Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products

Editors

Digvir S. Jayas

Chandra B. Singh

Fuji Jian

August 18–23, 2024

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Preface

The spread of COVID-19 in 2019 forced the organizers of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF) to postpone the gathering to 2021 and caused its realization online. Now in 2024, the globe has faced two wars—the war between Russia and Ukraine and the war in the Middle East. These events have influenced airlines to increase their costs. On the verge of these events, we are confident that participants coming to the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024) are doing their utmost to take part in the CAF2024 at the Delta Hotel in Winnipeg, Canada.

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 a 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. These conferences cover the treatment of products such as cereal grains, oilseeds, legumes, root crops, dried fruits, nuts and vegetables, and other agricultural products including bulbs and flowers. However, the CAF conferences do not cover controlled atmosphere treatments for the quality preservation of fresh agricultural products.

The major highlight of the CAF2024 was the preparation of a book edited by Digvir S. Jayas based on 18 invited chapters summarizing knowledge from the past 40 or so years on topics dealing with different aspects of controlled atmospheres and fumigation. A copy of the book was provided to each participant of the CAF2024. Other highlights of the CAF2024 based on the submitted papers were: a) the increased use of hermetic storage in bags for coffee and pulses in warm climates worldwide; b) the increased use of TranSafeLiners (TSL) (hermetic bags the size of container) that has become a big commercial success for cocoa beans transported around the world; c) niche applications of modified or controlled atmospheres in private houses, food industries, and museums; d) increased use of vacuum technology; and e) the increased use of ethyl formate as a significant alternative to methyl bromide.

It should be noted that statistics on the use of hermetic storage are not available. This information is based on personal communications. The use of hermetic storage in bags, in warehouses, or in TSLs in tropical countries reveals that it also controls infestation, taste, aroma, and free fatty acids (FFA) very well.

The establishment of the International Steering Committee followed the first Symposium held in Rome in May 1980 organized by Assoreni and the first Co-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, was the title of "International Conference on Controlled Atmospheres and Fumigation in Stored Products" 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); New Delhi, India (2016), and virtually from Winnipeg (2021).

The 12th International Conferences on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), next in the series, was held in Winnipeg, Canada, from August 18 to 23, 2024. The Local Organizing Committee included members from the CAF International Permanent Committee under the leadership of Dr. Digvir S. Jayas. The Local Organizing Committee members were from the University of Manitoba, Agriculture of Agri-Food Canada, and the Canadian Grain Commission, Canada. The CAF Conference Local Organizing Committee did a great job in organizing the conference and in producing high-quality refereed proceedings.

The chapters of the commemorative book were presented as the keynote presentations during the CAF2024. In addition to the book, the proceedings were edited by Prof. Dr. Digvir S. Jayas, Prof. Dr. Chandra B. Singh, and Prof. Dr. Fuji Jian, including 29 full papers and 43 abstracts, all of which were presented orally during the conference held over four days (Monday, Tuesday, Thursday, and Friday). Technical tours and a banquet were organized in the middle of the conference (Wednesday). Two workshops entitled "Dynamic Phosphine Fumigation - Precise Monitoring for Guaranteed Fumigation Success" and "Phosphine Fumigation" were organized by world leading experts. The technical support provided by Judith Mate in preparation for the book of proceedings is greatly appreciated; the assistance of Lisa Neufeld, Gage DeSteur, and Vimala SK Bharathi of the University of Lethbridge, AB, Canada in completing many organizational tasks is also 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, CAF2024 Local Organizing Committee
Prof. Dr. Chandra B. Singh, Treasurer, CAF2024 Local Organizing Committee
Prof. Dr. Fuji Jian, Secretary, CAF2024 Local Organizing Committee

International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF)

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An appreciation of Chris Bell (1945–2022)

Dr. Bell was born on November 21, 1945 in Southall, London. He pursued his passion for Biology and Entomology at Brunel University in Hillington/Uxbridge, London. Following his graduation, he embarked on his scientific journey at the Pest Infestation (Control) Laboratory (PICL), Slough, Buckinghamshire (UK), under the mentorship of W. Burns-Brown and John Freeman. His early research interests gravitated towards studying stored-product pest insects and their control using fumigants. Guided by H. D. Burgess and T. G. Onions, he successfully completed his PhD on the "Effects of Diapause of 4 Moth Pests on the Toxicity of Methyl Bromide and Phosphine."

I had the pleasure of meeting Dr. Bell for the first time in 1978 at the Second International Working Conference on Stored-Product Entomology in Ibadan, Nigeria. Our paths continued to intersect at various international conferences such as IWCSPP, CAF, IOBC, and MBAO, where his kindness and profound knowledge always shone through.

Dr. Bell grew to become a globally respected expert in stored products research, actively participating in national and international conferences on the subject. His contributions were invaluable, including his involvement in the permanent committee of the Controlled Atmosphere and Fumigation (CAF) conferences and his pivotal role in the publishing of the proceedings of the IWCSPP in York in 2002. Additionally, he lent his expertise to the MBTOC of the UNEP, showcasing his exceptional skills in English writing and editing. Over the years, he authored numerous papers in highly regarded scientific journals and held editorial positions at the Journal of Stored Products Research, culminating in his tenure as Editor in Chief from 2011–2015. Notably, he co-edited the renowned book "The Methyl Bromide Issue" in 1997 with Mr. Bishu Chakrabarti and Prof. Dr. Nick Price, featuring contributions from researchers around the globe.

Beyond his professional achievements, in 1998, Dr. Bell embraced his spiritual side and joined the church alongside his wife. He later assumed roles on the Parochial Church Council and as Churchwarden at All Saints, actively participating in the Diocesan and Deanery Synods.

Dr. Bell peacefully passed away on January 24, 2022, in York, leaving his beloved wife Vivien, and son Andy, daughter Alison, and grandchildren Isaac and Reshana. While his absence is deeply felt by his many friends, his legacy as a gifted specialist in stored products and an excellent scientist will endure in our memories.

This heartfelt tribute is based on the information shared in the JSPR in 2022 by Prof. Dr. Christoph Reichmuth and the edits made by Prof. Raul Narciso C. Guedes, Universidade Federal de Viçosa, Brazil.

Shlomo Navarro
Post-Harvest Research Entomologist, CEO of Green Storage Ltd.
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Stored Product Insects: Biology and Management Relevant to Controlled Atmospheres and Fumigation

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ABSTRACT

This is an introductory chapter summarizing the biology, diversity, and pest management of insects and mites of durable stored product. General insect biology including morphology, anatomy, and physiology is covered as the basics of entomology. Then the numbers, diversity, ecology reproduction and life history of over 100 species is covered. Some storage pests are short lived and produce large numbers of progeny, while others are long lived and produce fewer progeny but with more strategies for survival. The concepts and practice of integrated pest management, IPM, is laid out as a decision-making process of preventing, pest monitoring, and action if needed to keep insect number low to avoid economic loss. Non-chemical and chemical forms of pest control are covered with a brief overview on the basics of modified atmospheres, controlled atmospheres, and fumigation. Challenges in our field of work and potential for future effective research are highlighted.

Keywords: Pest management, Grain, Life history, Coleoptera, Lepidoptera

Paper No. CAF2024-K02

Nayak M, Jagadeesan R (2024) Insect resistance to fumigants in postharvest commodity protection: monitoring and management. Page 2. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Insect Resistance to Fumigants in Postharvest Commodity Protection: Monitoring and Management

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ABSTRACT

As insects cause a significant proportion of postharvest losses, a range of control strategies are in practice among which chemical treatments are at the forefront. Due to stricter regulatory requirements and consumer sensitivity towards pesticide residues, there has been a gradual decline in the use of contact insecticides, resulting in heavy reliance on fumigants to disinfest stored commodities. Among the sixteen fumigants registered for postharvest protection, the phasing out of the ozone-depleter, methyl bromide, has left only a few fumigants meeting industry criteria. Currently, phosphine and sulfuryl fluoride are the commonly used fumigants, though the former is always the first choice, considering the range of benefits it offers. These include its universal acceptance as a residue-free treatment, excellent efficacy, inexpensive, versatility in use, and application in a range of storage structures. However, over-reliance on phosphine has led to the development of resistance in major storage pests. In this chapter, we critically analyze the recent research advances made in resistance to phosphine, with special emphasis on monitoring, and management. These include our understanding of factors responsible for resistance development, its genetic basis, various methods used for detection, the types of monitoring, and their importance and implications. We also discuss the key tactics of using alternative treatments such as hermetic control and co-fumigation using phosphine with either sulfuryl fluoride and CO_2 in an integrated system to manage pests and resistance. In conclusion, we proposed some potential future directions in research towards maintaining the sustainability of phosphine as a critical disinfestant for stored commodities.

Keywords: Stored commodities, Fumigants, Resistance management, Phosphine, Sulfuryl fluoride, Hygiene, Rapid test, Molecular diagnostics, Monitoring, Co-fumigation

Paper No. CAF2024-K03

Navarro S, Navarro H, de Bruin T, Inbari N (2024) Insect biology – Re: controlled atmospheres, modified atmospheres, and hermetic storage. Page 3. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Insect Biology – Re: Controlled Atmospheres, Modified Atmospheres, and Hermetic Storage

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ABSTRACT

The alternative control method to the use of residue leaving toxic chemicals to control stored product pests is the sustainable and environmentally user-friendly application of modified or controlled atmospheres and hermetic storage technologies. For the successful application of these technologies, it is essential to know the biological responses of insects. In this chapter, insects' response to low O_2, to high CO_2, to relative humidity, the effect of air relative humidity on desiccation and insect mortality, effects of combinations of low O_2 and high CO_2, insect response to temperature, and insect movement in changing atmospheric composition is presented and discussed. In addition, insects' response to hermetic storage, to restricted O_2 supply, insect respiration at low O_2 concentrations, and the effects of hermetic storage on mortality of grain insects, are discussed. At the range of 20-29°C, 1% O_2 is needed to kill storage insects in 20 d. Atmospheres containing about 60% CO_2 rapidly kill stored-product insects. At 26°C, about 4 d of exposure would be sufficient to kill all stages (including eggs) of most stored-product insects. The successful applications of hermetic storage and the available nine storage systems based on the hermetic principles are reviewed. Challenges in the application of hermetic storage are reviewed and discussed. Future research in the application of hermetic storage includes discussions on gas tightness, condensation, liner permeability, recyclable liners, remote sensing, modeling storability, and the future of hermetic storage.

Keywords: Stored-product insects, Carbon dioxide, Low oxygen, Relative humidity, Modified atmospheres, Hermetic storage, Storage systems, Insect respiration, Desiccation, Insect mortality

Paper No. CAF2024-K04

Bartosik R, Cardoso L, Urcola H (2024) Silo bag storage. Page 4. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Silo Bag Storage

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ABSTRACT

Silo bags are a flexible, hermetic storage system made of polyethylene, available in a variety of sizes and can be used to store grains and their by-products. Silo bags have gained extensive adoption as a hermetic storage solution in Argentina. Annually, this method is employed for the storage of roughly 50 million tonnes (Mt) of grains across various levels, including farms, grain elevators, industries, and even at port facilities. Moreover, silo bags have gained recognition as a viable storage alternative in over 50 countries globally, ranging from cold climates like Canada and Russia to tropical regions such as Brazil and Colombia. In addition to the plastic bags themselves, the silo bag system involves other essential components, including bagging and extracting machines, as well as grain carts. These pieces of equipment have been specially designed with a high working capacity, enabling them to handle impressive volumes of 300–400 t per hour. Furthermore, silo bag monitoring systems have been developed based on CO_2 concentration measurements and airtightness evaluations through a pressure decay test. In general, when dry grain is stored in silo bags, the CO_2 levels range from 1 to 3%, while the O_2 levels range from 18 to 16%. As the moisture content (m.c.) and temperature of the grain increase, the modification of the interstitial atmosphere becomes more pronounced, resulting in CO_2 concentrations up to 30% and O_2 levels of 5 to 0% for moist grain. Few instances of insect presence in silo bags have been reported, with data analysis indicating that unfavorable environmental conditions hinder insect development. Nevertheless, suitable pest control strategies, based on phosphine fumigation and controlled atmospheres, have been successfully implemented. The quality of grains stored in silo bags is influenced by the interaction between m.c. and temperature. When the m.c. is sufficiently low to inhibit microbiological activity, the temperature itself has minimal impact, allowing for storage even during the summer without deterioration in quality. When the m.c. is high enough to allow microbial activity, in winter time quality parameters deterioration is slowed down due to the combination of low temperature and the modified atmosphere. However, during spring and summer, microbial activity and other detrimental processes intensify, leading to a decline in quality parameters that cannot be compensated for by the modified atmosphere.

Keywords: Hermetic storage, Flexible, Modified atmosphere, Quality, Grains, Oilseeds

Paper No. CAF2024-K05

Navarro S, Navarro H, Inbari N (2024) High-nitrogen and high-carbon dioxide systems. Page 5. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

High-Nitrogen and High-Carbon Dioxide Systems

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ABSTRACT

In high-nitrogen (N_2) and high-carbon dioxide (CO_2) systems, the objective is to attain a composition of atmospheric gases rich in CO_2 and low in O_2, or a combination of these two gases within the treatment enclosure, for the exposure time necessary to control the storage insects. In this chapter, the applications of high-N_2 and high-CO_2 technologies for the treatment of dry cereal grain stored in bulk in silos and ship fumigation, and some other applications to control insect pests are considered. In the section on technical aspects before applying high-N_2 and high-CO_2, topics related to achieving gastightness, testing gastightness in rigid and flexible structures, comparative results with variable pressure tests, use of pressure relief valve, and use of a breather bag are discussed. In the section on the generation of high-N_2 and high-CO_2 concentrations, the supply of gas from tankers, exothermic gas generators, and onsite N_2 generators is considered. Other aspects of applying atmospheric gases like the need to recirculate CO_2 and the sorption of CO_2 by the commodity are also discussed. In two separate sections, the experience gained applying high-N_2 concentrations, as well as the experience gained applying high-CO_2 concentrations, is reviewed. In the section, data on CO_2 treatment of cereal grain, ship fumigation using CO_2, CO_2 treatment of museum pests, CO_2 treatment of bed bugs, and CO_2 treatment of wax moths are given. In the last section of the chapter, future research topics applying high-N_2 and high-CO_2 concentrations include sustainability and environmental impact, economic viability and scalability, integrated pest management strategies, and consumer acceptance and sensory quality.

Keywords: Storage insect control, High-nitrogen, High-carbon dioxide, Testing gastightness, Nitrogen generators, Sorption of carbon dioxide, Ship fumigation, Museum pests, Bed bugs, Wax moths

Paper No. CAF2024-K07

Athanassiou CG, Sakka MK, Gourgouta M, Agrafioti P (2024) Extreme Temperatures for Insect Control. Page 6. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Extreme Temperatures for Insect Control

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ABSTRACT

The utilization of extreme temperatures for insect control at the post-harvest stages of durable agricultural commodities has been long regarded as a viable alternative over the use of traditional control methods. In this context, both cold and heat have been exploited towards this direction, in various application scenarios, in different types of facilities and commodities. Based on the data available so far, heat treatment is mostly applied on processing facilities and not on the product itself, while cold treatment is usually applied on the product. The purpose of this chapter is to address the different aspects of the use of extreme temperatures in stored product protection for the control of insects. Most data concur that extreme temperatures are a reliable and fast disinfestation technique, which can be used with good results in an extremely wide range of cases.

Keywords: Heat treatment, Cold treatment, Non-chemical control, Extreme temperatures, Stored product insects, Heat stress, Stored products, Quality, Grains, Cold hardiness, Commodities

Paper No. CAF2024-K08

Buenavista RM, Manivannan S, Xinyi E, Siliveru K, Subramanyam B (2024) Chlorine dioxide for insect and pathogen control in stored commodities. Page 7. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Chlorine Dioxide for Insect and Pathogen Control in Stored Commodities

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ABSTRACT

Chlorine dioxide is a strong oxidizing agent that can be used for insect and pathogen control for stored commodities. This chapter discusses the physical and chemical properties of chlorine dioxide; mechanisms of its insecticidal and antimicrobial actions; its efficacy on microorganisms and insect species commonly found in stored commodities; its effects on the quality of stored commodities; and its toxicity and health effects.

Keywords: Chlorine dioxide, Pathogen, Insect control, Decontamination

Paper No. CAF2024-K09

Stevens K, Thalavaisundaram S (2024) Ethanedinitrile (EDN) – a new broad-Spectrum fumigant for forest products. Page 8. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Ethanedinitrile (EDN) – A New Broad-Spectrum Fumigant for Forest Products

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ABSTRACT

The unanimously accepted ban on the use of the ozone-depleting fumigant, methyl bromide, led to the investigation and development of old and new fumigant chemistries for quarantine phytosanitary uses. Treatment of forest products is especially critical due to the elevated risk of transferring invasive pest species to new locations via international trading of these products. Alternative fumigants should be efficacious, commercially viable, and safe for workers and the environment. Ethanedinitrile is a new broad-spectrum fumigant for the replacement of methyl bromide as a forest products disinfectant. The extremely high vapor pressure and low boiling point of ethanedinitrile allow it to move throughout the log profile at concentrations required to control a wide range of timber pests, especially those associated with phytosanitary restrictions.

Keywords: Ethanedinitrile, Phytosanitary, Quarantine, Timber products

Paper No. CAF2024-K10

Jian F, Jayas DS (2024) Aeration and chilled aeration for insect, mite, and microflora management and grain quality preservation. Page 9. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Aeration and Chilled Aeration for Insect, Mite, and Microflora Management and Grain Quality Preservation

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ABSTRACT

Aeration is a process of forcing ambient air at less than 0.31 $m^3min^{-1}t^{-1}$ (0.25 cfm/bu) airflow rate to cool stored grain and eliminate temperature differences or gradients in stored grains, so the grain can be stored at 5 to 10℃ with reduced possibility of natural convection currents. This chapter reviews the function of the aeration, aeration system configuration and control, and mathematical modelling to optimize aeration. The focus of this chapter is on the use of aeration as a tool to minimize the adverse impacts of insect, mite, and microflora in grain and to maintain grain quality. In very few countries, such as Canada, aeration with ambient air can be used to kill insects in stored grains. The fundamental scientific background for these functions is also reviewed.

Keywords: Aeration, Chilled aeration, Insect control, Mold control, Grain quality management, Mathematical modeling, Aeration strategy

Paper No. CAF2024-K11

Bharathi VSK, Jayas DS (2024) Sulfuryl fluoride and propylene oxide as fumigants for stored-product pest control. Page 10. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Sulfuryl Fluoride and Propylene Oxide as Fumigants for Stored-Product Pest Control

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ABSTRACT

Globally, fumigation stands out as a highly effective method for the control of pests in stored products. The phase out of methyl bromide (MB), as mandated by the Montreal Protocol and growing resistance development of insects to phosphine, has prompted for the exploration of alternative fumigants. Sulfuryl fluoride and propylene oxide emerge as promising alternatives due to their elevated toxicity levels at shorter exposure time and reduced environmental risks. This chapter synthesizes research on use of sulfuryl fluoride and propylene oxide for fumigation treatments of stored products by discussing their mode of action, effectiveness against stored product pests, and the dynamics of sorption, desorption, and residues in treated food products. The chapter also explores a way to overcome the drawbacks of limited ovicidal effect of sulfuryl fluoride and flammability of propylene oxide through co-fumigation and sequential fumigation. Sulfuryl fluoride and propylene oxide are often used in combination with carbon dioxide, phosphine, and vacuum. These strategies are designed to enhance the effectiveness of fumigation treatments while concurrently reducing the fumigant concentrations. Moreover, the adoption of integrated pest management approaches contributes to diminishing the dependence on a single chemical as a fumigant, thereby strengthening overall pest control measures.

Keywords: Sulfuryl fluoride, Propylene oxide, Stored products, Fumigation, Pest management, Grain, Insect infestation, Alternative fumigants, Co-fumigation, Control measures

Paper No. CAF2024-K12

Ren Y, Navarro H (2024). Ethyl formate for the treatment of invertebrate pests. Page 11. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Ethyl Formate for the Treatment of Invertebrate Pests

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ABSTRACT

Ethyl formate is an old liquid fumigant with a boiling point of 54°C and vaporizes readily at normal temperatures. It occurs naturally in soil, water, vegetation, animal products, and a range of raw and processed foods. Therefore, ethyl formate is Generally Recognized as Safe (GRAS) as a food additive and is used as a flavoring agent with no evidence that it is hazardous to the public. The work safety related Threshold Limit Value (TLV) is 100 ppm, which is the concentration that a worker can be exposed to day after day for a working lifetime without adverse health effects. Ethyl formate has a long history as a fumigant for stored products in general cargo, packaged foods, and dried fruits, in particular. Its vapor has been shown to be toxic to stored product insects and has been used successfully to eliminate a broad range of insect pests without leaving poisonous residues. Ethyl formate was re-evaluated for grain protection in the 1980s and has been useful as a rapid fumigant for grains and similar durable commodities. However, the concentration of ethyl formate required to control internal developmental stages of insects, is higher than the Lowest Flammable Limit (LEL). It is therefore necessary to reduce the flammability by mixing ethyl formate with an inert gas. Unlike most other fumigants, ethyl formate kills insects rapidly and it breaks down to two naturally occurring products formic acid and ethanol. Therefore, ethyl formate has great potential as an alternative fumigant for methyl bromide quarantine fumigation and management of phosphine resistance.

Keywords: Fumigant, Ethyl formate, Alternative fumigant, Fumigation, Stored product insect pest control.

Paper No. CAF2024-K13

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Progress and Prospect of Nitric Oxide Fumigation for Postharvest Control of Pests and Microorganisms

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ABSTRACT

Nitric oxide (NO) was discovered a decade ago as a potent fumigant for postharvest pest control. Nitric oxide fumigation has been demonstrated to be effective against all of 14 pests tested to date, including external and internal pests (insects and mites) of fresh and stored products. However, because NO reacts with O_2 spontaneously to form nitrogen dioxide (NO_2), NO fumigation must be conducted under ultralow oxygen (ULO) conditions in airtight fumigation chambers. It also needs to be terminated with N_2 flush to prevent exposure of fumigated fresh products to NO_2. When properly conducted, NO fumigation is safe to all fresh fruit and vegetables. In addition, NO fumigation preserves postharvest quality and reduces mold development on fresh products. NO fumigation may leave increased nitrate and nitrite as residues if not terminated properly with N_2 flush. Nitric oxide fumigation has both NO and NO_2 which is from reaction of NO with O_2 in ULO atmosphere, and NO_2 levels, therefore, can be regulated by initial O_2 level in ULO atmosphere. Recently, we demonstrated that NO_2 fumigation was effective against the fungus *Aspergillus flavus* and microbes on almond and unshelled peanuts. Stored products often have both pest and microbial contamination problems. Nitric oxide fumigation has potential to control pests and microbes in a single treatment. Nitric oxide fumigation has advantages of high efficacy against postharvest pests, enhancing postharvest quality of fresh products, absence to toxic residues, and control of microbes. However, the disadvantages of complex fumigation procedures, requirement of airtight fumigation chambers, and nitrogen source are also apparent when compared with other fumigants. Therefore, the prospect of commercial use of NO fumigation is not clear. Potential expanded applications in microbial and mycotoxin control will be critical for future development of NO fumigation into a practical alternative method for pest and microbial control. This article reviews NO fumigation research covering NO fumigation methodology, efficacy data on pests and microbes, residues on fresh and stored products, benefits on fresh product quality preservation, and its advantages, and provides technical advice for future NO fumigation research and development.

Keywords: Nitric oxide, Nitrogen dioxide, Fumigation, Pest control, Microbial control, Stored product, Residue, Postharvest quality

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Contact Insecticides: Chemicals, Diatomaceous Earth, and Amorphous Silica for Stored-Grain Protection

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ABSTRACT

Stored-grain insects account for 5 to 20% loss in grain storage by making it unfit for human consumption. This is a huge loss and needs to be tackled appropriately. Fumigants and contact insecticides or grain protectants are some of the ways used to control insects. As the name suggests fumigants work by releasing toxic fumes or gases that penetrate the target area and kill the insects. For fumigant to work in its full potential, a well-sealed environment is required. In contrast to fumigants, contact insecticides are a group of insecticides that are designed to kill insects by coming into direct contact with them. They work by physically damaging the insect's exoskeleton and causing it to dehydrate and die. They are applied as liquids or dusts to the grain for protection against stored-grain pests. This chapter provides an overview on three types of contact insecticides used in grain protection: chemical insecticides, diatomaceous earth, and amorphous silica. Under each section, a detailed explanation on their usages and application, factors affecting their efficacy in grain protection, their advantages and limitation, mechanism of action and their physicochemical properties with a concluding section on combination treatment has been provided.

Keywords: Grain, Stored-grain insects, Contact insecticide, Diatomaceous earth, Amorphous silica, Dust, Control, Protectants, Fumigants.

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Natural Products for Fumigation and Treatment

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ABSTRACT

Synthetic fumigants like phosphine, carbonyl sulphide, and methyl iodide have high penetration ability, suitability for bulk products, and efficacy against different growth stages of arthropods. Despite the listed advantages, the search for alternatives is ongoing because some synthetic products are lethal to beneficial organisms, evoke pest resistance, and deposit residues in treated produce. Natural products from botanical, marine, and microbial sources are effective alternatives for fumigation and treatment. This chapter focuses on the pesticidal properties of natural products, the impact of natural products on the quality of the stored products, and challenges of natural product bioprospecting. Natural products are effective on arthropods and microbial pests; and can increase the level of antioxidants, maintain the level of ascorbic acid, act as sprout suppressants for tubers and improve the firmness of fruits in modified atmosphere packages. Challenges that limit their bioprospecting for pest control include product instability, insufficiency of raw materials for commercial production, and in some places, lack of appropriate government legislations, policies, and regulations. Research into formulations including encapsulation of natural products using nanotechnology would prevent degradation, improve water solubility, and enhance a moderated release of volatiles. The development of Integrated Pest Management (IPM) for storage pests must target the conservation of their biocontrol agents. Legislation and government policies should ensure preservation of the potential sources of natural products. Toxicity of natural products to mammals and other non-target beneficial organisms should be periodically evaluated. In conclusion, efforts towards commercialization of the newly discovered natural products should consider efficacy against storage pests, cost efficiency, eco-friendliness, and produce quality preservation.

Keywords: Bioprospecting, Biopesticides, Botanicals, Antimicrobial, Pesticidal, Fumigants, Essential oil, Pest management, Storage Pest, Marine Products

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Engineering Aspects of Grain Fumigation

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ABSTRACT

Engineering elements of fumigations that produce high efficacy as well as fumigation efficiency are defined and illustrated, covering four engineering processes which ensure successful fumigations. The base elements include: 1) Practical, affordable sealing of all major and most minor leaks in fumigated structures; 2) 'Closed Loop Fumigation' (*CLF*) recirculating fumigant through multiple structures simultaneously; 3) Phosphine (PH_3) gas supplied from alternative ground level generation or delivery devices; and 4) Phosphine concentrations monitored continuously by accurate sampling, recording, reporting devices during fumigations, including final gas ventilation.

The range of *CLF* system options is illustrated by a 70 700 t (2.6 M bu) 'Model *CLF* Elevator' with one central blower manifolded to 20 concrete silos, four steel bins, and one large warehouse, with all storages fumigated in sequence, simultaneously. Another example of *CLF* diversity is a system installed in 1998–2000 in China to fumigate all 'Squats' and warehouses in 19 new 'State Grain Depots'.

Solar thermosiphon fumigation in Western Australia is included to illustrate alternative engineering methods of recirculation fumigation in storage regions without electric power. Monitoring PH_3 concentrations during four alternative fumigation methods in France using PhosCapt-MP monitors highlights its 200 m (650 ft) gas sampling tube range, with continuous remote data logging and transmission.

Keywords: CLF, Monitoring, Solar thermosiphon, Affordable sealing, Ground level phosphine delivery

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Modeling the Concentration of Phosphine and Insect Mortality with Computational Fluid Dynamics

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ABSTRACT

Phosphine, a widely used fumigant for stored product insect control, faces challenges such as resistance development and inefficient monitoring methods. This chapter presents how Computational Fluid Dynamics (CFD) simulations can investigate phosphine distribution in diverse storage structures: cylindrical silos, containers, and railcars. The research aims to correlate phosphine distribution patterns with insect mortality to enhance precision fumigation strategies. Recent studies have evaluated the accuracy of CFD models by comparing predictions with sensor data, highlighting their utility in predicting gas distribution and insect mortality during fumigation processes. Factors impacting fumigant concentrations, including environmental conditions and sorption by commodities, are discussed. Best management practices and resistance management protocols are emphasized to ensure effective insect control. Future research opportunities include expanding the model's applicability to various storage structures and adopting advanced computational techniques for enhanced efficiency.

Keywords: Computational fluid dynamics, Mathematical modeling, Phosphine fumigations, Sorption, Insect Mortality, Lesser grain borer, Red flour beetle, Silos, Shipping containers, Wireless sensors

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Safety During Fumigation

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ABSTRACT

Fumigation with phosphine products and sulfuryl fluoride 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 and workers who need to enter the structure because of unsafe atmospheric conditions, possible grain entrapment, and the use of a potentially dangerous insecticides. Carefully reading the Safety Data Sheet and labeling before using a fumigant is required because of safety precautions for using severely toxic insecticides. Lockout tagout procedures are necessary to de-energize equipment prior to entering a structure. Knowledge about grain condition, how entrapment occurs and ways to avoid it, and detecting spoiled grain using carbon dioxide (CO_2) sensors is important. Thoroughly sealing structures to retain fumigant gas is critical and efficiency can be improved by using a closed loop system. Protective clothing and respiratory protection are vital in keeping applicators safe. Atmosphere and fumigant monitors are needed to test the air and keep applicators aware of the amount of fumigant in the area during all fumigations. Placing placards on structures and in areas to be fumigated is essential to provide a safe environment for all personnel. Fumigants must be stored safely and securely, away from personnel and occupied buildings. 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, Fumigation, Lockout tagout, Entrapment, Sealing, Gas monitors, Respiratory protection, Placards, Storage, Education

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Hydrogen Cyanide as an Alternative for Phosphine-resistant Pests and for Fumigations That Used Methyl Bromide in the Past

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ABSTRACT

The regulatory loss of methyl bromide, recent concern over sulfuryl fluoride as a greenhouse gas, and phosphine (PH_3) resistance in many pests raises the need for alternative stored-product fumigants. We investigated hydrogen cyanide (HCN), a well-known and highly effective fumigant. Laboratory experiments with PH3-resistant and susceptible red flour beetles (RFBs), *Tribolium castaneum*, and lesser grain borers (LGBs), *Rhyzopertha dominica*, found no differences in their susceptibility to HCN based on PH3 resistance. Toxicity experiments on these two species showed that RFBs were more tolerant of HCN than LGBs—being more susceptible—such that RFB adults required over 1000 ppm for 24 h at 25° C to achieve a 100% kill, while LGB adults could be killed at 50 ppm. Comparisons of life-stage mortality due to HCN found that eggs were very easy to kill in both species, while adults, larvae, and pupae required higher concentrations of gas. Mixed life-stage colonies were treated with different concentrations of HCN for 24 h so that all immature stages and adults of different ages were exposed at the same time. A total lack of adult emergence over time after fumigation signified a 100% kill of adults and all immatures by HCN. Treatments ranging from 636 to 1500 ppm killed colonies of both species, with no adult emergence after treatment. Similar experiments with HCN were conducted on the beetle species *Trogoderma variabile*, *Cryptolestes ferrugineus, Oryzaephilus surinamensis, Lasioderma serricorne*, and the moth *Plodia interpunctella*. *Lasioderma serricorne* was by far the most HCN-tolerant species among them, requiring over 2500 ppm HCN to prevent any live adults from emerging from mixed life-stage cultures. Other experiments reveled that HCN headspace concentrations went down markedly in the presence of grain and other foods. Effective applications will require careful attention to sorption and the amount of gas used.

Keywords: Cyanide, Phosphine, Methyl bromide, Stored grain, Beetles, Moths, Fumigant sorption

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Deoxynivalenol (DON) Reduction by Ozonation in Stored Grain

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ABSTRACT

Deoxynivalenol (DON) is a toxic secondary metabolite produced by the *Fusarium* spp. and can be found in stored grain such as wheat, barley, oats, and corn. Contamination by DON in grains causes significant quality and financial losses and poses health risks upon consumption. Hence, several approaches, including physical, chemical, and biological techniques, have been employed to control and reduce the production of DON. Among these methods, chemical methods exhibit notable advantages in decontamination efficacy, cost-effectiveness, and the feasibility of industrial-scale implementation. However, chemical residues from such methods degrade the quality and value of grains. Ozone is a powerful fumigant used to detoxify mycotoxins and control insects and mold in stored grain without leaving residues. However, the application of ozone at high doses may affect grain quality. This review focuses on the effectiveness of ozone in degrading DON, the degradation mechanism, and ozonation's industrial-scale feasibility. It also delves into intrinsic and extrinsic factors crucial to the DON degradation process and the effects on the quality and toxicity of ozonated products. Furthermore, the paper discusses the challenges of ozone application in the grain industry.

Keywords: *Fusarium*, Ozone, Mycotoxins, Decontamination, Oxidation

INTRODUCTION

Mycotoxins are toxic secondary metabolites synthesized by fungi such as *Aspergillu*s, *Claviceps*, *Fusarium*, and *Penicillium* in various food and feed substances. Some commonly found mycotoxins in grains are aflatoxins (AF), zearalenone (ZEN), fumonisins (FMN), ochratoxin (OTA), and trichothecenes (T-2, HT-2, deoxynivalenol (DON), and nivalenol (NIV) toxins). Deoxynivalenol, also known as vomitoxin, is a sesquiterpene type B trichothecene produced by the *Fusarium culmorum* and *Fusarium graminearum* species. Deoxynivalenol is the most prevalent and economically significant mycotoxin in stored grains (Feizollahi and Roopesh, 2021). Wheat crops are highly susceptible to *Fusarium* infection during the flowering phase. Under favorable climatic conditions, *Fusarium* infection causes Fusarium Head Blight (FHB) disease in wheat plants. The prevalence of FHB is mainly correlated with DON accumulation in harvested grains. Climatic changes such as excessive rainfall or drought conditions favor DON production in field crops. Further, the interaction of various factors such as temperature, relative humidity,

mechanical damage, insect activity, oxygen and carbon dioxide concentrations, level of field infection, substrate susceptibility, and fungal strain decides the level of DON accumulation in stored grains (Gab-Allah et al., 2023).

Deoxynivalenol contributes significantly to losses in grain quality, leading to discoloration, unpleasant odor, and changes in protein and starch properties. Moreover, consumption of DON-contaminated grains poses health risks to humans and animals. Deoxynivalenol is deemed a Group 3 carcinogen by the International Agency for Research on Cancer (Wang et al., 2016a). The toxicity of DON mainly affects the gastrointestinal tract and immune system in the animal body (Li et al., 2014). Research on animal specimens has demonstrated that DON can impede protein synthesis, induce anorexia, and have immunosuppressive effects. Animals consuming DON have exhibited symptoms, including vomiting and reduced weight gain.

In contaminated food and feed, DON can be found in acetylated forms such as 3-ADON, 7-ADON, and 15-ADON and in masked forms such as deoxynivalenol-3-glucoside (DON-3G) (Gab-Allah et al., 2023). Given this, many countries and international organizations have formulated acceptable limits for DON in grains. For instance, the United States (US) Food and Drug Administration (FDA) has set the maximum permitted level of DON in finished wheat products at 1000 µg/kg, with the allowable limit of DON not exceeding 2000 µg/kg in wheat flour. Similarly, the EU has set a limit of 1250 µg/kg in unprocessed cereals, 750 µg/kg for cereal flour for human consumption and 200 µg/kg for infant foods (Gab-Allah et al., 2023). Therefore, it is important to reduce the DON concentration in contaminated food and feed.

Various decontamination techniques have been employed to mitigate DON contamination in grains. These techniques include: physical approaches like gamma and ultraviolet radiations, adsorbents, and cold plasma; chemical methods such as ammoniation, ozonation, and the use of acids and bases; and biological treatments like microbial adsorption, biotransformation, and plant extracts. Among these, one of the emerging and novel techniques is ozonation. Ozone is a strong oxidant that can be used to control fungal growth in cereals, fruits, and nuts (Savi et al., 2014). Utilizing ozone as a grain fumigant presents a viable option for DON reduction from economic and environmental perspectives (Tiwari et al., 2010). Notably, it leaves no toxic residue on the grain surface as any excess ozone decomposes into oxygen. Ozone oxidizes the alkene bonds and allylic carbon in the DON structure, reducing its toxicity. Although high doses of ozone are effective in DON reduction, these elevated doses may affect wheat quality in several ways as ozone can cause lipid and starch oxidation, discoloration, protein modification, and germination losses (Savi et al., 2014). To effectively reduce the DON concentration in contaminated grain, the mechanism of DON degradation and factors that affect ozone decontamination efficiency should be understood. Therefore, this review discusses the mechanism of DON degradation, factors that affect ozone decontamination efficiency, the efficacy of ozone in DON degradation in stored grain, and the challenges of ozone application in grain processing.

OZONATION

Ozone is a natural gas with a triatomic oxygen structure and high oxidation potential of 2.07 V, surpassing that of other food oxidants such as hydrogen peroxide (1.78 V), oxygen (1.23 V), and chlorine (1.36 V). It has a pungent odor, a density of 2.14 kg/m^3 at room temperature, and a molecular weight of 48 g/mol (Afsah-Hejri et al., 2020). Ozone is a better alternative to chlorine and pesticides as it leaves no residue after application. It has been accorded the status of Generally Recognized as Safe (GRAS) by the US FDA. It is unstable at room temperature and rapidly decomposes into oxygen. Further, the stability of ozone depends on the temperature, pH, and pressure of the environment. Ozone can be used in gas, aqueous, and mist forms depending on the type of food. Gaseous ozone is more stable than aqueous ozone at room temperature and 6.5 pH (Afsah-Hejri et al., 2020).

Ozone can be generated by exposing atmospheric air to high-energy electric fields or corona discharge, ultraviolet (UV) radiations, electrolysis, or by chemically converting oxygen to ozone. The most common methods used by food industries are corona discharge (also called plasma treatment) and UV radiation. The commercially used ozone generator uses a corona discharge unit consisting of a high electric field between two electrodes wherein dried gases or oxygen is passed. It is important to note that ozone generators are mostly equipped with electrode cooling systems to control temperature. On passing oxygen through the electric field, it splits into reactive singlet oxygen atoms and radicals, which generate ozone in combination with the remaining oxygen (Brodowska et al., 2017). As ozone cannot be stored, it must be continuously produced at the treatment site. Yvin et al. (2001) developed a patented method aiming to enhance the microbial safety of flour derived from ozonated grains. Similarly, Dubois et al. (2007) patented the Oxygreen® process, which represents a significant advancement in the utilization of ozone for treating food grains. This method entails preconditioning grains in a closed batch reactor before subjecting them to ozonation.

Ozone has been traditionally used to disinfect drinking water. Due to its high oxidizing potential (2.07 V), it has been used for pesticide removal in grains, fruits, and vegetables; sterilization of equipment; and inactivating fungi, yeast, protozoa, and pathogenic bacteria (Xue et al., 2023). Further, it has been used to disinfect vegetables, fruits, meat, dairy, and packaging materials and to prevent microbial contamination. The primary inactivation mechanisms include attacking the microbial cell membrane and inactivating enzymes and genetic material, leading to leakage and cell lysis.

Recently, ozone has been used as a fumigant to control insects, pests, and fungi in stored grains to prevent further mycotoxin production. Fungal and mold infections in stored grains are common due to the formation of localized hotspots when the stored grain is not properly dried or aerated. Fungal growth over time might produce mycotoxins. Various studies have reported that ozone is effective in DON reduction in contaminated grains and reduces toxicity in grains for human consumption. The primary mechanism behind the ozonolysis of DON is the Criegee reaction. Ren et al. (2020) explained the degradation pathway of DON by ozonolysis. The double bond at C9-C10 is cleaved on ozone exposure to form carbonyls (ketone, aldehyde) or carboxylic acid. The ozone molecule undergoes a 1-3 dipolar cycloaddition with the alkene bond in DON, resulting in the formation of ozonides, which then form ketone or aldehyde oxides as intermediates (Savi et

al., 2014). Also, the hydroxyl group at C7 undergoes oxidation to form a carbonyl group, while the epoxy group is oxidized to yield a double bond.

FACTORS AFFECTING OZONE EFFICACY ON DON DEGRADATION IN STORED WHEAT

Ozone is applied to grains in storage bins. It has been reported as efficacious in the degradation of mycotoxins such as OTA, AF, patulin, and ZEN in grains. Within a storage bin, initially ozone interacts with the surface constituents of grains and other organic materials resulting in a reduction of ozone concentration (Tiwari et al., 2010). This limits the movement of ozone into the grain bulk. Subsequently, once the reactive sites are depleted, ozone movement is unhindered through the grain surface. Adsorption of ozone on grain surface also depends on grain moisture, temperature, bed thickness, flow rate, concentration, treatment time, and contamination level of grains (Alexandre et al., 2017).

Grain Moisture Content

Ozone is highly unstable in water and rapidly decomposes into oxygen and free radicals that provide ozone with its oxidative capability. Alexandre et al. (2017) demonstrated improved DON degradation (80%) with increasing moisture in whole wheat flour and wet milling effluents at a 65 mg/L ozone concentration for 180 min. Similarly, Zhuang et al. (2020) found that grains treated with ozone gas after tempering showed a greater reduction in DON concentration (33%) than those treated before tempering and ozonated water. It was observed that ozone decomposes into hydroxyl ions in water, which are more potent in oxidatively degrading DON. Comparable results were obtained by Wang et al. (2016), who showed maximum DON degradation at 20% m.c. wheat as compared with 11.8 and 16.3% m.c. In another study, Li et al. (2014) showed that at a 60 mg/L ozone concentration for 12 h, DON degradation increased from 19.5% to 57.3% in scabbed wheat with 9.5 and 17% m.c, respectively. Shuai et al. (2022) studied the effect of low ozone concentration (3 mg/L) in wheat and corn samples stored under barn and lab conditions, and they reported a significant decrease in fungal load but lower maximum values of DON degradation rates in barn samples than in the lab samples. Three reasons affecting ozone diffusion in grain piles were outlined: 1) a barn is a dynamic system, and the combined effects of temperature, humidity, insects, tightness, and impurities can affect ozone diffusion; 2) possible inward migration of DON in grains (during an equilibrium period of 30 d); and 3) a decline in moisture during 30 d might prevent ozone permeation in grains and reaction with DON. However, while Trombete et al. (2016) found no notable difference between the moisture content of wheat samples exposed to ozone (9.3%) and the control (11.3%), they did find a 64.3 and a 48% reduction of DON and AF, respectively, using a 60 mg/L ozone concentration for 300 min and thus reported that the most influential factors in mycotoxin degradation were ozone concentration and treatment time.

Ozone Concentration and Treatment Time

Increasing the ozone concentration and processing time improves the reduction of DON (Krstović et al., 2021; Patrícia et al., 2018; Wang et al., 2016a). Wang et al. (2016a) reported a twofold increase in DON degradation, from 26% to 53%, when naturally contaminated wheat samples were exposed to 75 mg/L of ozone for 30 and 90 min, respectively. Similarly, Li et al. (2014) also

showed a positive relation between ozone concentration and treatment time with the degradation rate of DON in scabbed wheat. In another study, Piemontese et al. (2018) found that wheat treated with gaseous ozone at 32.5 g/h for 12 or 16 h and at 48 g/h for 12 h exhibited a reduction in DON levels ranging from 31–48%. However, these treatments adversely affected the chemical and rheological parameters of the wheat. Contrarily, a concentration of 55 g/h for 6 h decreased DON and DON-3-Glc levels by 29% and 44% , respectively, without any detrimental effects on grain properties. Hence, the exposure of grains to low ozone concentrations for longer durations could be more detrimental than shorter exposures to high doses. Further, the chemical structure of mycotoxin can also determine the ozone concentration sufficient to break the chemical bonds.

Chemical Structure and Concentration of DON

Deoxynivalenol has a 12,13-epoxytrichothec-9-ene ring as its basic structure with a keto substitution at C-8 (Feizollahi and Roopesh, 2021). Patrícia et al. (2018) reported that the reduction in ZEN concentration in wheat bran after 15 min of ozonation was approximately twice as much (52%) as that observed for DON (29%). The lower degradation rate of DON than ZEN was attributed to the difference in their molecular structure. Besides the chemical structure of toxins, the initial concentrations of mycotoxin could also affect ozone efficacy in detoxifying grains. Li et al. (2014) reported higher degradation rates at a 1 µg/mL initial DON concentration in solution compared with a 5 µg/mL concentration when 2–8 mg/L of ozone was applied for 30 s and explained that lower DON contamination levels have a greater chance of reacting with ozone and other reactive ions in solution. However, a reduction of 29% and 53% in DON concentration was obtained in wheat with initial contamination levels of 0.294 mg/kg and 1.69 mg/kg in studies conducted by Piemontese et al. (2018) and Wang et al. (2016b), respectively. This variation in results could be explained by the difference in ozone concentrations (i.e., 55 mg/L and 75 mg/L) used in the former and latter studies, respectively.

Substrate Characteristics

It has been observed that the effect of ozone on DON was more pronounced in solution than in dry cereal grains (Li et al., 2014). Ozone generates more reactive ions in solution, so it has a greater effect on DON degradation. Li et al. (2014) demonstrated that within 30 s, 10 mg/L of ozone degraded 93.6% of the deoxynivalenol (DON) in the solution. However, when treating infected wheat with 80 mg/L of ozone for 2 h, only 26.11% of the DON present in the grains was removed. This discrepancy is attributed to ozone's higher reactivity and availability to DON in solution than in wheat grains. In a similar study, Sun et al. (2016) reported treatment with 80 mg/L of gaseous ozone effectively reduced DON levels by 83% within 7 min when applied to a 10 mg/L DON solution, while detoxification rates of 74.86%, 70.7%, and 76.2% for contaminated wheat, corn, and bran, respectively, were observed upon applying saturated aqueous ozone (80 mg/L) for 10 min. This reveals that ozone has a notable impact on reducing DON levels in solution. Furthermore, some studies have reported that ozone has more effectiveness in decontaminating wheat flour than wheat kernels due to greater surface area and better ozone penetration in wheat flour. However, small particle size and high ozone doses can lead to more oxidation of flour constituents, resulting in a loss in sensory properties.

Synergistic Action of Ozone

As high ozone concentration can adversely affect grain quality, some studies have proposed a combination of ozone with UV radiation, eliminating the need for high ozone doses. Combined UV and ozone treatments have been used for wastewater treatment. The disinfection mechanism involves the generation of hydroxyl radicals by photolysis of ozone by UV radiations (He et al., 2021). Li et al. (2019) demonstrated a 79% reduction in aflatoxins by exposing contaminated peanuts to 5 mg/L of ozone and 30 min UV radiation. In a similar study, László et al. (2008) showed a significant reduction in the microbial count and an increase in the whiteness of wheat flour upon the application of UV and ozone. A recent study by Babaee et al. (2022) found that synergistic treatment of citric acid, ozone, and UV reduced AF by 90% in pistachio nuts. Further, combining ozone with high-pressure processing, freezing, and hydrogen peroxide may reduce DON contamination in grains.

EFFECT ON GRAIN QUALITY

The oxidative power of ozone can have both desirable and adverse effects on grain properties. The positive effects include improved whiteness and starch properties in flour, while in some cases, adverse effects can be oxidation of food constituents, undesirable odor, and changes in color. Wang et al. (2016a) showed that an application of 75 mg/L of ozone on wheat kernels for 30–90 min showed no significant variance ($P > 0.05$) in fatty acid value, carbonyl and carboxyl content, protein content, amino acid content, and starch content of ozone-treated samples. Moreover, higher tenacity and whiteness were observed in the ozone-treated samples compared with the control. Similarly, Li et al. (2014) reported no adverse changes in the starch pasting properties of ozone-treated wheat grains and only a slight increase in dough development time, indicating improved flour quality after ozone treatment. Zhuang et al. (2020) demonstrated that applying ozone gas and ozonated water with a concentration of 60 mg/L for 2 h did not negatively impact the quality of wheat kernels, likely due to the protective epidermis layer present on the grains. However, when subjected to the same treatment conditions, wheat flour exhibited reductions in wet gluten content, sedimentation volume, and gluten index, along with decreased stability of the flour gel and a shorter dough development time. Similar results were observed by Patrícia et al. (2018), who reported changes in swelling capacity, starch resistance, pasting properties, and gluten properties of wheat flour treated with 65mg/L of ozone for 180 min.

TOXICITY OF OZONATED PRODUCTS

Some studies on cytotoxicity assays of DON on human cells and mice have revealed that ozone treatment can greatly reduce cytotoxicity. At varying concentrations of ozone and DON, six major degradation products ($C_{15}H_{18}O_7$, $C_{15}H_{22}O_9$, $C_{15}H_{18}O_8$, $C_{15}H_{18}O_9$, $C_{15}H_{20}O_{10}$, and $C_{15}H_{20}O_9$) were found by Li et al. (2019). They explained that a de-epoxidation reaction, an addition reaction at the C9–10 double bond, and changes in steric hindrances in the C3 position all significantly reduced the toxicity of most degradation products compared with DON. Similar results were shown by Ren et al. (2020), who performed a cytotoxicity test on ozone-treated DON. The authors confirmed a substantial decrease in the toxicity of DON following ozone treatment. Wang et al. (2017) performed an in vivo test of DON-contaminated wheat after ozone degradation. They found

that feeding DON-contaminated wheat to mice increased toxicity in liver, thymus, and kidney. However, feeding the same wheat after ozonation mitigated the adverse effects of DON.

CHALLENGES

Although ozone is effective in decontaminating mycotoxins, it has some limitations in practical application. The instability and quick decomposition of ozone limits its application in industries. Ozone exposure to the operator's skin could be extremely harmful and lead to irritation in the eyes, bare skin, and respiratory tract. Due to oxidation, corrosion on processing equipment can occur over continuous ozone application. Also, using ozone at higher concentrations can adversely affect the sensory quality of food. Further, as discussed above, the nutritional quality of wheat flour is adversely affected by high ozone concentrations. Alexandre et al. (2017) suggested a potential solution to mitigate alterations in the characteristics of whole wheat flour by applying ozone to the entire wheat grain prior to milling. However, this approach may result in decreased effectiveness in decontaminating DON. Another major limitation in the ozonation of grains is the inhomogeneous distribution of DON in naturally contaminated grains. This results in high DON variability in replicates, making it challenging to identify significant differences between the control and the treated samples.

CONCLUSIONS

Safe storage of food grains is essential for reducing both quality and quantity losses as well as for mitigating health risks. Ozone is an effective green alternative that disinfects grains against molds, fungi, insects, and mycotoxins. Increases in ozone concentration, treatment time, and grain moisture all augment the effectiveness of ozone in DON degradation. Furthermore, ozone is more effective in DON solutions than on grain surfaces. This review highlights ozone's significant potential in reducing DON contamination in grains with minimal effect on grain quality. The ozonated products have reduced toxicity as compared with the control. However, there are some challenges associated with ozone application in grains. Firstly, the high capital cost of an ozone generator may limit its use by small-scale industries. Secondly, ozone is a poisonous gas that, if used at high concentrations for longer durations, might accumulate and put the safety of personnel at risk. Thirdly, the high oxidative potential can adversely affect grains' sensory and chemical properties. Hence, more research is needed to address these challenges and to consider consumer acceptance and the safety of ozonated products. Ozonation alone cannot maximize DON reduction without affecting grain quality (Pandiselvam et al., 2017). Hence, the combination of ozone with other decontamination techniques should be taken into consideration in future studies.

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A Combined Efficacy of Phosphine and Methyl bromide on *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motschulsky in Wood Pellets as a Quarantine Treatment

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ABSTRACT

The international wood pellet market has been growing steadily, with Korea importing 10.3 Mt over a three-year period starting in 2020. However, processed wood pellets, unlike raw wood, have been found to be infested by stored-product beetles during the quarantine process in Korea. They are fumigated with only methyl bromide (MB). Processed wood pellets exhibit increased adsorption of MB, causing a need for re-fumigation which then leads to an elevated consumption of MB. Methyl bromide is an ozone-depleting substance and a highly toxic fumigant. Therefore, there is a need for alternative treatments to replace or reduce MB use.

In this study, we evaluated the practicality of using MB and phosphine (PH_3) for fumigating stored-product beetles in wood pellets. The efficacy of MB and MB+PH_3 mixtures against the pupae and adults of *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motschulsky was assessed by calculating LC_{50} and LC_{99} values.

Pupae of both pests were found to be more tolerant than their adults to MB. The LC_{99} of MB on pupae of *T. castaneum* and *S. zeamais* was 31.485 and 24.315 mg/L for 24 h at 20°C, while on adults, the LC_{99} was 634.5 and 502.1 mg h/L.

The LC_{99} of MB+PH_3 on pupae of *T. castaneum* and *S. zeamais* was 4.064+0.040 and 4.675+0.046 mg/L, respectively, at the same duration and temperature as the evaluation of MB treatment alone, which demonstrated a significant reduction of MB usage, indicating enhanced efficacy with the MB+PH_3 mixture.

These results could be used as a basis for the adoption of combined fumigation in wood pellets, which would help to protect the ozone-layer by reducing MB and accelerate the use of wood pellets as an eco-friendly fuel.

Keywords: Methyl bromide and Phosphine mixture, Grain pests, MB alternatives, Quarantine treatment, Wood pellets

INTRODUCTION

Wood pellets are biofuels made from compressed wood fiber (Naturalllywood, 2023). The global wood pellet market size was estimated at USD 8.23 billion in 2021 and is expected to expand at a compound annual growth rate of 5.5% from 2022 to 2030 (Grand View Research, 2023). Since they are recognized by scientists and global agencies as a biofuel that offers climate benefits as compared to fossil fuels, the rising global demand for renewable energy is driving the demand for wood pellets (Ireland, 2022). Specifically, Korea imported 10.3 Mt of wood pellets from 2020 to 2022, indicating the wood pellets were imported at a rate of almost 3.4 Mt annually (APQA, 2022a). Upon arrival at the import ports in Korea, the pellets are inspected for exotic pests and fumigated with methyl bromide (MB) (APQA, 2022b). Although wood pellets are made from wood fiber, stored-product beetles (*Carpophilus obsoletus* Erichson, *Ahasverus advena* (Waltl), *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst)) and ants (*Paratrechina longicornis* Latreille, *Solenopsis geminate* Fabricius) are among the main groups of pests intercepted from imported pellets (APQA, 2022a) and accounted for approximately 73% of wood pellet pest interceptions from 2020 to 2022. From 2020 to 2022, approximately 4% of the total imported amount—about 382,000 t of wood pellets—was fumigated during the interception process (APQA, 2022a).

Although the use of MB has been discontinued due to its adverse effects on ozone layer depletion and human health, a critical use exemption by UNEP still allows MB fumigation for quarantine and pre-shipment (QPS) purposes (Suwanlaong and Phanthumchinda, 2008; MBTOC, 2010; UNEP, 2018; Park et al., 2020; Choi et al., 2021). This is due to a general lack of feasible alternative treatments for pest disinfection in numerous commodities (MBTOC, 2014; UNEP, 2018) and the need for MB to avert trade disruptions. However, even approved MB treatment for a commodity can be highly sorptive; hence, the development of alternative treatments to replace or reduce MB has become crucial. Although MB is currently being used for the disinfestation of imported wood pellets in Korea, the MB guideline is not meant for wood pellets but rather for wood (APQA, 2022b). Processed wood pellets have had their physical properties changed, and as such, their MB sorption ability has become greater than that of wood and has thus decreased the concentration of MB during fumigation, which has caused a need for re-fumigation on the same commodity in order to disinfest the pests completely.

Phosphine is a traditionally established effective alternative disinfectant to MB for stored products (Hole et al., 1976; Aulicky et al., 2015). However, the effective use of phosphine generally requires a long fumigation time of at least > 48 h (Hole et al., 1976), which may not be ideal for wood pellets that require quicker treatment of 24 h to reduce expenses for logistics in the ports. Another option is a combination of control methods. Concurrent treatment of methyl bromide and phosphine have been evaluated on grain insects in Canada or fruit flies in China, confirming that efficacy was enhanced by a mixture of two fumigants (Bond and Morse, 1982; Li et al., 2020). Even though MB is included in the method, a new guideline is urgently needed in order to supply wood pellets sustainably and be able to produce electricity without affecting climate change in Korea.

Among the stored-product beetles, *Tribolium castaneum* and *Sitophilus zeamais* Motschulsky are widespread across the world, including in Korea. In particular, *Tribolium castaneum* is recognized as relatively tolerant to MB (Obata ,1998) as is *Sitophilus zeamais* to PH_3 (Hole et al., 1976).

In this study, we evaluated the feasibility of using methyl bromide and phosphine for the fumigation of the stored-product beetles of wood pellets. Pupae and adults of *Tribolium castaneum* and *Sitophilus zeamais* were selected as representative invasive beetle species, respectively. We evaluated the efficacy of MB and of a mixture of MB and PH_3 on the larvae and adults of *T. castaneum* and *S. zeamais* by calculating the LC_{50} and LC_{99} of both insects to MB alone and to a mixture of MB and PH_3.

MATERIALS AND METHODS

Insects and fumigants

Individuals of *T. castaneum* and *S. zeamais* were provided by the Plant Quarantine Technology Center, Republic of Korea. These stored-grain pests were reared in the laboratory at 26 ± 1°C and 60–70% r.h. with a 16:8 h (L:D) photoperiod. Flour (800 g), wheat bran (200 g), and dry yeast (70 g) were mixed to serve as a food source, and the beetles were reared in plastic containers (20 cm W × 7 cm L × 8 cm H). Mortality of the adults was assessed at the end of fumigation. Pupae were assessed 10 d after the end of fumigation to determine whether or not they had emerged. Methyl bromide (purity: 98.0 %) was supplied by the Animal and Plant Quarantine Agency (Gimcheon, Korea). Phosphine (2% PH_3 + 98% CO_2) was purchased from the Korea Nano Gas Co. (Yeoju, Korea).

Sensitivity test of MB and MB+PH_3 against *T. castaneum* and *S. zeamais*

Adults and pupae of *T. castaneum* and *S. zeamais* were placed in a 20 L desiccator at 20℃ and treated with different concentrations of MB and of MB+PH_3 for 24 h. The efficacy of the treatments against the adults was assessed at the end of the final day of fumigation. The pupae were checked for emergence at 10 d; they were determined to have been disinfected.

Determination of MB and PH_3 concentration and concentration and time (Ct) product

To calculate the Ct product, the concentrations of MB and PH_3 in fumigation chambers were determined at 0.1, 1.0, 2.0, and 24.0 h after fumigant injection into chambers. The collected gas concentration was analyzed with gas chromatography (GC) (Agilent Technology 6890N and 7890A, Santa Clara, CA, USA). The detector used for MB was a flame ionization detector (FID), and the column was a DB5-MS (30 m × 0.25 mm i.d. x 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). The oven temperature was maintained at a constant 100°C, and injector and detector temperatures were 250 and 280°C, respectively. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. The detector used for PH_3 was a nitrogen phosphorus detector (NPD). The GC conditions were as follows: the GC NPD injector temperature was 250°C; the oven temperature was 240°C; the detector temperature was 320°C; and the column was an HP-5 (0.53 mm × 15 m, Agilent Technology, Santa Clara, CA, USA).

The concentrations of MB and PH_3 were calculated based on peak areas using external standards. The calibration curve standards were prepared by spiking a known volume of MB and PH_3 into a 1 L Tedlar® gas sampling bag (SKC Inc., Eighty Four, PA, USA).

The Ct products were calculated based on the following equation as described in Ren et al. (2011):

$$Ct = \sum \frac{(C_i + C_{i+1})(t_{i+1} - t_i)}{2},$$

where C = concentration of fumigant (mg/L), t = time of exposure (h), i = order of measurement, and Ct = concentration × time product (g h/m^3) (Ren et al., 2011).

Data analysis

The dose–response effects of MB and MB+PH_3 on *T. castaneum* and *S. zeamais* were estimated through Probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

The pupae of both insects were more tolerant to MB than the adults, showing that the LD_{50} of pupae of both insects was almost twice that of adults. The LC_{50} of pupae and adults of *S. zeamais* was 17.935 and 8.091 mg h/L at 20°C, respectively. This result is consistent with previous research on MB to *Sitophilus granaria*, in which pupae were more tolerant than adults, indicating that the LD_{50} of pupae and adults was 1.375 and 1.232 log mg h/L at 25°C, respectively; its LD_{50} is 25.118 and 16.62 mg h/L, respectively, without alteration of log scale (Howe and Hole, 1966). The LC_{99} of *T. castaneum* pupae was 31.485 at 20°C while the CT product was calculated with 634.5 mg h/L. The LC_{99} of *S. zeamais* pupae was 24.315 mg/L at 20°C while the Ct product was calculated with 502.1 mg h/L.

Table 1. Probit analysis of MB efficacy against *T. castaneum* and *S. zeamais* for 24 h fumigation at 20°C.

Insect	Stage	LC_{50} (95% CL, mg/L)	LC_{99} (95% CL, mg/L)	LCT_{99} (mg h/L)
T. castaneum	Adult	10.660 (10.597-10.725)	11.758 (11.595-11.975)	-
	Pupa	27.402 (25.728-31.556)	31.485 (28.648-39.312)	634.5
S. zeamais	Adult	8.091 (7.309-8.803)	11.316 (10.150-14.528)	-
	Pupa	17.935 (17.076-18.622)	24.315 (22.896-26.844)	502.1

We evaluated the mortality of both insects with relevant concentrations of MB and PH_3 for 24 h. The pupae of both insects were more tolerant to MB+PH3 than the adults. The LC_{99} of MB+PH_3 of the *T. castaneum* pupae was 4.046+0.040 mg/L at 20°C while the Ct product was calculated as 75.91±0.76 mg h/L with an amount ratio of 100:1 of MB:PH_3. The LC_{99} of the *S. zeamais* pupae was 4.675+0.046 mg/L at 20°C while the Ct product was calculated with 81.12±0.80 mg h/l with an amount ratio of 100:1 of MB:PH_3. Considering that the LC_{99} of MB of the pupae of *T. castaneum* and of *S. zeamais* was 31.485 and 24.315 at 20°C, respectively (Table 2), the MB concentration needed in the MB+PH_3 mixture was determined to be significantly less in order to achieve 99% mortality, with 4.046 and 4.675 mg/L on *T. castaneum* and *S. zeamais* (Table 3), respectively, as described above. The efficacy of the MB and PH_3 mixture also revealed greater improvement than when the fumigants acted independently (Bond and Morse, 1982). Future research should be conducted at the middle or commercial scale with a large number of insects to confirm the concentration and time, including the Ct product, according to the temperatures suggested in this study.

In conclusion, our results showed a combined efficacy of MB+PH_3 that is greater than if they acted independently. This could be used as a basis for adopting a treatment schedule with MB+PH_3 for wood pellets, which will help protect the ozone layer by reducing MB and help prevent climate change by accelerating the usage of wood pellets for producing electricity instead of using fossil fuels.

Table 2. Probit analysis of MB+PH_3 efficacy against *T. castaneum* for 24 h fumigation at 20°C.

Fumigant	Stage	LC_{50} (95% CL, mg/L)		LC_{99} (95% CL, mg/L)		LCT_{99} (mg h/L)	
		MB	PH_3	MB	PH_3	MB	PH_3
MB+PH3 (100:1)	Adult	1.953 (1.799-2.176)	0.019 (0.017-0.021)	2.342 (2.129-2.679)	0.023 (0.021-0.026)	-	-
	Pupa	2.698 (2.394-3.148)	0.026 (0.023-0.031)	4.046 (3.509-4.972)	0.040 (0.035-0.049)	75.91	0.762

Table 3. Probit analysis of MB+PH_3 efficacy against *S. zeamais* for 24 h fumigation at 20°C.

Fumigant	Stage	LC_{50} (95% CL, mg/L)		LC_{99} (95% CL, mg/L)		LCT_{99} (mg h/L)	
		MB	PH_3	MB	PH_3	MB	PH_3
MB+PH3 (100:1)	Adult	1.603 (1.431-1.768)	0.016 (0.014-0.017)	2.990 (2.672-3.515)	0.029 (0.026-0.035)	-	-
	Pupa	1.767 (1.340-2.097)	0.017 (0.013-0.020)	4.675 (4.017-5.875)	0.046 (0.040-0.058)	81.12	0.800

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Concurrent Treatment of Sulfuryl Fluoride and Ethyl Formate for Disinfection of *Reticulitermes speratus*

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ABSTRACT

Methyl bromide (MB), designated as an ozone-depleting substance following the Montreal Protocol, is recommended for substitution and reduction by the International Plant Protection Convention (IPPC). Methyl bromide has been predominantly used in South Korea for wood, with 322 t having been imported in 2021. However, due to its ozone-depleting properties and health concerns related to human exposure, there has been a gradual phase-out of MB, leading to an increased demand for alternative fumigants.

Sulfuryl fluoride (SF) and ethyl formate (EF) are considered safer fumigants due to their higher Threshold Limit Values (TLV) compared with MB. However, using SF or EF alone is challenging, either due to SF's low efficacy against grain pest eggs or EF's limited ability to penetrate agricultural commodities. This study aims to evaluate the efficacy of a combined treatment of SF and EF against the most prevalent wood pest, the termite *Reticulitermes speratus*, to find a feasible treatment to replace MB.

Preliminary tests on SF and EF were conducted to determine whether the combined treatment resulted in an enhanced efficacy, by comparing the efficacy of individual treatments with SF and EF with the efficacy of their combined treatment against the termites. The combined treatment enhanced efficacy against the termites at both 23℃ and 5℃. The synergistic effect of the combined treatment was particularly significant, especially considering that the cumulative mortality rate of the individual fumigants was below 50%. Further large-scale trials are necessary to validate the efficacy and assess the effectiveness of the combined treatment against the termites. The study should also encompass various wood pests to evaluate its overall efficacy. Nevertheless, it is expected that the results of this study will contribute to the development of an alternative to MB treatment, enhancing worker safety and preserving the Earth's atmospheric environment.

Keywords: Methyl bromide alternative, Sulfuryl fluoride and ethyl formate mixture, MB alternatives, Quarantine treatment, Wood destroying pest

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Tobacco Fumigation with ECO2FUME® Phosphine Fumigant for the Control of Cigarette Beetles

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ABSTRACT

The tobacco industry has experienced significant losses due to cigarette beetle infestations worldwide. The primary chemical control method used today is via thorough fumigation with the solid formulations of metal phosphides. According to the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) standards, fumigation protocols require 4–12 d of exposure depending on the tobacco temperature and phosphine concentration. We investigated the efficacy of ECO2FUME®, a gaseous mixture of phosphine and carbon dioxide, for controlling cigarette beetles in cured tobacco. Tests were carried out in temperature-controlled cargo containers of 6.2 and 12.4 m (20 and 40 ft). Cured tobacco bales were put into containers. In the center of the tobacco bales, small polyvinyl chloride (PVC) vials equipped with wire mesh caps for air passage were inserted, each containing 40 individuals from one life stage only. The fumigation procedures comprised exposing all life stages (i.e., eggs, larvae, pupae, and adults) to phosphine concentrations of 1000 ppm within temperature ranges of 6°C to 26°C, with exposure periods ranging from 1 to 4 d. Tests were repeated twice, each with two replicates. Results showed that ECO2FUME could be used effectively for the control of cigarette beetle infestations in cured tobacco.

Keywords: Tobacco fumigation, ECO2FUME®, Phosphine fumigant, Cigarette beetle, *Lasioderma serricorne*, Life stages, Mortality

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is one of the most economically important crops worldwide, serving as a vital component in the production of cigarettes, cigars, and various tobacco products. Dating back centuries, tobacco has been cultivated, processed, and commercialized, shaping

economies and societies worldwide. Tobacco usage traces back to ancient civilizations, with archaeological evidence suggesting its cultivation as early as 5000 BC in the Americas (Gately, 2007). However, it was during the European Age of Exploration that tobacco gained widespread popularity, eventually becoming a global commodity. Colonial powers heavily promoted tobacco cultivation, leading to its integration into trade networks and the emergence of large-scale production systems (Proctor, 2012). The Industrial Revolution further propelled the tobacco industry forward, with advancements in manufacturing techniques and mass production.

Today, the tobacco industry remains a significant player in the global economy. The global tobacco market generated revenues exceeding USD 800 billion in 2022, growing at a CAGR of 3% from 2023 to 2032 (Anonymous, 2023). The economic ramifications extend beyond direct revenues, encompassing employment, taxation, and trade implications for both producing and consuming countries (Chaloupka et al., 2012).

Throughout its cultivation, processing, and storage, tobacco faces challenges, with insect pests posing a significant threat to its quality and economic value. Among the most destructive species are the cigarette beetle (*Lasioderma serricorne* Fabricius) and the tobacco moth (*Ephestia elutella* Hübner) (Ryan, 1999); both have profound impacts on cured tobacco manifesting through direct losses in yield and quality, increased costs associated with pest management, and potential rejection of infested tobacco lots by buyers and manufacturers (Edde, 2019). Additionally, infestations may lead to contamination by insect fragments and excreta, further diminishing the marketability of affected tobacco. Furthermore, insect activity can accelerate the degradation of tobacco constituents, affecting its aroma, flavor, and burning characteristics.

Effective management of insect pests during the storage and processing of cured tobacco is essential for preserving its quality and economic value. Common practices employed in tobacco storage facilities encompass the utilization of pheromone traps, sanitation protocols, application of contact pesticides (which are applied via space or surface sprays within the facilities without direct contact with the commodity), and controlled atmosphere and fumigation applications (Edde and Phillips, 2022). However, insect management in stored tobacco has relied mostly on phosphine fumigation.

Phosphine is the only fumigant allowed for use in tobacco fumigation (CORESTA, 2019). The use of solid phosphine formulations leads to an extension of the fumigation period in tobacco. Moreover, the target concentration can not be restored quickly in case of any gas leakage. Therefore, the use of cylinderized phosphine formulations instead of solid phosphine preparations may be beneficial in shortening the application period (Tumambing, 2012). Another advantage of cylinderized gas phosphine formulations is that they allow fumigation at low temperatures (Tumambing, 2012).

Therefore, the objective of this study was to evaluate the efficacy of ECO2FUME, a cylinderized phosphine formulation containing a gaseous mixture of 2% phosphine (by weight) in carbon dioxide, against all life stages of *L. serricorne*, under several temperatures and exposure times.

