CZECH UNIVERSITY OF LIFE SCIENCES Prague (CZU)

Faculty of Agrobiology, Food and Natural Resources

Department of Plant Protection

Controlled Atmospheres, Physical Disturbances and Repellents as Alternatives to Chemical Pesticides for Control of Stored Product Pests

Doctoral dissertation thesis

Author: Jawad Ali Shah

Supervisor: prof. Ing. Pavel Ryšánek, CSc.

Consultant: Ing. Václav Stejskal, Ph.D.

Praha, 2022

DECLARATION

I hereby declare that the present dissertation work **“Controlled Atmospheres, Physical Disturbances and Repellents as Alternatives to Chemical Pesticides for Control of Stored Product Pests”** is my own research work and I have properly acknowledged all the sources of materials used in this dissertation.

In Prague: 11.21.2022 Ing. Jawad Ali Shah

ACKNOWLEDGEMENTS

Words cannot express my gratitude to my Supervisor, Prof. Pavel Rysanek, for his support, kindness, trust, and hospitality. I also could not have undertaken this journey without the help of my Consultant Ing. Václav Stejskal, who provided me with space in their lab. Additionally, this endeavor would not be possible without the generous support of Dr. Tomas Vendl, knowledge, expertise, help, and friendship. It will not be fair not to acknowledge the help and support of Ing. Radek Aulický, Ph.D., for sharing his expertise and knowledge for the Modified atmosphere experiments. Thanks, should also go to the technicians of Crop Research Institute Šárka Matatkova and Bc. Zdeňka Hájková who helped me along during the experiments despite the language barrier.

I am also grateful to my classmates, faculty, and workers of the Plant protection department. I dedicate my Ph.D. to my father, Late Fazal Dayan, and my teacher Late Dr. Sajjad Ahmad, whose dream was to see me with a Ph.D. title. I would be remiss in not mentioning my mother, sisters, brothers, nephews, and nieces. Their belief in me has kept my motivation and spirit high during this process.

Thanks to all the old and new friends, those of a lifetime, and the latest discoveries, as their presence in my life, is vital. Among all, I want to thank Arshad Ali, Arshad Hayat, Waheed, Javed and Azam for embodying the qualities of a real friend and always finding a way to be right there where they are needed.

Finally, I would like to thank Almighty Allah for letting me through all the difficulties and giving me the strength and patience to complete my dissertation.

TABLE OF CONTENTS

TABLE OF CONTENTS……. 1-2

1. INTRODUCTION……..…… 3

1.2 The biology of insects used for this research is summarized as follows……..…**4**

1.3 Insects control measures……..…… **10**

2. LITERATURE REVIEW……12-20

2.1 Controlled atmosphere…….... **12-14**

2.2 Physical disintegration…….… **14-17**

2.3 Botanical extracts as repellents……... **17-19**

3. HYPOTHESES & OBJECTIVES……..… 20

4. LIST OF PUBLISHED STUDIES……..… 21-84

4.1 Study 1: Control of stored agro-commodity pests *Sitophilus granarius* and *Callosobruchus chinensis* by nitrogen hypoxic atmospheres: laboratory and field validations…….….22-40

4.2 Study 2: Frass produced by the primary pest *Rhyzopertha dominica* supports the population growth of the secondary stored product pests *Oryzaephilus surinamensis*, *Tribolium castaneum*, and *T. confusum*……..….41-47

4.3 Study 3: Effect of grain excavation damages by *Sitophilus granarius* on the Efficacy of grain protectant insecticides against *Cryptolestes ferrugineus* and *Tribolium castaneum*……..…….48-56

4.4 Study 4: Gel Carriers for Plant Extracts and Synthetic Pesticides in Rodent and Arthropod Pest Control: An Overview……..……...57-74

4.5 Biorational Control of *Callosobruchus maculatus* (Coleoptera: Bruchidae) in Stored Grains with Botanical Extracts……..…...75-84

5. SUMMARY & DISCUSSION……..…….85-90

5.1 Nitrogen hypoxic atmospheres……...…85-87

5.2 Physically disturbed/destructed kernels impact storage pests, biology, and control.....87-88

5.3 Botanical extracts as repellent……..89-90

6 CONCLUSIONS……..…… .91-92

7 LIST OF USED LITERATURE……..…….. .93-107

1. INTRODUCTION

Grains are the primary food source for people and farm animals globally, with production rising to approximately 7 million tons in 2014 (FAOSTATS, 2017). The world's most widely cultivated food crops are grains, including corn, wheat, and rice. Such crops frequently serve as the foundation for essential foods. According to the Organization for Food and Agriculture, cereals account for most of the estimated 1.3 billion tons of yearly food waste. The causes of the fall, which start with the initial reduction in agricultural production and ends with the ultimate drop in household consumption, are variable. Surprisingly, in the case of grains, food wastes are as significant in industrialized countries as they are in developing countries, but ultimately, in developed countries, over 40% of losses happen at the sales and user levels, commonly depicted as wasted food. In contrast, more than 40% of losses occur in developing countries' post-harvest and handling stages. (Gustavsson *et al.,* 2011).

Documented insect infestation in grain storage dates to 3000 BC, according to historical records and archaeological discoveries (Buckland, 1981). Since insects have the potential to destroy large quantities of food, especially when it is kept for an extended period, some precautions must be taken to discourage insect infestation (Pimentel, 1991). Pests (as a whole) represent health hazard when they are present in the commodity (Hubert *et al.,* 2018).

Severe losses are made by the insect pests on stored grains as well as to their products, across the globe (Talukder *et al.,* 2004). Grains and pulses are at high risk of infiltration by a huge group of insects and pests of stored goods through their storehouse (Teshome and Tefera, 2011). Some moths (70 species) and beetles (more than 600 species) attack agricultural and animal-based stocked goods which causes quantitative as well as qualitative losses of these products (Eliopoulos, 2019). In the overall production of stored grains, the yearly post-harvest losses vary from 10 to 20% by various biotic factors (Phillips and Throne, 2010). When discussing emerging countries, the extent of certain insects created damages vary from 9 to 43% (Jacobson, 1982; Pimentel, 1991). Those regions with low moisture and high temperature are more fancied by the insect pests (Ghanem and Shamma, 2007).

Therefore, particular areas might experience more shocking events. A loss of 76 percent of maize kept without pesticide spraying occurred in Zimbabwean fields (Giga *et al.,* 1991). Pests are present at every step of the industrial process. However, the storage period is the most critical and frequent point vulnerable to insect invasion (Riudavets, 2017).

The agro-food sector takes into account over 1,500 insect species, 150 of which are serious pests, even though food production is progressively paying attention to high-quality standards (Hagstrum and Subramanyam 2009; Hagstrum and Phillips, 2017; White *et al.,* 2011). In food sector, it is unacceptable to have live beetles, insect body pieces, or metabolic byproducts like webbings or feces (Athanassiou *et al.,* 2017). Sadly, grain storage's optimum temperature and moisture provide a highly protected environment for pest infestations (Reichmuth, 2000). This allows certain organisms to disperse locally and globally through networks of cereal shipment and marketing (Nopsa *et al.,* 2015).

Beetle respiration rate may direct to a rise in cereal moisture level, which can result in fungal development and likely mycotoxin growth, decreasing the security and safety of the food. Beetles' feeding results in insect-damaged grains, frass, and subsequent weight loss (Birch, 1947; Gram *et al.,* 2002; Vendl *et al.,* 2022; Sinha and Sinha, 1992).

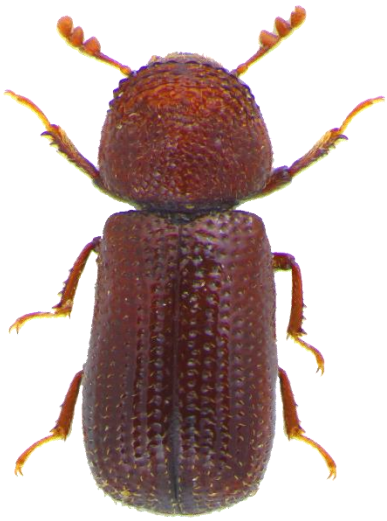
Only 10-15 species are usually present in the stored grain (White *et al.,* 2011). Weevils *Sitophilus* spp. (*S. granarius*, *S. oryzae* and *S. zeamais*), *Rhyzopertha dominica*, and *Sitotroga cerealella* cause significant cereal damage, not only since they are cosmopolitan organisms but mainly because they develop within the grain kernel until they emerge as adults, evading detection by conventional sampling techniques (Sola, 2018; Brader *et al.,* 2002; Hubert *et al.,* 2009).

Aside from inside eaters, stored-grains moths are one of the worst problems in Europe's factories, warehouses, food processing facilities, and private residences (Parkin, 1956; Trematerra and Gentile, 2010). Moths lay eggs on the surface of the grains, and the emerging larvae grow primarily on the top 50 cm of the grain (Schöller *et al.,* 1997). Moths produce a web that prevents the machine from properly grinding the material.

1.2 The biology of the insects used for this research is summarized as follows.

Rhyzopertha dominica: The lesser grain borer is well-known as the foremost damaging insect of stored grain goods as well as various other related starch-containing commodities across the globe

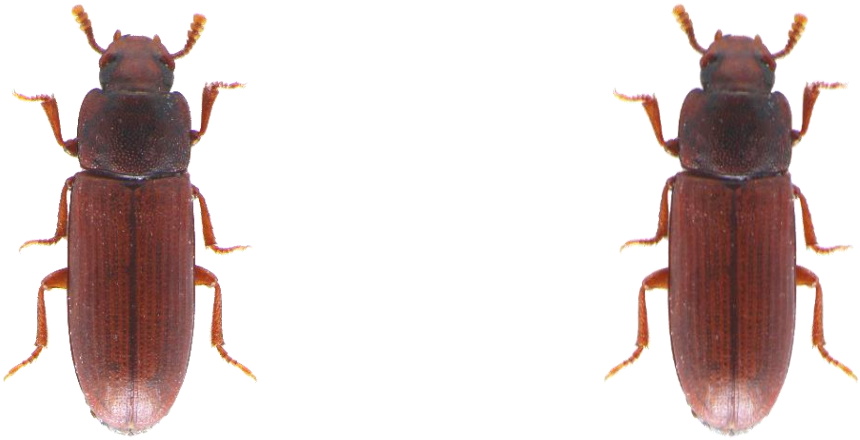
(Rao and Wilbur, 1972; Flinn and Hagstrum, 1994). India is the origin of the lesser grain borer (Schwardt, 1933). Larval stages and adults can attack sound and healthy grains (Mayhew and Phillips, 1994). Adults of *R. dominica* are active fliers, long-lasting and females lay about 1 to 7 eggs/day up to several months (Flinn and Hagstrum, 1994) and on average, roughly 200 to 500 eggs are deposited by a female through its entire life (Begum and Huda, 1974). The female lays her eggs on the exposed surface of the wheat grains. Initially the hatched larvae feed on powdered wheat, and later they penetrate the entire grains because of their internal feeding habits and further pupate inside the grains (Rao and Wilbur, 1972). The adults stay within the grains for a few days after emergence (Flinn and Hagstrum 1994; Mayhew and Phillips, 1994). *Rhyzopertha dominica* remains identified as a primary pest or primary feeder because it develops inside whole grains and creates random shape holes through the boring (Aitken, 1975). Amongst all stored grains beetle pests, this is the only insect species whose grubs can sustain and grow in cereals at only 8% moisture contents of the grains (Birch, 1945). Adults of *R. dominica* feed upon the endosperm by reducing the grain carbohydrate contents while all larval stages favor feeding upon seed germ (Dal Bello *et al.*, 2000).



Rhyzopertha dominica (Photo Credit: Vendl Tomas CRI, Prague)

Tribolium castaneum: The red flour beetle is distinguished from other *Tribolium* species by distinctive three-segmented club antennae. The space between the eyes and the area of the eyes (as seen from below) are equal. It is a member of Tenebrionidae family, the majority of which are saprophagous, and until now, about 80 species have been identified as pests of stored products (Hagstrum and Subramanyam, 2006). Eggs are arranged singly throughout the substrate and are typically covered with flour particles that stick to the gelatinous outer layer (Good, 1936). The larvae are white to yellowish and have distinct projections or spikes at the end of abdomen. Larvae feed on the flour and pass through six or more instars. Fresh pupae are yellow and turn into a coffee color when older. The male and female pupae can be differentiated by the presence of two small appendages on the abdomen of females. Although *T. castaneum* adults fly quickly in warm weather, the dissemination of this insect relies entirely on passive movement in trade. The red flour beetle is the most common of all stored grain beetles, a worldwide pest. Adults can live up to one year. In their lifespan, females produce hundreds of eggs. The red flour beetle is typically a supplementary pest of many different types of grains, breakfast cereals, legumes, oilseeds, cakes, nuts, and animal products. It is a very proficient omnivore that is especially prevalent in warm and dry circumstances. Quinones, which the adults secrete, leave behind a musty odor.

The confused flour beetle is a kind of darkling beetle called ***Tribolium confusum***. The name "confused" specifically relates to how it resembles the red flour beetle, not how it walks (Walter, 1990). The species are tiny, reddish-brown, and their size ranges from 3-6 mm (1/8-1/4 inch). The shape of their antennae is the primary physical distinction between the *T. castaneum* and *T. confusum*. In contrast, the confused flour beetle's antennae steadily extend, while the red flour beetle has club composed of three antennal segments. Additionally, the confused flour beetles do not fly, while red flour beetles can fly.



Tribolium castaneum (Photo Credit: Vendl Tomas CRI, Prague) *Tribolium confusum*

https://e- insects.wageningenacademic.com/tribolium_confusum

***Oryzaephilus surinamensis*:** The saw-tooth grain beetle, *O. surinamensis*, belongs to the family Silvanidae, and is among the most abundant cereal and food pests globally (Champ and Dyte, 1976). Due to its inability to harm whole grains, it is categorized as a secondary pest. However, this condition has altered due to mechanical challenges during harvest and drying, which result in shipping cracked and fragmented grain to warehouses, where this pest accumulate and produce severe infestation issues (Pricket *et al.*, 1990). In storage facilities, it is challenging to control this insect with insecticides because it can hide in numerous locations and has developed resistance to many chemicals (Wallbank & Collins, 2003). Due to their global invasion of packaged foods, adults and larvae often devour wheat flour, cereal bars, feedstock, copra, nuts, and dried fruits (Highland, 1991; Mowery *et al.*, 2002). Many grain storage facilities in Brazil are infested by the saw-toothed grain beetle, which results in grain quality and quantity losses and deterioration (Ahmed *et al.*, 2022).

Oryzaephilus surinamensis (Photo Credit: Vendl Tomas CRI, Prague)

***Sitophilus granarius*:** is among different storehouse pests; that occurs more often and is an important pest of stored grain in Europe (Boniecki *et al.*, 2020). This pest can be found across the world in temperate zones, but only in chilly upland places in tropical climates. By consuming cereal grains, this beetle reduces a grain's mass, nutritional value, and ability to germinate (Stejskal and Kucerova, 1996). During the winter season, adult hibernates inside the hole and cracks of the storage area or warehouse. The female makes a dip on the grain with their mandibles and places eggs in it, one egg in one grain. A female can produce up to 250–400 eggs in one season. In 6-7 days after the egg hatch, the larvae begin boring into the whole grains. After the larval stage, they appear in a covering called puparium inside the grain. During the pupal stage, they stop eating. The pupal stage continues for 6 to 14 days after this adult emerges from the puparium. On emergence, the adult weevil passes out of the grain and lives for 4-5 months. The grain weevil completes 3-4 generations in a year.

