by diffusion, resulting in a significant proportion of the gas loss. Therefore, it is suggested to use a higher Ct value in the presence of grain.

Table 1. Mean mortality (%) ± SE for *P. interpunctella* and *T. castaneum* eggs treated using 600 ppm ozone exposed to 9 h.

Species	Type of treatment	Mean %Mortality ± SE
Plodia interpunctella	Control with food	59.67 ± 8.32^{a}
	Control without food	56.00 ± 7.81^{a}
	Treatment with food	98.74 ± 1.69^{b}
	Treatment without food	99.54 ± 0.91^{b}
Tribolium castaneum	Control with food	23.00 ± 4.59^{a}
	Control without food	23.33 ± 3.91^{a}
	Treatment with food	68.33 ± 9.59^{b}
	Treatment without food	78.11 ± 8.45^{b}

*$n = 6$ for *P. interpunctella* and $n = 9$ for *T. castaneum*

Data were analyzed separately for each species using ANOVA and the mean numbers with different letters for a given species are significantly different (LS means at $\alpha = 0.05$).

Evaluating the efficacy of ozone when exposed to various surfaces

Susceptibility of *P. interpunctella* eggs on five surfaces with or without diet was tested in laboratory-fabricated arenas. Studies clearly showed, regardless of whether diet present or absent in the testing arena, eggs exposed to ozone failed to hatch compared to corresponding controls (Fig. 1). Treated surfaces did not influence the toxicity of the ozone, mortality remained high and above 95% for all surfaces. Studies have demonstrated that the movement of ozone through a grain mass is restricted and most of the gas reacts with the grain or food surfaces. Results from this preliminary study show, construction materials made of glass, aluminum, cement, vinyl, or wood behave in a similar manner in reacting to ozone. These surfaces appear to be not absorbing a significant proportion of the gas, leaving the ozone to react with the insects.

Overall, our study has shown that variability in egg susceptibility to ozone gas depended on the dose, exposure time, species, and presence or absence of food during the treatment. The study also showed common construction materials used in grain bins, mill equipment, processing, and storage plants absorbed insignificant proposition of the ozone gas, allowing ozone to be used in these facilities. Detailed further studies are ongoing.

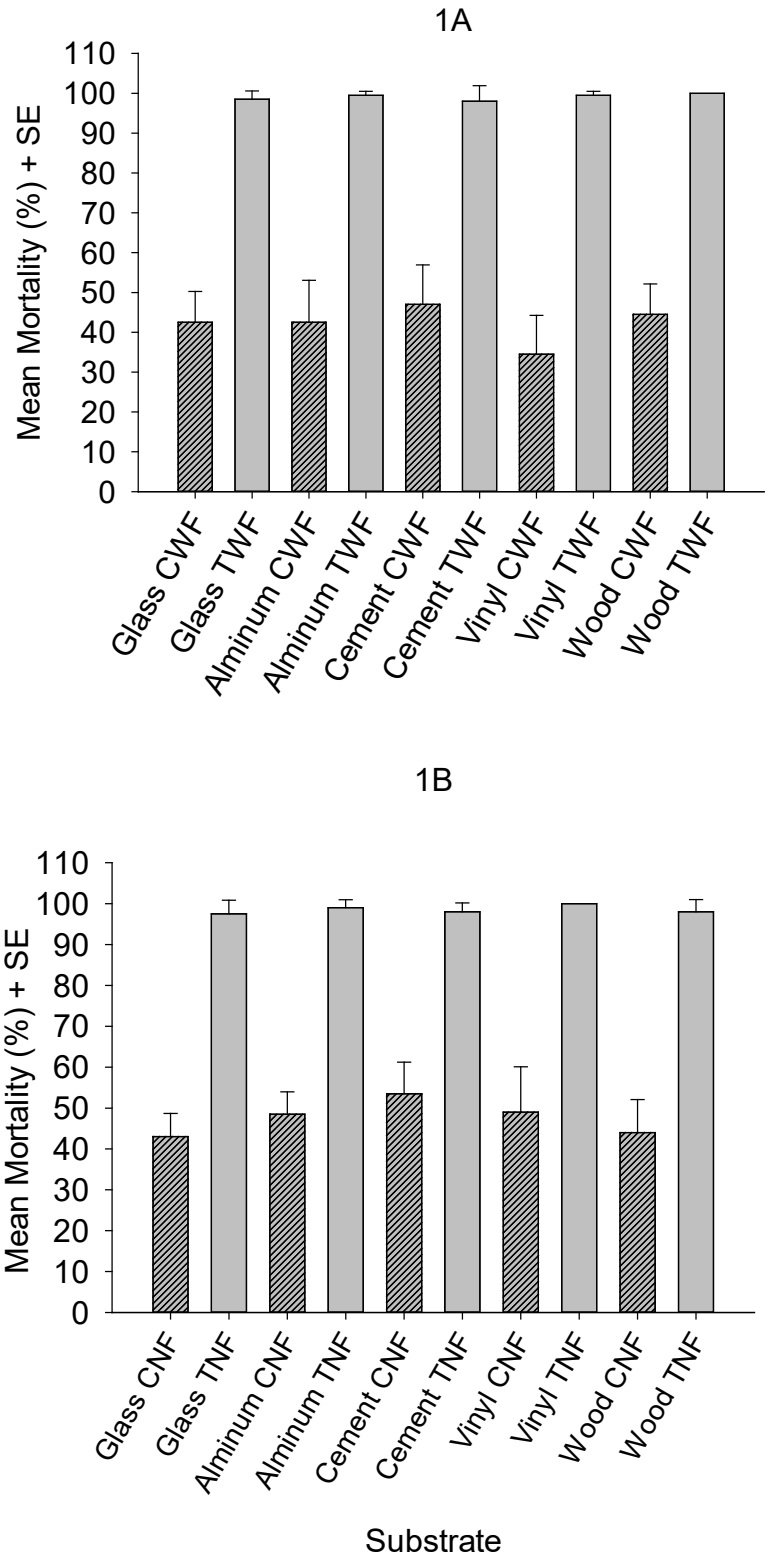

Fig. 1. Mean mortality (%) (+ SE) of *P. interpunctella* eggs exposed to 600 ppm of ozone for 9 h treated or control (no ozone treatment) with (1A) or without (1B) diet in various surfaces (n=6).

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Management of insects in Canada – status update

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Abstract

Canadian prairies being in a temperate region, provide cooler temperatures and drier air conditions throughout the year. In some months, ambient temperatures are low enough to control insects. Thus, insect infestations are not a major concern in Canadian grain. However, some infestations do occur on farms and in elevators when warm summer and fall temperatures occur after harvest or during spring and summer months in carried over grain from a previous year harvest. Thus, occasionally control of insects in grains using physical or chemical methods becomes a necessity. This paper summarizes the insect control methods within the context of the climate on the Canadian prairies, and essential requirements for the fumigation, and regulatory aspects of insecticide application.