MATERIALS AND METHODS

Lasioderma serricorne adults were collected from a local tobacco warehouse in 2023 and reared in 1 L glass jars containing whole wheat flour—known for providing optimal development and reproduction conditions (Hagstrum et al., 1997). The rearing took place at a temperature of 27°C and 60–65% r.h. in incubators (Binder KB 720, Germany) for approximately 6 mo to obtain sufficient numbers of each life stage.

Tests were conducted within temperature-controlled cargo containers of around 28.4 m^3 (L*W*H: 6 m × 2.4 m × 2.6 m) in Izmir, Turkey. Within these containers, cured tobacco bales were placed to simulate real-world conditions for the experiments. Cigarette beetles (*L. sericorne*) in all life stages—eggs, larvae, pupae, and adults—were utilized for the experiments. Samples of each life stages were put into respective small perforated lid PVC vials in batches of 20 individuals per vial. Test vials were placed within the core of the tobacco bales together with temperature and PH_3 loggers. Then, the doors of the containers were closed firmly and sealed from the exterior using airtight adhesive tape (Fig. 1.)

The required amount of phosphine from ECO2FUME® cylinder was injected inside containers using a stainless steel quick dispensing hose with gun type gas injector. The amount of dispensed ECO2FUME® was determined by the weight change of the cylinder on the top of a digital weighing scale accurate to 0.01 kg. The experiments started when the concentrations reached 1000 ppm PH_3 and the temperatures reached the test temperatures (Fig. 1.).

Fig. 1. Containers (left), test vials (middle), and ECO2FUME cylinder (right).

Temperature and PH_3 levels (Table 1) were continuously monitored remotely using temperature and PH_3 loggers placed next to the test vials within the core of the bales throughout the entire fumigation process. When the phosphine concentration fell below the target level, ECO2FUME was added to restore the concentration to 1000 ppm. At the end of the exposure periods, the containers were opened and aerated. Then, the test vials were removed from the tobacco bales, brought to the laboratory, and kept at 25°C and 65% r.h. Mortality of adults and larvae was recorded daily, while eggs and pupae were observed for 15 d to monitor hatching and adult emergence, respectively. Experiments were repeated twice, each with two replicates containing 20 individuals each.

RESULTS AND DISCUSSION

This study investigated the potential of utilizing the ECO2FUME cylinderized phosphine formulation (2% PH_3 + 98% CO_2, by weight) for fumigating tobacco to control *L. serricorne*. Temperatures and PH_3 concentrations recorded in the tests were within the target levels; however, PH_3 needed to be topped up when the concentrations dropped below the target level of 1000 ppm (Figs. 2–7).

We obtained complete mortality of all stages of *L. serricorne* exposed to 1000 ppm phosphine at all temperatures and for all exposure periods tested (Table 1). The results indicated that complete mortality occurred in all stages after 24 h exposure at 26°C, 48 h exposure at 16–25°C, 48 h exposure at ≥21°C, 72 h exposure at 12–15°C, and 96 h exposure at 6–10°C.

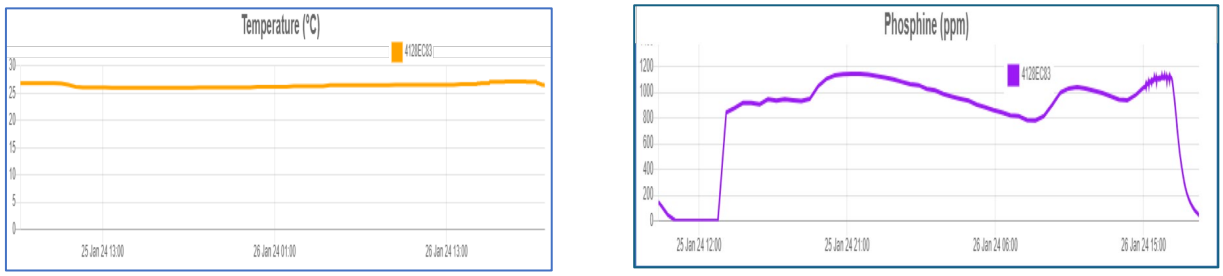

Fig. 2. Temperatures and PH_3 concentrations recorded for the 24 h exposure at 26ºC.

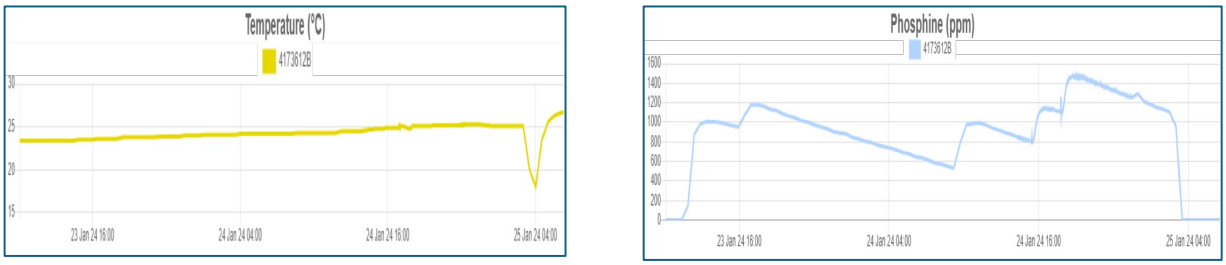

Fig. 3. Temperatures and PH_3 concentrations recorded for the 36 h exposure at 26ºC.

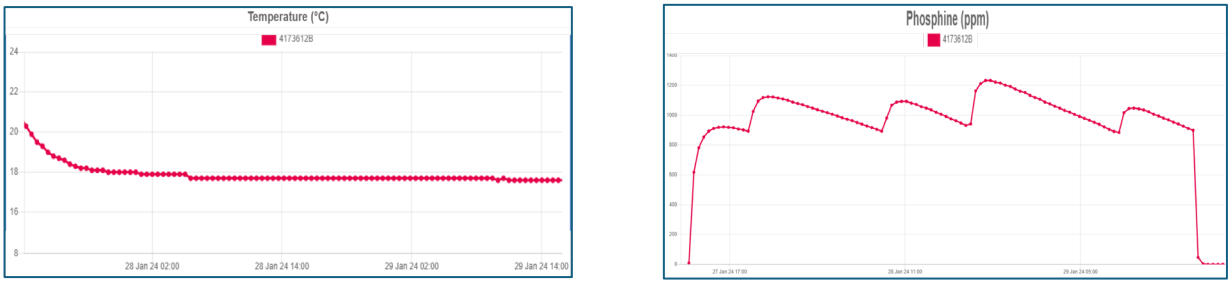

Fig. 4. Temperatures and PH_3 concentrations recorded for the 48 h exposure at 16–25ºC.

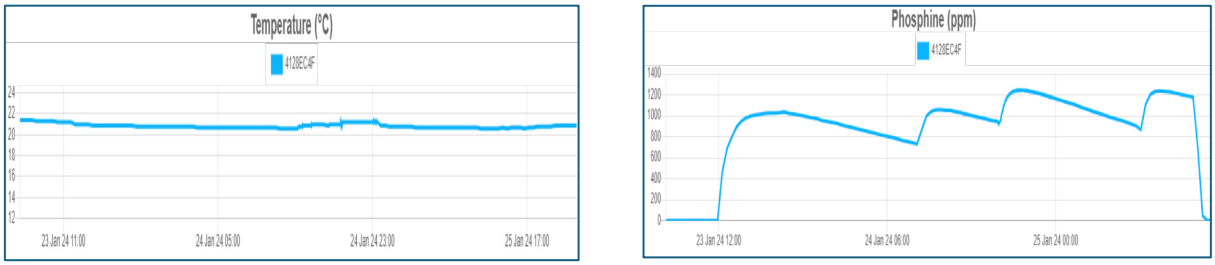

Fig. 5. Temperatures and PH_3 concentrations recorded for the 48 h exposure at or above 21ºC.

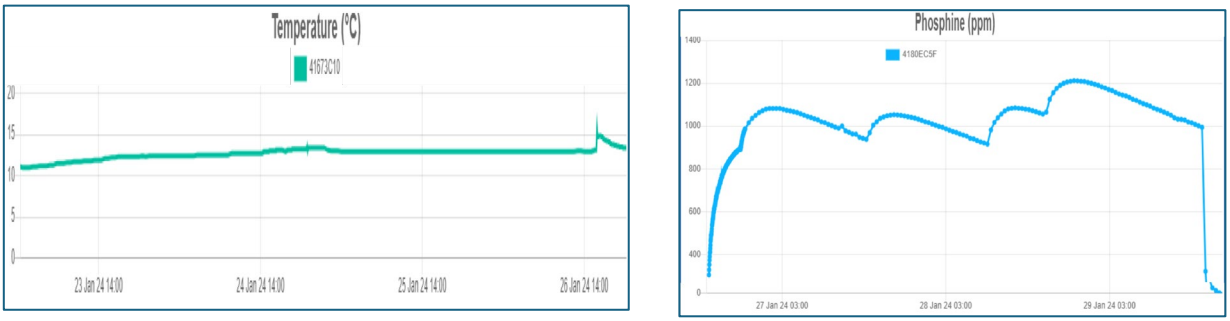

Fig. 6. Temperatures and PH_3 concentrations recorded for the 72 h exposure at 12–15ºC.

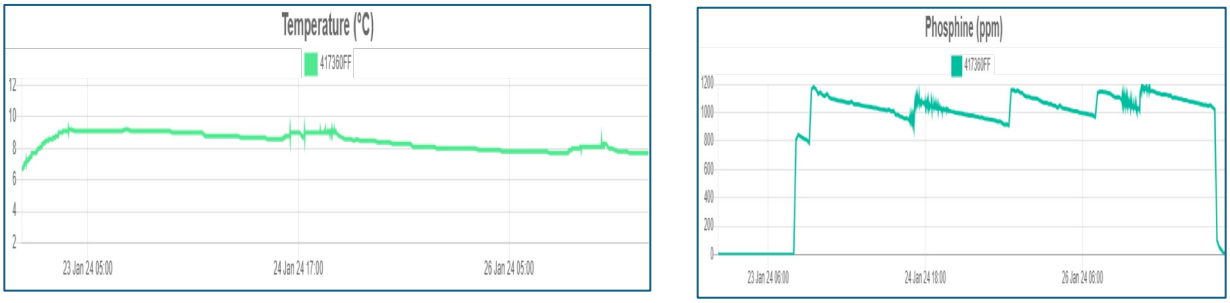

Fig. 7. Temperatures and PH_3 concentrations recorded for the 96 h exposure at 6–10ºC.

Hole et al. (1976) tested various phosphine concentrations at different temperatures and exposure periods against all life stages of *L. serricorne*. They found that the concentrations required for complete mortality in the most tolerant stage (i.e., pupae at 15°C, eggs at ≥25°C) decreased as the exposure time increased. Thus, the concentrations required for complete mortality of all stages were reported as 0.93 and 0.36 mg/L at 15°C for 2 d and 4 d exposure, respectively. At 25°C for 2 d and 4 d exposure and at 30°C for 4 d exposure, complete mortalities were reported to be 1.60 mg/L, 0.32 mg/L, and 0.17 mg/L, respectively (Hole et al., 1976). Similarly, Childs et al. (1973) found that at 26.7°C and with a 72 h exposure, eggs of *L. serricorne* were the most tolerant stage, followed by pupae and then larvae.

Kim et al. (2020) found that the treatment of *L. serricorne* with PH_3 at a concentration of 1.0 mg/L for 20 h showed a mortality rate of 86.36% (pupae) or higher (eggs, larvae, and adults).

Fukazawa and Takahashi (2015) studied the fumigant efficacy of PH_3 against resistant *L. serricorne* populations with different phosphine resistance levels under a range of concentrations (1–2,000 ppm) at 25°C for 24, 72, 120, or 168 h. As estimated from the graphs they presented, susceptible *L. serricorne* adults were completely killed between 24 h and 168 h as the PH_3 concentrations decreased from 100 ppm to 10 ppm at 25°C, respectively. However, adults from resistant populations exhibited a mortality rate of 42–82% when exposed to a concentration of 1000 ppm of PH_3 for 24 h. Complete adult mortalities in resistant populations were reported to be achieved at PH_3 concentrations of 300, 430, and 720 ppm for 168, 120, and 72 h of exposure, respectively (Fukazawa and Takahashi, 2015). Rajendran and Narasimhan (1994) suggested the use of 2 g of PH_3 per cubic meter at 30°C for 10 days in well-sealed structures to control PH_3-resistant strains of *L. serricorne* in stored tobacco.

Table 1. Mortality (%) of all life stages of *Lasioderma serricorne* exposed to 1000 ppm PH_3 at different temperature and exposure periods.

Temperature (°C)	Exposure period (h)	Stage	Mortality (%)*
26	24	Eggs	100
		Larvae	100
		Pupae	100
		Adults	100
	36	Eggs	100
		Larvae	100
		Pupae	100
		Adults	100
16–25	48	Eggs	100
		Larvae	100
		Pupae	100
		Adults	100
21	48	Eggs	100
		Larvae	100
		Pupae	100
		Adults	100
12–15	72	Eggs	100
		Larvae	100
		Pupae	100
		Adults	100
6–10	96	Eggs	100
		Larvae	100
		Pupae	100
		Adults	100

*Mortalities in controls were negligible; therefore, no correction was made.

Numerous studies indicate that exposure time in phosphine fumigations has a greater effect on efficacy than concentration (Rajendran and Narasimhan, 1994; Fukazawa and Takahashi, 2015; Nayak et al., 2020). Insufficient fumigation duration in phosphine applications is one of the factors leading to the development of resistance in insect pests (Nayak et al., 2020). Therefore, continuous screening of PH_3 resistance in *L. serricorne* populations remains very crucial regarding the efficacy of PH_3 applications in the tobacco industry.

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Navarro H, Ren Y (2024) Exploration of ethyl formate as a biosecurity fumigant for management of *Trogoderma Granarium*. Pg. 44ï 51. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Exploration of Ethyl Formate as a Biosecurity Fumigant for Management of *Trogoderma Granarium*

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ABSTRACT

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is a major stored-product pest categorized among the 100 worst invasive species worldwide. Therefore, its control has a quarantine significance, especially with increasing international trade. The efficacy of liquid ethyl formate (EF) was tested on the diapausing and non-diapausing larval stage of *T. granarium* in 2.65 L empty glass desiccators at a dose rate of 0ï 111 mg L^{-1}. The early larval stage (L2) and late larval stage (L5) of *T. granarium* were exposed to EF at 26Ñ1.2ºC and 55Ñ5% relative humidity for 6 h. Mortality was determined 24 h after the treatment for the non-diapausing larvae and after 7 d for the diapausing larvae. Before each trial, the half-time pressure decay test was carried out in order to ensure the gastightness of the desiccators. The effective doses for LD_{50} and LD_{99}, with lower and upper limits of each tested larval stage of *T. granarium*, were analyzed using Probit analysis ($P<0.05$). Results indicate that the second instar non-diapausing larva was the most susceptible stage to EF, requiring only 44.01 mg L^{-1} to obtain LD_{99} mortality, followed by its diapausing stage at 49.46 mg L^{-1} and then the non-diapausing late larval stage at 76.57 mg L^{-1}. The most tolerant life stage was the diapausing L5, which required 107.75 mg L^{-1} to obtain LD_{99} mortality. Further investigations will be carried out to demonstrate the lethal effect of the fumigant to control all of the non-diapausing stages and all diapausing larvae, as well as the effect of sorption in the presence of commodities to be fumigated.

Keywords: *Trogoderma granarium*, Khapra beetle, Pest management, Fumigation, Ethyl formate, Diapausing larvae, Quarantine pre-shipment treatment, Stored-product insects

INTRODUCTION

Trogoderma granarium, known as the khapra beetle, is the most significant dermestid pest of stored products due to its destructive feeding habits on grains and seeds. Originating from India, this insect has become a major worldwide stored-product pest, having spread to Africa, Europe, South America, and eastern Asia (Harris, 2006). Throughout the tropics, this pest is cosmopolitan,

multivoltine, and polyphagous (Odeyemi, 1989) and has been recorded to infest over 100 commodities (Athanassiou et al., 2016; Hagstrum and Subramanyam, 2016; Pasek, 1998); it has also been considered an A2 quarantine-level organism by the European and Mediterranean Plant Protection Organization (EPPO, 1981). *Trogoderma granarium* larvae feed mainly on wheat grain—as a result, the nutritive quality of the wheat decreases along with its weight, which leads to a lower marketing price (Ahmedani et al., 2009). This pest damages many more kernels than it consumes, and it causes heavy contamination of the commodities with body parts and cast skins (Myers and Hagstrum, 2012; Stibick, 2007). Therefore, the commodities infested with *T. granarium* are a major cause of concern worldwide. Diapause is a physiological state of suspended development that helps insects survive unfavorable conditions. In stored-product insects, diapause can occur as a response to environmental cues like temperature and food availability, allowing them to withstand long periods when resources are scarce.

Diapause occurs in 46 stored-product insect species where most diapausing beetle species are from the families Bruchidae, Dermestidae, or Ptinidae (Bell, 1994). Diapause can make insects less susceptible to pest treatment and make predicting the time of adult eclosion (larval diapause) or reproduction (adult reproductive diapause) more difficult (Hagstrum and Subramanyam 2016). *Trogoderma granarium* larvae can undergo a facultative diapause and are stimulated to diapause in response to adverse conditions: lowered temperature, inadequate food, isolated rearing, restricted space, or larval crowding. Moreover, its ability to enter diapause seems to assist the larvae in surviving adverse conditions; it may also promote dispersal, as diapausing larvae are frequently found on movable objects or transport equipment, such as freight containers, ships' holds and other forms of transport, and allow infestations to be spread all over the world. However, when food becomes available, regrowth occurs with successive molting (Beck, 1971). In *T. granarium,* behavior associated with the diapause-like arrest is characterized by seeking cracks and crevices in which larvae may remain inactive for lengthy periods (Burges, 1960). In this state of very low metabolic activity, they are extremely resistant to the effects of contact insecticides or fumigants; complete disinfestation may thus be difficult. Regardless of the method of induction, diapause in *T. granarium* can be terminated by a substantial temperature rise or the renewal of food (Burges, 1960, 1962; Nair and Desai, 1973). Therefore, larval diapause is the key characteristic that greatly contributes to its range expansion (Hadaway, 1956; Athanassiou et al., 2019).

The important fast-acting fumigant methyl bromide (MB) has been phased out worldwide, except for its use in quarantine pre-shipment treatments (QPS) (UNEP, 2006). Therefore, there is an urgent need to develop alternatives for the management of quarantine pests, such as the khapra beetle. Ethyl formate (EF) has been suggested as an alternative candidate to MB (Ryan and Dominiak, 2020).

Ethyl formate has been re-evaluated in the past 25 years for its use as an alternative fumigant to MB (Hilton and Banks, 1997; Damcevski and Annis, 1998; Haritos et al., 2006; Ruess et al., 2001). It was found to be successful due to its low mammalian toxicity, rapid loss of residues, solubility in water, natural presence in foodstuffs, benignity to the environment (Desmarchelier, 1999; Ren and Mahon, 2006), and its rapid effect (Ryan and Dominiak, 2020). Therefore, this work aims to investigate and compare the effect of liquid EF to control the larval stages of the diapausing and non-diapausing larvae of *T. granarium*.

MATERIALS AND METHODS

Non-Diapausing Larvae: Cultures of *T. granarium* were reared on wheat (12.5% m.c. wet basis) originating from Israel at 29ºC and 65±5% relative humidity (r.h.). Trials of each life stage of *T. granarium* were carried out in empty 2.65 L glass desiccators and replicated four times. Each replicate included at least 40 individuals of each life stage. Treatments were conducted at 26±1.2ºC and 55±5% r.h. by applying liquid EF (Alfa Aesar - Thermo Fisher Scientific Avocado Research Chemicals, 97% purity, balanced with ethanol) using 0.5 mL syringes (Pressure-LOK, USA) at various dosages of 0.0–91.2 mg L^{-1} for 6 h of exposure time. A magnetic stirrer was placed on the bottom of the desiccator to allow for the even distribution of EF for the first 5 min of exposure at each trial. Mortality counting was carried out 24 h after the treatment.

Diapausing Larvae: To induce the larval stage into diapause, cultures of mixed larvae—reared on wheat (12.5% m.c. wet basis) at 29ºC and 65±5% r.h.—were separated from their mediums and transferred to 15ºC for at least 1 mo. Trials of each larval stage of *T. granarium* were carried out in empty 2.65 L glass desiccators in four replicates. Each replicate included at least 50 individuals of each life stage. Treatments were carried out at 26±1.2ºC and 55±5% r.h. by applying liquid EF using 0.5 mL syringes at various dosages of 0.0–111 mg L^{-1} for 6 h of exposure time. A magnetic stirrer was placed on the bottom of the desiccator to allow EF to be evenly distributed within the desiccator for the first 5 min of exposure at each trial. Mortality counting was carried out after 7 d of incubation at 29ºC and 65±5% r.h., with the addition of wheat grains as a food source.

Before each trial, the half-time pressure decay test was carried out to ensure the gastightness of the desiccators. Only desiccators that held a half-life pressure (pa) decay of >5 min were used. Pressure measurements were carried out using a digital differential pressure gauge handheld barometer (Manometer HT-1890 Auto-off). Concentration measurements were taken at 6 h of exposure time with a calibrated Microtector II G460 equipped with an EF sensor (GFG, Dortmund, Germany). The data were analyzed by PoloPlus software (LeOra software version 1.0).

RESULTS AND DISCUSSION

Table 1 shows the different effective doses of EF (mg L^{-1}) needed to obtain LD_{50} and LD_{99} ($P<0.05$), with lower and upper limits of two instar larval stages of *T. granarium* for both non-diapausing and diapausing and treated and untreated larvae at 26±1.2ºC and 55±5% r.h. at 6 h exposure time. The early instars of the non-diapausing larval stages (L2) are much more susceptible to liquid EF than the pre-pupal stage (L5), requiring 44 mg L^{-1} and 76.6 mg L^{-1}, respectively, in accordance with achieving the LD_{99} values (Table 1). Whereas the difference in dosage needed to reach LD_{99} values between the non-diapausing and the diapausing larvae at the early larval stages represents only an 11% increase, in comparison, the required dosage to obtain LD_{99} values between both types in their respective late larval stages is much greater—representing a 28% increase.

Table 1. The effective dose of EF (mg L^{-1}) for LD_{50} and LD_{99} ($P<0.05$) with lower and upper limits for two instar larval stages of *T. granarium* both non-diapausing and diapausing along with their chi-square, degrees of freedom, heterogeneity, the Slope (T-ratio) obtained and the number of subjects treated and untreated at 26±1.2°C and 55±5% RH and 6 h exposure time.

Larval Stage	LD_{50} Dose Rate (mg L^{-1})	LD_{50} lower	LD_{50} upper	LD_{99} Dose Rate (mg L^{-1})	LD_{99} lower	LD_{99} upper	$\chi 2$	Df	Hetero-geneity	Slope (T-ratio)	Subject	Controls
L2 *ND	14.403	12.16	16.67	44.024	37.112	54.62	5.13	7	0.733	4.794± 0.421	937	173
L2 †D	35.570	34.65	36.48	49.465	46.85	53.40	7.63	8	0.954	16.245 ±1.529	1193	188
L5 ND	38.086	32.08	36.64	76.573	66.95	91.29	8.70	9	0.967	6.640± 0.497	664	124
L5 D	48.628	42.652	54.050	107.175	87.715	157.80	113.96	12	9.497	6.778± 0.347	1358	237

*ND = Non-Diapausing; †D = Diapausing

Quarantine treatments are needed to prevent the export of quarantined pests to regions or countries that are free of these insects. Without food, diapausing larvae may survive about 9 mo; with food, larvae may live as long as 8 yr (Burges, 1962). The high potential for the spread of *T. granarium* through international trade makes this species a continued threat. During diapause, intermittent feeding can give rise to increases in larval weight beyond those attained by non-diapausing larvae (Gothi et al., 1984). Post-diapausing adults may thus be larger, with females laying more eggs as a result (Karnavar, 1984). If *T. granarium* were to become established in the United States, such a situation would create market accessibility problems for several commodities in different climates (Howe and Lindgren, 1957; Banks, 1977; Viljoen 1990) that may pose a barrier for grain-exporting countries such as Australia, the United States, and Canada (Day and White, 2016).

The recommended dosages of fumigants for *T. granarium* are usually two times higher compared with those for other stored-product pests (Bell, 1984). One of the alternative fumigants that has been investigated is phosphine, which was found to be very effective in controlling diapausing larvae as well when exposed for 6 d at 20ºC with 1.5 g m^{-3} (1080 ppm) (Bell, 1984).

However, many recent studies point to the strong resistance of the beetle to phosphine that requires excessive exposures to achieve quarantine levels of security (e.g., more than 14 d of 800 ppm phosphine at 34ºC and 65% r.h.) (Sarfraz et al., 2000). Another alternative fumigant that has been investigated is sulfuryl fluoride (SF), which has been in use to control storage insects since 2004 (Prabhakaran, 2006). However, since SF is known to be a poor ovicide (Myers at al., 2021; Walse et al., 2009), the combination of SF with propylene oxide (PPO) was investigated.

Other control methods such as modified/controlled atmospheres, vacuum conditions, and high CO_2 concentrations are impractical for quarantine purposes since they require long exposure periods and expensive infrastructure. Also, temperature manipulations require special treatment designs due to the low specific heat of stored commodities under commercial-scale applications (Navarro, 2006) and thus are not in use. Therefore, although MB was phased out due to its association with the depletion of the ozone layer (UNEP, 2006), it is still the traditionally preferred choice for quarantine security worldwide due to its versatility and fast action.

In this study, the initial dosages of 44–76.5 mg L^{-1} correspond to the 264–459 ghm^{-3} concentration × time (Ct) product needed to obtain complete mortality of the larval stages of the non-diapausing larvae. However, in order to obtain the LD_{99} values for the diapausing larvae, higher dosages were required ranging from 49.5 to 107.17 mg L^{-1}, which corresponds to a Ct product of 297–643 $g \cdot h \cdot m^3$.

Coetzee et al. (2019) tested EF application in shipping containers. Adults of *Sitophilus oryzae* (L.), *Rhyzopeertha dominica* (F.), *Tribolium castaneum* (Herbst), *Trogoderma variabile* (Ballion) and *Lasioderma serricorne* (F.) were exposed to 90 gm^{-3} of EF mixed with N_2 (1:5 ratio) for 6 h exposure time. Complete mortality was achieved, which correlates to a 440 $g \cdot h \cdot m^{-3}$ Ct product with a range of 437.54–449.19 $g \cdot h \cdot m^{-3}$. Previously reported bioassays suggested that a Ct product for EF of approximately 450 $g \cdot h \cdot m^{-3}$ will kill egg stages of insects (Desmarchelier et al., 1999; Muthu et al., 1984; Xin et al., 2008; Mahon et al., 2003). Results in this study are in correlation with other common stored-product pests that were reported to be fully controlled at the larval stage (Coetzee et al., 2019) with a Ct product of 450 $g \cdot h \cdot m^{-3}$.

In a previous study (Finkelman et al., 2012), *T. granarium* non-diapausing larvae were exposed under laboratory conditions to Vapormate™ (16.7% EF in liquid CO_2) supplied from a pressurized cylinder at 420 g m^{-3} (corresponds to 70 g m^{-3} EF) at 30ºC and 70±2% r.h. The average mortality obtained after 12 h of exposure time was 76.3%; after 16 h, mortality was 97.5%. Only once the exposure time was extended to a full 24 h was the target mortality of 100% achieved. This indicates that their results significantly differ from this study. The reasons for such a difference might be that the work of Finkelman et al. (2012) was carried out using Vapormate™ and that the reported EF concentrations were calculated but not actually measured—while in our study, we used the vapor phase of EF, and the concentration was measured.

From the present study, it appears that EF is highly effective in controlling *T. granarium* diapausing and non-diapausing larvae within short exposure times.

CONCLUSIONS

According to the available literature, *T. granarium* is a naturally tolerant pest to fumigants and physical control methods. However, contrary to the available literature, the results in this study indicate a high sensitivity of the beetle to ethyl formate (EF). This study strongly supports the approach of the application of EF as a quarantine fumigant due to its rapid action and great effectiveness in controlling the beetle. Further research is needed to obtain additional LD_{99} values on all stages of this notorious pest, *T. granarium*.

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Paper No. CAF2024-A07

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The Role of Antioxidant Enzymes in *Liposcelis entomophila* (Enderlein) against PH_3 Fumigation and Hypoxia Stress

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ABSTRACT

Psocids or booklice are a kind of tiny stored-product pests that seriously threaten the safety of stored grain and food security. Among them, *Liposcelis entomophila* has developed resistance to phosphine and grain protectants. The antioxidant enzyme system plays crucial role in insect resistance and adaptation to environmental stress. To clarify the role of antioxidant enzymes in hypoxic adaptation of *L. entomophila*, the changes of reactive oxygen species (ROS) level and antioxidant enzyme activity in PH_3 resistant *L. entomophila* after PH_3 fumigation and hypoxia stress were determined. Results showed that both PH_3 fumigation and hypoxia treatment induced lipid peroxidation in *L. entomophila*, and PH_3 fumigation caused greater oxidative damage and H_2O_2 accumulation, but hypoxia stress had no such effect. The activities of Catalase (CAT), Superoxide dismutase (SOD) and Peroxidase (POD) increased significantly after hypoxia treatment. These results provided a basis for exploring the resistance of *L. entomophila* to oxidative damage caused by PH_3 fumigation and hypoxia. This study also identified 10 genes in the antioxidant enzyme system of *L. entomophila* and clarified their expression profiles under PH_3 fumigation and hypoxia stress. Genes SOD2, PRDX1, PRDX3 and PRDX6 responded significantly after hypoxia stress, indicating that they played an important role in eliminating oxidative damage caused by hypoxia. These results laid a foundation for understanding the role of antioxidant enzyme system in the adaptation of *L. entomophila* to adverse environmental stress, and also provided a theoretical basis for studying the molecular mechanism of hypoxia adaptability and PH_3 resistance, formulating scientific and effective fumigation and controlled atmosphere schemes.

Keywords: Phosphine (PH_3), Hypoxia, Catalase (CAT), Superoxide dismutase (SOD), Peroxidase (POD), Antioxidant enzymes, Booklice, Psocid

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Sotiroudas V, Agrafioti P, Sakka M, Athanassiou CG (2024) Recent advances on the distribution of phosphine in different application scenarios. Page 53. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Recent Advances on the Distribution of Phosphine in Different Application Scenarios

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ABSTRACT

The distribution of phosphine during its application has been examined in detail in recent years, due to the introduction of sensors that can provide remote real-time monitoring. In this context, some studies show that phosphine distribution within large structures, such as silos, is mostly uneven, which may allow insect survival in certain areas that are receiving low concentrations. Moreover, temperature plays a key role in both distribution and concentration of phosphine, while there are studies that show extreme variations within the same day, even in small structures, such as shipping containers. At the same time, it has been found that sorption of phosphine from the commodity is an important factor that determines the overall concentration and distribution, with extreme variations among different products (e.g., grains, legumes). In the case of silos, the utilization of a J-system (phosphine recirculation system) can drastically contribute towards a uniform distribution of phosphine, but additional sealing improvements are considered necessary to maintain a desired concentration level. The use of low concentration sensors has shown that phosphine concentration may exceed the safety limits for several hours, and often days after the termination of the fumigation and the degassing of the treated area, with the concomitant safety risks. These parameters are analyzed in detail in the current presentation, using paradigms from commercial fumigation applications throughout the globe.

Keywords: Phosphine, Stored product insects, Mortality, Distribution, Concentration

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Panigrahi SS, Senthilkumar T, Singh CB (2024) Automated aeration of canola in southern Alberta (Canada). Pg. 54–60. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Automated Aeration of Canola in Southern Alberta (Canada)

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ABSTRACT

Canola poses a unique storage challenge due to its high rate of respiration and risk of self-heating. Even dry canola must be regularly monitored and aerated to keep it cool for safe storage. Therefore, an aeration study was conducted in three 544 t flat bottom bins equipped with 7.5 kW low-speed centrifugal fans and fully perforated floors. Canola with 6–9.2% moisture content (wet basis) was loaded into the bins and the initial canola temperature was 20–26°C. All three bins were equipped with moisture and temperature monitoring cables. Canola moisture and temperature were regularly monitored, and automated aeration fan control was used to cool the canola to 5°C. Airflow distribution at the top of the grain was measured. Airflow in partially filled grain bins (264–438 t) ranged from 0.22–0.33 $m^3min^{-1}t^{-1}$ (0.17–0.26 cfm/bu). Aeration results showed that canola was effectively cooled to 1.5–4.5°C with fan operation of 116–175 h. No significant change in moisture was observed.

Keywords: Canola storage, Respiration, Grain bin, Automated aeration control, Airflow, Spoilage

INTRODUCTION

In-bin aeration is widely used to cool warm grain stored in grain bins. It is assumed that due to low airflow during aeration and relatively faster cooling, there is negligible grain drying. Therefore, most grain terminals and farmers prefer this operation to accomplish uniform desired grain temperature and alter the ecosystem to reduce insect and mite development. Aeration is typically conducted utilizing the cooling potential of ambient air, often exceeding the required timeframe due to several reasons. Firstly, airflow rates within the storage bin are unevenly distributed, particularly in peaked grain, necessitating more time to adequately ventilate areas with lower airflow rates compared to other areas. Secondly, the completion time of aeration cycles is usually estimated roughly, leading to potential extensions beyond optimal durations. Additionally, fluctuations in ambient temperature and relative humidity create multiple temperature zones and fronts within the bin, complicating the estimation of aeration cycle completion times, particularly

in bins with varying airflow rates across different spatial locations. Moreover, multiple aerations are conducted for grain stored for periods exceeding one year. These prolonged and repeated aerations may result in over-drying or re-wetting of stored grain, thereby leading to a loss of grain quality.

Western prairie provinces in Canada produce canola that is almost 99% of the national production (Arnason, 2021). Canola is typically harvested after the harvesting of most of the cereal grains around late fall, but this may vary based on regions. The crop is either swathed or direct combined based on weather and canola conditions and sold directly to elevators or stored on-farm for better market value opportunity later.

Recently (2021–2023), due to adverse weather conditions, prairie farms experienced significantly lower yields due to low grain moisture. As per the Canola Digest (Whetter, 2023), the 2021 year was regarded as a drought year, resulting in a 51.8% fall in production from the 2020–2016 average. A similar effect persisted until 2023, especially in the southwest prairies, mostly impacting the southern Alberta growers. Canola stored in the bins was extremely dry with high temperatures, demanding fast and efficient aeration to limit potential hotspots due to respiration or insect development.

Although Alberta has diverse weather, as categorized based on grain growing regions, with the Peace region being the wettest and the south being the driest, these recent years were seen to be dry throughout the province. Canola harvested during the daytime would have a significantly higher temperature than the nighttime harvest. This difference could initiate moisture migration from warmer to colder areas of the bin. Friesen and Huminicki (1989) reported that significant migration could be observed at moisture levels as low as 8% (wb) when stored at high temperatures and not aerated properly. Meanwhile, in warm weather, it is not customary to operate the aeration fan continuously as that would create different layers of cool and warm front across the storage bins.

Singh et al. (2014) investigated the potential of automating in-bin drying of wheat grain in 14 locations of the western prairies. They found that initial grain moisture, airflow rate, operation start date, and ambient conditions were the critical factors affecting efficiency. Their study also showed that automation based on evolving grain conditions was more efficient than continuous fan operation in terms of cost and quality. The Canola Council of Canada (2018) suggested that cooling could be executed once the ambient temperature is 5 to 10°C below the temperature of the stored canola. However, this approach may require constant monitoring and could lead to over-wetting/over-drying of canola at the bottom of bins mostly during prolonged operation. Therefore, this study was conducted to analyze the effect of an automated fan control system on the aeration efficiency of stored canola in three farm bins with a capacity of 544 t. Furthermore, the effect of coring was analyzed on the rate of cooling of the stored canola.

MATERIALS AND METHODS

In-bin Storage System and Canola Conditions

Three fully perforated 544 t flat-bottomed bins (7 tiers/rings) at the Lethbridge College Research Farm were used. Approximately 1073 t of canola was received from a nearby farm to fill the three bins. The grain loading started from September 25 to 29, 2023. Bin 1 was filled with 264 t followed by 326 and 483 t in bins 2 and 3, respectively. Bins 2 and 3 had the same canola variety. All the bins were spout filled, generating a peaked configuration. Bin 3 was filled up to 7 rings (eave was at the top of 7^{th} ring), while bin 1 and 2 were filled up to 4 and 5 rings, respectively. Each bin had 7.5 kW low-speed centrifugal fan. The initial canola temperature and equilibrium relative humidity (r.h.) in bin 1 ranged from 21–24℃ and 61–68% r.h., respectively. In bin 2, the readings ranged from 20–24℃ and 50–71% r.h., while in bin 3, the readings ranged from 22–26℃ and 65–75% r.h.

Monitoring and Fan Control Systems

All bins were equipped with temperature and r.h. monitoring cables and automated fan control systems provided by OPI Systems Inc. (Calgary, AB). The cables were connected wirelessly to the fan control system over dedicated nodes and a gateway. This promoted the transfer of evolving grain conditions data to the cloud, executed processing to calculate moisture content (m.c.), and commanded the fan control system to operate accordingly. Bin 1 had four cables, including two r.h. and temperature cables and two temperature cables, respectively. Bin 2 and 3 had one r.h. and temperature cable and two temperature cables, respectively. The r.h. and temperature cable installed at the middle of bin 1 had eight sensors, while the rest of the cables in all the bins had seven sensors. The fan control system installed on each fan comprised a weather station, fan radio, a relay system that connects with the fan motor, and a plenum sensor that monitors pressure, temperature, and r.h. These components were linked to a fan node that, along with a cable node, communicates data over to the cloud using a gateway. The OPI Systems uses proprietary grain isotherm curves to determine canola moisture content using the measured temperature and r.h. In bin 1, the initial moisture content ranged from 7–8% (wb); in bin 2 and 3, it ranged from 6–8.4% and 8.3–9.2%, respectively.

Automated Aeration Control Strategy

The automatic aeration control mode was selected to execute cooling of the stored canola. To allow for rapid cooling, the desired grain temperature range was set at 5–15℃, and the moisture content range was set to 7.5–12% wb to allow for the controlled re-wetting of the dry grain received from the farm. As the grain dropped below 15℃ and the average moisture reached 10% at the end of October, the temperature range was decreased to 0–4℃, and the lower moisture limit was decreased to 5%. The hypothesis was to allow drying for any higher canola moisture levels within the 1.5 m height from the floor.

The control mechanism executed during the aeration process was that the fan would turn on when:

$$Ambient\ temperature + Fan\ warming < Average\ grain\ temperature - Grain\ temperature\ offset$$

and

$$7.5\%\ wb < Plenum\ equilibrium\ MC < 12\%\ wb$$

The control was set in such a way that the fan would shut off when the ambient temperature got below 0°C. The fan warming was opted to be 2°C for a 7.5 kW fan, and the offset temperature was set to vary from 3 to 1°C as cooling progressed in order to bring the grain temperature within the set limits.

Airflow Measurement and Coring Strategy

Airflow measurement was conducted using a conical funnel and an Omega wind vane anemometer (Model: HHF11A, Omega, St-Eustache, QC). The funnel amplified the airflow by a factor of 31.3:1, putting it in the range of the anemometer. Using a continuity equation, airflow from the surface of the canola bed was calculated at multiple points along the radius of the bin. Airflow in bin 1 and 2 was 0.33 and 0.26 $m^3min^{-1}t^{-1}$, respectively. Airflow from bin 3 was below the desired 0.1 $m^3min^{-1}t^{-1}$ with 483 t of canola; therefore, coring was executed. The grain was unloaded until the inverted peak was nearly coinciding with the eave. This resulted in 45 t of grain being transferred to bin 2. This was 9.3% of the total filled capacity. Panigrahi et al. (2021) also determined that around 9% grain of the total filled capacity should be cored to achieve desired airflow throughout the 1,000 t bin. Airflow measurement was conducted again after coring and was observed to be approximately 0.22 $m^3min^{-1}t^{-1}$.

Statistical Analysis

The temperature and moisture content data were statistically analyzed using a t-test assuming unequal variance at a significance level of 95% confidence interval.

RESULTS AND DISCUSSION

Canola stored in the three bins took different times to be cooled due to the differences in storage capacity. The fans for bins 1, 2, and 3 took 116, 148, and 175 h, respectively, to bring down the stored canola to 0–4°C. This observation was corroborated by the Friesen and Huminicki (1989) study which hypothesized that around 150–200 h could be expended to cool canola. Bin 1 showed a final temperature range of 1.5–2.2°C (Fig. 1); bin 2 showed a range of 2.1–4.4°C (Fig. 2); and bin 3 showed a range of 2.8–4.0°C (Fig. 3). However, the moisture profiles within the bins were not significantly different ($p>0.05$) between the initial and final values. Bin 1 showed a moisture profile of 6.6–7.7%; bin 2 had a moisture profile of 6.4–10.6%; and bin 3 had a moisture profile of 7.0–7.7%.

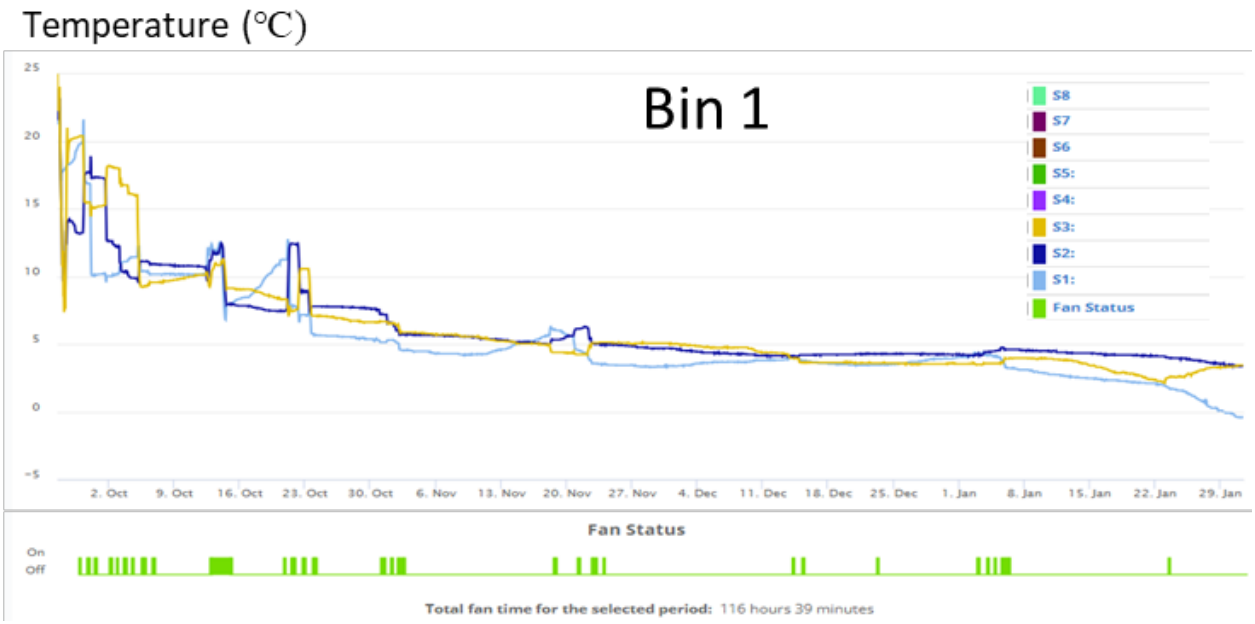

Fig. 1. Aeration profile for bin 1 stored canola.

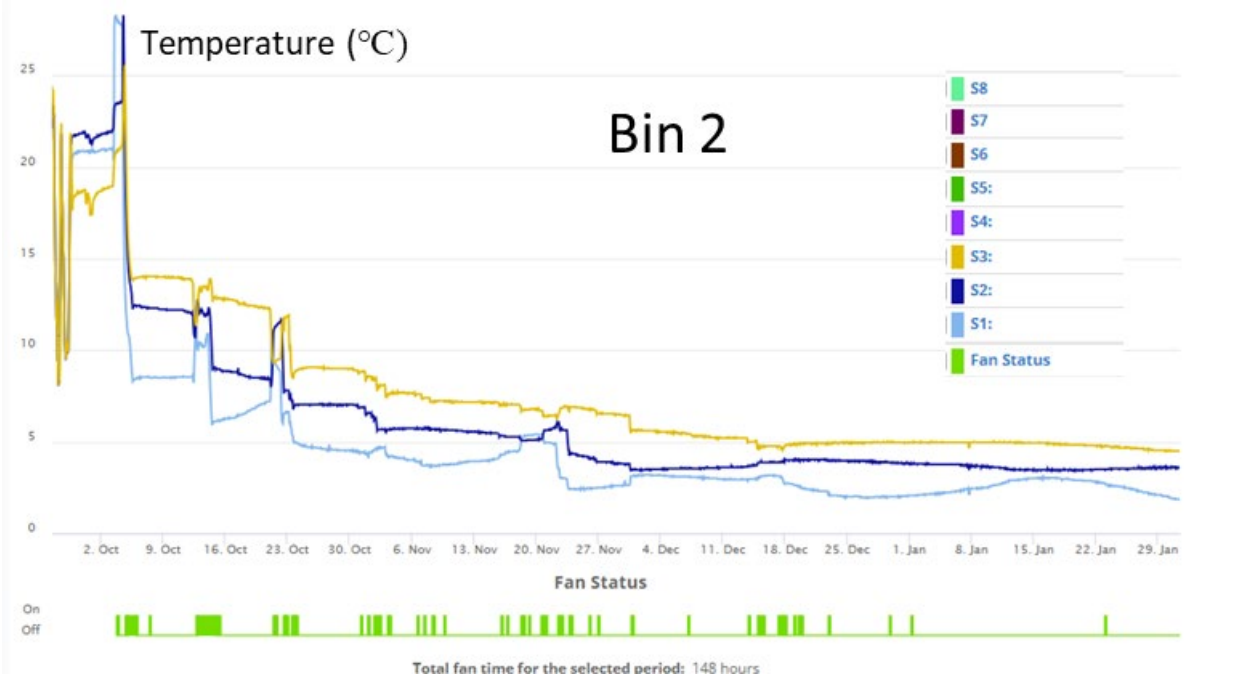

Fig. 2. Aeration profile for bin 2 stored canola.

Fig. 3. Aeration profile for bin 3 stored canola.

The consumption of fewer hours of fan use over the course of 4 mo shows the benefit of aeration automation in southern Alberta. This approach can be extrapolated to other adverse climatic regions to investigate the impact of automation in grain conditioning.

CONCLUSIONS

This study investigated the efficacy of automated fan control systems in cooling stored canola in farm-sized bins, alongside evaluating impact of coring on airflow. Results showed that the cooling times needed to reach the target temperature range of 0–4°C for bins 1, 2, and 3 were 116, 148, and 175 h, respectively. Moisture levels remained stable throughout. Coring notably improved airflow, enhancing cooling efficiency. These findings underscore the value of automated systems and coring techniques in optimizing aeration and preserving grain quality during storage. Further research can refine control strategies and assess long-term impacts, including economic viability.

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Paper No. CAF2024-A11

Doran GS (2024) Phosphine circulation in stored wheat in a thermosyphoning silo. Page 61. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Phosphine Circulation in Stored Wheat in a Thermosyphoning Silo

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ABSTRACT

Stored grain requires fumigation to prevent damage from pests, such as insects. A 50 t thermosyphoning silo was outfitted with gas sampling lines at three depths in the grain body and also in the silo headspace. Fifty tonnes of wheat were loaded and allowed to settle for 1 wk. The silo was sealed, pressure tested, and fumigated with aluminum phosphide tablets using an external, ground-level, fumigation chamber attached to the base of the thermosyphoning pipe. Phosphine concentrations were measured each morning using handheld meters. Once the minimum fumigation standards of 200 ppm for 10 d or 300 ppm for 7 d were met, the silo was vented using an integrated blower fan. This process was repeated three times in each calendar season, for a total of 12 fumigation cycles. Results showed that phosphine concentrations rapidly increased and tended to be slightly greater in the lower level of grain compared to other levels, due to a density of phosphine greater than air. Maximum concentrations in a cycle ranged from 400-800 ppm across the 12 fumigation cycles, and phosphine concentration standards were reached and exceeded in all 12 fumigation cycles throughout the year in 12.5 to 22 d, regardless of weather conditions. The silo remained sealed in the final fumigation cycle after the minimum fumigation standards were met, to characterize the sealing ability of the silo. Results showed the phosphine concentration did not fall below 200 ppm until 106 d after fumigation. These results demonstrate the ability of thermosyphoning to rapidly circulate fumigant throughout the grain body and maintain fumigant concentration for periods well in excess of Australian fumigation standards. The thermosyphoning silo also reduced the risk of explosion and improved operator safety by allowing ground-level fumigation to occur.

Keywords: Thermosyphoning, Phosphine, Silo, Grain, Storage

Paper no. CAF2024-A12

Buenavista RM, Casada ME, Phillips TW, Siliveru K (2024) Sorption kinetics and equilibrium isotherms of phosphine gas into wheat kernels. Page 62. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Sorption Kinetics and Equilibrium Isotherms of Phosphine Gas into Wheat Kernels

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ABSTRACT

Phosphine (PH_3) is the most used fumigant in the U.S. due to its low price, ease of use, and wide accessibility. With the growing concerns of phosphine-resistant insect pests, the sustainability of PH_3 as an effective fumigant has been put at risk. Sorption equilibrium data are critical for improving the accuracy of modelling studies for phosphine fumigation systems and would clarify PH_3 uptake and sorption capacity of the fumigated products. The objectives of this study were to determine the effect of initial concentration (from 400 to 2400 ppm) on the equilibrium concentration of PH_3 in wheat kernels and cumulative and daily PH_3 sorption through time. Kinetic data showed the sorption process was time-dependent and occurred in two phases—an initial faster adsorption phase followed by a slower sorption rate as the grain and PH_3 reached equilibrium. Pseudo-first and pseudo-second order models were fitted to PH_3 concentrations versus time experimental data. The pseudo-first order model provided better equilibrium estimates and was used for the sorption isotherm analysis. Langmuir, Freundlich, and Redlich-Peterson sorption isotherm models were fitted to the plot of equilibrium headspace gas concentration versus sorbed PH_3 quantity. All three models had low standard errors of prediction (0.46-0.47). These PH_3 sorption kinetics and values of total sorbed quantity at equilibrium are valuable for predicting rate and maximum quantity of PH_3 uptake in wheat.

Keywords: Phosphine, Sorption Isotherm, Wheat, Fumigation, Kinetics

Paper No. CAF2024-A13

Fukazawa N, Takahashi R (2024) Development of effective fumigation parameters for the control of phosphine-resistant *Lasioderma serricorne* (F.). Page 63. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Development of Effective Fumigation Parameters for the Control of Phosphine-resistant *Lasioderma serricorne* (F.)

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ABSTRACT

The cigarette beetle, *Lasioderma serricorne* (F.), is the most economically significant pest of stored tobacco. For many years, this pest has been successfully managed via phosphine fumigation. However, fumigation failures have been reported in various parts of the world, possibly because of the development of phosphine resistance. Recently, we collected *L. serricorne* individuals from a tobacco warehouse in Malaysia where fumigation failures had been frequently observed. We experimentally confirmed that the resistant population was able to survive the industry-recommended fumigation protocol (6 d of exposure to 600 ppm phosphine at 25°C). This study was conducted to develop phosphine fumigation parameters for the control of highly resistant populations. Fifty individuals at the larval stage, which was determined to be the most resistant stage to phosphine, were exposed to a range of phosphine concentrations (200–2,000 ppm) at 25, 30, and 35°C for 7 d. Four weeks after exposure, their viability was assessed. The concentration of phosphine required to achieve 99% lethality (LC_{99}) was then calculated. The LC_{99} values were 1,436, 959, and 683 ppm at 25, 30, and 35°C, respectively. To confirm the efficacy of treatment at the LC_{99} levels, mixed-age populations containing all stages were exposed to 1,500, 1,600, and 1,700 ppm phosphine at 25°C; 1,000, 1,100, and 1,200 ppm phosphine at 30°C; and 700, 800, and 900 ppm phosphine at 35°C for 7 d. The results showed that 7 d of exposure to 1,700, 1,100, and 700 ppm phosphine was required for the complete control of this highly resistant *L. serricorne* population at 25, 30, and 35°C, respectively. The results of this study would aid future revisions of the industry-recommended phosphine fumigation protocol.

Keywords: Cigarette beetle, Phosphine resistance, Phosphine fumigation, *Lasioderma serricorne*, Insecticide

Paper No. CAF2024-A14

Morokuma D, Fukazawa N, Takahashi R, Shinjo A, Hamano K, Imai T (2024) Identification of phosphine resistance-related gene mutations in the cigarette beetle, *Lasioderma serricorne* (F.). Page 64. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Identification of Phosphine Resistance-related Gene Mutations in the Cigarette Beetle, *Lasioderma serricorne* (F.)

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ABSTRACT

The global increase in insecticide-resistant insects is one of the major challenges associated with the management of insect pests, including pests of agricultural crops and stored products. The cigarette beetle, *Lasioderma serricorne* (F.), is one of the most economically serious pests of stored tobacco. Phosphine fumigation has long been used to control this pest in the tobacco industry. However, the development of phosphine resistance in the cigarette beetle has become a major problem in recent years. A laboratory strain of the cigarette beetle was collected in a tobacco warehouse in Malaysia, and this strain showed high phosphine resistance, including the ability to survive the industry-recommended fumigation protocol for resistant beetles. The objective of this study was to identify genetic mutations related to the strong phosphine resistance of the strain. In other insect species, mutations in two genes (*rph1: Cyt-b5-r* and *rph2: DLD*) are responsible for resistance to phosphine. We analyzed the sequences of these two genes in cigarette beetle strains with different levels of phosphine resistance. Three mutations in *rph1* and *rph2* (*rph1*Q130E, F301C, and *rph2* N505Y) specific to the highly resistant strain were detected. The relationship between these mutations and the level of resistance was evaluated using fumigation tests and forward genetics. The *rph2* N505Y mutation, a recessive homozygous trait, was shown to contribute significantly to the high phosphine resistance of this cigarette beetle strain.

Keywords: Cigarette beetle, *Lasioderma serricorne*, Phosphine resistance, Insecticide

Paper No. CAF2024-A15

Miao S, Lu Y, Xing Y, Ren Y (2024) Characterization of dihydrolipoamide dehydrogenase gene (DLD) from Liposcelis entomophila (Enderlein) a marker for strong phosphine resistance diagnosis. Page 65. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Characterization of Dihydrolipoamide Dehydrogenase Gene (DLD) from *Liposcelis entomophila* (Enderlein) a Marker for Strong Phosphine Resistance Diagnosis

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ABSTRACT

In past decades, widely spread and strong phosphine (PH_3) resistance has been detected in many major stored grain insect pests, including *Rhyzopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae*, and *Cryptolestes ferrugines.* However, what should not be neglected is that minor and tiny psocids seem to have developed strong PH_3 resistance, which is becoming increasingly serious in tropical and subtropical countries. Psocids are too small for detecting and monitoring PH_3 resistance, and traditional bioassay methods are not well suited for them as for other beetles. Thus, it is urgently needed to develop a rapid PH_3 resistance detection method. Previous studies found a core metabolic enzyme Dihydrolipoamide Dehydrogenase (DLD) mediated resistance to PH_3 in *T. castaneum* and *R. dominica*. Later, rapid PH_3 resistance test by molecular biology methods were developed based on this theory. Hence, in this study, the DLD gene (*LeDLD*) was characterized in *Liposcelis entomophila*, and the SNP of *LeDLD* was also analyzed. Also, PH_3 resistance of *L. entomophila* from the areas with serious occurrence of resistance in south China were investigated. It was found that 8 strains of *L. entomophila* had developed strong PH_3 resistance, the average mortality of the 8 strains was below 30% under a strong resistance discriminate dose (180 ppm for 20 h), and the strongest resistance was LeZJ strain, with an average mortality of 8.7%. By analyzing the polymorphism of the DLD gene, it is found that N505H was the most prevalent mutation site of LeDLD in resistant strains, and the strong PH_3 resistance of *L. entomophila* was highly correlated with this mutation. In conclusion, *L. entomophila* had developed strong PH_3 resistance, and the mutation of DLD closely related to PH_3 resistance of *L. entomophila*, which might develop a potential molecular biomarker for rapid PH_3 resistance diagnosis.

Keywords: Phosphine (PH_3), Resistance, Booklice, Psocid, Gene mutation, Dihydrolipoamide Dehydrogenase (DLD)

Paper No. CAF2024-A16

Wang K, Tang P (2024) Insight into the molecular mechanism of phosphine toxicity provided by functional analysis of *cytochrome b5 fatty acid desaturase* and *dihydrolipoamide dehydrogenase* in red flour beetle, *Tribolium castaneum*. Page 66. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Insight into the Molecular Mechanism of Phosphine Toxicity Provided by Functional Analysis of *Cytochrome b5 Fatty Acid Desaturase* and *Dihydrolipoamide Dehydrogenase* in Red Flour Beetle, *Tribolium castaneum*

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ABSTRACT

Phosphine is the dominant chemical used in postharvest pest control. Widespread and highly frequent use of phosphine has caused high resistance in many pest insects, including *Tribolium castaneum*. Lipid peroxidation and reactive oxygen species (ROS) are two major factors determining phosphine toxicity; however, the mechanisms of production of these two factors in phosphine toxicity are still unknown. Here, we first determined the time course of phosphine-induced lipid peroxidation and ROS production in *T. castaneum*. Our results showed that lipid peroxidation occurred before ROS in the process of phosphine toxicity, and fumigated beetles with higher resistance levels were associated with weaker activity on lipid peroxidation and ROS. A significant decline in lipid peroxidation was observed in fumigated individuals after knockdown of *cytochrome b5 fatty acid desaturase* (*Cyt-b5-r*) via RNA interference (RNAi), indicating that *Cyt-b5-r* was critical for triggering phosphine-induced lipid peroxidation. Moreover, significant decreases in both ROS and mortality were detected in fumigated *T. castaneum* adults fed melatonin for 7 days, an inhibitor of lipid peroxidation. *Cyt-b5-r* RNAi also inhibited ROS production and mortality in phosphine-treated beetles. Meanwhile, a significant decrease in ROS production (68.4%) was detected in *dihydrolipoamide dehydrogenase* (*DLD*) knockdown individuals with phenotypes susceptible to phosphine, suggesting that lipid peroxidation initiated ROS with the expression of *DLD*. However, a significant increase in ROS (122.1%) was detected in the *DLD* knockdown beetles with strongly resistant phenotypes, indicating that the *DLD*-involved pathway might not be the only mechanism of ROS generation in phosphine toxicity and the existence of a moonlighting role in downregulating ROS in strongly resistant *T. castaneum*.

Keywords: *Tribolium castaneum*, Phosphine toxicity, Resistance genes, RNAi, ROS

Paper No. CAF2024-A17

Khan SA, Saeed M, Shah JA (2024) Bioassay for detection of phosphine resistance in major storage insects. Page 67. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Bioassay for Detection of Phosphine Resistance in Major Storage Insects

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ABSTRACT

This study aimed to determine the level of phosphine resistance in four major storage insect pests, *Sitophilus granarius*, *Sitophilus oryzae*, *Tribolium castaneum*, and *Lasioderma serricorne*, collected from Khyber Pakhtunkhwa, Pakistan. Twenty beetles from the selected insects were exposed to phosphine gas concentration for five different time intervals, ranging from 1 h to 48 h. The probit analysis was performed to determine the LC50 level. Sitophilus oryzae was the most resistant species after 1 h with an LC50 of 3.769 ppm. *Lasioderma serricorne* was the most resistant insect after 3 h, with an LC50 of 3.706 ppm. The 95% confidence intervals for all four species do not overlap, suggesting that their LC50 values are not significantly different within the provided intervals. After 24 h of exposure, *S. oryzae* had the highest level of resistance, followed by *L. serricorne* and *S. granarius*. *Tribolium castaneum* had the lowest resistance level, with an LC50 value of 3.294 ppm. When the insects were exposed for 48 h, *S. oryzae* was the most resistant insect species, followed by *L. serricorne* and *S. granarius*. *Tribolium castaneum* showed the lowest level of resistance (3.181 ppm) to phosphine gas. In conclusion, the study suggests that phosphine application alone may not be an adequate and sustainable control approach for the management of these insect pests in Pakistan.

Keywords: Fumigation, Phosphine resistance, *Lasioderma serricorne*, *Sitophilus granarius*, *Sitophilus oryzae*, *Tribolium castaneum*, Confidence intervals

Paper No. CAF2024-A18

Jagadeesan R, Dembowski B, Warwick C, White B, Nayak M, Biggs I (2024) Combating pest and resistance in the tropics: a supply chain approach and its implications for the industry. Page 68. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Combating Pest and Resistance in the Tropics: a Supply Chain Approach and Its Implications for the Industry

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ABSTRACT

The warm and humid climate of the tropics favors higher pest multiplication and insect infestations, threatening the safe storage of food grains. While the industry relies on chemical treatments, lacking region-specific information could cause treatment failures, jeopardizing the country's food biosecurity and market access. Considering this, a comprehensive research project was rolled out across the tropical belt of northern Australia, addressing four major components: [1] pest and resistance profiling, [2] understanding the movement of insect pests (ecology), [3] establishing effective pest intervention strategies, and [4] ensuring the delivery and adoption of key findings. Overall, the project aimed to design and deliver integrated pest and resistance management (IPRM) practices for the region.

Two hundred samples, representing selected grain-growing regions across tropical northern Australia were collected and screened for insect pests and their resistance to fumigants and other supporting treatments. Results indicated that the red flour beetle, *Tribolium castaneum* (Herbst) is the predominant pest species followed by the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), and the lesser grain borer, *Rhyzopertha dominica* (Fabricius). Resistance to phosphine was prevalent among these species; however, no substantial variation in susceptibility to sulfuryl fluoride was observed. The observed infestation and resistance trends differed from the temperate or sub-tropical regions of Australia, indicating that tropical environments play a critical role in pest and resistance development in this region. Mass trapping of insects also confirmed the intense insect movement within the grain supply chain and warrants the timely need for implementing information-based IPRM practices. Validation of these practices in collaboration with the industry is progress to ensure practice change and a knowledge-based transition for the region.

Keywords: Tropics, Stored grains, Insect pests, Resistance, Integrated management, Fumigants and insecticides

Paper No. CAF2024-F19

Götze M, Gourgouta M, Maria K., Sakka MK, Athanassiou CG (2024) When reality kicks in: The challenge of transferring knowledge from laboratory trials to real treatments with phosphine. Pp. 69–76. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

When Reality Kicks in: The Challenge of Transferring Knowledge from Laboratory Trials to Real Treatments with Phosphine

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ABSTRACT

Phosphine is the most abundant fumigant used in stored-product protection worldwide. Its main advantages are numerous, ranging from various application areas to high efficacy against all life stages of major postharvest pests. While the use of all fumigants implies certain challenges, phosphine in all its forms is still a fairly easy-to-handle substance in the hands of professionals. With arising claims of decreased susceptibility having spread around the world over the last decade, one key question has concerned stakeholders: can phosphine still be successfully used as a valid option in integrated pest management systems or as part of quarantine treatments?

In ongoing studies, we evaluated the effectiveness of phosphine against eggs in various stored-product insect species, i.e., the red flour beetle, *Tribolium castaneum* (Herbst); the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.); the lesser grain borer, *Rhyzopertha dominica* (F.); the confused flour beetle, *Tribolium confusum* Jacquelin du Val; and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). For each species, populations with different levels of phosphine susceptibility were used; additionally, two different egg ages of 1 and 2 d old were investigated. Furthermore, different concentrations ranging between 50 and 2,000 ppm with various exposure times were applied. Moreover, some of these populations were tested in "real-world" applications with phosphine. Fumigations were conducted in different storage facilities (e.g., containers and silos), and phosphine concentration was constantly monitored with phosphine sensors. The preliminary data showed egg hatching was decreased with the increase of the concentration and the exposure interval. In most of the "real-world" applications, complete mortality was achieved for all species, life stages and strains, regardless of their susceptibility to phosphine. This study indicates that proper handling of this fumigant can still achieve sufficient insect control.

Keywords: Phosphine, Eggs, Egg hatching, Dose response, Fumigation, Tolerance

INTRODUCTION

Phosphine is a highly efficacious and broadly accepted substance in curing stored commodities from insect infestation. The application can be done via various metal phosphide formulations or directly through inlet of the gaseous form. It is also accepted as a quarantine measure in multiple countries, enabling trade even when sensitive species are apparent on a certain commodity. Raw or simply processed cereals and pulses represent the largest area of application. Even though the handling and application can be done safely and with great results, application practices and regulation vary highly on a global basis. This, coupled with a heightened interest in investigating stored-product protection measures have led to major concerns about phosphine being a feasible solution to insect infestation in the diverse field of commodities and storage types. Uncovering changes in phosphine susceptibility have been part of past and ongoing research projects, focusing in laboratory evaluation of the tolerance and resistance status of stored-product insects.

So far, most methods, which are widely accepted and can mostly be used both in the field and in the lab, have had a focus on investigating the adult stage. This has two major advantages: this developmental stage has a high grade of visibility through its mobility and it can be identified much better by experienced personnel responsible for protection measures. Unfortunately, experience and feedback show that most problems in storages do not occur by the unsuccessful erasure of adults but rather by the delayed appearance of newly hatched adults from the egg stage a couple of weeks after a treatment. The egg stage has been widely accepted to be the most problematic life stage to be caught by a fumigation with phosphine (Bell et al., 1984; Rajendran and Muthu, 1991; Bell and Wilson, 1995; Nayak et al., 2020). Bearing the past research results in mind which show elevated tolerance and resistance levels of stored-product insects, the question arises of whether this explicit reaction to exposure is:

a) also expressed in the egg stage,
b) and if yes, how this influences actual treatments.

This research is still ongoing at the University of Thessaly, which is conducting various real-world treatments with different life stages, species, and susceptibility levels against phosphine.

OUTLINE AND METHODS OF THE TRIALS

Breeding and Preparation of Lab Tests

The test insects utilized in the present study were reared in the Laboratory of Entomology and Agricultural Zoology (LEAZ), within the Department of Agriculture, Crop Protection, and Rural Environment at the University of Thessaly. They were maintained under constant conditions of 25 ± 0.1°C and 65 ± 0.5% relative humidity (r.h.) with 24 h of darkness. *Tribolium castaneum* and *T. confusum* strains were bred in wheat flour, while *Rhyzopertha dominica* and

Oryzaephilus surinamensis strains were reared in whole wheat kernels and oat flakes (Quaker Oats Company, Chicago, Illinois, USA), respectively.

Each species was represented by a susceptible (unexposed to any chemical treatments) and a phosphine-resistant strain. The susceptible strains, originally sourced from Greece, have been under maintenance at LEAZ for over two decades (Athanassiou et al., 2019). The resistant strains included *T. castaneum* TC931, which traces its origins to Australia (Sakka et al., 2020, 2024), as well as *R. dominica* GA6, originating from Greece (Sakka et al., 2024). Additionally, *O. surinamensis* Roca Defisan, originally from Spain, and *T. confusum* GA12, originating from Greece (Agrafioti et al., 2019) were utilized.

To obtain 1-day-old eggs for each species (*T. castaneum*, *T. confusum*, *O. surinamensis*, and *R. dominica*), a stock culture of 100 adults was utilized. Each group was placed in separate 500 mL jars containing finely sieved flour. These jars were then incubated at 26ºC and 65% r.h. for 24 h. After this incubation period, the adults were removed from the jars and the 1-day-old eggs were collected using a 212 µ sieve. For the 2-day-old eggs, a similar process was followed. However, after placing the adults in the jars with flour and incubating them for 24 h, adults were removed as before. Then, the eggs were left for an additional 24 h before being collected using the same 212 µ sieve.

Experimental Setup for Lab Scenarios

The eggs were subjected to various concentrations of phosphine gas, including 0 ppm as the control, as well as 50, 200, 500, and 1,000 ppm. Different exposure times of 2.5, 5, and 7 d were employed. Each set of eggs was delicately placed in glass vials measuring 1.3 cm in diameter and 4 cm in height, along with a small quantity of flour using a fine paintbrush. Subsequently, these vials were carefully transferred into separate compartments within airtight 1 L glass jars, with each combination of concentration, exposure interval, and age of eggs having its designated vial. To generate the phosphine gas, a 5 L plastic canister was utilized, containing 2 tablets and 50 mL of water in accordance with the Phosphine Tolerance Test (Detia Freyberg GmbH, Laudenbach, Germany). Phosphine concentration was measured with quantitative gas chromatography (GC) using a GC-2010Plus (Shimadzu, Kyoto, Japan) instrument equipped with a GS-Q column (30 m long × 0.25 mm i.d., 0.25 µm film thickness; MEGA S.r.l., Milan, Italy) and a flame photometric detector set into phosphorous mode as suggested by Cato et al. (2017) and also using glass tubes (Draeger 25A; Draeger Safety AG & Co., Lübeck, Germany). Using a syringe, the appropriate volume of gas was injected into the 1 L glass jars housing the vials with the eggs. The experiment encompassed three replicates, each with three sub-replicates, resulting in a total of 9 vials for each combination of concentration, exposure interval, and egg age. Post exposure, survival rates (in terms of egg hatching) were recorded separately at 7 and 14 d for each combination of species, concentration, exposure time, and egg age.

Field Trials

Seven trials were carried out in 2023 at different storage facilities (warehouses, containers, silos) in Greece. The study aimed to utilize insect cohorts of all susceptible and resistant strains for each of the trials; however, in some cases, certain strains could not yield adequate numbers for testing, so they were not used. For each species and population, insects were placed in cylindrical plastic vials (3 cm in diameter, 8 cm in height, Rotilabo Sample tins Snap on lid, Carl Roth, Germany). The vials were perforated in the upper part for facilitating air flow. The day before phosphine fumigation, ten individuals were taken from the cultures and placed in vials and left in incubators set at 25°C and 65% r.h. (different vials for each insect species and strain). In each vial, 1 g of diet was placed. White flour was used for *T. castaneum*, and *T. confusum*, *E. kuehniella*, and *P. interunctella*; wheat kernels for *S. oryzae*, *T. granarium*, and *T. varabile*; oat flakes for *O. surinamensis*; and maize kernels for *P. truncatus*. Vials with insects were placed in different locations near phosphine sensors within the storage facility. For each species and strain, three different vials (replicates) were used. A separate series of replicated vials from corresponding strains for each insect species was maintained outside of the treated area and served as control. Phosphine concentration was measured by the use of wireless sensors provided by Centaur Analytics (Centaur Analytics Inc., CA, USA) (Agrafioti et al., 2020).