Sitophilus granarius (Photo Credit: Vendel Tomas CRI, Prague)

Cryptolestes ferrugineus: belongs to the family Laemophloeidae, and the common name is rusty grain beetle. It has a wide geographic distribution that extends from the tropics to temperate areas. It is preadapted to live and breed in silos. Every day, females lay two to three eggs upon grain. The life cycle takes about 21 days, at 70% relative humidity and 35 °C (Smith, 1965). It has a 6 to 9 months lifespan and an extreme resistance to freezing conditions when related to other stored-grain insects (Fields, 1992). Adults are the most cold-tolerant and able to withstand 15 °C for four weeks and stop movement below 2 (Smith, 1970). However, they favor the warm temperature and reach the warmer region of the grain bulk (Jian *et al.*, 2004).

Cryptolestes ferrugineus (Photo Credit: Vendel Tomas CRI, Prague)

Callosobruchus chinensis: Azuki bean weevil is a worldwide pest of legume seeds such as cowpea, mung bean, and azuki bean (Arora and Singh, 1970). Due to extensive studies of *C. chinensis*, it is considered a model organism for new research (Chen *et al.*, 2007). Females deposit one egg at a time on top of suitable host seeds. The first instar larvae emerge from the egg after several days and dig through into the grain, which it nourishes until adulthood. In optimal conditions, adult *Callosobruchus* beetles only live for around 12 days and do not feed on stored goods. In this stage, females produce many eggs (up to 70 in *C. chinensis*); however, if the seeds are already infested, it may affect the oviposition (Chavan *et al.*, 1997; Parr *et al.*, 1998). The most economically important bruchid species has a relatively short life cycle. Under ideal circumstances, the lifecycle can take only 22-25 days.

Source: https://link.springer.com/chapter/10.1007/978-981-10-8687-8_10

1.3 Insect Control Measures:

Since 1940, the preferred way to control insect pests has been chemical treatment using a variety of insecticides (Barratt *et al.,* 2017; King *et al.,* 1985; Van Lenteren, 2012; Zettler and Arthur, 2000). But, due to limitations set by domestic consumers, the state, and importing nations, the supply of effective insecticides has significantly reduced (Niedermayer and Steidle, 2013; Parrella *et al.,* 1992). Environmental issues, such as the presence of chemical remains in edibles, influence non-target organisms. Still, the most critical problem is that insects have developed resistance to most of the pesticides used to control stored grain pests. Besides the above concerns, it also affects the health of the food workers involved. (Zettler and Arthur, 2000; King *et al.,* 1985).

Pesticides can be divided into two categories: they are contact insecticides and fumigants. Among fumigants, methyl bromide, phosphine, or sulfuryl fluoride are three formulations that are frequently used. They immediately kill all stored product insect life stages in a structure or a commodity. The most common insecticide was methyl bromide. However, because of ozone layer depletion, it was restricted in developed nations starting in 2005 and most developing nations beginning in 2015(Sola, 2018). The most common fumigant in use worldwide is phosphine. However, the usage of this fumigant is restricted because of its high lethality and pest resistance (Hagstrum and Phillips, 2017; Phillips and Throne, 2010; Wilkin, 2000).

Additionally, sulfuryl fluoride is only marketed in a few countries and is primarily used to disinfect structures. The most widely used contact insecticides in Europe are deltamethrin and chlorpyriphos-methyl. These are sprayed directly onto grain or buildings, providing several months of pest protection (Bell, 2000; Fields and White, 2002; Zettler and Arthur, 2000).

The unavailability of insecticides and the emergence of insect resistance have motivated scientists to begin research on potential replacements. Phillips and Throne evaluated the alternatives for using pesticides in (2010). They divided them into methods for influencing the physical environment and practices for controlling biological products. The first involves cleaning the structure and keeping insects out, manipulating temperature and relative humidity or controlled atmosphere. On the other hand, the biologically based approaches are divided into four categories: pheromones that attract insects; biopesticides or plant-based insecticides based on plant extracts that repel insects; resistance variates; and finally, the predators and parasitoids called natural enemies (Sola, 2018).

Controlling insect pests in storage and food factories needs a high grade of proficiency connected with knowledge and experience. Pest management plans should integrate various practical and cost-effective methods to prevent food contamination. These solutions must target immediate pest problems, avoid future infestations, and adapt to daily needs while remaining flexible enough to handle emergency pest control scenarios. Using insecticides judiciously for stored-product pest control techniques tends to prioritize non-chemical pest control components (Abdel-Aziz, 2011).

Ineffective pest control results in tainted items, which can have negative effects on one's health, finances, legal situation, and aesthetics.

Financial losses may result from the following factors:

1. The appearance of live or dead insect pests in goods and bins.
2. The occurrence of smells, webbing, and frass in commodities and bins.
3. The customer's loss of trust in the company resulted from these circumstances.
4. Actual weight loss due to insect feeding can lead to economic losses.

Considering the abovementioned concerns, the current research was carried out to discover a non-chemical method of managing stored grain insect pests. According to the types of insects, the study is separated into three parts. Applying a modified or controlled environment is the first step in controlling internal grain feeders. The second step involves determining the impact of primary pests' physically disturbed/destructed kernels on the biology and control of the secondary pests. The third step involves determining the repellency effect of botanical extracts against the stored grains insects.

2. LITERATURE

2.1 Modified atmosphere: The development of the designated gases (N2 or CO2) supplied from the pressurized tanks creates an altered gas composition that is intentionally created and maintained. Modified atmosphere (MA) offers a secure and environmentally friendly substitute for using usual residue-producing chemical fumigants for managing pest species invading stored cereals, oilseeds, packaged commodities, and grocery items. The use of a modified atmosphere to control stored grain insects has been studied for over three decades (Navarro, 2006). Insects contain a well-developed respiratory design that allows immediate air intake from the environment through spiracles. Insects regulate the inflow and outflow of respiratory gaseous exchange through the opening and closing of these spiracles. They use muscular contractions to ventilate their tracheal system (Matthews and White, 2011). During the process of respiration exchange of gases happens. The insects take the fumigants from the environment through the respiratory system factors that affect insect respiration and influence fumes uptake. Modifications in the concentrations of N2, O2, and further gas concentration can affect the respiration rate and therefore the rate and biochemistry of metabolism and, finally, the effectiveness of fumigants (Lu *et al.,* 2009).

The losses caused by stored grain insects during storage need the application of various methods for their control (Gbaye and Odeyemi, 2005). During the recent past, the preservation of grains and other long-lasting agricultural products massively depends on pesticides to control the storage pests. The toxicity of these fumigants and insecticides to the environment, non-target organisms, and the development of resistance by insects restrict their application for stored grain pests (Flora *et al.,* 2006; Nayak *et al.,* 2020). The present trend and demand are the use of non-toxic control methods, which are safe for all the producer, consumer, and the environment (Donahaye *et al.,* 1996). The rise of this technique has addressed the concern of the public over the traces of chemical in food and the environment. Nitrogen application to prevent stored grain insects in storage under controlled or modified atmospheres is among the most effective non-toxic options for pesticides (Aulicky *et al.,* 2017). The storage system must be air-tight and have no leakage for an effective result of the nitrogen-controlled atmosphere and ensure the constant concentration of nitrogen. The application of N2 controlled atmosphere also inhibits mold and maintains the nutritional value of stored grain (Moncini *et al.,* 2020). Temperature directly affects the controlled atmosphere

treatment period. High temperature is more effective and needs less exposure length (Donahaye *et al.,* 1996). A controlled atmosphere must be air-tight to hold oxygen concentration under 1%. As the concentration of oxygen increases, it may take more time to kill all the life stages of pests. With 2% oxygen hundred percent mortality was achieved in 20 days exposure (Navarro *et al.,* 2012) and with 1% or less oxygen in ten days (Aulicky *et al.,* 2017). About 78% of nitrogen is available in the air and can be introduced to the chamber or controlled atmosphere area with the help of the nitrogen dynamos or pumps (Navarro *et al.,* 2012). The level of oxygen worked in such an application is 1% or less (Jay and Cuff, 1981). Nitrogen treatment for controlling various development stages of *Ephestia elutela, Tribolium confusum*, and *Oryzaephilus surinamensis* was effective following increased temperature (Athanassiou *et al.,* 2017). The atmospheric nitrogen can be used in different kinds of structures, varying from silos to chambers (Navarro, 2006, 2012). The use of nitrogen-controlled atmosphere gives many benefits, including financial and environmental. The insect/pest's cultures do not develop resistance and are safe for application in silos because nitrogen does not react with metals. Eventually, there is no need for product registration and ventilation before marketing (Ren *et al.,* 2012). The beginning acquisition in the construction or modification of a permanent warehouse creates CA costly, various prosperous applications are comprehended (Carvalho *et al,* 2012).

There are two types of responses of insects to low oxygen levels: regulating and conforming (Hochachka, 1991; Makarieva *et al.,* 2006). During the exposure, individuals in regulating type increase metabolism and consume a lot of nutrients to sustain the energy needed for standard processing. Conversely, the conforming class decreases energy, respiration, and carbohydrate consumption without using glycolytic pathways. Conforming organisms often tolerate prolonged low O2 exposure more than regulating species. However, damage repair mechanisms are necessary for hypoxia survival. Remediation processes occur continually in the regulating group and only after recovery to normoxic conditions in the conforming class. Insects' aerobic metabolism slows down in hypoxia (or anoxia), and research on the housefly Musca domestica has shown that this prevents rapid cold hardening (RCH) (Coulson and Bale, 1991).

Similar findings were made by Nilson *et al.,* (2006). They discovered that lack of oxygen contained no impact at quicker exposure duration but reduced rapid cold hardening in *Drosophila melanogaster* beyond 1 hour of exposure.

A non-lethal low-temperature "shock" part of RCH protects against five more deadly low-temperature vulnerabilities (Lee *et al.,* 1987). However, Yocum and Delinger (1994) discovered that RCH was not observed in anoxic settings in the flesh fly (*Sarcophaga crassipalpis*). In conclusion, gas conditions may impact how some species react to low-temperature stress. Low oxygen levels during 48 and 72 hours of exposure did not affect the number of egg masses, while 2 and 5% oxygen had a negative effect on the total amount of oviposition. The lifetime reproductive output was significantly reduced when the exposure length expanded from 48 to 72 hours. The exposure of the female and eggs to hypoxia reduced the number of mature offspring. It has been demonstrated that eggs laid in low oxygen conditions with extended exposure times have reduced adult emergence rates. These results suggest that hermetic environments hinder cowpea bruchids' capacity to lay eggs and produce healthy offspring (Yan Yan *et al.,* 2016).

T. castaneum adults, pupae, and larvae were treated for 72hrs with different levels of CO2 and N2 at 27 °C with 50% relative humidity. In general, 58% CO2 was more efficient on pupae, whereas 58% CO2 killed more adults than 99% N2. Although 99% N2 caused higher deaths after 48 hours, there was no noticeable difference in the death rates of larvae subjected to the two atmospheres. The variations in death rates for immature stages treated to 58% CO2 and 97% CO2 were minor, although 97% CO2 caused much greater adult mortality than 58% CO2. Weight loss and death were minimal once the beetles were introduced to a 91% N2 environment, while weight loss and mortality were significant when the beetles were introduced to a 97% CO2 atmosphere. When the weight reduction of insects exposed to 99% N2 and 58% CO2 was compared, the adult's weight loss was higher in the N2 environment. In contrast, pupal weight reduction was higher in the CO2 atmosphere (Edward and Wilfred, 1981).

According to the finding of the trials, nitrogen, controlled atmosphere application stands as a viable option for phosphine Siroflo/ECO2FUME and contact pesticides for controlling stored pests (Navarro *et al.,* 2012).

2.2 Physical disintegration:

Based on cereal's damage pattern, insects that attack it are categorized into secondary and primary pests. Groups of arthropods (mites and insects) that feed outside and primarily harm the grain germ are called "secondary pests."Secondary pests can damage seeds in one of two ways: either by

simply eradicating the embryo from the exterior (as in the case of large species like *Plodia* spp., *Ephestia* spp., and *Tribolium* spp.) or by creating cavities in the germ (as in the case of microscopic arthropod species like mites and *Cryptolestes* spp.). Psocids often pierce the outer layer with tiny holes to reach the embryo, which they can then entirely eat (Kucerova 1999; Gautam *et al.*, 2013). They might potentially cause additional harm to the germplasm based on how long and severe the infestations are. However, numerous secondary pests, such as beetles, mites, and psocids, prefer to consume broken kernels (Athanassiou *et al.*, 2010). Internally eating insects that produce undetected infestations within kernels, such as *Sitophilus* species, *Rhyzopertha dominica*, bruchids and moths *Sitotroga* species, are the principal pests. In many situations, they only destroy the endosperm. Primary pests are largely accountable for damaged final cereal goods since they originate within the grains (Trematerra *et al., 2011*). Rodent chewing causes a definite form of grain damage in legumes and cereal grains that have been preserved for use as feed. According to Stejskal and Hubert (2008), primary pests instead of secondary pests are the focus of most chemical treatments used on publicly stored grains in the Czech Republic.

On the other hand, enterprises that produce seeds have a different sense of the risk posed by primary and secondary pests. Both pest categories are equally important economically to these businesses, as secondary pests can severely affect the germination capacity of stored seeds. In recent years, it has become increasingly challenging to protect seeds from pests due to a surge in secondary pests around the planet (e.g., Arthur, 2012), a development in pest resistance, and a decrease in the active components in pesticides. Methyl bromide, organophosphate-based sprays, and dichlorvos vapor bands, which have since been prohibited in the European Union, are the three most common methods of seed treatment over pests.

Sitophilus zeamais showed more attraction towards the insect-damaged kernel than the other two kernel groups. There was no clear differentiation in the attractiveness of unharmed versus manually broken kernels. The sequence of the grains' "attractiveness" to an adult *S. zeamais* was arthropod damaged and manually broken. While comparing newly manually broken and stored broken grains with freshly arthropod and stored arthropod damaged grains, significant differences in attractions to adult *S. zeamais* were discovered (Trematerra *et al.,* 2000).

Even though these attractants' properties are typically well-studied, less information is available about the commodity's function, like an adjuvant **(**Landolt and Phillips, 1997). For example, an individual insect's activities may be influenced by the shape of certain grains. *T. castaneum, T. confusum,* and *O. surinamensis* attracted to wheat grains damaged by *S. oryzae* more than undamaged or manually broken grains (Trematerra *et al.,* 2000). However, storage may coincide with insect-damaged, cracked, and whole grains. One of the most important inquiries regarding this co-occurrence may be that some kernel-kernel interactions can control insect behavior, as Pickett *et al.,* (2003) demonstrated for plant-plant interactions. For example, if insects damaged kernel (IDK) affects the "attractiveness" of entire grains, it would be beneficial to precisely comprehend the procedure.