Keywords: Canada prairies, Insect control method, Fumigation, Regulation

Introduction

Canada annually produced about 98 million tonnes of cereal grains, oilseeds and pulses (together referred to as grains) which are initially stored on farms (Statistics Canada 2021) except that a small quantity (less than 5%) may be delivered directly at harvest to elevators (grain handling facilities) or processing factories. Grains on farms are typically stored in flat bottom corrugated galvanized steel bins or welded steel hopper bins of different diameters and heights. Most of these bins have an air introduction system installed to aerate or dry grain using ambient or near-ambient air. The term near-ambient is used when air is pulled over a fan and frictional heat of fan adds 3-5°C to ambient air. Sometimes supplemental heat may be added to increase air temperature by 5 to 10°C for low-temperature grain drying (usually referred to as natural air drying with heater). Over the years, bin diameters have increased on farms mainly to reduce unit cost of storage and to accommodate larger harvested volumes. Bins with 10 m diameter are common currently on Canadian farms. The capacity of bins and grain handling rates have also increased considerably in elevators, while the number of grain elevators on the Canadian prairies has decreased (Fig. 1). This has also resulted in increased storage capacity on farms where grains maybe stored for up to two years depending on the demand of Canadian grains in the export markets.

While stored on farms, grains are exposed to diurnal and seasonal changes in weather parameters such as temperatures, relative humidity, velocity of air, solar radiation, and precipitation as well

as by the location of the bin with respect to surrounding structures and its orientation if bin is rectangular in shape. These weather parameters affect internal grain conditions, particularly temperature and moisture content, two important parameters affecting development and growth of fungi and insects. The effect of weather parameters is pronounced in the 15 cm of grain near bin walls and at the top surface of grain if head space is large. The interactions of biological, chemical, and physical parameters are best understood by treating stored grain mass as a man-made ecosystem and can be mathematically modelled (Jayas et al., 1995; Jian et al., 2005).

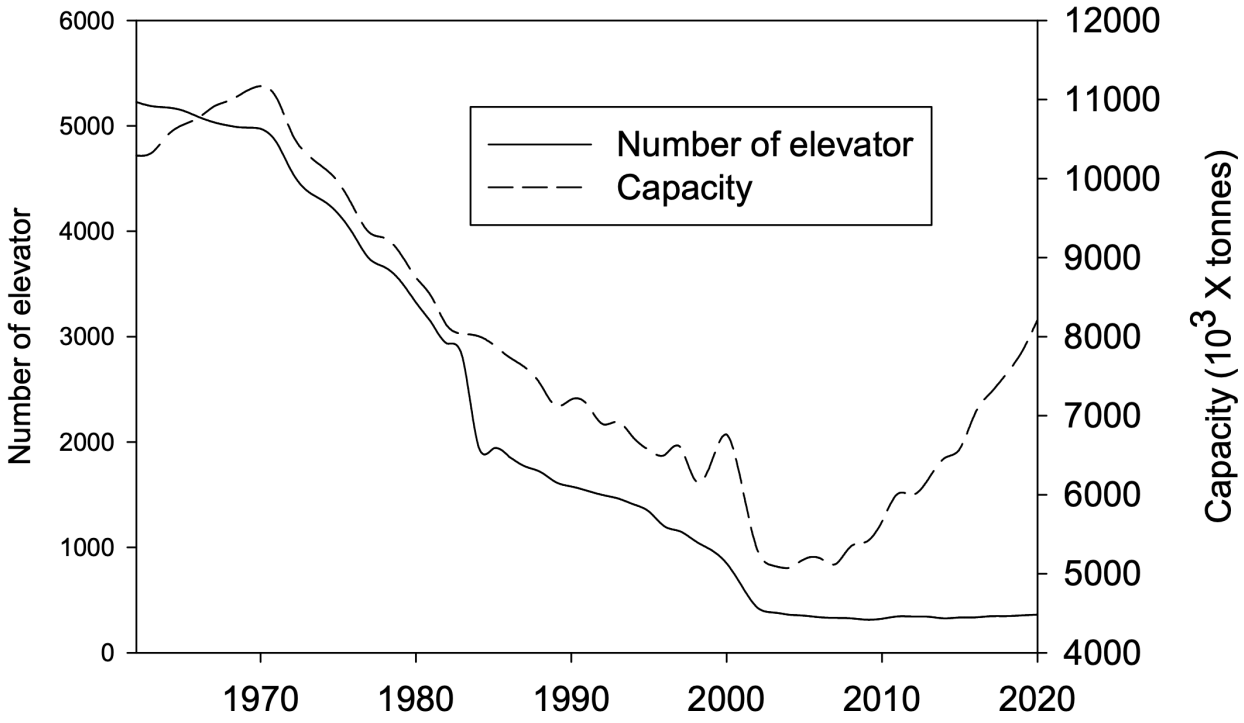

Fig. 1. Total elevator number and handling capacity of the elevators in Canada.

The Canadian prairies are considered to be a temperate region that provides cooler temperatures and drier air conditions throughout the year (Fig. 2). In the period between December and March, ambient temperatures can be low enough to control insects in grains in small diameter bins, while aeration to lower grain temperatures in large diameter bins is needed due to thermal properties of stored cereals. Even during the months when temperatures are not low enough to kill insects, temperatures are often low enough to keep grains at temperatures below 10°C where insect activity as well as fungal development is minimal. Thus, insect infestations are not a major concern in Canadian grains. However, some infestations do occur on farms and elevators after harvest with a warm summer and fall or during following spring and summer. Thus, control of insects in grains using physical or chemical methods occasionally becomes a necessity. Therefore, purpose of this paper is to provide an overview of status of methods used to control insects in Canada.