After the termination of each fumigation, the vials were transferred to the laboratory and mortality was evaluated for the mobile life stages (adults and larvae). For eggs and pupae, the hatching or emergence rate was examined respectively, 7 d following the termination of the exposure period. In the case of vials containing adults, after recording adult mortality, all individuals were removed from the vials, and the vials were kept in incubators at the conditions above for an additional period of 65 d. After this incubation period, the vials were opened and progeny production was recorded.

RESULTS

Lab Trials

In general, the findings of the lab trial suggest a higher susceptibility of 2-day-old eggs, irrespective of their pre-defined sensitivity against phosphine. As observable in Fig. **1** displaying the results of resistant strain treatments, a set-up of >200 ppm from 5 d of exposure led to no hatching in the post exposure observation. For the 1-day-old eggs of *R. dominica* and *T. castaneum*, a 7 d exposure did not result in full control at the lowest concentration tested (50 ppm).

Overall, every set up resulted in no survival of normally susceptible strains (Fig. 1).

Fig. 1. Overall assessment of hatched eggs in reaction to the exposure scenarios in both susceptibility statuses of *T. castaneum*, *O. surinamensis*, *R. dominica*, and *T. confusum*

Field Trials

Due to differing availability of species in the LEAZ laboratory, a divergent selection of species was used to investigate their behavior in real fumigations. These treatments were highly variable regarding storage object, commodity type, fumigation duration (3–21 d), product used, and application rate. All in all, most of the selected strains showed a very low to no survival rate in all tested developmental stages. If survivors were apparent, it was the egg stages of resistant populations in the test (2) (Fig. 2). Thus, the results of the lab trials were proven to be representative of behavior against phosphine in the field.

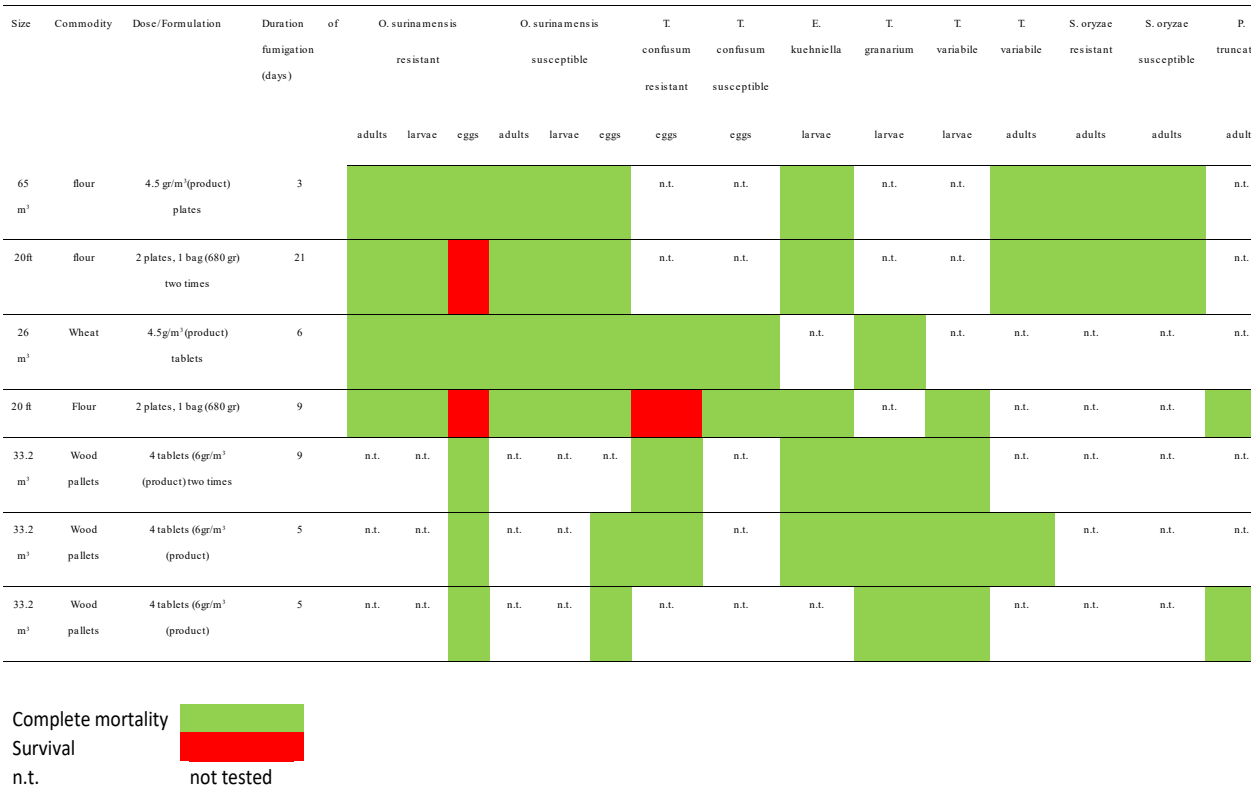

Fig. 2. Evaluation of various field trials conducted in Greece, 2023. Red field displays scenarios with surviving insects, while green field represents complete mortality. Fields marked with n.t. display species and life stages not tested in the scenario.

Unfortunately, field trials with phosphine, even though it is a treatment conducted indoors, include the uncertainty of attaining a successful fumigation. Gas tightness, monitoring, dosage, commodity type, and exposure time, as well as environmental conditions, all have a high extent of influence on the quality of the treatment. While laboratory trials can be ultimately controlled, this is most likely not the case for the broadest application areas in postharvest storages. This results in highly varying concentration levels (Fig. 3) risking efficacy of the treatment and increasing the probability of failures (Fig. 4).

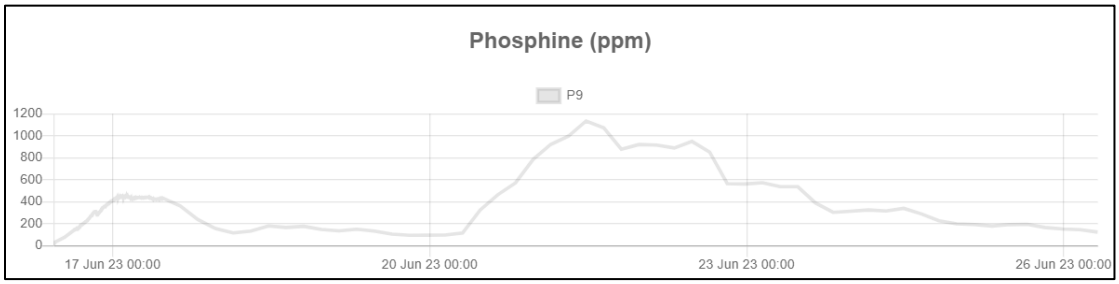

Fig. 3. Treatment of container containing wood pellets in Greece, 2023, monitoring data of phosphine in ppm over 9 d exposure period; successful treatment (no surviving life stages found).

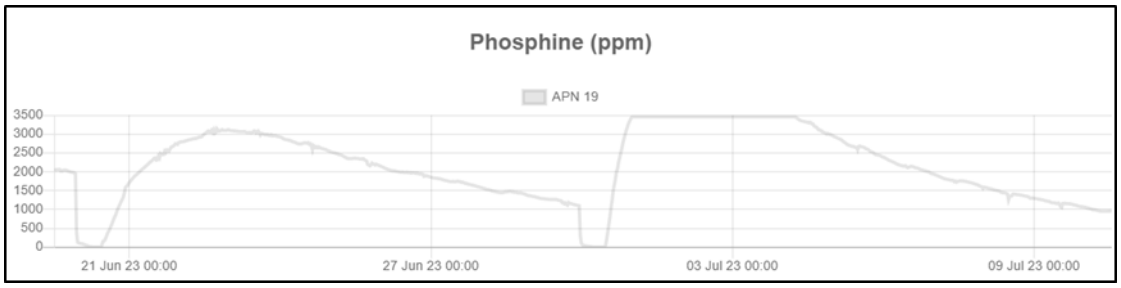

Fig. 4. Treatment of a container containing bagged flour in Greece, 2023, monitoring data of phosphine in ppm; treatment was not successful (alive eggs of resistant *O. surinamensis* and *T. confusum*).

CONCLUSIONS

Phosphine fumigations are not yet at risk of being discontinued as an overall application type, but phosphine should be considered carefully and only be used as a last resort. In that particular connection, the quality of the treatment determines its destiny. Laboratory trials have proven to be a good representation of the worst-case scenario, and eggs do exhibit the decreased susceptibility status noted in hypotheses. Concentration levels over 200 ppm and a 5 d exposure period are benchmarks for laboratory scenarios. However, it should be considered carefully as to whether these benchmarks should be used in actual treatments, as achieving these benchmarks is highly unlikely due to the various influential factors during fumigations.

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Paper No. CAF2024-A20

Ignacio MCCD, Maier DE (2020) Development of decision support tool for predicting hermetic bag performance during grain storage. Page 77. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Development of Decision Support Tool for Predicting Hermetic Bag Performance during Grain Storage

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ABSTRACT

Hermetic technology is a popular alternative and chemical-free storage method because it effectively controls insect activity in stored grain. It works under the principle of a bio-generated modified atmosphere where oxygen (O_2) concentration dramatically decreases. At the same time, carbon dioxide (CO_2) levels proportionally increase in the stored product enclosed within an airtight gas barrier. Numerous studies have demonstrated that hermetic storage bags effectively inhibit insect infestation, thus preserving the quality of grains. What has not been evidenced in most studies is the effect of initial insect infestation level (insects/kg grain) and barrier property on how quickly the change in bio-modified atmosphere is established. This study developed a decision support tool that predicted O_2 depletion in hermetic storage bags as a function of insect and grain kernel respiration and liner O_2 transmission rate (OTR). Results confirmed that insect respiration dominated O_2 depletion in maize stored at safe storage moisture contents of 13–14%, while grain respiration was negligible. Bags with low OTR liners reduced O_2 below 5% to asphyxiate adult insects in maize (13–15% MC w.b.) 2 to 14 d faster than bags with high OTR liners at low initial infestation levels (2 adults of *S. zeamais*/kg) stored at 27–33°C. Moreover, high initial insect infestation (10 adults of *S. zeamais*/kg) reduced oxygen levels inside bags in less than 30 d, regardless of the OTR value of a gas barrier liner.

Keywords: Decision support tool, Grain Storage, Hermetic bag, Insect infestation, Oxygen, Respiration

Paper No. CAF2024-A21

Zhao Q, Du X, McKirdy S, Ren Y, Zhan G (2024) Radioprotective effects on late third-instar larvae of melon fly (Diptera: Tephritidae) irradiated under low-oxygen atmospheres. Page 78. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Radioprotective Effects on Late Third-Instar Larvae of Melon Fly (Diptera: Tephritidae) Irradiated Under Low-Oxygen Atmospheres

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ABSTRACT

Ionizing radiation creates free radicals, and its efficacy is enhanced by the presence of oxygen (O_2). Modified Atmosphere Packaging (MAP) is employed to preserve the quality and extend shelf-life of fresh fruits and vegetables. However, low- O_2 level can induce radioprotective effect for insects compared with irradiation in ambient air. Therefore, there is a need to determine the critical O_2 levels that can result the radioprotective effect. In this study, the late third-instar larvae of melon fly, *Zeugodacus cucurbitae* (Coquillett), were reared on pumpkin fruits, and put into MAP bags filled with low-oxygen air (ranging from 0 to 5% O_2, balanced with nitrogen) or ambient air. The bags were then exposure to X-ray irradiation at dose varied from 16 to 100 Gy with 12 Gy increments, and efficacy was assessed based on the emerged adults. Dose-response data on mortality underwent analysis through two-way analysis of variance (ANOVA) and probit analysis. The variance of radio-tolerance was significant in 0% O_2 atmospheres according to two-way ANOVA. Consequently, the 95% confidence limits of lethal dose ratios at LD_{90} and LD_{99} were utilized to identify significant differences between treatments at individual O_2 levels. The distinctions of radio-tolerance were observed at 0, 1, 2, and 3% O_2 levels but insignificant at 4 and 5% O_2 levels and ambient air. The critical threshold of the radioprotective effects for *Z. cucurbitae* larvae was determined to be 4% O_2 level. An estimated maximum radiation dose of 15 Gy was proposed to compensate for this effect during phytosanitary irradiation treatment (probit-9 mortality). Furthermore, we are currently investigating the mechanism of irradiation under low-O_2 levels induces radio-protective effects.

Keywords: Irradiation, Modified atmospheres, Low oxygen radiation, Radioprotective effects, Critical threshold, Antioxidant enzymes

Paper no. CAF2024-A22

Mompremier R, Bern C, Bowers E, Brumm T, Maier D (2024) Field testing of Purdue Improved Crop Storage (PICS) bag for maize storage in Haiti. Page 79. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Field Testing of Purdue Improved Crop Storage (PICS) Bag for Maize Storage in Haiti

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ABSTRACT

Maize is widely grown by Haitian farmers and its sale is the only income for some. The average farmer produces less than 100 kg per year and postharvest losses average 30%. Purdue improved crop storage (PICS) bags use hermetic storage to decrease postharvest losses in grain stored on smallholder farms. Our objective was to test PICS bags for long-term, on-farm storage of maize in Haiti. Three each of 50 kg PICS bags and control bags (pre-used polypropylene rice or bean sacks) were tested. Bags were each loaded with 50 kg of maize and then stored without opening for 170 d. Data recorded before and after storage included live maize weevil counts, aflatoxin levels, maize moisture contents, and bag weights. Live weevil counts in the PICS bags did not change significantly from the initial five weevils/kg maize but increased significantly from five to 199 weevils/kg in the control bags. Aflatoxin levels were mostly <3 ppb before and after storage. No maize moisture changes were significant. Average weight of PICS bags did not change significantly, but control bags, on average, lost 5% (2.5 kg) of weight, which was significant. The PICS bags effectively protected maize over 170 d, while control bags allowed unacceptable maize weevil infestation and weight loss.

Keywords: Purdue Improved Crop Storage (PICS) bags, Maize storage, Haiti, Aflatoxin, Postharvest loss

Paper No. CAF2024-A23

Jie Y, Shi T, Yang D (2024) Application of controlled atmosphere agents and technology in insect control and grain quality preservation. Page 80. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Application of Controlled Atmosphere Agents and Technology in Insect Control and Grain Quality Preservation

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ABSTRACT

The innovative development of green grain storage technology is a significant research topic in the field of grain storage, with controlled atmosphere storage being one of its methods. Controlled atmosphere agents and technology regulate the gas composition and relative humidity within a sealed space, achieving pest and mold prevention and extending the shelf life of grains. These agents can rapidly reduce oxygen levels, lowering the oxygen concentration in a limited sealed space to below 0.5% within 3 d and maintaining this low-oxygen environment over the long term. Additionally, these agents can keep the relative humidity within the stack at 70%. During a storage period of 480 d, this technology can limit grain moisture content loss to below 0.1 percentage point and slow the increase in rice hardness, adhesiveness, and chewiness, thereby preserving its original texture.

Keywords: Controlled atmosphere agents, Pest prevention, Control quality

Paper No. CAF2024-F24

Bartosik R, Cardoso ML, de la Torre D, Abadía, MB, Maciel G (2024) Implementing CO_2-based controlled atmosphere treatments in big bags with inexpensive liners. Pp. 81–88. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Implementing CO_2-Based Controlled Atmosphere Treatments in Big Bags with Inexpensive Liners

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ABSTRACT

The production of specialty grains such as quinoa, amaranth, teff, popcorn, peanuts, and different organic crops involves meeting specific safety requirements, including the necessity to be free of insecticide residues. Consequently, in recent years there has been a growing interest in alternative controlled atmosphere (CA) treatments. On the other hand, market opportunities have required the adaptation of CA application to big bags and raffia containers typically holding 1 m^3 of product. Normally, the CA treatment system consists of a gas application system (CO_2 or N_2), an internal polyethylene bag with specific design and gas barrier properties, and a closure via heat sealing. However, these systems can prove costly, limiting their usability for numerous products. The purpose of this experiment was to assess the feasibility of implementing a CA treatment (with CO_2) through the design of simpler and more cost-effective technologies, aiming to expand the user base of CA treatments. The experiment involved analyzing the effectiveness of a simple and economical polyethylene bag (70 microns, without a gas barrier) in combination with two closure systems: a simple one (twisting-folding-knot) versus the control system (heat-sealing). The control condition was established when a concentration × time product (Ct product) of 12,000 %h was reached (minimum allowed concentration of 40%). The initial injection created an internal atmosphere of 90% CO_2. Overall, it was observed that treatments with a heat-sealed closure achieved satisfactory control conditions with a single initial injection, reaching the target Ct product while consistently maintaining the CO_2 concentration above 40%. In contrast, treatments with the knot-closure method did not ensure adequate sealing, requiring gas reinjections in some cases to achieve the control condition. In conclusion, this study demonstrates the feasibility of implementing a successful CA treatment in raffia big bags using low-cost polyethylene liners. However, it is crucial to employ the heat-sealed closure system to ensure the efficacy of the treatment.

Keywords: Specialty grains, Controlled atmosphere, Carbon dioxide, Big bag, Liners

INTRODUCTION

Argentina boasts a large expanse of organic agricultural land (Research Institute of Organic Agriculture FiBL, 2021), encompassing approximately 100,000 ha dedicated to crops. Among these hectares, 33% are specifically dedicated to the cultivation of cereals and oilseeds (SENASA, 2022). This cultivation encompasses a variety of crops, including traditional cereals such as wheat, corn, barley, and oats, as well as oilseeds like sunflower and soybean. Additionally, Argentina cultivates various other organic crops, such as white sorghum, amaranth, and teff.

Despite their differential price premiums, organic crops typically yield lower outputs compared with conventional crops (Seufert et al., 2012), require strict protocols for segregation, and have severe restrictions on the use of chemical inputs (Research Institute of Organic Agriculture FiBL, 2021). Organic crops encounter significant challenges in controlling insects during storage, primarily due to the restricted availability of alternatives to conventional insecticides. Among the few options utilized are diatomaceous earth and essential oils (IICA, 2009). In this context, the use of modified atmospheres (MAs) emerges as an alternative to traditional insecticides (Pons et al., 2010). Modified atmospheres function by altering the storage environment to create conditions for insect control without relying on insecticide application, thereby minimizing pesticide residues (Banks et al., 1990). Such atmospheric modifications require airtight storage conditions and can either be self-generated through biological processes within the stored bulk (auto-modified atmosphere) or externally induced by enriching the intergranular atmosphere with nitrogen (N_2) or carbon dioxide (CO_2) (controlled atmospheres) (Navarro and Donahaye, 1990).

The investigation into MAs with flexible liners has extended from small-scale airtight storage technologies (usually 30–100 kg capacity), which are designed for low-resource users (Baributsa and Ignacio, 2020), to notably large-capacity polypropylene containers known as big bags, boasting a volumetric capacity of 1 m^3 (700–800 kg). These containers (plain raffia bags, without airtight conditions) have garnered extensive use in Argentina for the storage and/or transportation of organic grains. One key advantage they offer is the optimization of logistics and operational efficiency, particularly in managing intermediate grain volumes ranging from 5 to 30 t. In contrast to smaller 30–100 kg bags, which necessitate manual handling, big bags can be efficiently managed with forklifts, thus reducing labor requirements.

Research in this field has employed internal polyethylene bags engineered with specific design and gas barrier properties (e.g., ethylene-vinyl alcohol copolymer (EVOH) or polyamide), sealed through heat-sealing techniques, thereby demonstrating effective insect control (Pons et al., 2010). In recent years, commercial products have been developed with a focus on providing packaging with high barrier properties and a high degree of automation in sealing and gas application. However, the widespread adoption of such advanced technologies remains hampered by associated costs, thereby confining its utilization to a minority of organic crop producers and/or marketers in Argentina and much of South America.

The utilization of liners lacking (cost-effective) gas barrier properties poses the challenge of establishing and sustaining an effective atmosphere. Previously, our research group investigated the feasibility of implementing MA systems with raffia big bags and cost-effective polyethylene liners using soaked grains as O_2 scavengers to maintain a hypoxic atmosphere (Bartosik et al., 2021; Taher and Bartosik, 2018). While our study demonstrated the conceptual feasibility of this approach, the practical utility was limited by the development of an unpleasant moldy grain odor, thus constraining its application in real-world scenarios.

The objective of this study was to evaluate the viability of implementing a controlled atmosphere (CA) treatment for organic grains utilizing CO_2 by developing simplified and economically viable technologies. The aim was to broaden the accessibility of this technology to a wider user base.

MATERIAL AND METHODS

The experiment was conducted at an organic grain storage facility located in Balcarce city, Argentina, utilizing eleven big bags containing various organic grains, including teff (6 bags), amaranth (2 bags), white sorghum (2 bags), and wheat (1 bag). Prior to the implementation of the CA treatment, the grain was transferred from the original containers to the raffia big bags, which were internally lined with polyethylene bags.

The polyethylene liners had a dimension of 2.4 m in length and 1 m in diameter, with the lower end bellows folded and heat sealed. The upper end remained completely open without any additional features for facilitating closure. The material composition of the bag comprised a standard 70 micron thick monolayer polyethylene liner lacking any barrier properties, commonly employed for holding construction material (such as sand) and available at a market price of about USD 3.

After filling, nine of the big bags were sealed using the twisting-folding-knot system (KS), with consistent adherence to a standardized procedure (Fig. 1). Conversely, the remaining two big bags were sealed using a heat-sealing system (HS), employing a portable heat sealer (La Pipiola, Argentina), which was connected to a 12 V power source. Subsequently, gas injection into the big bags was carried out using compressed CO_2 from 120 kg cylinders. Each cylinder, equipped with a pressure regulator, was connected to a flexible hose. This hose was inserted into the grain mass through a 5 cm incision in the upper side of the liner, aiming to reach the bottom of the bag with the assistance of a metal rod. This incision also served as a gas purge point during injection. The CO_2 concentration during injection was continuously monitored at the purging point using a portable gas analyzer (Motomco, USA), with measurements taken at 30 s intervals until a concentration of 90% in the exhausted gas was achieved. Finally, the incision utilized for CO_2 injection and gas purging was sealed with a special rubber-based tape (Rivamar, Argentina).

Fig. 1. Left: Big bag closed using the twisting-folding-knot system. Right: Big bag closed using the heat-sealing method (indicated by arrow).

Following gas application, CO_2 concentration inside the big bags was monitored every 24 h throughout the treatment duration. Gas samples were extracted by inserting a histological needle through a septum placed in the liner of the big bag. The goal was to maintain a minimum CO_2 concentration of 40%, with a reinjection procedure implemented whenever the CO_2 concentration dropped below this threshold, aiming to restore the internal concentration to 90%. The criterion employed to determine the duration of treatment entailed calculating the cumulative sum of the products derived from the concentrations attained (%) and the duration of exposure (h), yielding the cumulative concentration × time (Ct product) metric. Treatment completion was defined as achieving a Ct product value equal to or greater than 12,600 %h (Alagusundaram et al., 1995).

RESULTS AND DISCUSSION

The injection time required to attain the target CO_2 concentration of 90% was less than 5 min per big bag. The injection procedure adhered to the guidelines outlined by Garcia (2020), which advocates for placing the purging point as far as possible from the injection point. This positioning facilitates an effective sweeping or piston effect within the intergranular air due to the injected gas, optimizing the distribution and dispersion of CO_2 throughout the grain mass.

The two big bags with the HS system achieved the control criteria with only one injection, indicating satisfactory airtightness. In contrast, only four out of the nine big bags utilizing the KS mechanism attained the same condition (Table 1). The remaining five big bags employing the KS system necessitated reapplications, as the CO_2 concentration fell below 40% within 2–3 d post injection (Fig. 2). Moreover, discrepancies were noted in the number of reinjection cycles required, ranging from 1 to 4.

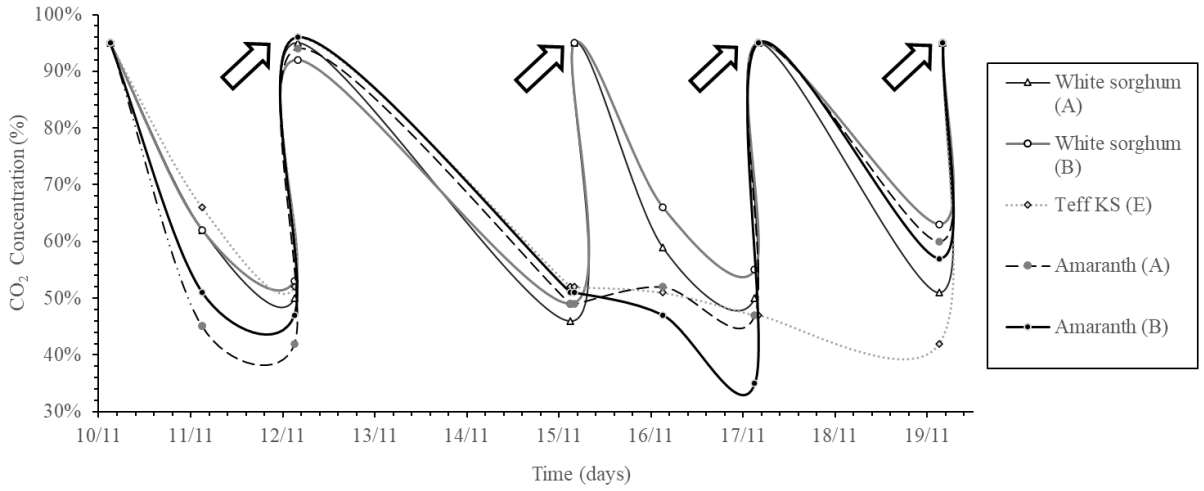

Fig. 2. Carbon dioxide concentration (%) over time (d) for big bags requiring gas supplementation (arrows indicate the moment of gas reapplication).

In the big bags where a single application was sufficient, it is noteworthy that the CO_2 concentration experienced a pronounced decline during the initial 24 h period, dropping from approximately 90–95% down to 60–70% (Fig. 3).

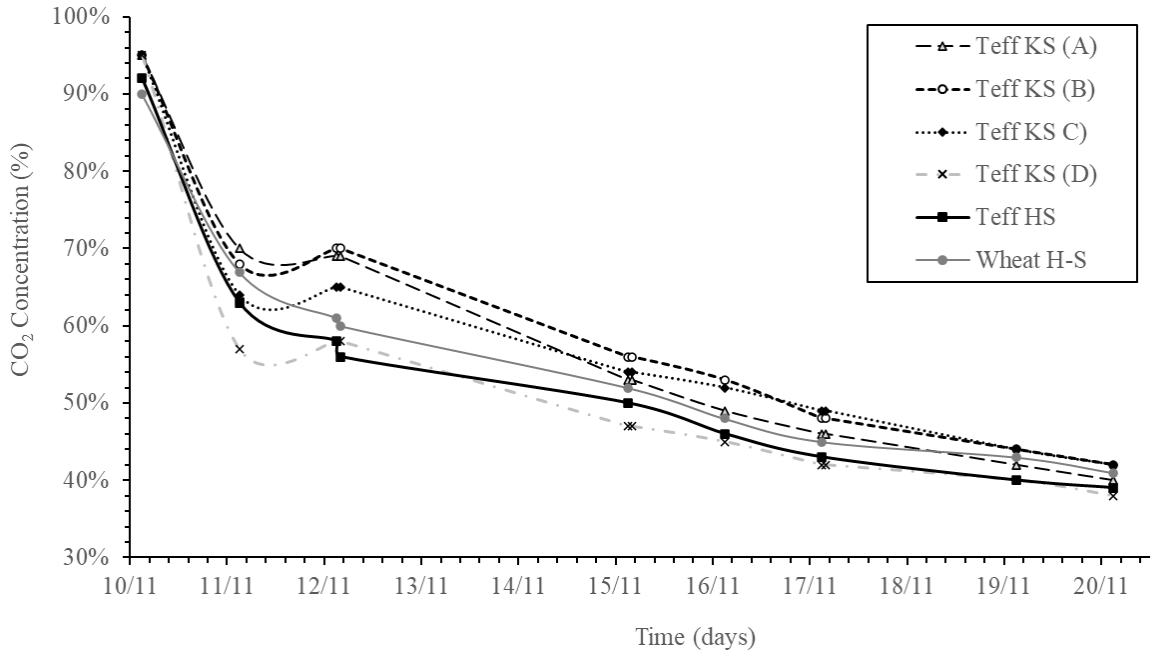

Fig. 3. Carbon dioxide concentration (%) over time (d) for big bags requiring a single gas application.

This phenomenon can be attributed to the sorption process, the extent of which varies depending on factors such as the grain type, moisture content, and temperature, and may exhibit partial reversibility (Yamamoto and Mitsuda, 1980). Subsequent maintenance or marginal augmentation

of gas concentration is indicative of the partial reversibility of this sorption phenomenon. Following the first 24 h, the CO_2 concentration exhibited a near-linear decline until reaching a level of 38–40% after 10–11 d post treatment. This gradual reduction in concentration is attributed to potential leakage through the sealing system or natural permeation through the plastic liner. It is pertinent to note that the sustainment of an effective intergranular CO_2 concentration is determined not only by gas leakage but also by CO_2 sorption by the grains themselves (Cofie-Agblor et al., 1998). This aspect assumes particular significance when considering the application of CA treatments in grains characterized by high sorption rates.

The target Ct product (12,600 %h) was reached earlier in the big bags that had undergone more reapplications (white sorghum, 8 d) (Table 1). The required exposure time gradually increased to a maximum (10–11 d) in the big bags with a single application; this is because the CO_2 values were frequently restored to levels above 90%. The concentration of CO_2 remained elevated in the hours following the application, thereby increasing the average concentration compared with the big bags with a single application.

Although the control criterion was also achieved in the big bags requiring multiple injections, the practical implementation of CA treatments under this condition could pose challenges. Continuous monitoring is essential to detect when reinjection is necessary. Additionally, the process of reinjection demands additional labor and consumes more CO_2. For instance, treatments requiring a single application were estimated to consume 1.3 kg of CO_2, while those necessitating four additional reinjections consumed a total of 4.2 kg (Table 1).

Table 1. Number of gas injection cycles required, total CO_2 consumed and time (h) to reach the required Concentration time (Ct) -product (12600 %h) for the different big bags with twisting-folding-knot and heat-sealing closure systems.

Big Bag	Closure system	Number of CO_2 applications	Total CO_2 consumption (kg)*	Time to reach target Ct (h)
White sorghum (A)	Twisting-folding-knot (KS)	5	4.2	200
White sorghum (B)				
Amaranth (A)		4	3.5	215
Amaranth (B)				
Teff (E)		3	2.8	220
Teff (A)		1	1.3	240-250
Teff (B)				
Teff (C)				
Teff (D)				
Teff	Heat-sealing (HS)			
Wheat				

*Estimate made based on initial and final CO_2 concentrations, an interstitial air volume of 0.4 m^3, and a purging efficiency of 50%.

Therefore, while multiple injections may achieve the desired outcome, they also entail increased resource consumption and labor, highlighting the importance of optimizing the sealing procedure to minimize these drawbacks.

CONCLUSIONS

Our results suggest that a single-shot treatment suffices when the big bag is adequately sealed, even with a non-barrier liner. Consistent with this, Navarro (2013) noted the efficacy of a solitary CO_2 treatment when an initial concentration exceeding 70% is established, maintaining a concentration above 35% for at least 10 d. However, the use of the KS did not consistently achieve the desired airtightness. Similar observations were made in a previous study evaluating the KS through a pressure decay test (Bartosik et al., 2021). Challenges in achieving consistent airtightness with the KS may stem from the larger size of the big bag's opening (2 m wide), which exceeds that of smaller bags (30–100 kg capacity with 0.5 m wide opening) employing the same closure mechanism (Arthur et al., 2022). Exploring alternative technological solutions, such as bags with smaller openings that can be easily sealed after filling, may mitigate these challenges despite potential increases in packaging costs. Conversely, the HS technology offers greater predictability in closure system outcomes.

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Effect on *Lasioderma Serricorne* Eradication and Flue-Cured Tobacco Quality Using Dynamic Controlled Atmosphere Technology

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ABSTRACT

Lasioderma serricorne (Fabricius) is a significant pest affecting stored tobacco worldwide, characterized by its complex feeding habits, robust reproductive abilities, and extensive damage range. This study employed dynamic controlled atmosphere technology (DCAT) to investigate varying durations of low oxygen (O_2) treatment on tobacco leaves, aiming to identify the optimal time required for effective lethality and insecticidal impact on the *L. serricorne*, as well as to assess the influence of this treatment on the quality of the tobacco leaves. The findings revealed that DCAT could rapidly decrease and stably maintain O_2 content within the tobacco stack below 0.5%. Within a span of 10 d, this method completely eradicated the adults of the *L. serricorne*, and within 20 d it eliminated all larvae and eggs of the *L. serricorne*. Furthermore, an analysis of the tobacco leaves before and after treatment indicated that short-term low O_2 insecticidal exposure had a minimal effect on their appearance, chemical composition, and sensory quality, thereby not compromising the aging quality of the tobacco leaves. This study completed essential data for the application of DCAT in managing pests in stored tobacco.

Keywords: Dynamic controlled atmosphere, Tobacco leaves, *Lasioderma serricorne*, Quality

INTRODUCTION

Insect infestations during storage can cause significant quality loss and quantity degradation in flue-cured tobacco. *Lasioderma serricorne* is one of the most destructive insect pests to stored tobacco leaves and cigarettes.

Phosphine fumigation is the most widely used chemical control method for various tobacco processing factories during tobacco storage (da Silva et al., 2018). However, its application is restricted to certain predetermined conditions due to its high toxicity, flammability, explosivity, and corrosivity to metals. The growing demand for eco-friendly technologies in tobacco storage has increased the need for developing new methods and technologies. Among these methods, nitrogen represents a promising alternative to traditional chemical control methods for storing pests (Navarro, 2006, 2012; Sakka et al., 2020). The use of nitrogen is environmentally friendly, leaves no residues, and does not require registration for use in food storage and processing (Sakka et al., 2020).

Our research focused on augmenting the data for the industrial application of nitrogen (N_2) gas for controlling pests in boxed tobacco leaves. By comparing with a normal atmospheric condition (control), we investigated the lethal time and effect of an N_2-enriched low-O_2 environment on *L. serricorne*, as well as its impact on the quality of flue-cured tobacco. Results from this study provide data support and practical guidance for the application and promotion of active low-O_2 modified atmosphere technology for controlling pests.

MATERIALS AND METHODS

Experimental Equipment and Materials

Samples

Lasioderma serricorne was collected from a tobacco factory located at Zhengzhou, Henan, China, and reared on a diet of 50% flour/yeast and 50% wheat kernels in weight ratio. Pupae of *L. serricorne* were obtained from the culture. The 2 d old eggs of *L. serricorne* were provided by the Henan University of Technology, Henan, China. The flue-cured tobacco for the experiment was provided by the Henan China Tobacco Golden Leaf Production and Manufacturing Centre.

Experimental equipment

Tianjin Sengluo Technology Co. provided the following equipment: CA-160Y nitrogen production equipment, CNDJ-901Y intelligent multi-point oxygen, a temperature and humidity remote monitoring device, a 201F oxygen and carbon dioxide detector, a high airtight flexible tent (air change rate 0.02/d), multi-functional film continuous sealing machine, brushes, and test bottles.

Methods

Tobacco stack setup

The trial was set up with 3 stacks of approximately 100 boxes each of tobacco, stacked in 4 layers and individually sealed using high barrier films. The control was set up with 4 boxes of tobacco stored naturally under normal atmospheric conditions.

Experimental pest placement

In each experimental group, one box of tobacco was selected from the upper, middle, and lower parts of the stacks, and one bottle of *L. serricorne* with air permeability was placed inside; all stages of *L. serricorne* were placed in each bottle. The bottles were placed at a depth of about 20 cm in the middle of the box.

Oxygen reduction in tobacco stacks and maintenance time of hypoxia

The oxygen reduction mode in the dynamic controlled atmosphere process was used to replace the air in the stacks with rapid nitrogen filling, so that the oxygen content in the tobacco box was reduced to less than 0.5%, and timing was started so that the oxygen content was continuously maintained in the range of 0.1% to 0.5% for 10, 15, and 20 d.

To ensure the accuracy of oxygen content, one set of wireless oxygen, temperature, and humidity sensors was placed in the tobacco box of each stack, and the diffusion rate of oxygen content in the tobacco box at different locations was monitored using real-time detection data.

Determination of insect mortality

After the polyethylene film which sealed the tobacco pile was removed, the bottles holding the test insects and tobacco leaves were removed, and the insects were transferred to petri dishes. The survival of the insects (larva, pupa, and adult) in the petri dishes was observed. The eggs in the petri dishes were incubated at 30℃ and 70% r.h. for 14 d.

RESULTS

Diffusion Rate of Low Oxygen in the Tobacco Box

As shown in Table 1, each box of tobacco had low-oxygen gas levels, but the rate of diffusion of the oxygen content went from 2% to 0.1% in 2.7 d. In the middle of the 2nd and 3rd layers, the tobacco boxes were piled up, thus affecting the oxygen content of the diffusion rate from 2% to 0.1% over the extended time.

Table 1. Statistics on the diffusion rate and maintenance of oxygen content in the tobacco box at different positions of the stacks.

Processing time (d)	Lower part of stack	Middle of stack	Upper part of stack
Oxygen sensor placement	Inside the 4th box from the top	Inside the 3rd box from the top	Inside the 2nd box from the top
Duration of hypoxic insecticide treatment	20 d	15 d	10 d
Oxygen level and maintenance time	2% to 1% (0.7 d) 1% to 0.1 % (2 d) ≤ 0.1 % (18 d)	2% to 1% (1.3 d) 1% to 0.1 % (3 d) ≤ 0.1 % (10 d)	2% to 1% (1.75 d) 1% to 0.1 % (6 d) ≤0.1 % (3 d)

Effectiveness of Different Hypoxia Action Times on Killing of *L. serricorne*

Under the conditions of 20℃~30℃ and 55% r.h.~65% r.h., the dynamic controlled atmosphere process was used to reduce the oxygen by filling nitrogen into the stacks, so that the oxygen content in the stacks was maintained within the range of 0.1%~0.5%. The bottled insects were taken out of different parts of the stacks on the 10, 15, and 20 d, respectively. As shown in Table 2, when compared with the normal atmospheric conditions control group, within a span of 10 d, this method completely eradicated the adults and pupae, and it could eliminate all larvae and eggs of the beetle in 20 d.

Table 2. Effect of the mixture on insect mortality.

Exposure time (d)	Mortality (mean ± standard deviation)			
	Adults	Larvae	Pupae	Eggs
10	100±0	95±5.0	100±0	80±10.0
15	100±0	98±2.0	100±0	90±5.0
20	100±0	100±0	100±0	100±0
Control (20 d)	15±2	0±0	0±0	40±10.0

Effect of Low Oxygen Environment on Quality of Flue-cured Tobacco

Effect on the appearance quality of flue-cured tobacco

Compared with the normal atmospheric condition (control), there was no significant difference in appearance before and after 10, 15, and 20 d of controlled atmosphere treatment (Table 3).

Table 3. Changes in appearance quality of flue-cured tobacco with different low oxygen levels.

Test groups	Sampling time (d)	Appearance evaluation score						
		Color purity	Oil content	Fullness	Uniformity	Brightness	Smell	Overall rating
Control	0	7.0	7.5	7.0	7.0	7.0	7.5	43.0
	20	7.0	7.0	7.0	7.5	7.0	7.5	43.0
Controlled atmosphere	0	7.0	7.5	7.0	7.5	7.0	7.5	43.5
	10	7.0	7.5	7.0	7.0	7.0	7.0	42.5
	0	7.0	7.5	7.5	7.5	7.0	7.5	44.0
	15	7.0	7.0	7.0	7.5	7.0	7.5	43.0
	0	7.5	7.5	7.0	7.5	7.0	7.5	44.0
	20	7.0	7.5	7.0	7.5	7.0	7.5	43.5

Effect on chemical composition of flue-cured tobacco

Compared with the normal atmospheric condition (control), controlled atmosphere insecticide had no effect on the chemical quality of flue-cured tobacco (Table 4).

Table 4. Changes in chemical composition of flue-cured tobacco at different controlled atmosphere times.

Test groups	Sampling time (d)	Chemical composition %						
		Total phytanine	Total nitrogen	Reducing sugar	Total sugar	Potassium	Chlorine	Starch
Control	0	2.82	2.05	20.51	22.23	1.41	0.22	2.17
	20	2.80	2.06	20.66	22.47	1.45	0.25	2.14
Controlled atmosphere	0	2.81	2.04	20.60	22.32	1.43	0.21	2.19
	10	2.77	2.07	20.59	22.56	1.4	0.23	2.12
	0	2.83	2.1	20.57	22.7	1.41	0.23	2.16
	15	2.78	2.08	20.60	22.48	1.47	0.26	2.15
	0	2.80	2.07	20.61	22.42	1.44	0.24	2.13
	20	2.79	2.05	20.58	22.73	1.40	0.22	2.2

Effect on sensory quality of flue-cured tobacco

Compared with the normal atmospheric condition (control), the evaluation score of 10, 15, and 20 d of controlled atmosphere insecticide was slightly improved (Table 5).

Table 5. Changes in sensory quality of flue-cured tobacco at different controlled atmosphere times.

Test groups	Sampling time (d)	Sensory score							
		Aroma	Aroma volume	Concentration	Off-odor	Strength	Irritation	Aftertaste	Overall rating
Control	0	6.5	6.0	6.0	6.0	6.0	6.0	6.0	42.5
	20	6.5	6.0	6.0	6.0	6.0	6.5	6.0	43.0
Controlled atmosphere	0	6.5	6.0	6.0	6.0	6.0	6.5	6.0	43.0
	10	6.5	6.0	6.0	6.5	6.0	6.5	6.0	43.5
	0	6.5	6.0	6.0	6.0	6.0	6.0	6.0	42.5
	15	6.5	6.0	6.0	6.0	6.0	6.5	6.0	43.0
	0	6.5	6.0	6.0	6.0	6.0	6.5	6.0	43.0
	20	6.5	6.0	6.0	6.0	6.5	6.5	6.5	44.0

CONCLUSIONS

Adopting dynamic low oxygen regulation insecticidal technology could quickly reduce the oxygen content in the stack to less than 0.5% at 20 to 30℃, which could kill 100% of the adults and pupae of *L. serricorne* in the tobacco box within 10 d and 100% of the *L. serricorne* larvae and eggs within 20 d. At the same time, by comparing quality of flue-cured tobacco before and after the test, it could be seen that the dynamic controlled atmosphere insecticidal process had basically no effect on the quality of flue-cured tobacco, such as appearance quality, chemical composition, and assessment of sensory quality. With the continuous development of controlled atmosphere technology and airtight enclosure processes, tobacco controlled atmosphere insecticide is gradually moving from static controlled atmosphere to dynamic controlled atmosphere development to achieve the whole stack of tobacco controlled atmosphere insecticide during the real-time monitoring of parameters, such as oxygen content and process automation and control. When the oxygen content is lower, the humidity control is more accurate, and the insecticide effect is better.

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Paper No. CAF2024-A26

Hertel F (2024) Experiments with a gastight to the Australian Standard AS 2628 retro sealed metal bin grain silo. Page 95. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Experiments with a gastight to the Australian Standard AS 2628 retro sealed metal bin grain silo

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ABSTRACT

After sealing a metal bin grain silo at the humid mountain region Vogtland in Germany in 2018, circumstances as the covid pandemic, the leak of qualified workers and bureaucracy caused delay to the project. We have restarted now in 2024 with installing the electronic monitoring system and testing protocol. A closed loop fumigation, suitable sensors for phosphine, carbon dioxide, and oxygen, ways to seal gate valves, the dewpoint in the headspace and a leakage test compared with a half-life pressure test are on the list. In the meantime; however, we have been able to find out the possible applications and limits of the system. I would like to share the first results and my experience on these topics.

Keywords: Hermetic storage, Controlled atmosphere, Australian Standard AS 2628, Grain silo, Gastight

Paper No. CAF2024-A27

Dong X, Tang P, Wu X, Chen E, Shao J (2024) Alleviating grain rancidity: exploring the impact of nitrogen-modified atmospheres on lipid metabolism in paddy storage. Page 96. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Alleviating Grain Rancidity: Exploring the Impact of Nitrogen-Modified Atmospheres on Lipid Metabolism in Paddy Storage

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ABSTRACT

Paddy (*Oryza sativa* L.), a widely cultivated grain, constitutes a primary dietary source for half of the world's population. Paddy is susceptible to quality losses during storage due to oxidation process, and lipid metabolism is a major contributing factor to quality deterioration. While nitrogen-modified atmospheres have been widely employed in paddy storage to delay grain aging, the mechanisms governing their impact on lipid metabolism remain elusive. Therefore, this study aims to analyze lipid metabolism, a determinant of rice quality rancidity, in paddy grain subjected to nitrogen-modified atmosphere storage.

Physiological, biochemical characteristics, and lipid substance metabolism in paddy grain were investigated during nitrogen-modified atmosphere (25°C with 96% N_2) and conventional storage (25°C with 78% N_2). The results demonstrated that nitrogen-modified atmosphere effectively preserved paddy quality by suppressing both lipase and lipoxygenase (LOX) enzyme activities. Additionally, it decelerated the decomposition of crude fat and generation of fatty acids compared to conventional storage conditions. Lipidomic profiling, conducted using a Ultra-High-Performance Liquid Chromatography coupled with an Q Extractive™ HF mass spectrometer, revealed alternations in lipids under nitrogen-modified atmosphere storage. Identified lipids could be classified into five main group: 612 Glycerolipids (GL), 520 Glycerophospholipids (GP), 230 Sphingolipids (SP), 65 Sterol lipids (ST), and 28 Fatty acids (FA). Pathway enrichment analysis revealed that during storage, paddy grains primarily underwent synthesis and hydrolysis of lipids, including triglycerides (TG), diglycerides (DG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and cardiolipin (CL), as well as oxidation and degradation of fatty acids, such as linoleic acid and α-linolenic acid during storage. Nitrogen-rich conditions exhibited inhibitory effects on the metabolism of major lipid substances, such as glycerides and glycerophospholipids, to maintain the lipid stability and mitigate paddy rancidity. This study provides valuable insights into understanding how nitrogen-modified atmosphere alters lipid metabolism in paddy storage, thereby alleviating grain rancidity.

Keywords: Paddy (*Oryza sativa* L), Lipidomic profile, Nitrogen-modified atmosphere, Storage, Rancidity, Quality

Paper No. CAF2024-A28

Athanassiou CG, Sakka M, Agrafioti P, Sotiroudas V (2024) Factors affecting the insecticidal effect of nitrogen against major stored-product insects. Page 97. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Factors Affecting the Insecticidal Effect of Nitrogen against Major Stored-product Insects

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ABSTRACT

The application of nitrogen has been thoroughly evaluated for the control of a wide range of stored product insects in different types of commodities, facilities, and application scenarios. The term "nitrogen" is an application to reduce the percentage of oxygen to 1% or less, which is lethal for insects. There is a wide range of biotic and abiotic factors that drastically affect the efficacy of the method, such as the temperature, the relative humidity, the commodity, as well as the target species. In principle, the increase of temperature drastically increases the efficacy of the method and can noticeably shorten the duration of the application. The application of nitrogen can be done in different structures that vary from chambers to silos, which are connected to an external nitrogen generator. Apart from its effect on insects, nitrogen can significantly reduce the concentration of molds in dried fruits, causing no negative changes in the major quality and sensory characteristics of the commodity. Also, nitrogen can serve as a "resistance breaker" in the case of populations that are resistant to phosphine, as resistant populations have been found to be equally susceptible to nitrogen with non-resistant populations. Finally, there are species, such as the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), that are less susceptible than others to nitrogen. In this presentation, we will illustrate all these parameters, in "real world" experiments.

Keywords: Modified atmospheres, Nitrogen, Resistance, Stored-product insects, Mortality

Paper No. CAF2024-A29

Chen E, Tang P, Wang K, Dong X, Wu X, Sun S (2024) RNA-seq analysis of gene expression changes in response to hypoxia stress in *Tribolium castaneum*. Page 98. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

RNA-Seq Analysis of Gene Expression Changes in Response to Hypoxia Stress in *Tribolium Castaneum*

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ABSTRACT

Low oxygen, controlled atmosphere technology can achieve efficient prevention and control of stored grain pests, which has been considered as the most effective way to solve the problems of traditional chemical fumigation resistance and pesticide residue in the field of grain storage. However, it has been reported that some important stored grain pests have developed strong hypoxic adaptability, which poses a huge threat to grain security. In this study, the hypoxia tolerances of *T. castaneum* from five different populations were measured, which showed different hypoxia tolerance among different populations. Then, the RNA-seq analysis was conducted to clarify the molecular mechanisms of hypoxia tolerance in *T. castaneum*, and we found 347 genes were differentially expressed (124 genes up-regulated and 223 genes down-regulated) between the hypoxia treatment and control groups. Afterwards, the Gene Ontology (GO) annotation analysis of differentially expressed genes (DEGs) showed hypoxia stress had significant effects on metabolic processes, cellular processes, cell components, membrane components, binding, and catalytic activities of *T. castaneum*. The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed that DEGs were mainly enriched in primary metabolism pathway including, starch and sucrose metabolism, protein digestion and absorption, fatty acid biosynthesis, and signaling pathways such as Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK), lysosome and autophagy under hypoxia stress. Besides, some genes that involved in autophagy and lysosomal mitochondrial autophagy pathways were identified to be significantly up-regulated, which may be responsible to clear damaged mitochondria that caused by the excessive production of Reactive Oxygen Species (ROS) under low oxygen stress. Importantly, the results of Ribonucleic Acid-seq (RNA-seq) and real-time Quantitative Polymerase Chain Reaction (qPCR) analysis showed that the 12 mitochondrial coding genes were significantly inhibited under low oxygen treatment, suggesting their critical roles in response to hypoxia tolerance. In conclusion, the present study provides us the comprehensive gene expression profiles under hypoxia stress, which lays a molecular basis to study roles of specific pathways and genes in hypoxic adaptability.

Keywords: *Tribolium castaneum*, RNA-seq, Hypoxia adaptability, Nitrogen modified atmospheres, Green prevention and control, Stored product pests

Paper No. CAF2024-A30

Tiwari A, Jian F (2024) Investigating the impact of sub-zero temperatures on stored canola seeds: moisture, germination, and beyond. Page 99. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Investigating the Impact of Sub-Zero Temperatures on Stored Canola Seeds: Moisture, Germination, and Beyond

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ABSTRACT

Survival of stored oilseeds at sub-zero temperatures is an under explored area. Limited knowledge is available regarding the characteristic changes that these oilseeds undergo in terms of viability and moisture migration when subjected to extremely low temperatures. This knowledge can become a valuable resource for agricultural practices, offering guidelines for the drying and storage of harvested oilseeds under challenging climatic conditions. The present study investigated the effects of sub-zero storage conditions on canola seeds, with a focus on understanding the interplay between moisture content variations and germination. Canola seeds were conditioned to 14% moisture content (w.b.) to represent as 'wet' sample. The experimental design involved subjecting these seeds to three sub-zero temperatures (-5, -15, and -25°C) at varying relative humidities (20, 40, 60, and 80%) until the moisture content comes in equilibrium with the storage condition. The samples, under all conditions except the ones stored at -25°C, showed subsequent decrease in the moisture content levels over time (reaching withing the safe storage moisture content levels) with minimal germination decrease of the seeds (over 80% germination at equilibrium). These results pointed towards the drying capacity of the cold temperatures along with preservation of the seeds germination capacity. Further studies on the changes in the status of water inside stored canola seeds under sub-zero conditions can bridge the gap in understanding in-depth about this drying effect and seed survival.

Keywords: Low temperature drying, Desorption, Isotherms, Viability, Equilibrium moisture content

Paper No. CAF2024-F32

Kolpa J, Christenson C (2024) The use of cylinderized phosphine gas for highly effective fumigations of DDGS exports. Pp. 100–107. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

The Use of Cylinderized Phosphine Gas for Highly Effective Fumigations of DDGS Exports

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ABSTRACT

The U.S. Grains Council states, "Distiller's dried grains with solubles (DDGS) are the nutrient rich co-product of dry-milled ethanol production. Utilization of DDGS as a feed ingredient is well documented as both an energy and a protein supplement. Combined, U.S. ethanol plants possess the capacity to produce more than 57 billion liters (15 billion gallons) of ethanol and 44 Mt of DDGS." As such, DDGS are also prone to insect infestations and result in numerous non-compliances on exports due to live insects. The situation is exacerbated when exporters fail to fumigate the container correctly or omit the fumigation altogether. Containers arriving with live insects in Vietnam and Thailand became so prevalent that special protocols were developed to address the concern and re-establish paused trade. While the initial pest of concern was the khapra beetle, (*Trogoderma granarium* (Leconte) (Coleoptera: Dermestidae)), countries were seeing violations from several stored-product pests. Containers found with live insects after shipping are often re-fumigated with methyl bromide, which, for obvious reasons, is undesirable. Use of the cylinderized phosphine fumigants $ECO_2FUME^{®}$ and $VAPORPH_3OS^{®}$ was optimized to reduce time while achieving 100% mortality across all life stages at various temperatures. Monitoring was performed throughout the fumigation at multiple points in the shipping containers.

Keywords: ECO2FUME, VAPORPH3OS, Phosphine, Fumigation, DDGS, Export, Grain, Khapra beetle

INTRODUCTION

Distiller's dried grains with solubles (DDGS) are the co-product of dry-milled ethanol production. According to the U.S. Grains council, U.S. ethanol plants possess the capacity to produce more than 57 billion liters of ethanol and 44 Mt of DDGS. Exports of DDGS have exploded since the early 2000s. The USDA AgTransfer website shows that U.S. exports of DDGS peaked in 2015 and have remained steady (Fig. 1). The growth of the DDGS market is due to ethanol's production as a fuel additive, especially in replacement of MTBE (methyl tert-butyl ether).

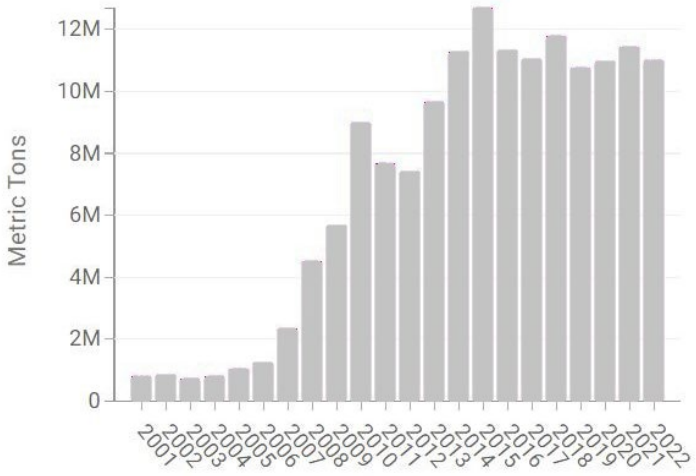

Fig. 1. U.S. annual exports of DDGS.

According to the U.S. Grains Council, U.S. DDGS exports totaled 10.4 Mt (4.1 million bushels in corn equivalent) in Marketing Year (MY) 2022/23, worth USD 3.3 billion and down 1.2 Mt, or 10.3% from MY 2021/22. Fifty-four countries purchased U.S. DDGS in MY 2022/23.

Mexico purchased the bulk of DDGS exports, consisting of more than 20 percent of the export market, while South Korea was the second largest importer. Vietnam, Indonesia, and Canada rounded out the top five importers for 2022/2023.

The USDA AgTransfer website estimates that in 2020 there were >3 Mt of DDGS shipped in containers via ship. The density of DDGS can vary, and a typical shipping container will range from 23 t to slightly more than 26 t of DDGS upon shipping. These figures match weights listed in export manifests as well. Using these figures as an estimate, 120,000 shipping containers of DDGS left the United States in 2020 via water. Using the export database, Datamyne, roughly 2 Mt of DDGS were exported using more than 80,000 shipping containers. Differences in these numbers are likely due to inefficiencies in reporting and export classifications.

Using the export database, Datamyne, 29,294 shipping containers of DDGS were shipped in 2020 to Vietnam, and 9,352 shipping containers were exported to Thailand. These are also the only two countries with a mandatory fumigation protocol, leaving up to 90,000 shipping containers at risk of infestation, degradation, and as a potential invasive insect vector.

In 2012, Vietnam discovered an infestation of *Trogoderma inclusum* (Leconte), the larger cigarette beetle, an actionable quarantine pest, in a shipment of DDGS from the U.S. After this find, all subsequent shipments to Vietnam were required to be fumigated. An additional violation occurred when a shipment of 12 containers to Vietnam was found to contain *Trogoderma variabile* (Ballion). These 12 containers of DDGS were shipped from Norfolk, VA on September 17, 2015,

and were inspected in Vietnam at arrival on October 27, 2015. It is assumed that these containers had been properly fumigated; however, results indicated otherwise. *Trogoderma variabile* and *T. inclusum* are considered to be close relatives and are both stored-pest products of concern (Phillips et al., 2018). In December 2016, Vietnam suspended imports of U.S. DDGS after the reported detections of these quarantine pests in U.S. shipments. Prior to the suspension, Vietnam was the third-largest market for American DDGS, with exports valued at more than USD 230 million in 2016.

Thailand in 2017 was the fourth-biggest overseas market for DDGS, importing 738,413 t of the high-protein feed used at poultry and livestock farms, as shown by USDA data. In August and November of 2018, Thai authorities found the *T. granarium* in two DDGS shipments from the U.S. In January of 2019, Thailand suspended the importation of DDGS from the United States until a fumigation protocol could be adopted.

Both bans were eventually lifted as the USDA worked with both countries to develop protocols. The Vietnam Plant Protection Department lifted the ban on imported DDGS from the U.S. effective September 2017 (Trung, 2017).

As per the USDA Reference #281 dated May 1, 2020, to the Federal Grain Inspection Service (FGIS) Policy Bulletin Board, the new protocols combine phosphine concentration, exposure time, and temperature (Table 1).

Table 1. Phosphine protocol for DDGS exports to Vietnam.

Fumigation Schedule for Containerized DDGS[1]

Commodity Temperature (°C)	Commodity Temperature (°F)	Minimum PH_3 Concentration Reading[2] (ppmv)	Minimum Exposure Period (d)
10 – 15	50-59	750	5
>15-20	60-69	750	4
>20	≥70	750	3

1. If applicant chooses to use cylinderized gas (e.g., $ECO_2FUME^{®}$) for corn, wheat, and sorghum, use this table for treatment schedule and note that mass monitoring is not required.
2. Phosphine concentration readings at center mass must not be less than 500 ppmv throughout duration of treatment. Also, no more than one 24 h interval reading at center mass is less than 750 ppmv.

The protocol as approved by the Thai Department of Agriculture to lift the ban is similar. As per the USDA Reference #284 dated April 13, 2021, to the FGIS Policy Bulletin Board, the new protocols combine phosphine concentration, exposure time, and temperature (Table 2).

Table 2. Phosphine protocol for DDGS exports to Thailand.

FUMIGATION SCHEDULE for Containerized DDGS[1]

Commodity Temperature (°C)	Commodity Temperature (°F)	Minimum PH_3 Concentration Reading[2] (ppmv)	Minimum Exposure Period (d)
10 – 15	50-59	750	5
>15-20	60-69	750	4
>20	≥70	750	3

1. If applicant chooses to use cylinderized gas (e.g., $ECO_2FUME^{®}$) for containerized DDGS, use this table for treatment schedule and note that center mass monitoring is not required.
2. If applicant chooses to use pellets, tablets, or plates, the concentration readings at 24 h and at completion of exposure period must meet or exceed these minimums.
3. Certification of treatment should rely on timing based on when doors are closed, unless doing so means that the minimum exposure time specified on the label is not met, in which case, the labeled exposure time should be followed.

Even with a new protocol in place, Thailand's DDGS purchases have declined from over 1 Mt in 2018 to just under 200,000 t in 2022 according to the U.S. Grains Council. During this time, Thailand found that up to 20% of shipments had live insects. In 2021, 68% of the violations came from two districts; in 2022, 63% came from two districts, with one common to both years. Since this time, the top two violators have changed fumigation companies. As the U.S. Grains Council presentation did not contain scientific names, the nomenclature from their report is used as follows. Thailand did find only two incidents of the cigarette beetle in each of 2022 and 2023; however, flat grain beetles and red flour beetles were found at much higher incidents. In none of the reports was *T. granarium* reported.

Distiller's dried grains with solubles are a co-product of the dry milling manufacturing process obtained after the removal of ethyl alcohol by distillation from yeast fermentation of a grain or a grain mixture by condensing and drying at least three-fourths of the solids of the resultant whole stillage by methods employed in the grain distilling industry. The DDGS are produced by blending corn distillers liquid solubles with wet corn distillers grains before being dried and can be produced from other grains as well, such as barley, rye, sorghum, and wheat, according to the Iowa Renewable Fuels Association.

Due to the highly processed nature, DDGS are pest free until put into storage and are moved from ethanol plants primarily by truck and rail; however, DDGS can be shipped by barge and eventually transported by ocean going vessels. The DDGS are shipped to transloading facilities where they are prepped for export. Insects can contaminate the commodity during these storage, transportation, or transloading periods.

Transloading and fumigation of DDGS can take place at the port before loading onto a vessel or along rail service where they are prepared and fumigated before the container is shipped to a port. Containers are not shipped holding gas. Using the aforementioned data, nearly 40,000 DDGS containers are fumigated in the United States each year before shipping to Vietnam or Thailand, which, on average, means that almost 110 containers are fumigated daily across the United States, with 300–500 containers sitting under gas at any given moment. Depending on the conditions, demurrage and detention fees can be applied to these containers. Space on the transload yard is also needed for containers during the fumigation and for the safe aeration of phosphine. Fumigators must be on site daily for the preparation, fumigation, monitoring, and aeration of the containers. These additional cost factors disincentivize fumigators from taking extra time to fumigate according to the protocol.

There are two ways to introduce phosphine into a fumigation space. The first method is using cylinderized phosphine, such as ready-for-use $ECO_2FUME^{®}$ or $VAPORPH_3OS^{®}$. $ECO_2FUME^{®}$ is introduced into a space via a cylinder over a relatively short period of time. $VAPORPH_3OS^{®}$ is introduced into a fumigation space with special equipment, diluting the phosphine with air below the flammability level (Tumambing et al., 2012). In both cases, the phosphine is expelled into the headspace in the container with the doors already closed. The second method to introduce phosphine into a container is via a metal phosphide, such as aluminum phosphide or magnesium phosphide. These metal phosphides react via a hydrogenation reaction to liberate phosphine (Bell, 2000; Chaudhry, 2000).

Fumigation with metal phosphides relies upon the speed of the chemical reaction based on temperature and humidity. As metal phosphides are generally located at the front of the container by the door, concentration will vary over time and location with no forced dispersion of the phosphine. Phosphine will disperse through the container based on ambient conditions such as temperature changes, solar radiation, and wind. In most testing scenarios, metal phosphides do not hit target concentrations until at least 24 h and, in some cases, up to 72 h for various locations in a container (Agrafioti et al., 2021).

Fumigations of DDGS using $ECO_2FUME^{®}$ start receiving center of mass readings within 30 min, and concentration measurements every 24 h indicate concentrations can be held for longer periods (Table 3).

Table 3. Example data from commercial fumigation of DDGS to Vietnam with ECO$_2$FUME®.

Doors Closed	Exposure Start	Air Temp (°F)	Commodity Temp (°F)	Dosage (g/1000 ft^3)	Total lbs Used	24 h (ppm)	48 h (ppm)	72 h (ppm)
6:06 PM	6:36 PM	75	81	45	13.5	945	809	559
6:01 PM	6:31 PM	75	80	45	13.5	825	799	602
5:59 PM	6:29 PM	75	79	45	13.5	843	762	611
5:57 PM	6:27 PM	75	79	45	13.5	927	815	611
5:50 PM	6:20 PM	75	79	45	13.5	890	766	537
5:43 PM	6:13 PM	75	77	45	13.5	954	799	631
5:28 PM	5:58 PM	75	80	45	13.5	801	753	529
3:30 PM	4:00 PM	75	81	45	13.5	913	788	591
5:48 PM	6:18 PM	75	77	45	13.5	909	826	545
5:44 PM	6:14 PM	75	78	45	13.5	923	789	599
5:24 PM	5:54 PM	75	77	45	13.5	888	766	507
5:46 PM	6:16 PM	75	78	45	13.5	885	790	617
5:30 PM	6:00 PM	75	81	45	13.5	848	767	577
5:32 PM	6:02 PM	75	80	45	13.5	969	777	606
5:26 PM	5:56 PM	75	81	45	13.5	924	827	578
4:42 PM	5:12 PM	75	76	45	13.5	923	896	520
4:40 PM	5:10 PM	75	76	45	13.5	817	785	632
4:28 PM	4:58 PM	75	78	45	13.5	793	790	587
4:41 PM	5:11 PM	75	80	45	13.5	808	799	590
4:45 PM	5:15 PM	75	78	45	13.5	1129	977	667

In both conditions, for the testing done using metal phosphides (Agrafioti et al., 2021) or using ECO$_2$FUME® (Table 3), gas loss was detected over time. Gas loss can generally be attributed to absorption, leakage, and half-life. Demonstrations in 2017 of fumigation with ECO$_2$FUME® highlighted gas loss in a shipping container loaded with DDGS. When concentrations drop, fumigators have the ability to use ECO$_2$FUME® to top up the concentration without opening the shipping container (Fig. 2).

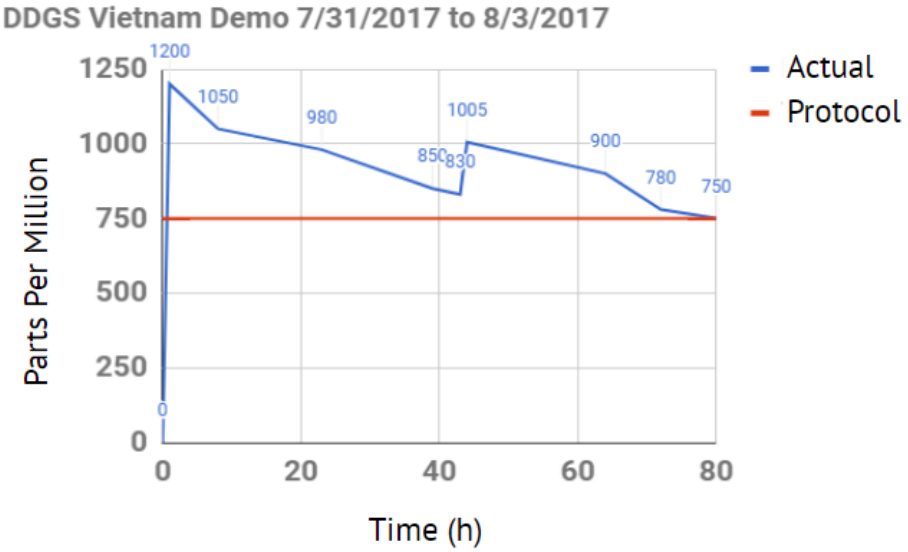

Fig. 2. Commercial application with additional phosphine added during the fumigation.

Examination of the efficacy against *T. granarium* (Gourgouta et al., 2021) indicates that the DDGS protocols can kill moving stages but are slightly less efficacious on eggs. For *T. inclusum* (Athanassiou et al., 2020), there is also an indication of efficacy against moving stages with less efficacy on eggs; however, the protocols were measured at longer times in this study compared with the stated protocols from Thailand and Vietnam. Studies of *T. variabile* (Banks and Cavanaugh, 1985; Walse et al., 2017) indicate that mortality is a function of phosphine concentration, temperature, and time, but control is expected to be similar to the other species. One note of caution is that too high of a concentration of phosphine is less effective than an optimized one (Walse et al., 2017).

In the optimization of efficacy, fumigations with metal phosphides will generally require an extra day before the center of mass concentrations achieve appropriate levels. Even with the extra time, concentrations in other points of the container may still be below desired levels depending on external conditions. In some cases, the metal phosphide may not have even fully reacted yet. Use of a cylinderized phosphine, such as $ECO_2FUME^{®}$, allows for closer adherence to the desired protocols for Thailand and Vietnam. $ECO_2FUME^{®}$ also allows for greater concentration control and shorter overall fumigation times.

It should be noted that even if using cylinderized phosphine strictly to the protocol compared with the efficacy testing of said species, a possibility of egg survival still exists. Because other species, such as *T. castaneum*, have been found in shipments, further investigation into the protocols is warranted.

Profitability of ethanol production requires DDGS to command value in the market. Optimizing the fumigation insures higher efficacy against target insects while balancing cost, time, and resources such as transportation and yard space.

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Ban J, Kim B, Moon Y, Kim J (2024) Single and combined treatments of ethyl formate and controlled atmosphere against the red flour beetle, *Tribolium castaneum*. Page 108. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Single and Combined Treatments of Ethyl Formate and Controlled Atmosphere against the Red Flour Beetle, *Tribolium castaneum*

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ABSTRACT

Development of proper fumigation method for grain pests became important because of recent resistance problem of phosphine and environmental hazard of methyl bromide (MB). Ethyl formate (EF) is emerging as a promising fumigant in plant quarantine, demonstrating high efficacy against various quarantine pests. However, reports indicate that controlling grain pests requires a higher dosage of EF. In this study, we applied both single and combined treatments of EF and controlled atmosphere (CA specifically, low-oxygen treatment, LOT) on the red flour beetle, *Tribolium castaneum*, to explore the potential for reduced fumigant usage. The study found that solely using low-oxygen conditions for 2 wk resulted in less than 10% mortality in adult beetles, though it completely controlled the egg stage. Treatment with EF alone led to less than 50% mortality in the egg stage, but fully controlled the adult stage. However, a combined approach of EF and LOT achieved 100% mortality in larvae, pupae, and adults, while controlling 47.5% of the egg stage. These findings suggest that a combined treatment of EF and LOT effectively manages grain pests, yet additional research is necessary to achieve control across all developmental stages of *T. castaneum*.