The host's attractivity may differ between the primary and secondary stored pests (Trematerra *et al.,* 2000). Broken rice kernels in pheromone traps may attract rice weevils more strongly than unbroken grains, according to Trematerra *et al.,* (1999). When a pheromone and an attractant are combined, there is a greater chance of seeing a colony with a diverse age group, which happens outdoors (Wakefield *et al.,* 2005). A similar phenomenon occurs with maize weevils: after finding an excellent place to invade, individuals release an aggregation signal that attracts other members of the identical kind (Levinson *et al.,* 1990). Additionally, the number of secretions or frass may boost the release of active substances from typical kernel components, such as the kernel's embryo or endosperm, that are incredibly appealing for *S. oryzae*. Further, broken grain provides the weevil better accessibility (Trematerra *et al.,* 1999).

According to Trematerra *et al*., (2000), secondary pests are more attracted to the grains that are damaged by *S. oryzae* than to undamaged or manually broken kernels. Trematerra *et al.,* (2007) got the same results for the *S. zeamais*. Therefore, adult *S. zeamais* feed on kernels contaminated with semiochemicals produced from kernels of the exact species that were infested as adults. Because of this, huge numbers can quickly gather close to tiny infestations. According to these findings, the active semiochemicals produced from insect-damaged kernels may be markedly different from those from undamaged or manually broken grains.

Many scientists investigated the relationship between grain and primary insects (Levinson and Kanaujia 1981; Stejskal and Kucerova, 1996; Steidle *et al.,* 2005). The olfactory samples indicate that such relationships have a particular impact on controlling arthropod behavioral patterns in

stored goods (Estabrook and Yoder, 1998; Pickett *et al.,* 2003). Damage from insects or mechanical damage affects other intact grains, directly impacting how infestations manifest in the stored grain ecosystem. Volatiles ingested during conditioning could trigger a reaction that alters the chemicals developed as a defense reaction. More research is required to investigate the signaling tracks activated in reaction to insects feeding on corn kernels and other factors of insect-grain relations.

2.3 Botanical Insecticides as repellents:

There is currently a lot of interest to the researcher in the employment of novel conventional techniques of prevention against stored grain insects, mainly where botanical extracts are involved. The reason is that most insecticides and fumigants used against stored grain pests are banned, or the insect develops resistance. Botanical extracts are the most researched compounds (Dales, 1996; Bohinc *et al.,* 2020). Although there are several studies on using plant extracts or whole plant materials for pest control, few of them are used commercially (Rajendran and Sriranjini, 2008). Whereas the history of utilizing botanical extracts have not been thoroughly documented, it is clear from various archaeological records that the usage of several botanicals for insect prevention in Europe extends back over three thousand years ago (Pavela, 2016). Grain silos were frequently fumigated with numerous phytochemical compounds in the roman Times. Additionally, fragrant herbs were placed close to the granaries' entrances. As a response, people became aware of the repellent properties of plant extracts (Dubey, 2011). It was discovered that nicotine derived from tobacco leaf could eradicate plum beetles in the seventeenth century, and this discovery led to the development of the first commercially plant pesticide. A brand-new botanical pesticide called rotenone was launched in 1850, prepared from the roots of the Derris plant (Abd El Ghany, 2012). Any continued development of commercially botanical insecticides was stopped in Europe. Following the Second World War, inexpensive pesticides replaced these botanical insecticides derived from organochlorines and organophosphates. (Ware and Whitacre, 2004).

Chrysanthemum cinerariifolium flowers produce pyrethrin-based phytochemicals, mainly pesticides that quickly enter insect cuticles, particularly moths and larvae (Casida, 2012). Storage containers must be cleaned for maximum effectiveness before getting loaded with grains.

A pyrethrum-based insecticide widely used to minimize pest infestation in tropical and temperate climates (Dayan *et al.,* 2009). Soluble or dry harvests, granules, liquid, volatile compounds, oils, or crushed pieces of plants can be used as a botanical insecticide (Rajashekar *et al.,* 2016; Rajashekar *et al,* 2012).

According to Jacobson (1982), botanical pesticides can be divided into six categories based on the physiological action of insects, i.e., antifeedants, attractants, repellents, chemosterilants, toxicants, and growth retardants.

Antifeedants, also known as "feeding deterrents," are substances that prevent or inhibit insect eating by making the treated items unpleasant (Munakata, 1997). *Gaultheria procumbens* essential oil worked as an antifeedant for *S. oryzae* and *R. dominica* (Kiran and Parkash, 2015).

The substances used in insect repellent are beneficial because they protect while having no adverse effect on the environment. By activating olfactory or other senses, the repellents repulse the insect pest from the treated substances. Insect pests attacking stored grain have been repelled by extracts, granules, and oils from various plants (Owusu, 2001; Xie *et al.,* 1995; Boeke *et al.,* 2003). For example, *T. castaneum* and *C. maculatus* were observed to be repulsed by the oil of *Artemisia annua* (Tripathi *et al,* 2000). A wide range of plant derivatives is poisonous to insects that feed on stored-product food, according to findings from around the globe (Rajendran and Sriranjini, 2008; Dubey *et al.*, 2010; Park *et al.,* 2003; Isman, 2006). According to Talukder and Howse, (1995) 43 plants possess toxicant impact against several species of stored-grain insects. In Spain, Pascual-Villalobos and Robledo (1998) investigated botanical extracts of 50 wild plants for their efficacy in killing the *Tribolium castaneum*. Four species were discovered as potential: *Asphodelus fistulosus*, *Bellardia trixago*, *Senecio lopezii*, and *Anabasis hispanica*. The oil from garlic contains toxicants and fumigants effects on *T. castaneum* (Huang *et al.,* 2000). The neem plant, mainly the crushed fruit, leaf, oil, and ground fruit, was used to save stored cereals from insects (Talukder *et al,* 2004; Jotwani and Sircar, 1965; Devi and Mohandas, 1982). At a 1 to 2% concentration, seed dust and neem oil provided significant cereal protection from *S. oryzae*, *T. cataneum*, *R. dominica*, and *C. chinensis* (Pereira and Wohlgemuth, 1982). The neem oil applied to the kernel creates a uniform layer about the grains protecting them for 180-330 days from insect infestation (Ahmed, 1994). The grain treated with particles of *Mesua ferrea*, *Acorus calamus*, and *Rauvolfia serpentina* served as grain protectants from the *R. dominica* (Tiwari, 1994). Orange peel contains some

elements that work as grain protectants against *Callosobruchus maculatus* (Don-Pedro, 1985). Applying coconut oil at the rate of 1% to *Vigna radiata* keeps *C. chinensis* away for six months from stored seeds (Doharey *et al.*, 1990). Menthol formulation was found to be effective in controlling *C. chinensis* in pulse grain (Singh and Mehta, 2010). Forty-three plant species were listed as having the power to prevent reproduction in the stored grain insects. Additionally, studies have found that natural compounds, such as plant oils, kill insect ova (Obeng-Ofori and Reichmuth, 1998). It was discovered by Pankey *et al.*, (2004) that garlic oil, *Eucalyptus globulus*, *E. stageriana*, and *E. citriodora* (Talukder, 2006) might reduce *egg laying capacity and offspring production in T. castaneum, C. maculatus, and Zabrotes subfaciatus*.

Botanical extracts contain some chemicals that work as repellents, which means keeping the treated product or grain unattractive and repulsive to the insect. Repellents are particularly more effective at inducing different species of insects to escape from sprayed grains. *Baccharis salicifolia* and *Curcuma longa* possessed some chemicals which can repel *S. zeamais* and *T. castaneum* from the treated grains (Pavela, 2005; Tiwari *et al.*, 1995).

3. Hypotheses and Objectives/Goals

3.1 Hypotheses the methodology includes three different hypotheses.

1. Decreasing nitrogen concentration and temperature will increase the length of exposure hypoxic atmosphere (to be 100% effective) differentially in various stored product pest species and their developmental stages.

2. The effect of the physical disintegration of grain by primary stored product pests associated with frass production can positively affect the development of various species of secondary stored product pests.

3. There exist botanical extracts that will be repellent for main stored product beetle pests for at least 48 hrs.

3.2 Objectives/Goals

1. Validation of the effects of abiotic factors on the effectiveness of controlled anoxic/hypoxic atmospheres with respect to the use against selected types of storage pests.

2. Analysis of the effects of intact grain feeding activity of the primary Coleoptera stored product pests on the biology of various species of secondary stored product Coleoptera pests.

3. Verification of repellent properties of botanicals to storage pests to protect small volumes of stored commodities at the level of smaller farmers and small food production companies.

4. LIST OF STUDIES

1. Control of stored agro-commodity pests *Sitophilus granarius* and *Callosobruchus chinensis* by nitrogen hypoxic atmospheres: laboratory and field validations.

2. Frass produced by the primary pest *Rhyzopertha dominica* supports the population growth of the secondary stored product pests *Oryzaephilus surinamensis*, *Tribolium castaneum*, and *T. confusum.*

3. Effect of grain excavation damages by *Sitophilus granarius* on the efficacy of grain protectant insecticides against *Cryptolestes ferrugineus* and *Tribolium castaneum.*

4. Gel Carriers for Plant Extracts and Synthetic Pesticides in Rodent and Arthropod Pest Control: An Overview.

5. Biorational Control of *Callosobruchus maculatus* (Coleoptera: Bruchidae) in Stored Grains with Botanical Extracts.

agronomy

MDPI

Article

Control of Stored Agro-Commodity Pests *Sitophilus granarius* and *Callosobruchus chinensis* by Nitrogen Hypoxic Atmospheres: Laboratory and Field Validations

Radek Aulicky [1,*], Jawad Ali Shah [2], Vlastimil Kolar [3], Zhihong Li [4] and Vaclav Stejskal [1,2]

[1] Crop Research Institute, Drnovska 507, 16106 Prague, Czech Republic
[2] Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 16500 Prague, Czech Republic
[3] Podravka-Lagris, 76323, Dolní Lhota, Czech Republic
[4] Department of Plant Biosecurity, College of Plant Protection, China Agricultural University (CAU), 100193 Beijing, China
* Correspondence: aulicky@vurv.cz

Abstract: Given the complexity of the practical usage of controlled atmospheres for the protection of agro-commodities, several researchers have pointed out that there is not enough robust scientific documentation regarding the usage of inert gases for their widespread practical application. Therefore, this work evaluated various regimes of hypoxic and anoxic nitrogen atmospheres for the control of two key stored-product pests, in laboratory and under field conditions in silos. *Sitophilus granarius* and *Callosobruchus chinensis* were selected as the tested species since they are important pests of grain/rice or legumes in Europe and Asia. Under laboratory conditions, we tested nitrogen (N_2) concentrations (from 95 to 100%) and exposure times (1–20 days) on the developmental stages of both pest species. In most developmental stages of *S. granarius* and *C. chinensis*, the shortest effective exposure was found for nitrogen concentration of 99%. Based on our laboratory tests, validation studies were subsequently carried out in semi-hermetic steel silos (25t) using continuous nitrogen saturation by on-site built swing pressure generators. It was found that a full control of all stages of *S. granarius* and *C. chinensis* was achieved in 11 days of nitrogen exposure, using concentrations ranging above 99% and below 100%. Our work shows that hypoxic nitrogen treatment can be effectively achieved in small steel silos under proper technological and environmental conditions.

Keywords: integrated pest management; controlled atmospheres; modified atmospheres; anoxia; hypoxia; stored product pests; phyto-sanitary treatment

Citation: Aulicky, R.; Shah, J.A.; Kolar, V.; Li, Z.; Stejskal, V. Control of Stored Agro-Commodity Pests *Sitophilus granarius* and *Callosobruchus chinensis* by Nitrogen Hypoxic Atmospheres: Laboratory and Field Validations. *Agronomy* **2022**, *12*, 2748. https://doi.org/10.3390/agronomy12112748

Academic Editor: Paraskevi Agrafioti

Received: 9 September 2022
Accepted: 3 November 2022
Published: 5 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Storage arthropods can cause multiple types of damage to agro-commodities during their storage [1], import and export [2]. Storage pests are also able to invade and spoil processed and packaged food [3–5]. In addition to economic quality damage, the infestation of commodities and processed foods by storage insects and mites is a problem in terms of their contamination of food with allergens [6,7]. Currently, storage pest risks are also increasing due to climatic temperature changes [8]. Another risk factor for effective pest control is the increasing resistance to phosphine, that is currently the most frequently used fumigant at a global scale [9–11]. The use of low temperatures [12], liquid nitrogen as freezing agents [13], alternative fumigants, ozone, or inert gases, are proposed as solutions [14,15]. In terms of societal requirements for residue-free exposure to products, yet effective pest control effects, inert gases and anoxic/hypoxic atmospheres are among the most promising [16,17]. Controlled atmospheres aim at creating a low-oxygen (hypoxia)

or zero-oxygen (anoxia) environment that is lethal to pest insects and mites. Anoxic and hypoxic atmospheres, as eco-friendly pest control solution, can be applied using different procedures or technologies in various types of stores and chambers [15,17,18], or commodity and food packaging [19–21]. Some types of anoxic and hypoxic atmospheres not only control pests, but they also may maintain the quality of the products [22,23]. The physiological effects of inert gases are in many aspects different from most other gaseous insecticides [24–26]. Thus, in addition to the ecological aspects, an advantage of inert gases is their effective action on pest populations that are already resistant to traditional insecticides. For example, Sakka et al. [27] and Agrafioti et al. [9], have recently demonstrated the high potential of hypoxic atmospheres to control populations of a number of phosphine-resistant storage pest species. In addition, controlled atmospheres are starting to be component of phyto-sanitary and phyto-quarantine treatment strategies [28,29].

Despite the above advantages of inert gases and controlled anoxic atmospheres, their use in practice may not be entirely straightforward, both from a technical and biological point of view. Regarding inert gases, and nitrogen in particular, a limited number of laboratory studies and practical applications are available. They describe their use in gas-tight chambers, laboratory experimental facilities and field stores and silos [30–37]. These works show the significant influence of different types of storage and the exposure regimes of inert gases, with respect to their effectiveness on different storage arthropod pest-species [38]. Commenting on the biological efficacy of anoxic atmospheres, Bailey and Banks [39] noted that the contemporary literature is apparently conflicting on the relative susceptibilities of various species, and the relative speed of action of N_2-oxygen or CO_2-oxygen mixtures. Even the more recent study by Liu [26] confirmed that there were considerable differences between stored product insect species and stages in susceptibility to low oxygen treatment. Not only pest species, but also various life stages of storage pests, may respond differently in terms of their susceptibility to various concentration of CO_2 and N_2 [26]. Contrary to what conventional intuition would suggest, Navarro [40] found that for certain storage pest species, a shorter controlled atmosphere (CA) exposure time is required if CA is composed of somewhat less than 100% nitrogen concentration. Therefore, depending on the particular pest species, effective nitrogen dosage and exposure must be maintained within a narrow range of effective concentrations [17]. The biological efficacy of CA is linked to the physical characteristics of the storage conditions and the technical characteristics of the stores. Differential gas tightness of various types of storage structures is among the key factors influencing procedures for the effective use of controlled atmospheres. Different construction types of commodity silos may also have different gas sorption characteristics, that influence the requirements for CA operational procedures. Technical structures and the method of filling inert gases affect the uniformity of their distribution within the treated objects [17,37]. Operational procedures of CA and exposure regimes can also be affected by the geographical location of the stores, as different areas have different thermal conditions. Areas with low ambient temperatures may require extended exposure times and increased gas consumption. Given the complexity of the practical usage of controlled atmospheres, Athanassiou [41] pointed out that there is still relatively little scientific documentation regarding usage inert gases for silo applications under field conditions. This implies that there is still a need to investigate the use of gases in different pest species and developmental stages, under different technical storage conditions and exposure regimes.