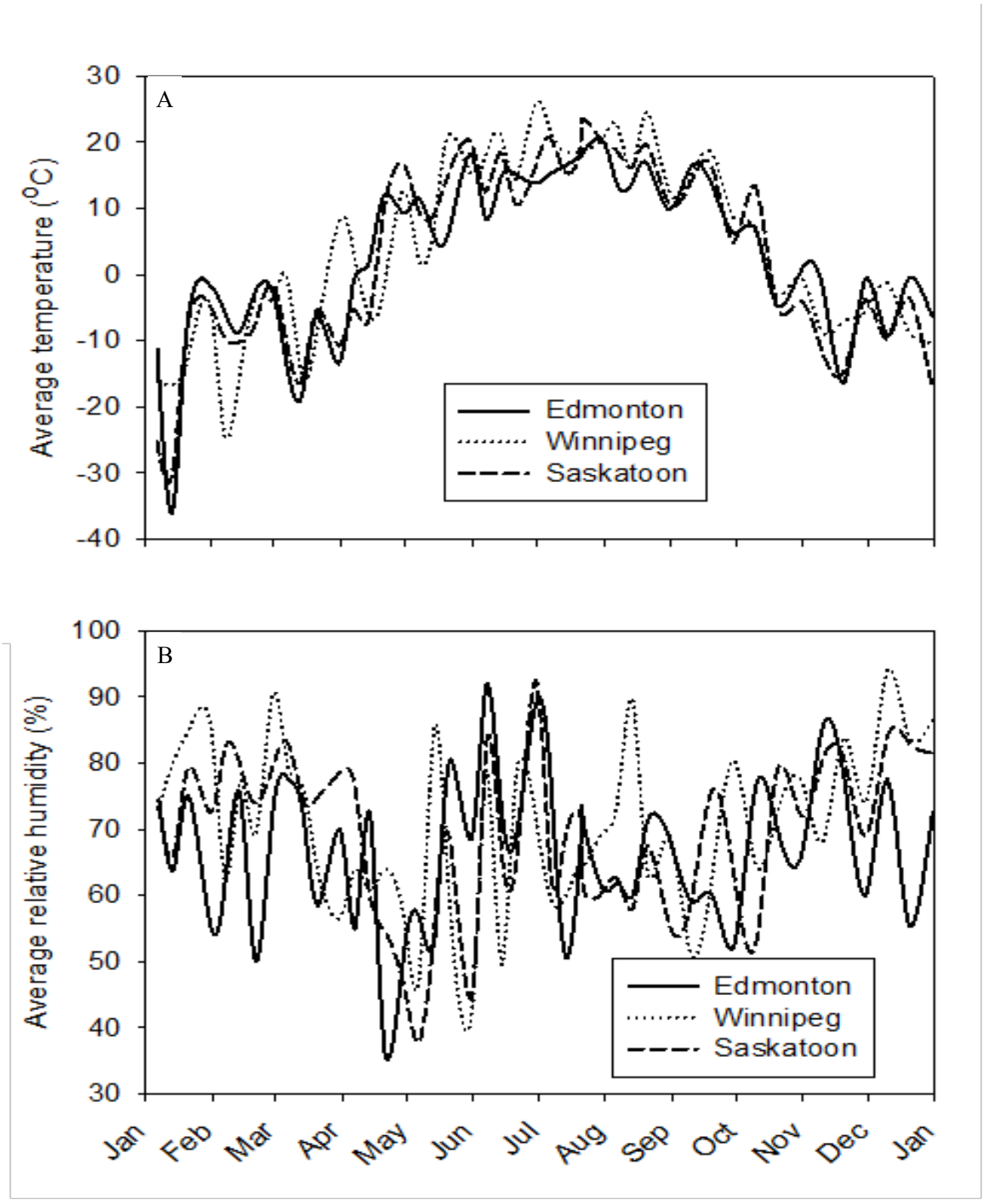

Fig. 2. Average temperatures (A) and relative humidity (B) at Edmonton, Winnipeg, and Saskatoon in 2020.

Insect control methods

Insect control methods can be broadly grouped into three categories: biological, physical, and chemical. Biological control methods are at the research stage in Canada and are not used to control insects in grain. Physical control methods that have been researched in Canada include impact, sieving, low temperature, high temperature, microwaves, infrared radiation, high carbon dioxide (CO_2) concentrations, high nitrogen (N_2)/low oxygen (O_2) concentrations, ozone, and irradiation using high energy electron beams or gamma rays from Cobalt-60 irradiator (Timlick et al., 2002; Neethirajan et al., 2007; Jian et al., 2016). Out of these only high CO_2 concentrations to control insects has been adopted by organic producers and grain industry. For example, an export terminal elevator in Vancouver is equipped to conduct high CO_2 fumigations. Chemical control methods can be grouped into two categories: contact insecticides and fumigants (Table 1). Contact insecticides can be used to treat structures (empty storage structures and cracks and crevices in buildings) and grains. The main contact insecticides are malathion, pyrethrins, cyfluthrin, and diatomaceous earth. Out of these, only diatomaceous earth is used directly on grain and all others are used for treatment of structures. Malathion, in dust or emulsifiable concentrate form is permitted for direct use on grain but its effectiveness is questionable and therefore is rarely used. Chemical fumigants available for use in Canada are Aluminum phosphide, Magnesium phosphide, Sulfuryl fluoride, and Methyl bromide. Cylinderized mixtures of phosphine and carbon dioxide are also sold for use.

On farm control of insects in stored grains is almost exclusively performed through aeration/temperature management. In years when harvest conditions are warmer, grain producers may choose to proactively apply malathion or diatomaceous earth as grain is being moved into storage. Contact insecticides such as pyrethrins are also often used to treat valves/venting to stop insects from entering bins when fresh grain is binned.

At export terminals, grain is received from railcar unloads that are typically in 90 tonnes in size. Grain is sampled using automatic crosscut sampling devices that typically takes 10 kg from each railcar. The collected samples are assessed for various aspects of quality including insect infestation. By the time grain has been determined as infested it may already be binned and the complete contents of the bin require fumigation. Large terminal silo bins are approximately 1000 tonnes. In the event infestation is discovered prior to binning the unloaded grain with the grain of same type and class, it may be diverted to smaller bins for independent fumigation.

Terminal elevators typically unload 8,000 to 12,000 tonnes within an 8 h working shift. Sampling systems used are for determining grain quality and therefore there are statistical limitations on the detection of insects from these systems. However, given that grain is sampled in a similar fashion at: farm delivery, country elevator loading, receival into an export terminal and when grain is being loaded onto vessels, the system is relatively robust.

Essentials for fumigation

Fumigants are toxic to humans, so the utmost care must be taken when handling these chemicals. To avoid accidental exposure to fumigant chemicals, treatments are only performed by registered applicators in Canada. Information that is helpful to regulators is generated through research and published literature.

Table 1. Chemicals used for controlling stored-product insects in Canada

Insecticides		Salient features
Fumigants	Aluminum Phosphide	Numerous forms, used for broad number of pests, temperature limitations for mortality
	Magnesium Phosphide	Fast acting, used for broad number of pests, temperature limitations for mortality
	Sulfuryl fluoride	Fast acting, used in cylinderized product for broad number of pests, insect stage limitations for mortality
	Carbon Dioxide	Used in commercial and organic production in elevators for broad range of pests, requires effective monitoring of concentration, logistical temperature limitations for mortality
	Methyl Bromide	Used in cylinderized product for broad number of pests, highly regulated - quarantine use only
	Phosphine / CO_2	Used in cylinderized product, fast acting, concentration highly controllable
Directly applicable contact insecticides	Malathion	Easy to use (Emulsifiable Concentrate (EC) or dust), low mammalian toxicity, levels of resistance/tolerance, organophosphates not well accepted
	Diatomaceous earth	Low mammalian toxicity, used in commercial and organic production, long residual activity, impacts flowability/test weight
Contact insecticides for structural application	Pyrethrin	Some residual activity, low mammalian toxicity, some concerns over resistance
	Malathion	Easy to use (EC), some residual activity, organophosphates not well accepted
	Diatomaceous earth	Long term activity, loses activity in the presence of organic dust and moisture

Necessary information required for proper fumigation includes: (i) insects to be eliminated and their response to fumigants; (ii) resistant stages of insects to be controlled; (iii) appropriateness of grain temperature (>10°C) and moisture content (>10% wb) for fumigation; (iv) commodity (cereals vs oilseeds) being treated; (v) application process (e.g., aluminum phosphide pellets added during grain loading or inserted using probes); (vi) any surfaces (copper) that can be corroded due to exposure from fumigants; (vii) structure airtightness for fumigation; (viii) the brand name (i.e., Aluminum phosphide is sold as Phostoxin, Phosfume, and Weevilcide) and other trade names for

formulation (i.e., tablets 5/8" dia, pallets 3/8" dia, pellets individually or held within in a sachet or porous bag); and (ix) appropriateness of fumigation time (i.e., avoid sunny, windy periods). For large structures it is advisable to use a closed loop system with recirculation capability to enhance uniformity of fumigant distribution. Monitoring of fumigant is critical during fumigation to ensure that right concentration (C) is maintained for the required period (t) to ensure desired Ct product.