Keywords: *Tribolium castaneum*, Ethyl formate, Controlled atmosphere, Low-oxygen treatment

Paper No. CAF2024-F34

Kim B, Kim J, Moon Y, Lee B (2024) Korea's transition to ethyl formate: pioneering sustainable alternative to methyl bromide. Pp. 109–112. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Korea's Transition to Ethyl Formate: Pioneering Sustainable Alternative to Methyl Bromide

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ABSTRACT

Since 2010, Korea has been at the forefront, under the guidance of the Animal and Plant Quarantine Agency (APQA), in developing innovative phytosanitary treatment guidelines aimed at phasing out the use of methyl bromide (MB) due to its detrimental role in ozone layer depletion and the significant health and safety concerns for workers handling the fumigant in some practical cases. Central to this initiative is the adoption of ethyl formate (EF) products, including both liquid and cylinderized forms, as a pivotal solution for the treatment of imported sub-tropical and tropical fruits often infested with exotic pests such as scales and mealybugs. The effectiveness and safety profile of EF mark a transformative shift in quarantine pest management, offering an environmentally friendly and worker-safe alternative. The continual update of these guidelines marks Korea's significant shift away from MB, advocating for sustainable methods that serve as a model for the worldwide adoption of phytosanitary disinfestation, thereby promoting eco-friendly practices and improving worker safety.

Keywords: Phytosanitary guideline, Methyl bromide alternative, Ethyl formate, Quarantine

INTRODUCTION

Amid global efforts to phase out methyl bromide (MB) due to its environmental and health hazards, as outlined by the Montreal Protocol, its use in quarantine and pre-shipment (QPS) remains an exception. Nonetheless, the International Plant Protection Convention (IPPC) strongly urges the exploration of viable alternatives to MB in QPS (IPPC, 2009). The urgency for alternatives is underscored by MB's acute toxicity to fumigators and the potential for chronic exposure in work environments, particularly at low temperatures. Recent studies in Korea have highlighted the more severe health impacts on fumigators compared to those with indirect exposure, evidenced by variations in heart rate variability (HRV) indices and bromide ion levels (Park et al., 2020).

Since 2010, under the Animal and Plant Quarantine Agency (APQA)'s leadership, Korea has pioneered the development of innovative phytosanitary measures, notably adopting ethyl formate (EF) for treating imported and exported fruits. The transition to EF aligns with environmental and safety priorities, making it a cornerstone of Korea's phytosanitary strategy and setting a precedent for global phytosanitary treatment. This new disinfestation guideline in Korea not only mitigates the reliance on MB but also exemplifies a commitment to sustainable and health-conscious phytosanitary solutions.

GUIDELINES OF EF REGISTRATION AND PHYTOSANITARY DISINFESTATION IN KOREA

Ethyl formate (EF) is recognized as a safe and environmentally friendly chemical in the food industry, attributed to its long-standing use and classification as Generally Recognized as Safe (GRAS). Its adoption in quarantine applications has been bolstered by its non-ozone-depleting properties and enhanced worker safety profile. Consequently, the Korea Pesticide Regulation Authority has approved EF for quarantine use, acknowledging its efficacy and safety.

The Animal and Plant Quarantine Agency (APQA) in Korea has been at the forefront of developing EF-based phytosanitary disinfestation protocols. Since 2008, APQA has methodically progressed in crafting detailed protocols that utilize the distinct advantages of EF to enhance phytosanitary effectiveness (Tables 1 to 6). These guidelines are meticulously designed to tackle the specific challenges presented by a variety of quarantine pests affecting both imported and exported goods, representing a notable shift from traditional disinfestation methods that depended on methyl bromide (MB). This effort not only underscores Korea's dedication to adopting safer and more environmentally responsible quarantine disinfestation approaches but also has the potential to influence phytosanitary practices worldwide.

CURRENT GUIDELINES

Table 1. Ethyl formate phytosanitary disinfestation guidelines for mealybugs, aphids, mites, thrips, and whiteflies on fresh fruits (apple, pear, persimmon, grape, peach) using PVC-Tarp or fumigation chamber.

Purity	Dosage (g/m^3)		Minimum Ct Products	Temp.	Exposure time	Loading ratio
(%)	Usage	Amount of EF	($g\ h/m^3$)	(°C)	(h)	(t/m^3)
99	70	70	107	>5	4	< 0.2

Table 2. Ethyl formate phytosanitary disinfestation guidelines on tropical/subtropical fruits using PVC-Tarp or fumigation chamber.

Commodities	Target Insects	Purity (%)	Dosage (g/m^3)		Minimum Ct Products ($g\ h/m^3$)	Temp. (°C)	Exposure time (h)	Loading ratio (t/m^3)
			Usage	Amount of EF				
Citrus (oranges, grapefruits)	Mealybugs, Weevils	16.6	420	70	105	>5	4	-
		99	70					
Banana	Mealybugs	16.6	210	35	19	>13		< 0.19
		99	35					
Banana	Ants, Termites	99	70	70	78	>13		-
Kiwi	Mealybugs	16.6	210	35	-	>13		-
Kiwi	Mealybugs, Aphids, Mites, Thrips, Whiteflies	99	70	70	105	>5		
Blueberry	Flies	99	70	70	208	>5		< 0.05
Tropical/subtropical without specific disinfestation guidelines	Mealybugs, Aphids, Mites, Thrips, Whiteflies	99	70	70	105	>5		

Table 3. Ethyl formate + phosphine (PH_3) phytosanitary disinfestation guidelines for mealybugs on pineapple using PVC-Tarp or fumigation chamber.

Fumigants	Purity (%)	PH_3, EF Dosage (g/m^3)	Amount (g/m^3)	Temp. (°C)	Exposure Time (h)	Loading Ratio (t/m^3)
PH_3 + Ethyl formate ($C_3H_6O_2$)	2	1	50	>8	4	< 0.15
	16.7	25.1	150			

Table 4. Ethyl formate phytosanitary disinfestation guidelines for mealybugs, aphids, mites, thrips, whiteflies, ants, and termites on imported nursery plants using PVC-Tarp or fumigation chamber.

Purity (%)	Dosage (g/m^3)		Minimum Ct Products ($g\ h/m^3$)	Temp. (°C)	Exposure Time (h)	Loading Ratio (t/m^3)
	Usage	Amount of EF				
99	35	35	76	>15	4	< 0.05

Table 5. Ethyl formate phytosanitary disinfestation guidelines for ants and termites on miscellaneous goods (stone products, used machines, plastics) using PVC-Tarp or fumigation chamber.

Purity (%)	Dosage (g/m^3)		Minimum Ct Products ($g\ h/m^3$)	Temp. (°C)	Exposure Time (h)	Loading Ratio (t/m^3)
	Usage	Amount of EF				
99	35	35	78	>2	4	-

Table 6. Ethyl formate phytosanitary disinfestation guidelines on vegetables using PVC-Tarp or fumigation chamber.

Commodities	Target Insects	Purity (%)	Dosage (g/m^3)		Minimum Ct Products ($g\ h/m^3$)	Temp. (°C)	Exposure Time (h)	Loading Ratio (t/m^3)
			Usage	Amount of EF				
Sweet pumpkin	Mites	99	70	70	108	5-10	4	< 0.15
					59	10-15		
					42	>15		
Tomato	Whiteflies, Thrips	16.6	50	8.35	-	>13		-
Paprika	Whiteflies, Thrips, Aphids, *Agrotis spp.*	16.6	100	16.6	-	>13	2	-

GUIDELINES UNDER DEVELOPMENT

The Animal and Plant Quarantine Agency (APQA) in Korea, in collaboration with other international research groups, is advancing phytosanitary treatments by integrating EF with physical methods such as low temperatures and controlled atmospheres. This approach is specifically designed to target key international quarantine pests. The goal is to enhance the efficiency and sustainability of disinfestation efforts, establishing new guidelines for environmentally friendly quarantine practices. These developments could represent a significant advancement in global phytosanitary efforts, providing a safer and more effective alternative to MB.

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Ethyl Formate: A Promising Phytosanitary Alternative to Methyl Bromide for Combating Hitchhiking Insect Pests on Imported Non-Food Commodities

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ABSTRACT

Methyl bromide (MB), traditionally preferred for its effective disinfestation of various quarantined commodities, is now restricted in Korea due to environmental and fumigator safety concerns, particularly in imported subtropical and tropical fruits. This has led to increased interest in alternatives such as ethyl formate (EF). This study investigated EF's potential as an alternative, focusing on its efficacy against surrogate hitchhiking pests like flies, hemipterans, moths, and ants. The notable effectiveness of EF, coupled with its rapid dissipation post fumigation, underscores its practicality. The successful implementation in commercial trials positions EF as a more environmentally friendly and viable alternative to MB, indicating a move towards safer, more sustainable pest control methods in Korea.

Keywords: Ethyl formate, Methyl bromide alternative, Imported goods, Phytosanitary treatment

INTRODUCTION

There has been a growing trend of increasing imports of miscellaneous goods in South Korea. Consequently, the detection of quarantine insect pests in these goods is on the rise (APQA, 2023). Although the variety of imported/exported miscellaneous goods is expanding, previous guidelines for phytosanitary disinfestation were established only for certain types, leaving a gap in the protocol. Therefore, there is a need to establish new phytosanitary disinfestation guidelines for individual miscellaneous goods. Methyl bromide (MB) has been traditionally used for disinfestation during quarantine processes, but its use has been increasingly restricted due to concerns over ozone layer depletion and worker safety issues (Yang et al., 2016; Park et al., 2021). Ethyl formate (EF) fumigation, as one of the alternatives to MB, has been evaluated for various applications, including fruits, vegetables, and even non-food commodities (Kim et al., 2023; Kwon et al., 2024). Compared to MB, EF offers several advantages, including shorter fumigation times,

lower mammalian toxicity, environmental friendliness, enhanced worker safety, and no residual issues due to its rapid breakdown into formic acid and ethanol (Park et al., 2020). In this study, commercial trials on imported miscellaneous goods (cocopeat, plant debris) were conducted targeting *Tribolium castaneum* (Herbst) and *Lycoriella mali* (Fitch), which have demonstrated higher tolerance to EF fumigation among hitchhiking insect pests such as flies, moths, ants, and stink bugs.

MATERIALS AND METHODS

Insects and Chemical

Lycoriella mali was collected at a mushroom farm in Yeongcheon, Gyeongbuk, and *Tribolium castaneum* was obtained from the Kyungpook National University in Daegu. The *Lycoriella mali* and the *T. castaneum* samples were maintained in an insect rearing room at 24℃ and 60–70% relative humidity (r.h.) with a ratio of 16:8 [L:D] h. Ethyl formate (Fumate, >99% purity) was supplied by Safefume Inc., Korea.

Commercial Trial

Commercial trial on cocopeat against Lycoriella mali

A commercial trial on miscellaneous goods (cocopeat) was conducted in a 76.4 m³ imported container at Gamman Port in Busan. The experiment targeted cocopeat (20,900 kg; 27.3% loading ratio; w/v) imported from Indonesia. Based on preliminary experiments, EF (liquid, 99%) was applied at a concentration of 70 g/m³ using an SFM-II vaporizer for 4 h at 25°C. More than 150 *L. mali* 3^{rd}–4^{th} instar larvae, inoculated in petri dishes containing 2% agar media, were used with three replicates.

Commercial trial on cocopeat against Tribolium castaneum

A commercial trial involving miscellaneous goods (cocopeat) was conducted in a 76.4 m³ imported container at Gamman Port, Busan. The experiment focused on cocopeat (18,500 kg; 24% loading ratio; w/v) imported from Indonesia. Ethyl formate (liquid, 99%) was applied at a concentration of 70 g/m³ using an SFM-II vaporizer for 24 h at 25°C. More than 150 *T. castaneum* adults were used with three replicates for trial.

EF gas sampling and bioassay

Ethyl formate gas sampling was conducted at intervals of 10 min, 1, 2, 4, and 24 h using a portable EF monitor (Industrial Scientific Corp., MX6-IBRID®, Pittsburgh, Pennsylvania, USA) after the start of fumigation. The insects that were treated with EF were then transferred to the laboratory for efficacy assessment. Mortality was investigated after 3 d.

RESULTS

When 70 g/m^3 EF was applied for 4 h at 25℃ in a cocopeat container targeting *L. mali* 3^{rd}–4^{th} instar larvae, it was confirmed that the sorption of EF reached approximately 75% by the end of

the 4 h fumigation (Figs. 1 and 2). Additionally, it was verified that *L. mali* 3rd–4th instar larvae were completely controlled (Table 1).

When 70 g/m^3 of EF was applied for 24 h at 25℃ in a cocopeat container targeting *T. castaneum* adults, it was confirmed that the sorption of EF reached approximately 85% by the end of the 24 h fumigation (Fig. 3). Furthermore, it was verified that *T. castaneum* adults were completely controlled (Table 2).

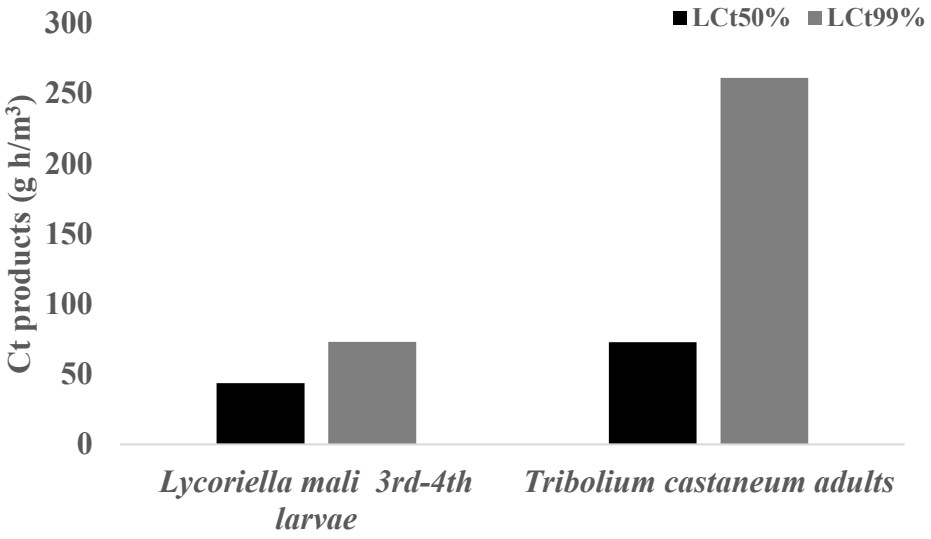

Fig. 1. Lethal Ct (Concentration × time) values of EF fumigation efficacy against hitchhiking insect pest *Lycoriella mali* based on 4 h fumigation and *Tribolium castaneum* based on 24 h fumigation at 25±2℃.

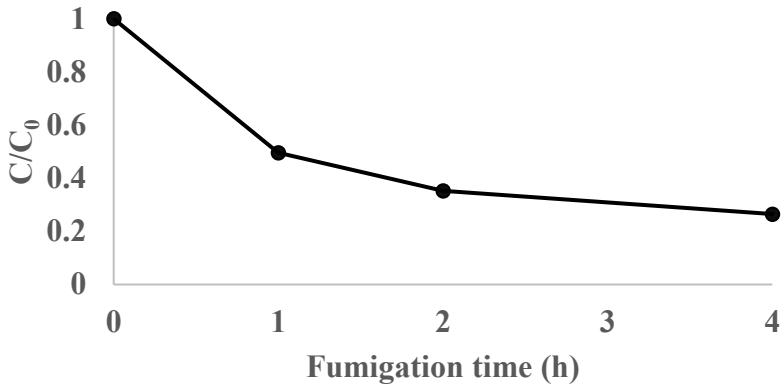

Fig. 2. Ethyl formate concentrations in imported cocopeat container to target hitchhiking insect pests *Lycoriella mali* 3rd–4th instar larvae (EF of 70 g/m^3 applied for 4 h at 25±2℃).

Table 1. Accumulated Ct products of EF and mortality of *Lycoriella mali* 3rd–4th instar larvae in 4 h EF fumigation on cocopeat container (76.4 m^3) at 25±2℃.

EF dosage (g/m^3)	Ct products ($g\ h/m^3$)	Total No. of tested	Total No. of dead	Mortality (%)
Untreated	-	276	0	0
70	151.3	611	611	100

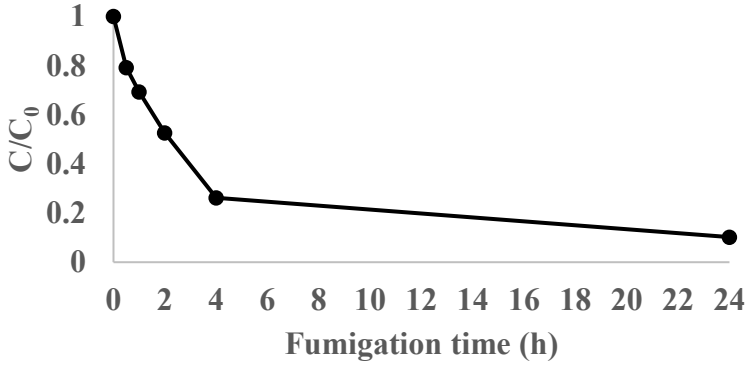

Fig. 3. Ethyl formate concentration in commercial cocopeat container to target *Tribolium castaneum* adults (EF of 70 g/m^3 applied for 24 h at 25±2℃).

Table 2. Accumulated Ct products of EF and mortality of *Tribolium castaneum* adult in 24 h EF fumigation on cocopeat container (76.4 m^3) at 25±2℃.

EF dosage (g/m^3)	Ct products ($g\ h/m^3$)	Total No. of tested	Total No. of dead	Mortality (%)
Untreated	-	356	0	0
70	797.5	1,333	1,333	100

CONCLUSIONS

The results indicate that an EF fumigation of 70 g/m³ is an effective phytosanitary disinfestation method for non-food commodities, such as cocopeat. A 4 h EF fumigation is sufficient to manage hitchhiking insect pests, while a 24 h EF fumigation is necessary for insects that are more directly embedded. Moreover, EF presents a viable alternative to MB for phytosanitary disinfestation against a variety of pests, including flies, moths, ants, and stink bugs. The development of guidelines for the phytosanitary use of liquid EF is advised to support its broader adoption toward further applications on non-food commodities.

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Paper No. CAF2024-A36

Nguyen KT, Han Y, McKirdy SJ, Du B, Ren Y (2024) Evaluation of ethyl formate fumigation coupled with cold storage to control Mediterranean fruit fly (*Ceratitis capitata*). Page 118. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Evaluation of Ethyl Formate Fumigation Coupled with Cold Storage to Control Mediterranean Fruit Fly (*Ceratitis Capitata*)

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ABSTRACT

Mediterranean fruit fly (Medfly), *Ceratitis capitata*, is one of the most destructive horticultural pests in the world for several decades. The Medfly has invaded the Mediterranean region, southern Europe, the Middle East, Western Australia, South and Central America, and Hawaii. More than 500 hosts, including fruits, vegetables, and nuts have been recorded being attacked by Medfly, which imposes significant economic losses to the horticultural industry globally. This study evaluated the effect of the combined treatment of ethyl formate (EF) and commercial temperature operation at 5°C for control of Medfly to reduce the chances of chilling injury incidences of fresh products during storage and quarantine. Different stages of Medfly including egg, 1st, 2nd, and 3rd instars were fumigated with EF at 15 to 45 mg/L in a purging flow system for 6 h in laboratory and then stored for 7 to 14 d at 1°C, 3°C, and 5°C. The combined treatment had significantly higher mortality than in EF fumigation alone. Furthermore, all stages of Medfly were completely controlled after combined treatment in 30 mg/L EF at 5°C for 14 d. These results suggest that the combined treatment of EF fumigation coupled with cold storage could offer a commercially available quarantine method for the management of Medfly.

Keywords: Fruit fly, *Ceratitis capitata*, Postharvest treatment, Quarantine, Fumigation, Ethyl formate, Cold storage

Paper No. CAF2024-F37

Ormanoglu N, Ferizli AG, Sen F, Emekci M (2024) Efficacy of high-pressure carbon dioxide applications against the dried fruit beetle, *Carpophilus hemipterus* (Coleoptera: Nitidulidae). Pp. 119–125. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Efficacy of High-Pressure Carbon Dioxide Applications Against the Dried Fruit Beetle, *Carpophilus hemipterus* (Coleoptera: Nitidulidae)

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ABSTRACT

Türkiye is the world's largest producer and exporter of dried fruits and nuts, such as figs, apricots, raisins, and hazelnuts, with a total annual export revenue of USD 1.5 billion. The control of insect pests in dried fruits and nuts relies heavily on the use of toxic fumigants. Increasing concerns about the adverse effects of pesticides on human, animal, and environmental health, as well as the need for shorter fumigation periods, are the driving forces for the development of alternative methods. Therefore, this paper focuses on high-pressure CO_2 treatments as an alternative to methyl bromide (MB) in Quarantine and Pre-shipment (QPS) applications, using *Carpophilus hemipterus* (L.) (Coleoptera: Nitidulidae), a major pest of dried figs, as the model organism.

All the experiments were conducted in a high-pressure chamber of 1 L volume under the constant temperature of 25°C and at 0.5 MPa (5 bar) pressures. The LT_{90} values for eggs, larvae, pupae, and adults were calculated as 6, 20, 6, and 9 h, respectively. At the longest exposure period of 21 h, statistically significant differences were found in terms of C* (brightness) and L* (color) values of the figs, without affecting the market value of the product negatively.

Keywords: Dried fruits and nuts, MB alternative, Dried fruit beetle, *Carpophilus hemipterus*, Life stages, LT_{50} values, High-pressure CO_2

INTRODUCTION

Türkiye is one of the countries with self-sufficiency and export potential in fruit production, both in terms of its production area and ecological structure. Preserving the market share of dried figs, one of the country's most significant export products, depends on obtaining the desired quality of the product and maintaining its quality throughout its shelf life. Since the prohibition of the potent fumigant methyl bromide (MB) in 2015 due to its adverse effects on the ozone layer, there has been no surge in research dedicated to exploring alternative methods for pest eradication in dried figs. A range of methodologies is at hand, notably featuring phosphine fumigation, sulfurylfluoride, high-pressure CO_2, modified atmosphere, freezing, and ozone applications (Donahaye and Navarro, 1989; Fields and White, 2002; Navarro et al., 2007; Şen et al., 2009; Hulasare et al., 2010; Aksoy et al., 2012; Navarro et al., 2014; Tütüncü and Emekci, 2014). Phosphine fumigation and freezing (shocking) techniques are commonly employed in Türkiye (Gençdağ et al., 2019). The primary objective of fumigating dried figs is to achieve comprehensive pest eradication while minimizing both dosage and duration. While MB fumigations typically require only 24 h for efficacy, achieving complete pest mortality with phosphine fumigations demands a longer application period, ranging from 3 to 15 d. Extensive research findings suggest that this prolonged exposure duration may accelerate the development of resistance in stored-product pests (Benhalima et al., 2004; Pimentel et al., 2009; Emery et al., 2011; Nayak et al., 2020). The deep freezing (shocking) method, which is a high-cost application, involves subjecting the product to temperatures of -15 to -20°C for 24 h (Yatkın and Emekci 2013).

As a viable alternative to toxic fumigants and extended post-harvest applications in dried fruits, carbon dioxide (CO_2) treatments represent a significant option. Carbon dioxide has a toxic effect on insects and many other pests when applied for sufficient time, at the appropriate temperature, and in high concentrations. Furthermore, its status as a safe gas is underscored by its absence of residue risk and non-flammable characteristics. When applied as a fumigant at atmospheric pressure, CO_2 exhibits a slower action compared with phosphine or MB. The marketing of dried figs typically commences around mid-August, with a significant portion of production, especially targeted towards European countries, being marketed until mid-November. Due to the pressure to enter the European market as early as possible, the duration of fumigation poses a significant concern for exporters (Sen et al., 2010). High-pressure CO_2 applications emerge as a significant alternative due to both the short duration of application and the ability to make the product available for consumption immediately after treatment (Riudavets et al., 2010).

The application of CO_2 gas under high-pressure conditions is utilized as a method particularly in combatting pests, especially in products with low moisture content. Due to the relatively high initial capital investment required for the equipment necessary for high-pressure CO_2 applications, its practical use is considered feasible for high-value products such as spices, nuts, figs, medicinal plants, and other specialty export items. Simultaneously, it can be deemed a viable alternative in circumstances necessitating short application durations, as seen in the case of figs. Additionally, the absence of residue risk and the non-use of any chemicals in these applications are anticipated to enhance the contribution of the product to the economy when exported as organic produce. In this study, the efficacy of 0.5 MPa (5 bar) high-pressure CO_2 for varying durations on *Carpophilus hemipterus* (Coleoptera: Nitidulidae), a pest affecting figs, was examined.

MATERIALS AND METHODS

Carpophilus hemipterus was reared in a climate-controlled chamber at 25°C and a relative humidity of 75±5%. The experiments involved utilizing the stages of egg, larva, pupa, and adult of *C. hemipterus*.

This study utilized a high-pressure reactor equipped with a stainless-steel vessel of 1000 mL capacity, capable of withstanding temperatures up to 50°C and pressures up to 50 MPa (5 bar). The device is equipped with two needle valves for gas inlet and outlet. Temperature and pressure can be digitally controlled. Tests were carried out in 25 mL test vials covered by mesh lids to facilitate air exchange. The experiments were set up with 5 replicates and 3 repetitions, each consisting of 20 individuals per replicate. The test vials introduced into the pressure system were subjected to CO_2 at a pressure of 0.5 MPa and 25°C. Test durations for all biological stages were extended at 1 h intervals starting from 1 h until the time when absolute mortality occurred. In all trials, the gas release time was set to 30 min. The control groups were placed in a climate chamber at 25°C without undergoing any further processing.

After the high-pressure CO_2 applications were completed across all biological stages, the effective duration of treatment for each pressure level was determined, and applications were conducted on dried fig fruits at those pressure and duration combinations. In trials conducted at 0.5 MPa pressure, the larval stage was observed to require the longest application duration, with a treatment period of 21 h being administered to achieve absolute mortality.

The experiments involving dried figs were carried out using 3 replicates and 3 repetitions, with each replicate containing 15 dried figs. Fifteen dried figs were placed into the high-pressure reactor, and a pressure of 0.5 MPa was applied for a duration of 21 h. The dried figs included in the experiment, along with the control group, were assessed for fruit color, firmness, moisture content, soluble solids content (SSC), titratable acidity (TA), and pH value. In the high-pressure experiments, exposure time x mortality values obtained for different biological stages were subjected to probit analysis using the POLO PC program (LeOra Software, 1994) to determine LT_{50}, LT_{90}, LT_{95}, LT_{99}, and LT_{P9} values. The data obtained from the fig quality analysis were subjected to analysis of variance using the IBM® SPSS® Statistics 19 (IBM, NY, USA) statistical package program, and differences among means were determined using the Duncan test ($P<0.05$).

RESULTS AND DISCUSSION

The impact of CO_2 applications at normal atmospheric pressure on all stages of the target pest varies depending on temperature, ranging from several days to several weeks (Banks and Annis, 1990; Fleurat-Lessard, 1990). However, when applied under high pressure, the application duration shortens. It has been observed that the solubility of gases increases under high pressure. Therefore, the toxic effect of CO_2 under high pressure is attributed to the increase in solubility in insect hemolymph, leading to a decrease in pH. Additionally, it has been noted that the rapid release of gas from the system also damages the insect's cell membranes, thus playing a role in the mortality of the pest (Stahl, 1985; Caliboso et al., 1994). Therefore, in this study, the gas release process was conducted uniformly for all applications, lasting for 30 min.

In the context of the effect of high-pressure CO_2 on *C. hemipterus*, there is a lack of existing literature. However, research conducted on other species indicates that the efficacy of high-pressure CO_2 treatments varies across different species and biological stages. According to Riudavets et al. (2010), research revealed that eggs of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) left for 8 h exhibited higher tolerance to 2 MPa CO_2 compared with eggs aged 1–4 d. Additionally, Ahmed and Hashem (2012) observed that eggs and pupae of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) displayed greater sensitivity to CO_2 in comparison to the larval stage. In their study evaluating the effect of high-pressure CO_2 on *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), Kutbay et al. (2011) reported contrary findings to other studies conducted on the same pest species, which suggested that the most tolerant stage was the egg. Instead, they found that larvae and pupae were more tolerant compared with eggs (Kutbay et al., 2011; Ahmed and Hashem, 2012). According to the probit data obtained in this study, it was observed that in applications conducted at 0.5 MPa pressure, the most tolerant biological stage was the larva (LT_{50} 11 h; LT_{90} 20 h). The values for other biological stages are as follows: eggs (LT_{50} 2 h; LT_{90} 6 h), pupae (LT_{50} 1 h; LT_{90} 6 h), and adults (LT_{50} 3 h; LT_{90} 9 h) (Table 1).

Table 1. The LT values (h) determined for all stages of *Carpophilus hemipterus* following the application of 0.5 MPa high-pressure CO_2.

Life Stage	Slope ± SE	LT_{50}[a] (hours)	LT_{90}[a] (hours)	LT_{95}[a] (hours)	LT_{99}[a] (hours)	LT_{P9}[a] (hours)	Chi-square	Heterogeneity
Egg	4.179± 0.116	2.992 (2.370-3.628)	6.061 (4.786-9.513)	7.404 (5.609-13.018)	10.778 (7.467-23.725)	27.105 (14.729-106.029)	453.09	41.19
Larva	4.977± 0.118	11.525 (10.800-12.279)	20.852 (18.908-23.695)	24.668 (21.940-28.837)	33.812 (28.912-41.810)	73.344 (56.590-104.757)	704,12	11,54
Pupa	2.536± 0.059	1.988 (1.488-2.438)	6.364 (5.123-8.779)	8.851 (6.781-13.541)	16.432 (11.242-31.161)	75.097 (37.663-249.303)	836.30	33.45
Adult	3.092± 0.105	3.523 (3.249-3.793)	9.148 (8.258-10.349)	11.990 (10.575-13.993)	19.915 (16.735-24.765)	69.257 (51.201-101.554)	84,029	3,00

[a] $P \leq 0.05$

In their study evaluating the effect of 0.01 and 0.05 MPa pressure CO_2 on the physical and chemical properties of dried apricot fruits after a 24 h application period, Sadeghi et al. (2021) found that increasing gas concentration had no significant effect on the color and firmness of dried apricots compared with the control group. They noted that color changes on the surface of the product were negligible and not perceptible to the naked eye (Sadeghi et al., 2021). In our study, however, it was determined that the L* value representing the lightness–darkness of the surface color and the C* value representing the brightness–vividness and dullness of the surface were lower in dried figs subjected to 0.5 MPa pressure (21 h) compared with the control group, and this difference was statistically significant (Table 2). Nevertheless, this difference was minimal, imperceptible to the naked eye, and thus would not diminish the market value of the product.

Regarding the chemical properties of dried fig fruits under high-pressure CO_2 at 0.5 MPa pressure (21 h), there was no statistically significant difference observed when compared with the control group in terms of firmness, moisture content, soluble solids content (SSC), titratable acidity (TA), and pH value (Table 3).

The quantity of research on high-pressure CO_2, both domestically and globally, remains limited. It is imperative to enhance the literature through further investigations into various pest species and dried fruits. In the current era, where eco-friendly approaches are favored, replacing traditional chemical-based pest control methods in agriculture, contributing to this field through research is of paramount importance. High-pressure CO_2 systems are associated with substantial initial investment costs, as systems capable of withstanding the typical application pressure of 2 MPa are typically imported. However, domestically manufactured systems resistant to lower pressures could offer a more cost-effective solution. Moreover, high-pressure CO_2 applications hold promise for increased future utilization, particularly in quarantine scenarios necessitating brief application durations and involving high-value exports like dried fruits.

Table 2. The effect of applying 0.5 MPa pressure for 21 h on the color (L*, a*, b*, C*, h°) of dried fig fruits.

Treatments	L*	a*	b*	C*	h°
Control	41.55±0.89 a†	7.46±0.96^{NS}	17.80±1.21^{NS}	19.30±1.35†	67.31±0.91^{NS}
0.5 MPa	36.66±1.08 b	6.71±0.28	14.90±0.82	16.34±0.85	65.76±0.39

†Significant, ^{NS}Not significant

Table 3. The effect of applying 0.5 MPa pressure for 21 h on the firmness (N), moisture content, soluble solids content (SSC), TA (g of citric acid 100 g^{-1} of dry matter) and pH value of dried fig fruits.

Treatments	Firmness (N)	Moisture Content (%)	SSC (%)	TA (%)	pH değeri
Control	7.59±0.51^{NS}	17.55±0.10^{NS}	64.89±0.38^{NS}	0.77±0.13^{NS}	4.70±0.14^{NS}
0.5 MPa	7.94±0.32	17.36±0.21	65.11±0.42	0.78±0.07	4.63±0.10

^{NS}Not significant

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Lourie AP, Walse SS, Corbett SM (2024) Hydrogen cyanide fumigation to control brown marmorated stinkbug *Halyomorpha halys* (Stål) (Hemiptera: Petatomidae). Page 126. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Hydrogen Cyanide Fumigation to Control Brown Marmorated Stinkbug *Halyomorpha Halys* (Stål) (Hemiptera: Petatomidae)

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ABSTRACT

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a generalist pest with numerous hosts that can cause significant agricultural damage. Moreover, BMSB has caused a substantial urban and commercial disturbance where it is found, as overwintering adults may seek refuge inside of dwellings, covered spaces, vehicles, and consignments. Because of their potential threat and ability to hitchhike overseas, BMSB are regulated as a quarantine pest in many countries. There is a high need for phytosanitary treatments of potential hosts and refuges to mitigate risk. Hydrogen cyanide (HCN) may serve a valuable role as another tool in postharvest fumigation protection of international borders and shows promising results as an alternative for methyl bromide (MB) fumigation. Exploratory HCN fumigations of nondiapausing and diapausing BMSB showed the effect of physiological state on the species' tolerance to fumigants. Effective treatments for nondiapausing BMSB occurred at lower exposures (Ct) and shorter lengths (1-1.5 mg/L h for 1 h and 2 h) than effective treatments for diapausing BMSB (20-30 mg/L h for 4 h and 6 h). Mortality assessments indicated HCN induced stupefaction of diapausing BMSB at the effective doses for nondiapausing BMSB. This phenomenon occurred when the specimens experienced paralysis for multiple days before slowly rebounding back to normal behavior. Stupefaction posed a barrier in analyzing diapausing BMSB response to HCN fumigations, but efforts were ongoing to better understand and analyze specimen mortality.

Keywords: Hydrogen, Cyanide, Stinkbug, Alternatives, Methyl Bromide, Postharvest, Fumigation

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Sakka MK, Gourgouta M, Athanassiou CG (2024) Insecticidal effect of low and high temperatures for the control of all life stages of *Oryzaephilus surinamensis* (L.) and *Ephestia kuehniella* Zeller. Pp. 127–133. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Insecticidal Effect of Low and High Temperatures for the Control of All Life Stages of *Oryzaephilus surinamensis* (L.) and *Ephestia kuehniella* Zeller

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ABSTRACT

Laboratory tests were carried out to examine the insecticidal effect of low and high temperatures on different life stages (adults, larvae, pupae, and eggs) of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). All individuals were exposed to different low and high temperatures ranging from -18 to -10°C and 30 to 55°C, respectively, and different exposure times from 1 to 168 h. Moreover, we assessed the efficacy of low/high temperatures outside the laboratory with small-scale experiments on artificially infested currants by both species. Regarding *O. surinamensis*, even though the adults, larvae, and pupae were less cold-tolerant than eggs, exposure to temperatures of -5°C for 168 h did kill all life stages of *O. surinamensis*, including the eggs. In contrast, the adults of *E. kuehniella* were not killed when exposed to -5°C for 168 h; however, exposure to temperatures of -15 °C at all exposure times did seem to kill all life stages of *E. kuehniella*. Regarding heat treatment, although the most heat-tolerant stage of *E. kuehniella* was shown to be the eggs, exposure above 50°C did kill all life stages of *O. surinamensis*, including the eggs, even after 1 h of exposure. Small-scale experiments showed low egg hatching at -15°C for low temperatures and above 38°C for high temperatures. The findings from this study indicated that species, temperature, and life stage are critical parameters for the effective control of the tested species. These results are expected to shed light on the use of low and high temperatures for stored-product disinfestation in realistic conditions.

Keywords: Cold, Heat, Stored products, Temperature, Exposure time, Moths, Coleoptera

INTRODUCTION

Postharvest insect species are responsible for qualitative and quantitative losses during storage of different commodities such as grains, nuts, and dried fruits (Athanassiou et al., 2016; Taddese et al., 2020; Berhe et al., 2022). Control strategies are mainly focused on the use of chemical treatments such as phosphine (Nayak et al., 2020; Machuca-Mesa et al., 2023).

However, the development of resistance to phosphine in many stored-product insects, including the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and the mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), has increased the use of alternative methods.

Various methods have been evaluated as alternatives to phosphine such as sulfuryl fluoride (Opit et al., 2016; Jagadeesan and Nayak, 2017), low oxygen (Sakka et al., 2020; 2022a), or extreme temperatures (Mahroof et al., 2003; Sakka et al., 2022b). For instance, Sakka et al. (2020) tested nitrogen treatment against phosphine-resistant and susceptible populations of *O. surinamensis*; the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and found that heat is effective regardless of the susceptibility to phosphine.

Extreme temperatures, as an alternative method to chemical controls, have been investigated by several studies (Athanassiou et al., 2018, 2021). However, most of the data available for extreme temperatures are based on laboratory experiments while there are few data on the efficacy of extreme temperatures in small-scale conditions. In an attempt to investigate the key parameters of cold and heat treatment, we evaluated all life stages of *O. surinamensis* and *E. kuehniella* at different exposure times and temperatures ranging from 5 to -18°C for cold and 30 to 55°C for heat treatment.

MATERIALS AND METHODS

Insects

Oryzaephilus surinamensis and *E. kuehniella* populations were reared at the Laboratory of Entomology and Agricultural Zoology at 25°C, 65% relative humidity (r.h.), and in continuous darkness. The diet used for the rearing was oats for *O. surinamensis* and a mixture of 600 g of wheat bran, 400 g cracked wheat, 50 g of brewer's yeast, 80 mL of honey, 80 mL of glycerin, and 60 mL of water for *E. kuehniella*. Eggs were 1–2 d old; pupae were <5 d old; larvae were used at their last instar; and adults were <1 wk.

Laboratory Tests

Cold tests

We evaluated six levels of cold (5, 0, -5, -10, -15, and -18°C) and six exposure intervals (1, 3, 6, 9, 24, and 168 h). The tests were carried out at the set controlled temperatures in a freezer that exists in the Laboratory of Entomology and Agricultural Zoology. Plastic cylindrical vials (3 cm in diameter, 8 cm in height, Rotilabo Sample tins Snap on lid, Carl Roth, Germany) were used for the tests. Within each vial, 10 individuals were placed (different vials for each species and life stage), along with a small quantity of food. Mortality of the exposed individuals was assessed after 1 d of the application, with the exception of eggs and pupae which were examined for hatch/emergence 7 d post exposure.

Heat tests
We evaluated four levels of heat (30, 45, 50, and 55°C) and four exposure intervals (60 min, 3 h, 12 h, and 24 h). The same procedure was followed, as described above, for mortality and replicates.

Field Tests

A series of field tests were conducted to evaluate low and high temperatures on insect control in the production facilities of the Agricultural Cooperatives' Union of Aeghion S.A. For each species, plastic cylindrical vials (7.5 cm in diameter, 8.8 cm in height) were used as experimental units. One day before the application of cold/heat, eggs of each species were placed in the vials filled with 20 g of Corinthian currants and were maintained in incubators set at 25°C and 65% r.h. In addition, separately for each trial, a series of vials were placed outside of the treated area and used as controls. At the end of the tests, the vials were placed in incubators set at 25°C and 65% r.h., and 65 d later, the emergence of adults was recorded. Three replicates and three sub-replicates for each temperature and exposure time were performed.

Data Analysis

The data were submitted to a two-way analysis of variance separately for each life stage (eggs, larvae, pupae, and adults) with exposure intervals and temperature as the main effects and mortality as the variable response. All analyses were conducted by using JMP 11 software (SAS Institute Inc., 2013). Means were separated by the Honest Significant Difference [HSD] test at 0.05.

RESULTS

Lab Trials

Low temperature tests
Table 1 presents part of the results of cold treatment against all life stages of *E. kuehniella* exposed to different temperatures and exposure intervals. In general, complete mortality was reported even after 1 h at -15°C in all life stages. At 0°C, low mortality was recorded after 1 and 3 h of exposure for *E. kuehniella*.

High temperature tests
Table 2 presents part of the results of heat treatment against all life stages of *E. kuehniella* exposed to different temperatures and exposure intervals. At 40°C and after 24 h of exposure, larvae and adults had not reached 100% mortality (like the pupae and eggs had). However, at 55°C, complete mortality was recorded for all life stages and exposure times.

Table 1. Mortality (% ± SE) of *Ephestia kuehniella* eggs, larvae, pupae, and adults exposed to different degrees of cold temperatures and exposure intervals (within each column, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05, *error df*=48).

Temperature ⟹ **Exposure (h) ⬇**	**0°C**	**-5°C**	**-10°C**	**-15°C**
Eggs				
1	15.6 ± 2.4C	81.1 ± 2.0B	100.0 ± 0.0	100.0 ± 0.0
3	47.8 ± 3.6B	82.2 ± 2.2B	100.0 ± 0.0	100.0 ± 0.0
6	48.9 ± 3.1B	84.4 ± 2.4B	100.0 ± 0.0	100.0 ± 0.0
9	51.1 ± 3.5B	100.0 ± 0.0A	100.0 ± 0.0	100.0 ± 0.0
24	100.0 ± 0.0A	100.0 ± 0.0A	100.0 ± 0.0	100.0 ± 0.0
168	100.0 ± 0.0A	100.0 ± 0.0A	100.0 ± 0.0	100.0 ± 0.0
Larvae				
1	0.0 ± 0.0D	4.4 ± 2.4E	93.3 ± 2.3B	100.0 ± 0.0
3	8.9 ± 2.6CD	6.7 ± 2.3DE	95.6 ± 1.7AB	100.0 ± 0.0
6	11.1 ± 2.6C	16.7 ± 4.4D	100.0 ± 0.0A	100.0 ± 0.0
9	16.7 ± 2.3C	61.1 ± 2.6C	100.0 ± 0.0A	100.0 ± 0.0
24	52.2 ± 3.2B	77.8 ± 3.2B	100.0 ± 0.0A	100.0 ± 0.0
168	100.0 ± 0.0A	100.0 ± 0.0A	100.0 ± 0.0A	100.0 ± 0.0
Pupae				
1	0.0 ± 0.0C	25.5 ± 2.4E	77.8 ± 2.2	100.0 ± 0.0
3	0.0 ± 0.0C	41.1 ± 2.0D	100.0 ± 0.0B	100.0 ± 0.0
6	5.6 ± 2.4BC	55.6 ± 1.7C	100.0 ± 0.0B	100.0 ± 0.0
9	10.0 ± 2.9B	65.6 ± 1.7B	100.0 ± 0.0B	100.0 ± 0.0
24	11.1 ± 3.1B	100.0 ± 0.0A	100.0 ± 0.0B	100.0 ± 0.0
168	100.0 ± 0.0A	100.0 ± 0.0A	100.0 ± 0.0B	100.0 ± 0.0
Adults				
1	56.7 ± 4.1D	67.8 ± 2.2D	83.3 ± 2.3B	100.0 ± 0.0
3	65.6 ± 2.9CD	70.0 ± 2.3D	100.0 ± 0.0A	100.0 ± 0.0
6	66.7 ± 1.7CD	78.9 ± 2.6C	100.0 ± 0.0A	100.0 ± 0.0
9	70.0 ± 2.9C	84.4 ± 1.7BC	100.0 ± 0.0A	100.0 ± 0.0
24	82.2 ± 2.2B	87.8 ± 1.5B	100.0 ± 0.0A	100.0 ± 0.0
168	100.0 ± 0.0A	100.0 ± 0.0A	100.0 ± 0.0A	100.0 ± 0.0

Table 2. Mortality (% ± SE) of *Ephestia kuehniella* eggs, larvae, pupae, and adults exposed to different degrees of hot temperatures and exposure intervals (within each column, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05, *error df=48*).

Temperature ⟶ **Exposure (h) ⇩**	**40°C**	**50°C**	**55°C**
Eggs			
1	0.0 ± 0.0	96.7 ±1.7B	100.0 ± 0.0
3	0.0 ± 0.0	100.0 ± 0.0A	100.0 ± 0.0
12	0.0 ± 0.0	100.0 ± 0.0A	100.0 ± 0.0
24	0.0 ± 0.0	100.0 ± 0.0A	100.0 ± 0.0
Larvae			
1	10.0 ± 3.7C	100.0 ± 0.0	100.0 ± 0.0
3	12.2 ± 4.0C	100.0 ± 0.0	100.0 ± 0.0
12	63.3 ± 3.7B	100.0 ± 0.0	100.0 ± 0.0
24	95.6 ± 2.4A	100.0 ± 0.0	100.0 ± 0.0
Pupae			
1	36.7 ± 1.7C	100.0 ± 0.0	100.0 ± 0.0
3	58.9 ± 2.0B	100.0 ± 0.0	100.0 ± 0.0
12	100.0 ± 0.0A	100.0 ± 0.0	100.0 ± 0.0
24	100.0 ± 0.0A	100.0 ± 0.0	100.0 ± 0.0
Adults			
1	6.7 ± 2.3C	100.0 ± 0.0	100.0 ± 0.0
3	10.0 ± 3.3C	100.0 ± 0.0	100.0 ± 0.0
12	71.1 ± 2.6B	100.0 ± 0.0	100.0 ± 0.0
24	100.0 ± 0.0A	100.0 ± 0.0	100.0 ± 0.0

Field Trials

Table 3 presents part of the results of cold and heat treatment against eggs of *O. surinamensis* exposed to different temperatures and exposure intervals. In general, low or no adult emergences were recorded even after just 1 h at -10°C. At -10°C, no adults were recorded at all exposure times. Regarding heat treatment, low numbers of adult emergences were recorded at all exposure times, and the emergence of adults was not eliminated by either of the hot temperatures.

Table 3. Adult emergence (adults/vial) of *Oryzaephilus surinamensis* eggs exposed to different degrees of cold and hot temperatures and exposure intervals (within each column and treatment, means followed by the same uppercase letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05, *error df*=26).

Exposure (h) ⇓	**Temperature** ⟶		
	-5°C	**Cold**	**-10°C**
1	1.4 ± 0.3A		0.0 ± 0.0
6	0.0 ± 0.0B		0.0 ± 0.0
12	0.6 ± 0.2B		0.0 ± 0.0
	35°C	**Hot**	**38°C**
1	2.0 ± 0.3A		1.8 ± 0.3A
6	1.8 ± 0.3A		0.3 ± 0.2B
12	1.3 ± 0.4A		0.9 ± 0.2B

DISCUSSION

The results of the present study indicate that extreme temperatures are effective against all life stages of *O. surinamensis* and *E. kuehniella*. In a similar study, Athanassiou et al. (2018) tested different life stages of *O. surinamensis* (i.e., adults, larvae, and eggs) and found that eggs were more cold-tolerant than larvae. Moreover, Andreadis et al. (2012) reported that all life stages of *E. kuehniella* could not survive at -12.5°C. Our results demonstrate that extreme temperatures can be used as a viable alternative method. We have also demonstrated that adults and larvae are the most cold-tolerant life stages and that larvae and eggs are the most heat-tolerant life stages. Finally, temperature, exposure time, and species and their life stage are critical parameters for the efficacy of extreme temperatures.

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Prospect of Sulfur Dioxide Fumigation for Postharvest Pest Control

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ABSTRACT

Despite sulfur dioxide (SO_2) being a very old pesticide, very few studies and very little practical use for SO_2 in postharvest pest control have been reported. This is likely due to its high sorption on stored products and phytotoxicity to fresh products. We studied SO_2 fumigation for controlling postharvest pests and concluded that SO_2 fumigation has good potential for postharvest pest control on fresh and stored products. Insect pests of both fresh and stored products were very susceptible to SO_2 fumigation. Western flower thrips were effectively controlled in 30 min fumigations with 0.3-0.5% SO_2 at a low temperature of 5°C without injuries to navel oranges or peppers. However, the treatment caused severe injuries to broccoli and increased darkened lenticles on apples. Fresh products with a smoothy wax layer had better tolerance to SO_2 fumigation. Longer SO_2 fumigations at low concentrations were also effective in controlling mealybugs on table grapes without phytotoxicity. For stored-product insects, 3 h fumigation with 2% SO_2 effectively controlled all life stages of the navel orangeworm. Effective control of navel orangeworm larvae in infested pistachios was also demonstrated in SO_2 fumigations of pistachios with continued injections of SO_2 to compensate for sorption. The confused flour beetle and rice weevil (at different life stages) were also effectively controlled in 3 h fumigations with 0.5-2% SO_2. There were considerable variations among different life stages and species in susceptibility to SO_2 fumigation. These studies show that SO_2 fumigation can be used safely and effectively on select fresh products with smooth waxy cuticles. For stored products, a relatively short SO_2 fumigation with continuous SO_2 injection is technically feasible and can be used to control stored-product insects. Therefore, SO_2 fumigation shows bright prospect as a safe and effective alternative treatment for postharvest pest control on fresh and stored products.

Keywords: Sulfur dioxide, Fumigation, Postharvest pest control, Phytotoxicity, Fresh fruit, Stored-product insect

INTRODUCTION

Elemental sulfur is believed to be one of the earliest chemical pesticides. Used as a liquid or powder, the acidic nature of sulfur inhibits the growth of mold. Solutions of lime sulfur were once used as dips to destroy lice. Sulfur dioxide (SO_2), which is generated by burning elemental sulfur, has been used to inhibit the respiration of insects and other small pests. However, most uses are in

food preservation and for microbial control (Green, 1976). Sulfur dioxide is registered as a fungicide and commonly used to control the gray mold fungus, *Boitrytis cinerea*, on table grapes, to prevent the spoilage and oxidation of wine, and to sanitize equipment used in wineries. Although SO_2 also has a long history of being used as a pesticide, it has not been used to control postharvest pests in modern times when other fumigants such as methyl bromide and phosphine became available. However, our recent and current studies, together with other studies over the past 30 years, all show that SO_2 has good potential to become a viable methyl bromide alternative for postharvest pest control on fresh and stored products. In this article, past and current SO_2 research is reviewed, and the prospect of SO_2 fumigation for postharvest pest control is also discussed. Specific recommendations are also made for SO_2 fumigation research and treatment development.

SULFUR DIOXIDE FUMIGATION METHODS AND EFFICACY

There are 13 pest species that have been reported to be affected by SO_2 fumigation (Table 1). Most studies show that SO_2 fumigation has good efficacy against postharvest pests. In the Mitcham et al. (2005) study, a 30 min fumigation with 1% SO_2 combined with 6% CO_2 at 19.5°C for quarantine treatment of the black widow spider (*Latrodectus hesperus*) on table grapes was studied for its safety on table grape quality without inclusion of the black widow spiders. The Pacific spider mite (*Tetranychus pacificus* McGregor) and the two-spotted spider mite (*T. urticae Koch*) are two pests of grape vines. Exposures of Pacific spider mites and two-spotted spider mites to very low levels (0.2-1.4 ppm) of SO_2 release pads for mold control on table grapes at 0.4-1.7°C resulted in over 95% mortality of both species in 6 and 3 wk, respectively (Yokoyama et al., 2001). These studies show that mites are very susceptible to SO_2 fumigation. Using SO_2 release pads for quality maintenance of harvest grapes can also control mites, thus making SO_2 fumigation practical for mite control.

The grape mealybug (*Pseudococcus maritimus (Ehrhorn)*) and the vine mealybug (*P. ficus* (Signoret)) are two major vineyard pests in California. Mitcham and Zhou (1998) reported that 30 min fumigations with 0.5 and 0.75% SO_2 at 20°C resulted in 73 and 100% control of grape mealybug crawlers, respectively. In the Liu (2019) study of the responses of grape mealybugs and vine mealybugs to SO_2 fumigation, different mixes of SO_2, N_2, and air were constantly released into fumigation chambers to maintain stable SO_2 concentrations under a normal oxygen level or under an ultralow oxygen (ULO) level of ≈30 ppm. Fumigations with 100 ppm (0.01%) SO_2 provided an effective control of eggs and nymphs/adults of both mealybugs in ≥3 d under normal oxygen or ULO. The difference between SO_2 fumigations under normal atmosphere and ULO was relatively small. Therefore, considering the additional cost to establish and maintain ULO conditions, ULO does not bring any benefits to SO_2 fumigation. Fumigations of 1 d with 0.04% SO_2 resulted in 89.8 egg mortality and 100% nymph/adult mortality for grape mealybugs and 100% mortality for vine mealybug eggs and nymphs/adults (Liu, 2019).

The omnivorous leafroller (*Platynota stultana (*Walsingham)) is a major pest of grapes. In the Vail et al. (1991) study of the omnivorous leafroller, 3rd instar larvae were exposed to SO_2 for 0.2, 0.4, 0.6, 0.8, and 1 h in a constant-flow system with continuous SO_2 monitoring. The treatments with 95% and complete control had CT products of 1,176 and 1,626 ppm h, respectively (Vail et al., 1991). In the Yokoyama et al. (1999) study, a combination of low temperature (0-1°C) storage

and 0.2-1 ppm SO_2 from SO_2 slow-release pads was tested against 2nd instar larvae of the omnivorous leafroller, and complete control was achieved in 3 wk (Table 1) (Yokoyama et al., 1999).

The western flower thrips (*Frankliniella occidentalis*) is native to North America and is a major pest on a wide variety of horticultural crops. Because the western flower thrips is quarantined in some countries, a quarantine treatment is needed on many exported fresh fruits and vegetables. In an early study, 1 wk exposure to 0.3 ppm SO_2 emitted from slow-release pads provided 100% control of this pest (Yokoyama et al., 2001). Short SO_2 fumigations were also demonstrated to be effective against western flower thrips. A 30 min fumigation with 0.3% SO_2 at 5°C caused 98.9% mortality of the thrips. Complete control of the pest was achieved in 30 min fumigations with 0.5% SO_2 and 1 h fumigations with 0.3% SO_2 at 5°C (Table 1) (Liu, 2024a).

The blueberry maggot (*Rhagoletis mendax* (Curran)) and the spotted wing drosophila (*Drosophila suzukii* (Matsumura)) are internal feeding insects of soft fruits that were subjected to SO_2 fumigation. The blueberry maggot is native to North America and infests ripening blueberries, and postharvest treatment is required for the export of blueberries to overseas markets (Abeli et al., 2021; Hubhachen et al., 2023). The spotted wing drosophila is a major pest of small fruits such as strawberries, cherries, and blueberries in Europe and North America, and it is also quarantined in some countries. Abeli et al. (2021) reported that 2 h fumigations with 2.2% SO_2 at 10°C followed by 21 d cold storage at 0.5°C resulted in the effective control of blueberry maggot larvae. However, cold storage alone also caused >99% mortality of blueberry maggot larvae in blueberries, indicating the importance of cold storage in the postharvest control of the pest (Abeli et al., 2021). In another recent study, spotted wing drosophilae and blueberry maggots in infested blueberry fruit were effectively controlled with a sequential combination of a 30 min fumigation with 1% SO_2 and 6% CO_2 followed by 12 d storage at 2°C or 6 d storage at -0.5°C (Hubhachen et al., 2023). However, longer fumigations of 3 d with 0.05% (500 ppm) SO_2 at 22°C caused 100% mortality of blueberry maggot larvae and spotted wing drosophila larvae in fruit without subsequent cold storage (Hubhachen et al., 2023). These results show that SO_2 fumigation is effective against internal feeding insects such as the blueberry maggot and the spotted wing drosophila and that cold storage is also an important factor in managing internal pests like the larvae of the blueberry maggot and the spotted wing drosophila in small fruits.

The confused flour beetle (*Tribolium confusum*) and the rice weevil (*Sitophilus oryzae*) are two major pests of stored products. Fumigations of 24 h with 1.5% SO_2 in 95% CO_2 atmospheres at 25°C had complete control of adults of both the confused flour beetle and the rice weevil (Riudavets et al., 2014). However, exposure to 95% CO_2 in controls also had 43.7 and 81.2% adult mortality for the confused flour beetle on wheat flour and the rice weevil on rice, respectively. Fumigations with a lower SO_2 dose of 0.15% resulted in only 39.4% mortality of confused flour beetle adults on wheat flour, and fumigations with 0.45% resulted in only 18.7% mortality of rice weevil adults on rice (Table 1) (Riudavets et al., 2014). In this study, the specific treatments cited above include 20 g wheat flour for the confused flour beetle and 50 g brown rice for the rice weevil in 200 mm diameter glass desiccators. The desiccators with insects and foods were flushed continuously with gas mixtures with proper levels of CO_2 and SO_2 to conduct fumigation treatments.

However, no data on the amount of SO_2 injected or the levels of SO_2 maintained in fumigation chambers were reported (Riudavets et al., 2014).

In a more recent study, 3 h SO_2 fumigations were demonstrated to be effective against the confused flour beetle and the rice weevil (Liu, 2024b). However, there are considerable variations in susceptibility to SO_2 fumigation between the two species and among different life stages (Table 1). The confused flour beetle is more susceptible to SO_2 fumigation than the rice weevil. Three hour fumigations with 0.5% SO_2 resulted in 100% mortality of confused flour beetle adults. For the rice weevil, 3 h fumigations with 1.5% SO_2 only resulted in 96.5% adult mortality. Confused flour beetle adults are also more susceptible to SO_2 fumigation than eggs. In contrast, rice weevil adults are more tolerant to SO_2 fumigation than eggs, as suggested by the almost equivalent mortality rates but higher SO_2 concentration needed for rice weevil adults than eggs (Table 1) (Liu, 2024b). Three hour fumigations with 2% SO_2 resulted in 100% mortality of all immature life stages of the confused flour beetle in the infested diet. In contrast, the same fumigation treatment only resulted in 87.5% mortality of all rice weevil immature life stages (Table 1) (Liu, 2024b). This study demonstrates that both species can be controlled effectively with 3 h SO_2 fumigations.

The Indian meal moth (*Plodia interpunctella*) is another major stored-product insect. Fumigations with SO_2 at 66–133 g/m^3 (2.3–4.7%) doses for 3–6 h at 28°C resulted in 81.4–94.3% mortality of eggs, larvae, and pupae of this pest, while controls had only 28.6% mortality (Table 1) (Rezanejad et al., 2022). Sulphur dioxide fumigations with a much-reduced concentration of 0.05% resulted in 100% control of Indian meal moth eggs in 24 h at 2 and 15°C. At 0.07% concentration, 24 h SO_2 fumigations at 2°C resulted in >95% mortality of Indian meal moth larvae and pupae (Table 1) (Liu unpublished).

The navel orangeworm (*Amyelois transitella*) is a major postharvest pest of tree nuts including almonds, walnuts, and pistachios. Sulphur dioxide fumigation is effective against the eggs, larvae, and pupae of the navel orangeworm (Table 1). However, there are considerable variations among these different life stages in susceptibility to SO_2 fumigation (Liu, 2023). Eggs were most susceptible to SO_2 fumigation, and 3 h fumigations with 0.2% SO_2 at 20°C resulted in 100% mortality of eggs. Pupae were more susceptible to SO_2 fumigation than larvae, and 3 h fumigations with 1% SO_2 at 20°C gave complete control of pupae. There were also variations among different ages of the larvae, and small larvae were more susceptible to SO_2 fumigation than large larvae. Complete control of large larvae in infested pistachios was achieved in 3 h fumigations with 2% SO_2 at 20°C (Liu, 2023).

Although a few pests were only subjected to long term fumigations with very low SO_2 levels, they are also likely to be controlled with short fumigations based on their susceptibility to SO_2 (Table 1). The effective control of insects and mites with short SO_2 fumigations of 30 min to 3 h indicates high efficacy of SO_2 fumigation against postharvest pests and good potential for postharvest pest control. Despite the fact that SO_2 has had a long history of being available for pest control, the number of studies on SO_2 fumigation is disproportionately lower than on most other pesticides. This is likely due to its phytotoxicity to fresh fruit and vegetables and high sorption on stored products.

Table 1. Select SO_2 fumigation treatments against postharvest pests of fresh and stored products.

Pest species	Life stages	SO_2 fumigation treatments	Mortality (%)	References
Black widow spider	adult	1% SO_2, 6% CO_2, 0.5h, 19.5°C	n/a	Mitcham et al. 2005
Pacific spider mite	nymph/adult	0.2-1.4ppm SO_2, 6wk, 0.4-1.7°C	98.0	Yokoyama et al. 2001
Two-spotted spider mite	nymph/adult	0.3-1.4ppm SO_2, 3wk, 0.4-1.7°C	95.9	
Grape mealybug	crawler	0.5% SO_2, 0.5h, 20°C	73.0	Mitcham and Zhou 1998
		0.75% SO_2, 0.5h, 20°C	100	
	egg	0.01% SO_2, 3d, 2°C	85.8	Liu 2019
		0.01% SO_2, 3d, 2°C, ULO[a]	99.5	
		0.04-0.05% SO_2, 1d, 2°C	89.8-95.8	
	nymph/adult	0.01% SO_2, 3d, 2°C	99.0	
		0.01% SO_2, 3d, 2°C, ULO	100	
		0.04-0.05% SO_2, 1d, 2°C	100	
Vine mealybug	egg/nymph/adult	0.01% SO_2, 3d, 2°C, ULO	100	Liu 2019
		0.04% SO_2, 1d, 2°C	100	
Omnivorous leafroller	larva	0.16% SO_2, 1h, 22°C	100	Vail et al. 1991
		0.2-1ppm SO_2, 3wk, 1°C	100	Yokoyama et al. 1999
		0.6-1.1ppm SO_2, 6wk, 0.4-1.7°C	100	Yokoyama et al. 2001
Western flower thrips	larva/adult	0.3ppm SO_2, 1wk, 0.4-1.7°C	100	Yokoyama et al. 2001
		0.3% SO_2, 1h, 5°C	100	Liu 2024a
		0.5% SO_2, 0.5h, 5°C	100	
Blueberry maggot	larva[b]	2.2% SO_2, 2h, 10°C→21d, 0.5°C[c]	>99	Abeli et al. 2021
		21d, 0.5°C (control)	>99	
		1% SO_2, 6% CO_2, 0.5h, 22°C→12d, 2°C	100	Hubhachen et al. 2023
		1% SO_2, 6% CO_2, 0.5h, 22°C→6d, -0.5°C	100	
		0.05% SO_2, 3d, 22°C	100	
Spotted wing drosophila	larva[b]	0.05% SO_2, 3d, 22°C	100	Hubhachen et al. 2023
Confused flour beetle	adult	0.15% SO_2, 95% CO_2, 1d, 25°C	39.4	Riudavets et al. 2014
		1.5% SO_2, 95% CO_2, 1d, 25°C	100	
		95% CO_2, 1d, 25°C	43.7	
		0.5% SO_2, 3h, 20°C	100	Liu 2024b
	egg	1% SO_2, 3h, 20°C	97.3	
		1% SO_2, 3h, 20°C	98.6	
	egg/larva/pupa	2% SO_2, 3h, 20°C	100	

Rice weevil	adult	0.45% SO_2, 95% CO_2, 1d, 25°C	18.7	Riudavets et al. 2014
		1.5% SO_2, 95% CO_2, 1d, 25°C	100	
		95% CO_2, 1d, 25°C	81.2	
		1.5% SO_2, 3h, 20°C	96.5	Liu 2024b
	egg	1% SO_2, 3h, 20°C	95.2	
	egg/larva/pupa	1% SO_2, 3h, 20°C	69.4	
		2% SO_2, 3h, 20°C	87.5	
Indian meal moth	egg/larva/pupa	66g/m^3, 3-6h, 28°C	92.8-94.3	Rezanejad et al. 2022
		133g/m^3, 3-6h, 28°C	81.4-82.9	
	egg	0.05% SO_2, 24h, 2&15°C	100	Liu unpublished
	larva/pupa	0.07% SO_2, 24h, 2°C	>95	
Navel orangeworm	egg	0.2% SO_2, 3h, 20°C	100	Liu 2023
	larva[d]	2% SO_2, 3h, 20°C	100	
	pupa	1% SO_2, 3h, 20°C	100	

[a]: ULO: Ultralow oxygen environment with ≈30 ppm O_2.
[b]: Larvae in blueberries.
[c]: The "→" denotes SO_2 fumigation was followed by cold storage.
[d]: Navel orangeworm larvae were at 6th instar in pistachios, and complete control was also achieved in large-scale fumigation of pistachios.

SAFETY OF SO_2 FUMIGATION TO POSTHARVEST QUALITY OF FRESH FRUIT AND VEGETABLES

Sulphur dioxide is commonly known to cause phytotoxicity to plants as based on the effects of atmospheric SO_2 pollutants on plants (Guderian, 1977). However, there is a severe lack of publications regarding the phytotoxicity of SO_2 fumigation to fresh fruit and vegetables. Several studies show that harvested grapes can tolerate long term exposure to low levels of SO_2 from slow-release pads to control mold as well as mites (Yokoyama et al., 2001), shorter exposures of 1–3 d to 0.01–0.04% SO_2 for controlling mealybugs (Liu, 2019), and very short treatment of 0.5 h with high levels of 1% SO_2 to control black widow spiders (Mitcham et al., 2005). These studies show that certain SO_2 fumigation treatments for pest control have no phytotoxicity to harvested grapes. In a more recent study, 30 min fumigations with 0.3–0.5% SO_2 for control of western flower thrips on select fresh fruit and vegetables had no phytotoxicity to bell peppers or navel oranges. The treatment caused severe discoloration of broccoli. For apples, the treatment caused stains to lenticles but did not cause discoloration of cuticles (Liu, 2024a). Based on the differences in phytotoxicity to different fresh products, it is concluded that a smooth waxy cuticle is a critical factor for tolerance of fresh products to SO_2 fumigation. Since many fresh products have smooth waxy cuticles, they likely can tolerate SO_2 fumigations for controlling certain external pests. Therefore, SO_2 has good potential to be used for postharvest pest control on fresh fruit and vegetables.

EFFECTS OF SORPTION ON SO_2 FUMIGATION DESIGN FOR STORED-PRODUCT INSECT CONTROL

High SO_2 sorption on stored products creates difficulties to maintain desired concentrations during fumigation for pest control. Therefore, there are needs for innovative methods for SO_2 fumigation research and applications in exploring the potential of SO_2 fumigation for postharvest pest control. To overcome the effects of sorption in evaluating insect susceptibility to SO_2 fumigation, a constant-flow system such as reported systems (Vail et al., 1991; Liu, 2019) is recommended in conducting SO_2 fumigation research. For small-scale fumigation tests without a constant-flow system, it is important to minimize the quantity of materials that will adsorb SO_2 in fumigation chambers and to use large fumigation chambers. Given the fact of an expected decline in SO_2 concentration during fumigation, monitoring SO_2 concentrations during fumigation is critical for ensuring that the desired SO_2 exposures are achieved and for preventing false outcomes of ineffectiveness of SO_2 fumigation treatments. Multiple injections may be needed to maintain desired SO_2 levels.

Past studies have shown that short fumigation treatments of 30 min to 3 h are sufficient to control all pests (Liu, 2023, 2024a, 2024b). Therefore, short SO_2 fumigation should be studied and developed for postharvest pest control. Shorter treatments are expected to have less SO_2 adsorbed on stored products than longer treatments. As demonstrated with the SO_2 fumigation of pistachios, SO_2 is continuously adsorbed on pistachios during 24 h fumigations (Liu, 2023). For practical uses of SO_2 fumigation on stored products, shorter fumigations are expected to have less SO_2 adsorbed and therefore shorter durations for desorption after fumigation. Sulphur dioxide sorption on pistachios is also significantly lower at a lower temperature of 5°C than at higher temperatures of 15 and 25°C. However, the magnitude of the difference is relatively small, suggesting that SO_2 fumigations can be conducted at a wide range of temperatures without substantial effects on sorption (Liu, 2023).

Active air circulation is also a critical factor in the SO_2 fumigation of stored products. Because stored products have a high capacity to adsorb SO_2, it is not expected that SO_2 will be able to permeate far throughout stored products to control insects in the absence of active air circulation, as this air movement is needed to carry SO_2 throughout the products being fumigated. Under conditions of inadequate air circulation, rapid sorption on fumigated products can lead to high SO_2 levels near SO_2 release points and no SO_2 at locations far from the SO_2 release points. In the SO_2 fumigations of pistachios for controlling the navel orangeworm, air in the fumigation chamber was circulated throughout the pistachios with an air blower, and the navel orangeworm larvae at different positions were all killed (Liu, 2023). On the other hand, SO_2 reacts with moisture in air to form sulfuric acid which is corrosive to certain metals. Therefore, special precaution needs to be taken in designing an air circulation system for SO_2 fumigation. Corrosion resistant air blowers or fans should be used, and exposed metal elements should be properly sealed or coated.

DISCUSSION

Past SO_2 fumigation studies have demonstrated high efficacy of SO_2 fumigation against all tested pests. The effective control of mealybug crawlers and western flower thrips in short fumigations of 30 min as well as the effective control of stored-product insects including the confused flour beetle, rice weevil, and navel orangeworm in 3 h demonstrated a higher efficacy of SO_2 fumigation than most other fumigants. The effective control of blueberry maggot larvae in blueberries and spotted wing drosophila larvae in blueberries showed that SO_2 fumigation is also effective against certain internal feeding insects. However, practical applications of SO_2 fumigation on fresh produce are largely dependent on whether a select SO_2 fumigation is safe for the postharvest quality of specific fresh products. The effective control of western flower thrips on bell peppers and navel oranges without phytotoxicity as well as the effective control of mealybugs without negative impact on postharvest quality of table grapes indicate good potential of SO_2 fumigation on certain fresh products (Liu, 2019, 2024a).

In three studies, SO_2 fumigations were conducted under high CO_2 tensions. However, there was no test on the synergism between SO_2 and CO_2 in toxicity against any pest (Mitcham et al., 2005; Riudavets et al., 2014; Hubhachen et al., 2023). High CO_2 tensions have been reported to be toxic to insects, and this toxicity could be due to CO_2 toxicity or O_2 depletion or both (Annis and Morton, 1997). It is unknown how much of a contribution the addition of CO_2 makes to insect mortality in response to combinations of SO_2 and CO_2. However, given the fast action and high efficacy of SO_2 fumigation (Vail et al., 1991; Mitcham and Zhou, 1998; Rezanejad et al., 2022; Liu, 2023, 2024a, 2024b), even if CO_2 does contribute to insect mortality, adding CO_2 into the SO_2 fumigation mix may only marginally improve the efficacy of the treatment and may not be cost-effective.