The present work was aimed at investigating the lethality of controlled hypoxic atmospheres on two species of primary pests of stored products, under laboratory conditions and in small silos. *Sitophilus granarius* L. (Curculionidae) and *Callosobruchus chinensis* L. (Chrysomelidae: Bruchinae) were selected as the tested species since they are important pests of grain/rice or legumes in Europe and Asia. Moreover, beetles of the genus *Sitophilus* and *Callosobruchus* are generally suitable model species for CA testing and validation, as they are among the less susceptible storage arthropods to anoxic atmospheres [42–45]. Based on the findings of Navarro [40], the specific objective of the laboratory part of the

work was to determine whether the tested pest species belong to the group of storage pests for whose control it is optimal to reach concentrations around 99%, or to the second group of species, for which the optimal control is 100% anoxia. Under laboratory conditions, we thus tested N_2 concentrations of gaseous nitrogen, on various developmental stages of *S. granarius* and *C. chinensis*. Jay [38] commented on the fact that there might not always be complete relevance between laboratory studies and the field application of CA to control stored-product insects. Therefore, based on our laboratory tests, validation studies were subsequently carried out in smaller semi-hermetic silos (25t) with continuous nitrogen saturation using on-site swing pressure nitrogen generators. Experiments were conducted in terms of the pest protection of imported agricultural commodities using standard shipping containers; therefore, tests were conducted in a set of small steel silos, each corresponding to the contents of one standard shipping container. The aim was to determine whether it was possible to achieve complete control of the tested pest species in 11 days under realistic operating conditions, or whether it was necessary to expose the pests for 21 days.

2. Materials and Methods

2.1. Tested Species and Cultures

The insects used for the study included two internally feeding stored-product Coleoptera species: *Sitophilus granarius* and *Callosobruchus chinensis*. Both populations (strains) tested were sensitive strains to commonly used insecticides, that were kept under laboratory conditions for at least 5 years. They were maintained in a rearing room at 25 °C and 75% RH. As feeding substrate was used wheat grain for *S. granarius* and mung beans for *C. chinensis*.

2.2. Laboratory Trials

2.2.1. Testing Apparatus

All pest species and their developmental stages were tested in a thermostatically controlled N_2 apparatus and the scheme is depicted in Figure 1. It is the modified version of the testing device proposed by Navarro et al. [45]. It consisted of 6 small plastic hermetic boxes (Lock and Lock HPL834, 2600 mL) in temperature 25 °C. These boxes were serially connected by transparent plastic hoses (PVC; diameter of 6/9 mm, Deutsch and Neumann GmbH; Hennigsdorf, Germany). The target nitrogen concentration (95%, 99% or 100%) was delivered from a pressurized metal cylinder (Linde Gas a.s.; Prague; Czech Republic) using a C200/2B-3SS outlet valve (Linde Gas a.s.; Czech Republic). A gas washing bottle containing an aqueous NaCl saturated solution was positioned between the metal cylinder and the initial small plastic box to humidity the nitrogen to 75% RH. The concentration of oxygen was determined using a GMH 3691 oxygen sensor (Greisinger Electronic GmbH; Regenstauf, Germany) connected behind the last experimental plastic box. The flow rate of nitrogen was measured using a Flow Sensor SFAB (Festo AG and Co. KG; Esslingen Germany).

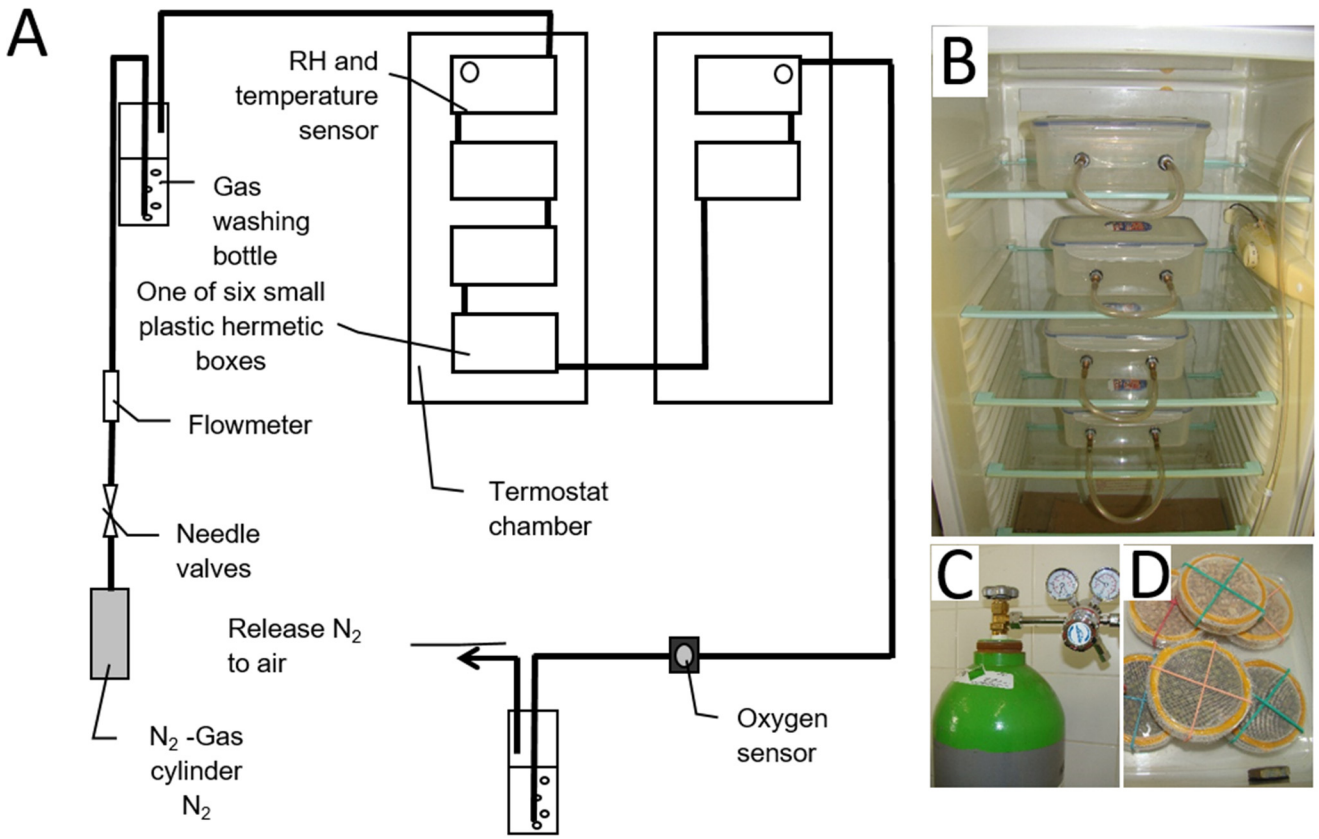


Figure 1. Testing apparatus for validation of nitrogen control atmospheres in laboratory conditions (**A**)—technical scheme; (**B**)—plastic boxes in a climatised thermostatic-chamber; (**C**)—source of N_2 (gas cylinders); (**D**)—bio-samples of pests and commodites in Petri dishes covered by mesh lid.

2.2.2. Bioassay Experimental Protocol

In the experiments, the efficacy was validated on two species, *S. granarius* and *C. chinensis*. Validation of efficacy was performed for all developmental stages (adult, pupa, larva and egg). Each development stage was tested separately. The immature development stages were prepared according to the modified methodology of Aulicky et al. [37] (certified methodology for controlling the effectiveness of controlled atmospheres and fumigation in silos using bio-tests), as follows: (i) Eggs—The adults of both species were placed in a breeding container with substrate for 72 h. Then, the adults were removed and the substrate with the deposited eggs was used for further testing. (ii) Larvae and pupae—Adults were placed in the breeding container with substrate for 1 week. Then, the adults were removed and the substrate was stored at 25 °C in a thermo-chamber. For *S. granarius*, the substrate was used for larvae between 21–35 days, and for pupae between 35–48 days. For *C. chinensis*, the substrate was used for larvae between 14–21 days and for pupae between 28–35 days.

The tested stages were placed together with a substrate weighing 15 g (*S. granarius*—wheat grain and *C. chinensis*—mung beans) in Petri dishes (diameter 60 mm) that had a mesh lid (diameter of the hole in the lid 50 mm). Fifty adult individuals were placed into each dish. The age of adults of *S. granarius* was 7–14 days and *C. chinensis* 1–5 days.

All pest species and stages were enclosed and exposed in small plastic hermetic boxes; each box contained 8 petri dishes; one dish from each species and stage. In each treatment, 6 small plastic boxes were connected in series and the application of nitrogen from a pressurized metal cylinder was started. After reaching the target oxygen concentration, the pests were separately exposed for 1, 4, 8, 12, 16 or 20 days. After each

exposure trial, the last small plastic box with the samples was always disconnected and the controlled atmosphere was vented. Subsequently, the treated box was placed in a thermostat with a temperature of 25 °C. Adult mortality was checked 4 days after the end of exposure. It was because it was previously described so called delayed mortality effect in some types of controlled atmospheres [44]. Check of efficacy for other developmental stages was performed using adult hatching. The inspections were carried out regularly once a week until the adults stopped hatching. The control samples were prepared in the same way as the experimental ones, but they were not exposed in boxes by hypoxic nitrogen atmosphere.

2.3. Field Silo Validation Trials

2.3.1. Silos and Source of N_2

Trials using N_2 were conducted in four identical steel silos with a capacity of 25 tons, fully loaded with a polished rice grain. Three silos were concurrently used as test silos filled with controlled nitrogen atmosphere (CA-N), whereas one silo was used as an untreated control without CA-N. The silos were shaded and thermally insulated by the roof and wall construction. The silos were not completely hermetic. However, they were sealed by glue (metal silo seals) and rubber (silo top and bottom covers) and they were equipped with a gastight press plenum through which the nitrogen was introduced. Due to these additional adaptations, we called the silos "semi-hermetic" further in the text. The silos were located inside the building of a food processing facility located in the Czech Republic (Podravka-Lagris a.s., south Moravia). The treated semi-hermetic silo bins included the discoid panel with a hole and plug for nitrogen filling at the top of the silo (Figure 2D). The hermetic discoid panel—located at the bottom of the silo—has a hole with a plug for nitrogen/oxygen concentration measurement (Figure 2B). For further technical description of semi-hermetic silos, see Aulicky et al. [37]. The nitrogen (N_2) controlled atmosphere was produced from two on-site installed sets based on Swing Pressure Nitrogen Generators (N_2—NITRO Source PSA, Parker) (Figure 2A). Each set consisted of the following units: compressor, air receiver, adsorption dryer, nitrogen buffer vessel, low-pressure N_2 storage unit and piping.

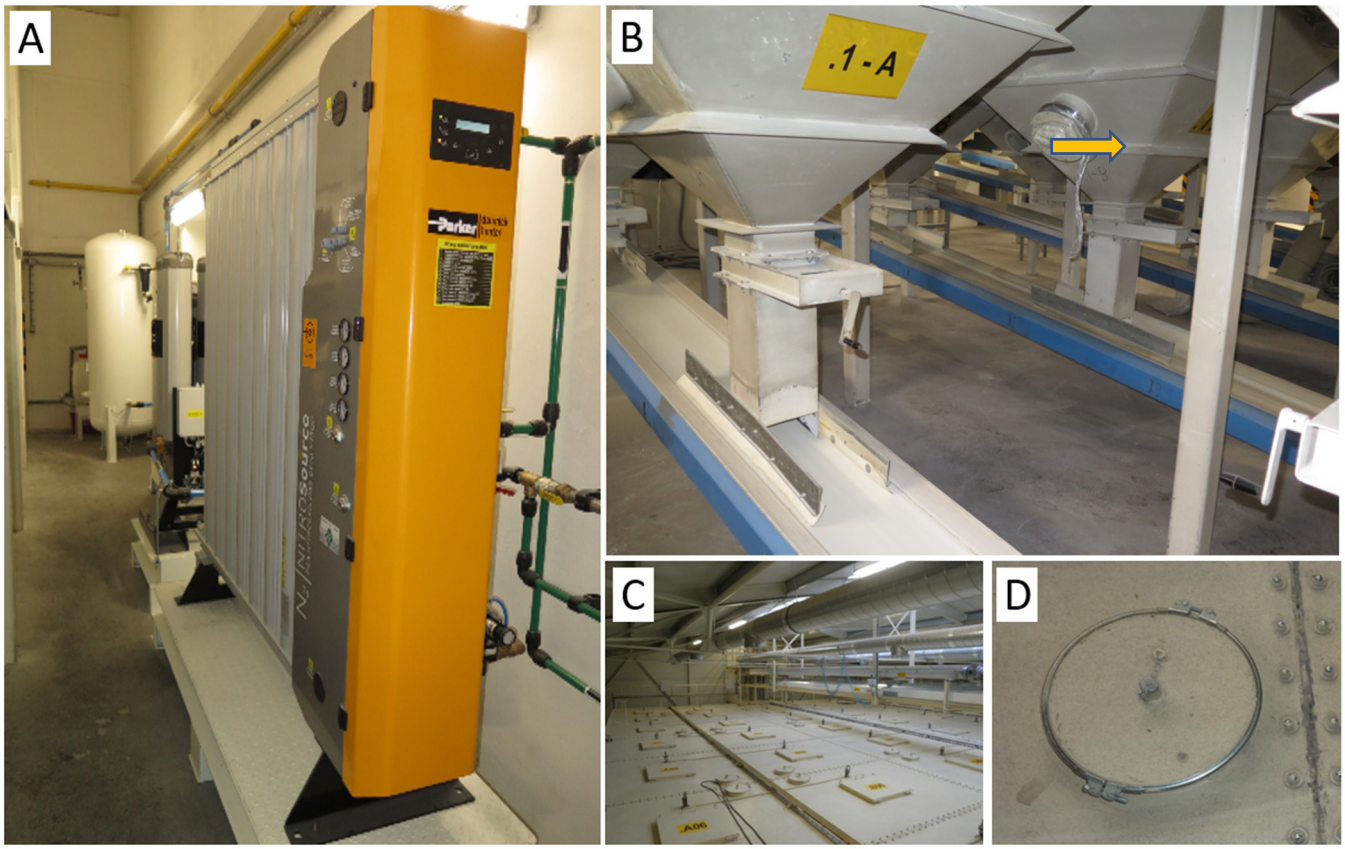


Figure 2. Visualisation of the validation of biological efficacy of nitrogen-controlled atmospheres in silos. (**A**)—the on-site- build pressure swing nitrogen generator (N_2—NITRO Source PSA—Parker) consisting of compressor, air receiver, adsorption dryer, nitrogen buffer vessel, low pressure N_2 storage unit and piping; (**B**)—hermetic removable discoid panel at the bottom of the silo with a hole and plug for nitrogen/oxygen measures; (**C**)—top board of silo bins with rectangular opening for loading the commodity and discoid panels; (**D**)—detail of a hermetic discoid panel with plug for nitrogen filling at the top of the silo.