Regulatory aspects for pesticide use on stored products in Canada

The use of pesticides in Canada on stored grains is governed by a number of authorities. The Pest Management Regulatory Agency (PMRA), as a department within the Health Canada organization is responsible for registering and reviewing the established submitted labels of pesticides that are allowed to be sold and used within Canada. Companies seeking to register pesticides in Canada must submit efficacy, toxicological and environmental impact assessment studies for the products being put forward for registration. Only when the PMRA is satisfied with all of the submitted data, will registration for a product be granted. Products registered by the PMRA may be used for prevention/control and the label is considered to be a legal document as it contains information on product efficacy, safety, dosage and application prerequisites.

Dosage is established by the registrant with the pesticide submission and this information must demonstrate effectiveness and data must show that residual activity is acceptable from a safety perspective but that it also is congruent with the information on the label.

The Canadian Grain Commission (CGC) is responsible for regulating standards and overseeing quality attributes associated with grain exported from Canada. The Research Laboratory within the CGC continuously monitors over 100 different chemicals that may be associated with grain and includes residues of insecticides, herbicides, fungicides in addition to heavy metals that may be picked up by plants on the fields and by grains during postharvest storage and handling. Information from the monitoring program is used to support grain quality statements required in contracts for grains to access various international markets. The CGC will provide pesticide residue information and compare it with levels established in Codex Alimentarius and other internationally established standards. The CGC also has the authority to stipulate grain treatment if inspection staff determine infestation of the grain being monitored.

The Canadian Food Inspection Agency (CFIA) is Canada's National Plant Protection Organization (NPPO) and like other countries' NPPOs, is responsible for provision of Phytosanitary certificates for grain being exported. As a part of the phytosanitary certification process, the CFIA's requirements include inspection programs that demonstrate that the grain, grain handling facility and the conveyance receiving the grain meet established protocols and are essentially free from various pests. In the event one of the criteria is not meeting compliance to established standards, the CFIA may withhold the issuance of the phytosanitary certificate. They may also order offloading or treatment of a commodity, a handling facility or a conveyance if deemed appropriate.

Conclusions

Usually insects are not a major concern in Canadian grains but when insects are detected in stored grains then mainly fumigants (Aluminum phosphide, Magnesium phosphide, Sulfuryl fluoride) and high CO_2 concentrations are used for insect control. Occasionally Diatomaceous earth is used on grain for insect control. Methyl bromide is only permitted for quarantine purposes. Pyrethrin,

malathion, cyfluthrin and diatomaceous earth are used for treating storage structures only. Canada has an excellent regulatory framework for registration, use of chemicals for treatment of grains and structures and for issuance of phytosanitary certificates. Also, chemical treatments are only conducted by trained personnel.

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Alternatives to the use of phosphine fumigation in Ghana

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Abstract

Presently in Ghana, fumigation in stored products is not well developed. The Government of Ghana has started constructing warehouses for storage of agricultural commodities under the Planting for Food and Jobs policy. Stored product insect pest management strategies are changing to: meet consumer's demand for food free of chemical residues, address concerns about safety of insecticides to humans, delay insecticide resistance development in insects, and comply with stricter pesticide regulations. Phosphine is predominantly registered and used for disinfestation of stored cocoa beans against insect infestation in Ghana. Although this chemical is efficient and effective; however, continuous usage of chemicals could lead to many problems such as resistance development in insects. Chemical fumigants such as sulfuryl fluoride, Dichlorvos, chloropicrin, formaldehyde, hydrogen cyanide, and ethylene oxide are effective against stored product insect pests. There is, therefore, the need to evaluate alternatives to and integrated combinations with the use of phosphine. Furthermore, the majority of farmers in Ghana are resource-poor and have neither the means nor the skills to obtain and handle fumigants appropriately. Further, Ghana is being encouraged to adopt new methods like controlled atmosphere, use of heat treatment, electromagnetic waves, and ionized gas like cold plasma as alternate stored product insect pest control methods. This paper focuses on the advances in stored-product protection in Ghana with emphasis on alternative chemicals to supplement the widespread use of phosphine in Ghana.

Keywords: Fumigation, Alternatives, Heat, Phosphine, Integrated pest management

Introduction

In Ghana, most of the stored products produced are annually lost to insects. Infestation by stored product insects results in major damage and causes drastic economic losses to farmers (Obeng-Ofori, 2008). The control of stored product insect in most sub-Saharan African countries like Ghana is a major challenge to farmers, and storekeepers. However, fumigation is a proven technique for effective control and management of the infestation by stored grain pests. Chemical fumigants are used primarily for insect pest disinfestation (Nambi et al., 2017). The use of chemical fumigants in Ghana has increased drastically. The frequent and indiscriminate use of insecticides has resulted in the failure of these chemicals to effectively control storage insect pests (Subramanyam and Hagstrum, 1996).

Use of phosphine (PH_3), one of the most used fumigant for fumigation, has increased globally. Phosphine is a colourless, odourless, flammable gas in its pure form but is detected as distinctive garlic or fishy odour due to impurities in the mixture (Chadda, 2016). Phosphine is becoming an essential fumigant for insect protection in storage goods. But few drawbacks, such as low temperatures and relatively long exposure periods, restrict the use of phosphine (Kostyukovsky et al., 2010). Phosphine is the only fumigant currently used in Ghana, because of its low cost, ease of availability and residue-free treatment. The efficacy of fumigation depends upon the combined effect of the exposure period and phosphine concentration (Chadda, 2016).

The continued use and concentration inadequacy of phosphine fumigations have resulted in resistance among targeted insect species (Chaudhry, 2000; Benhalima et al., 2004; Collins 2006; Pimentel et al., 2010; Obeng-Ofori, 2010; Phillips et al., 2012; Chen et al., 2015; Bajracharya et al., 2016) which has caused a significant increment in the incidence of the heavy product pest infestations. The indiscrimate use of insecticides is the major reason causing these heavy infestations. There is a serious concern because the only alternative fumigant currently in general use is methyl bromide. Methyl bromide fumigation requires more equipment and skill than those needed for phosphine (Price and Mills, 1988). Due to the development of resistance to phosphine by most of the stored product pests, there is a need for searching for an alternative solution to control storage pests in Sub-Saharan Africa countries like Ghana (Obeng-Ofori, 2010). Nevertheless, Nambi et al. (2017) have noted that fumigation methods like controlled atmosphere storage, hermetic storage, and CO_2 are used for disinfestation at a commercial scale. There is little information on the use of these alternative methods in controlling insect pest in our stored commodities. Concerning that, there is an urgent need to look at the effectiveness and availabilities of alternatives to the use of phosphine fumigants in Ghana.

Uses of phosphine for fumigation

Notable studies have been conducted on the use of phosphine as a fumigant for the storage of agricultural produce to prevent the infestation of stored product pest. Some of the successful use of phosphine fumigations that have been conducted include the use of polyethene bags containing ground nuts (Proctor and Ashman, 1972), disinfestation of kiwifruits (Jamieson et al., 2012), wheat in silo bins (Boland, 2012), and wheat in silo bags (Ridley et al., 2011). Jamieson et al. (2012) observed that the phosphine gas was effective in the control of oleander scale, long-tailed mealybug and greedy scale insects.