Signs of phytotoxicity of SO_2 fumigation to fresh produce include color bleaching and darkening discoloration as SO_2 can act as both a reducing and oxidizing agent. Sulphur dioxide is hydrophilic. Moisture on product surfaces and condensation will attract SO_2, and this deposited SO_2 then causes bleaching. Sulphur dioxide may also oxidize exposed tissues to form brown spots such as darkened lenticles on apples (Singh et al., 2016; Liu, 2024a). Plant surfaces typically have wax layers to retain moisture and prevent photoirradiation damage. The wax layers also serve as barriers to SO_2 and, thereby, prevent or reduce phytotoxicity. Fresh fruits and vegetables with smooth waxy cuticles, as demonstrated with bell peppers and navel oranges, are more tolerant to SO_2 fumigation than products with rugged surfaces such as broccoli (Liu, 2024a). In SO_2 fumigation studies of fresh products, phytotoxicity can be recognized early on by the extent of SO_2 concentration declines, as based on our experience. When there is very little to no decline in SO_2 concentration during fumigation, it is most likely that there is very little sorption of SO_2 on the fresh products and that the fumigation treatment is safe to the products.

Either multiple injections or a constant flow of SO_2 are recommended to maintain relatively stable SO_2 concentrations during SO_2 fumigation. Short fumigation durations are recommended to reduce amount of SO_2 adsorbed on fumigated products. Fumigations at low temperatures are preferred over high temperatures. Active ventilation should also be mandated in SO_2 fumigations of fresh and stored products, as the postharvest processes of fresh fruit and vegetables may include transporting, sorting, packaging, cooling, cold storage, and shipping. Sulphur dioxide fumigation is best used at low temperatures during cold storage.

Sulphur dioxide fumigation has several advantages over other major alternative fumigants, such as phosphine and sulfuryl fluoride. Sulphur dioxide fumigation has a much higher efficacy than both phosphine and sulfuryl fluoride, as judged by its much shorter fumigation durations against same pest species. Phosphine fumigation may take over 10 d to control some stored-product insects (Hole et al., 1976), and some stored-product insects have also developed resistance to phosphine (Benhalima et al., 2004). Sulfuryl fluoride fumigation not only takes longer to control stored-product insects, as compared with SO_2 fumigation, but is also not effective against insect eggs (Bell et al., 1998).

Sulfur is a macronutrient, and SO_2 is a GRAS (Generally Recognized As Safe) compound. Therefore, SO_2 fumigation is compatible with organic products and has better safety to human health as compared with most other fumigants. For large-scale commercial applications of SO_2 fumigation, scrubbing devices are available for use to remove SO_2 exhaust in order to mitigate any concerns about excessive SO_2 release into the atmosphere. Since SO_2 fumigation is already used widely as a preservative on food products, there is also a favorable regulatory environment for its commercial use in postharvest pest control. Therefore, the prospect of SO_2 fumigation for postharvest pest control is bright, and SO_2 has the potential to become a preferred alternative fumigant to methyl bromide for postharvest pest control. More research and development efforts are needed to accelerate the practical use of SO_2 fumigation for postharvest pest control.

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The Progression of Phosphine Toward a Quarantine Tool

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ABSTRACT

A perspective on the past, present, and future of phosphine was reviewed. This work reflected on phosphine research, starting with its use on durables, transitioning through the "doomsday blackeye" of resistance – or so was thought, and finishing with how motivation to use phosphine in a quarantine capacity resulted in an improved understanding that serves the future of phosphine application well. Examples of correct and incorrect interpretations of phosphine use were presented and discussed, at least from the perspective of the USDA-ARS (United States Department of Agriculture – Agricultural Research Service). Regulatory elements important to the continued use of phosphine were addressed. Finally, several contemporary examples of Quarantine Pre-Shipment (QPS) applications were detailed, including its use to control warehouse beetle on dried distiller grain exports, bean thrips on citrus exports, and key fruit fly pests of fresh fruit.

Keywords: phosphine, Quarantine, methyl bromide alternatives

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Phosphine for Storage Insect Pest Management of Perishables in India

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ABSTRACT

The efficacy of phosphine against the storage insect pests of perishables including vegetables (bitter gourd, chilies), fruit (mango), and flowers (chrysanthemum, garden roses) and the effect of the fumigant on the quality of these perishables were studied. Phosphine residue levels in the treated commodities were also monitored. In laboratory bioassays, infested samples of bitter gourd, chilies, mango, chrysanthemum, and garden roses collected from the experimental fields of the Indian Agricultural Research Institute (IARI), New Delhi, were fumigated with ammonia-free phosphine generated from 77.5% QuickPhos® granular aluminum phosphide preparation for the control of *Bactrocera cucurbitae* (fruit fly) in bitter gourd, *Scirtothrips dorsalis* (thrips) in chilies, *Bactrocera dorsalis* (fruit fly) in mango, *Macrosiphoniella sanbornii* (aphids) in chrysanthemum, and *Rhipiphorothrips cruentatus* (thrips) in garden roses. The laboratory studies were conducted under different exposure periods (4 to 15 h) at a range of 0 to 2400 ppm phosphine concentrations with three replicates for each set of doses and exposure periods with an equal number of untreated controls. Complete mortality at respective phosphine concentrations and exposure periods was achieved as follows: aphids on chrysanthemum flowers at 2400, 2200, 1400, and 1200 ppm levels for 4, 6, 8, and 10 h; thrips in roses at 65, 55, 50, and 40 ppm for 4, 6, 8, and 10 h; fruit fly in bitter gourd at 1400, 1200, 1100, 800, and 600 ppm for 4, 6, 8, 10, and 15 h; thrips in chilies at 60, 50, 40, and 30 ppm for 4, 6, 8, and 10 h; and fruit fly in mango fruits at 1500, 1200, 1000 and 800 ppm for 4, 6, 8 and 10 h. The quality parameters of the phosphine-treated perishables did not significantly change, and phosphine residues were noted in trace amounts (<0.3 ppm) after 2 h of aeration.

Keywords: Perishables, Phosphine, Fumigation, Quality parameters, Residue levels, India

INTRODUCTION

Due to decentralized global production and the globalized economy, there has been a significant increase in the international trade of commodities, including perishables, in recent years. From a

supply and demand standpoint, India has a great chance to increase its exports of perishable goods like fruits, vegetables, and flowers. For a pest-free supply of produce, refrigerated containers containing horticultural products need to be treated for disinfestation. Because of its penetration deep into the treated commodity achieving 100% pest control, methyl bromide (MB) is the most widely used and effective fumigant for quarantine and pre-shipment (QPS) disinfestations of various horticultural commodities, including perishables. However, MB has substantial potential to deplete the ozone layer and was designated as an Ozone-Depleting Substance under the Montreal Protocol in 1992. Consequently, there has been an increasing need to determine the best substitute for this fumigant.

Additionally, phosphine fumigation has been observed as a feasible and successful replacement for MB in the treatment of perishables to stop the international spread of non-native insect pests. Its rising popularity is attributed to its affordability, simplicity of use, and lesser chance of leaving stable residues in the products. Because of its activity on broad spectrum of insects, it can be used before perishables are exported to avoid pest infestations.

Though phosphine fumigation using traditional AlP tablets has been reported with promising results in terms of insect mortality, the impact of the ammonia present in the tablets made the quality of the treated fruit unacceptable. Nevertheless, cylinderized phosphine (VAPORPH3OS®) and phosphine produced by on-site generators (QuickPHlo-RTM) are examples of pure formulations that have advanced to the point where phosphine can be used as a strong fumigant for perishables.

The availability of these substitute sources of phosphine without ammonia makes it easier to fumigate fresh produce at low temperatures without compromising fruit quality or the shelf life of perishables. Since this process slows down the fresh produce's metabolism, the fruit's quality is unaffected. Usually, controlled atmosphere chambers, cooling chambers, or fumigation chambers can be used for this. There has never before been a sorption and residue analysis study on phosphine-treated perishables in India. The main aims of the study were to determine the efficacy of phosphine against stored insects on perishables and the impact of phosphine on the quality parameters of these perishables, followed by residue analysis of the treated commodities.

The perishables in this study were exposed to pure phosphine at certain dosages and durations of exposure that accordingly produced 100% insect mortality. For the estimation of residues, phosphine concentration in the treated perishable commodities was analyzed using gas liquid chromatography after the fumigation and aeration processes to make sure the residues were below the Threshold Limit Value (TLV) of phosphine (0.3 ppm). The Maximum Residual Levels (MRLs) of phosphine are not reported for the perishable commodities.

MATERIALS AND METHODS

As per the seasonal availability and needs, the perishable infested samples, which included fruits (mango, Amarpali variety), vegetables (bitter gourd, S-2 and chili, NS 1101 varieties), and flowers (rose, Virangna and chrysanthemum, Jaya varieties), were collected from the experimental fields of the Indian Agricultural Research Institute (IARI), New Delhi. Pure phosphine (93%, free of Ammonia) was produced in the laboratory using QUICKPHLO-R® 77.5% granular (UPL Ltd.,

Mumbai, India) formulation of aluminum phosphide and a 5% (v/v) aqueous sulphuric acid solution following the FAO (1975) method. The granules were wrapped in a thin cloth, securely immersed into the solution, and positioned carefully beneath an inverted glass funnel. Through the inverted funnel's neck, the gas generated during the reaction was collected in the upper vessel. To stop any gas from escaping, a septum was used to seal the upper vessel's opening. The gas for the bioassay experiments was withdrawn using gastight syringes manufactured by Hamilton, Reno, Navada, USA.

The laboratory bioassay experiments were conducted in several 2-3 L gastight glass desiccators. A known weight of infested perishable samples was placed in the desiccators, followed by the sealing of the lids. Additionally, a glass tube assembly, with an inlet and outlet for the monitoring of gas concentration and a center tube with septa for injection of the gas, was inserted into the lids. Three replicates of each set of doses and exposure periods, along with an equal number of untreated controls, were used to expose the infested samples to be treated under different exposure periods (4 to 15 h) using phosphine concentrations in the range of 0 to 2400 ppm. The laboratory bioassay experiments were conducted in controlled conditions at temperatures ranging from 15 to 29°C, and relative humidity (r.h.) in the range 44–90% depending on the weather conditions during the experiments on various perishable commodities. The gas concentrations were monitored using a FumiSense Pro Gas Monitor (measuring range of 0–2000 ppm), manufactured by Uniphos Envirotronic Pvt. Ltd, Vapi, India. The samples were removed after 2 h of aeration following the respective exposure periods, for monitoring of the insect mortality. The insect mortality was counted and monitored by dissecting the fruits (in the case of vegetables and fruits) and by removing the petals (in the case of flowers). However, a few of the treated samples were taken out undisturbed and subjected to an analysis of physio-chemical quality parameters, including firmness, moisture content, titratable acidity, total soluble solids (TSS), ascorbic acid, total carotenoids, anthocyanin, phenolic content, chlorophyll content, water uptake, and antioxidants.

The firmness of chilies, bitter gourd, and mango fruits was determined by the Texture Analyzer (TAxT2, Stable Micro Systems, Godalming, United Kingdom) using a 2 mm cylindrical stainless steel (SS) probe with a pre-test of 5 mm/s, a test speed of 0.5 mm/s, and a post-test speed of 10 mm/s to a depth of 10 mm. Maximum force during the puncture, expressed in Newton (N), was used to denote the fruit firmness. The moisture content was determined by comparing the initial and final weight of the samples. For shelf life, the treated and untreated samples of fruits and vegetables were kept for 6–7 d before they became rotten. In the case of the vase life of flowers, the flowers were kept for 1–2 d until they became semi-dried. The water uptake of flowers was measured by keeping the flowers in a known amount of water and measuring the volume of water after 1–2 d of having taken the flowers out of the vase. The TSS content in the fruits and vegetables was measured using a handheld refractometer based on the principle of refraction of light. For chlorophyll content, the optical density (OD) of the samples incubated in dimethyl sulfoxide (DMSO) was measured at 648 and 655 nm wavelengths following the methodology of Manolopoulou et al. (2016). The anthocyanin content in the flowers was analyzed by putting the samples in 80% ethanol and recording the OD observations at 510 and 700 nm wavelengths; the antioxidant content was estimated using CUPric Reducing Antioxidant Capacity (CUPRAC) method. For ascorbic acid analysis in bitter gourd and chilies, the samples were dissolved in metaphosphoric acid and titrated against the dye (Tillmans et al., 1932). For titratable acidity in these vegetables, a few drops of phenolphthalein dye was added to the aqueous solution of the

samples and titrated against a solution of NaOH (Nielsen, 2017). The samples of mango fruits were taken in acetone and transferred to a layer of petroleum ether, followed by recording the observations of OD at 452 nm for carotenoid content (Hsieh and Karel, 1983). The residue analysis of phosphine-treated vegetables, fruits, and flowers under different exposure periods was carried out at the UPL laboratory, Vapi, Gujarat, using the gas chromatography (GC) method (Nowicki, 1978) to check if any residues were left by having applied pure phosphine for insect pest management during the storage of the perishables.

RESULTS AND DISCUSSION

A series of phosphine concentrations were standardized for storage insect pest management of perishable commodities under different exposure periods. Table 1 provides the effective phosphine concentrations leading to 100% insect mortality in perishable commodities while applying various phosphine concentrations over different exposure periods, along with the lethal toxicity levels of phosphine.

Table 1. Phosphine efficacy against storage insects of perishable commodities and impact on the quality parameters.

Crop	Commodity	Exposure Period (h)	Effective Concentration (ppm)	Lethal Toxicity LC_{50} (ppm)	Residues (ppm)	Quality Parameters		Remarks
						Physical	Biochemical	
Vegetables	Bitter gourd	4 6 8 10 15	1400 1200 1100 800 600	762 547 514 298 293	0.1 0.09 0.1 0.1 0.07	Moisture content, Color, Texture	Chlorophyll content, Ascorbic Acid (AA), Total Soluble Solids (TSS)	No significant difference was observed in any quality parameter between treated and untreated control samples
	Chilies	4 6 8 10	60 50 40 30	31 18 14 13	0.01 0.01 0.015 0.02	Moisture content, Color, Texture	Chlorophyll content, AA, TSS, Antioxidants	
Fruits	Mango	4 6 8 10	1500 1200 1000 800	**577** 451 377 262	0.03 0.019 0.03 0.024	Texture, and Physiological loss in weight (PLW)	Titratable Acidity (TA), Carotenoids	-do-
Flowers	Chrysan-themum	4 6 8 10	2400 2200 1400 1200	1281 845 691 687	0.24 0.21 0.21 0.17	Water uptake, vase life, Initial and final Dry weight	Anthocyanin content	-do-
	Roses	4 6 8 10	65 55 50 40	37 25 21	0.02 0.003 0.004 0.008	-do-	-do-	

The phosphine concentrations at 2400, 2200, 1400, and 1200 ppm were observed to give complete mortality of aphids (*Macrosiphoniella sanbornii*) on chrysanthemum flowers for 4, 6, 8, and 10 h of exposure periods, respectively; likewise 65, 55, 50, and 40 ppm of phosphine resulted in 100% mortality of thrips (*Rhipiphorothrips cruentatus*) in roses for 4, 6, 8, and 10 h of exposure periods, respectively; concentrations of 1400, 1200, 1100, 800, and 600 ppm against the fruit fly (*Bactrocera cucurbitae*) in bitter gourd for 4, 6, 8, 10 and 15 h of exposure periods, respectively; concentrations of 60, 50, 40, and 30 ppm against thrips (*Scirtothrips dorsalis*) in chilies for 4, 6, 8, and 10 h of exposure periods, respectively; and concentrations of 1500, 1200, 1000, and 800 ppm against the fruit fly (*Bactrocera dorsalis*) in mango fruits for 4, 6, 8, and 10 h of exposure periods, respectively. The concentration of phosphine gas can be changed depending on the desired exposure periods for complete management of the storage insect pests of the perishables.

Up until now, data on phosphine concentrations under different exposure periods have never been reported for fruits, vegetables, and flowers. The data of quality parameters under all the exposure periods were subjected to an Analysis of Variance (ANOVA) for statistical analysis and are presented in Table 2.

Table 2. Statistical analysis of quality parameter data of the phosphine treated perishables using ANOVA.

Calculated F Values for the Perishables Under Different Exposure Periods								
		Quality Parameters						
Perishable	Exposure Period (h)	Chlorophyll	Ascorbic Acid	Antioxidants	TSS	Titratable Acidity	Carotenoids	Anthocyanin
Bitter Gourd	4	0.49 (4,10)	1.51 (4,10)	NA	NA	NA	NA	NA
	6	9.17* (4,10)	0.43 (4,10)	NA	NA	NA	NA	NA
	8	1.29 (4,10)	3.55* (4,10)	NA	NA	NA	NA	NA
	10	0.44 (4,10)	1.22 (4,10)	NA	NA	NA	NA	NA
Chili	4	1.84 (4,10)	2.07 (4,10)	1.17 (4,10)	NA	NA	NA	NA
	6	0.38 (4,10)	0.28 (4,10)	0.36 (4,10)	NA	NA	NA	NA
	8	1.91 (4,10)	1.57 (4,10)	1.62 (4,10)	NA	NA	NA	NA
Mango	4	NA	NA	NA	0.11 (2,6)	0.93 (2,6)	4.11 (2,6)	NA
	6	NA	NA	NA	0.75 (2,6)	0.07 (2,6)	0.64 (2,6)	NA
	8	NA	NA	NA	0.04 (2,6)	0.64 (2,6)	0.82 (2,6)	NA
	10	NA	NA	NA	0.09 (2,6)	1.93 (2,6)	7.7 (2,6)	NA

Chrysanthemum	4	NA	NA	NA	NA	NA	NA	1.59 (5,12)
	8	NA	NA	NA	NA	NA	NA	0.86 (5,12)
	10	NA	NA	NA	NA	NA	NA	1.92 (5,12)
Roses	4	NA	NA	NA	NA	NA	NA	0.24 (5,12)
	6	NA	NA	NA	NA	NA	NA	0.27 (5,12)
	8	NA	NA	NA	NA	NA	NA	2.1 (5,12)
	10	NA	NA	NA	NA	NA	NA	5.38* (5,12)

The degrees of freedom are shown in parenthesis. All treatments are observed to be non-significant from each other, while only three treatments were shown to be significantly different, i.e., one in bitter gourd for chlorophyll under 6 h; the second one for ascorbic acid under 8 h of exposure; and the third for anthocyanin in roses for 10 h of exposure periods. All significant different treatments are shown with an * sign. NA – Not Applicable

Almost all quality parameters of phosphine-treated and untreated commodities are non-significant in nature, which clearly implies there is no significant impact of phosphine treatment on the quality of the perishable commodities. However, in exceptional cases of chlorophyll and ascorbic acid content in bitter gourd samples, a significant difference was observed under the exposure periods of 6 and 8 h, respectively. A similar significant difference was observed in anthocyanin content of the treated and untreated roses under 10 h of exposure periods. This effect may be due to the difference in the stages of the fruits or flowers plucked from the experimental fields; however, no such visual effects were observed. The overall study showed no significant differences in any of the physical or biochemical parameters of phosphine-treated and untreated control samples of the perishable commodities.

The perishable samples treated with phosphine showed traces of residues in the range of 0.003–0.1 ppm, which is significantly less than the phosphine's TLV of 0.3 ppm. Since there is no information available regarding the maximum residue limits (MRLs) of phosphine for fruits, vegetables, or flowers, we used the TLV of phosphine as the standard.

Fumigants are used to prevent pest infestations in food and other stored commodities. The quality of the commodity may be affected by fumigant treatment in several ways. Residues of unchanged fumigant may remain in the commodity. The commodity's flavor, taste, odor, nutritional value, and processing abilities could all be impacted by the fumigant's reaction with its individual chemical constituents, changing the commodity's chemical or physical characteristics. Methyl bromide, ethylene dibromide, ethylene dichloride, carbon tetrachloride, phosphine, and ethylene oxide are the major fumigants used for the treatment of grains, vegetables, and fruits (Plimmer, 1977).

However, pure phosphine without ammonia is observed to be an effective fumigant for insect pest management of fruits, vegetables, and flowers without compromising the quality of the produce and for leaving no residues in the treated perishables. Therefore, phosphine can be used as a fumigant to effectively manage storage insect pests of perishable commodities

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The Different Internal Life-Stages of Naked Mediterranean Fruit Fly *Ceratitis Capitata* (Diptera: Tephritidae) Responses to Continuous Purging Flow of Ethyl Formate Followed by Low Oxygen Storage

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ABSTRACT

In the context of the globalization of fruit trade, fruit fly on the harvested fruit is challenging safeguarding trade, particularly, under the methyl bromide phasing out. Therefore, it is urgently required to develop environmentally friendly and efficient fruit fly control methods based on export advantages for the international market, such as phytosanitary requirements and quality.

In this study, we evaluated responses of different internal life-stage of Mediterranean fruit fly (*Ceratitis Capitata*) (Medfly) to ethyl formate (EF) fumigation alone and post its treatment then followed with low oxygen storage. The results showed that, after 6 h of fumigation, significant efficacy to control all life stages of Medfly in laboratory diets was obtained at EF dose range of 15–45 mg/L and room temperature (25±1°C). Moreover, the insecticidal effect was enhanced by further a nitrogen controlled atmosphere treatment.

Overall, our study showed that compared with EF fumigation alone, the combination of EF fumigation and a nitrogen controlled atmosphere can achieve effective control of Medfly at low concentrations of EF. Therefore, using the purging-flow system to apply food-grade ethyl formate fumigation followed by nitrogen or hypoxia storage techniques could be a solution of system approach to managing biosecurity threats - Medfly and fruit quality.

Keywords: Plant biosecurity, Fruit fly, Medfly, Quarantine treatment, Alternative fumigant, Ethyl formate, Controlled atmosphere

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Ethyl Formate – Update on Applications and Potential Uses

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ABSTRACT

Liquid ethyl formate (EF), used to fumigate dried fruit in 1927, is making a comeback. The patented non-flammable mixture of EF in carbon dioxide (CO_2) has expired. Grain fumigation is now the focus after early involvement with horticulture and quarantine applications. Early stored-grain insect laboratory R&D efficacy testing was followed by successful 50 t silo trials. Upscaling to larger 2000 t storages is planned. Benefits of ethyl formate include rapid action, no withholding period, lower toxicity (TLV=100 ppm), and no residue issues. The breakdown products are US FDA-approved GRAS (Generally Regarded as Safe) food additives.

Reviews of EF over recent years recommended its use as a methyl bromide (MB) alternative. Both gases are rapid-acting fumigants but differ with aeration requirements. Ethyl formate breakdown on grain can happen in days and requires minimal aeration. Post aeration requirement for MB fumigation can take up to 5 d. Aeration of MB is complicated by slow release and restrictions related to health and safety concerns. Restricted MB releases around shipping terminals in proximity to populated urban areas extends the total fumigation time. Quicker turnaround could be achieved using EF.

The eMate fumigant, liquid 99% EF product, is registered in Australia and recently registered in New Zealand. The NZ Biosecurity has approved EF (20 g/m^3, Ct 65 g.h/m^3) plus >3% CO_2 to control the brown marmorated stink bug (BMSB), yellow spotted stink bug (YSSB), and ants.

Ethyl formate is effective against PH_3 resistant insects. Of particular interest is the highly PH_3 tolerant but EF susceptible *Cryptolestes ferrugineus* (Stephens). Ethyl formate with PH_3 (co-fumigant) can manage PH_3 resistant insects. High EF efficacy was achieved on *Cryptolestes sp*. without the use of a CO_2 synergist. Control of the highly tolerant *Sitophilus oryzae* (Linnaeus) was achieved by extending the exposure time or by using a co-fumigant, e.g., EF with PH_3.

Keywords: Ethyl formate, Fumigation, Efficacy, No-withholding period, Quarantine, Brown marmorated stink bug, Grain storage, Vaporizing, Dispensing equipment

HISTORY

Fumigation of stored products with hydrogen cyanide (HCN) and carbon disulfide (CS_2) was a well-known practice in the late 1800s. Post World War I (WWI) insurance companies and railroads ruled against the use of CS_2 due to the risk of explosion and fire. Because of this situation, the United States Department of Agriculture conducted tests on many potential alternative chemicals. The first results published (Shepard et al., 1937) included EF with results on its control of stored-product pests in wheat (Neifert et al., 1925). This study tested more than 100 chemicals and reported that EF and ethyl acetate were the only promising grain fumigants of box cars (railway cars). As the historical fumigant of dried fruit, the uses of EF extended over time to include horticulture and cereal grains (Simmons and Fisher 1945). Muthu et al. (1984) conducted large-scale tests on cereals, pulses, spices, dried fruits, nuts, and dried tubers and recommended EF as a safe general fumigant for stored food. With treated commodities, EF was not found to adversely affect the quality or flavor. Ethyl formate is naturally present in a range of plant and animal products. These products include cereal grains, fruits, vegetables, beer, wine and spirits, tuna, meat, mussels, milk, cheese, and bread (Desmarchelier et al., 1999; Ren and Mahon, 2003; Ryan and De Lima, 2012).

For its use as a food additive, EF holds the "generally regarded as safe" (GRAS) status with the US Food and Drug Administration (FDA) (Haritos et al., 2006). Advantages of EF include short fumigation exposure time, low toxicity to mammals and the environment, and a rapid breakdown with minimal or no residues (Coetzee et al., 2019; Haritos et al., 2003). The vision of the Commonwealth Scientific and Industrial Research Organization (CSIRO) and the Grain Research and Development Corporation (GRDC) to replace dichlorvos and phosphine for insect control in farm storages initiated the interest in EF as a fumigant. In addition, EF enjoys a ruling of no requirement for a maximum residue limit (MRL). In field trials, wheat treated with ethyl formate at 90 g ethyl formate per tonne of grain was assessed in a milling and baking trial (Haritos et al., 2003). The ethyl formate treatment did not cause detrimental effects to the milling and baking qualities of wheat; nor were EF residues detected above background levels in whole or processed fractions. The patented non-flammable mixture of 16.7% EF in CO_2 has expired (Ryan and Bishop 2003). The 99% EF (eMate Fumigant) is registered by the Australian Pesticide and Veterinary Medicine Authority (APVMA) and the New Zealand Ministry for Primary Industries under the Agricultural Compounds and Veterinary Medicines Act (ACVM).

EFFICACY

Rapid dispensing of EF (e.g., dispensed using an aeration fan) minimizes sorption issues (Haritos, 2006). Due to the lower toxicity EF, a relatively high dose (70 g/m^3) is usually required; however, only short exposure times (4-24 h) are needed for EF to be effective. Ethyl formate is registered in Australia to control some 41 different pests.

The efficacy of EF is well documented with extensive coverage of stored-product pests. An issue is the reported survivors of immature stages of *Sitophilus oryzae* (Linnaeus). Fumigation of the lesser grain borer (*Rhyzopertha dominica* (Fabricius), highly phosphine-resistant field strain) and flour beetles (*Tribolium spp*., laboratory strain, moderately tolerant to fumigants) achieved 100%

mortality at 70 g/m^3 EF (25ºC, 20% CO_2, 24 h). However, fumigating the rice weevil (*Sitophilus oryzae*, highly tolerant, laboratory strain) only achieved 91% mortality at 156 g/m^3 EF (25ºC, 42% CO_2, 3 h) but achieved >99% mortality at 156 g/m^3 EF (25ºC, 42% CO_2, 72 h). The explanation of this tolerance was narrowed down to the *S. oryzae* pupa (Haritos, 2005).

Export grain terminal trials using EF (Allen and Desmarchelier, 2000) disinfested psocids (*Liposcelis spp.*) as proven by a grain silo sampling system. No residues of EF could be detected in the grain following treatment. Insect bioassay at 25ºC, 70 g/m^3 of EF with 7% CO_2 for an exposure time of 30 min achieves 100% mortality with *Liposcelis entomophila* (psocids), *O. surinamensis* (saw-toothed grain beetle), *R. dominica* (lesser grain borer), *S. oryzae* (rice weevil), and *Tribolium castaneum* (Herbst) (red flour beetle).

Insects infesting hay fodder exported to Japan were disinfested using laboratory and field research into EF / CO_2 mixtures (De Lima, 2006). In fumigating 67 m^3 shipping containers filled with approximately 30 t of compressed hay bales and EF applied at 120 g/m^3 for 24 h in the winter temperature range of 8 to 19°C, all stages of *Sitophilus* spp. and *T. castaneum* (>12,000 of each spp.) placed in vials in the hay bulk were killed. The final free gas concentration (58 g/m^3 EF +8% CO_2) was sufficient to provide longer-term protection from re-infestation in transit.

Wright et al. (2002) achieved 100% mortality with all life stages of *S. oryzae* at 85 g/m^3 EF (32ºC, 0% CO_2, 2 d); Mahon et al. (2003) achieved 100% mortality with all life stages of *S. oryzae* at 26 g/m^3 EF (32ºC, 7% CO_2, 2 d). No adverse effect on germination of the treated seeds was reported.

If a tolerant form of *S. oryzae* is encountered, complete control should be achieved by extending the exposure time or by applying a co-fumigant, i.e., simultaneous application of EF with PH_3 which eliminates PH_3 resistant insects and all stages of *Sitophilus* sp. (Gu, 2020).

DISPENSING

The safe application of fumigants requires dispensing a non-flammable gas/vapor mixture. From the history of fumigation, it is clear that flammability is a significant issue with most fumigants. While the intergranular space will mitigate the risk of flammability, there is always the possibility of the headspace volume being filled with an explosive mixture. The industrial experience of gas safety engineers warns of the ever-presence of an ignition source, e.g., static electricity. Flammable gas and vaporized liquids in open space do not burn but explode. An additional concern is the possibility of fumigants being blamed for dust explosions.

Dispensing EF has its challenges, especially with the fumigation of large storages at grain export terminals. Currently, MB is allowed under a QPS (quarantine pre-shipment) exemption to fumigate export grains. While MB and EF are rapid fumigants with required exposure times of less than 24 h, there is an additional time involved with the aeration of MB post fumigation. In addition, the Environmental Pollution Authority (EPA) instructs the amounts to be vented with various weather conditions. The overall result is that MB fumigation time can take up to 5 d while EF could be completed in 1 d. Ethyl formate has a 'No Withholding Period', and EF breaks down on the grain in days. The issue with dispensing EF is the need to vaporize the liquid EF into a non-flammable

vapor, CO_2, and ambient air mixture. Heat is required to vaporize the liquid EF. Mixing with an inert gas is needed to achieve a non-flammable gas/vapor mixture. The EF gas mixture must be non-flammable prior to entry into the storage to be fumigated. An option is to replace the storage air space with an EF mixture of typically 2.2% EF with CO_2 greater than 3% and oxygen (O_2) less than 16%. Publication by Haritos et al. (2006) determined the efficacy benefits of added CO_2 and reported to have no additional benefit above 5%. While there are options using merchant CO_2 or both merchant nitrogen (N_2) or on-site N_2 generators, the use liquified petroleum gas (LPG) burner gas is being evaluated. The burner gas is clean, uses up excess air, and delivers the required >3% CO_2 and heat required to vaporize the liquid EF. The volume of the storage air to be replaced is less than 50% of the storage volume, which includes air in the headspace and in the intergranular space.

From a design and construction perspective, there are considerations which are necessary to enable EF to be used safely in fumigation, which do not relate to its efficiency as a fumigant but, rather, to factors addressing hazardous properties to be controlled in any fumigation. Ethyl formate is flammable but must not combust during a fumigation. The standard method of eliminating flammability is the addition of an inert gas, which is usually CO_2 and/or N_2. From a knowledge of the flammable limits of EF, a 'nose curve' can be constructed to determine the required level of the added inert gas. As CO_2 is itself a product of combustion, there is the possibility of producing it on-site. Merchant CO_2 is both cumbersome (e.g., liquid storage) and expensive. Readily available LPG can be burned to produce the required CO_2. Obviously, LPG burns at a flame temperature of around a 1000°C. This can be reduced by burning at the LEL (Lower Explosive Limit) using extra air and absorbing heat in vaporizing liquid EF. Even so, the temperature of the gas stream is still likely to be around 400°C, which is very hot. The cooling of the feed gas requires external cooling which involves a heat exchange with a colder element. This is the least productive method involving large surface areas. If forced convection is considered on the cool side as it already exists on the hot side, then an enclosure might be needed on the cool side with the possibility of pressure build-up needing over-pressure protection. Water is the obvious liquid to be used as a coolant. The enclosure on the liquid side seems obvious with pressure build-up (steam) and protection (pressure relief). Non-enclosure is possible if the hot gas stream simply runs through a water bath. However, this involves water vapor / steam loss, with consequent scaling only avoided with an extra system of condensate return. This is the most powerful cooling with the latent heat of water-steam being harnessed. There is flexibility in the amount of atmospheric air that can be added which will also assist in reducing the temperature of the gas stream being delivered to the silo. Even diluting the burner gas with 50% ambient air will give a CO_2 level 6% (double the minimum 3%). While the purity of merchant CO_2 is food grade because of its application to put the fizz in beverages, the LPG burner gas is burnt in excess air which oxidizes impurities; thus, there should be no issue with the gas /solid external contact for 24 h. The burner with excess air negates significant impurities and reduces flame temperature.

DISCUSSION

The potential for EF includes the replacement of MB used at export grain terminals for the fumigation of cereal grains. The benefit of EF is on “time saving”—MB fumigation takes 5 d whereas EF can be completed in 1 d. Although the EF dose is at least double that of MB and EF

fumigant costs are higher than MB, it is the grain storage time at export grain terminals which is a critical factor in operating efficiency, with fumigation costs being a lower priority. The total cost needs to include labor, efficient operation, and environmental issues.

The adoption of co-fumigants needs to be considered to ensure treatments are available for dealing with insect resistance issues. The ability to deal with PH_3 resistant insects, especially the *Cryptolestes sp.*, is becoming a requirement.

CONCLUSIONS

Following success with horticulture and quarantine applications, the current EF focus is on the fumigation of grain to control stored-product pests. The volatile liquid EF is a potential alternative to MB. Both MB and EF are rapid acting fumigants but differ with aeration requirements.

Potential EF applications include export terminal fumigations (to replace MB), use as a resistance "break", and satisfying the need for an on-farm rapid lower toxicity fumigant. On-farm use of PH_3 has been of concern related to insect resistance development.

Dispensing EF has its ongoing challenges, especially with the fumigation of large grain storage compartments. Heat is required to vaporize the liquid EF, and this vapor must first be treated in order to become a non-flammable mixture before being delivered to the storage container.

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Fumigants: Lethal Gas / Vapor – Options and Issues

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ABSTRACT

Pesticide treatment of stored-product pests uses fumigants (gases/volatile liquids) and grain protectants to meet the requirements of insect-free export and domestic grain. Insect resistance is a serious efficacy issue. Grain protectants have had a long list of pesticides rendered ineffective and discarded due to insect resistance. The fumigant, phosphine (PH_3), has ongoing resistance issues but is the preferred grain fumigant. As most current fumigants suffer specific weaknesses, there is a need for additional fumigants. An immediate solution for fumigants, learning from the usage of grain protectants, is the dispensing of multiple registered fumigant products simultaneously (Co-Fumigant). An existing example of a Co-Fumigant is the dispensing of PH_3 and sulfuryl fluoride (SF), as well as the dispensing of ethyl formate (EF) and PH_3 as an alternative option.

In 1963, the Australian Government reacted to excessive insect infestations by promulgating the "Export (Grains) Regulations", which prohibited the export of grain unless it was free of insect pests. The grain protectant, malathion, was adopted in Australia in 1960/61 to protect wheat for export and domestic use; however, malathion resistance was detected in 1968. To negate insect resistance, mixtures of grain protectant products are currently in use.

Over the intervening 60 years, significant advances have occurred related to efficacy, sorption, flammability, vaporization, and dispensing. Efficacy is impacted by non-uniform distribution related to leaky storage and by sorption. Sorption may be mitigated using a high flow rate of the vaporized fumigant diluted in air. The flammability issue for phosphine has been resolved by using a cylinderized liquid phosphine/carbon dioxide mixture or by the onsite mixing of pure phosphine and air. Vaporization is required for liquefied gases and liquid fumigants. Dispensing systems are required to maximize efficacy and ease of use. All fumigants currently used are noted in the original 60-year-old Monro list.

Keywords: Stored-product pests fumigant, Grain protectants, Phosphine, Ethyl formate, Sulfuryl fluoride, Hydrogen cyanide, Methyl bromide, Co-Fumigant

INTRODUCTION

This review of fumigants (gases/volatile liquids) also refers to issues encountered by grain protectants (insecticide mixtures) used to control stored-product pests. Grain protectants in liquid form are sprayed onto moving grain to obtain a uniform distribution of the insecticide. These synthetic insecticide chemicals were not marketed prior to World War II but were in commercial use in the 1960s. Grain protectants are applied when sheds, bunkers, and silos are not gastight. The Australian Standard, AS2628 (2010), defines the acceptable leak rate for gastight grain storages. When there is no sealed storage available to allow for the use of fumigants, grain protectant liquid insecticides can be sprayed onto the moving grain at receival into storage to prevent insect infestation. Grain protectant doses are not effective as a knockdown treatment post insect infestation. Each product can only be applied once to a parcel of grain, and accurate application is essential for residues to avoid maximum residue limits (MRL).

In 1948, the Wheat Stabilization Act created the Australian Wheat Board (AWB) as the only licensed marketer of wheat. The AWB provided a centralized decision process to address the choice of pesticides, rate of application, and compliance with health and safety issues. In 1963, the Australian Government—reacting to excessive insect infestations—promulgated the "Export (Grains) Regulations", which prohibited the export of grain from Australia unless it was free of insect pests (Graver and Winks, 1994). Pesticide residue issues, related to grain protectants, also became a grain marketing issue. Eastern Australia grain handlers, in their use of grain protectants, were unable to meet the increasing demand for low-residue or residue-free grain initiated during the late 1980s. The Western Australia (WA) grain authority, WA Cooperative Bulk Handling Ltd. (CBH); however, was able to supply residue-free grain during the late 1980s because of its decision to install sealed gastight storages suitable for fumigation (Barry, 1984). This capability is established within CBH and has been extended to WA on-farm storages. Up to 1994, no grain protectants had been used by CBH (Winks and Ryan 1994). The other states were not as fortunate because few of their storages met the requirements of AS2628.

Initiated by the development of insect resistance, the AWB set up a committee to investigate Australia's ongoing technical requirements of the industry. In 1973, the Stored Grain Research Laboratory (SGRL)—located within the CSIRO Division of Entomology, Canberra—was established. Using levies, the grain industry funded the SGRL and met annually to consider pest control issues and to plan pest control strategies for the coming season. Post SGRL, ongoing support continues with input from the Grain Research and Development Corporation (GRDC), the Queensland Department of Primary Industries (QDPI), the New South Wales Department of Primary industries (NSW DPI), Murdoch University, chemical manufacturers, and grain industry specialists. From the outset of the malathion era, the question of residues had been an important consideration, and efforts were made to ensure that the grain exported from Australia complied with maximum residue levels (MRLs). It was not always found to be an easy task to meet MRLs or the nil residue standard. To cope with the multiple treatments from the farm onwards, handling authorities needed to supplement their application technology with residue analysis. The ability to control export grain residue levels using analytical laboratories led to the National Residue Survey (NRS). The NRS monitors the levels and associated risks from pesticide residues and contaminants in Australian food products.

GRAIN PROTECTANTS

Grain protectants, including organophosphates, carbamates, synthetic pyrethroids, bacterium, and insect growth regulators, were not available until post World War II. The "Export (Grains) Regulations", which prohibited the export of grain from Australia unless it was free of insect pests, led to the introduction of grain protectants. The grain protectant, malathion, was adopted in Australia in 1960/61 as the solution to insect-free status for export grain and domestic use. Based on the life cycle of various species, a period of up to 6 wk from application to shipment was needed to ensure that all insects present at time of treatment had emerged and had been killed; reaching this needed 6 wk period was accomplished by having started treatments at the rural grain receival storages. So commenced the "golden age of malathion and tin sheds". However, insect resistance to malathion was detected in 1968, which thus began an ongoing list of pesticides (organophosphates, carbamates, synthetic pyrethroids, bacterium, and insect growth regulators) being made redundant with the onset, yet again, of insect resistance (Winks and Ryan, 1994).

Current grain protectant recommendations by the Grain Research Development Corporation (GRDC, 2024) advise: 1st step—choose a Spinosad or deltamethrin-based stored-grain protectant and rotate every year to prevent resistance. Spinosad or deltamethrin (which will have s-methoprene included) provide effective protection against four of the five grain storage pests, including the lesser grain borer, *Ryzopertha dominica* (Fabricius); the rust-red flour beetle, *Tribolium castaneum* (Herbst); the saw-toothed grain beetle, *Oryzaephilus spp.*; and the flat grain beetle, *Cryptolestes spp.*). As for the 2nd step—choose a mixing partner to include as protection against the rice grain weevil, *Sitophilus oryzae* (Linnaeus)—the fifth grain storage pest. The choice will be between stored-product protectants containing either fenitrothion or chlorpyrifos-methyl. The simultaneous dispensing of multiple mixtures supports the concept of a Co-Fumigant as suggested by research groups in the USA (Muhareb et al., 2009) and Australia (Jagadeesan et al., 2018). Other lessons learned from the grain protectants experience include replacing active ingredients because of insect resistance, using selected chemicals as resistance breaks, and monitoring for resistance outbreaks. All the above apply equally to fumigants.

FUMIGANTS

While fumigants have been used for more than a century, the efficacy depends on dispensing a non-flammable gaseous/vapor mix, achieving uniform distribution, leaving no significant chemical residue, and causing mortality within all the insect life stages. A critical requirement is a gastight storage (tested according to the Australian Standard AS2628). A silo is only truly sealed if it passes the half-life pressure decay of >5 min. While it may be possible to claim "pesticide-free grain" using fumigants, it is not possible to guarantee "insect-free" status in an unsealed storage.

Early fumigants composed of industrial chemicals such as sulfur dioxide (SO_2), carbon disulfide (CS_2), and hydrogen cyanide (HCN) predate other fumigants of stored products. Methyl bromide (MB) was discovered as a fumigant by Le Goupil (1932); liquid ethyl formate (EF) was used in the niche fumigation of dried grapes in 1927 (Simmons and Fisher, 1945); and phosphine (PH_3) patents were granted in Germany (Freyberg, 1935) and the USA (Freyberg, 1938) for aluminum phosphine tablets which generated PH_3 by reacting with atmospheric moisture. In the 1960s,

Australia had access to four fumigants (MB, PH_3, HCN, EF). All these fumigants are flammable, with MB gas requiring a high-energy ignition to demonstrate explosive limits: LEL 10% and HEL 16%. On the other extreme, PH_3 requires a very low-energy ignition for the LEL 1.6% and HEL 100%. The non-flammable sulfuryl fluoride (SF), initially used as a structural fumigant for dry wood termites (Vikane), was renamed Profume and was approved for food fumigation in 2004. The preferred fumigant PH_3 has insect resistance issues. Sulfuryl fluoride is an alternative fumigant used in the PH_3 insect resistance break strategy. There are environmental issues associated with some fumigants. The preferred fumigants are MB and PH_3; however, MB—an ozone depletor—is restricted to Quarantine Pre-Shipment fumigation because of its Montreal Protocol listing. Phosphine is the fumigant of choice because of cost considerations, superior efficacy, and environmental acceptance. The major user of fumigants—the cereal grain industry—is determined to maintain PH_3 as the priority grain fumigant. Phosphine has been used as a fumigant for 90 years and is available as solid tablets (e.g., aluminum phosphide tablets that react with atmospheric moisture to generate the gas) or in gas cylinders as 99% PH_3 or a non-flammable PH_3/CO_2 mixture. The 99% pure PH_3 is mixed on site with air to deliver a non-flammable <1.6% PH_3 in an atmospheric air mixture. Most fumigants are flammable and must be diluted/mixed to become non-flammable mixtures prior to dispensing into the storages to be fumigated.

The H.A.U. Monro "Manual of Fumigation for Insect Control, 2nd Edition" FAO Rome (Monro 1969) was the "Fumigators' Bible" (1st Edition, 1961) and became globally popular with eight reprints plus an updated book edited by Bond (1984). Both Hector Monro and Edwin Bond were entomologists at the Research Institute, Agriculture Canada, London, Ontario. Comparison with the Monro List shows the five current fumigants in use in Australia (EF, HCN, MB, PH_3, SF) on the 60-year list (Table 1).

Table 1. Fumigants used in Australia for over 60 years.

Fumigant*	**Period of Use**
Ethyl Formate [$HCOOC_2H_5$; EF = 2%].	Current / ML
Hydrogen Cyanide [HCN = 1%].	Current / ML
Methyl Bromide [CH_3Br; MB = 1%].	Current / ML
Phosphine [PH_3 = 0.1%].	Current / ML
Sulfuryl Fluoride [SO_2F_2; SF = 3%].	Current / ML
Acrylonitrile [CH_2CHCN]	Old/ML
Carbon Disulfide [CS_2 = 1%]	Old/ML
Chloropicrin [Cl_3CNO_2]	Old/ML
Dichlorvos [$Cl_2C{=}CHOPO(OCH_3)_2$] DDVP = 2 ppm	Old/ML
Ethylene Dibromide [CH_2Br-CH_2Br]	Old/ML
Ethylene Dichloride [CH_2Cl-CH_2Cl]	Old/ML
Ethylene Oxide [CH_2-O-CH_2]	Old/ML
Paradichlorobenzene [$C_6H_4C_{l2}$]	Old/ML
Trichloroethylene [$CHCl{=}CCl_2$]	Old/ML
EDN/Cyanogen [C_2N_2; CN]	Potential
Carbonyl Sulfide [COS]	Potential
Co-Fumigant:	
Propylene Oxide [PO]/ [SF]	Potential
[PH_3] / [SF]	Potential
[PH_3] / [EF]	Potential

*Reproduced from Monro (1969); ** ML is Monro List

Current fumigants include: EF—rapid acting (4–24 h), flammable, effective in controlling cereal insects, sorption issues, no EF residues, pre- and post-mix with CO_2 to eliminate flammability and to enhance the EF efficacy, and potential yet to be realized; HCN—restricted to empty storages (24 h), flammable liquid, and potential yet to be realized; MB—rapid acting (4–24 h), an ozone depletor being phased out because of its Montreal Protocol listing, and current use is restricted to Quarantine / Pre-Shipment (QPS); PH_3—slow acting (5 d exposure time), flammability hazard, priority grain fumigant because of its efficacy and cost-effectiveness, and outbreaks of strong insect resistance issue; and SF—slow acting (>2 d), non-flammable, controls PH_3 insect-resistant outbreaks, efficacy (insect eggs), fluoride residues, and global warming issues.

Early research and years of development raised expectations for two potential fumigant gases, viz: cyanogen (C_2N_2/EDN) and carbonyl sulphide (COS). The EDN fumigant has had its registration approved for soil and timber/log fumigation but not for stored-product insects. There has been no obvious activity of seeking approval for COS as a fumigant for stored-product insects.

Other potential fumigants from the Monro List include: carbon disulfide (CS_2), dichlorvos (DDVP), and methyl formate (MF). A historical fumigant, CS_2 was once popular as an on-farm fumigant, but fatal accidents related to its flammability resulted in it being withdrawn by the pesticide registration authorities. However, it is now possible to deliver a non-flammable mixture of CS_2. Dichlorvos vapor at toxic concentration can be moved through grain for up to 4 m using aeration fans (Desmarchelier et al., 1977), and 0.4 ppm for 4 h exposure has been shown to kill 95% of the confused flour beetle (Harein et al., 1970). Optimistically, there may be revivals of CS_2, DDVP, and methyl formate (MF)—particularly as Co-Fumigants.

The concept of Co-Fumigation, like mixed grain protectants, could be a practical solution for some fumigant issues. Reported Co-Fumigation examples include the MBAO publications detailing the paper reporting on the combination of propylene oxide [PO], SF, and CO_2 studied by Muhareb et al. (2009). This mixture balanced the poor SF efficacy on insect eggs with the effectiveness of PO on eggs plus the high efficacy of SF on post embryonic insect stages. Insects studied were the warehouse beetle (*Trogoderma variabile* (Ballion)), the Indian meal moth (*Plodia interpunctella* (Hubner)), and the red flour beetle (*Tribolium castaneum)*. The published paper reported both eggs and post embryonic stages showed reduction by a factor of 3x in the Ct products required. The addition of synergist CO_2 allows the combination to be delivered as either a non-flammable pre or post mix (onsite mixing). More appropriate for the Australian grain industry is the study by the Department of Primary Industries Queensland (DPIQ) laboratory. The DPIQ study was initiated over the concern of insect resistance to PH_3 in stored grain "because there is no suitable replacement for PH_3, as most of the available alternatives suffer from specific weaknesses". The DPIQ study controlled the strong insect resistance to PH_3 and reduced the time required for a successful fumigation by using the Co-Fumigation combination of PH_3 and SF to achieve this goal (Jagadeesan et al., 2018).

Flow-Through Fumigation—SIROFLO® (Winks and Ryan, 1990; Winks, 1993)—is an option for non-gastight grain storages that currently involves a continuous flow of a low PH_3 (~100 ppm) concentration for an extended exposure period (3 wk) in storages which are partially sealed (such as a bucket, which has a sealed bottom but a non-sealed top). The flow-through fumigation is conducted in both vertical and horizontal storages in Eastern Australia. This technique is of use

with fumigant applications in non-gastight storages and with alternative fumigants of high sorption stored products (e.g., oilseeds).

DISCUSSION

The use of fumigants is to achieve insect-free and pesticide residue-free food. The aim of this paper was to evaluate options and issues with existing fumigants. With the demise of MB, PH_3 is the preferred fumigant. The preferred fumigant PH_3 does need relief where strong insect resistance occurs. Currently identified strong PH_3 resistance is controlled by a follow-up SF fumigation. Learning from the grain protectants model of spraying multiple registered products simultaneously supports the adoption of mixed fumigants, i.e., Co-Fumigant. The DPIQ has demonstrated that Co-Fumigation using PH_3 and SF simultaneously gives the dual benefits of controlling all insects in a shorter time. There would be more confidence and security in the grain industry if additional backup fumigants for strong resistance were available. The historical Monro List includes the potential backup fumigants of EF and HCN (registered fumigants) and of CS_2, DDVP, and MF (not currently registered). In addition, there are two potential stored-product fumigants—EDN and COS. Volatile fumigants are more likely to be small molecules and commodity chemicals which could negate any commercial protection for the time and expenses involved in the registration and marketing of a new fumigant.

Earlier reviews included “Fumigation - an endangered technology” (Banks, 1994) and “Return of the Fumigant” (Ducom, 2006). Banks (1994) concern of the extinction of fumigants was related to the projected demise of MB and global high insect resistance to PH_3. Ducom (2006) review was optimistic with planned new fumigant molecules in the pipeline. While new fumigants are needed, an intermediate option is adopting Co-Fumigant applications.

While issues of ozone depletion and global warming are not resolved, community health and safety issues are an ongoing focus. Efficacy data has multiplied but continues to be impacted by non-uniform distribution related to leaky storage and sorption. Sorption can be so significant that fumigants concentration is reduced and no longer effective. Counter measures to overcome sorption should be a high priority for industry and research laboratories. Flammability issues can usually be overcome, e.g., PH_3 flammability were resolved using cylinderized liquid PH_3 /CO_2 mixes and the onsite mixing of pure PH_3 and air. Vaporization is a requirement for liquefied gases and liquid fumigants to ensure no liquid is deposited on grain surfaces. Dispensing systems are becoming more sophisticated as is seen with the global technology transfer of onsite mixing producing non-flammable gaseous PH_3 mixtures.

CONCLUSIONS

The history of grain protectants highlights major issues with insect resistance, resulting in the demise of a long list of no-longer-effective insecticides. The current grain protection treatment protocol uses two grain protection products simultaneously. The advantage of grain protectants is the ability to treat and store grain in non-gastight storages; however, the disadvantage is pesticide residues.

The effective practice of applying two separate registered grain protectant insecticides simultaneously should be adopted with existing fumigants to eliminate issues of insect resistance, flammability, and efficacy. All current fumigant gases have some issues. The preferred fumigant, phosphine (PH_3), has issues of strong insect resistance. Sulfuryl fluoride (SF), the resistance break fumigant for PH_3, has the drawbacks of incomplete efficacy (insect eggs), global warming, and fluorine residue with repeat fumigations. Hydrogen cyanide (HCN), a veteran fumigant, is currently limited to the treatment of empty grain storages; and the potential stored-grain fumigant, cyanogen (C_2N_2), is yet to obtain approval. Another veteran fumigant, methyl bromide (MB)—known to be an ozone depletor—has been retained for quarantine and pre-shipment (QPS) purposes only. While ethyl formate (EF) is making a comeback, it has yet to demonstrate success as a fumigant of bulk grain.

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Progress and Benefits of UltraPhos Compressed Liquefied Phosphine Gas

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ABSTRACT

Compressed liquefied 99% phosphine (PH_3: UltraPhos / U-Phos in the USA) shipped in high pressure cylinders (~4 MPa or 40 bar), is a fumigant used to control stored-product pests. UltraPhos has been successfully used in Australia for the last 10 yr and is now used in other countries.

UltraPhos has benefits over the commonly used solid metallic phosphide PH_3-generating products: the PH_3 flammability hazard is controlled; accurate control of PH_3 concentration is regulated; PH_3 is rapidly dispensed; uniform distribution within the grain mass is quickly achieved; and the handling and disposal of the solid PH_3 generating products is avoided and requires less labor.

Successful fumigations depend on the quality of the cylinderized phosphine and the dispensing machine design/operation. Gaseous PH_3 reacts with oxygen in dispensing equipment, producing polymer dust and oily phosphoric acid which affects control valves. This issue demands pre- and post-purging of PH_3 dispensing systems with inert gases such as carbon dioxide (CO_2) or nitrogen (N_2) gas. The critical impurities of cylinderized phosphine are diphosphine (P_2H_4) and white phosphorus (P_4), which are pyrophoric and can cause fires in dispensing equipment. The manufacturing process and specifications determine the level of impurities present.

The flammable UltraPhos is mixed onsite and rapidly diluted to ~10,000 ppm (LEL =18,000 ppm) with atmospheric air using sophisticated dispensers. Two sizes of PH_3 gas dispensers are currently manufactured—the HiFlo (PH_3: 6 kg/h) and the LoFlo (PH_3: 0.2 kg/h)—to complement the different doses and exposure times in the treatment of gastight storage (up to 1 wk exposure) and leaky grain storage (up to 4 wk exposure), respectively.

Keywords: Phosphine gas, UltraPhos, U-Phos, Cylinderized phosphine, High pressure cylinders, Diphosphine, White phosphorus, Flammability hazards, Dispensing fumigants machine, Onsite mixing

INTRODUCTION

Gaseous PH_3 has applications in industries as diverse as the manufacturing of silicon semiconductor products and of flame-retardant treatments for industrial clothing. Compressed liquefied PH_3 gas was initially investigated as a fumigant for the control of the fruit fly (Ryan, 1997). The initial PH_3 fumigant was patented in Germany (Freyberg, 1935) and soon after in the USA (Freyberg, 1938). These patents were related to a solid aluminum phosphide (AlP) formulation which generates PH_3 gas on exposure to moisture in the atmosphere.

Insect control was initially achieved using grain protectants; however, insect resistance and the requirement for insect and pesticide-free products led to the increased use of PH_3 fumigation. The major threat to the on-going use of PH_3 is the outbreaks of strong insect resistance. Fumigation also needs to satisfy marketing requirements. Australia exports about 80% of the grains it produces, and to maintain market reputation, the grain must be free of live insects and pesticide residues. 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 proven way to minimize resistance is by using PH_3 correctly in gastight sealed storages and by achieving the minimum Ct product (Concentration × time) to ensure effective fumigation. Many grain storages are not "gastight". The Australian Standard, AS2628 (2010), states that sealable storages must be able to pass a 5 min, half-life pressure test. Modern "bunker" bulk storages can be sealed for fumigation. Specialists from bulk grain storage sealing companies can achieve gastight status in many storages. Most "non-sealed" storages can be partially sealed and adapted to flow-through fumigation which uses low PH_3 levels (~150 ppm) with extended exposure times (4 wk). Flow-through PH_3 fumigation has been used in Australia for over 25 yr in tall vertical silos and has the lowest treatment dose (~3 g/t).

PHOSPHINE PRODUCTS

There are two types of PH_3 products differentiated as solid and compressed liquid. The "solid" formulation refers to the slow-release solid AlP tablets while "liquid" product refers to the non-flammable mixture of 2% PH_3 (wt) in carbon dioxide (CO_2) and compressed liquefied 99% PH_3 gas both in high-pressure cylinders. The AlP formulation continues to dominate the global fumigation market, albeit with an increasing use of cylinder PH_3 products.

The advantages of the solid formulations are portability, relatively low cost, and versatility of application under a variety of conditions. Disadvantages of the solid formulations include the inability to "top-up" PH_3 concentration and control/maintain the optimum PH_3 concentration; operator safety during PH_3 handling; disposal of unreacted residues; flammability issues (rely on slow release over days to diffuse and be diluted in the surrounding air); disposal costs of unreacted powder residues; and longer exposure times to generate the gas and achieve uniform distribution.

The initial liquid formulation was a high-pressure (4 MPa) non-flammable mixture of 2% PH_3 (wt) in carbon dioxide (CO_2) kept in gas cylinders. This patented non-flammable PH_3/CO_2 mixture (Ryan and Latif, 1989) progressed to the onsite mixing patent of PH_3 and air (Ryan and Shore, 2005). The advantage of these gaseous PH_3 products is the 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 with the ability to "top up"; a more rapid delivery of PH_3 gas; better distribution in the grain mass without disturbing grain; and a controlled flow and dose maintenance for long periods. In addition, cylinder PH_3 avoids exposure and requires less labor. The compressed liquefied UltraPhos 99% PH_3 gas has critical specifications for pyrophoric impurities such as P_2H_4 and P_4, which are pyrophoric (Fluck, 1973). Large volumes can be fumigated using compressed liquefied 99% PH_3 gas (>20,000 m^3/cylinder, which is equivalent to ~30 cylinders of the 2% PH_3/CO_2 mix). UltraPhos has been successfully used in Australia for the last 10 yr, and the identical product U-Phos is registered in the USA.

FLAMMABILITY

The solid AlP formulation generates flammable PH_3 gas when exposed to moisture in the atmosphere. However, because the flammable PH_3 is released slowly over days and diluted by diffusion into the surrounding air, this potential of generating a flammable gas is mitigated. The high-pressure (4 MPa) non-flammable mixture of 2% PH_3 diluted in CO_2 was developed to overcome these flammability issues (Ryan and Latif 1989).

Over the past 30 yr, new technology has enabled 99% PH_3 to be supplied as a compressed liquefied gas under pressure (4 MPa) in industrial gas cylinders and mixed onsite with atmospheric air. The process involves rapid onsite dilution in atmospheric air to less than the 1.8% flammability limit prior to dispensing into the storages being fumigated. All PH_3 products are manufactured from P_4, which is pyrophoric (any unreacted P_4 is a flammability hazard). The critical impurities of cylinderized phosphine are diphosphine (P_2H_4) and P_4, which are pyrophoric and can cause fires in dispensing equipment. The manufacturing process determines the level of impurities (Ryan and Nicolson, 2014). It is critical to ensure that the PH_3 level being dosed into the storages is non-flammable in order to avoid fire or explosion.

DISPENSING

Gaseous PH_3 reacts with atmospheric oxygen in dispensing equipment, producing polymer dust and oily phosphoric acid which affects control valves (Ryan and Shore, 2012). To prevent this from happening, a pre- and post-purging of PH_3 dispensing systems with an inert gas, e.g., CO_2 or N_2, is required (Ryan and Nicolson, 2014).

The flammable 99% PH_3 formulation (UltraPhos/U-Phos) is dispensed onsite by rapid dilution in air (Ryan and Shore, 2005) to less than 18,000 ppm using sophisticated dispensers. Dispensers currently being manufactured have different capacities. For example, GasApps Australia has the HiFlo (PH_3: 6 kg/h) and the LoFlo (PH_3: 0.2 kg/h) to complement the different dose and exposure times in the treatment of gastight storages (up to 1 wk exposure) and leaky grain storages (up to 3 wk exposure), respectively. The unique design of the dispenser and its resulting benefits include being easy to set up and safe to use with multiple safety interlocks. The HiFlo dispenser is usually supervised as the applied dose is quickly delivered, while the LoFlo (with an exposure period of 2 wk) can operate at unmanned isolated sites. Both dispensers communicate any issues on-line, and both can be interrogated remotely.

As most of the fumigation equipment is used at rural sites, robust reliable dispensing equipment design is required to withstand the rugged rural “road” transport (Fig. 1-5).

Fig. 1. Bunker fumigation Australia.

Fig. 2. Installing UltraPhos dispensing hose.

Fig. 5. UltraPhos cylinders.

Fig. 3. Installing UltraPhos dispensing hose.

Fig. 4. UltraPhos HiFlo dispenser.

DISCUSSION

The main issues associated with PH_3 application are ineffective fumigation and insect tolerance/resistance. The expression “If you are not measuring, you are not fumigating” is an important reminder. Other issues include non-gastight storages and/or failure to top up the PH_3 concentrations.

Effective fumigations should be carried out in validated gastight storages. The Australian Standard, AS 2628 (2010), details the use of a decaying pressure test (pressure drop in 5 min from 25 mm to 12 mm using a U-tube liquid manometer). Many grain storages fail this test; however, all can be fumigated using PH_3 flow-through fumigation. The liquid PH_3 formulations support CSIRO’s flow-through fumigation process, SIROFLO (24/7 flow and 28 d exposure), in non-gastight grain storages (Winks, 1987). The flow-through fumigation enables the fumigation of grain in “leaky” (non-gastight) storages.

The flow-through fumigation maintains a small level of 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 storages (Winks and Ryan, 1990). The low PH_3 concentration (~150 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). Any released PH_3 is short-lived because it reacts with the atmospheric oxygen, forming phosphoric acid (food acid, fertilizer).

Another major issue is outbreaks of strong PH_3 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’, ‘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 using sulfuryl fluoride (SF) has been implemented. Sulfuryl fluoride is being used in the effective management of strong resistant *C. ferrugineus* populations in bulk storages; however, SF does have an issue with efficacy against the egg stage of storage pests, particularly at lower temperatures (Nayak et al., 2010). The option of Co-Fumigation, e.g., simultaneous PH_3 and SF addition has benefit.

Kashi and Bond (1975) showed that, in 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 used. 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.

CONCLUSIONS

The compressed liquefied UltraPhos 99% PH_3, shipped in high pressure cylinders (4 MPa), has been successfully used in Australia for the last 10 yr and is now used in other countries. U-Phos is identical to UltraPhos and was renamed when recently registered in the USA.

Dispensing PH_3 has progressed to onsite mixing with atmospheric air. The process involves the rapid mixing of PH_3 with high velocity air to deliver a non-flammable mixture of 1% PH_3 in air (as previously shown in Figs. 1–5). The compressed liquefied UltraPhos 99% PH_3 has benefits over the commonly used metallic phosphides, including onsite mixing to eliminate any flammability hazard as well as regulation and maintenance of PH_3 concentration during fumigation.

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Fumigation of Wheat with Ozone

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ABSTRACT

Ozone is a strong oxidant with the advantage as a fumigant of rapid decomposition and leaving no residue. Although ozone has been widely used in the food industry in recent years, research on it for control of stored grain insect pests is limited. All life stages of *Rhyzopertha dominica* (lesser grain borer) and *Tribolium castaneum* (red flour beetle) in wheat were completely killed at 700 ppm ozone and 25±1°C and 55–60% r.h. for 16 h treatment. The pupae stage is the most tolerant stage to ozone. The end-point of mortality for adults is 8 d after ozone treatment. Ozone can penetrate and evenly distribute through bulk wheat silo after a short purging period under vacuum. There was no effect on seed germination, consistent across all the ozone treatments from the untreated control to treated grain for 16 h ($p<0.05$). Physiological effects on wheat and its quality were investigated without negative impact on soluble protein, gluten, moisture content, and hardness. Two new chemical compounds, nonanal and nonanoic acid, were generated after ozone treatment, both of which are naturally produced in plant and contribute ross, coconut, and fresh grain odor. Therefore, ozone is an environmentally friendly fumigant that offers rapid 24 h fumigation and management of phosphine resistance.

Keywords: Stored product insect control, *Rhyzopertha dominica*, *Tribolium castaneum*, Ozone fumigant, Fumigation, Phosphine resistance

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Walse S (2024) Ethyl formate applications for fresh fruit in the United States. Page 175. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Ethyl Formate Applications for Fresh Fruit in the United States

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ABSTRACT

Ethyl formate is a valuable alternative to methyl bromide for controlling surface-feeding insects on fresh fruit. Here we detail efforts over the last several years to gain (Federal Insecticide, Fungicide, and Rodenticide Act) FIFRA-registration as a bio-pesticide, with attention to efficacy, residues, applicator/bystander exposure data, as well as key market opportunities. We will present fumigation experiences across a wide variety of fruits, citing similarities and differences, as well as likelihood of adoption by respective industries. Of particular focus will be the use of ethyl formate to control key insect pests of table grapes, for both exports from California and imports from Chile. Finally, we will discuss how the initial work of De Lima on "field-temperature fruit" has evolved to play a critical role for the California citrus industry, whereby it addresses a domestic quarantine need to control Asian citrus psyllid.

Keywords: Ethyl formate, Bio-fumigant, Methyl bromide alternative

Paper No. CAF2024-F49

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Enhancing Grain Storage Safety: Investigating the Efficacy of Temperature Reduction Using Grain Chillers to Mitigate Insect Infestation

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ABSTRACT

It is well known that insect pests thrive within specific temperature ranges, and grain storage facilities typically create favorable conditions for their growth. The situation is aggravated by the prohibition of highly efficient fumigants, resistance to available fumigants, and the proliferation of invasive insect species, underscoring the need to identify alternative methods to protect stored grain from infestation. This study addresses the potential of temperature reduction in preventing insect infestation, acknowledging the well-documented phenomenon of a significant slowdown in insect development at lower temperatures. The research conducted involved a trial in a continental climate near New Delhi, India. Two small-scale silos, each with approximately 800 kg storage capacity were utilized, alongside a sample stored in a standard plastic gunny bag to resemble traditional storage. One silo followed conventional industry practice, aeration with ambient air for six hours nightly, while the other was equipped with a small-scale grain chiller. Both silos stored freshly harvested, pre-cleaned, and dried corn of the same batch. The study assessed the level of insect-damaged kernels in the initial product, as well as after 3 and 6 mo of storage. Results revealed a notable difference in insect damage among the storage methods. The product stored in the gunny bag exhibited over 22% insect-damaged kernels, the aerated silo displayed over 5%, while the chilled silo remained at 0%. Average product temperatures during the trial were 29.7°C, 29°C, and 16.8°C for bag storage, aerated silo, and chilled silo, respectively. In conclusion, this study demonstrates the effectiveness of using a grain chiller in preventing insect infestation, presenting a viable, sustainable alternative for preserving stored grains in a cost-effective manner.

Keywords: Grain chilling, Grain cooling, Safe storage, Grain storage, Cool conservation, Infestation mitigation, Fumigation alternative

INTRODUCTION

The pivotal role of grain temperature in managing insect infestation and preserving the quality of stored grains is well established (Li et al., 2018). Maintaining control over temperature is critical, given the inability of insects to thrive outside the range of 13–35°C (Fields, 1992). High-temperature treatments, while effective in pest control, often come at the cost of quality degradation, particularly in the context of heat treatments using hot air (Paul et al., 2020). Cooling

grain has emerged as an effective strategy against insects, leading to the development of grain chillers that can be used in scenarios where ambient conditions are insufficient for cooling (Maier and Navarro, 2002). Over the past six decades, the successful use of chilled aeration in grain storage has been demonstrated, with grain chilling recognized as the primary method for preventing damage from molds and insects, as early as 1989 (U.S. Congress, 1989).

The application of grain chilling involves utilizing a mobile refrigeration system that independently regulates the temperature and relative humidity of the aeration air, ensuring optimal conditions for stored grains (Maier, 1994). Existing studies suggest that maintaining grain temperature below 20°C significantly reduces the development rate of insects compared to higher temperatures (Morales, 2017). Industrial silo complexes utilizing grain chilling have showcased the ability to keep stored grain insect-free for extended periods, even under extreme weather conditions (Lazzari et al., 1994, 2010). The insulating properties of the grain itself contribute to the efficiency of grain chilling, necessitating only occasional re-chilling to sustain optimal storage conditions (Maier, 1994). Simulations conducted for paddy rice silos indicate that a brief period of cooling can maintain low average grain temperatures over an extended storage period, potentially reducing or eliminating the need for chemical control of stored-product insects (Morales, 2017).

Chilling grain below 15°C within a week has proven effective in preventing most insect species from completing one life cycle, making chilled aeration a technically feasible strategy for reducing or eliminating the need for chemical control of stored-product insects (Rees, 2004). Economically, grain chilling emerges as a viable solution for chemical-free pest control, even in tropical conditions, with operational costs found to be lower than alternative methods such as aeration with ambient air and fumigation combined (Morales, 2017).

Noteworthy is the broader impact of grain chilling, extending beyond pest control to mitigate quality losses in cereal storage and prevent product deterioration (Adler, 2007). Proper cleaning of storage facilities before grain chilling is emphasized to ensure successful chemical-free and insect-free storage (Lazzari et al., 2010). In instances where living insects are present before storage, a one-time phosphine fumigation cycle followed by grain chilling to 1°C has proven effective for insect-free long-term storage.

To contribute further insights into the practical implications of these findings, our study investigates the influence of storage methods (chilling, aeration, and traditional bags) and the resulting temperature on corn infestation in a continental climate near New Delhi, India. This research aims to bridge existing knowledge gaps and enhance our understanding of the interplay between storage conditions and insect infestation in a real-world context.

MATERIALS AND METHODS

In this study, we employed a carefully designed experimental setup to investigate the influence of storage methods on corn infestation in a continental climate near New Delhi, India (Fig. 1). The key components of our setup included two small-scale silos, a chiller, an aeration fan, product stored in a standard gunny bag, and laboratory equipment (Fig. 1).