2.3.2. Temperature, Humidity and Oxygen Level

Oxygen concentration measurements were performed with a Dräger X-am 7000 portable detector (Dräger, GmbH, Stuttgart, Germany). Temperature and RH were measured during treatment in a selected cell at the top using a TinyTag Ultra 2 digital data logger (TinyTag Ultra 2; Gemini Data Loggers Ltd., Chichester, UK). During the shorter exposure period (10 + 1 day), the mean temperature was 19.2 ± 0.1 °C (range: 18.8–20.8 °C) and RH was 52.1 ± 0.1 % (48.0–52.8 %), and during the longer exposure period (20 + 1 day), the mean temperature was 21.0 ± 0.1 °C (20.3–21.8 °C) and RH was 50.0 ± 0.1 % (34.3–50.8%).

2.3.3. Bioassay Exposure

Validation of the effectiveness of the controlled atmosphere with nitrogen was carried out in four metal silos (each capacity of 25 tonnes) containing polished rice -silos are described above. All developmental stages (adults, pupae, larvae and eggs) of the two primary pest species (*S. granarius* and *C. chinensis)* were used in the tests. Biological samples were prepared following the same procedure as for the laboratory tests (Section 2.2.2). In the case of adults, 20 individuals were used per dish. Samples were located in two positions in each silo. One group of samples was placed at the top and the other at the bottom of the silo unit. A total of 32 dishes (4 dishes for each species and developmental stage) were placed at each position in the case of CA-N treated cells. A

total of 48 dishes (6 dishes for each species and developmental stage) were placed in the control silo unit without CA-N treatment. Treatments were based on N_2 purging and N_2 maintenance phases. After the start of nitrogen application, there was a so-called purging or filling phase, which lasts 24 h. The target oxygen concentration was below 1% through the entire silo profile, including the bottom of the silo unit. Reaching this concentration, an exposure period of 10 or 20 days was calculated. Thus, the total exposure periods were either 11 days (1 day N_2 purging; 10 days of N_2 maintenance) or 21 days (1 day N_2 purging; 20 days of N_2 maintenance). During the treatment period, the oxygen concentration at the bottom of all silos was checked at regular intervals. At the end of each of both exposure periods, the differentially exposed samples were removed and transferred to the laboratory. The procedure for evaluating the biological efficacy of CA-N on the tested pest species and developmental stages was the same as described above for the laboratory trials.

2.4. Statistical Procedures

Data from the laboratory test were analyzed as a multifactorial experiment design for each species separately. In adults, concentration and exposure times were assessed for survival and mortality. For the other developmental stages (eggs, larvae and pupae), concentration, exposure time and developmental stage for adult pupation were evaluated. For statistical purposes, each species was evaluated separately. Exposure time and concentration were entered as factors in the ANOVA. Concentrations also included controls without nitrogen. Each exposure time included separate evaluation of two concentrations. Data were transformed and evaluated using parametric multi-factorial ANOVA test (Statistica statistical software, version 12; StatSoft CR s.r.o., Prague, Czech Republic). The homogeneity of the groups was further evaluated using the Tukey—Kramer HSD post-hoc test at the 0.05 level of significance.

3. Results

3.1. Laboratory Trial

Laboratory results showed that the effectiveness of three different concentrations of nitrogen atmospheres varied for the tested pest species and their developmental stages, depending on the duration of exposure. Parameters of ANOVA for the main effects of controlled atmospheres in *Sitophilus granarius* and *Callosobruchus chinensis* are summarized in Table 1. Table 2 shows the effect of various exposure times on adult mortality of *S. granarius* adults at three concentrations of nitrogen atmosphere. In the controlled atmosphere of 100% N_2, the complete adult mortality was achieved at an exposure time of 8 days, whereas for 99% N_2 atmosphere it required 12 days' CA-N exposure for the same biological efficacy. The lowest used concentration of nitrogen atmosphere (i.e., 95% N_2) failed to deliver complete adult mortality in even 20 days. Table 3 presents data on the emergence of adults from previously treated grain containing eggs, larvae, or pupae of *S. granarius* by two concentration of nitrogen atmosphere. Controlled atmosphere of 95% CA-N did not lead to suppression of all treated stages even within 20 days, except one case regarding pupae treatment. Both N_2 concentrations (i.e., 99 and 100%) completely suppressed the development of eggs and larvae equally rapidly. The complete inhibition of egg and larval emergence was achieved at 12 day exposure time at both concentrations. However, higher concentration of N_2 (100%) requires longer exposure time (16 days) to suppress development of pupae than the slightly lower N_2 concentration (99%) (12 days).

Table 1. Parameters of ANOVA for main effects related to mortality of the exposed adults of *Sitophilus granarius* (df = 180) and *Callosbruchus chinensis* (df = 180) or to *S. granarius* (df = 315) and *C. chinensis*—(df = 315). adult emergence following their previous N_2 exposure in sub-adult stages (egg, larva and pupa).

Species	Stages	Source	*df*	*F*	*p*
S. granarius	Adults	Exposure time	5	284.9	<0.001
		Concentration	5	3042.4	<0.001
		Exposure time × Concentration	25	77.8	<0.001
C. chinensis	Adults	Exposure time	5	981.2	<0.001
		Concentration	5	201.9	<0.001
		Exposure time × Concentration	25	77.1	<0.001
S. granarius	Eggs, larvae, pupae	Exposure time	6	696.2	<0.001
		Concentration	2	127.9	<0.001
		Stage	2	4.2	<0.016
		Exposure time × Concentration	12	15.5	<0.001
		Exposure time × Stage	12	11.4	<0.001
		Concentration × Stage	4	19.6	<0.001
		Exposure time × Concentration x Stage	24	2.1	<0.003
C. chinensis	Eggs, larvae, pupae	Exposure time	6	711.0	<0.001
		Concentration	2	150.8	<0.001
		Stage	2	104.2	<0.001
		Exposure time × Concentration	12	7.8	<0.001
		Exposure time × Stage	12	24.4	<0.001
		Concentration × Stage	4	54.6	<0.001
		Exposure time × Concentration x Stage	24	16.1	<0.001

Table 2. Laboratory efficacy (% of mean adult mortality; Av. % ± SE) of six exposure times (ranging from 1 to 20 days) and three concentrations (ranging from 95% to 100% N_2) nitrogen-based controlled atmospheres on the exposed adults of grain weevil (*Sitophilus granarius*). (Different letters indicate statistically significant differences between variables).

		Concentration		
Exposure Time		**100% N_2**	**99% N_2**	**95% N_2**
1 day	Exposed	73.7 ± 2.0 Aa	15.0 ± 2.1 Ba	4.3 ± 1.4 Ca
	Control	1.0 ± 1.0 Ab	0.7 ± 0.4 Ab	1.3 ± 1.0 Aa
4 days	Exposed	93.7 ± 2.0 Ac	79.3 ± 1.8 Bc	10.3 ± 2.3 Ca
	Control	2.3 ± 1.1 Ab	0.7 ± 0.4 Ab	3.0 ± 1.1 Aa
8 days	Exposed	100.0 ± 0.0 Ac	95.3 ± 1.4 Ad	20.7 ± 2.8 Bb
	Control	4.0 ± 1.6 Ab	4.7 ± 2.0 Ab	4.7 ± 1.4 Aa
12 days	Exposed	100.0 ± 0.0 Ac	100.0 ± 0.0 Ad	32.3 ± 4.3 Bc
	Control	4.0 ± 1.6 Ab	6.0 ± 1.8 Aab	6.0 ± 1.4 Aa
16 days	Exposed	100.0 ± 0.0 Ac	100.0 ± 0.0 Ad	65.7 ± 5.5 Bd
	Control	4.70 ± 1.5 Ab	6.7 ± 1.4 Aab	10.0 ± 1.9 Aa
20 days	Exposed	100.0 ± 0.0 Ac	100.0 ± 0.0 Ad	82.3 ± 2.0 Be
	Control	4.7 ± 1.5 Ab	6.7 ± 1.4 Aab	10.3 ± 2.2 Aa

Table 3. Laboratory efficacy (Average No. of adults emerged following their treatment in subadult stages; Av. No. ± SE) of six exposure times (ranging from 1 to 20 days) and three concentrations (ranging from 95% to 100% N_2) nitrogen-based controlled atmospheres on eggs, larvae and pupae of grain weevil (*Sitophilus granarius*). (Different letters indicate statistically significant differences between variables).

Development Stage	Exposure Time	Concentration		
		100% N_2	**99% N_2**	**95% N_2**
Eggs	1 day	27.8 ± 1.8 Aa	32.7 ± 2.8 Aa	41.8 ± 1.6 Ba
	4 days	22.7 ± 1.9 Aa	29.0 ± 2.6 ABab	35.5 ± 2.4 Bab
	8 days	19.5 ± 1.1 Aa	17.2 ± 3.4 Ab	33.3 ± 1.1 Bab
	12 days	0.0 ± 0.0 Ab	0.0 ± 0.0 Ac	26.8 ± 1.3 Bb
	16 days	0.0 ± 0.0 Ab	0.0 ± 0.0 Ac	17.8 ± 2.7 Bbc
	20 days	0.0 ± 0.0 Ab	0.0 ± 0.0 Ac	10.5 ± 1.3 Acd
	Control	41.0 ± 1.5 Ac	38.7 ± 2.7 Aa	45.5 ± 1.7 Aa
Larvae	1 day	37.5 ± 2.6 Aa	47.2 ± 1.4 Aa	39.2 ± 2.7 Aa
	4 days	22.0 ± 2.1 Ab	23.7 ± 2.4 Ab	35.8 ± 2.7 Ba
	8 days	2.5 ± 0.6 Ac	4.3 ± 1.2 Ac	25.8 ± 2.6 Bab
	12 days	0.0 ± 0.0 Ac	0.0 ± 0.0 Ac	21.5 ± 1.8 Bbc
	16 days	0.0 ± 0.0 Ac	0.0 ± 0.0 Ac	10.2 ± 1.6 Acd
	20 days	0.0 ± 0.0 Ac	0.0 ± 0.0 Ac	3.8 ± 1.5 Ad
	Control	46.5 ± 1.8 Aa	54.5 ± 1.3 Aa	45.7 ± 3.6 Aa
Pupae	1 day	45.2 ± 5.6 Aad	44.3 ± 1.9 Aa	42.0 ± 2.1 Aa
	4 days	37.5 ± 1.9 Aa	29.8 ± 3.2 Ab	35.8 ± 2.1 Aa
	8 days	18.5 ± 3.8 Ab	11.2 ± 2.4 Ac	20.8 ± 1.7 Ab
	12 days	3.8 ± 1.9 Abc	0.0 ± 0.0 Ac	18.3 ± 2.9 Bbc
	16 days	0.0 ± 0.0 Ac	0.0 ± 0.0 Ac	6.7 ± 0.9 Acd
	20 days	0.0 ± 0.0 Ac	0.0 ± 0.0 Ac	0.0 ± 0.0 Ad
	Control	52.2 ± 4.7 Ad	47.2 ± 1.9 Aa	45.3 ± 2.6 Aa

Table 4 shows adult mortality and Table 5 shows the emergence of adults from previously treated eggs/larvae/pupae of *C. chinensis* following their exposure to three concentration of controlled nitrogen atmosphere (CA-N). For 99% and 100% CA-N the complete *C. chinensis* adult mortality was reached in 8 days, whereas 95% CA-N required 12 days of exposure. However, the latter was associated with 100% mortality in the untreated control since *C. chinensis* adults are short-lived. Controlled nitrogen atmosphere (CA-N) achieved more rapid action (12 days) at a slightly lower CA-N concentration (99% N_2) than at the highest concentration (100% N_2) (16 days) in all immature stages except pupae. At a CA-N concentration of 99% N_2, 100% larval mortality was attained at the 12 day exposure, while at a higher concentration up to the 16 day exposure. Similar to *S. granarius*, the lowest used concertation of CA-N (95%) did not lead to the suppression of all treated stages of *C. chinensis* within 20 days of exposure, except in one case regarding pupae treatment.

Table 4. Laboratory efficacy (% of mean adult mortality; Av. % ± SE) of six exposure times (ranging from 1 to 20 days) and three concentrations (ranging from 95% to 100% N2) nitrogen-based controlled atmospheres on the exposed adults of adzuki bean weevil (*Callosobruchus chinensis*). (Different letters indicate statistically significant differences between variables).

Exposure Time		Concentration		
		100% N_2	99% N_2	95% N_2
1 day	Exposed	97.0 ± 1.7 Aa	40.0 ± 2.1 Ba	27.7 ± 3.8 Ca
	Control	76.3 ± 3.7 Ab	26.0 ± 1.8 Bb	18.3 ± 3.3 Bb
4 days	Exposed	100.0 ± 0.0 Aa	84.0 ± 2.3 Bc	44.0 ± 3.3 Cc
	Control	87.7 ± 3.2 Ac	78.7 ± 1.4 Ac	36.0 ± 3.6 Bbc
8 days	Exposed	100.0 ± 0.0 Aa	100.0 ± 0.0 Ad	90.0 ± 2.8 Bd
	Control	100.0 ± 0.0 Aa	100.0 ± 0.0 Ad	87.0 ± 2.4 Bd
12 days	Exposed	100.0 ± 0.0 Aa	100.0 ± 0.0 Ad	100.0 ± 0.0 Ae
	Control	100.0 ± 0.0 Aa	100.0 ± 0.0 Ad	100.0 ± 0.0 Ae
16 days	Exposed	100.0 ± 0.0 Aa	100.0 ± 0.0 Ad	100.0 ± 0.0 Ae
	Control	100.0 ± 0.0 Aa	100.0 ± 0.0 Ad	100.0 ± 0.0 Ae
20 days	Exposed	100.0 ± 0.0 Aa	100.0 ± 0.0 Ad	100.0 ± 0.0 Ae
	Control	100.0 ± 0.0 Aa	100.0 ± 0.0 Ad	100.0 ± 0.0 Ae

Table 5. Laboratory efficacy (Average No. of adults emerged following their treatment in subadult stages; Av. No. ± SE) as of six exposure times (ranging from 1 to 20 days) and three concentrations (ranging from 95% to 100% N_2) nitrogen-based controlled atmospheres on eggs, larvae of adzuki bean weevil (*Callosobruchus chinensis*). (Different letters indicate statistically significant differences between variables).