Alternatives to the use of phosphine fumigation in Ghana

The use of phosphine gas as fumigant can be alternated with the use of hermetic storage (triple-layer hermetic storage bags), diatomaceous earth, controlled atmosphere storage, high concentration of CO_2, heat treatment, steam, electromagnetic waves such as microwave, gamma rays, infrared, ultraviolet, and ionized gas like ozone.

Hermetic storage

Hermetic storage is reported to be an effective method for the protection of agricultural produces in the absence of the use of fumigants for the control of insects and the preservation of the quality of the agricultural produce (Obeng-Ofori et al., 2015). The hermetic storage is a sustainable alternative to other methods of storage that protects commodities from different range of pests such as the stored product insects and moulds. Hermetic storage consisting of a sealed storage system containing a modified atmosphere has been adopted recently. This means that, as a result of respiration effects, there is generally depletion of oxygen (O_2) and production of high carbon dioxide (CO_2) atmosphere which can lead to a drastic reduction in pest population (Jonfia-Essien *et al.,* 2008).

The Triple-layer hermetic bags have been used in the control of different kinds of stored product beetles such as cowpea bruchids, *Callosobruchus maculatus* (F.) (Murdock et al., 2003), *Dinoderus spp* and *Prostephanus truncates* (Horn) on cassava chips (Hell et al., 2010) with very promising results. In Ghana, Ansah et al. (2015) assessed the storability of two maize cultivars in hermetic triple-layer biodegradable bags. In their study, it was observed that the biodegradable hermetic triple-layer bag reduced insect reproduction and maize sprouting, and could effectively maintain grain quality. Also, Anankware et al. (2013) distinguished that the triple-layer hermetic bag protected maize against *P. truncatus* and *Sitophilus zeamais* Motschulsky. The effectiveness of hermetic storage in insect control and quality preservation of cocoa beans in Ghana was studied by Jonfia-Essien et al. (2008). Through the study, it was observed that there was 100% mortality of the insects under study using the Cocoon^{TM} storage and it seemed to be the most promising method for storing cocoa beans. This shows that the triple hermetic layer bag has a promising use as a replacement of phosphine in Ghana. The hermetic layer bag is effective in the control of storage pests and it does not cause any harm to the food products, the environment, and the consumers. The use has resulted in pesticide-free commodities, and safe products for consumption. Hermetic storage improves grain quality and seed viability because it maintains the original grain moisture content and reduces pest damage.

Diatomaceous earth (DE)

The diatomaceous earth is a formulation made from natural substances of the earth. Diatomaceous earth is formed from the fossils of diatoms and is mainly composed of amorphous hydrated silica (Ebeling, 1971). There are many widely available DE formulations that have been tested effectively as grain protectants against a wide variety of storage pests (Arthur, 2000; Fields and Korunic, 2000; Subramanyam and Roesli, 2000; Athanassiou et al., 2008; Stathers et al., 2008; Kavallieratos et al., 2010; Arthur and Fontenot, 2013). Several notable studies have been conducted on the use of different types of diatomaceous earth on the control of stored product insects as an alternative to the use of phosphine gas in Ghana (Badii et al., 2013; Adarkwah et al., 2017). Badii et al. (2013) studied the efficacy of diatomaceous earth formulations against *C.*

maculatus in Kersting's groundnut (*Macrotyloma geocarpum* Harms) and characterized the influence of dosage and relative humidity. It was observed that the DEs were effective in suppressing the growth of the stored product insects. Also, the authors recommended that the DEs could serve as a potential alternative method to protect the Kersting's groundnut against the destructive *C. maculatus*, for smallholder farmers in tropical Africa. In the study conducted by Adarkwah et al. (2017), they assessed the insecticidal efficacy of botanical food by-products against selected stored-grain beetles by the combined action with modified diatomaceous earth. The combination of powders of the botanical food by-products and diatomaceous earth controlled the beetles faster compared to the plant products only. The DE blocks the insect spiracles and insects die from asphyxiation and the lodging of dusts between cuticular segments increases water loss through abrasion of the cuticle. The use of diatomaceous earth in the control of storage pests is promising in Ghana. It is worth to know that the use of diatomaceous earth formulation in the control of storage pests and the protection of grains have been on the increase. It is of low toxicity, is eco-friendly, and the effect on human health is minimal as compared to other protectants. The use of diatomaceous earth as a natural inert dust to control insect pests in stored grains as an alternative to insecticides should be encouraged to limit pest infestation (Arthur, 2003). Various diatomaceous earth formulations can be registered as a cocoa protectant in Ghana. This can help to reduce the level of stored pest infestations.

Natural enemies

Commercial application of natural enemies against stored product beetles can be adopted to prevent beetle infestation. The main application in grain storage is still for small-scale organic farms (Hansen, 2005). Females of the pteromalid wasps *Lariophagus distinguendus*, *Anisopteromalus calandrae* and *Theocolax elegans* lay their eggs on host larvae or pupae inside grains or cocoons. For this purpose, the ovipositor is inserted and the host larva is paralyzed prior to oviposition. After emergence from the egg, the parasitoid larva feeds on the host larva from the outside, thereby killing it. The bethylid wasp *Cephalonomia tarsalis* parasitises larvae of *Oryzaephilus* spp., the eggs are laid externally on host of the larva after paralyzation (Hansen, 2005).

Modified Atmospheres

Beside fumigants, use of modified atmospheres (MAs) seems to be the best bet for pesticide free organic storage. However, the technology of MAs can be well adapted where cheap sources of nitrogen or carbon dioxide are available and the storage structure is well sealed. Biogas, produced from the cow dung at farm level in many households has shown promising results to control the insect-pests in stored grains in some countries like India without affecting pulses' germination and quality. Also, Jayas (2012) noted that the latest approaches to controlling dry cocoa insect infestation in Ghana were the use of modified atmospheres (MAs) in conjunction with other IPM strategies.

Conclusions

Ghana faces a lot of challenges with the issue of stored product pests which has been a burden on the country in achieving the sustainable development goal of zero hunger. The government of Ghana, through the Planting for Food and Job policy, is putting measures in place for the country to be one of the hubs for food in Africa. The use of alternative methods aside the use of chemicals

in preventing the infestation of insect pests is a great area of concern. Many farmers lack understanding about use of chemical fumigants and their detrimental effects. However, several means of disinfesting grains from insect pests have been observed in our review which includes the use of triple-layer hermetic storage bags, diatomaceous earth, natural enemies, and modified atmospheres. Farmers need to be educated on the use of these technologies to enhance their productivity in a positive manner.