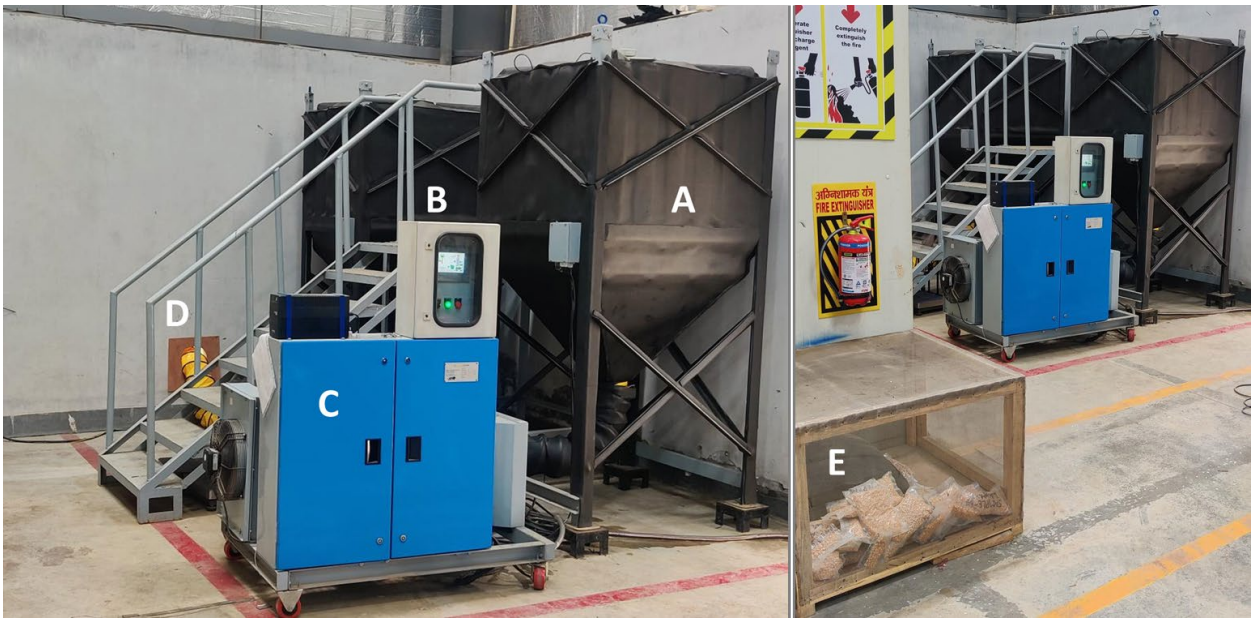

Fig. 1. Experimental setup. In the graph, A and B are the two small silos, C is the chiller, D is the aeration fan, and E is the product stored in a standard gunny bag.

The silos used in the experiment were locally purchased and made from high-density polyethylene (HDPE), each with a capacity of 1 m³. To ensure proper insulation, the silos were equipped with 19 mm thick closed-cell nitrile rubber, with a thermal conductivity of 0.037 $Wm^{-1}K^{-1}$. The silo bottom was cut open in-house and fitted with an aeration floor opening covering the full floor area of 1.2 m². Both silos were equipped with three PT100 sensors each, mounted in the silo center and continuously measuring the product temperatures in the bottom, middle, and top layers, respectively. One PT100 sensor was fitted in the middle of the storage bag.

The chiller was specially designed for the study, featuring a semi-hermetic 2-cylinder compressor (Bitzer 2FES-3Y-40S) with a rated cooling power of 10.9 kW, operating at a condensing temperature of 40°C and an evaporating temperature of 10°C. The refrigerant used was R-407C, and the chiller was powered by a 3-phase 415 V/50 Hz supply. Capacity control was achieved through a Danfoss variable speed drive, and a 2-kW electric heater with Radix thyristor control provided additional temperature control. The chiller system was managed by a PLC (Siemens S7-1200) and an HMI (Siemens KTP700), both supported by a battery-buffered UPS. The maximum air flow of the chiller was 1800 m³/h, with a constant air flow and static pressure of approximately 350 m³/h and approximately 300 Pa maintained during the trials. The supply fan was a Hicool CFBE-225S (Centrifugal fan, forward-curved, rated voltage 230 V/50 Hz), and the condenser fan was a Ziehl-Abegg FN035-VDK.0F (axial fan with external rotor motor, rated voltage 3 phase 400 V/50 Hz). The chiller was fitted to supply cold air to silo A via a hose at the silo bottom. Silo B was connected accordingly to an aeration fan, supplying approximately 350 m³/h of ambient air to the silo. Both silos were set up next to each other inside a hall to assure identical ambient conditions. The ambient air for silo B was sucked in from outside the hall to achieve realistic results, with the aeration fan being protected from rain and direct sunlight. The air for the chilled

silo A was sucked in directly from the hall, since the silo supply air conditions were controlled independently of the ambient conditions. Since grain is stored in bags that are usually piled up, the storage bag was covered with the same insulation material as the silos to give realistic results. The filled bag was placed inside a mesh cage to protect it from rodents.

The silos and the storage bag were loaded with freshly harvested, pre-cleaned, and dried corn of the same batch, purchased from Bihar, India. Different storage conditions were applied to the silos, with one following conventional industry practice utilizing aeration and the other equipped with the grain chiller. The Siemens S7-1200 PLC and Siemens KTP700 HMI were utilized to control and monitor both the aeration as well as chilling conditions. The aeration fan ran for 6 h daily, 3 h in the late evening between 21:00 and 00:00 and 3 h in the early morning between 3:00 and 6:00. The chiller in silo A was started automatically once any of the three temperatures measured in the silo reached 21°C and was henceforth running continuously until the target temperature of 15°C was reached in all layers. The product temperatures, air temperature, and humidity conditions in both silo supply air flows, as well as the silo outlets, were recorded throughout the trials.

Sampling was carried out at regular intervals using a Green Agritech Equipment sampling spear (Ambala, India) with a length of 1.5 m and six openings. Insect damage was evaluated in the fresh product and after 3 and 6 mo of storage as described by Mutungi et al. (2020). Moisture content was determined using the oven drying method according to ISO/DIS 712-2 standard.

RESULTS AND DISCUSSION

In the physical examination of the silo surfaces after 3 mo, flying insects were already observable in the aerated silo B. Silo A showed no apparent changes compared to the trial start. After 6 mo, only a few flying insects were observed in the headspace of silo A, and the appearance of the corn remained unchanged from the trial start (Fig. 2A). In contrast, the conditions in silo B had significantly worsened, with the top layer covered in webbing, dead moths, and other insects (Fig. 2B).

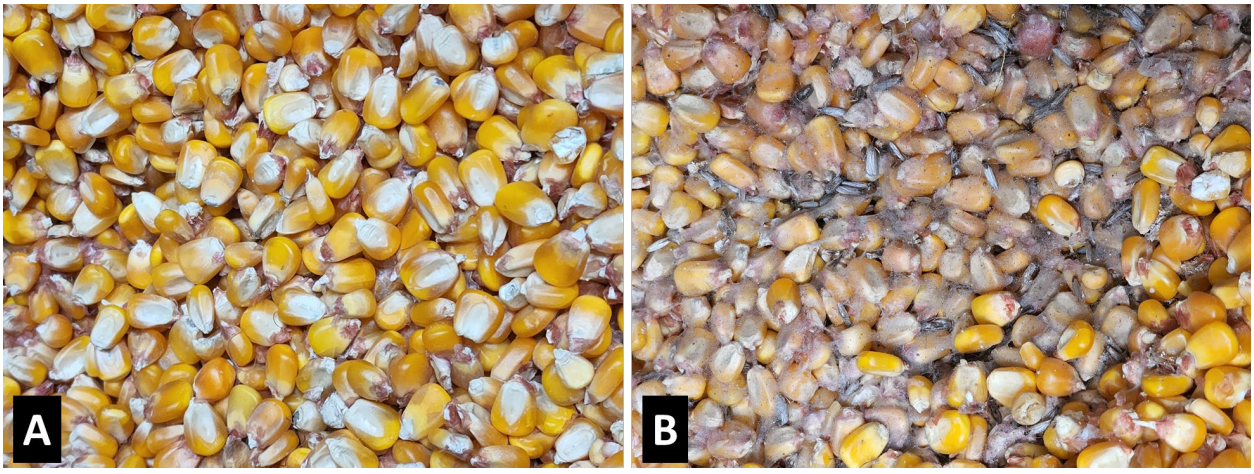

Fig. 2. Silo surface after 6 mo of storage in the chilled silo (A) and aerated silo (B).

Figure 3 depicts the monthly average product temperatures in chilled silo A, aerated silo B, and the gunny bag. The temperature in the chilled silo dropped to the target temperature of 15°C within a couple of days and remained at a constant low value with an average of 16.8°C during the trial period. The product temperatures in aerated silo B, as well as the gunny bag, only reduced after 4 mo of storage, with the average product temperature in the storage bag being the highest at 29.7°C, closely followed by silo B with an average product temperature of 29°C.

Figure 4 shows the changes in moisture content during the trial for the three storage methods. The product moisture was stable in the storage bag in the first 3 mo of the trial until the drop in ambient temperature in autumn led to a decrease in product temperature and hence a slight increase in moisture content. The moisture content in the chilled silo A increased in the first month of the trial and remained stable at a value just below 15%. This increase in moisture content seen when using chilling is attributed to two factors. Due to the small scale of the trial, the after-heating in the chiller was slightly too low during the trial, leading to a higher relative humidity in the supply air of approximately 80%, as opposed to the standard 65%. This increase in relative humidity led to moisture reabsorption in the corn due to an increase in equilibrium moisture content, which was now around 15%. Due to the small scale of the silo and hence increased thermal losses, the chiller runtime was slightly higher than it would be in a regular-sized silo. This meant that more air of high humidity was blown into the silo, leading to an increase in moisture content that would not be seen to this extent in industrial scale. Since the product temperature in silo A was at a low average of 16.8°C, this slightly higher moisture content was of no concern for the product quality, as could be seen when evaluating the corn quality after the trial. The moisture content in silo B changed very frequently in the first month of the trial, drying down to close to 11% before stabilizing at 14% for a month and then drying down again to just over 13%. Apart from the fact that a storage moisture content of 14% is very high for the prevailing temperature of more than 30°C, the frequent changes in moisture content can lead to stresses in the grain kernels, leading to an increase in broken corn. This not only lowers the product quality by itself but also facilitates the entry of secondary insect pests.

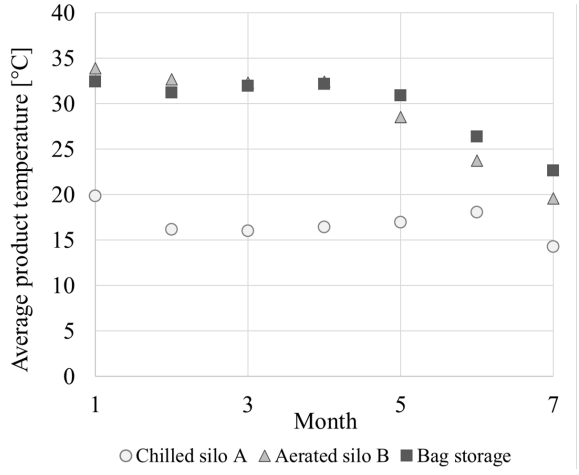

Fig. 3. Product temperatures during trial.

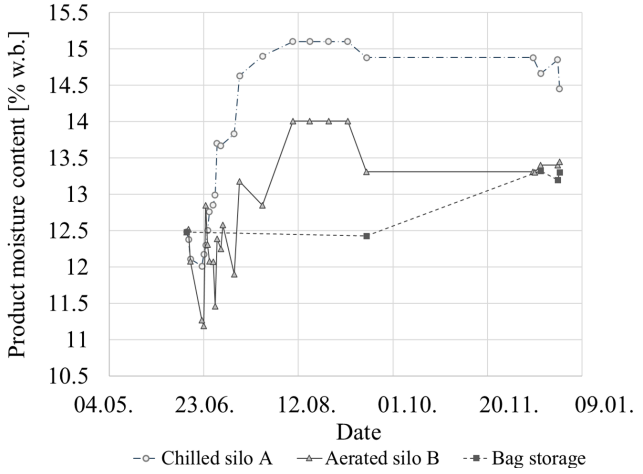

Fig. 4. Product moisture contents during trial.

The results of the quality determination revealed a notable difference in insect damage among the storage methods (Fig. 5). A first tendency could already be seen after 3 mo of storage when the number of insect-damaged kernels rose to 0.86% and 2.17% in the aerated silo B and the gunny bag, respectively. At the end of the trial, after 6 mo, the product stored in the gunny bag exhibited over 22% insect-damaged kernels, in the aerated silo over 5%, while in the chilled silo remained at 0%. Although a few flying insects were spotted at the top of the chilled silo A, there was still no noticeable insect damage observed in the grain bulk. Both the samples from the aerated silo B, as well as the storage bag, showed significant insect damage, and at least six different species of living as well as dead insect pests could be identified.

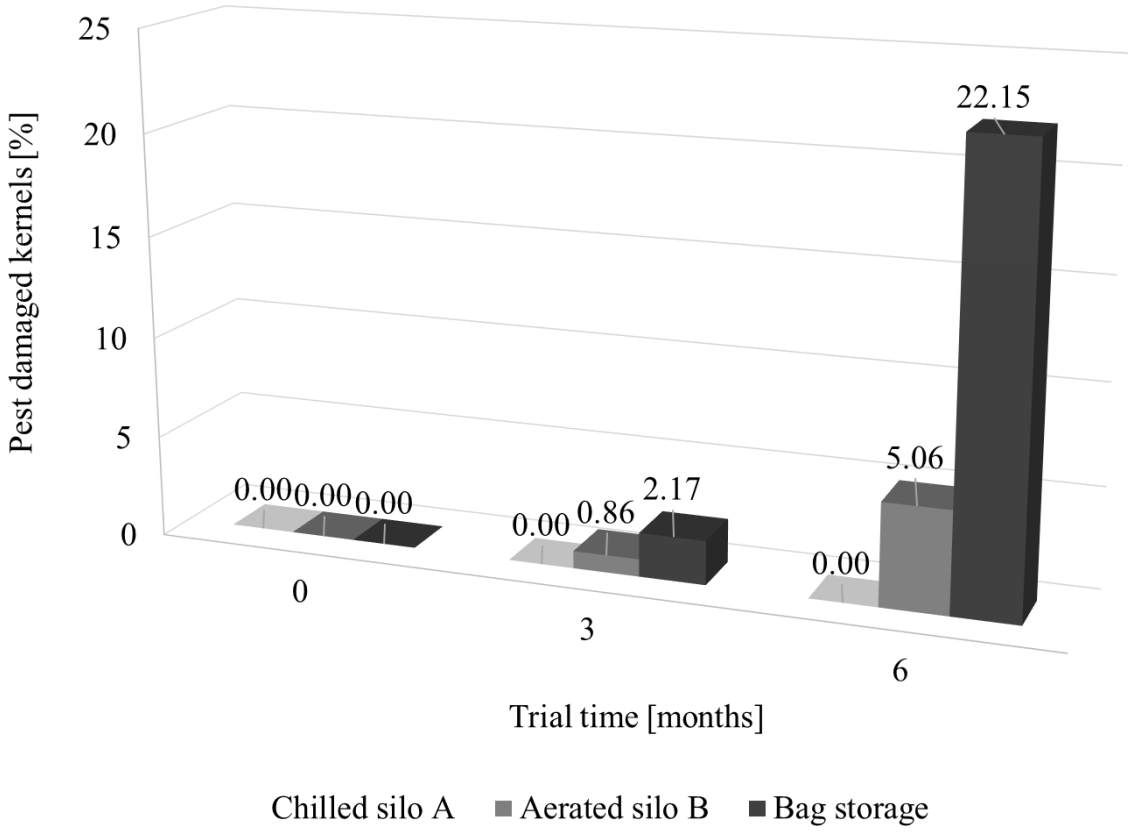

Fig. 5. Percentage of pest damaged kernels for all storage methods after 3 and 6 mo.

Our study highlighted the tremendous effect of the storage method on the product temperature, moisture content, and subsequent insect infestation in stored corn. It was demonstrated that a moderate but immediate temperature reduction could make a significant contribution to infestation control, with the corn showing no signs of insect damage even after 6 mo of storage without any chemical pest control. In conclusion, this study underscored the effectiveness of using a grain chiller in preventing insect infestation, presenting a viable, sustainable alternative for preserving stored grains in a cost-effective manner.

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Yang X, Qiu J, Xi J, Shen H (2024) Analyze the attractant effect of fragrance monomers of tobacco on tobacco beetles. Page 183. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Analyze the Attractant Effect of Fragrance Monomers of Tobacco on Tobacco Beetles

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ABSTRACT

Lasioderma serricorne (Tobacco beetle) is an important global storage pest which threatens the storage life of tobacco and other products. Different from sexual attraction, which can only attract males, food attractants can attract both males and females. This study focused on the attractant effect of several fragrance monomers of tobacco on tobacco beetles. The result showed that: 1) seventeen fragrance monomers of tobacco were initially screened from the tobacco fragrance library, comprising alcohols, lipids, ketones, and hydrocarbons. Five monomers with lure indices reaching 60%–65% were selected by olfactometer, which were propanoic acid 2-methyl-3-phenylpropyl ester, 3-phenylpropyl butanoate, 9E, 12Z-Tetradecadien-1-yl-acetate, phenethyl acetate, and 3-phenylpropyl acetate; 2) after conducting lure effect tests through compound combinations, two compound formulas with lure indices exceeding 70% were identified; and 3) combining two monomers and two compound food attractants with sex pheromones showed that their relative lure indices for tobacco beetles were higher than when using sex pheromones alone.

Keywords: *Lasioderma serricorne* (Tobacco beetle), Fragrances monomers, Lure index, Food attractant, Complex synergism

Paper No. CAF2024-A52

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Freezing out Flour Mills to Control Red Flour Beetles

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ABSTRACT

Low temperatures during the winter months on the Canadian prairies can be used to control insects in buildings. This low cost and environmentally friendly technique can provide an alternative solution to fumigation and is compatible with organic grain storage and processing facilities. To investigate its effectiveness, we conducted three freeze outs in two mills near Winnipeg, Manitoba, in the winters of 2014, 2015, and 2019. *Tribolium castaneum* (Herbst), a common insect pest in flour mills in Canada, was used in bioassays. Bioassays consisted of 50 adult *T. castaneum* with 15 g of media (95% white flour + 5 % brewer's yeast) placed in ventilated plastic vials throughout the facilities. Effects of cold acclimation (adults were acclimated in the lab for 1 mo at 15°C), acclimation on site (adults placed on site 1 mo prior to freeze out, temperatures ~15°C), and adult ages were investigated. Freeze outs lasted 69.5 h (2014), 76 h (2015), and 166 h (2019). During these times, outside temperatures averaged -15°C (2014), -20.3°C (2015), and -5.4°C (2019). In all three cases, temperatures inside the facilities remained higher than outside temperatures by ~15°C at 24 h and by ~10°C at 48 h after initiation of freeze outs. Mortality in bioassays ranged from 0% to 100%. In 2015, 100% mortality was observed in non-cold-acclimated insects, and 91-95% in lab and mill cold-acclimated insects, respectively. All the bioassays that did not reach 100% mortality had been placed in a non-ventilated narrow gap between two walls. In 2014, 89–90% mortality was observed for the lab cold-acclimated and non-cold-acclimated insects but only 24% mortality on average for insects acclimated on site. Again, overall percent mortality was reduced by survival in bioassays placed between walls. In 2019, due to relatively high temperatures, mortality was only 3% for cold acclimated adults, 10% for non-cold-acclimated young adults, and 23% for non-cold-acclimated old adults. These results show that *T. castaneum* can be successfully controlled by freezing out facilities if outside temperatures are sufficiently low, and cold air can be blown into narrow gaps.

Keywords: Low temperature, Cold treatment, Pest control, IPM, Alternative to fumigation, Stored-product insects

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Banks HJ, Sheppard M (2024) Improvised heat treatment of solid wood floor components in transport crate for exporting giraffe to New Zealand. Page 185. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Improvised Heat Treatment of Solid Wood Floor Components in Transport Crate for Exporting Giraffe to New Zealand

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ABSTRACT

A giraffe was to be sent (road and sea transport) from Perth (Australia) zoo to a zoo in New Zealand (NZ). The purpose-built transport crate contained an untreated hardwood (Jarrah, 40 mm thickness) floor. It was an import requirement of the NZ biosecurity authorities that the floor be treated against insect pests and other pests of biosecurity concern to appropriate standard prior to loading on the initial transport (road).

The 2.8 t crate was made with steel framework, walls and doors of sheet metal, lined with plywood with internal covering of synthetic turf. The roof was open, with PVC tarpaulin covering. Heat treatment to equivalent of International Standards for Phytosanitary Measures ISPM15 was chosen as the only method that would fit within the operational considerations and constraints of the zoo management and also likely to be acceptable to NZ authorities at import. Treatments had to be completed in situ next to the giraffe enclosure, within the working day of the zoo and to carry negligible risk to the zoo animals. These animals could be within the typical risk area of a fumigation. Fumigation with toxic gases were judged unacceptable, as they were too slow.

The crate, with doors folded back, was enclosed in 25 mm bubble wrap thermal insulation, with a controllable vent in the roof and the side covering loosely sealed to the ground with sand. Heat, as hot air at 75–80ºC, was supplied into the plenum below the floor from a fan-forced 20 kW electric heater. The heated air emerging from the plenum circulated around the walls of the crate, before venting close to the tarped roof. Some controlled venting also occurred directly from below the floor distant to the input site.

Temperatures were monitored with Type K thermocouples at 4 critical points on the upper surface of the floor and various other points in the enclosure. The solid wood components were shown to be heated to >56ºC for more than 30 min continuous exposure, equivalent to the requirements for ISPM15 for solid wood packing materials. A heat treatment certificate accompanied the giraffe and shipment. It was accepted by the NZ biosecurity authorities.

Keywords: Heat , Disinfestation , Transport , Fumigation , Wood , Quarantine

Paper No. CAF204-A54

Abshire JL, Ranabhat S, Brabec D, Bingham GV, Zhu KY, Morrison III WR (2024) Integration of long-lasting insecticide-incorporated netting improves fumigation efficacy enhancing the protection of bulk storage of commodities. Page 186. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Integration of Long-Lasting Insecticide-Incorporated Netting Improves Fumigation Efficacy Enhancing the Protection of Bulk Storage of Commodities

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ABSTRACT

Innovative approaches to pest management, such as long-lasting insecticide-incorporated netting (LLIN), have been used to impair mobility and prevent infestation of stored products by insect pests. Integrated pest management (IPM) plans rarely rely on one tactic alone to provide durable control. However, integration of LLIN with existing IPM tactics, such as phosphine fumigation, has not been well studied. To evaluate this interaction, we placed 60 perforated buckets (i.e., miniature silos) filled with 500 g of uninfested wheat, inside three 3-t grain bins. Buckets were protected by either LLIN (0.3% α-cypermethrin, Carifend, BASF), control netting (without insecticide), or no netting (negative control). All buckets were routinely evaluated for fumigation, but 30 buckets from each bin were never treated. To determine if fumigation was necessary, monthly samples of 100 g of grain were taken from 12 buckets from each of the three grain bins between June–October in 2022 and 2023. Dispersing insect life stages were sieved and recorded, while grain quality measures were evaluated. Based on the Federal Grain Inspection Service defect guidelines, if the threshold (e.g., two live insects or 16 IDK (insect damaged kernels) per 100 g) was met in any fumigated bucket during the month, fumigation was triggered for the 30 treated buckets in that bin. We recorded and compared the length of protection of each fumigation. Overall, the buckets protected with LLIN showed an 83–99% and 89–99% reduction in both insect dispersal and progeny production compared to insecticide-free netting and no-netting controls, respectively. Additionally, damage in LLIN-protected buckets was reduced by 50–99% compared to controls. Importantly, the total number of fumigation sessions were reduced by 68–91% when using LLIN compared to controls. Our results demonstrate that LLIN can be reliably used to enhance the efficacy of phosphine fumigation in bulk storage.

Keywords: Long-lasting insecticide netting, Integrated pest management, Fumigation, Bulk storage, Lesser grain borer, Red flour beetle, Wheat, Synergy, Stored products

Paper No. CAF2024-F55

Tumambing J, Huangmak P, Depalo M (2024) Poultry house fumigation with VAPORPH3OS® phosphine fumigant for effective control of darkling beetles and rodent infestations. Pp. 187–194. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Poultry House Fumigation With VAPORPH3OS® Phosphine Fumigant for Effective Control of Darkling Beetles and Rodent Infestations

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ABSTRACT

The broiler chicken industry in many parts of the world has serious issues with darkling beetles and infestations by rodents. In severe cases, these insects and rodents contribute to chicken deaths and higher feed conversion ratios. In lesser but still serious situations, they can prolong the chicken growing period, causing fluctuations in chicken weight. Darkling beetles are carriers of many types of microorganisms and indirectly substantially increase the bacteria/virus load in chicken meat. They can also damage chicken shed structures. Most treatment options for darkling beetles only control the adults and larvae rather than the immature eggs and pupae. Because traditional pesticide sprays do not reach and penetrate immature beetles, the darkling beetle population continues to rise. In the past several years, SYENSQO explored the use of fumigation, a novel approach to beetle and rodent control in empty poultry houses, as a more effective method for darkling beetle management. In SYENSQO's collaboration with research institutions and broiler chicken farms in Australia, a phosphine fumigation protocol of 350 ppm initial dose and 100 ppm minimum for 2 d at 20°C or higher was established for 100% mortality of all stages of darkling beetles. Rodents are completely killed using a dose of 200 ppm for 2 h. Field demonstrations in selected chicken farms in Australia, Indonesia, and Thailand showed that fumigation offered value-added benefits of significant savings in annual operating costs due to lower feed conversion ratios, lower chicken mortality rates, and in only having to perform one treatment instead of six times a year. Boot swab tests before and after fumigation in Thailand showed that the Salmonella count was reduced to zero after the first 6 wk growing cycle and up to 6 growing cycles. Additional benefits of fumigation include having healthier chickens that mature more quickly with uniform weights at harvest, lower maintenance costs of structures, and more hygienic environments due to greatly reduced water consumption.

Keywords: Poultry house fumigation, VAPORPH3OS®, Phosphine fumigant, Darkling beetle, Rodent, Disinfestation

INTRODUCTION

The darkling beetle, (*Alphitobius diaperinus* (Panzer)), is a common cosmopolitan insect pest of broiler poultry houses and is capable of transmitting a range of poultry diseases, including Newcastle disease, Marek's disease, Avian flu, Infectious *Laryngotracheitis*, Salmonellosis, and Fowl pox, as well as parasites such as tapeworms and protozoans. Also, both adult and larval darkling beetles can cause intestinal obstruction in poultry since these birds lack enzymes to digest chitin (Elowni and Elbihari, 1979), and this may eventually cause microscopic lesions along the bird's intestinal wall. The warm and humid conditions present in poultry houses provide a favorable environment for the growth and multiplication of darkling beetles (Lambkin, 2001). Darkling beetles are uniquely attracted to ammonia-rich environments (Rowland et al., 2007). The adult stage life span is 3–12 mo, during which time, a female may typically lay up to 200–400 eggs or even up to 2000 (Dunford and Kaufman, 2006; NSW DPI, 2012). The larval stage is hatched from the egg stage within 4 to 7 d and develops through the 1 to 6 instars toward complete development into the adult stage within 40 to 100 d, depending on temperature and food quality (Dunford and Kaufman, 2006; Rueda and Axtell, 1996).

Aside from the spread of diseases and parasites by darkling beetles, when the birds are removed from the sheds, the beetles will migrate from under the feed lines and feed pans to the walls, where they burrow into the insulation and structural wood to pupate. The resulting structural damage reduces insulation efficiency and increases heating and cooling costs as well as maintenance costs for the grower (NSW DPI, 2012). Moreover, community relations can suffer when adult darkling beetles fly from litter spread on farms to the lights of private houses (Dunford and Kaufman, 2006). The broiler poultry meat industry is concerned about increasing darkling beetle numbers in poultry houses and the pest's potential to breach farm biosecurity; insecticide resistance is also likely (Lambkin, 2001; NSW DPI, 2012).

The current treatment options, such as the pesticide sprays used for darkling beetles, only control the adults and larvae rather than the immature eggs and pupae. Because traditional pesticide sprays do not reach and penetrate immature beetles, the darkling beetle population continues to rise and insecticide resistance eventually develops. Rodent infestation is also a big concern in broiler poultry houses, particularly the old structures, as rodents also damage the insulation and the whole structure by creating burrows as their habitat and residence for the young. Rodents can disrupt the water and feed supply from several hours to a whole day by cutting the power supply cables with their teeth.

In response to the above issues on darkling beetles and rodent infestations, SYENSQO—in collaboration with research institutions and broiler chicken farms in Australia—developed a fumigation approach as a more effective method of managing darkling beetles and rodents. A phosphine fumigation protocol was developed by laboratory efficacy studies, followed by field validation trials and actual fumigation demonstration.

LABORATORY EFFICACY STUDIES

The first approach in adopting fumigation as an effective method of treatment for darkling beetles is to develop a phosphine fumigation protocol that can achieve 100% mortality of the four stages of development. Laboratory efficacy studies conducted by the Postharvest Grain Protection unit of the Queensland Department of Agriculture and Fisheries (QDAF) came up with a phosphine fumigation protocol of 350 ppm initial dose and 100 ppm minimum or 200 ppm average for 2 d at 20°C or higher for all stages of the darkling beetle (Ridley and Collins., 2017). Rodents are completely killed using a dose of 200 ppm for 2 h. Residues of phosphine in darkling beetles fumigated at 350 ppm for 2 d at 20°C were far below the maximum residue limit (MRL) levels of 0.01 mg/kg of phosphine after only 2 h of ventilation after the conclusion of the fumigation.

FIELD VALIDATION TRIALS

To verify the commercial validity of the developed phosphine fumigation protocol under laboratory scale studies, two sets of field validation trials were conducted by the South Australia Research and Development Institute (SARDI) and SYENSQO at two different broiler poultry farms in South Australia.

The first field validation trial tested the efficacy of the developed protocol using VAPORPH3OS® phosphine fumigant. The trial included an industry standard spray treatment (Spinosad as active ingredient), an untreated control, and VAPORPH3OS® fumigation treatment. The three treatments were applied as two replicates of 8,600 m^3 broiler poultry houses. The adult and larval darkling beetles were sampled using litter/soil scrapes of 120 mL volume collected from the shed floor directly below feeder units.

The results demonstrated that the SYENSQO VAPORPH3OS® phosphine treatment provided significant control of the darkling beetle population at the three post-treatment sample assessments, which contrasted with the results of the ineffective industry standard treatment. At the third post-treatment assessment (6 wk growing period), live adult and larval abundances in the fumigation treated sheds were significantly reduced by about 90% average (P=0.002) relative to the control treatment areas. The primary source of post-treatment darkling beetle recruitment in the fumigated sheds is likely to have been eggs and possibly pupae that survived the hygiene and fumigation treatments, but some re-infestation by adult immigrants from outside the sheds may have contributed (Bakeret et al., 2016). There was significantly less bird mortality in the fumigated sheds (3.61%) compared with the non-fumigated sheds (4.31%). VAPORPH3OS® also completely eradicated rodents inside the poultry sheds within the first two hours of fumigation.

A second field validation trial was conducted to compare the efficacy of VAPORPH3OS® against industry standard spray treatment (cyfluthrin as active ingredient) and untreated control using the same methodology as in the first trial. The results of the second trial demonstrated that the VAPORPH3OS® phosphine treatment provided significant control of the darkling beetle population for the six-week post-treatment assessment period, which contrasted with the results of the ineffective industry standard treatment.

At the third post-treatment assessment, both live adult and larval abundances in the fumigation treated sheds were significantly reduced by about 70% relative to the control treatment areas. The insect mortality count results also showed that the standard spray treatment and the untreated control treatment are similar in terms of ineffectiveness (Baker et al., 2017).

DEMONSTRATION FUMIGATION IN AUSTRALIA, THAILAND, AND INDONESIA

Actual fumigation treatments of selected broiler poultry houses in Australia, Thailand, and Indonesia were conducted to demonstrate the efficacy of fumigation treatment and assess the value-added benefits of fumigation in terms of cost savings in lower feed conversion ratios and lower chicken mortality rates as compared with the standard pesticide spray treatment.

In Australia, two broiler poultry farms in Mangrove Mountain (NSW) with a total empty space volume of 10,000 m^3 and 5,000 m^3 broiler poultry houses, respectively, were selected for actual fumigation treatment. Both broiler poultry houses were of a modern-type design with automated temperature control and ventilation systems. Sealing preparation of the two poultry houses was conducted at the ventilation fans, front doors, side wall vents, and evaporative coolers to minimize gas leakage during the 2 d fumigation. An initial dose of 350 ppm phosphine was introduced uniformly into the whole empty space volume through a water snake gas distribution system composed of 120 m length by 50 mm diameter plastic lay flat tube with 1 cm diameter holes every 5 m.

Even though there were only minimal gas losses due to gas leakage and gas sorption of the ground, some top up was required after 24 h of fumigation in order to maintain a minimum phosphine concentration of 100 ppm for 2 d.

As shown in Figure 1, the demonstration fumigation of the two empty poultry houses confirmed lab trial and field validation trial results. Fumigation treatment using VAPORPH3OS® phosphine gas was significantly more effective and prevented considerable reinfestation at all life cycle stages compared with pesticide sprays. The percentage of live adult and larval darkling beetles during the last week of the chicken growing period was much lower in the fumigated poultry house (24% live insects) compared with the untreated house (354% live insects) and the pesticide-sprayed house (324% live insects). The presence of the 24% live insects in the fumigated poultry house after 6 wk of treatment was likely due to reinfestation from beetles outside the house. The fumigation treatment also resulted in a lower feed conversion ratio (1.68 kg feed/kg chicken) and chicken mortality rate (2.92%) compared with the pesticide spray treatment (feed conversion ratio of 1.72 kg feed/kg chicken and chicken mortality rate of 3.61%). With a minimum volume of 25,000 birds per 5,000 m^3 poultry house, these lower values translate to a monetary savings of approximately AUD 13,000 per poultry house per year.

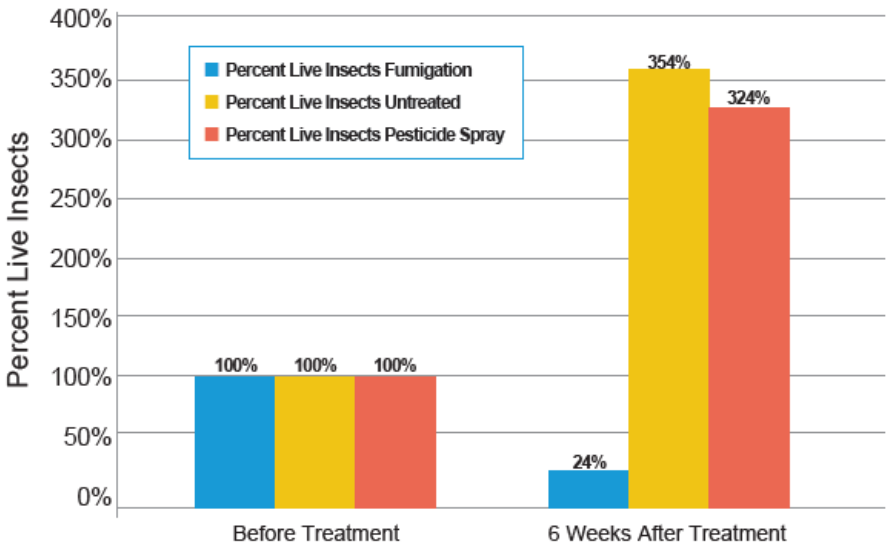


Fig. 1. Average population of adult and larval darkling beetles in the fumigated and pesticide-sprayed poultry houses at different insect sampling periods.

Figures 2 to 5 show the setup of the gas injection using VAPORPH3OS® and the HDS 200 phosphine/air mixer, sealing of the poultry house, and the gas distribution system using a lay-flat plastic hose.

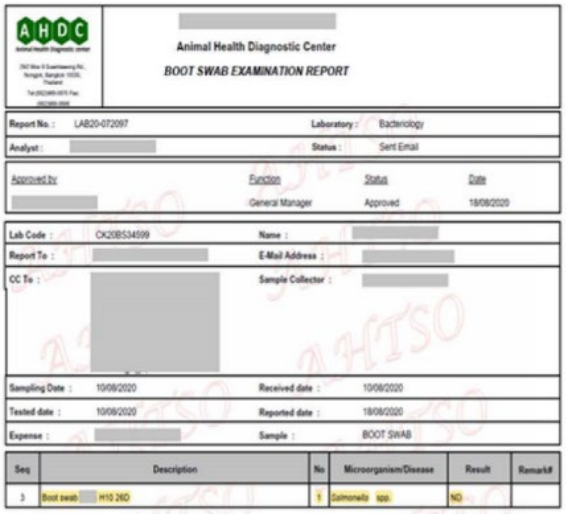
Fig. 2. Setup of the VAPORPH3OS® cylinder and the HDS 200 phosphine/air mixer in a trailer and hose connection into the middle side of the poultry house.

Fig. 3. Setup of the gas distribution system using a 10 cm diameter lay-flat plastic hose.

Fig. 4. Sealing of the ventilation fans at the back of the poultry house.

Fig. 5. Sealing of the evaporative cooler (cooling pad) with thick plastic sheet inside the poultry house.

In Thailand and Indonesia, a demonstration fumigation of selected poultry houses with an empty space volume of 5,000 m^3 effectively reduced populations of darkling beetles, rodents, and other animal pests. As shown in Figure 6, a boot swab test conducted during the fumigation in Thailand showed that the Salmonella count, which was detected before fumigation, became undetectable in the first to fifth growing cycles. Unlike pesticide spray, fumigation is carried out in a dry environment, resulting in a significant reduction in water consumption (AHDC, 2000).



Crops no.	Fumigation status	Lab Boot Swab Result
no. 623 6/8/2019 - 17/9/2019	Before	C
no.624 8/10/2019 - 18/11/2019	Before	C
no. 625 19/12/2019 - 30/1/2020	Before	C
no. 631 24/2/2020 - 4/4/2020	After 1st crop	ND
no. 632 6/5/2020 - 16/6/2020	After 2nd crop	ND
no.633 16/7/2020 - 25/8/2020	After 3rd crop	ND
no. 634 17/9/2020 - 29/10/2020	After 4th crop	ND
no. 635 26/11/2020 - 6/1/2021	After 5th crop	ND

Fig. 6. Boot swab test results before and after fumigation for the five growing cycles (crops).

CONCLUSIONS

During field trials and demonstration fumigation, the use of VAPORPH3OS® to fumigate empty broiler poultry houses significantly reduced the populations of darkling beetles and rodents. This is because the fumigant can reach areas that are often missed during spray treatment application. Lower darkling beetle populations have been proven to result in cost savings by reducing the feed conversion ratio and chicken mortality rate. As a result, the chickens become healthier, mature more quickly, and have a more uniform weight at harvest.

Additionally, fumigation treatment remains effective for up to six growing cycles, requiring only one treatment per year compared with the current six treatments per year with pesticide spray. Other advantages of fumigation include healthier chickens, lower maintenance costs for the structure, and a more hygienic environment due to significantly reduced water consumption.

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Single Application Shiphold Fumigation of Export Logs in New Zealand Using VAPORPH$_3$OS® Phosphine Fumigant

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ABSTRACT

For the past two decades, the majority of pine logs exported to China from New Zealand have been treated in transit utilizing phosphine. The protocol adopted involves the use of aluminum phosphide (AlP) with an initial phosphine dose of 2.0 g/m^3 at the port followed by top up of 1.5 g/m^3 after 5 d while in transit in order to maintain a minimum of 200 ppm phosphine for 10 d. This approach utilizes a total phosphine dose of 3.5 g/m^3 and achieves 100% mortality of all stages of target insects. However, there are increasing concerns around the overall operation including: risks of fire, explosion and exposure during initial and top up operations, safety issues for the on-board technician to navigate the vessel while in transit, and storage and handling issues of AlP, along with the costs and complexity of removing and disposing spent residue from the shiphold at the destination port.

To address the above concerns, a single application approach at the load port applying the same phosphine dose of 3.5 g/m^3 utilizing VAPORPH$_3$OS® was proposed and subjected to efficacy studies. Scientific efficacy trials conducted by Plant and Food Research New Zealand showed the single application with pure phosphine from VAPORPH$_3$OS® effectively controlled all developmental stages of golden-haired bark beetles and other major wood insect species. The fumigation protocol of 200 ppm minimum for 10 d (240 h) equivalent to a concentration × time (Ct) product of 48,000 ppm–h was also exceeded with the single application fumigation. The above results were then validated successfully during field trials conducted by the partnership of SYENSQO, Genera, and Plant and Food Research. The Ministry for Primary Industries of New Zealand has reviewed the scientific and field validation data and has recommended the use of single application fumigation with VAPORPH$_3$OS® for commercial application on in-transit treatment of export logs to China.

Keywords: Single shot approach, Shiphold fumigation, Export logs, VAPORPH3OS®, Phosphine fumigant

INTRODUCTION

A majority of the pine logs (*Pinus radiata*) exported from New Zealand to China requiring phytosanitary treatment is treated with phosphine in shiphold (below deck) while in transit. The phosphine fumigation protocol that has been used for about 20 yr is a combination of an initial dose of 2.0 g of phosphine per m^3 from aluminum phosphide (AlP) blankets at the port and a top-up of 1.5 g of phosphine per m^3 from AlP tablets after 5 d during sea journey to maintain a minimum of 200 ppm phosphine concentration for 10 d (Zhang et al., 2004), which has been accepted by China's quarantine regulations for over two decades. This treatment, with a total phosphine dose of 3.5 g/m^3, has been effective against all developmental stages of bark beetles, particularly *Hylurgus ligniperda*. However, there are associated risks and safety issues in the above approach and concerns around the overall operation. These include: 1) risks of fire, 2) explosion and exposure during initial and top up operations, 3) safety issues for the on-board technician to navigate the vessel while in transit, and 4) storage and handling issues of AlP, along with the costs and complexity of removing and disposing spent residue from the shiphold at the destination port.

The current risks and safety issues with the AlP protocol pose a high probability of this approach becoming unsustainable, causing the export of logs from New Zealand to China to become uneconomical considering the alternatives available. To support the New Zealand forest industry and continue the log trade to China, there is a need to develop and implement a new approach to meet the current phytosanitary requirements. This new approach should also future-proof the in-transit vessel fumigation, a critical component for the future of the New Zealand forest industry.

The above concerns are proposed to be mitigated by introducing a "single shot" approach of cylinderized phosphine gas (VAPORPH$_3$OS® phosphine fumigant) in place of the "double application" approach utilizing AlP. VAPORPH$_3$OS® is a cylinderized formulation of 99.3% average phosphine content by weight and is safely applied using phosphine/air mixing equipment that safely blends phosphine and air and delivers a non-flammable mixture of 1% (10,000 ppm) of phosphine with air into the sealed structure. An approved phosphine/air mixer that can be used for the above approach is an HDS 800 unit. The HDS 800 unit is capable of delivering about 12 kg/h of phosphine and is manufactured and supplied by Horn Technologies based in Santiago, Chile.

The single shot approach with VAPORPH$_3$OS® and the HDS 800 offers real benefits in that it can:

- Remove the risk of AlP residue fires
- Remove the need for collecting and disposing AlP residue after fumigation
- Remove the phosphine exposure risk (of AlP that has not fully reacted) to the ship's crew and stevedores when they are discharging the holds.
- Remove the need for an in-transit technician (ITT and crew-assist) during top-up operations in transit
- Remove the risk of fire and explosion from AlP in contact with moisture (due to high humidity environments in shipholds)
- Minimize the risk for storage and handling of fumigants (metal phosphides vs. cylinderized phosphine).

LABORATORY EFFICACY STUDIES

A scientific efficacy study on the single shot approach was first conducted by Plant and Food Research New Zealand (Zhang et al., 2007) for CYTEC Australia, which showed promising results. This laboratory-scale study made use of actual fresh logs collected from a local New Zealand mill and cut into 60 cm long blocks to fit into a 90 L sealed fumigation chamber. Results of the study showed that a single application of 3.5 g/m^3 with cylinderized phosphine gas at 15°C effectively controlled all developmental stages of golden-haired bark beetles (*Hylurgus ligniperda*) and other major wood insect species (*Hylastes ater* and *Arophalus ferus)*. As fresh logs were used in this study, it was also shown that phosphine depletion during log fumigation is a common issue due to high phosphine absorption by logs, particularly the highly porous and fibrous bark layer, and due to very high moisture content (>85% wet basis). It was recommended that the promising results of this study be verified under commercial field trial conditions to confirm their validity.

COMMERCIAL FIELD VALIDATION TRIALS

With the increasing operational concerns posed by the AlP protocol, field validation studies were conducted by Plant and Food Research for Genera and in partnership with SYENSQO (Estefandi et al., 2023). This scientific study tested the efficacy and validity of a single dose cylinderized phosphine gas fumigation of 3.5 g/m^3 and of 4.5 g/m^3 for complete control of both mature and immature stages of golden-haired bark beetles, one of the primary insect pests and the most tolerant insect species of New Zealand pine logs. The actual setup of the commercial scale fumigation trials was composed of insect-infested fresh logs fully covered with bark loaded into 40 ft (12.2 m) shipping containers. Pure cylinderized phosphine from VAPORPH$_3$OS® was introduced into the shipping containers using an HDS 800 phosphine/air mixer from Horn Technologies.

From testing over 50,000 insects, results of this field validation study showed that fumigation with a single dose of 3.5 g/m^3 pure phosphine gas effectively controlled all developmental stages of *Hylurgus ligniperda*, with high mortality rates (>99%) achieved in all treatments and three replicates (Table 1). The study also showed that the currently accepted protocol of 200 ppm phosphine concentration minimum for 10 d, equivalent to a concentration × time (Ct) product target value of 48,000 ppm–h, was achieved and was well above this minimum Ct product. An initial phosphine concentration of over 3500 ppm was achieved after the first hour of fumigation; the concentration drastically decreased to below 2000 ppm after 24 h and then remained at or above 200 ppm after 10 d.

Table 1. Mean mortality (± SD) in three replicates combined of *Hylurgus ligniperda* larvae, pupae, and adults in logs after fumigation with single shot phosphine gas of 3.5 g/m^3 and 4.5 g/m^3 as compared to commercial control (2 g/m^3 initial dose + top up of 1.5 g/m^3) and control (no treatment) in 40-ft (12.2-m) shipping containers containing commercial-grade filler logs (Estefandi et al., 2023).

Life stage	Treatment	Mean mortality (± SD)	Total number of insects
Larvae	Control	3.79 ± 7.38	2718
Larvae	Commercial Control	100	5596
Larvae	3.5 g/m^3	100	8579
Larvae	4.5 g/m^3	99.99 ± 0.04	5568
Pupae	Control	9.03 ± 11.84	3505
Pupae	Commercial Control	100	7170
Pupae	3.5 g/m^3	99.83 ± 0.49	11024
Pupae	4.5 g/m^3	99.53 ± 1.24	4972
Adult	Control	1.46 ± 1.62	13991
Adult	Commercial Control	100	2155
Adult	3.5 g/m^3	100	4088
Adult	4.5 g/m^3	100	999

DEMO SHIPHOLD FUMIGATION TRIALS

In order to confirm operational effectiveness, further field validation trials of the "single shot" approach were conducted at the Port of Tauranga through the fumigation of export logs in the shiphold of an actual ship destined for China. As part of preparation on the safety and efficiency of this approach prior to the actual fumigation, a comprehensive risk assessment of the gas delivery system was conducted covering: the transport of the cylinders and the Horn Diluphos System (HDS 800) machine; setup of the HDS 800 in a truck with flexible hose connections at the wharf; setup of transition plate covers, gas delivery hose connections with recirculation, and hose disconnection; and the transfer of gas delivery hoses to other shipholds, along with boundary and operational gas level monitoring. This risk assessment was done to satisfy the operational safety requirements of the port. A standard operating procedure (SOP) was prepared, and this SOP served as the step-by-step guide during the actual fumigation.

Setup of the HDS 800 and Gas Delivery System

An HDS 800 was installed inside a truck together with four VAPORPH_3OS® cylinders and one nitrogen cylinder. This facilitated ease of movement of the HDS 800 and the cylinders together for gas injection into each shiphold along the wharf. A 3-phase power source available along the wharf every 50 m supported the movement of the truck along the wharf and enabled a power connection at each dosing point.

A flexible hose connection was used to deliver phosphine gas safely inside the shiphold. At the outlet side of the HDS 800, a 40 m flexible hose was connected to a transition plate cover over the manhole. This cover included a flexible plastic dust to deposit phosphine gas close to the bottom of the shiphold. At the opposite end of the shiphold, a 60 m flexible hose was used to join the exhaust manhole to the inlet of the HDS 800 machine to allow for gas recirculation (Tumambing, 2019). This setup required 3.5 h to complete and injected an average of 35 kg of phosphine gas per shiphold at a dose rate of 3.5 g PH_3/m^3.

Phosphine Gas Monitoring

For each the five shipholds, three gas sampling lines were installed at the bottom, middle, and top sections for manual gas monitoring. About a 1 m length of each sampling line protruded outside the manhole for use in actual manual gas monitoring. A new model of SILOCHEK3, capable of gas readings up to 3000 ppm, was used for manual gas monitoring at 2–3 times a day. The gas readings for both the 10 d of fumigation and the aeration were recorded. Figures 1–6 show the setup of the HDS 800 and the VAPORPH$_3$OS® gas delivery system in fumigating an actual ship with export logs in the shiphold (below deck).

Fig. 1. Panoramic view of a 50,000 m^3 ship and fumigation setup with VAPORPH$_3$OS® and HDS 800.

Fig. 2. Setup of the HDS 800, VAPORPH$_3$OS®, and nitrogen cylinders inside a truck and flexible hose connections.

Fig. 3. Closer view of the HDS800, VAPORPH$_3$OS®, and nitrogen cylinders inside a truck.

Fig. 4. Setup of a transition piece cover with flexible hose on top of a manhole.

Fig. 5. Close up view of a transition piece cover strapped on top of a manhole.

Fig. 6. A manhole cover without a transition piece cover.

CONCLUSIONS

Through laboratory studies, field validation trials, and commercial fumigation trials, the "single shot" application approach with VAPORPH$_3$OS® has been shown to be a safe, efficient, and effective alternative to the current "double application" approach with AlP. The Ministry for Primary Industries of New Zealand has reviewed the efficacy reports and recommended the use of single shot application fumigation with VAPORPH$_3$OS® for commercial application on the in-transit treatment of export logs to China.

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Controlling Spotted Lanternfly Using Tyratech Formulations

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ABSTRACT

The spotted lantern fly (SLF), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), is an invasive insect first introduced to the United States in 2014. Approaches are critically needed to control this pest from infesting commodities and consignments that must be moved through quarantines. Of particular concern is the overwintering egg life stage, which is laid in clusters on the surface of logs, sawn timber, and other outdoor items. Microscopic investigations revealed chorionic microstructures permeable to both gas and liquids. The efficacy of conventional fumigants (methyl bromide, sulfuryl fluoride, or phosphine) will be briefed and can be used to limit its spread in commercial channels of regulated articles. Non-fumigation control measures are also needed, particularly for homeowners and business owners without access to fumigation. Various Tyratech formulations, using insecticides exempt from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) registration under the 25(b) authorization, were evaluated to address this need. These formulations target the tyramine receptors with promising efficacy toward all life stages, including eggs. The applied formulations resulted in the complete mortality of all egg masses subject to the treatment. In total, 231 SLF eggs remained unhatched using the commercially available formulation and 3,219 SLF eggs remained unhatched using the non-commercially available formulations.

Keywords: Spotted lanternfly, Essential oils, Tyramine receptor, Fumigation

INTRODUCTION

The spotted lanternfly (SLF) (*Lycorma delicatula*) is an invasive insect first introduced to the United States in 2014. It is a leafhopper that feeds on the phloem of over 70 different species of plants including grapes, apples, hops, and black walnuts (Urban and Leach, 2023). Although SLF do not typically kill their host plants, they secrete a honeydew-like substance which is susceptible to mold growth (Urban, 2020). The widespread horticultural distribution of the tree of heaven, *Ailanthus altissima* (Mill.), could spread SLFs from the northeastern United States (Barringer and Ciafré, 2020; Jones et al., 2022; Murman et al., 2020).

The spotted lanternfly is currently treated in the field using a variety of methods, including sticky bands to trap the insects, circle traps, scraping egg masses into alcohol, or using insecticides such as Dinotefuran or Imidacloprid applied as a soil drench, trunk spray, or trunk injection (Spotted Lanternfly Management Guide, n.d.). Natural insecticides such as pyrethrins and other botanical oils have proven to be initially effective, but they lose their effectiveness over time (Spotted Lanternfly Management Guide, n.d.). Conventional fumigations like methyl bromide, sulfuryl fluoride, or phosphine will serve to limit its spread in commercial channels of regulated articles, such as logs, sawn timber, and vehicles. Non-fumigation control measures are also needed, particularly for homeowners and business owners without access to fumigation.

Various studies have highlighted the use of essential oils to control insects (Lee et al., 2019). The primary benefit of using these products is a targeted spray on egg masses, nymphs, or adults to limit mortality to non-targets and beneficials. Additionally, many of these compounds are exempt from FIFRA registration under the 25(b)-authorization due to the fact that they are Generally Recognized As Safe (GRAS). The effectiveness and low cost of these compounds make them an ideal option for controlling SLFs in the field by homeowners and organic farmers.

MATERIALS AND METHODS

All procedures were conducted in the BSL-III quarantine facility greenhouse located at the UC Davis Contained Research Facility (CRF) (University of California Davis, Davis, CA). Greenhouse conditions were set to 23°C and utilized a light cycle of 16:8 h (L:D). An enclosure for SLFs was built with the purpose of determining the conditions necessary for SLF egg mass production as well as for harvesting egg masses in order to test the ability of various fumigants and essential oil-based insecticides to prevent the SLF eggs from hatching.

Aeroponics System

A High-Pressure Aeroponics (HPA) system (Current Culture H2O, Fresno, CA) was installed and planted with *A. altissima* and grape vine (*Vitis Vinifera* (L.)) purchased from Seed World in accordance with the aeroponics manual. The unit was set to mist the roots of the plants for 15 s every 7 min. A quantity of 10 mL of the fertilizer NPK (1.3, 2, 5.9) (Cultured Solutions® VEG B, Current Culture H2O, Fresno, CA) was also added once a week to the watering tank to provide nutrients to the growing plants.

Rearing Enclosure Construction

Two enclosures to house the SLFs were designed, built, and fixed atop the HPA system (Current Culture H2O, Fresno, CA). Each enclosure was approximately 61 cm (24 in) wide, 86 cm (34 in) long, and 127 cm (50 in) tall with four 33 cm x 33 cm (13 in x 13 in) sealable apertures in the front for manipulating the insects and plants. The enclosure was constructed from 1.3 cm (½ in) poly vinyl chloride (PVC) (Charlotte Pipe, Charlottle, NC) and utilized 1.3 cm (½ in) PVC elbows and tees to create the overall shape. It was then wrapped with 183 cm (72 in) wide charcoal fiberglass screen mesh 18x14 (Phifer; Tuscaloosa, AL) and glued into place using 10 1.3 cm (½ in) long

all-purpose glue sticks (Arrow; Saddle Brook, NJ). Once constructed, pool noodles were cut to the appropriate lengths for both the length and width of the bottom of the enclosures and cut lengthwise to wrap around the 1.3 cm (½ in) PVC pipe. The excess screen mesh was secured to the aeroponics reservoir housing using 4.8 m x 3.2 cm (16 ft x 1.25 in) ratchet tie down straps (Husky; Atlanta, GA). One enclosure was designated for nymphs while the other cage would be utilized for mature adults. A completed version of the enclosures is shown in Fig. 1.

Fig. 2. A completed version of the enclosures.

Enclosure Maintenance

Enclosure maintenance, such as trimming vegetation and collecting dead insects, was conducted through large access apertures on the front of the enclosure. Trimming excessively tall vegetation and branches that pressed against the enclosure was vital to prevent damage.

Insect Collection, Transport, and Rearing

The SLF colony was started in the summer of 2023 and replenished with eggs collected in Kearneysville, WV by Dr. Tracy Leskey (USDA-ARS-Kearneysville, WV). An example of a recently collected egg mass is shown in Fig. 2.

Fig. 3. Egg mass on tree bark.

After collection, the eggs were kept refrigerated using ice packs and sent to the CRF at UC Davis. Once the eggs were received, they were treated for mold using a potassium sorbate solution (5% w/v), allowed to dry, and then placed into cold storage (4°C) until needed. Once removed from cold storage (4°C), treatments were conducted within 48 h. Treated egg masses, as well as non-treated controls, were placed back into cold storage (4°C) to simulate treatment during "wintertime" and natural spring emergence. Once removed from cold storage (4°C) for "springtime" efficacy evaluation, the treated egg masses and respective controls were placed in 30.5 cm x 30.5 cm x 30.5 cm (12 in x 12 in x 12 in) aluminum cages with a cloth aperture on one side and maintained in the greenhouse until hatching, approximately 21 to 28 d later. Thereafter, efficacy data were collected as described below, and survivors were transferred to the enclosures to propagate the colony. In addition, to evaluate the potential to rear SLFs in the enclosures from hatchling to adulthood, a "rearing" experiment was conducted in which 150 eggs (that were never treated or removed from the cold storage) were transferred directly into the enclosures.

Once nymphs were in their 4th instar, they were transferred from the nymph cage to the adult cage to reduce mortality from dew-covered leaves. The adults were left in the adult cage for approximately 30 d, at which point, the adults were separated into 2 groups. The first group remained in the adult SLF rearing cage which maintained a temperature of 23°C and utilized a light cycle of 16:8 h (L:D) while the second group was transferred to a growth chamber (Conviron, Pembina, ND) that was set to 15°C and utilized a light cycle of 16:8 h.

Formulations

Testing was conducted using a commercially available insecticide (Zevo, Cincinnati, OH) primarily containing 1.3% geraniol and 0.2% cinnamon oil. Further testing using not yet commercially available formulations with unknown concentrations of essential oils was also performed on the SLFs. No dilutions or changes were made to the formulations, which were applied as directed.

Insecticidal Efficacy

Egg masses allocated from the above shipment were used to determine initial effectiveness. Egg masses were placed into groups of five and randomly chosen to be subjected to the treatment or serve as a control. Three trials were performed for each control and treatment type. A control group of 10 egg masses was placed in a cage and allowed to develop naturally in the controlled greenhouse. The treated samples were placed on top of separate paper towels and sprayed with the appropriate test formulation for approximately 2 s as per the manufacturer's instructions and then placed into a container that was identical to the control. Both the controls and the treated egg masses were allowed to develop naturally in the controlled greenhouse at 23°C and a light cycle of 16:8 h (L:D) for approximately 4 wk.

RESULTS AND DISCUSSION

Rearing

The initial "rearing" trial resulted in approximately 120 SLF nymphs that hatched from the 150 eggs. Approximately 15 individuals survived to adulthood (8 females, 7 males). This low number was primarily due to early mortality from an overabundance of honeydew secretions trapping the nymphs and preventing their movement. The surviving individuals were separated by gender and randomly assigned to remain at 23°C (4 females, 3 males) or be transferred to the growth chamber set to 15°C (4 females, 4 males). Of the two groups, the only group that began to lay egg masses was that of the group set to 15°C (4 females, 4 males). These initial results combined with other successful rearing studies suggest that the SLF needs colder temperatures to stimulate laying egg masses. Additionally, to decrease mortality, cages should have leaves with excess honeydew secretions removed in order to increase the probability of nymphs reaching adulthood.

Insecticidal Efficacy

After applying the formulation to the egg masses and waiting 4 wk, the egg masses were counted to determine the number of hatched and unhatched nymphs in both the controls and treated egg masses. As seen in Table 1, the control groups had approximately 146 out of 323 nymphs hatch while the egg masses treated with the commercially available formulation as well as the unavailable formulation had 0 nymphs hatch. These results suggest an overwhelmingly positive ability to control SLFs using natural formulations.

Table 1. Results of control and treatment type on SLF egg masses.

SLF Egg Masses					
Control (No Treatment)		Zevo Treatment		Unavailable Commercially Treatments	
Hatched	Unhatched	Hatched	Unhatched	Hatched	Unhatched
146	177	0	231	0	3219

Although this is primarily an exploratory study, future testing will rely on a greater number of egg masses to improve statistical analysis as well as serve as a strong foundation for supporting the implementation of these types of products by small-scale farmers and the general public.

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Impact of Different Fumigants on the Storage Quality of Corn Used for Popcorn Production

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ABSTRACT

The maintenance of quality in cereal grains, such as corn, depends on the provision of optimal storage conditions. This is essential as it directly impacts the quality of derived products, including popcorn. The present study aims to examine the effects of three distinct fumigants, namely phosphine, carbon dioxide, and nitrogen, on the storage quality of corn utilized for popcorn manufacturing within a duration of three months. The variables that require monitoring encompass the volumetric expansion during popping, moisture content, physical damage, and fungal infestation of the stored grains. The hypothesis posits that the selection of fumigant can have a substantial impact on the aforementioned variables, which can ultimately impact the quality of corn and the resulting popcorn outcomes. The study employed a controlled storage environment for each fumigant and rigorous data collection protocols were followed to ensure precise outcomes. Data provided significant findings that stakeholders in the popcorn production chain may use to make informed decisions about the kind of fumigant to use for storage, possibly enhancing the quality of the popcorn and lowering loss from spoilage.

Keywords: Corn, Popcorn, Fumigation, Storage conditions, Control

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Overview of Three-Dimensional Insect Movement and Its Impact on Fumigation Strategies

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ABSTRACT

Stored grain facilities remain an integral component of global food security, yet they are continually infested by insect pests that compromise the grain quality and quantity. For devising effective fumigation strategies, understanding the influence of insect movement on the effectiveness of fumigants within the grain storage facility is crucial. Insects exhibit diverse movement behaviors, including horizontal and vertical movements, as well as deep burrowing within grain masses. Their movements create diverse spatial patterns, challenging the conventional pest management methods. The current work highlighted the importance of understanding insect movement and behavior and consequently addressing how insect movement pattern influences the fumigation strategies based on their behavior. This review also discussed the efficacy of fumigation methods in managing stored pests. As the global landscape of agriculture and pest control continues to evolve, this review emphasized the indispensable role of studying insect movement in shaping effective fumigation planning and execution strategies.

Keywords: Pest infestation, Insect movement and behavior, Fumigation strategies

INTRODUCTION

Losses of stored grains are a significant challenge to global food security as they affect both the economic stability and availability of food resources. Several factors contribute to the grain degradation and loss, as the grains pass through the postharvest stages of transportation and storage. Infestation by pests such as insects, rodents, and fungi are the primary cause. Insects like beetles, weevils, and moths contribute to damaging the grains by consumption, whereas fungi may produce mycotoxins which can cause health risks when consumed (Jayas et al., 1995). Insects pose a major risk to the quality and integrity of stored products, resulting in significant economic losses and potential health hazards. To address this problem, various pest control techniques have been utilized as a primary approach for pest control. These methods include, chemical fumigants, heat treatment, and controlled atmosphere strategies, where they aim to eliminate the pest population

while reducing the negative impact on the environment and human health (Guru et al., 2022). Fumigation in the grain bins plays a vital role in eliminating the pests and safeguarding the grains from deterioration. Conventionally, fumigants such as phosphine and methyl bromide have been the evident choice. However, insects have developed resistance to phosphine over the years, and methyl bromide has been phased out due to its global warming potential (Rajendran and Sriranjini, 2007). The developed resistance is related to insect movement behavior and their distribution in stored bulks and the fumigation application practices (Jian, 2019). Therefore, to eradicate insects and obtain maximum efficacy in fumigation, understanding the insect movement and behavior within the grains is crucial.

INSECT MOVEMENT BEHAVIOR

Numerous research studies have been undertaken over the years to explore how insects move and respond to varying environmental conditions (Jian et al., 2011, 2012a, b, c; Bharathi et al., 2023). Insects navigate through the grain bulk in search of suitable conditions for feeding and reproduction (Hagstrum et al., 1996). The structural design of storage facilities, including the presence of aeration systems and grain handling equipment, can affect insect dispersion within the storage space (Fields and Muir, 1996). The spatial and temporal movements of the insects within the storage bin determine the distribution of infestation—which has a significant influence on grain storage practices and pest management. Insects form clusters often in the region with optimum moisture contents and temperatures and also exhibit distinct activity patterns because of the seasonal variations throughout the storage period (Bharathi et al., 2023).

A spatiotemporal distribution study on insects and mites in a horizontal storage facility storing 41 t wheat, conducted by Athanassiou et al. (2005), showed diverse spatial and temporal patterns for different insect and mite species. The experiment extended over 9 mo with sampling conducted at 10 d intervals at several locations from the facility. A total of nine insect and twenty mite species were identified from the samples. The most common insects included *Tribolium castaneum* (Herbst), *Cryptolestes ferrugineus* (Stephens), and *Rhyzopertha dominica* (F.); the most common mites included *Lepidoglyphus destructor* (Schrank), and *Acarus siro* L., as well as the predator *Cheyletus malaccensis* (Oudemans). Many insects and mites were concentrated in the upper section of the bulk, whereas *C. malaccensis* was evenly distributed in the upper and middle sections. In another study examining the movement of *C. ferrugineus* and *T. castaneum* within 300 t wheat stored inside a corrugated steel bin, it was observed that there was minimal insect activity near the bin walls during periods of low temperatures and that *C. ferrugineus* exhibited a downward vertical movement immediately after being introduced into the bin and then dispersed throughout the grain. In contrast, *T. castaneum* were mostly observed in the top layers of the grain; however, they were also observed at other locations inside the bin (Bharathi et al., 2023).

Understanding the three-dimensional spatial distribution of the stored grain pests is a crucial step in the formulation of fumigation strategies. Targeted interventions, such as localized insecticide application and strategic placement of monitoring devices, can be implemented based on the observed insect distribution patterns (Jian, 2019). Three-dimensional temporal and spatial distribution studies in stored wheat at different temperatures (20, 25, and 30°C), moisture contents (11, 13, and 15%), and different insect densities—such as 0.1, 1, and 5 adults/kg for *T. castaneum*

(Jian et al., 2012b), *Oryzaephilus surinamensis* (L.), and *Sitophilus oryzae* (L.) (Jian et al., 2012a); 1, 5, and 10 adults/kg for *R. dominica* (Jian et al., 2012c); and 0.1, 1, and 10 adults/kg for *C. ferrugineus* (Jian et al., 2011)—all revealed that adult insect aggregation decreased with increasing adult insect density. *Sitophilus oryzae, R. dominica*, and *T. castaneum* exhibited a continuous temporal pattern, whereas such consistency was not observed for *C. ferrugineus* and *O. surinamensis*. The decrease in aggregation suggests heightened competition for resources, leading to more dispersed distributions. This dispersal pattern poses significant challenges for fumigation treatments, as these dispersed insect populations are harder to target effectively with fumigants compared with densely aggregated populations (Flinn et al., 2010). Dispersed insects may be situated in inaccessible areas or shielded within the grain mass, reducing their exposure to fumigants and making them less susceptible to control measures (Toews et al., 2006). The continuous temporal pattern indicates the consistently active nature of insects, ensuring the need for sustained fumigant exposure throughout treatment. Failure to maintain prolonged exposure could allow insects to survive and rebound post treatment (Campbell and Arbogast, 2004). At times, heightened activity of insects may allow them to evade treatment and move to unexposed areas. In such cases, careful monitoring and adjustment of fumigation protocols is required to ensure effective pest control measures (Malekpour et al., 2020).

INFLUENCE OF DISTRIBUTION ON RESISTANCE DEVELOPMENT

Uneven distribution and insect movement behavior might help insects to develop resistance to fumigants. A prevailing challenge in controlling insect infestations in agricultural commodities through fumigation is the ability of the insect to move within the infested regions. Insects possess the ability to navigate and find refuge in hidden areas, which compromises the efficacy of the fumigation treatment (Jian, 2019). Insects often establish localized populations inside the complex environments of storage facilities. Their diverse distribution patterns are influenced by various environmental factors such as grain moisture content, temperature gradients, and availability of food resources. The interspecific interactions among the insects such as competition for food resources and mating behavior can further influence their distribution within storage bins (Jian, 2019). Dominant individuals will take over resource-rich areas leading to localized aggregation; this influences the fumigation treatment and aids in resistance development. The top layer or the center region of the grains inside a bin can both provide optimal conditions for insect development, leading to higher population in these areas. This uneven distribution creates spatial variation in exposure to fumigation treatments. Areas with high fumigant concentrations, such as the top layers, exert stronger selection pressure for resistance. Insects located in these areas are prone to encounter lethal concentrations of fumigants, encouraging them to develop resistance mechanisms as a means of survival. In the areas of less resistance selection pressure, such as the bottom layers, insects can prevail and maintain their genetic diversity. The fumigant-resistant individuals disperse into the bin, contributing to the spread of spatial resistance. As they colonize untreated areas, their now-resistant populations continue to prevail; this aids the gene flow movement between generations and increases the resistance (Shi et al., 2012).

EFFICACY OF FUMIGATION IN MANAGING STORED PESTS

Fumigation has been the commonly practiced method for controlling insect infestations. The efficacy of a fumigation technique depends on several factors, including the insect species, their growth stage, temperature, humidity, and the properties of the stored product (Bell, 2000). While chemical fumigants like phosphine are highly effective against a wide range of pests, resistance development remains a concern (Schlipalius and Ebert, 2020). Additionally, ensuring uniform distribution of fumigants throughout the storage facility is essential for successful results (Kumar, 2017).

Resistance occurs when pests develop mechanisms to tolerate and survive any exposure to fumigants which would otherwise be lethal. This situation occurs because when pests are exposed to the same fumigant repeatedly, it encourages the growth of individuals that naturally resist the fumigant's effects. Therefore, repeated exposure to the same fumigant over time, along with genetic variations, leads to resistance development (Nayak et al., 2020). Numerous studies have reported the emergence of resistance in stored-product pests to commonly used fumigants such as phosphine, methyl bromide, and sulphuryl fluoride (Rajendran, 1992; Daglish et al., 2018; Jagadeesan and Nayak, 2023). Research on *T. castaneum* throughout the years revealed high levels of resistance to phosphine across the globe, indicating its widespread nature and significant implications for grain storage management. Prolonged exposure, genetic mutation, and increased metabolic detoxification mechanisms have been identified as the key factors contributing to this resistance (Pimentel et al., 2008; Schlipalius and Ebert, 2020; Zakladnoy, 2020). A similar pattern for resistance has also been observed in *C. ferrugineus* (Nayak et al., 2013; Venkidusamy et al., 2018).