Development Stage	Exposure Time	Concentration		
		100% N_2	99% N_2	95% N_2
Eggs	1 day	9.2 ± 1.2 Aa	18.7 ± 1.2 a	34.7 ± 1.6 Bad
	4 days	5.2 ± 1.4 Aa	16.5 ± 2.2 Ba	29.5 ± 1.8 Cab
	8 days	6.0 ± 1.3 Aa	9.5 ± 1.8 Aab	22.7 ± 1.8 Bb
	12 days	3.7 ± 1.4 Aa	0.0 ± 0.0 Ab	10.0 ± 1.3 Ac
	16 days	0.0 ± 0.0 Aa	0.0 ± 0.0 Ab	7.7 ± 1.1 Ac
	20 days	0.0 ± 0.0 Aa	0.0 ± 0.0 Ab	1.0 ± 0.5 Ac
	Control	21.3 ± 2.7 Ab	33.00 ± 0.9 Bc	43.3 ± 2.1 Bd
Larvae	1 day	54.3 ± 4.5 Aa	29.0 ± 1.3 Ba	37.0 ± 2.3 Ba
	4 days	22.5 ± 2.3 Ab	21.7 ± 1.3 Aa	30.0 ± 1.8 Aab
	8 days	18.7 ± 2.3 ABb	8.7 ± 3.9 Ab	21.5 ± 0.4 Bbc
	12 days	1.5 ± 0.62 Ac	0.0 ± 0.0 Ab	14.0 ± 1.6 Bc
	16 days	0.0 ± 0.0 Ac	0.0 ± 0.0 Ab	6.7 ± 1.0 Acd
	20 days	0.0 ± 0.0 Ac	0.0 ± 0.0 Ab	5.8 ± 1.1 Acd
	Control	63.2 ± 3.5 Aa	55.7 ± 2.1 ABc	51.5 ± 1.7 Be
Pupae	1 day	31.5 ± 5.3 Aa	15.2 ± 1.4 Ba	29.3 ± 0.9 Aa
	4 days	47.2 ± 3.6 Ab	6.3 ± 0.7 Bab	25.0 ± 1.7 Ca
	8 days	11.8 ± 1.9 Ac	2.5 ± 0.8 Ab	22.8 ± 2 Ba
	12 days	0.8 ± 0.5 Ad	0.0 ± 0.0 Ab	8.5 ± 0.9 Ab
	16 days	0.5 ± 0.4 Ad	0.0 ± 0.0 Ab	3.2 ± 0.6 Ab
	20 days	0.0 ± 0.0 Ad	0.0 ± 0.0 Ab	0.00 ± 0.0 Ab
	Control	52.7 ± 2.8 Ab	30.8 ± 4.7 Bc	49.3 ± 1.3 Ac

3.2. Field Trials in Silos

The initial N_2 purging phase of nitrogen into the commodity-filled silos required 24 h for both types of exposures, i.e., 1 + 10 or 1+ 20 days. The average nitrogen consumption

was 148.5 L*min^{-1} in the shorter exposure time and 147.3 L*min^{-1} in the longer exposure time. From Figures 3 and 4, it is apparent that in all silos oxygen concentrations below 2% was achieved (at the bottom of each silo unit) within 12 h, and it took a further 12 h to achieve oxygen concentrations below 1%. In the subsequent saturation and maintenance phase, despite slight diurnal fluctuations in temperature and atmospheric pressure, oxygen concentrations between 0% and 1% were maintained for both the 10 day exposure (Figure 3) and the 20 day exposures (Figure 4).

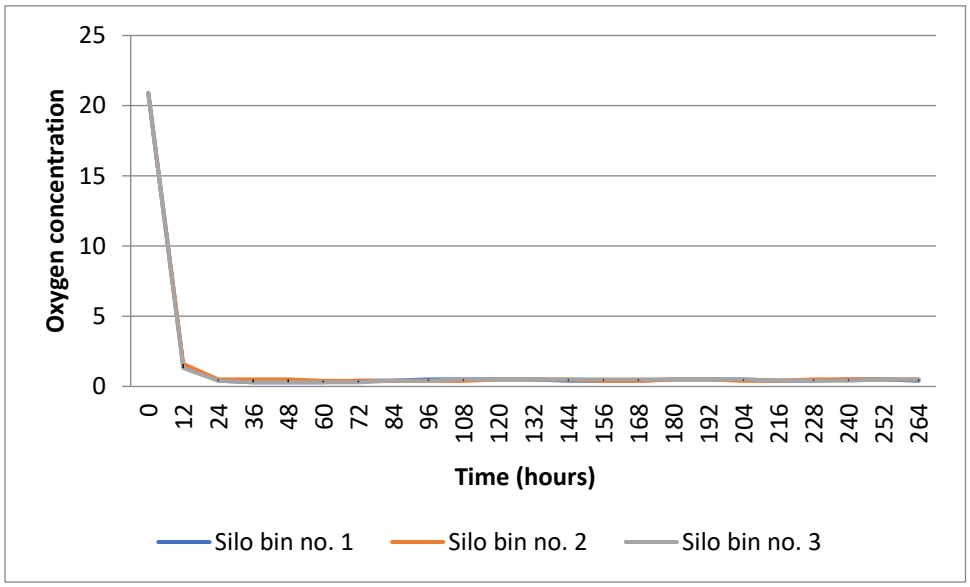


Figure 3. Oxygen concentration during field test—10 + 1 days of exposure (n = 3 silo units); 1 day N_2 purging; 10 days of full N_2 concentration exposure. The average oxygen concentration after initial 12 h nitrogen purging was 1.5 ± 0.1%; the average oxygen concentration for the 10 days of nitrogen maintenance was 0.4 ± 0.0%.

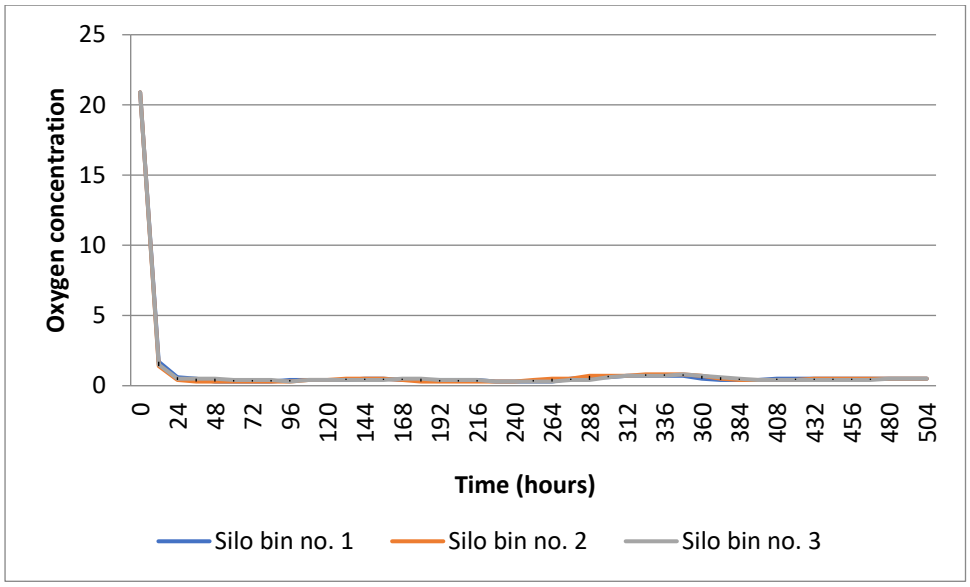


Figure 4. Oxygen concentration during field test—20 + 1 days of exposure (n = 3 silo units); 1 day N_2 purging; 20 days full N_2 concentration exposure. The average oxygen concentration after initial 12 h nitrogen purging was 1.5 ± 0.1%; the average oxygen concentration for the 20 days of nitrogen maintenance was 0.5 ± 0.0%.

The data summary on the mortality of the controlled nitrogen's atmospheres (below 1% of oxygen) on adults of *S. granarius* and *C. chinensis* in metal silos, under two exposure

regimes of 10 or 20 days, can be found in Table 6. Table 7 shows the efficacy of the tested nitrogen atmospheres on eggs, larvae and pupae of both tested species. All field validation tests, carried out in small semi-hermetic silos, showed 100% mortality (Table 6) or 100% development suppression (Table 7) in both exposure regimes and all tested species and their developmental stages under given conditions.

Table 6. Field efficacy (% of adult mortality; Av. % ± SE) of controlled nitrogens atmospheres (below 1% of oxygen) on adults of the tested species *(S. granarius, C. chinensis*) in metal silos under two exposure regimes 11 (1 day N_2 purging; 10 days full N_2 concentration exposure) or 21 days. For each treated silo units (n = 3 silos) 4 bio-assay-dishes with insects were used.

			Exposure Time	
Species	**Sample**	**n**	**11 Days**	**21 Days**
S. granarius	TS Upper	3	100.0 ± 0.0	100.0 ± 0.0
	TS Lower	3	100.0 ± 0.0	100.0 ± 0.0
	Control—Lower position	1	6.7 ± 2.8	1.7 ± 1.1
C. chinensis	TS Upper	3	100.0 ± 0.0	100.0 ± 0.0
	TS Lower	3	100.0 ± 0.0	100.0 ± 0.0
	Control—Lower position	1	96.7 ± 1.1	99.2 ± 0.8

Table 7. Field efficacy (Average No. of adults emerged following their treatment in subadult stages; Av. No. ± SE*)* of controlled nitrogens atmospheres (below 1% of oxygen) on eggs, larvae and pupae of the tested species in metal silos under two exposure regimes of 11 or 21 days. For each treated silo units (n = 3 silos) 4 bio-assay-dishes with insects were used.

				Exposure Time	
Species	**Stage**	**Sample**	**n**	**11 Days**	**21 Days**
S. granarius	Pupae	TS Upper	3	0.0 ± 0.0	0.0 ± 0.0
		TS Lower	3	0.0 ± 0.0	0.0 ± 0.0
		Control—Lower position	1	4.7 ± 0.5	9.3 ± 1.5
C. chinensis		TS Upper	3	0.0 ± 0.0	0.0 ± 0.0
		TS Lower	3	0.0 ± 0.0	0.0 ± 0.0
		Control—Lower position	1	29.7 ± 1.9	22.0 ± 3.8
S. granarius	Larvae	TS Upper	3	0.0 ± 0.0	0.0 ± 0.0
		TS Lower	3	0.0 ± 0.0	0.0 ± 0.0
		Control—Lower positon	1	5.2 ± 0.6	23.0 ± 1.9
C. chinensis		TS Upper	3	0.0 ± 0.0	0.0 ± 0.0
		TS Lower	3	0.0 ± 0.0	0.0 ± 0.0
		Control—Lower position	1	16.3 ± 1.4	36.3 ± 5.6
S. granarius	Eggs	TS Upper	3	0.0 ± 0.0	0.0 ± 0.0
		TS Lower	3	0.0 ± 0.0	0.0 ± 0.0
		Control—Lower position	1	6.8 ± 1.0	9.3 ± 1.3
C. chinensis		TS Upper	3	0.0 ± 0.0	0.0 ± 0.0
		TS Lower	3	0.0 ± 0.0	0.0 ± 0.0
		Control—Lower position	1	124.3 ± 27.9	33.0 ± 9.7

4. Discussion

Good technological practice for commodity storage on farms, and their onward export and import, requires the availability of robust, rapid and environmentally safe pest control procedures. Compared to toxic fumigants, the use of modified atmospheres (= controlled atmospheres or hermetic storage) is considered challenging in terms of the requirements for relatively long exposure times [16,17,46,47]. For a long-term storage of agricultural commodities at large farms or national strategic reserves facilities, the length of exposure to controlled atmospheres used in silos is usually not a major technological

constraint [26]. However, the length of exposure to controlled atmospheres can play a significant role in the short-term storage of commodities on small farms with limited storage capacity, in ports [39], or in commercial organizations that process agro-commodities (cereals, rice, legumes, dry/dried fruits) into small food packages for retail sales [44,48]. For such companies, minimizing concentrations and exposure duration while achieving high efficacy is an essential constraint for the implementation of controlled atmospheres for routine practical use. This work therefore addressed the problem of optimization of the length of exposure by hypoxic nitrogen atmospheres for the control of two important storage pests, under laboratory and small-silos conditions.

4.1. Laboratory Trials

In the laboratory part of our work, new biological data were obtained regarding the biological efficacy of controlled nitrogen atmospheres on *C. chinensis* and *S. granarius* species under different exposure lengths (1–20 days) and different concentrations (95–100%). From a practical point of view, an important finding of our work was that for most of the tested exposures up to 20 days, the concentrations 99% and 100% nitrogen were highly effective for the control of all developmental stages (eggs, larvae, adult pupae) of both species. In contrast, at 95% nitrogen concentrations, 100% mortality was not achieved for all life stages of both species tested for most of the tested exposure times. The published work on laboratory exposures of *C. chinensis* and *S. granarius* with controlled atmospheres is relatively sparse [49–55]. As a result, a direct comparison of our results with data from other authors is also relatively limited. Cui et al. [49] investigated survival, development time delay and metabolomics changes in larvae (4th instar) of *C. chinensis* under two hypoxia regimes (2% O_2 = hypoxia or 2% O_2 + 18% CO_2 = hypoxia/hypercapnia). They found that the development of *C. chinensis* was significantly suppressed by both hypoxia conditions and showed profound differences in metabolites between the treatment and control groups. Cui et al. [49] concluded that *C. chinensis* has high tolerance to hypoxia since some proportion of 4th-instar larvae survived (i.e., emerged as adults after 24 days) more than 20 days under hypoxia/hypercapnia. In our experiments, we achieved 100% mortality of *C. chinensis* larvae when exposed in nitrogen atmosphere 99% for 12 days. However, the larval survival and adult emergence of *C. chinensis* was observed for all tested exposure times at 95% nitrogen atmosphere. Regarding *S. granarius*, Lindgren and Vincent [50] reported 95% mortality of *S. granarius* adults after 5.3 day exposure by 100% nitrogen exposure (70 °F and 60–70% RH). Adler [54] observed 99% mortality from 10 strains of *S. granarius* in 6.6 days (20 °C and 75–99% nitrogen and 1% oxygen). The results by Adler [54,55] are comparable with the results obtained later by Conyers and Bell [56] on the sensitive and resistant strains of *S. granarius* (99.3–100% mortality, in 8 days at 99% nitrogen atmosphere- 50% RH and 20 °C), and with the results obtained in our study (100 % mortality in 8 days at 100% nitrogen atmosphere, in 12 days at 99% nitrogen atmosphere).

Although direct comparison with available literature on the tested species is limited, it is still interesting to compare our results with abundant publications concerning the effectiveness of controlled atmospheres on the related pest species. They mainly include *Callosbruchus maculatus* [44,57–62], *Acanthoscelides obtectus* [57,63], *Sitophilus zeamais* [34], and *S. oryzae* [33,35]. Comparisons of data on *C. chinensis* and *S. granarius* with data on related species allow further generalization of the biological effects of inert gases on pests [24,64], and thus the construction of generally applicable atmosphere-controlled technologies and procedures for stored product pests [17]. Our laboratory data show that *C. chinensis* and *S. granarius* adults were the most susceptible developmental stages, which is in agreement with many findings obtained by other authors for several species of storage pests. For example, Hashem et al. [63] found that hypoxia/hypercapnia effects were more severe in adult bruchids *A. obtectus*, which led to 100% mortality after 3 days of exposure, but the full hatchability suppression required 7 days. However, for *S. zeamais,* Williams et al. [35] found that not only adult stages but also eggs were more sensitive to

anoxia than larval and pupal stages. The comparison of our data with the previously published results also suggested that the tested species of internal-feeding pests might be less sensitive to controlled atmospheres compared to some species of externally-feeding pests, such as *Tribolium* sp. and *Oryzaephilus* sp. [37,44,56,65,66]. For example, Sakka et al. [44] found that *C. maculatus* is more tolerant to nitrogen atmosphere than *T. castaneum*. They hypothesized that the difference in their sensitivity could be because the immature development of *C. maculatus* occurs in the inner part of the seeds, where nitrogen penetration may be more gradual, and the *T. castaneum* individuals are more directly exposed to nitrogen. However, several authors have shown that some externally developing pests, such as *Cryptolestes* spp. and *Trogoderma* genera [29,44,46,56,66], can exhibit tolerance to controlled hypoxic/anoxic atmospheres. For example, Conyers and Bell [56] found that adults of *C. ferrugineus* appeared to be most tolerant to an atmosphere with 1% oxygen, followed by *S. granarius*, while adults of *O. surinamensis* were the most sensitive. Moncini et al. [33] reported that an atmosphere containing 98.5% N_2 caused the complete mortality of *S. oryzae* adults after 3 days of exposure on wheat kernels, whereas the complete mortality of *T. confusum* adults required 7 days on flour. This is surprising, because it was previously reported [65] that externally developing *T. confusum* was very sensitive to nitrogen atmosphere. In this case, the question is whether sensitivity may be influenced not only by pest species, but also by the type of food commodity, such as grain or flour [33]. All the discrepancies found in the above-mentioned published works suggest that the affiliation of a particular species of a storage pest to an ecotype, with development inside or outside the seed commodity, is not a completely reliable predictor of its sensitivity or tolerance to anoxic/hypoxic atmosphere. In addition, the complex biotic and abiotic relationships discussed above indicate that practical procedures and exposure regimes for the use of a controlled atmosphere in practice should be more or less specific to each pest species.