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Capacity building of smallholder farmers on postharvest management of grain crops using SuperGrainBags in Malawi

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ABSTRACT

Grain storage losses contribute significantly towards household food insecurity in sub-Saharan Africa (SSA), where production is smallholder farmer dominated. Farmers have been reliant on synthetic pesticides for grain protection against storage pests. Limited efficacy and health risks associated with synthetic pesticide use have led to increased awareness and adoption of pesticide-free options like hermetic storage. Knowledge gaps on proper use of hermetic storage options exist among users. Training of users on recommended hermetic storage practices is essential for optimum performance which enable users to fully enjoy benefits associated with the technology. However, hermetic storage alone cannot be a complete postharvest loss reduction solution because poor pre-storage crop handling can lead to enhanced bio-deterioration during storage. It is in this regard that GrainPro conducted crop postharvest management training in Malawi to equip smallholder farmers with relevant skills and knowledge. The training focused on recommended practices for the various postharvest stages. The storage training was centred on the use of SuperGrainBags (SGBs), one of GrainPro Inc's hermetic storage products. There were three phases; Phase I involved training of representatives from 54 farmer organisations (training for trainers) (n = 104), Phase II was a community-based training where the trainees from Phase I were assisted to train fellow farmers in their respective communities (n = 2094) and Phase III which involved setting up demonstration learning centres in selected 10 farmer organisations (n = 301) to test performance of SGBs in comparison to conventional synthetic pesticides. The farmers appreciated the training and were satisfied with the field performance of the SGBs. The paper shared findings from the initiative which highlighted socioeconomic factors affecting adoption of postharvest technologies by smallholder farmers and potential solutions.

Keywords: Household food security, Smallholder farmers, Hermetic storage, Postharvest loss reduction, Learning centres

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Grain storage systems and insect management in Punjab (north India)

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Abstract

A food bowl of India, Punjab contributes 17% to wheat and 12% to rice production per year from the country's 3% of the net sown area. The grains thus produced are either moved along the supply chain to meet the market demands or are held under storage for future use. The types and conditions of storage structures are the most important factors in handling and storage of food grains. In Punjab, a part of the produced grains is generally stored at farm level, in structures like Bukhari (3.5 to 18 tonnes) made of mud and bricks, earthen egg shaped Bharola (40-80 kg) and galvanized metal bins (PAU model - 0.15 to 1.5 tonne). The large amount is stored commercially by government agencies or hired storage structures that accounts for 14.6 million tonnes (Mt) in warehouses, uncovered Cover and Plinth (CAP) structures and silos. Major storage is done by agencies such as: Punjab State Warehousing Corporation (covered: 5.22 Mt and uncovered: 1.03 Mt), Food Corporation of India (covered: 4.43 Mt and uncovered: 2.41 Mt), Central Warehousing Corporation (1.26 Mt) and silos (FCI and private sector 0.25 Mt). About 97% of the commercial storage is done in bags (made of jute or polypropylene woven) under covered or uncovered conditions either inside warehouse or CAP storage as compared to 3% in bulk modernized silos. The biotic agents like beetles (Coleoptera) and moths (Lepidoptera) are the common insects attacking grains under storage deteriorating the quality and affecting the quantity of stored grains. The effective management of these insects found under the prevailing storage conditions is a major challenge in Punjab, presently relying on earlier developed storage protocols for fumigation (Aluminum phosphide at 3 tablets of 3 g each/tonne with polythene cover on grain stack, for shed fumigation at 21 tablets of 3 g each/28 m^3); along with prophylactic sprays, i.e., every 15 d with Malathion 50 EC (Emulsifiable Concentrate), and every three months with Deltamethrin 2.5 WP (Wettable Powder). The structures are built in such a way that aeration is carried out by opening opposite doors of the warehouse, side wall vents, and roof turbo vents. In farm level small scale storages, plant extracts such as from neem, black pepper, turmeric, and sweet flag are being used (dosage at 10 g extract/kg of grains). Research on the effect of abiotic, biotic factors, physical methods, and stored product entomology in specific grain storage of Punjab is meagre, which limits insect management and results in significant use of chemical fumigants and insecticides.

Keywords: Fumigation, Grain storage, Insects, Insecticides, Storage structures, Punjab, India, Aluminum phosphide

Introduction

In Punjab, a province (state) of India, about 75% of the state population depends directly on agriculture and about 97% of the total cultivable area is used for crop production. Punjab is popularly known as the 'Food Basket of the Country' or 'Granary of India', because of its contribution to wheat (17%) and rice (12%) production from the country's 3% of the net sown area. Punjab produces 2% of the world's wheat and 1% of rice. India's position as the second largest producer of food grains for decades has been possible due to the contribution of Punjab. Punjab produced 30.73 million tonnes (Mt) of food grains in 2020, which was 57% of India's wheat, 41% rice, and 4% other grains like pulses and oilseeds (Anonymous, 2020). The record food grain production in Punjab is driven by having the greatest area of fertile land, availability of water (99% of Punjab area is successfully irrigated), and higher adaptation of agricultural mechanisation compared to other Indian states. A national study conducted by ICAR-CIPHET, Ludhiana reported post-harvest losses of 4.7% in food grains (Nanda et al. 2012); however, losses can be much higher and even 100% of the grain output could be unfit for consumption by humans and animals in case of poorly managed grain storage in Punjab. On average, 75% of food grains stored in Punjab is mainly by central and state government agencies, and 25% is stored at farm level by farmers for consumption and seed purposes. The sound food grain storage management of procured grains is a very important activity, but is often neglected due to insufficient storage capacity in Punjab whereby grains become more vulnerable to the vagaries of climate and insects, which in turn causes huge post-harvest losses. This paper discusses the status of food grain storage facilities, common insects found in storages, and their existent management in Punjab.

Status of grain storage in Punjab

Stored grain is a living entity that respires and decays with time and, hence, demands safe storage systems and processes. The types and conditions of storage structures are the most important factors in the handling and storage of food grains. In Punjab, a smaller volume of produced grains is generally stored at farm level, and the larger volume is stored commercially by central and state government agencies in warehouses, CAP or in hired storage structures (Fig. 1).

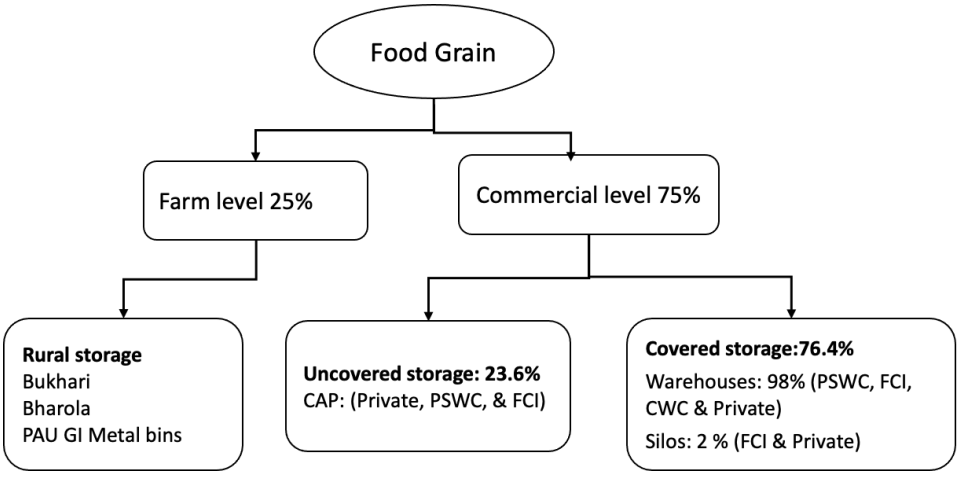

Fig. 1. Status of food grain storage in Punjab.