Fumigation planning involves careful consideration of several factors for successful execution. These factors include selection of appropriate fumigants, determination of concentration, exposure time, and implementation of safety measures (Hagstrum, 2012). Research conducted on *R. dominica* demonstrated that applying a prolonged and consistently lethal concentration of fumigant led to a significant reduction in their population, regardless of their migration rate. Moreover, ensuring a well-structured silo and implementing proper sealing measures were found to be effective strategies in minimizing infestation levels (Shi et al., 2013). Another study investigated the possibility of a co-fumigation technique using phosphine and sulfuryl fluoride for controlling four types of stored-product pests and found that each species reacted differently regardless of the gas concentrations used. Among the insects tested, *C. ferrugineus* showed a stronger response to the mixture, followed by *S. oryzae*, *R. dominica*, and *T. castaneum* (Jagadeesan et al., 2018). Similarly, a mixture of phosphine and carbon dioxide can also be used to control *C. ferrugineus* populations (Constantin et al., 2020). These findings highlight the capability of combining different fumigants to enhance their effectiveness in controlling these pests. Also, these studies underscore the importance of selecting the appropriate fumigant for pest management, taking into special consideration its efficacy against specific insect species with the concentration and exposure time required for achieving optimal results.

CONCLUSIONS

Studying insect movement is crucial for effective fumigation planning. Understanding how the insects move within the infested areas helps in identifying the potential hiding spots and areas of high activity, which are essential for targeted fumigation treatments. By knowing where the insects seek refuge and how they navigate through the environment, we can develop strategies to ensure proper fumigant distribution inside the silos and maximize their treatment efficacy. Moreover, insights into insect movement helps in assessing the effectiveness of fumigation protocols and identifying areas that may require additional attention or reapplication of fumigants. Thus, integrating insect movement studies into fumigation planning enhances the precision and success of pest control efforts, leading to improved results in managing insect infestations in various agricultural aspects.

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Phytosanitary "Systems Approach" for the Control of Insect Pests in Citrus

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ABSTRACT

This project was designed to evaluate various treatments and strategies for the control of insect and microbiological pests to ensure pest-free and high-quality California fresh citrus enters key export marketing channels. Recently market access to Korea, China, and Australia/New Zealand, three key export markets for California fresh citrus, have been critically impacted by pest related trade barriers. Work was spent to identify critical events in the citrus production and marketing chain and evaluate the efficacy of each event toward the suite of pests per respective market. These events tracked the efficacy of key insects (flat mites, thrips, and scale) and decays (green, mold, sour rot, brown rot, Septoria spot) on oranges and lemons that were subject to standard packing operations with fungicide treatment, followed by a 21 d cold storage to simulate oceanic transit to export markets. These treatments suppressed decay throughout the cold storage as expected and yielded complete control of the insect pests. Research also quantified ethyl formate fumigation of bulk citrus prior to packing, as well as a 12 h phosphine fumigation of packed, palletized citrus prior to shipping.

Keywords: Ethyl formate, Phosphine, Systems approach, Citrus, Total utilization, Bean thrips, Asian citrus psyllid, California red scale, *Brevapalpus californicus*

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Nead-Nylander BA, Walse SS, Corbett S, Rowley J (2024) Effective removal of sulfuryl fluoride following fumigation using a commercially available liquid-air scrubber. Page 218. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Effective Removal of Sulfuryl Fluoride Following Fumigation Using a Commercially Available Liquid-Air Scrubber

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ABSTRACT

The Global Warming Potential (GWP) is a benchmark easily understood by the public and regulators. It is used to develop policies for managing gas emissions including establishing agreements between countries. While there is disagreement among climate scientists around the validity of using GWP to establish policy, GWPs continue to be reported when climate change and associated policies are discussed publicly. Sulfuryl fluoride (SF) has a GWP of 4740 but comprises a very small portion of the atmospheric gases. The contribution of SF to climate change is negligible based on radiative forcing, measurement of the capacity of a gas to contribute to climate change based on factors including GWP and amount of the gas in the atmosphere. Regardless, SF's GWP has raised concerns over its long-term use as a fumigant resulting in increased interest in developing methods for it to be captured or scrubbed.

In 2016, Douglas Products (registrant for ProFume® gas fumigant, 99.8% sulfuryl fluoride) partnered with Dr. Spencer Walse to explore the potential use of existing technology to capture SF. Results from a pilot study indicated that SF could be effectively scrubbed. Half-loss times for SF (~15 min) were significantly lower than those for methyl bromide (100 min.) with no impact from temperature. Additional studies confirmed that adding a catalyst to the solution improved scrubbing efficiency to $\geq$ 96%. Results of these studies determined the amount of SF captured per L of solution used leading to the development of a tools to predict the amount of SF remaining within the fumigated space and when the solution is no longer effective, critical information for process to be viable commercially. Studies were conducted in 2024 to confirm previous study results and to identify best practices for disposal of spent solution. This presentation will provide results of previous and current studies.

Keywords: Sulfuryl fluoride, Liquid-air scrubber, Climate change, Greenhouse gas, Global warming, GWP

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Hall 4th WA, Jones T, Sonon R (2024) Considerations and progress in developing multiresidue analysis methods for the residues of postharvest and preplant fumigants. Page 219. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Considerations and Progress in Developing Multiresidue Analysis Methods for the Residues of Postharvest and Preplant Fumigants

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ABSTRACT

The inclusion of preplant and postharvest fumigants in multiresidue pesticide screens (i.e., QuEChERS based methods) is not possible due to the volatility, and in some cases (i.e., propylene oxide and ethylene oxide), the reactivity of the analytes. We present a multiresidue method for fumigants in agricultural commodities based on cryomilling the sample in the presence of iodide salt followed by headspace solid phase microextraction (SPME) sampling and analysis by gas chromatography coupled with time-of-flight mass spectrometry. The fumigants: sulfuryl fluoride, methyl bromide, ethylene oxide (with degradant chloroethanol), propylene oxide (with degradants propylene chlorohydrin and propylene bromohydrin), 1,3-D and dimethyl disulfide were all quantitated with limits of quantitation in the 0.01–0.05 mg/kg range. The method is also shown to be effective as a screen for the presence of hydrogen cyanide, methyl isothiocyanide, metam sodium / potassium and dazomet.

Keywords: MRLs, Fumigant analysis, Methyl bromide, Sulfuryl fluoride, Propylene oxide, Ethylene oxide, SPME

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Senthilkumar T, Panigrahi SS, Singh CB (2024) Continuous carbon dioxide monitoring in a commercial bin filled with canola. Pp. 220–226. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Continuous Carbon Dioxide Monitoring in a Commercial Bin Filled with Canola

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ABSTRACT

Carbon dioxide (CO_2) concentration was continuously monitored for 5 mo in a commercial 544 t (20,000 bushels) bin filled with canola. The canola harvested with an initial moisture content between 7-8% (wb) was stored in a bin installed with CO_2 sensors in both the headspace and the plenum. The bin also had a continuous monitoring and automated fan control system comprising moisture cables, temperature cables, a weather station, and a plenum sensor. The fan control system activated the fan to cool down the stored canola based on the canola moisture content, temperature, and ambient weather. The initial CO_2 concentration in the bin at both the headspace and the plenum were at the safe levels of below 400 ppm and remained so throughout the initial 5 mo storage. The CO_2 concentration increased up to 2000 ppm in the headspace and the plenum when the fan started operating for the first time and then reduced to a normal concentration of below 400 ppm. The plenum concentration increased above 500 ppm whenever the fan was under operation, whereas the headspace was below 400 ppm. The relationship between CO_2 concentration, moisture content, and the temperature of canola was also explored.

Keywords: Canola storage, Respiration, Carbon dioxide, Moisture content, Temperature

INTRODUCTION

Canola (*Brassica napus* L., *Brassica rapa* or *Brassica juncea* cultivars of low erucic acid) is the highest cash value crop in Canada with annual cash receipts of CDN 13.6 billion (Statistics Canada, 2024), and its total economic value stands at CDN 30 billion (LMC International, 2020). The presence of pests inside stored canola will degrade the quality of canola; therefore, it is important to store the harvested canola at proper moisture content and temperature levels to avoid spoilage. Fungi and mites are major sources of spoilage in stored canola (Senthilkumar et al., 2015). The drying process can eliminate the pre-harvest fungi, but post-harvest fungi can grow even when the average moisture content and temperature levels are maintained at desired levels. Continuous monitoring of stored canola can detect spoilage at an early stage, and then corrective actions can be taken immediately to avoid further damage to the canola.

Modern storage structures are equipped with temperature and relative humidity (r.h.) sensors, weather stations, fans, and fan control systems for effective conditioning of stored grains. Carbon dioxide sensors can be a good addition to the existing sensors to indicate early spoilage. Carbon dioxide (CO_2) is one of the early indicators of spoilage. Inside the storage structures, the oxygen levels decrease, and carbon dioxide levels increase due to metabolic activity and other oxidative reactions happening inside the storage structures (Reuss et al., 1994; Singh (Jayas) et al., 1983). Carbon dioxide concentration more than 1000 ppm inside the storage structures indicates spoilage in stored grains. Carbon dioxide concentration at lower levels can cause respiratory issues, headaches, and nausea while at higher levels exceeding 100,000 ppm can be lethal to human beings (Mallinger, 1996).

Carbon dioxide sensors are available in the market, and these can easily be integrated into the current grain monitoring platforms which monitor the moisture content and temperature of the grain; however, suitable placement of CO_2 sensors inside the grain storage structures is very important (Singh (Jayas) et al., 1983). Carbon dioxide sensors were installed in the headspace of the stored corn to detect hotspots (Ileleji et al., 2006), and Maier et al. (2006) detected early spoilage and pests in stored corn by installing CO_2 sensors at both the headspace and the plenum. However, there is limited evidence on CO_2 distribution in bins stored with canola. The CO_2 sensors available in the market need to be placed without direct contact with the grain as any contact with the grain will have dust that will affect the sensors. Carbon dioxide, due to its higher density than atmospheric air, can settle down at the plenum, and, during the fan operation, the carbon dioxide can move to the headspace. Therefore, there are three options for where to place the CO_2 sensors: 1) only in the headspace; 2) only in the plenum; and 3) in both the headspace and the plenum. This study focused on detecting the distribution of CO_2 by placing sensors at the headspace and at the plenum, as well as the changes in concentration over the storage period of 5 mo. How CO_2 was diffusing inside the grain bin during the fan operation was also studied.

MATERIALS AND METHODS

Storage and Initial Canola Conditions

A fully perforated 544 t grain bin (7 rings/tiers), located at the Lethbridge College Research Farm, Alberta, was used in this study. Canola (371 t) was procured from a local farm and stored in the bin, which was filled up to the 5th ring out of 7 rings. The initial temperature of the canola in the bin was in the range of 20–24°C, and the equilibrium relative humidity was in the range of 50–71% r.h. The canola was loaded from the top into the bin. The storage bin was fitted with a 7.5 kW centrifugal fan for conditioning the grain. The canola was loaded into the grain bin starting on September 25, 2023, and ending on September 29, 2023.

Monitoring and Automated Fan Control Systems

The storage bin was equipped with 3 temperature cables, 1 r.h. cable, 1 plenum pressure sensor, and an automated fan control system provided by OPIsystems Inc. (Calgary, AB). The bin also had a dedicated fan control system comprising a weather station to monitor the ambient conditions, a fan radio to control fan operation, and a plenum sensor to monitor pressure and temperature. The monitoring cables and weather station were connected remotely to the fan control system. The data

from the monitoring cables and fan control system were transmitted continuously by channel nodes and gateways to the cloud environment, where the data were processed and the fan controls were operated accordingly. The r.h. and temperature cable installed at the middle of the bin had seven sensors. The grain isotherm curves developed by OPI were utilized to determine the moisture content using the temperature and r.h. data obtained from the sensors. The initial moisture content ranged from 6–8.4% (wb).

Carbon dioxide Sensor

Two CO_2 sensors with 50 ppm accuracy were received from OPI and were installed in the headspace and the plenum, respectively. The data from the CO_2 sensors were transmitted via gateways to the same cloud environment where the other sensor data were stored. The data from the CO_2 sensors were used only to monitor the grain condition. The data from the other temperature and r.h. monitoring cables were used only to control the fan operations.

Automated Aeration Control Strategy

The automated aeration control mode was used to aerate the canola in order to achieve the desired temperature between 5 and 15°C and the desired moisture content between 7.5 and 12% and to allow for the controlled rewetting of the canola. As the temperature of the canola reached below 15°C and the average moisture content reached 10% at the end of October, the temperature range was changed to 0–4°C, and the lower moisture limit was brought down to 5%. The fan control system would shut down the fan when the ambient air temperature was below 0°C. The fan warming was set at 2°C for the fan and the offset temperature range between 3 and 1°C as the aeration progressed to bring down the grain temperature to within the desired limits.

Statistical Analysis

The temperature and moisture content data were statistically analyzed using a t-test assuming unequal variance at a significance level of 95% confidence interval.

RESULTS AND DISCUSSION

Temperature and Moisture Content

The fan attached to the storage bin ran for 148 h to bring down the stored canola to 0–4°C. This observation was similar to the observations made by Friesen and Huminicki (1989) who estimated that around 150–200 fan hours were needed to cool the canola. The storage bin temperatures observed were in the range of 2.1–4.4°C (Fig. 1). The moisture content measurements within the bin were not significantly different ($p>0.05$) between the initial and the final values. The observed moisture content of the storage bin ranged from 6.4–10.6% (Fig. 2).

Carbon dioxide Measurements

The initial CO_2 concentration inside the canola bin was 2000 ppm at the headspace and 1500 ppm at the plenum. The higher CO_2 concentration was mainly due to the initial high grain moisture content at the bottom of the bin (as shown by the S1 sensor), which resulted in higher metabolic

activity (Figs. 3 and 4). The higher headspace value was attributed to a pressure gradient between the headspace and the environment caused by diurnal temperature fluctuations (Ileleji et al., 2006). The CO_2 concentrations reached the normal level of below 400 ppm at the headspace once the grains cooled down to the desired temperature and moisture content levels (Fig. 3); however, the CO_2 concentrations at the plenum fluctuated between the normal 400 ppm and the higher limit of 1500 ppm (Fig. 4) over the storage period.

Fig. 1. Temperature (°C) profile of the bin with stored canola over the storage period.

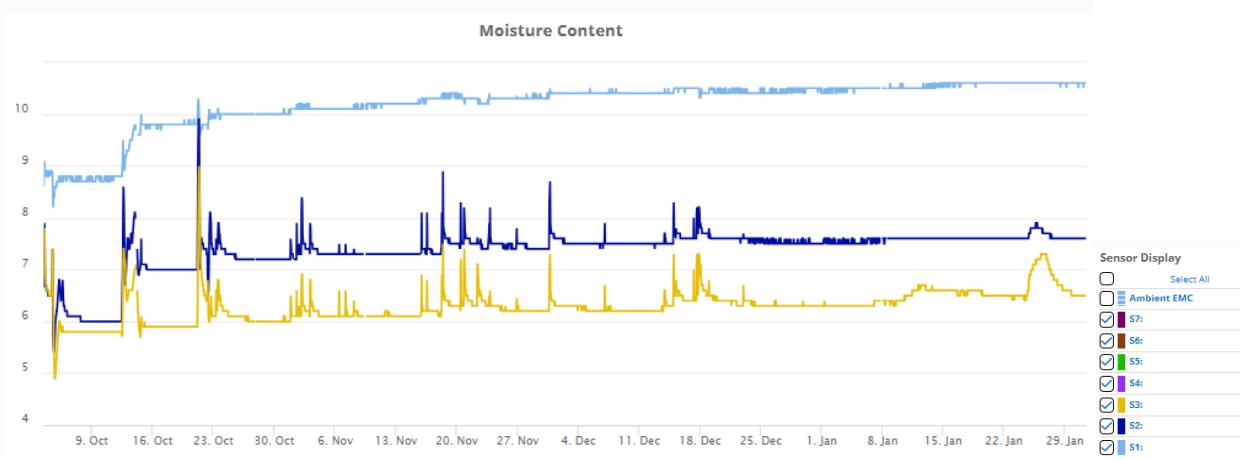

Fig. 2. Moisture (% wb) profile of the bin with stored canola over the storage period.

Fan Operation and Carbon Dioxide Concentration

The headspace CO_2 concentration was constantly below 400 ppm, except for the initial period when the headspace temperature was low. The fan operation cooled down the grain temperature, and the headspace concentration was also reduced to the normal level of below 400 ppm. It could be assumed that the pressure gradient normalized after fan operation. There was no change in CO_2 concentration even during the fan operations in the headspace over the storage period (Fig. 3). To be more specific, the CO_2 concentration at the plenum was higher whenever the fans were running. This can be attributed to the significantly large temperature distribution ($p<0.05$) within the silo (Fig. 1). Because the bottom-level canola was cooler and had a higher moisture content relative to the upper-level stored canola, the interstitial air tended to sink, resulting in CO_2-rich air accumulating in the plenum. Figure 4 clearly shows the higher CO_2 concentration levels whenever the fans were running.

Fig. 3. Headspace CO_2 concentration levels (ppm) and fan operation (vertical green lines indicate fan operation) over the canola storage period.

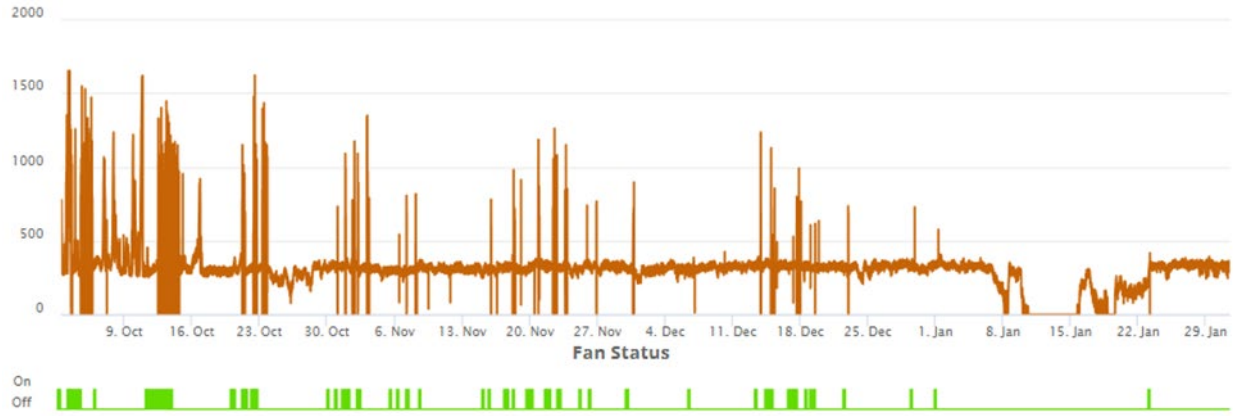

Fig. 4. Plenum CO_2 concentration levels (ppm) and fan operation (vertical green lines indicate fan operation) over the canola storage period.

These results show that CO_2 sensors need to be placed in the plenum and the headspace of a typical on-farm silo because of the influence of pressure gradients on the headspace and the grain temperature gradient. For canola storage, placing the CO_2 sensor in the plenum has more value than placing in the headspace; however, if the hotspot occurs near the top of the grain bin, then placing the CO_2 sensor in the headspace would likely be helpful in detecting any spoilage at an early stage before the CO_2 can even reach the plenum area (due to its higher density characteristics).

CONCLUSIONS

This study investigated the best placement for the CO_2 sensors inside the canola bin and the changes in CO_2 concentrations based on the grain condition. The CO_2 concentrations at both the headspace and the plenum were higher when the temperature and moisture content levels of the canola were also high. The CO_2 concentrations reduced to the normal level (below 400 ppm) once the grain temperature and moisture content were reduced to the safe storage levels. The CO_2 concentrations at the plenum reached higher levels whenever the fans were running.

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Dynamic Phosphine Fumigation Using Continuous Monitoring

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ABSTRACT

Phosphine gas is an essential means of controlling grain pests. However, for it to be effective, a certain gas concentration threshold must be held for a specific time period relative to grain temperature. It is therefore necessary to monitor the phosphine concentration throughout the fumigation process, as it can vary over time as a result of various phenomena like leakage, sorption, and chimney effect. Until the 1980s, colorimetric tubes were the only way to measure phosphine in the field. In 1986, the discovery that an electrochemical cell could measure phosphine made it possible to automate multi-point measurements and record and transmit data in real-time. After three decades of research and several prototypes, CaptSystemes created the PhosCapt®-MP, an automatic, connected multi-point device, designed to work in harsh conditions and offer high accuracy over a wide measurement range. The challenge for phosphine fumigations in a group of irregular-sized storages of variable sealing tightness is to achieve and maintain gas concentration uniformity at an effective level throughout the group. Thanks to multi-point monitoring with data transmission, concentrations can be precisely adjusted in any location using a variety of techniques, for example, by adapting the gas flow to different parts of the storage, or by re-dosing. In this way, the effectiveness of the techniques used can be verified. Four examples of dynamic fumigation using a PhosCapt-MP are highlighted in this paper: four 10,200 m^3 concrete silos without recirculation, eight 2,300 m^3 concrete silos with recirculation, a SIROFLO®-like system fumigation without tarpaulin in three 13,000 m^3 metal silos and another of 100,000 m^3 in two domed sheds. Continuous multi-point phosphine monitoring has proven to be an essential tool for guaranteeing fumigation efficiency, monitoring degassing, combatting the development of phosphine-resistant strains, and enhancing safety next to fumigated sites.

Keywords: Fumigation, Phosphine, Monitoring, Electrochemical cell, Efficiency, safety, PhosCapt, Phosphine resistance

INTRODUCTION

Today, grain pest control faces a number of new challenges. Alternatives to phosphine are disappearing. Contact insecticides are being phased out due to their residues. Anoxia is too complicated to implement on a large scale since it needs totally gastight sealing. The use of cold

treatments is not cost-effective due to increasing energy costs. With global warming, insect infestations no longer stop in winter. Phosphine fumigations in large or non-gastight structures pose challenges in obtaining and maintaining a uniform gas concentration at an efficient level for a minimum amount of time defined by grain temperature. Yet, we cannot be sure whether the fumigation was successful without measuring the gas in key locations. The important advantage of continuous gas concentration measurements is the opportunity to adjust, in real-time, the amount of gas in all locations to guarantee a successful fumigation, even in the most complicated situations. "PhosCapt®" phosphine monitors (Fig. 1B), the result of more than 30 yr of research, have enabled the development of innovative dynamic fumigation techniques.

HISTORICAL BACKGROUND

For a long time, phosphine measurement methods were either too cumbersome, expensive, or difficult to implement in the field. Until the 1980s, the only measurement method in the field relied on Draeger® or Auer® colorimetric tubes. In 1986, for the first time, it was discovered that because phosphine is a reducing gas, an electrochemical cell could react to it (Ducom and Bourges, 1986). There were electronic devices measuring the reducing gas CO, and the manufacturer, Herrmann-Moritz, suggested that Ducom and Bourges (1986) modify a CO device to measure phosphine. This discovery opened the way for precise, easy-to-use, inexpensive measuring devices. It was finally possible to take a lot of measurements at a low cost. Thanks to the first microcomputers in the 1980s, it became possible to experiment with multi-point measurements, automation, data recording, and real-time data transmission. In 1987, the first automated prototype for taking multi-point phosphine measurements was developed at the LNDS laboratory in Bordeaux, France (Fig. 1A). Its data could be accessed remotely using a Minitel connection (the ancestor of the Internet in France). The PhosCapt®-MP phosphine monitor, commercialized since 2015, resulted from this research (Fig. 1B).

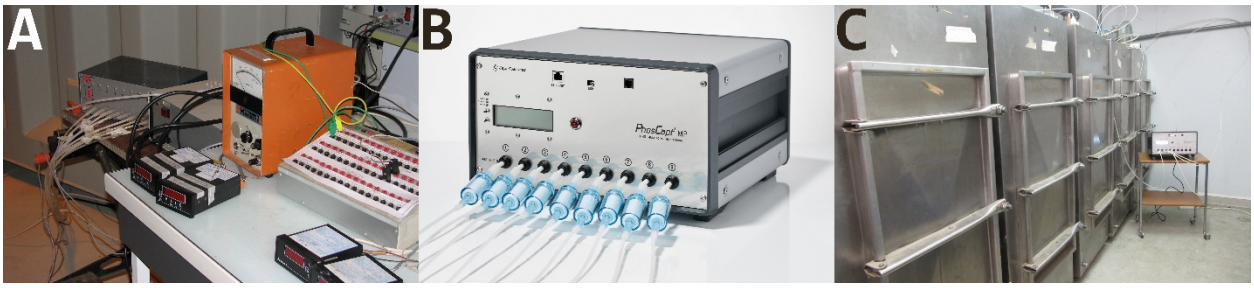

Fig. 1. A–Prototype in lab; B–PhosCapt®-MP first model in 2015; and C–AlP hydrolysis speed.

PhosCapt®-MP MONITORING SYSTEM

The system works autonomously and automatically to measure phosphine concentrations on twelve 4 mm (or ¼") ID lines of up to 200 m (650 ft) long several times a day on a user-defined periodicity (1, 3, 6, 8 h, or continuous). It was created to be easy to transport and deploy, to be accurate with a wide measurement range (0 to 15,000 ppm and a minimum detection of 0.02 ppm PH_3), and to transmit the results in real time via email to the fumigation operator and to a server

for online concentration monitoring. A copy of the readings is recorded as a spreadsheet file in its non-volatile memory for traceability. It is made to resist harsh conditions like dust and large temperature amplitudes. In large-scale fumigations, multiple devices can be used together to increase the number of sampling points. When they are connected to an Ethernet network, one computer can configure and monitor them all locally. The configuration consists of identifying each line by assigning it a label and selecting its measurement mode from three different modes: *Efficacy (E), Clearance (C)* or *Security (S)*. Unused lines are deactivated, but they can be activated anytime. Then, the user selects the measurement series frequency and starts the measurement cycle. To receive email data, the user enters an email address. An optional alarm can be configured to trigger an email if a concentration threshold is reached. Depending on the settings and the line measuring mode, the device will trigger an alarm when the concentration falls under a low threshold (i.e., < 250 ppm) on an '*Efficacy (E)*' line or exceeds a high threshold on a '*Security (S)*' line (i.e., > 0.3 ppm).

To ensure maximum precision on a wide measurement range, two sensors are used. One sensor measures high concentrations in the 0 to 15,000 ppm PH_3 range with 1 ppm precision, and a second sensor measures low concentrations from 0 to 20 ppm PH_3 with 0.01 ppm precision. In lines configured in *Efficacy (E)* mode, only the high-concentration sensor is used. In lines configured in the other two modes (*Clearance (C)* and *Security (S)*), firstly, the high concentration sensor is used and then, if the concentration is lower than 20 ppm, a second sample is taken using the low concentration sensor with greater precision. The *Security (S)* mode is used in lines that check for leaks around the fumigation area. These lines are sampled first at each measurement cycle. The *Clearance (C)* mode is used in lines that monitor high concentrations for fumigation efficacy; when the concentration drops at the end of the fumigation, it automatically switches to the low concentration sensor to monitor degassing and to ensure that the area is safe for access. The *Efficacy (E)* mode is used in lines that only monitor high concentrations for efficacy.

Monitoring concentration ensures fumigation effectiveness and prevents having to re-fumigate too frequently. It enables a precise adjustment of the amount of gas used. It also allows for the fumigation of certain storage spaces that would be too expensive to seal.

For research use, the standard sampling settings can be adapted for special conditions like working with small bins or carrying out more frequent measurements. The PhosCapt®-MP is used in a wide variety of experiments in a laboratory or in the field as, for example, an AlP hydrolysis speed study (Fig. 1C), a PH_3 sorption study in wet wood, or in gas diffusion studies in containers or silos.

DYNAMIC FUMIGATION COMPONENTS

The principle of dynamic fumigation is that three components (storage sealing, gas delivery, and the circulation system) can be adjusted dynamically according to the real-time data from the gas monitoring system.

- Storage structures that are sealed using practical, affordable sealing methods of all major and most minor leak sources, so that fumigation gas leakage is minimal.

- A ground-level dosage generation delivery system, which safely delivers an efficient quantity of phosphine gas with the ability to re-dose easily (e.g., dry release cabinets as shown in Figure 2).
- An optional modular multi-storage unit gas circulation system using manifolds. This can be a closed-loop circuit like CLF (Noyes et al., 1998) or thermosiphon (Newman et al., 2012) or even an open SIROFLO®-like system. In large storage facilities, valves are used to enable a fine-tuning of gas routing and flow regulation in the different areas to adjust concentrations in all locations. Motorized gas circulation is an option.
- A reliable phosphine monitoring system, which allows fumigators to 'react in real time' by receiving continuous gas concentration measurements at key locations in all fumigated storage facilities and surrounding areas where leaks can occur. At the end of fumigations, monitoring helps determine when grain bins and work areas are safe for entry (Fig. 1B).

LARGE STORAGE STRUCTURE FUMIGATIONS IN FRANCE

In France, very few silos are sufficiently gastight to ensure good fumigation, i.e., to have uniform gas concentration and keep it in place. One exception is concrete silos that are generally sufficiently gastight if they are closed at the top. The silos can be inspected, and any leak sources such as manholes, extractors, vents, and grain inlets can be sealed. Traditionally, phosphine generators are placed at the top of the cell or sometimes at the bottom because, even though gas diffusion is slower, concentrations are more uniform (Ducom et al., 2021). Active recirculation can help, but might lead to more leakage, so it should be used with moderation. Metal silos are generally not made to be gastight, as they often have openings between the walls and the roof to allow dust and moisture to escape. There are also silos or flat storages, where cells are often impossible to keep gastight, even with tarping, which may also be too complex or expensive. In these conditions, it is necessary to implement a fumigation system that does not require sealing. A solution was proposed at CSIRO by Winks (1992) known as SIROFLO®. It is a continuous pressurized phosphine distribution system, which distributes gas upward throughout the grain. Winks (1992) showed that exposure time is far more critical than gas concentration. To control major grain-infesting species, it is essential to significantly prolong the usual exposure time of 5 d. In these conditions, high phosphine concentrations are not needed; in fact, the gas is more effective at lower than usual concentrations. Gas release must be continued long enough to overcome egg and pupal survival, so that sufficient gas is still available when the insects hatch. To avoid phosphine resistance, it is important to maintain a lethal concentration in all locations. In situations where sealing is possible but still not totally gastight, a recirculation system is often preferred, like CLF, which is one of the most advanced recirculation systems, as it combines multiple storage entities in one system.

GROUND-LEVEL DOSAGE GENERATION DELIVERY SYSTEM

Liquid phosphine in cylinders has not yet been certified for use in France. A technique has been developed which assists in the passing of a controlled flow of air into a dry release cabinet containing metal phosphide blankets (Fig. 2).

This enables gas to be injected with or without recirculation. The phosphine concentration leaving the cabinet varies depending on the air humidity, the fan flow rate, the quantity of AlP product loaded in the cabinet, and the use of recirculation or not.

Fig. 2. Two PH_3 dry release cabinets (MFD. Valérie Ducom).

CONTINUOUS MONITORING SYSTEM

To take advantage of these gas diffusion techniques, it is essential to have precise and continuous gas concentration monitoring in key locations: within the grain load at different levels, in the headspace, and near the openings (extractors, ventilation doors). Throughout the fumigation, gas concentration fluctuates due to leaks, sorption, thermal effects (Ducom et al., 2021), or other atmospheric events like pressure drops or wind. To maintain the minimum required concentrations in every location, real-time data is essential to dynamically adjust the different parameters: the fan flow rate, quantity of phosphine from the gas generator, recirculation system valve adjustment, and sealing—if needed and if possible. In this way, the effectiveness of the techniques used can be verified, which makes it possible to fumigate silos or warehouses that cannot be totally sealed. Outside the fumigated enclosure, monitoring helps detect possible leaks in critical areas like galleries that can be ventilated to protect the electrical installation from gas corrosion.

FOUR CASE STUDIES IN FRANCE

Fig. 3. Four treated storage systems located in France: 1–Baziège, concrete bins, durum wheat, no active diffusion or recirculation; 2–Roncenay, conical bottom concrete silos, peas, active gas diffusion with recirculation; 3–Pomacle, metal bins, wheat, active diffusion, no recirculation; and 4–Buchères, ground sheds, wheat, injected gas, no recirculation, and no tarping.

1–Baziège (Aug. 2019): 30,000 t of durum wheat in four 10,200 m^3 concrete bins.

Four silos were fumigated in passive mode, meaning that no active diffusion or recirculation was used, as shown in Figure 3(1). This fumigation was the subject of a study (Ducom et al., 2021) aimed at characterizing the differences in phosphine penetration and distribution into a grain mass using two types of applications—one from the top of a silo (Fig. 4A, 4B) and the other from the bottom (Fig. 4C, 4D). Ten PhosCapt®-MP monitors took a total of 30,000 measurements from 104 locations over 37 d. Data showed that gassing from the bottom gave total efficacy at all levels. On the other hand, gassing from the top gave no efficacy throughout the silo, even at double the dose. For the first time, it was possible to 'visualize' PH_3 distribution and its complexity.

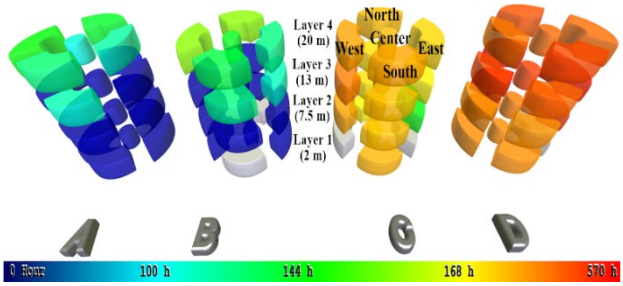

Fig. 4. 200 ppm PH_3 exposure time in 3D cartography.

2–Roncenay (Aug. 2023): Peas in eight 2,300 m^3 conical bottom concrete bins.

As shown back in Figure 3(2), active gas diffusion was used with four dry release cabinets and recirculation in eight silos with conical bottoms infested with pea weevils. Valves on the ventilation ducts were used to manually adjust the gas flow. One PhosCapt®-MP monitor with one line in the grain at the top of each cell and one line in the upper gallery to detect leaks took 1000 measurements at nine locations, eight times a day, for 14 d. The concentrations were relatively uniform after the first five days, except for cells C55 and C77, but all cells remained well above 200 ppm during the long gas exposure time, which exceeded 10 d (Fig. 5). Leakage in the gallery was very low, which is consistent with good results in the cells.

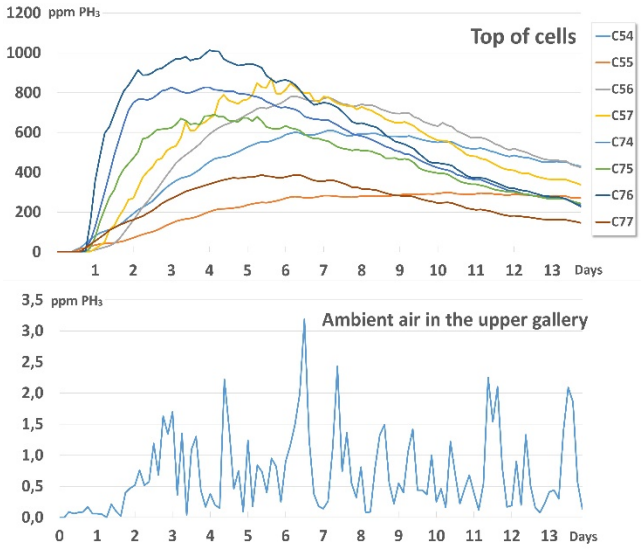

Fig. 5. PH_3 concentrations in Roncenay.

3–Pomacle (Aug. 2023): Wheat in three 13,000 m^3 non-gastight metal bins.

Three out of eight bins, as shown in Figure 3(3), were fumigated without roof sealing (as each bin had a roof–sidewall air gap) using active gas diffusion without recirculation, with one dry release cabinet per bin. The 16 ventilation ducts of each cell were connected to a manifold using flexible tubing from the dry release cabinet. Valves on the ventilation ducts were used to manually adjust gas flow. One PhosCapt®-MP monitor took a total of 1,000 measurements at six locations within the three bins (Fig. 6). One line was placed below the grain surface at the top of each cell, and one line was in each of the bottom galleries to detect leaks. The objective was to obtain as long of a continuous gas exposure time as possible (over 15 d in this case). The graphs in Figure 7 show that the gas concentrations were relatively uniform, remaining above 200 ppm in all three cells (CB2, CB3, and CB4) for more than 10 d. In the galleries, the PH_3 concentrations were quite high, especially at the start of the hydrolysis: 150 to 200 ppm. In fact, the sealing around the CB4 air entry doors was not tight enough. When the gallery doors were tightened, it took 2 d for the gas concentration in CB4 to reach the target level. After 5–6 d, concentrations in the galleries stabilized at around 40–60 ppm. After the fumigation, no live rice weevils or any other insects were found.

Fig. 6. A PhosCapt®-MP in a cabinet outside the bin.

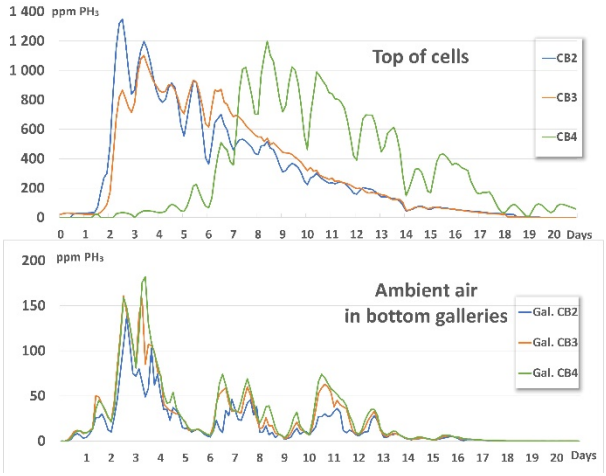

Fig. 7. PH_3 concentrations in Pomacle.

4–Buchères (Oct. 2022): 75,000 t of wheat in 150,000 m^3 in two ground sheds

Eight dry release cabinets connected to the ventilation system slowly injected gas inside 100,000 m^3 of grain without tarping or recirculation (as previously shown in Figure 3(4)). The ventilation flow was modulated based on air humidity and the PH_3 gas concentration measurements in each cell. In Cell 7, the concentration was maintained above 200 ppm for 10 d. Re-dosing on the 10th day prolonged this gas exposure to 20 d (Fig. 8).

In Cell 10, even after re-dosing, the gas concentration was still close to zero. It was necessary to adjust the ventilation duct valves to route enough gas to Cell 10 to reach the target value and maintain a good concentration for 8 d.

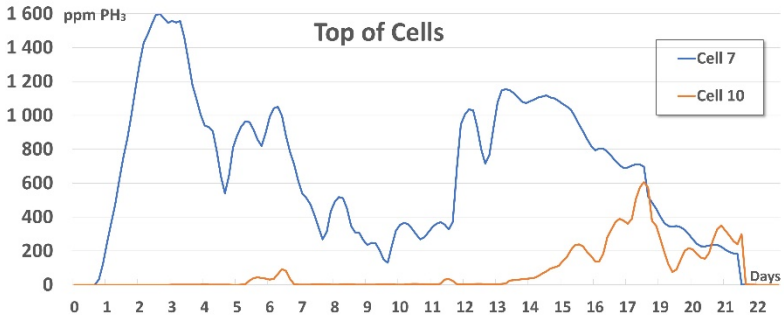

Fig. 8. PH_3 concentrations in Buchères.

Before the fumigation, there was a very significant rice weevil infestation in all the cells. After fumigation, sieving revealed no living insects. In the following 7 mo, no insects were found during quality checks. Three PhosCapt®-MP monitors took more than 5,000 gas concentration measurements at 29 locations over 25 d (Fig. 9).

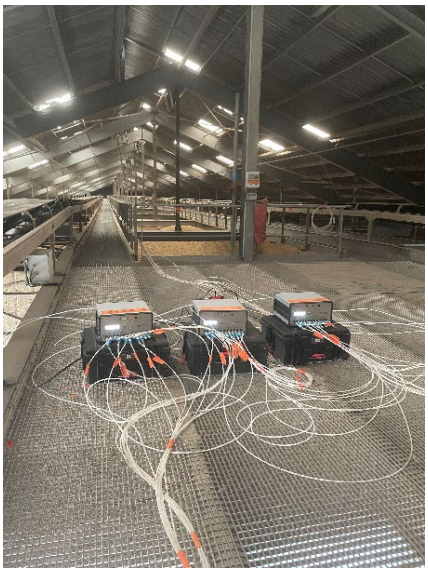

Fig. 9. Three PhosCapt®-MP monitoring 29 locations.

CONCLUSIONS

Phosphine fumigation remains almost the only economical and completely effective method for eliminating grain insects that does not produce residues. Continuous multi-point phosphine monitoring enables dynamic adaptation of the technique to each situation to guarantee an effective and safe fumigation. It introduces the precise control and maintenance of fumigation

concentrations; thus, monitoring pays off through efficient dosage/concentration management, with much more predictable efficacy, and results in higher grain product quality and value. In the longer term, these techniques help prevent the emergence of phosphine-resistant insects since each fumigation eliminates all stages.

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Investigating Hazardous Chemicals Inside Shipping Containers Before and After Fumigation Treatment with Ethyl Formate

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ABSTRACT

International trade continues to drive the increasing throughput of shipping containers throughout major ports. Fumigation remains a popular biosecurity treatment for shipping containers. The air trapped inside shipping containers could be contaminated with fumigant residuals and hazardous airborne chemicals. Seemingly harmless cargo and packaging materials could contribute to the release of chemicals. Exposure to these airborne chemicals may be hazardous to the health of workers and impact the environment. This research aims to (1) investigate the airborne chemicals in 20 ft (6.1 m) shipping containers relative to the cargo, and (2) observe the inter-reactions between the airborne chemicals and the ethyl formate fumigant. Cargo-based categories were visually assigned: *Machinery and Parts*, *Woods and Packaging,* and *Dangerous Goods*. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) solid phase microextraction SPME fiber was placed inside each shipping container and analyzed onsite with gas chromatography mass spectrometry (GC-MS). A total of 81 containers were investigated, and 55 unique compounds were identified. Twenty volatile compounds were found to contribute to the ten highest medians. Three of these compounds were common to all categories (*formaldehyde, ethyl formate, and m-xylene*). The absolute peak areas of compounds compared pre-fumigation and post-fumigation for *Machinery and Parts* showed a significant difference for 7 compounds, *Woods and Packaging* showed a significant difference for 12 compounds, and *Dangerous Goods* showed a significant difference for 2 compounds. This is the first-time the systematic monitoring of hazardous airborne chemicals for pre-fumigation and post-fumigation has been studied and reported. In addition, we evaluated the inter-reaction of ethyl formate, with the chemicals released from the cargo. This work has potential to support policies and procedures for occupation, health, and safety.

Keywords: Occupation and environment safety, Work safety, Occupational exposure, Fumigant residue, Air sampling, Chemical identification, Cargo classification, SPME-GC-MS

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Phosphine-Fumigation Monitoring of Railcars

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ABSTRACT

Railcars are a common method of transporting grain and processed commodities in the USA. Treatment of railcars with fumigants during transit is allowed because the train routes are not considered public roads. Electronic technologies have become available for monitoring phosphine gas (PH_3) fumigation on railcars. These fumigation sensors were used to collect and store phosphine concentration and local temperature data during rail transport. The sensors were configured to collect data points every 4 h for 84 data points or 14 d total. When the railcar reached its destinations, the electronic sensors were placed into shipping cases and sent back to the originating laboratory via UPS shipping. The fumigation events were downloaded to a local computer at the laboratory. Many fumigation events were assessed for both bulk and freight railcars. The fumigations per railcar were evaluated by charting the phosphine concentration changes over the 4–8 d of fumigation. Fumigation charts from both bulk and freight railcars were prepared. Also, the data were summarized into two parameters: concentration × time (Ct) value and the holding time in days. Two bulk railcars which contained corn grits had fumigation Ct values of 115,00 and 125,00 ppm×h over 8 d for sensors that were located at the top of the corn grits. Phosphine penetration into lower levels of corn grits was tested in separate laboratory trials where concentration was found to be ~300 ppm at 2 m below the surface after 8 d and Ct was ~58,000 ppm×h. Fumigation events for many freight railcars were evaluated and found to have a range of Ct values from 7,400 to 52,000 ppm×h over 2–6 d. Additional laboratory tests demonstrated that a strain of phosphine resistant lesser grain borer adults had over 90% mortality with 25,000 ppm×h Ct after 4 d of containment.

Keywords: Wi-Fi sensors, Calibrations, Concentration, Sealing

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Visualization of Phosphine Fumigant Dispersion through Grain in a Steel Bin Affected by Application Method

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ABSTRACT

Effectiveness of fumigation with phosphine is often compromised by suboptimal distribution of the gas. Non-uniformity in fumigant gas distribution can be caused by several factors such as leaks in the grain bin envelope, foreign material in the grain, and phosphine placement. Many workers in fumigation have stressed that the most important factor for a successful fumigation is the ability of the grain storage structure to retain the fumigant gas. Phosphine concentration and distribution was studied during field tests, with two replications, in U.S. corrugated steel bins containing 95 t of hard red winter wheat to document uniformity. The bins were sealed following bin sealing recommendations, except where the eave to sidewall seal had aged, and phosphine was applied by conventional probe-only and probe and tarp techniques and by closed-loop fumigation (CLF). Contour plots of phosphine movement showed leakage and uneven distribution patterns over time with conventional probed tablets, which resulted in some areas in the lower half of the grain mass receiving a zero dose and additional locations remaining below the target phosphine concentration of 200 ppm for the entire period of fumigation. With the CLF fumigation, phosphine concentrations were uniform throughout the bin levels at each time step, but average concentration levels were lower than those measured in the conventional fumigations. Since the same bins and wheat were used in the conventional fumigations, this indicates that more leakage occurred with CLF which is likely due to the CLF recirculation fan pressure. CLF delivers on its potential to produce more uniform fumigant distribution and would be expected to produce effective concentration levels when implemented in a well-sealed structure.

Keywords: Phosphine fumigation, Closed-loop fumigation, Grain storage, Insect pests, Dispersion, Concentration

INTRODUCTION

Phosphine is a widely used fumigant for bulk-stored cereal grains, oil seeds, and other bulk dried commodities due to its low cost and relative ease of use. It is widely used for the control of insect pests in stored grain or grain products, whether stored in bulk or in sacks, and it has good efficacy at reducing pest population levels when properly applied. Probe-only or probe and tarp fumigation have been the conventional methods of applying the fumigants in stored grain for decades. In probe-only fumigation, the fumigant dosage in the form of aluminum phosphide (AlP) pellets or tablets are probed from one to five feet into the top surface of the grain mass in storage. Additionally, 1/4 of the dosage could be placed in aeration ducts beneath the grain bin floor to help increase gas concentration into the lower portion of the grain. This application becomes probe and tarp when the grain surface is covered with a flexible sheeting material, such as a poly tarpaulin, to keep the phosphine gas from escaping from the grain mass. When AIP pellets are used in tall grain silos, where probing is not possible, these can be added into grain bulk as it is transferred from the silo into a bin, a process sometimes termed automatic dispenser application. Others have applied the entire dosage in aeration ducts below the grain bin floor (Cook, 2016; Flinn and Reed, 2008) or by distribution into the grain when filling the bottom half of a bin. Closed-loop fumigation (CLF) uses low-volume airflow induced by fans or natural convection, moving fumigant/air mixtures between the headspace and bottom of the bin in a closed-loop cycle (thermo-siphon).

The success of fumigation for control of insect pests in bulk stored grain is affected by the concentration and uniformity of phosphine gas distribution achieved by the foregoing methods of applying the fumigation along with the gas retention properties of the grain storage structure. For fumigation with phosphine to be successful in controlling insect pests in bulk grain, concentrations must remain at 200 ppm for a minimum of 100 h across the grain mass to kill all life stages of grain insects (Noyes and Phillips, 2007). However, a minimum of 72 h is generally mentioned in extension publications on phosphine fumigation. Effective fumigation of bulk stored grain can be hampered by poor distribution of the gas through the bulk volume. To ensure efficacy of fumigation, it is essential to monitor gas concentration over space and time. However, this would require multipoint monitoring, which is difficult to achieve in practice because of the very limited capability to deploy monitoring hardware. The question is: what single or finite number of measurements of phosphine concentration should be used in assessing fumigation effectiveness and where should these be measured within the geometry of a grain bulk. The objective of this study was to evaluate the distribution of phosphine in temporarily sealed grain storage bins during conventional fumigation with probed tablets in comparison to distribution of phosphine using CLF.

MATERIALS AND METHODS

Fumigation experiments were conducted in two corrugated steel grain storage bins (Model CB/CBU 7-4, Chief Agri, Kearney, NE, USA) at the USDA-ARS Center for Grain and Animal Health Research in Manhattan, Kansas, over the course of 2016 to 2018 (Casada et al., 2018). Each cylindrical corrugated metal bin was 6.6 m in diameter with 4.2 m eave height and 6.0 m peak height with a perforated floor for grain aeration. The bins were temporarily sealed following standard recommendations using 4 mil plastic sheets covering all openings using contact adhesive.

The sidewall to the eave joint had been previously sealed with caulk but was not updated for the tests. The bins were loaded from the central top bin hatch with 95 t (3490.3 bu.) of hard red winter wheat (~11.5% moisture content) which was then leveled at a height of 3.6 m. Phosphine concentration levels within the grain bulk after fumigations were monitored by sampling the interstitial air at uniformly spaced points across the cylindrical grain bulk through sampling tubes at 45 tubes within the grain mass and 3 in the headspace (Fig. 1). The depths at which the sampling tube inlets were defined as five 0.72 m thick layers of grain referenced to the grain surface with the topmost 0.72 m layer designated as the first level. A Dräger X-am® 5000 (Drägerwerk AG & Co., Lübeck, Germany) personal monitoring instrument with a (0–2000) ppm phosphine sensor was used to measure phosphine concentrations at 4 h to 8 h intervals.

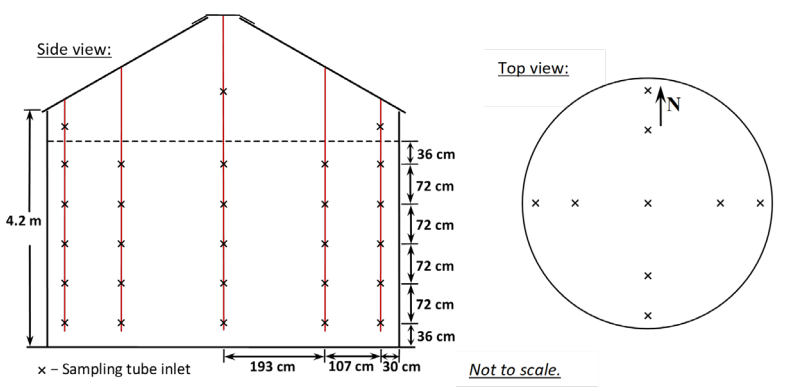

Fig. 1. Sampling tube locations within a test bin.

A subset of the experiments comparing conventional fumigant application by "probe" and "probe and tarp" methods with closed-loop fumigation (CLF) conducted under similar ambient temperature conditions (~25°C) during late spring/early summer in the U.S. were selected for visualization of phosphine gas dispersion (Table 1). Phosphine gas concentrations were averaged for all nine sampling points at each level in the grain, and the resulting means were graphed versus time. Point measurements across the five depths and across the N–S and E–W bin vertical sections were graphed into contour plots (Surfer, Golden Software, Golden, CO, USA).

Table 1. Fumigation tests.

Test No.	Date	Descriptor	Fan Sched.	Notes
2B	6/27/2017	Probe High No Tarp 2	N.A.	648 Probed w/o tarp test 2, Probe 9 hole locations and 3 depths 1.22 m, 0.61 m, 0.30 m (4 ft, 2 ft, 1 ft), 648 tablets per bin
5A	5/30/2018	Probe High Tarp 1	N.A.	648 Probed w/ tarp test 1, Probe 9 hole locations and 3 depths 1.22 m, 0.61 m, 0.30 m (4 ft, 2 ft, 1 ft), 648 tablets per bin
3D	7/17/2018	CLF High 5	Sched. 2b	648 Recirculation test 3, Closed Loop 648 tablets per bin, fans on at the first noon time, 23 min. maintenance cycle every 6 h

RESULTS

Distribution of phosphine gas through grain storage volumes has typically been presented as line charts of phosphine concentration versus time after the application of fumigation. Results from the experiments examined are shown in Figure 2. Probing phosphine tablets into the top 1.2 m of the grain (probe-only application) resulted in 1000 ppm or more of phosphine at the upper two levels (0.36 and 1.08 m), with sampling points near the tablets, maintained for about 50 h. Phosphine was detected in the middle of the grain mass at a level higher than 200 ppm by 3 h after the fumigation was initiated when application was probe-only, but it was not detected in the case with probe and tarp until the second reading at about 10 h. Additionally, no phosphine dispersed into the lower two levels of the grain mass using these two conventional application methods.

In the CLF application, peak readings never exceeded 1000 ppm, but the concentration at all levels of the bins were similar and the variation with time after fumigation had a similar decreasing trend after the AlP tablets were used up. Moreover, the 2000 ppm readings seen with the probe only treatment were not observed in CLF, even though the tablets were also spread on the top grain surface near the sampling probes located in the headspace. The CLF graphs show that the phosphine was not retained in the bins for more than 70 h. The larger number of fluctuations in concentrations over time and generally lower concentrations measured in the different grain levels, especially the bottom layer, compared with the measurements in bin B suggest that bin D had greater leakage through its structure than bin B.

With the probe and tarp application, phosphine levels at the upper two levels were recorded at or near the maximum measurement possible with the Dräger until about 66 h after fumigation due to the proximity of upper-level sensors to the tablets, as mentioned above. From hours 24–72 after fumigation, phosphine gas was measured in the 300 to 600 ppm range in the third level in bin B. Phosphine measurements in the 200 ppm range were recorded for the fourth level with this method of application but not when there was no tarp placed over the grain surface in the case of probe-only application.

As shown in Figure 2, the phosphine measurements for bin D exhibited similar trends in decreasing phosphine concentration after the fumigation. There was more fluctuation in measurements in D compared to B, which is probably due to the difference in the sealing of the two bins to keep phosphine from leaking out.

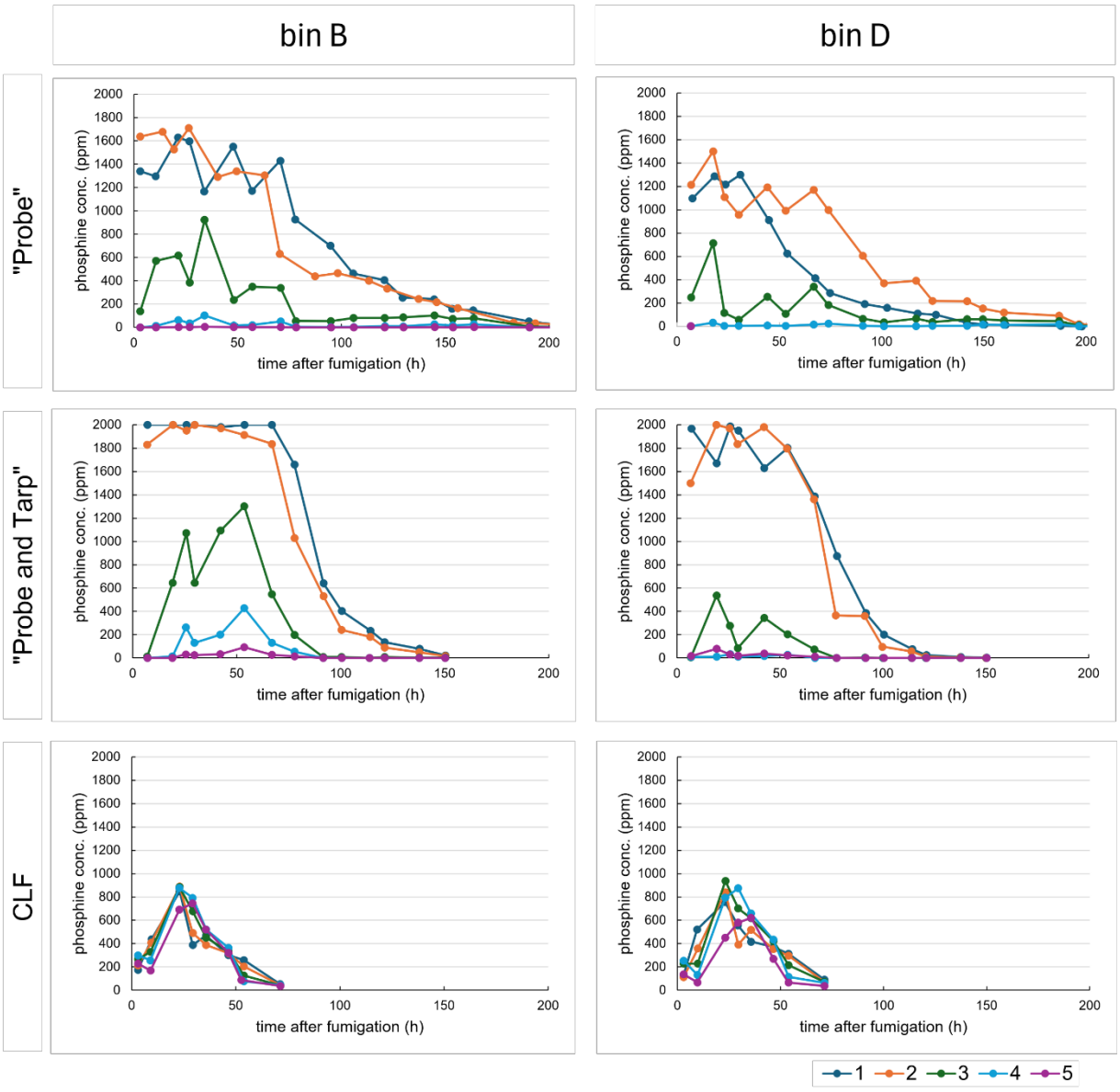

Fig. 2. Variation in phosphine concentration at different bin levels with time after fumigant application by different methods.

When the phosphine concentration measurements are averaged over the whole grain mass (45 points), the resulting trends are presented in Figure 3 (color scale is phosphine concentration in ppm). The graphs show that the concentration level for each bin remained above 200 ppm for 72 h, which falls in the often-recommended (200–300) ppm range for a minimum of 3 days.

However, these averages do not identify specific areas within the bin that did not receive sufficient concentrations. Phosphine levels with CLF peaked at around 45 h after fumigation, then gradually dropped to marginal levels before the critical timepoint of 72 h. Thus, the phosphine level in this instance of CLF was not maintained for the 72 h and generally forwarded much less than the 100 h recommended by researchers in Australia. In general, the phosphine concentration averaged across each layer of grain, as presented in Figure 3, illustrated how effectively the fumigant moved downward from the tablet application on or near the top surface into the lower layers of the bin. However, averaging the results over the two dimensions (average across the five levels and then averaging those layer means for the whole bin) masks any non-uniformity of phosphine dispersion over three dimensions.

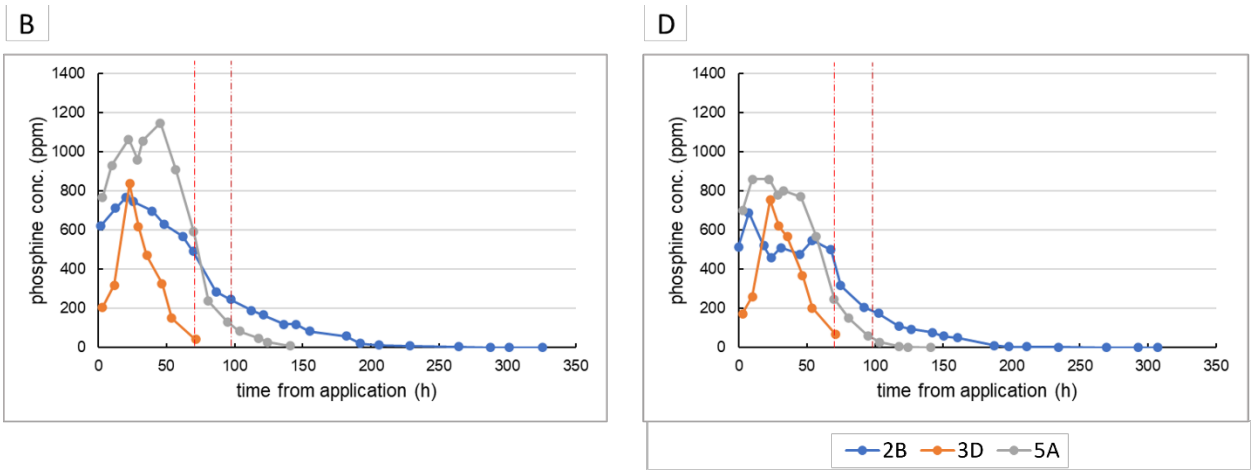

Fig. 3. Variation in the mean phosphine concentration for each bin after fumigant application by different methods.

This additional variance is more evident in the contour plots displaying phosphine dispersion across the (0.4, 1.8, and 3.6 m) horizontal planes (top, middle, and bottom grain layers, respectively) and N–S and E–W vertical planes through the bin center line when peak concentration was recorded for the three methods of phosphine fumigation tested, as shown in Figure 4. The average of the total number of zero phosphine concentration measurements up to 72 h for the conventional fumigations (74 for probe-only and 23 for probe and tarp) heightens the concern about consistency in phosphine dispersion and the efficacy of fumigation.

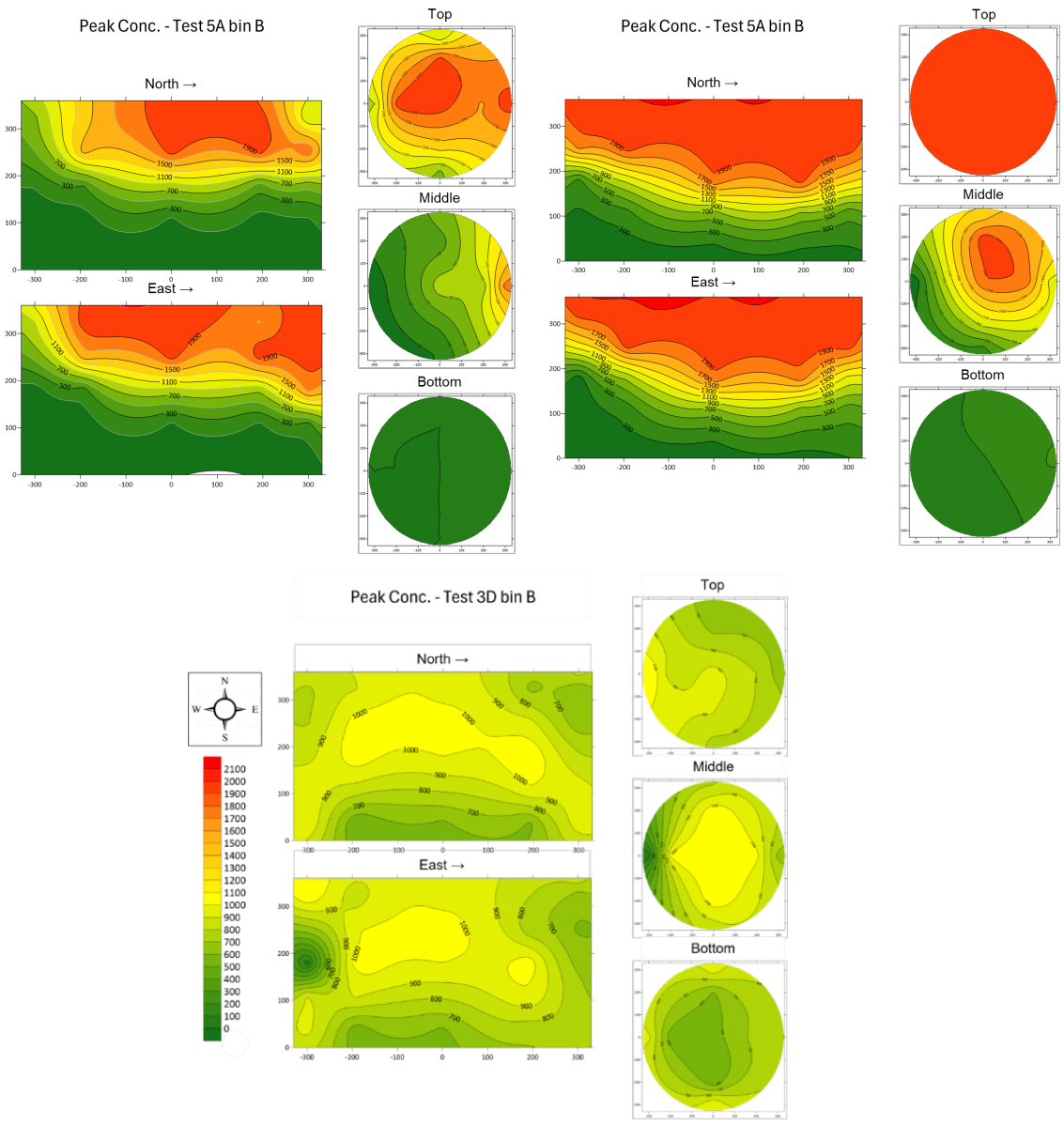

Fig. 4. Phosphine concentration and movement across vertical and horizontal planes within bin B at peak concentration as affected by fumigant application method.

DISCUSSION

This study shows that phosphine concentration and, indirectly, movement of phosphine throughout bulk grain presented uneven phosphine distribution patterns when fumigating with conventional techniques. Additionally, effects of leakage over time were observed with both conventional and CLF techniques. In bins that were not well sealed, conventional probed tablet fumigation resulted in an uneven distribution of phosphine gas at the maximum label rate throughout the grain mass. As a result, effective doses were only reached for the top levels of the binned grain close to where the phosphine tablets were probed. However, the distribution of phosphine gas was much more uniform throughout the bins when using CLF. All methods of fumigation were affected by leakage that was likely wind-driven and tended to be excessive (Casada et al., 2018). With CLF, the measured phosphine concentration levels, including the average level for the whole bin, suggested that leakage was higher than when conventional fumigation was used. This is likely due to the high pressures in the bin and ductwork induced during the intermittent fan operation. Leakage driven by recirculation fan pressure and wind effects in these temporarily sealed bins prevented lethal phosphine gas dosages for the recommended length of time in all or part of the bins in all tests. These results showed that CLF is advantageous as a phosphine fumigant application method because it distributes phosphine gas uniformly in the storage bin but can have the disadvantage of causing faster escape of phosphine when the bin has excessive leakage points during fumigation. CLF delivers on its potential to produce more uniform fumigant distribution when it is implemented in a bin that is built as a sealed structure; this is more evident in contour plots of the data collected by Cook (2016).

With this widespread use and lofty expectations for fumigation with phosphine, the effectiveness of phosphine fumigation is a prime concern for all users. Ideally, phosphine levels would be monitored throughout the storage structures once the recommended dosage has been applied and allowed to progress through the recommended holding time. Real-time measurements are often not taken or are limited in scope even though monitoring of fumigant concentration over time is critical for determining the effectiveness of fumigations. This predicament is caused by the lack of phosphine sensors that can be deployed into the grain mass with acceptable cost and retrieval considerations or other practical methods of comprehensive monitoring for fumigators. There are wireless phosphine sensors now available, but cost requires strategic placement of a limited number of these sensors. The alternative is to install a limited number of gas monitoring lines as accomplished in this study for research purposes. As indicated in this study the lower portion of a grain bin is the critical point for monitoring during conventional fumigation with tablets probed in the top layer of the grain.

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The mention of trade names is solely for the purpose of providing specific information and does not constitute nor imply recommendation or endorsement by the USDA or KSU. USDA is an equal opportunity provider and employer.

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Phosphine Concentration Monitoring as a Critical Success Factor in Fumigation Processes

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ABSTRACT

Gas monitoring is utilized as one of the primary tools for assessing the success of the fumigation processes. Having more frequent measurements over time enables the visualization of important fumigation phenomena for adequate and real-time management of the processes, verifies effectiveness, and provides traceability. In this work, different monitoring methodologies and fumigant applications were evaluated, allowing for the assessment of the value of proper monitoring during fumigation.

Keywords: Monitoring, Fumigation, Phosphine, HDS®, Liquid phosphine

INTRODUCTION

The success of a grain fumigation process depends on multiple factors, including the type of fumigant used, the injection strategy, the state of conservation of the facility and sealing process, the condition of the treated product, packaging materials, and weather conditions.

All these factors are dynamic and change over time, combining in a unique way in each fumigation process, potentially generating different results. When analyzing the whole fumigation work, even though it may appear to be an artisanal process, the truth is that it is a quite complex one which must be properly analyzed to guarantee good results and generate the process of continuous improvement.

"If you are not monitoring, you are not fumigating" (van Someren Graver, 2004). Fumigant gas monitoring is a fundamental tool for the correct evaluation of fumigation processes (Ducom et al., 2021), which shows the result of the interaction of all fumigation success factors. Although there are different techniques for measuring phosphine concentration, such as colorimetric tubes or electronic handheld detectors, these usually represent just a snapshot of a dynamic situation, which by itself does not enable evaluation of the success of the fumigation process.

By increasing the density of measurements over time, it is possible to move from this snapshot to a dynamic view—a movie of how the fumigation evolves—allowing for the evaluation of the

different work strategies and even for taking corrective actions along the fumigation process in the case of having real-time concentration measurements (Horn et al., 2010).

In this work, four different methods of phosphine application in vertical metal silos with pistachio nuts were evaluated, and the measurement of phosphine, through real-time electronic monitoring, represents a fundamental tool to effectively evaluate fumigation processes and allow adequate decision making.

MATERIALS AND METHODS

The study was carried out in a pistachio processing plant located in California, USA. The plant had metal silos with a diameter of 15 m and a height of 12.5 m, with a capacity of 1,000 t of in-shell pistachios.