Another practical objective of our laboratory work was to explore the mortality-concertation relationship in the tested species for the 95%-100% range of atmospheric nitrogen concentrations. Navarro and Navarro [17] pointed out that effective nitrogen dosage and exposure must be relatively precise and within a certain range of concentrations, because mortality may not have a positive linear relationship with increasing nitrogen concentration over the entire range of values. The basis for this caveat was previous laboratory experiments conducted by Navarro [40]. He found that, contrary to intuitive expectation, the necessary exposure time for some species increases when the atmospheric oxygen concentration approaches zero. In other species, however, 100% anoxia may be more effective than various levels of hypoxia. For example, Navarrro [40] described that *Ephestia cautella* pupae and *Tribolium castaneum* adults reacted to increasing concentrations of N_2 in a similar pattern, whereas *S. oryzae* adults reacted in a different way. The lower the oxygen concentration was, the shorter the exposure time needed to produce 95% mortality of *E. cautella* pupae and *T. castaneum*. However, *S. oryzae* adults showed greater sensitivity at 1% oxygen than at the zero level of oxygen concentration. In our study, we found that responses to different oxygen concentrations differed not only between the two species tested, but also among developmental stages within a species. In *S. granarius*, lethal exposure was shorter at 100% nitrogen concentration than at the lower concentration (99%), but the opposite was true for the pupal stage of this species. For the species *C. chinensis*, the exposure time leading to 100% mortality was the same for the 99% and 100% concentrations, whereas for the immature stage, the exposure time leading to 100% mortality was higher for the lower of the two concentrations tested, 99%.

4.2. Field Validations

The required effective pest control exposures of commodities to hypoxic atmospheres can exceed one month, depending on the technological and environmental conditions in commodity stores and silos [35]. However, such long exposure times may not be acceptable for many companies for operational and economic reasons [44]. Users

of controlled atmosphere usually balance between minimizing the length of the hypoxic exposure of infested commodities and maximizing its biological efficacy on pests. Unfortunately, practitioners frequently do not have detailed information to optimize these control processes for many pest species when controlling them in small silos. Most practical validations are either from large silos [67,68], laboratory small silos [34], mini-silos [35], glass desiccators [56] or hermetic chambers [44,48]. Published data regarding the use of controlled atmospheres for the treatment of commodities in smaller silos (25t) is lacking, or is very limited [35,37,69]. At the same time, detailed technological information is very important because the treatment of small silos is challenging; even small gas leaks cause a significant reduction in the concentration of inert gases [17,70]. For potential users, compensating for nitrogen leakage from silos by continuously saturating the silo-atmosphere with inert gases may seem economically and operationally demanding. However, our fieldwork has shown that good results can be achieved even in small metal silos in the normal operating conditions of a commercial commodity store (Podravka-Lagris, CZ). Our validations in these silos demonstrated that these types of silos could be saturated by the 98% nitrogen within 12 h, and a target hypoxic concentration above 99% can be well achieved within 24 h. It was further demonstrated that under a period of 10–20 days, a relatively stable level of over 99% nitrogen can be maintained; oxygen concentration oscillating between 0.3–0.7%. However, it should be noted that the treated silos were located indoors. Thus, the large fluctuations in temperature and the effects of direct sun-glare were reduced, that likely buffered large fluctuations in controlled atmosphere concentrations. Indeed, other authors have documented that the method of application, the type of facility and/or temperature/sunshine fluctuations can cause concentration fluctuations and uneven distribution of inert and toxic gases in treated stores, chambers and transport vehicles [11,44,71,72]. Regarding the importance of the necessity of an even concentration distribution of nitrogen in the treated chamber, Sakka et al. [44] stressed that: "pest location is a crucial parameter that may determine the insecticidal effect of nitrogen. In our study, we saw that despite variations in insect survival in the different locations, some of the insects that survived we found in the vials that had been placed in the "heart" of the pallet. This is somehow expected, as nitrogen penetrates into the pallet from the outside to the inside, and thus, "oxygen nests" can be created in the internal part of the product mass." From this point of view, it would be interesting to compare our results (concerning the stability and concentration variations of a controlled hypoxic atmosphere) with experiments carried out in small silos located outside shielded and tempered spaces, in the future experiments.

Concurrently with the validation of nitrogen filling and concentrations maintenance in the silos, bio tests were placed in the upper and lower parts of the silos. This is because our previous work [37] revealed that there might be differences in the biological efficacy achieved between the top and bottom of the silo when filling silos by nitrogen from top to bottom, and using very short exposures. However, in the current validation experiments, it was shown that both exposure regimes (11 and 21 days) resulted—under given environmental conditions—in 100% control of all developmental stages of *C. chinensis* and *S. granarius* at both the top and bottom of the treated silos. Thus, these tests yielded optimistic results, acceptable for the use of relatively short exposure times of controlled atmospheres in small silos. On-site nitrogen production using a swing-pressure-generator is economically challenging; especially in the current global energy crisis. Thus, the energy savings made possible by short exposure times, and thus reduced nitrogen production needs, is a very important and timely result of this work for practice. However, we believe that the results obtained for *C. chinensis* and *S. granarius* still need to be validated for other species. Indeed, there is an experimentally supported suspicion that some species, such as *T. granarium,* may be highly tolerant to hypoxic [44,46] and hypercarbic [46] atmospheres. To control resistant species such as *T. granarium*, it will likely be necessary to increase the length of exposure, manipulate temperature/pressure [48,73], or

use a combination of stressors such as radiation and controlled atmospheres [29]. Other aspects that merit future research attention may be effects caused by differences in the sensitivity of different geographic strains of pests to anoxic and hypoxic atmospheres. The differences in the tolerance of different strains of pests to controlled atmospheres has been noted by Adler [54] and Conveyers and Bell [56], who reported differences in the responses to hypoxia of *S. granarius* populations from different countries and geographic regions.

5. Conclusions

Hypoxic or anoxic atmospheres belong among promising and environmentally acceptable methods of effective stored product pest control, both for farmers and for international phyto-quarantine services [16,74,75]. Moreover, they have the potential for the effective control of pest populations resistant to traditional fumigants, such as phosphine. In this paper, data were presented regarding both the laboratory and field efficacy of hypoxic atmospheres on different developmental stages of two primary key pest species (*C. chinensis* and *S. granarius*) with intra-grain immature development. The laboratory data indicated that 10–20 days' exposure resulted in 100% control of all developmental stages of *C. chinensis* and *S. granarius* when nitrogen concentrations was maintained between 99% and 100%, under defined temperature and humidity. Hypoxic nitrogen concentrations of 99% had even higher efficacy than completely anoxic atmospheres in the laboratory conditions tested. However, the 95% nitrogen concentration atmosphere did not result in 100% control of both species at the exposures given, even under controlled laboratory conditions for exposures of up to 20 days. Experimental validation in the silos, located indoors without large temperature fluctuations and direct sunshine-warming effects, showed that a targeted hypoxic concentration above 99% could be well achieved within 1 day of nitrogen initial purging, and stably maintained for the consequent 10–20 days. Bio tests indicated that both exposure regimes (10 + 1 and 20 + 1 days) resulted in 100% control of all developmental stages of *C. chinensis* and *S. granarius* under the given environmental conditions. Therefore, the use of nitrogen atmospheres in smaller silos for relatively short exposure periods can be considered a promising effective method for controlling *C. chinensis* and *S. granarius* species in small silos equivalent to the capacity of a standard shipping container. Thus, the results are of interest both for the protection of stored commodities on small farms, and for commercial organizations storing/treating separately a supply of commodities corresponding to the size of standard shipping containers. However, it is recommended to extend the tests and validations to other pest species and geographical strains/populations as they may have different sensitivities [56,66].

Author Contributions: conceptualization—V.S., R.A., Z.L. and V.K.; methodology—R.A., V.K., Z.L. and V.S. laboratory and field validation, R.A., Z.L., V.K. and V.S.; resources—V.S., Z.L., writing—original draft preparation, V.S., R.A., J.A.S., Z.L. and V.K.; writing—review and editing, V.S., R.A., J.A.S., Z.L. and V.K.; supervision—V.S. and Z.L.; project administration—R.A.; funding acquisition V.S., Z.L., V.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the following projects: VZ-RO014 provided by Ministry of Agriculture of the Czech Republic—R.A., V.S; International Collaboration project Inter-Action—LTACH19029 provided by MSMT of the Czech Republic and—R.A., J.S., V.K., V.S. and Key Program of National Key Research and Development Project in China (No. 2018YFE0108700) Z.L. JAS was supported as a PhD student by the Czech University of Life Sciences Prague. Part of processing expenses of the MS were covered HR Award project: Strengthening strategic management of science and research in the CRI, p.r.i., project registration no.: CZ.02.2.69/0.0/0.0/18_054/0014700.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable

Data Availability Statement: Not applicable.

Acknowledgments: We thank T. Vendl and M. Frankova for critical reading of the MS. We also thank C. Moravčík (Lagris Podravka) and S. Matatkova (CRI) for their excellent technical help.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Stathers, T.; Lamboll, R.; Mvumi, B.M. Postharvest agriculture in changing climates: Its importance to African smallholder farmers. *Food Secur.* **2013**, *5*, 361–392.
2. Stejskal, V.; Vendl, T.; Kolar, V.; Li, Z.; Aulicky, R. First population quantification of the infestation of legumes by stored-product bruchids imported in freight containers into Europe. *Bull. Insectol.* **2020**, *73*, 233–239.
3. Limonta, L.; Savoldelli, S.; Suss, L.; Locatelli, D.P. Pest detected in packed food: Ten years of analysis. *Ital. J. Food Sci.* **2016**, *28*, 440–447.
4. Trematerra, P.; Stejskal, V.; Hubert, J. The monitoring of semolina contamination by insect fragments using the light filth method in an Italian mill. *Food Control* **2011**, *22*, 1021–1026.
5. Stejskal, V.; Bostlova, M.; Nesvorna, M.; Volek, V.; Dolezal, V.; Hubert, J. Comparison of the resistance of mono- and multilayer packaging films to stored-product insects in a laboratory test. *Food Control* **2017**, *73*, 566–573.
6. Hubert, J.; Erban, T.; Nesvorna, M.; Stejskal, V. Emerging risk of infestation and contamination of dried fruits by mites in the Czech Republic. *Food Addit. Contam. Part A* **2011**, *28*, 1129–1135.
7. Hubert, J.; Stejskal, V.; Athanassiou, C.G.; Throne, J.E. Health Hazards Associated with Arthropod Infestation of Stored Products. *Annu. Rev. Entomol.* **2018**, *63*, 553–573.
8. Adler, C.; Athanassiou, C.; Carvalho, M.O.; Emekci, M.; Gvozdenac, S.; Hamel, D.; Riudavets, J.; Stejskal, V.; Trdan, S.; Trematerra, P. Changes in the distribution and pest risk of stored product insects in Europe due to global warming: Need for pan-European pest monitoring and improved food-safety. *J. Stored Prod. Res.* **2022**, *97*, 101977.
9. Agrafioti, P.; Kaloudis, E.; Athanassiou, C.G. Utilizing low oxygen to mitigate resistance of stored product insects to phosphine. *J. Sci. Food Agric.* **2022**, *102*, 6080–6087. https://doi.org/10.1002/jsfa.11960.
10. Nayak, M.K.; Daglish, G.J.; Phillips, T.W.; Ebert, P.R. Resistance to the Fumigant Phosphine and Its Management in Insect Pests of Stored Products: A Global Perspective. *Annu. Rev. Entomol.* **2020**, *65*, 333–350.
11. Aulicky, R.; Stejskal, V.; Frydova, B. Field validation of phosphine efficacy on the first recorded resistant strains of *Sitophilus granarius* and *Tribolium castaneum* from the Czech Republic. *J. Stored Prod. Res.* **2019**, *81*, 107–113.
12. Stejskal, V.; Vendl, T.; Li, Z.; Aulicky, R.; Li, Z. Minimal Thermal Requirements for Development and Activity of Stored Product and Food Industry Pests (Acari, Coleoptera, Lepidoptera, Psocoptera, Diptera and Blattodea): A Review. *Insects* **2019**, *10*, 149.
13. Li, H.Y. Potential Use of Liquid Nitrogen Aeration on Wheat Disinfestation. Master's Thesis, Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada, 2008.
14. Fields, P.G.; White, N.D.G. Alternatives to Methyl Bromide Treatments for Stored-Product and Quarantine Insects. *Annu. Rev. Entomol.* **2002**, *47*, 331–359.
15. Stejskal, V.; Vendl, T.; Aulicky, R.; Athanassiou, C. Synthetic and Natural Insecticides: Gas, Liquid, Gel and Solid Formulations for Stored-Product and Food-Industry Pest Control. *Insects* **2021**, *12*, 590.
16. Adler, C.; Corinth, H.G.; Reichmuth, C. Modified atmospheres. In *Alternatives to Pesticides in Stored-Product IPM*; Springer: New York, NY, USA, 2000; pp. 105–146.
17. Navarro, S.; Navarro, H. Advances in insect pest management in postharvest storage of cereals: Use of controlled atmosphere and temperature control. In *Advances in Postharvest Management of Cereals and Grains*; Maier, D.E., Ed.; Burleigh Dodds Science Publishing Limited: Cambridge, UK, 2020; p. 478.
18. Carvalho, M.O.; Pires, I.; Barbosa, A.; Barros, G.; Riudavets, J.; Garcia, A.C.; Navarro, S. The use of modified atmospheres to control *Sitophilus zeamais* and *Sitophilus oryzae* on stored rice in Portugal. *J. Stored Prod. Res.* **2012**, *50*, 49–56.
19. Riudavets, J.; Castañé, C.; Alomar, O.; Pons, M.J.; Gabarra, R. Modified atmosphere packaging (MAP) as an alternative measure for controlling ten pests that attack processed food products. *J. Stored Prod. Res.* **2009**, *45*, 91–96.
20. Kucerova, Z.; Kyhos, K.; Aulicky, R.; Stejskal, V. Low pressure treatment to control food-infesting pests (*Tribolium castaneum*, *Sitophilus granarius*) using a vacuum packing machine. *Czech J. Food Sci.* **2013**, *31*, 94–98.
21. Kucerova, Z.; Kyhos, K.; Aulicky, R.; Lukas, J.; Stejskal, V. Laboratory experiments of vacuum treatment in combination with an O_2 absorber for the suppression of *Sitophilus granarius* infestations in stored grain samples. *Crop Prot.* **2014**, *61*, 79–83.
22. Ren, Y.; Yang, C.; Newman, J.; Agarwal, M.; Kostas, E.; Cheng, H. Nitrogen application offers for both control of insect and grain quality. In Proceedings of the 9th International Conference on Controlled Atmospheres and Fumigation in Stored Products, Antalya, Turkey, 15–19 October 2012; Navarro, S., Banks, J.H., Jayas, D., Bell, C.H., Noyes, R.T., Feizli, A.G., Emekci, M., Isikber, A.A., Alagusundaram, K., Eds.; ARBER Professional Congress Services: Ankara, Turkey, 2012; pp. 471–477.
23. Carvalho, M.O.; Barbosa, A.; Barros, G.; Magro, A.; Adler, C.; Navarro, S.; Riudavets, J.; Timlick, B. Quality preservation of stored rice using modified atmospheres in Portugal. In Proceedings of the 9th International Conference on Controlled Atmospheres and Fumigation in Stored Products, Antalya, Turkey, 15–19 October 2012; Navarro, S., Banks, J.H., Jayas, D., Bell, C.H., Noyes, R.T., Feizli, A.G., Emekci, M., Isikber, A.A., Alagusundaram, K., Eds.; ARBER Professional Congress Services: Ankara, Turkey, 2012; pp. 15–19.