There are three different categories of storage structures (temporary, semi-permanent, and permanent) available in the state for storage of produced food grains. The classification of structures is done based on the duration of storage, types, design, and utility. The storage structures used at farm level and CAP fall in the category of temporary to semi-permanent, and the warehouse or silos structures fall under the category of permanent structures.

Covered type storage

The storage of food grains under protected structures (with partial or full control of environment) is called covered storage. Types of covered storages used for grain are warehouses, bins, and silos which all fall under the permanent storage category. These structures are managed by three different agencies: Punjab State Warehousing Corporation (PSWC), Central Warehousing Corporation (CWC), and Food Corporation of India (FCI).

The PSWC is one of the oldest state warehousing corporations in the country and has 120 operational warehousing centres across the state, including 133 owned and 348 hired warehouses, equivalent to the storage capacity of 5.5 Mt (Anonymous, 2021). Despite having these large storage facilities, demand for storage is not met in most years; therefore, temporary CAP storages are also created to supplement the capacity. A majority of the warehouses (5,000-50,000 tonnes) in Punjab and in most Indian states are still managed by an unorganized sector and only about 7–8% of modern warehouses have adequate size, racking systems, palletization and standardization, and out of the total warehousing space, almost 82% is not mechanized (Gurpreet and Chaudhary, 2014). All warehouses present in the state are based on old designs and standards because the establishment and management of warehouses had been done by government agencies like CWC, SWC and FCI in the 1960s (Fig. 2).

Fig. 2. Types of conventional warehouses (top and middle left); Modified single span with common road loading-unloading and with rail loading-unloading (top right); Dome shaped single span (bottom left); Gable shape single span (bottom right).

The overall size of a 5000-tonne godown is 130.6 m length x 21.67 m width x 5.4 m height with plinth height of 0.6 to 0.8 m, which can accommodate 12 bag stacks in each occupying area of 6.10 m x 9.14 m. The ventilation of godowns (2% of covered area) is carried out by windows, wall ventilators, and rolling doors of Size; 0.60 m x 0.60 m, 1.50 m x 0.60 m, and 1.83 m x 2.44 m, respectively (Anonymous, 1962).

Under the Jute Packaging Materials (JMMA) Act, 1987, it is mandatory to use jute bags as the packaging material for the storage and transportation of food grain in India. Therefore, jute bag storage is a very common form of storage used in these warehouses (godowns) and their layouts and management is done as per standards (Fig. 3). The grain is stored at a recommended safe moisture level, generally of less than 14% for long term storage for wheat. The standard dimension recommended for each bag (50 kg) stack is 9.14 m x 6.09 m x 4.5 m. The minimum gap of 0.75 m between two stacks, 0.8 m between walls and stack, and 1.5 m between top of stack and roof is used. Storage in bags offers many advantages like easy movement and handling by unskilled labour, ease of fumigation, ease of sampling, reusability of bags, and good mechanical properties. There are some disadvantages like grain spillage during improper handling, prone to rodents and insect attack, fluctuation of grain moisture due to atmospheric moisture absorption or drying, and easy infection by mould.

Fig. 3. Stack arrangement of food grain bags inside a conventional warehouse: lengthwise (left); and widthwise (right).

Silos require about 1/3 of the land as compared to existing conventional warehouses (1.75 acres) and offer many advantages over conventional warehouse storage like mechanized handling and efficient storage management. Therefore, PSWC and FCI have constructed steel silos of 0.15 Mt and 0.2 Mt capacity (2% of present covered storage capacity), respectively under Public Private Partnership (PPP) mode in Punjab to modernize the storage infrastructure and improve the shelf-life of stored food grains (Fig. 4). Constructed silos offer numerous advantages such as reduction of post-harvest losses and savings on gunny bags, wooden crates, tarpaulin covers, and labour wages. It forms an integrated supply chain and logistics model that procures food grains from farmers and local mandis, and supplies to the Public Distribution System (PDS) in Punjab. The losses caused by rodents, insects, fungi, and handling in a silo system is about 0.2% compared to 8% in a godown system (Sawant, 1985).

Uncovered storage

Present infrastructure for grain storage in Punjab does not meet the demand of buffer storage of food grains, thus uncovered storages have been used. Although grain in such structures is highly prone to damage by biotic and abiotic factors, agencies prefer their use for short term storage to bridge the gap between supply and demand. The CAP storage is a type of uncovered storage in which grains are stored in an open space on wooden dunnage over rat and moisture proof raised plinths, and then the stacks are covered by 250-micron LDPE sheets from the top and along the four sides (Fig. 5). FCI and PSWC has 3.44 Mt of owned and leased CAP storage capacity at present in Punjab, which constitutes about 23% of total storage capacity. Weather is the major grain damaging factor, which can cause huge post-harvest losses during bad weather like heavy rains and higher humidity.

Fig. 4. Modern silo facility at Moga, Punjab.

Fig. 5. Cover and Plinth (CAP) storage of food grains.

For safe and proper storage under Indian conditions, research is required for quality management for local climatic conditions with careful selection of the storage site, storage structure, implementation of Integrated Pest Management (IPM), and with proper aeration of grains followed by regular inspection of grain stock (Sharon et al., 2014).

Rural farm storage

This is another form of temporary type, uncovered or partially protected form of storage found in rural Punjab. Punjab is an agrarian state, and therefore most of the farmers produce and store their

food grains at farms for their own consumption and seed purposes after selling surplus grains. They use locally available raw materials to develop traditional structures which are different in design, shape, size, and capacity (Dhaliwal and Singh, 2010; Fig. 6). Singh (2002) evaluated post-harvest losses in these storages of Punjab and found that about 94% of food grain could be lost due to damage by biotic and abiotic factors.

Major insects and management

The common classification of the storage insects is based on their feeding habit as primary pests (generally internal feeders) and secondary pests (external feeders). Common insects found in stored grain in Punjab are: Rice weevil, *Sitophilus oryzae* (L.); Wheat weevil, *S. granarius* (L.); Lesser grain borer, *Rhyzopertha dominica* (F.); Saw-toothed grain beetle, *Oryzaephilus surinamenis* (L.); Flat grain beetle, *Laemophloeus pusilloides;* Khapra beetle, *Trogoderma granarium;* Rice moth, *Corcyra cephalonica* (Stainton); and Paddy moth, *Sitotroga cerealella* (Olivier). These insects thrive in stored grain and can live 25 to 300 days (Tyagi et al., 2019).

Fig. 6. Farm level small scale storage structures: Kupp (left), Bharola (middle), and PAU Bin (right).

Chemical management

About 90% of food grains are stored in conventional godowns (warehouses) in Punjab, therefore the most used preventive and curative method is chemical treatment. These methods are popular for insect management in private mandis, PSWC, and FCI warehouses because they are inexpensive, easily accessible and can be managed by unskilled workers. Fumigant is a chemical with higher vapour pressure, is toxic to insects and is able to penetrate through the commodity. Unlike chemical sprays, fumigants require airtight conditions to achieve full efficacy. Relative humidity and temperature in storage, as well as the moisture content of seeds and air tightness were important factors influencing the efficiency of fumigation.