Each silo had an aeration system with two fans in the lower part of the silo that injected air into a chamber under the silo floor, which had perforations to allow air to pass through during the aeration process. The upper part of the silos was airtight and had ventilation hatches that could be opened and closed hermetically. Each silo had three gas measuring points pre-installed and equipped with 6 mm diameter hoses, located at 1, 6 and 11 m above the silo floor. The fumigation protocol required that a minimum concentration of 200 ppm for 72 h be maintained.

Case 1. Aluminum Phosphide Fumigation with Discrete Gas Monitoring

Fumigation was carried out using aluminum phosphide tablets. After sealing the silo, aluminum phosphide tablets, in the required total for fumigation, were placed in a tray and then introduced into the aeration chamber under the perforated floor of the silo. The applied dose of phosphine was equivalent to 1.57 g/m^3.

Gas measurements were performed using a gas sampling pump and a Drager® Xam 5000 electronic detector measuring up to 2,000 ppm phosphine. Gas readings were taken daily and recorded manually on a log sheet.

Case 2. Aluminum Phosphide Fumigation with Real-Time Gas Monitoring

The same procedure as above mentioned was conducted and the dose of phosphine was 1.57 g/m^3.

For gas measurement, a FOSFOQUIM® brand gas monitor (model CertiPH3os®) was used, with the capacity to measure up to 4,000 ppm of phosphine. The system had an integrated sampling pump for continuous measurements at 15 min intervals during the whole fumigation.

Case 3. Fumigation with Liquid Phosphine in Cylinders with Real-Time Gas Monitoring

Fumigation was carried out in silo No. 3 by applying liquid phosphine (Phosphine 97%) using Horn Technologies® equipment (model HDS® 200), with a capacity of 47 g/min of injected phosphine mixed with air. The application was made directly into the aeration chamber under the perforated floor of the silo. The dose of phosphine was 1 g/m^3.

Gas monitoring was performed using the monitor and procedure used for Case 2.

Case 4. Fumigation with Liquid Phosphine in Cylinders plus Recirculation with Real-Time Gas Monitoring

Fumigation was carried out in silo No. 4 by applying liquid phosphine (Phosphine 97%) using Horn Technologies® equipment (model HDS® 200) with a capacity of 47 g/min of injected phosphine mixed with air. The application was made directly in the aeration chamber under the perforated floor of the silo. The dose of phosphine was 1 g/m^3. After gas injection, recirculation was provided using a J System® brand blower (model B9) with a flow rate of 900 m^3/h during the exposure time.

Gas monitoring was performed using the monitor and procedure used for Case 2.

RESULTS AND DISCUSSION

Case 1. Aluminum Phosphide Fumigation with Discrete Gas Monitoring

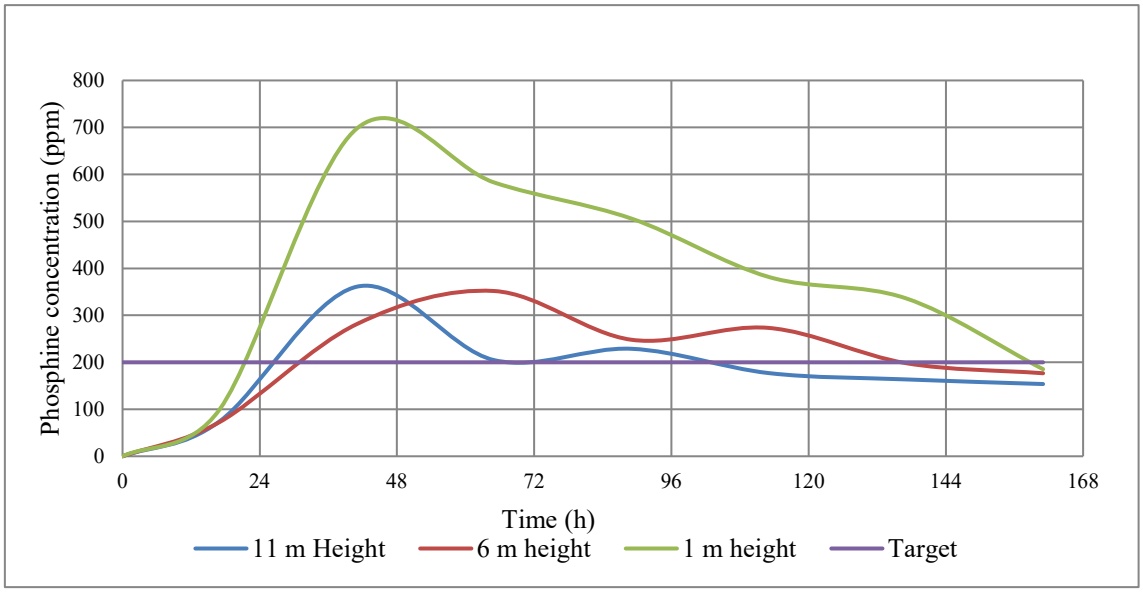

Fig. 1. Monitored gas concentrations with aluminum phosphide fumigation with discrete gas monitoring (Case 1).

As shown in Figure 1 above, gas concentrations slowly evolved from the beginning of the fumigation, reaching the minimum concentration limit in all the measurement zones approximately

40 h after the beginning of the process. The measurement zones located lower down in the silo exhibited higher concentrations than in the higher zones due to the placement of the aluminum phosphide in the aeration chamber under the floor. With the data obtained, the process could be considered successful since the objective of maintaining 200 ppm for an exposure time of more than 72 h was achieved.

Case 2. Aluminum Phosphide Fumigation with Real-Time Gas Monitoring

Using the same fumigation procedures as in Case 1, by increasing the density of measurements using real-time measurements, it was possible to observe a very different fumigation, with more irregular concentration levels and with cycles of approximately 24 h between concentration peaks (Fig. 2). This behavior may be due to a combination of factors, such as the dependence of the aluminum phosphide hydrolysis reaction on temperature, air movements inside the silo, and structural gas tightness. With this higher density of measurements, it can be clearly observed that the fumigation protocol was not achieved since the concentrations fell below 200 ppm in the highest areas of the silo.

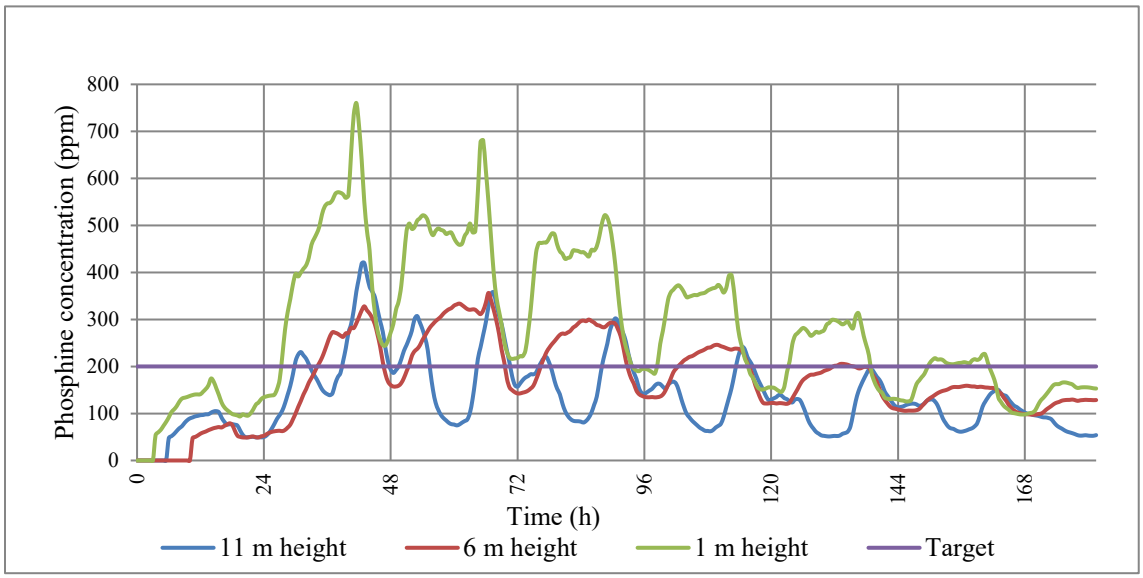

Fig. 2. Monitored gas concentrations with aluminum phosphide fumigation with real-time gas monitoring (Case 2).

Case 3. Fumigation with Liquid Phosphine in Cylinders with Real-Time Gas Monitoring

As liquid phosphine was applied, gas concentrations increased very rapidly in the silo, reaching lethal concentrations in all areas in less than 2 h. During the injection process, it was detected that the silo presented several leakages, especially in the lower part of the silo (Fig. 3). Consequently, the gas concentration dropped rapidly in the silo, requiring a gas top-up before 48 h to maintain the concentration. A top-up equivalent to 0.27 g/m^3 of phosphine was applied. Despite the reinjection process, it was not possible to maintain the necessary gas concentrations due to the poor hermeticity of the silo.

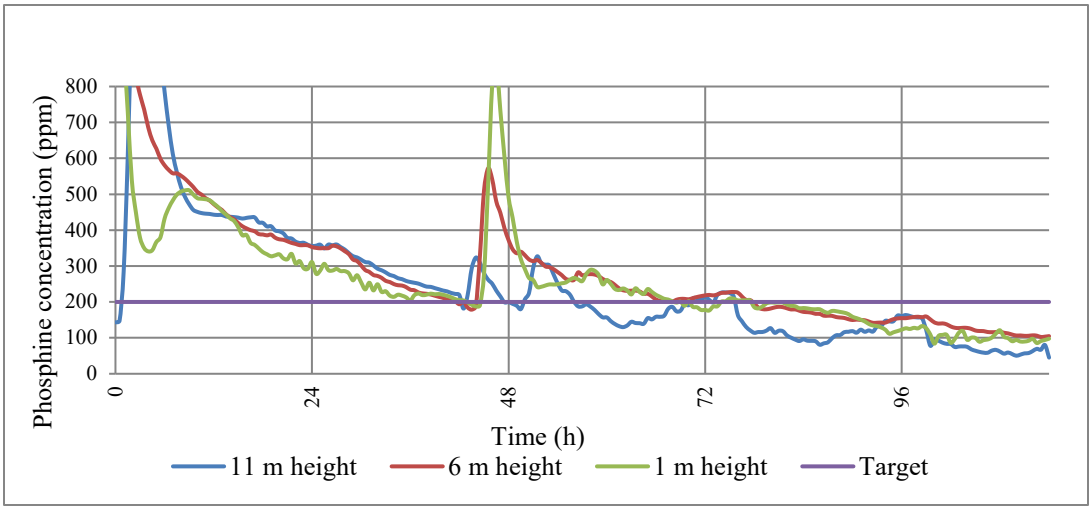

Fig. 3. Monitored gas concentrations with fumigation with liquid phosphine in cylinders with real-time gas monitoring (Case 3).

Case 4. Fumigation with Liquid Phosphine in Cylinders plus Recirculation with Real-Time Gas Monitoring

Gas concentrations increased rapidly in the silo by applying liquid phosphine, achieving lethal concentrations in the whole silo in less than 2 h. It was observed that the use of the recirculation system generated an even distribution of the gas throughout the silo. In this case, gas concentrations remained above the required 200 ppm for more than 96 h, meeting the requirements (Fig. 4).

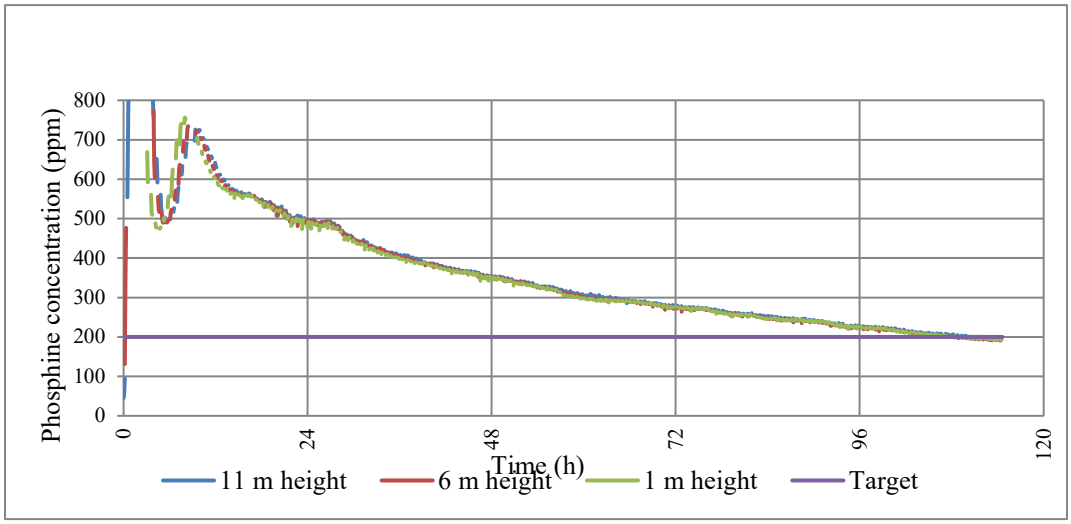

Fig. 4. Monitored gas concentrations with fumigation with liquid phosphine in cylinders plus recirculation with real-time gas monitoring (case 4).

ADVANCED PHOSPHINE MONITORING AND INTEGRATION WITH PHOSPHINE INJECTION EQUIPMENT

With the technological advances of electronic phosphine monitors, including remote communication and other user interfaces, new opportunities have become available for phosphine applications in structural and flour mill fumigations; however, corrosion produced by phosphine has become a severe problem for the application mechanisms.

Because phosphine produces corrosion in copper, gold, and silver, the electronic equipment inside the fumigated facilities might suffer corrosion and thus malfunction after the fumigation. The corrosive effect is highly dependent on the phosphine concentration and exposure time. In addition, aluminum phosphide commercial products also contain ammonium carbamate, which generates ammonia as a byproduct—which is also highly corrosive to these metals.

By using liquid phosphine applied through the Horn Technologies HDS equipment in conjunction with electronic phosphine monitors, most of the structural and flour mill application problems related with phosphine can be overcome.

By using an interface between the HDS phosphine injection equipment and the monitoring equipment, it is possible to precisely control the phosphine concentration during the fumigation (Fig. 5) and to keep it between 200 and 250 ppm during 72 h of exposure time, avoiding high concentration peaks (which could lead to more corrosion), allowing for the optimization of phosphine consumption, and adapting the phosphine injection rate dynamically during the fumigation period depending on structural or weather-induced (i.e. wind) leakage (Rogers et al., 2014).

In addition, when using liquid phosphine, only phosphine is applied, eliminating the corrosive contribution of ammonia.

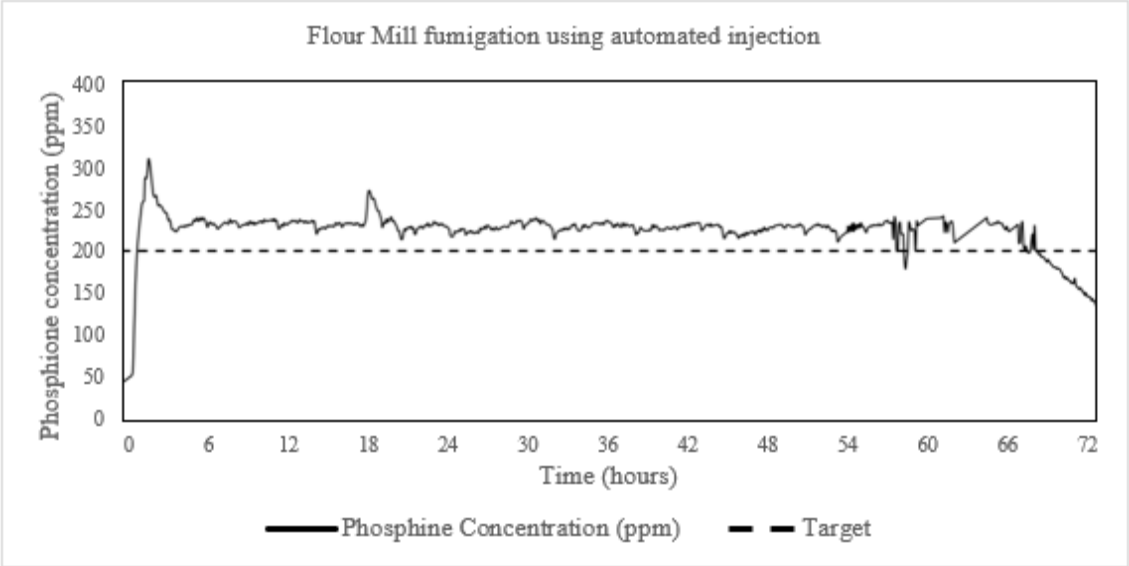

Fig. 5. Phosphine concentration during fumigation of a flour mill use automatic injection.

Based on the observed results, the following was concluded:

- Implementing good monitoring procedures was essential for the correct evaluation of the fumigation process. Gas measurements performed in greater frequency provided accurate and timely information for decision making, such as fumigant top-up, when required.
- Good monitoring procedures helped pest control professionals and end-users to utilize and preserve the active ingredients of the fumigant, avoiding sub-lethal concentrations in the fumigation process, which might lead to the development of insect resistance over time. Few or too distant gas readings can hide relevant phenomena, as seen when analyzing cases 1 and 2, generating erroneous evaluations of the processes.
- The application of liquid phosphine enabled reaching gas concentrations in very short times (< 1 h compared with the application of metal phosphides (> 24 h), thus reducing fumigation times.
- The use of recirculation had an important effect on gas distribution throughout the silo profile, ensuring homogeneous gas distribution.
- The integration of advanced monitoring with phosphine injection equipment allowed for new methods of application for phosphine, such as in structural or flour mill fumigations, as the fine control of phosphine concentration during the exposure period helped minimize corrosion to electronic equipment.

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Faridi H, Jian F (2024) Bulk density of dry wheat mixed with different sizes and percentages of dockage. Page 254. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Bulk Density of Dry Wheat Mixed with Different Sizes and Percentages of Dockage

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ABSTRACT

A steady and reliable flow of bulk grain is crucial for effective handling, processing, and storage of grain bulks. The storage of wheat is particularly susceptible to the influence of impurities. This study examined the impact of three dockage sizes (smaller than 1.1 mm, between 1.1–2 mm, and larger than 3.3 mm) at four dockage percentages (0, 2.5, 5, and 10%) on the bulk density of wheat with a moisture content of 7.5% (wet basis). The application of Artificial Neural Network (ANN) techniques was employed to predict and model wheat density based on various dockage sizes and percentages. The analysis revealed that the ANN created through the Scaled Conjugate Gradient algorithm, achieving a determination coefficient value of 0.99 and exhibiting a difference of less than 9.4 kg/m^3 between predicted and measured bulk densities, emerged as the most fitting model when compared to other alternatives. Furthermore, a basic exponential growth model with two parameters served as a regression model, resulting in a difference of less than 11.3 kg/m^3 between predicted and measured bulk densities. The comparison between the ANN models and the regression model demonstrated the superiority of ANN model in predicting wheat bulk density, supported by robust statistical parameters. This research enhanced our understanding of the intricate dynamics between dockage characteristics and wheat's physical properties, particularly in the controlled atmospheres of wheat silos, contributing valuable insights to the optimization of storage conditions.

Keywords: Dry wheat bulk, Impurities, Densities, Porosity, Regression modeling, Artificial neural network

Paper No. CAF2024-A72

Zhang H, Ren Y, Mckirdy S, Thomas M, Du X (2024) IoT-based AI deep learning Technology in real-time fumigation monitoring and audit. Page 255. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

IoT-based AI Deep Learning Technology in Real-time Fumigation Monitoring and Audit

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ABSTRACT

Monitoring and recording accurate levels of fumigant gas during application, holding period, and after fumigation, is of vital importance for validating fumigation efficacy and for health and safety purposes. However, the presence of complex container gases generated passively from materials used in the construction of the container, and the cargo itself, can interfere with the accurate reading of fumigant levels within shipping containers. An innovative solution that integrates Internet of Things (IoT) technology with artificial intelligence (AI) and deep learning (DL) has been introduced in fumigation monitoring and audit processes to significantly enhance measurement accuracy, specificity, and sustainability in pest control.

In a demonstrated case study, this integrated technology provided real-time monitoring and predictive analytics and ensured compliance with ethyl formate fumigation in shipping containers. An industrial prototype ethyl formate monitoring and assurance device was developed for shipping container fumigation. This cutting-edge integration not only elevates the precision and effectiveness of fumigation processes but also aligns with broader objectives of environmental sustainability and regulatory compliance. The associated Ethyl Formate Assurance Tool (EFAT) software APP application was customized for reporting ethyl formate concentrations at fumigation and residue levels without interference from the container gases. The prototype system demonstrates significant potential for adaptation in monitoring various fumigants and pesticides.

Keywords: Internet of things (IoT), Artificial intelligence (AI), Deep learning (DL), Fumigation monitoring, Real-time audit, Ethyl formate

Paper No. CAF2024-F73

Murugesan KM, Kim M, Kozyniak G, Gui Y, Hu C, Jian F (2024) Detection of *Tribolium castaneum* adults using a novel microwave sensor: preliminary study. Pp. 256–263. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Detection of *Tribolium castaneum* Adults Using a Novel Microwave Sensor: Preliminary Study

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ABSTRACT

A microwave frequency-based sensor is a novel technology for insect detection in stored grains that has advantages in terms of enhanced efficiency and easy installation, as well as lower operational costs than conventional insect detection methods. The microwave sensor works on the principle of resonance. A shift in resonance frequency that occurs upon a disturbance or intervention (such as by insect movement in grain bulks) in the measuring cavity is used to detect the insect presence and movement. A microwave resonator sensor with an operating frequency of around 1.3 GHz was tested to detect red flour beetle (*Tribolium castaneum* (Herbst)) adults in stored wheat with 14.5% moisture content (wet basis). Three insect densities of 1, 5, and 10 insects/kg in 10 kg of wheat were tested. The peak resonating frequency for each sensor was evaluated before and after the insect introduction into the wheat bulks. The shift in peak frequency was directly related to the presence of insects in the stored bulks. The setup also used a software-defined radio peripheral, the HackRF One, as well as an open-source software GNU Radio companion to control and analyze the microwave signals—which, together, performed as a digital signal processing component replacing the conventional bulky vector network analyzers. The results indicated that the sensor had the potential to detect even one single insect in the stored wheat bulks. Overall, the application of this microwave frequency sensor helped in determining the insect activity inside a bulk grain. The fact that it could be placed inside silos and allow for continuous real-time monitoring brings huge scope and potential in stored grain insect management for the grain industries.

Keywords: Microwave sensor, Stored grain insects, Red flour beetles, Resonance frequency, FFT, Real-time monitoring, Silos

INTRODUCTION

The current methods of detecting insects in grain bins include manually or mechanically retrieving samples and using traps to capture insects (Zhu et al., 2022). Even though many methods (such as sieving, near infra-red spectroscopy, electrical conductance, chemical analysis, and soft X-ray imaging) can be used to detect insects from samples, these methods are time consuming and cannot automatically detect insects, monitor insect activity, or estimate insect density in situ (Jian et al., 2016) . When traps are used, it is necessary to frequently enter the bins and check for captured insects. However, entering bins is forbidden because a stored grain bin is defined as a confined space (due to low O_2 concentration and, in some cases, the presence of toxic gases). In the market, there is only one commercial insect detection device (an electrical trap called the Insector®) that can be used to detect insects inside grain bins (Zhu et al., 2022). However, the electronic trap must be installed vertically, and it must be carefully and frequently cleaned of dust and dead insects—which requires entering the bin. Furthermore, the trap is 1 m long and would be destroyed during grain loading and unloading (Jian et al., 2012), and any possible broken parts of the trap would damage the grain unloading auger and block the grain flow during grain unloading. Therefore, this type of electric trap can only be used on the grain surface (at no more than a 1 m depth) and needs to be removed from the bin before grain unloading. Insects can infest grain at any place inside of a bin. Therefore, a miniaturized sensor, which can non-destructively monitor insect activities at any stages of insect infestation and not be influenced by grain operation or the storage environment, is highly demanded.

In this study, we are proposing a novel compact and inexpensive microwave resonator sensor that can be placed at any depth of grain bulk and can provide continuous and real-time monitoring of insects. Any changes in resonant properties of the cavity could be directly related with the properties of the object under testing, which, in our case, is the insects. Reimer et al. (2018) proved that a resonator sensor could detect insects without grain. However, it is not known whether insects inside grain bulks can be detected by using this developed resonator sensor. This study explored the application of the microwave sensor in a wheat grain bulk in order to understand its response to varying insect densities.

MATERIALS AND METHODS

Wheat

Canada Western Red Spring Wheat conditioned to 14.5% moisture content (wet basis) was used in this study. The moisture content of the grain was determined using the standard oven drying method by drying 8–10 g of samples at 130°C for 19 h in triplicates (ASABE, 2014)). The wheat was cleaned by using # 8 sieves before using.

Insects

The adults of *Tribolium castaneum* were reared on wheat flour and brewer's yeast prepared in the ratio of 95:5 w/w. The cultures were kept at 30 ± 1°C and 70 ± 5% r.h. in darkness. A mixed-sex sample of adults with ages up to 2 mo was selected from the culture.

Microwave Resonator and Sensor Setup

An in-house fabricated microstrip array-based active resonator was designed on a RO4350B substrate (Rogers Corporation Pvt. Ltd., Arizona, USA). The substrate (resonator) was covered by two copper layers of 35 μm thickness on its top and bottom. The substrate was 0.76 mm thick with a permittivity value of 3.48 ± 0.05. The resonator, along with its covers, was referred to as a sensor. The sensor was designed to have the operating resonance frequency of 1.3 GHz to 1.32 GHz with a high quality factor of more than 10^5. Figure 1(A) shows the sensor having an array designed at the center of the substrate for capturing the insect movement. As the insect moves across the sensor surface, its perturbation introduces the change of resonance frequency according to the cavity perturbation theory. The sensor was designed in such a way that even trace movements of insects could be detected. The sensor had an overall sensing area of about 2 cm^2 and was covered by a plastic cylindrical enclosure (3.2 cm diameter and 3 cm height with perforated sides) as shown in Fig. 2(B). The purpose of the enclosure was to prevent signal loss from the grain's dielectric absorption. The perforation along the sides of the enclosure allowed the insects to enter and exit the enclosure so that their activity inside the enclosure could be detected. The sensor setup consisted of various components, including a HackRF One, the microwave resonator with enclosure, a low noise amplifier (LNA), and a PC system. The HackRF One is a software-defined radio (SDR) peripheral and is controlled by an open-source GNU radio environment. The HackRF One has an operating frequency ranging from 1MHz to 6 GHz. The microwave signals generated at the sensor through a feedback loop are amplified by the LNA. The radiated microwave signals are captured by the HackRF One, and the time–domain signals are transferred to the PC through a USB channel. The sample rate for the experiment was set high enough to satisfy the Nyquist criterion. The GNU Radio Companion (GRC) application, through its various flow graphs and blocks, receives the digitalized time–domain signal samples for processing. The different components were connected as shown in Fig. 1(C) such that the continuous spectrum details were collected over the study period.

Experimental Setup and Data Collection

The overall experimental setup shows the continuous monitoring of signals (Fig. 1). A 10 kg sample of wheat conditioned to 14.5% moisture content was transferred to a plastic cylindrical container with a dimensionally equal height and diameter (30 cm). The spectrum containing the resonance frequency was fixed at the center of the screen to be recorded continuously. This continuous spectral response was recorded and later processed with MATLAB to monitor the shift in resonance frequency. To maintain its uniformity of placement, the sensor was buried in half of the total grain height inside the storage container.

Different insect densities of 1, 5, and 10 adults/kg (A/kg) were tested, and adults of *T. castaneum* were introduced at the top of the grain bulk. The experiment was run for at least 24 h, and the recording was manually stopped when an insect was detected. The following parameters were characterized: captured insect frequency; the average time spent by the insect inside the enclosure; and the maximum shift in resonance frequency observed during insect contact.

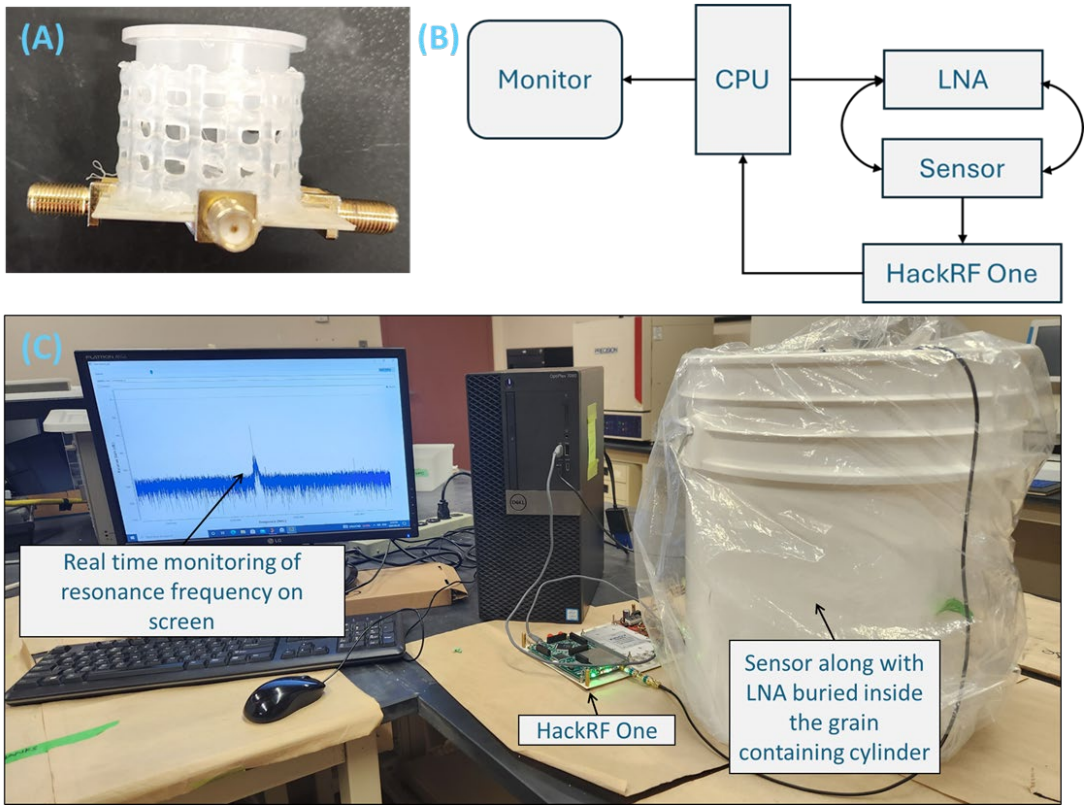

Fig. 1. Experimental set up. (A) Active microwave resonator. (B) Connection of sensor. (C) Microwave sensor setup consisting of different components.

Data Analysis

Effect of bulk grain (only) on sensor response

The difference of dielectric properties among air, grain, and insects is the detection principle used for the RF or microwave-based measurement techniques (Ding et al., 2008). The resonator sensor exhibited a characteristic resonance frequency in its natural state when it was not influenced by any interventions like grain or insects. This frequency could be identified as a peak on the screen, as shown in Figure 1(C); whenever there was an intervention across the detection area of the sensor, the resonance frequency decreased. This could be better understood from Figure 2, in which, the effect of bulk grain (without insects) on the resonance frequency is represented. Region A indicates the response when the sensor was in its natural state without grain and shows that the resonance frequency was 1.31×10^9 Hz. Later, when the sensor was buried inside the grain bulk,

the resonance frequency reduced sharply, as seen in Region B. Region C indicates the resonance frequency of value 1.308 $\times 10^9$ Hz becoming stabilized over time inside the grain bulk, as there was no movement of grain. During this sensor placement, there was a significant shift (shift = difference between the resonance frequency values before and after sensor placement inside the grain bulk) calculated to be around 2 MHz in resonance frequency.

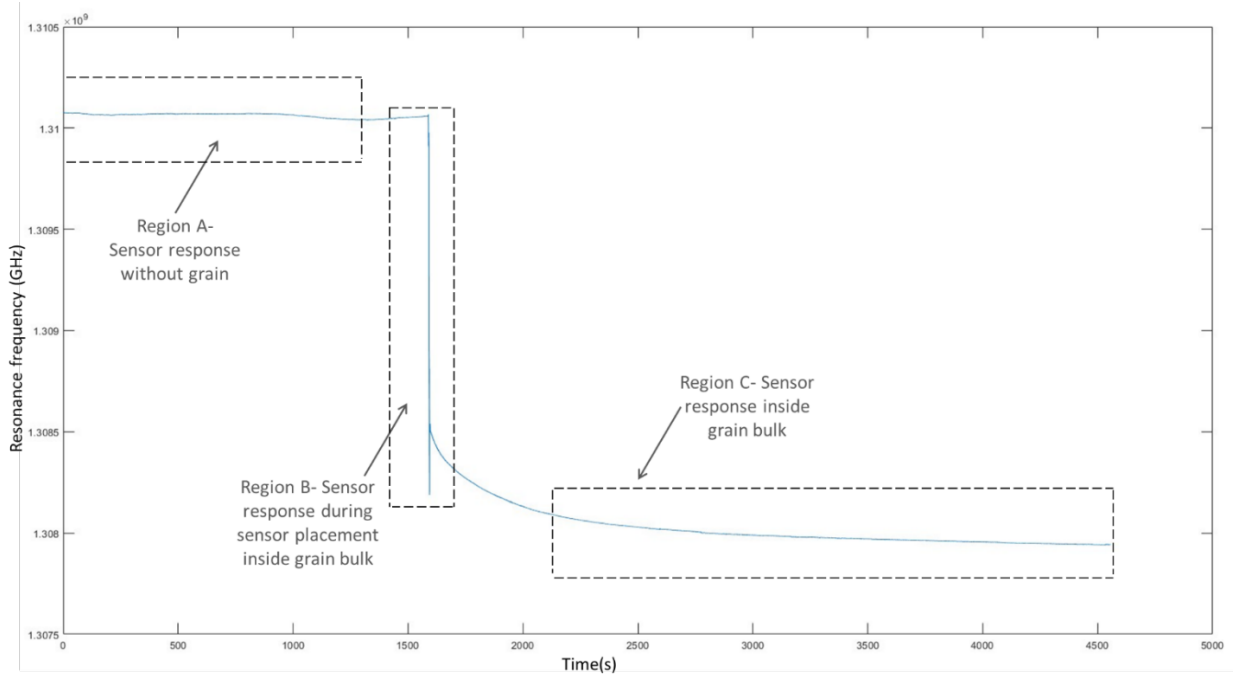

Fig. 2. Sensor response before and after its placement inside the grain bulk.

Effect of insect (only) on sensor response

When an insect was moving inside the enclosure, the electromagnetic wave traveled through the insect body and got attenuated depending on the dielectric properties of the insect (Ding et al., 2009). This attenuation triggers peak shifts. As an insect moved on the sensor, the resonance frequency steadily shifted from its base straight line (Fig 3). Figure 3 shows a straight line again after 1200 s, indicating the insect moved out of the enclosure. At 1400 s, the insect entered the enclosure again (Fig. 3). The gradual shifting of resonance frequency indicated the steady changes in the electric field of the resonator due to insect movement, while the maximum shifts correspond to the stronger field regions and insect positions over those regions. The shift value depends significantly on the type of insect because its size and activity can influence the shift in resonance frequency. These characterized frequency shiftings were used to count the number of captured insects in the sensor enclosure.

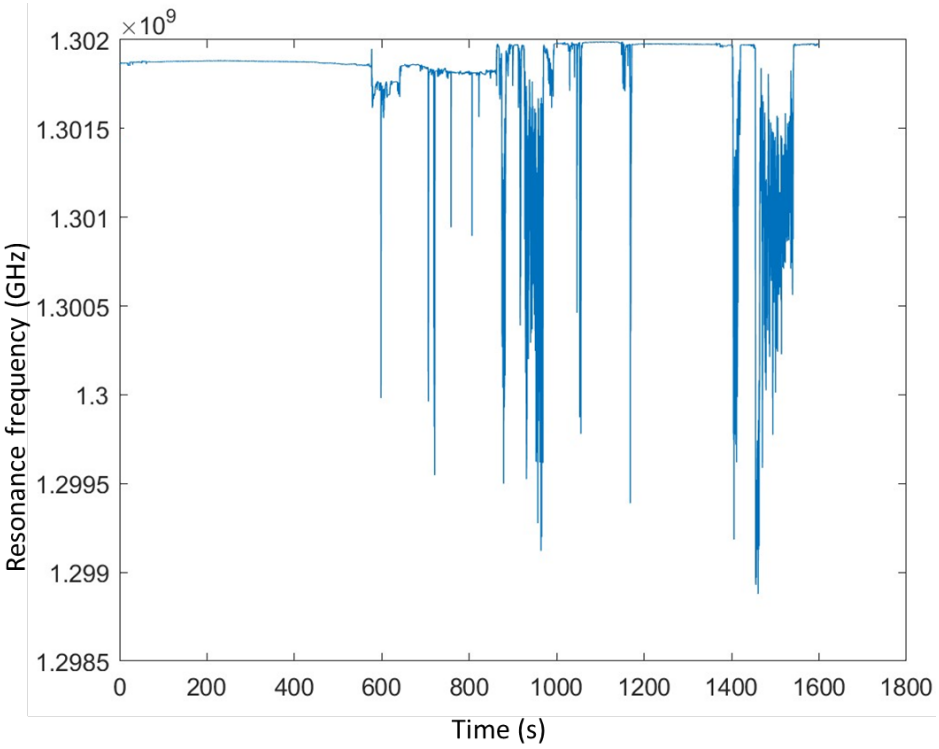

Fig. 3. Sensor response on the detection area of the sensor after introduction of one adult of the red flour beetle.

Insect monitoring inside the grain bulk

Different insect densities of 1, 5, and 10 A/kg were tested by introducing 10, 50, and 100 insects on top of the grain bulk (10 kg) surface and were studied for at least 24 h continuously. During these experiments, the following parameters were measured: captured insect frequency; the average time spent by the insect contacting the sensor inside the enclosure; and the maximum shift in resonance frequency observed during insect contact. The results were obtained when the spectrum response was started for recording after the sensor had been placed inside the grain. For each insect density, triplicates were performed.

RESULTS AND DISCUSSION

Figure 4 shows how different insect densities can influence the insect detection process when performed under the same conditions. From the recorded sensor response, various parameters were evaluated and are presented in Table 1. The time taken for the first detected insect and the captured insect frequency depended on insect density, whereas the average time spent by an insect inside the enclosure and the maximum shift during each detection did not significantly depend on insect density but rather on insect behavior. A single red flour beetle adult spent 8.21 ± 5.27 min inside

the enclosure and triggered a maximum shift of 2.84 ± 0.48 MHz. This high deviation indicates that movement and activity are associated with the biological variances of insects.

The greatest frequency of captured insects was found to be in the higher insect density sample of 10 A/kg (Table 1). For lower insect densities (1 and 5 A/kg), the capturing of insects was generally determined to be less frequent (Table 1). Therefore, the developed sensor could be used to monitor insect activity based on their density.

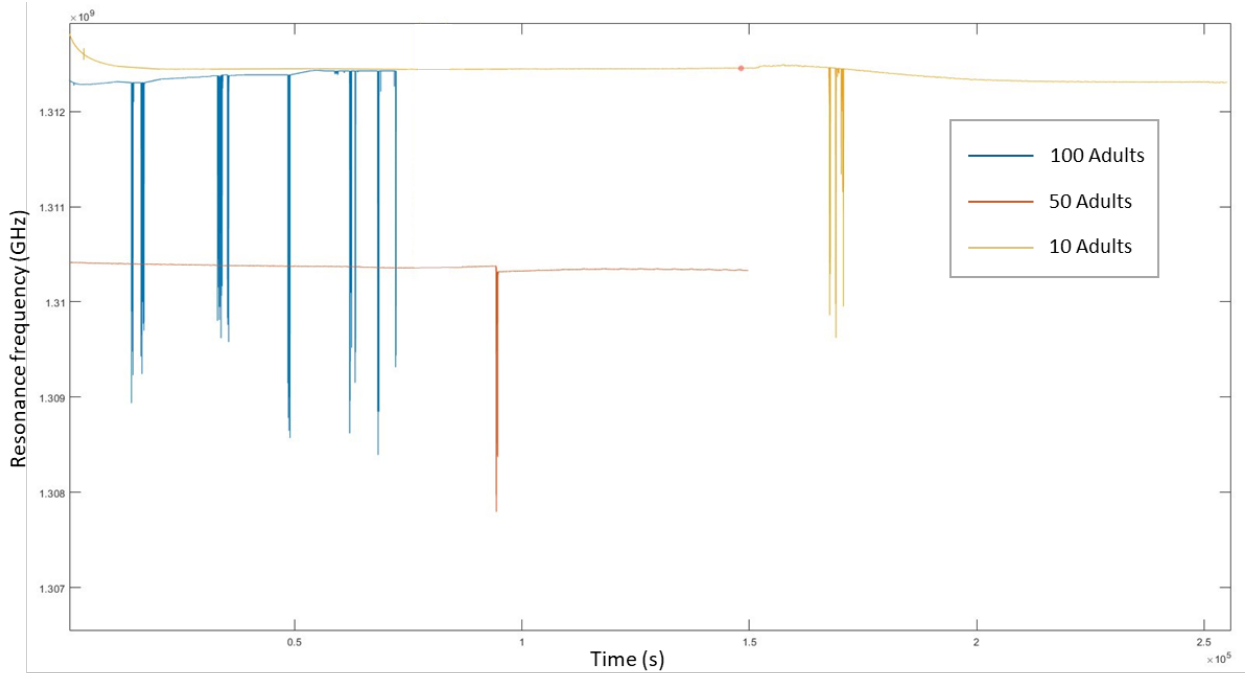

Fig. 4. Sensor response for the detection of insects at different insect densities.

Table 1. Estimation of different parameters during insect detection at different insect densities.

Insect density (A/kg)	Time taken for first insect detection (h)	Captured insect frequency (No. of insects/h)	Time spent by insect inside enclosure (min)	Maximum shift (MHz) during each detection
10	1.91 ± 1.83	0.67 ± 0.81	6.95 ± 5.36	2.95 ± 0.53
5	9.77 ± 14.23	0.1 ± 0. 09	7.67 ± 5	2.56 ± 0.69
1	16.25 ± 26.22	0.06 ± 0.04	10 ± 5.45	3.02 ± 0.23

ACKNOWLEDGEMENTS

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New SmartProbe Technology for Early Detection of Insect Pests and Environmental Monitoring in Stored Products

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ABSTRACT

Insect infestation occurs in the entire supply chain of food and agricultural systems. It causes significant losses in product quality and economic value and results in a large amount of toxic chemical use for disinfestation, which is expensive and harmful to the environment and human health. The current human visual inspection method is labor intensive and cannot find insects in the early stage of infestation in food and agricultural products.

A new SmartProbe Technology, based on artificial intelligence (AI) and the Internet of Things (IoT), was recently developed at the University of California, Davis, and commercialized by AIVision Food Inc. This SmartProbe Technology aims to reduce food losses caused by insect pests through detecting them in the early stage of infestation and monitoring the environmental conditions of foods in storage, processing, handling, and transportation. The smart insect detection system captures the insects through a novel probe when insects emerge. It remotely monitors insect activities, temperature, and relative humidity and sends notifications alerting pest control managers to take appropriate actions. Data regarding environmental conditions and the quality of the products are used for the prediction of insect occurrences based on AI-trained models in order to achieve better management. The insect images and related information can be stored locally and in the cloud and accessed through mobile devices and computers using the SmartProbe app.

This study demonstrated that the chemical cost of pest management in rice storage was reduced by 74% by using this new technology—which also provides an innovative solution to the problems caused by insect infestation, including product and quality losses, chemical use, food safety concerns, and high management costs. It replaces the current human inspection method and brings significant economic and social benefits. The SmartProbe technology has been widely used in warehouses and processing facilities of rice, almonds, and walnuts in the United States and other countries.

Keywords: Insects, Detection, Monitoring, Stored product, Control, Wireless

INTRODUCTION

Insect infestation during the storage, handling, processing, and shipping of food and agricultural products causes serious problems, including loss of quality, quantity, and food safety, and directly results in a significant loss in the economic value of food and agricultural products. According to the Food and Agriculture Organization (FAO) of the United Nations, 10–20% of global food production today is destroyed by insect infestation during the postharvest, storage, and handling process (Rajasri and Kavitha, 2015; Ioannis et al., 2023). It has been reported that the pests in stored grain cause considerable postharvest losses, ranging from 9% in developed countries to 20% or more in developing countries (Ahmad et al., 2021; Chitra and Subramanian, 2016). Insect pests, pathogens, and mites pose serious threats and cause severe damage to grains by producing certain enterotoxins and mycotoxins (Morgan and Aldred, 2007). Additionally, the presence of insects in food products can discredit the image of the product or the manufacturer of the product, make the food non-compliant with established food regulations, and may have adverse economic impacts to the manufacturer in terms of product replacement and lawsuits (Christos et al., 2011).

Khir and Pan (2021) reported that infestation caused about 2.5% of the total and head rice losses of medium grain rice by the time the insects were found by the human inspection method. This quality loss meant a loss of value of approximately USD 5.50 per tonne of paddy rice based on the average rice price in 2021. Another example, as reported by the authors, determined that infestation in tree nuts during processing, handling, shipping, and storage could cause an estimated 3-4% product loss, and their lab results also showed that the presence of just one red flour beetle in 100 kernels of almonds caused damage to 13 kernels during 14 d of storage and 23 kernels during 22 d of storage. The damaged product lost about 90% of its economic value (Khir and Pan, 2023). If the insect infestation could be detected in the early stage, product loss would be avoided or significantly reduced.

The aforementioned problems associated with insect infestation force producers, processors, and handlers to use a large amount of toxic chemicals for fumigation, causing concerns about environmental pollution, worker health, and food safety. In addition, insect infestation is also a problem during shipping for international trade due to the lack of monitoring methods. Therefore, a new and effective detecting and monitoring method for insect infestation in agricultural products is urgently needed and can help eliminate the infestation risk, minimize food loss, improve sustainability, and lead to many benefits.

The current insect detection methods, including human visual inspection, probes, and traps, are subjective, destructive, inaccurate, time-consuming, costly, and unable to detect insect infestation in the early stage. Insects cannot be discovered until the population is large, and, by then, the damage has already been done. In addition, traps used in processing facilities and warehouses need human inspection regularly, and infestation status may not be identified in a timely manner. Some of the new techniques, such as acoustic detection, near-infrared spectroscopy, and X-ray methods, have been studied for their feasibility to be implemented for insect detection in stored grain (Rajendran, 2005; Neethirajan et al., 2007). However, these methods have not been adopted in commercial operations due to the high costs of the technologies and over concerns in labor and reliability. Therefore, there is an urgent need for new and effective methods for detecting and monitoring insect infestation in agricultural and food products to: minimize the infestation risk;

reduce chemical use in order to mitigate health and environmental concerns; maintain storage longevity, quality, and safety of agricultural products; and lower inspection and treatment costs.

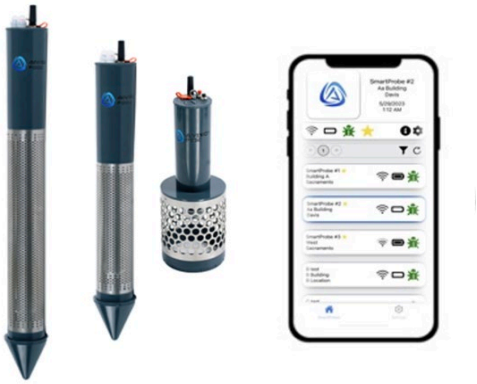
The objective of this study was to develop and commercialize the SmartProbe Technology for its use in reducing food losses caused by insect pests through the probe's ability to detect insects in the early stage of infestation and monitor the environmental conditions of foods in storage, processing, and handling and transportation without human inspection.

MATERIALS AND METHODS

SmartProbe Technology

SmartProbe Technology (SPT) was recently developed at the University of California, Davis (Fig. 1). The SPT has the capability of early detection of various types of insects once they emerge in food and agricultural products. The SPT remotely monitors the activities of insects and alerts the pest control managers of storage and processing facilities to take proper actions for controlling the insects. It also monitors the temperature and relative humidity (r.h.) of stored products. The system has two main components: a smart probe for insect detection and environmental monitoring and software with functions of cloud artificial intelligence (AI) computing, data storage, and alerting.

The smart probe attracts insects, allows them to enter a collecting chamber, and then takes photos to capture their images. It is programmed to take images periodically through the mobile device application or via computer. The probe also has sensors to record temperature and relative humidity. The images and recorded data are then sent automatically to a server where the images are saved and processed with machine learning to count the captured insects. The data relating to the number of insects, temperature, and relative humidity are then sent to the user interface to be viewed at any time, and the alerting notifications are transmitted to the facility managers when necessary. Three models (L, S, and H) of the SmartProbe were developed for use in bins/boxes and silos/stockpiles and for hanging in processing and storage facilities (Fig. 1). Now the probes are commercially available from AIVision Food Inc.



L S H SmartProbe APP

Fig. 1. Three different models of the SmartProbe Technology and the SmartProbe App

SmartProbe Technology Tests and Demonstration

Rice: The SmartProbe Technology was successfully demonstrated at seven commercial rice warehouses, which included Lundberg Family Farms, Erdman Warehouse, INC, Framers Rice Cooperative (Hi & Dry), Framers Rice Cooperative (Stegman), Tyndall Mound Warehouse, and Butte County Rice Growers Association. These warehouses included seven flat storage facilities and one silo. The smart probes were installed in each storage facility from April 2021 to August 2021 and remotely monitored. The rice was also inspected for insects by human visual inspection when the probes were installed and during the test period until the probes were removed.

Almonds: The three models of the SmartProbe Technology were demonstrated in 2021, 2022, and 2023 at four commercial processing facilities, which included Wonderful Pistachios & Almonds, California Grown Nut Co., West Valley Hulling Co., and Silver Creek Almond Co. The probes were demonstrated for early detection of insect pests and for monitoring the environments in stockpiles, processing rooms, and storage warehouses. The probes were set to take images of insect activity and to record the temperature and relative humidity (r.h.) data every 8 h, while being remotely monitored through the SmartProbe App and WiFi or Starlink installed in the stockpile area. The insect monitoring results from conventional traps were used for comparison as well.

Walnuts: The smart probes were demonstrated in 2021 and 2022 at Carriere Family Farms to monitor insect activity in walnut kernels in bins and inshell walnuts in silos. The probes were set to take images of insect activity and to record the temperature and r.h. data every 8 h.

RESULTS AND DISCUSSION

Monitoring Insect Infestation in Rice

The SmartProbe Technology was able to provide early detection of insect activities in all tested storage facilities and to simultaneously monitor temperature and r.h. The results showed that the first insect was detected the next day after the probe installation in the tested storage facilities. All the probes detected insects within 18 d. Figure 2 provides examples of the types of flat concrete and metal silo storages used in this study, and Figure 3 shows the number of insects detected in these storages. The number of insects continued to increase during the installation period. However, the human inspection in all tested storage facilities did not find any insects during the entire test periods. The new technology was able to catch and detect different types of insects. Additionally, the results indicated that the insect infestation started in the top layer of rice and the top layer disinfestation treatment had about 16% of chemical cost compared with the typical treatment of fumigating the entire products.

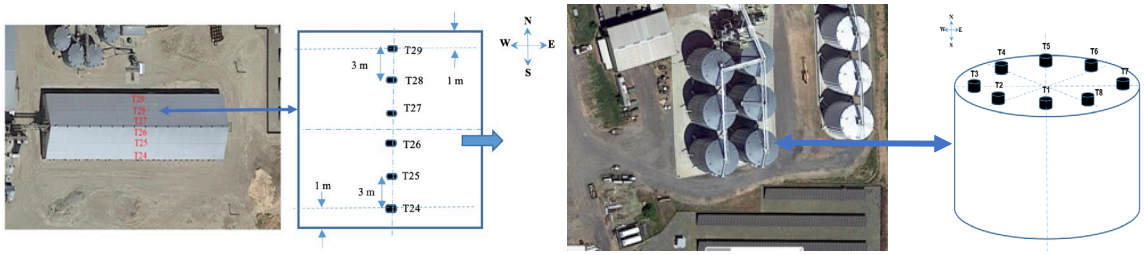
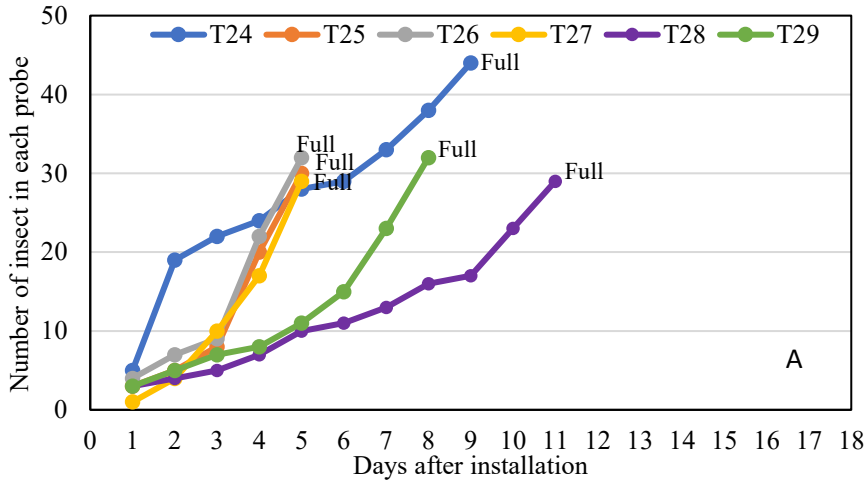
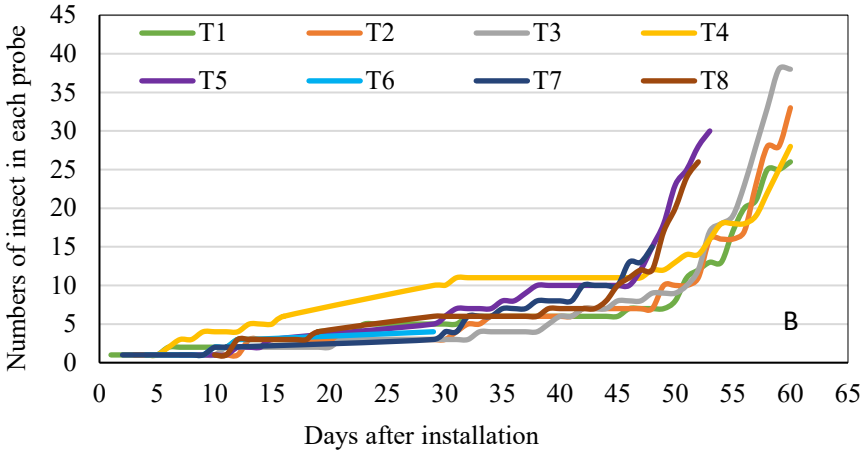


Fig. 2. Smart probes installed in flat concrete and metal silo storage facilities.



Insects detected in probe # T29 after 8 d of installation



Insects detected in probe # T4 after 7 d of installation

Fig. 3. Number of insects detected in rice in (A) a flat concrete storage and (B) a metal silo.

The results showed that the SmartProbe Technology was an effective tool that detected the insects in the early stage of the insect infestation in storage bins, boxes, almond stockpiles, packaging rooms, and storage warehouses (Figs. 4–7), even though no insects in the products were expected or detected by human visual inspection. The insects detected by the probes included red flour beetles, moths, bugs, and nets. Particularly, the probes detected insect pests much sooner and had better catching ability compared with the conventional traps in an almond packaging room (Fig. 8), which indicated that the new technology was more effective and timelier in identifying the insects. Additionally, it was found that the insect activities started in the surface layer in the top location in the stockpile due to the warmer temperature. The new technology successfully detected insect activity in walnut kernels in bins and inshell walnuts in silos at Carriere Family Farms. Insects were detected when no insects were even expected (Fig. 9). The probes measured the temperature and r.h. of the almonds and monitored the storage conditions of the processing room. The technology can also monitor the effectiveness of a fumigation treatment to achieve precision treatment. The results confirmed that the significant insect damage of almonds could be reduced by using the early detection technology and precision pest control.

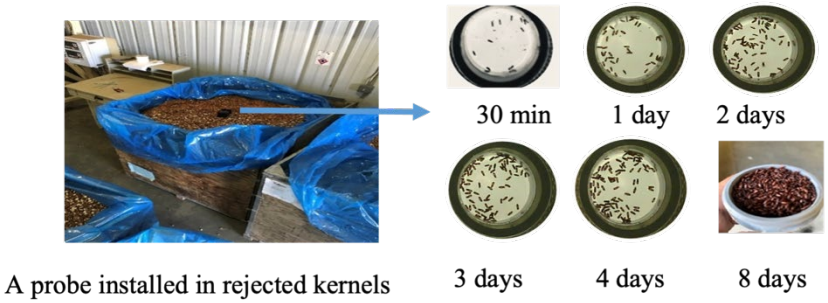


Fig. 4. Insects detected by SmartProbe Technology in rejected almond kernels.

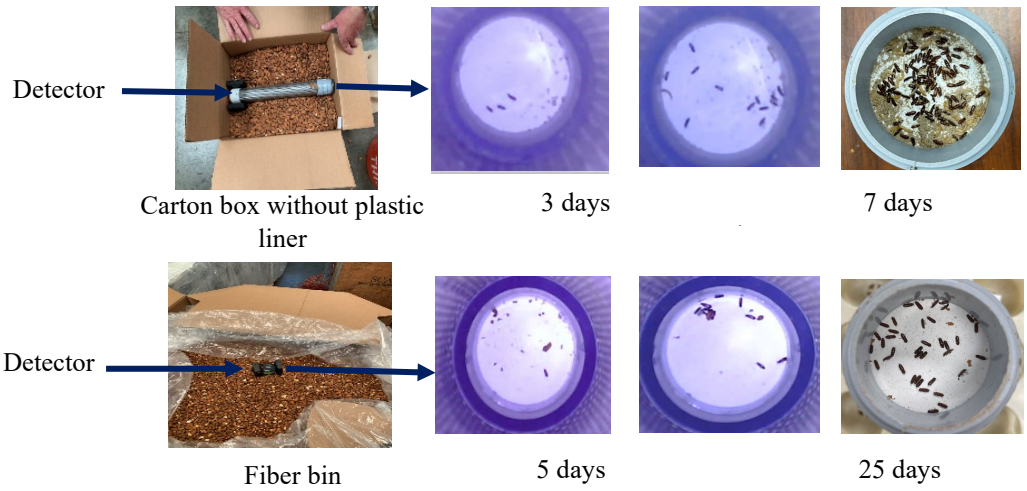


Fig. 5. Insects detected by SmartProbe Technology in a carton box and fiber bin of final products of almonds.

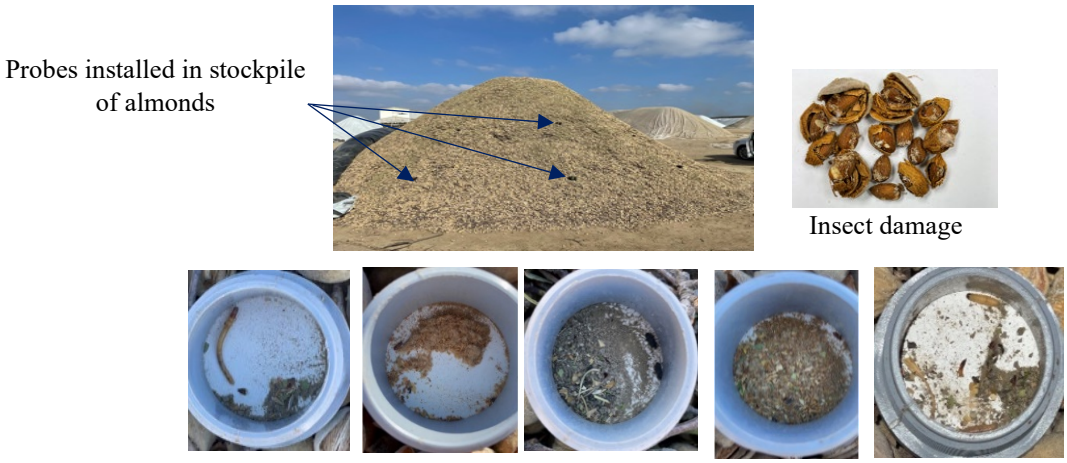


Fig. 6. Insects detected by SmartProbe Technology in stockpiled almonds.

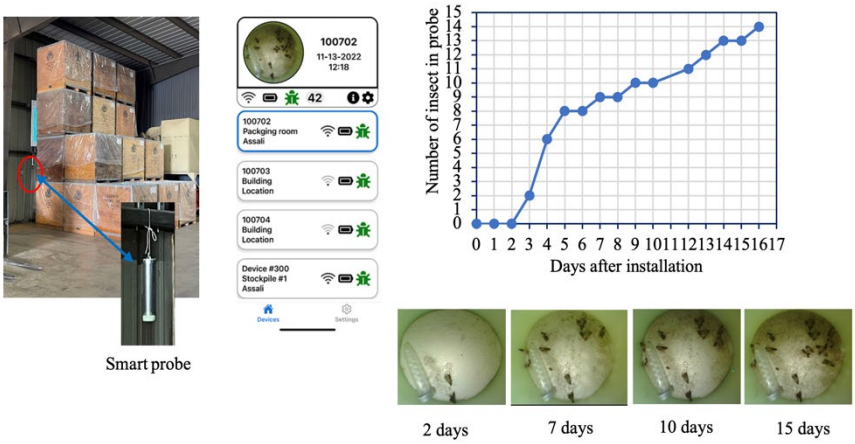


Fig. 7. Insects detected by SmartProbe Technology in a processing room of almonds.

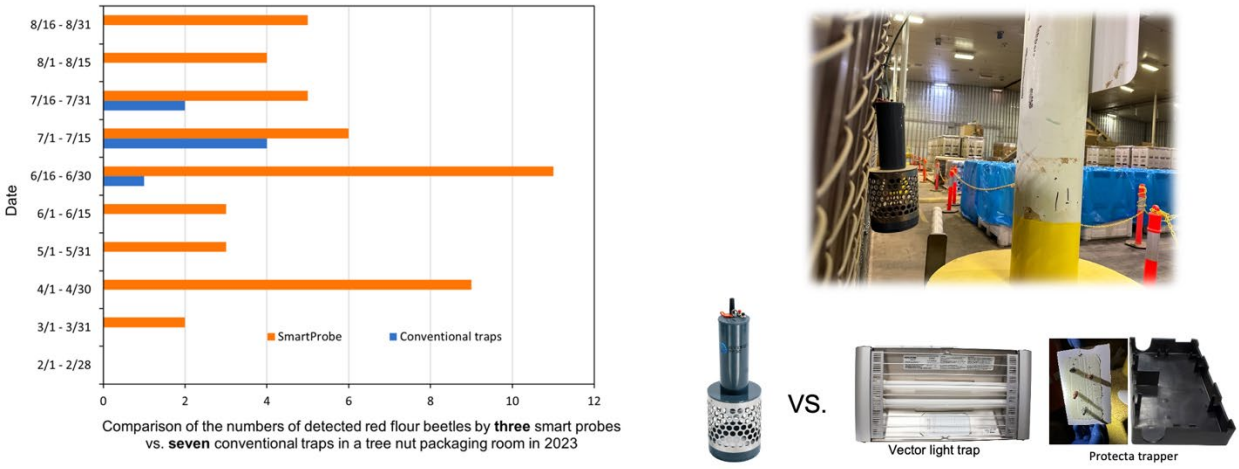
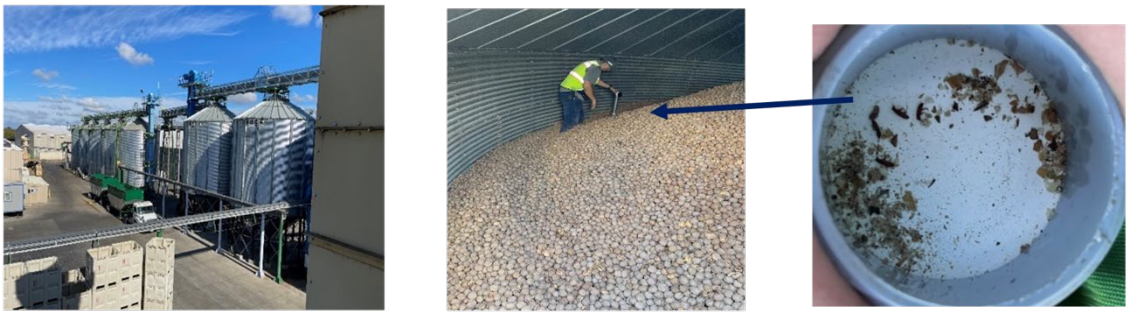


Fig. 8. Comparison of the number of red flour beetles detected by three models of the SmartProbe Technology and seven conventional traps in a processing room of almonds.



Metal storage silos for walnuts

Installing detectors in walnuts in metal storage silo

Insects detected after 2 days of installation

Fig. 9. Insects detected by SmartProbe Technology in in-shell walnuts in a metal storage silo of walnuts.

CONCLUSIONS

This study revealed that the SmartProbe can be used to effectively detect insects and monitor environmental conditions in various food and agricultural products during storage, processing, handling, and transportation/shipping, eliminating the cost of human inspection and associated risks remotely and early. It can replace the current paper and light traps in warehouse and processing facilities. The technology is the only fully demonstrated and commercially available technology for remote insect monitoring. The application of this technology can prevent unnecessary fumigation treatment and ensure effectiveness of disinfestation treatment, eliminating the guess in fumigation time or late fumigation causing product loss. The monitoring of environmental conditions of products using SmartProbe technology provides important information for avoiding product quality deteriorations. The technology recently received the AE50 Outstanding Innovation Award from the American Society of Agricultural and Biological Engineers for its innovation, significant engineering advancement, and impact on the market served. The SmartProbe Technology system is commercially available at AIVision Food Inc.

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Glennon DJ (2024) Web-based phosphine edge AI monitoring combats infestation in stored grains. Page 273. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Web-based Phosphine Edge AI Monitoring Combats Infestation in Stored Grains

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ABSTRACT

Stored grain pests' resistance to phosphine fumigations is a serious concern among major grain growing countries. Phosphine gas is widely used to protect stored commodities from insect damage. After decades of use and misuse, evidence of insect resistance to phosphine is showing up in many parts of the world. It is believed that ineffective fumigations from low gas concentrations are the driving force that contributes to phosphine resistance. Recent studies have shown that phosphine resistance has increased in both frequency and strength of resistance. Continued use of phosphine as an effective fumigant requires accurate phosphine concentration levels to be recorded and reported for required concentration × time (Ct) kill values.

Innovative sensors delivering validations (accuracy, repeatability) for phosphine, carbon dioxide, relative humidity, and temperature values are presented in real time via multiple communications platforms. An encrypted web portal provides remote internet access worldwide via any secure web browser. Real-time monitor diagnosis of onboard sensors and data display affords Edge AI validation with this Patented Spectros Instruments Blockchain Control-Alert-Report Web Platform.

Keywords: Phosphine, Fumigation, Edge AI, IoT, Large data, Grains, Validation, Compliance

Paper No. CAF2024-A77

Guo A, Zhang Y, Ji Y, Chen X, Zhang W, Lui X, Yan D, Fang W, Li Y, Cao A, Wang Q (2024) The potential for reducing aflatoxin B1 contamination of stored peanuts by soil disinfection. Page 274. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

The Potential for Reducing Aflatoxin B1 Contamination of Stored Peanuts by Soil Disinfection

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ABSTRACT

Aflatoxins from the fungus *Aspergillus flavus* that contaminate stored peanuts is a major hazard to human health worldwide. Reducing *A. flavus* in soil can decrease the risk of aflatoxins in stored peanuts. In this experiment, we determined whether peanuts grown on soil fumigated with dazomet (DZ), metham sodium (MS), allyl isothiocyanate (AITC), chloropicrin (PIC) or dimethyl disulfide (DMDS) would reduce of the quantity of *A. flavus* and its toxin's presence in peanuts. The results of bioassays and field tests showed that PIC was the most effective fumigant for preventing and controlling *A. flavus*, followed by MS. PIC and MS applied to the soil for 14 d resulted in LD_{50} values against *A. flavus* of 3.558 and 4.893 mg kg^{-1}, respectively, leading to almost 100% and 98.82% effectiveness against *A. flavus*, respectively. Peanuts harvested from fumigated soil and then stored for 60 d resulted in undetectable levels of aflatoxin B1 (AFB1) compared to unfumigated soil that contained 0.64 µg kg^{-1} of AFB1, which suggested that soil fumigation can reduce the probability of aflatoxin contamination during peanut storage and showed the potential to increase the safety of peanuts consumed by humans. Further research is planned to determine the practical value of our research in commercial practice.

Keywords: Fumigation, *A. flavus*, AFB1, Peanuts, Health hazard

Paper No. CAF2024-A78

Lin H, Chen X, Zhao X, Cao A, Wang Q, Teng C (2024) Effect of sodium dichloroisocyanurate and chlorine dioxide on postharvest preservation of mango at ordinary temperature storage. Page 275. In: Jayas DS, Singh CB, Jian F (eds) Proceedings of the 12th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2024), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Effect of Sodium Dichloroisocyanurate and Chlorine Dioxide on Postharvest Preservation of Mango at Ordinary Temperature storage

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ABSTRACT

Mangoes often face the challenges of easy decay and short shelf life during storage. Sodium dichloroisocyanurate and chlorine dioxide are commonly used as efficient and broad-spectrum disinfectants. In this experiment, we used these two disinfectants to fumigate and soak the harvested mangoes, to characterize the preservation effect of these two disinfectants on mangoes. The results showed that compared with the control treatment, both disinfectants significantly reduced the disease index and yellowing of mangoes. From 0–10 d, the hardness of mango fruits treated with disinfectants was significantly higher than the control treatment. After being stored at room temperature for 3–10 d, the total soluble solid (TSS) content of mangoes treated with sodium dichloroisocyanurate fumigation was significantly lower than the control treatment. Both disinfectants delayed the rate of decrease in TA content. After 10–25 d, the vitamin C (VC) content of mangoes treated with both disinfectants was significantly higher than the control treatment. Our results indicated that although both sodium dichloroisocyanurate and chlorine dioxide can reduce the disease index during mango storage, considering the overall quality of mango fruits, and sodium dichloroisocyanurate as a fumigant has a better preservation effect during mango storage.

Keywords: Fumigant, Mango, Preservation, Sodium dichloroisocyanurate, Chlorine dioxide