Presently, aluminum phosphide (ALP) is the formulation available in India for large scale fumigation to control stored product pests effectively. It is the first line of defence in grain storages of Punjab, and generally recommended at 3 tablets per tonne of food grains (each tablet is 3 g and can release 1 g of phosphine gas).The fumigation period is 7 d. Wherever *Trogoderma* larvae are present then doses may be raised by 50%. The detailed applications, dosages and schedules are given in Tables 1 and 2.

Table 1. Prophylactic treatment to stacks/godowns for disinfestation of food grains in Punjab

Chemical	Dilution	Dosage of prepared solution	Remarks
Malathion 50EC	1:100	3/100 m^2	Spray on stack surface on in 15 d
		1L/270 m^3	For aerial spray once a week or as the situation warrants
Deltamethrin 2.5WP	40 g/L	3 L/100 m^2	Spray on stack surface once in 90 d
DDVP 100EC	1:150	3 L/100 m^2	To be sprayed only 20% for godown disinfestation. Not to be sprayed on food grain bags

(Source: FCI Quality Control Handbook, 2018)

Table 2. Pest control plan in storage by Food Safety and Standards Authority of India

Schedule	Frequency	Treatment	Area
Daily	Three times	Integrated fly management	In and around building, entry points (doors and shutters) and breeding grounds
	Four times	Electric flying insect control	Food grain godown, bran collection, coding room, packing room
	Once	Rodent check	Near storage zone
Weekly	Once	Non-selective disinfestation	In and around building, entry points (doors and shutters) and breeding grounds
	Once	Lizard management	Outside storage area
Fortnightly (Mar – Oct)	Once	Prophylactic sprays	Crawling insects
Monthly (Nov - Feb)	Once	Prophylactic sprays	Insect hiding places
Monthly	Once	Cockroach management	Cracks and crevices
45 d (need based)	Once/as and when required	Fumigation	Grain, equipment and others

(Source: Food Industry Guide 2017)

Table 3. Commercially available products based on botanical pesticides

Active Ingredient	Dose (ml per litre of water)
Oil of neem (*Azadirachtin*)	5.0 to 10.0
Essential oil of garlic (*Allium sativum* L.)	5.0
Citrus cinensis L. oil (limonene and linalool)	2.0 to 5.0
EO of thyme (*Thymus vulgaris*)	2.0 to 5.0
Rotenone	5.0
Nicotine (*Nicotiana tabacum* L.)	10.0

(Source: Guru P.N. and Mridula D. 2021)

Use of plant derivatives for stored product protection is an age-old practice and still in use under rural farm storages of Punjab. Because of the active ingredients present in them, botanicals help in managing insects through several actions as: repellents, antifeedants, toxicants, chemosterilants, and growth regulators. Plant families like meliaceae, myrtaceae, apiaceae, lamiaceae, lauraceae, poaceae, and pinaceae are reported to contain insecticidal secondary metabolites/volatiles like terpenoids, alkaloids, and phenolics. The dosages range from 2 to 15 g/kg of food grains; however, sometimes use can be >20 g/kg. The active stages, especially those of adult insects, are generally more susceptible than eggs. Botanical neem kernel powder at 2% (w/w) was found to be effective against S. oryzae on stored wheat in Punjab with no significant adverse effect on seed germination (Singh et al., 2017). Commercially available products are given in Table 3, which are being used by farmers at their farms for grains stored in metal bins.

Conclusions

The network of covered conventional godowns and uncovered CAP storages, along with rural farm structures, has been playing an important role in Punjab's grain storage and India's national food security. Presently, insect management in commercial storage is largely relying on earlier developed storage protocols and fumigation by Aluminum Phosphide, Malathion, and Deltamethrin. The research on abiotic and biotic factors, physical methods, and stored product entomology in grain storage of Punjab is meagre, which limits the insect management and results in significant use of chemical fumigants and insecticides. Improved versions of rural farm structures with standardized plant-based formulations have the potential to reduce post-harvest losses and ensure local food security in rural areas. Timely repair and maintenance of structures, frequent monitoring of grain, aeration, personnel training, and shifting from uncovered to covered storage are all important measures that can be implemented in both the organized and un-organized sectors which are involved in grain handling and storage for enhancing safe storage capacity and reducing post-harvest losses.

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Survival of 10 stored product insects on canola

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ABSTRACT

Canola production is important to the Canadian economy, contributing $27 billion each year. Approximately 20 million tonnes are harvested annually in Canada, with 90% destined for export. Insects often attack stored cereals and pulses but we do not know if insects can also attack stored canola. The purpose of this study was to assess whether ten stored product pest species, commonly found on cereal products, are able to develop from egg to adult (one generation) on canola at 25°C. Two tests were conducted. The first test assessed the ability of 10 stored product insects to develop onto seven treatments: (1) wheat flour with 5% brewer's yeast (control), (2) whole canola at 8% m.c. (moisture content), (3) canola with 10% broken seed at 8% m.c., (4) whole canola at 12% m.c., (5) canola with 10% broken seed at 12% m.c., (6) pure dockage and (7) dockage with broken canola seed. The second test (ongoing) has assessed so far the ability of four species to develop onto three treatments at 8% m.c.: (1) whole canola seeds, (2) broken canola seeds, and (3) control (same as previous). Preliminary results show that *Tribolium confusum* Jacquelin du Val, *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), *Oryzaephilus Mercator* (Fauvel), *Stegobium paniceum* (L.), and *Lasioderma serricorne* (Fabricius) are able to develop on canola seeds. Presence of dockage, broken seeds, and different moisture contents had little or no effects, except for *Lasioderma serricorne*, which produced more offspring when dockage was present. *Oryzaephilus merator* was the only species to produce significantly more offspring on canola than on the control diet. *Lasioderma serricorne, Stegobium paniceum, Oryzaephilus surinamensis,* and *Cryptolestes ferrugineus* (Stephens) displayed longer development time on canola than on control diet. *Cryptolestes ferrugineus* produced very few offspring on the canola treatments. *Dermestes maculatus* De Geer, *Ephestia kuehniella* Zeller, and *Trogoderma variabile* Ballion, all did not appear to be able to develop on canola.

Keywords: Insect fitness, Canola storage, Oilseeds, Canola pests

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Three-dimensional movement detection of *Tribolium castaneum* and *Sitophilus zeamais* in wheat flour using X-ray Micro-Computed Tomography

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ABSTRACT

Insect infestation of cereals and their products (i.e., flour) affect their chemical and physical qualities and can cause significant damages and losses. During the storage of bulk wheat or wheat flour, it is very important to understand and predict the movement of insects as many pest control strategies depend on insect movement behavior. Several research studies have been reported in this area, however, most of the previous studies were limited to two-dimensional mapping due to the challenges in monitoring insect behavior in three-dimensional space. The present work aimed to evaluate the feasibility of implementing an X-ray microcomputed tomography technique for tracking insect movement in grain powder. The movement of two insect species namely *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motschulsky were tested in wheat flour. The experimental results showed that upon movements of the insects within flour, the length, direction and three-dimensional pattern of movement could be clearly identified. These observations indicated that X-ray microcomputed tomography imaging was a promising advanced tool for non-invasive, non-destructive detection of insect movement in three dimensions and it could offer a new pathway in improving existing pest control management programs.

Keywords: Insect movement, X-ray micro-computed tomography, 3D modelling