24. Mitcham, E.; Martin, T.; Zhou, S. The mode of action of insecticidal controlled atmospheres. *Bull. Entomol. Res*. **2006**, *96*, 213–222.
25. Zhou, M.; Zhang, X.; Pan, B.; Zhu, J.; Qian, X.; Li, X.; Xu, K.; Tang, B.; Li, C. The endogenous metabolic response of *Tribolium castaneum* under a high concentration of CO_2. *Agriculture* **2022**, *12*, 979.
26. Liu, Y.B. Effects of long-term low oxygen storage treatment on survival of rice weevil (*Sitophilus oryzae*) and confused flour beetle (*Tribolium confusum). J. Econ. Entomol.* **2022**, *115*, 1712–1718. https://doi.org/10.1093/jee/toac106.
27. Sakka, M.K.; Gatzali, F.; Karathanos, V.T.; Athanassiou, C.G. Effect of nitrogen on phosphine-susceptible and-resistant populations of stored product insects. *Insects* **2020**, *11*, 885.
28. Ren, L.L.; Peng, C.Y.; Liu, B.; Li, X.Y.; Li, B.S. Research progress of controlled atmosphere treatment for phytosanitary use. *Plant Quar*. **2019**, *33*, 1–5.
29. Zhao, Q.-Y.; Li, T.-X.; Song, Z.-J.; Sun, T.; Liu, B.; Han, X.; Li, Z.-H.; Zhan, G.-P. Combination of modified atmosphere and irradiation for the phytosanitary disinfestation of *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Insects* **2021**, *12*, 442.
30. Person, N.K.; Sorenson, J.W. Use of gaseous nitrogen for controlling stored-product insects in cereal grains. *Cereal Chem.* **1970**, *27*, 679–686.
31. Njoroge, A.W.; Mankin, R.W.; Smith, B.; Baributsa, D. Effects of hypoxia on acoustic activity of two stored-product pests, adult emergence, and grain quality. *J. Econ. Entomol.* **2019**, *112*, 1989–1996.
32. Navarro, S. The use of modified and controlled atmospheres for the disinfestation of stored products. *J. Pest Sci*. **2012**, *85*, 301–322.
33. Moncini, L.; Sarrocco, S.; Pachetti, G.; Moretti, A.; Haidukowski, M.; Vannacci, G. N2 controlled atmosphere reduces postharvest mycotoxins risk and pests attack on cereal grains. *Phytoparasitica* **2020**, *48*, 555–565.
34. Locatelli, D.P.; Girgenti, P.; Moncini, L.; Limonta, L. Semifield study on the effect of membrane-based nitrogen production for the control of the eggs of *Sitophilus oryzae* (L.) and *Tribolium confusum* J. du V. *J. Entomol. Acarol. Res.* **2020**, *52*, 9278.
35. Williams, P.; Minett, W.; Navarro, S.; Amos, T.G. Sealing a farm silo for insect control by nitrogen swamping for fumigation. *Aust. J. Exp. Anim. Husb.* **1980**, *20*, 108–114.
36. Timlick, B.; Dickie, G.; McKinnon, D. Nitrogen as a major component of a controlled atmosphere to manage stored product insect pests in large vertical storage. *IOBC WPRS Bull.* **2002**, *25* 193–197.
37. Aulicky, R.; Kolar, V.; Plachy, J.; Stejskal, V. Field efficacy of brief exposure of adults of six storage pests to nitrogen-controlled atmospheres. *Plant Protect. Sci.* **2017**, *53*, 169–176.
38. Jay, E.G. Imperfections in our current knowledge of insect biology as related to their responses to controlled atmospheres. In *Controlled Atmosphere and Fumigation in Grain Storages*; Ripp, B.E., Banks, H.J., Bond, E.J., Carlverley, D.J., Jay, E.G., Navarro, S., Eds.; Elsevier: Amsterdam, The Netherlands, 1984; pp. 493–508.
39. Bailey, S.W.; Banks, H.J. A review of recent studies of the effects of controlled atmospheres on stored product pests. In *Controlled Atmosphere Storage of Grains*; Shejbal, J., Ed.; Elsevier: Amsterdam, The Netherlands, 1980; pp. 110–118.
40. Navarro, S. The effects of low oxygen tensions on three stored-product insect pests. *Phytoparasitica* **1978**, *6*, 51–58.
41. Athanassiou, C.G. Is Low Oxygen Effective against Insects in Silos? 2019. Available online: https://millermagazine.com/blog/is-low-oxygen-effective-against-insects-in-silos-3249 (accessed on 31 August 2022).
42. Storey, C.L. Mortality of *Sitophilus oryzae* (L.) and *S. granarius* (L.) in atmospheres produced by an exothermic inert atmosphere generator. *J. Stored Prod. Res.* **1975**, *11*, 217–221.
43. Annis, P.C. Towards rational controlled atmosphere dosage schedules—A review of the current knowledge. In Proceedings of the 4th International Working Conference on Stored-Product Protection, Tel Aviv, Israel, 21–26 September 1986; Donahaye, J., Navarro, S., Eds.; Maor-Wallach Press: Jerusalem, Israel, 1987; pp. 128–148.
44. Sakka, M.K.; Gatzali, F.; Karathanos, V.; Athanassiou, C.G. Efficacy of low oxygen against *Trogoderma granarium* Everts, *Tribolium castaneum* (Herbst) and *Callosobruchus maculatus* (F.) in commercial applications. *J. Stored Prod. Res.* **2022**, *97*, 101968.
45. Navarro, S.; Lider, O.; Gerson, U. Response of adults of the grain mite, *Acarus siro* L. to modified atmospheres. *J. Agric. Entomol*, **1985**, *2*, 61–68.
46. Vassilakos, T.N.; Riudavets, J.; Castañé, C.; Iturralde-Garcia, R.D.; Athanassiou, C.G. Efficacy of modified atmospheres on *Trogoderma granarium* (Coleoptera: Dermestidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae). *J. Econ. Entomol.* **2019**, *112*, 2450–2457.
47. Kuyu, C.G.; Tola, Y.B.; Mohammed, A.; Mengesh, A.; Mpagalile, J.J. Evaluation of different grain storage technologies against storage insect pests over an extended storage time. *J. Stored Prod. Res.* **2022**, *96*, 101945.
48. Athanassiou, C.G.; Chiou, A.; Rumbos, C.I.; Sotiroudas, V.; Sakka, M.; Nikolidaki, E.K.; Panagopoulou, E.A.; Kouvelas, A.; Katechaki, E.; Karathanos, V.T. Effect of nitrogen in combination with elevated temperatures on insects, microbes and organoleptic characteristics of stored currants. *J. Pest Sci.* **2017**, *90*, 557–567.
49. Cui, S.; Wang, L.; Qiu, J.; Liu, Z.; Geng, X. Comparative metabolomics analysis of *Callosobruchus chinensis* larvae under hypoxia, hypoxia/hypercapnia and normoxia. *Pest Manag. Sci.* **2017**, *73*, 1267–1276.
50. Lindgren, D.L.; Vincent, L.E. Effect of atmospheric gases alone or in combination on the mortality of granary and rice weevils. *J. Econ. Entomol.* **1970**, *63*, 1926–1929.
51. Krishnamurthy, T.S.; Spratt, E.C.; Bell, C.H. The toxicity of carbon dioxide to adult beetles in low oxygen atmospheres. *J. Stored Prod. Res.* **1986**, *22*, 145–151.

52. Fleurat-Lessard, F.; LeTorch, J.M. Practical approach to purging grain with low oxygen atmosphere for disinfestation of large wheat bins against the granary weevil, *Sitophilus granarius*. In Proceedings of the 4th International Working Conference on Stored-Product Protection, Tel Aviv, Israel, 21–26 September 1986; pp. 208–217.
53. Fleurat-Lessard, F.; LeTorch, J.M. The influence of oxygen content at atmospheres enriched with carbon dioxide on the mortality of hidden stages of *Sitophilus* spp. in wheat grains. *Entomol Exp. Appl.* **1991**, *58*, 37–47.
54. Adler, C. Efficacy of controlled atmospheres on ten strains of the granary weevil *Sitophilus granarius* (L.) from different places of origin. In Proceedings of the 5th International Working Conference on Stored-Product Protection, Bordeaux, France, 9–14 September 1990; Fleurat-Lessard, F., Ducom, P., Eds.; Imprimerie du Médoc: Bordeaux, France, 1991; pp. 727–735.
55. Adler, C. A comparison of the efficacy of CO_2-rich and N_2-rich atmospheres against the granary weevil *Sitophilus granarius*. In Proceedings of 6th International Working Conference on Stored Product Protection, Canberra, Australia, 17–23 April 1994; Highley, E., Wright, E.J., Banks, H.J., Champ, B.R., Eds.; CAB International: Wallingford, UK, 1994; pp. 11–15.
56. Conyers, S.T.; Bell, C.H. A novel use of modified atmospheres: Storage insect population control. *J. Stored Prod. Res.* **2007**, *43*, 367–374.
57. Ofuya, T.I.; Reichmuth, C. Control of two bruchid pests of stored grain legumes in a nitrogen atmosphere. *Crop Prot*. **1993**, *12*, 394–396.
58. Ofuya, T.I.; Reichmuth, C. Effect of level of seed infestation on mortality of larvae and pupae of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in some controlled atmospheres. *J. Stored Prod. Res.* **1994**, *30*, 75–78.
59. Ofuya, T.I.; Reichmuth, C. Effect of relative humidity on the susceptibility of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) to two modified atmospheres. *J. Stored Prod. Res.* **2002**, *38*, 139–146.
60. Ingabire, J.P.; Hategekimana, A.; Bhuvaneswari, K.; Mohan, S.; Ganapathy, S. Management of pulse beetle, *Callosobruchusmaculatus* (F) population by nitrogen based modified atmosphere. *J. Entomol. Zool. Stud.* **2013**, *1*, 48–52.
61. Yewle, N.; Swain, K.C.; Mann, S.; Guru, P.N. Performance of hermetic bags in green gram [*Vigna radiata* (L.) R. Wilczek] storage for managing pulse beetle (*Callosobruchus chinensis*). *J. Stored Prod. Res.* **2022**, *95*, 101896.
62. Morsy, M.M. Nitrogen-based modified atmosphere technique for controlling pulse beetle, *Callosobruchus maculatus* (F) in stored white beans. *J. Plant Prot. Pathol*. **2021**, *12*, 883–888.
63. Hashem, M.Y.; Ahmed, S.S.; Naroz, M.H. Comparative effects of hypoxia, hypercapnia, and hypoxia/hypercapnia on the mortality and survival of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae). *J. Stored Prod. Res.* **2022**, *98*, 101988.
64. Cao, Y.; Xu, K.; Zhu, X.; Bai, Y.; Yang, W.; Li, C. Role of modified atmosphere in pest control and mechanism of its effect on insects. *Front. Physiol*. **2019**, *10*, 206.
65. Chiappini, E.; Molinari, P.; Cravedi, P. Mortality of *Tribolium confusum* J. du Val (Coleoptera: Tenebrionidae) in controlled atmospheres at different oxygen percentages. *J. Stored Prod. Res.* **2009**, *45*, 10–13.
66. Aulicky, R.; Li, Z.; Kolar, V.; Stejskal, V. Control of quarantine pest of *Trogoderma granarium* in silos using non-residual controlled atmospheres and ten days' exposure. *DDD* **2022**, *31*, 69–72. (In Czech with English abstract)
67. Shejbal, J.; Tonolo, A.; Careri, G. Conservation of wheat in silos under nitrogen. *Ann. Tech. Agric. Paris* **1973**, *122*, 773–785.
68. Navarro, S.; Athanassiou, C.G.; Varnava, A.; Vroom, N.; Yiassoumis, D.; Leandrou, I.; Hadjioannou, S. Control of stored grain insects using nitrogen in large concrete silos in Cyprus. In Proceedings of the 9th International Conference of Controlled Atmospheres and Fumigation in Stored Products, Antalya, Turkey, 15–19 October 2012; ARBER Professional: Ankara, Turkey, 2012; pp. 478–487.
69. McGaughey, W.H.; Akins, R.G. Application of modified atmospheres in farm grain storage bins. *J. Stored Prod. Res.* **1989**, *25*, 201–210.
70. Bibby, I.P.; Conyers, S.T. Numerical simulations of gas exchange in leaky grain silos, using measured boundary conditions. *J. Stored Prod. Res.* **1998**, *34*, 217–229.
71. Brabec, D.; Kaloudis, E.; Athanassiou, C.G.; Campbell, J.; Agrafioti, P.; Scheff, D.S.; Bantas, S.; Sotiroudas, V. Fumigation monitoring and modeling of hopper-bottom railcars loaded with corn grits. *J. Biosyst. Eng*. **2022**, *47*, 358–369.
72. Aulicky, R.; Stejskal, V. Efficacy and limitations of phosphine "spot-fumigation" against five Coleoptera species of stored product pests in wheat in a grain store—Short note. *Plant Prot. Sci*. **2015**, *51*, 33–38.
73. Navarro, H.; Navarro, S. Efficacy of controlling two stored cashew nut insects in 1% O_2 and 99% N_2 at 43 °C. In Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products, Winnipeg, MB, Canada, 23–28 August 2020; Jayas, D.S., Jian, F., Eds.; CAF Permanent Committee Secretariat: Bonn, Germany, 2021, pp. 153–159.
74. Navarro, S. Modified atmospheres for the control of stored-product insects and mites. In *Insect Management for Food Storage and Processing*; Elsevier: Amsterdam, The Netherlands, 2006; pp. 105–146.
75. Navarro, S.; Timlick, B.; Demianyk, C.J.; White, N.D.G. Controlled and modified atmospheres. In *Stored Product Protection*; Hagstrum, D., Phillips, T., Cupperus, G., Eds.; Kansas State Research and Extension: Manhattan, KS, USA, 2012; pp. 1–25.

Bulletin of Entomological Research

cambridge.org/ber

Research Paper

Cite this article: Shah JA, Vendl T, Aulicky R, Stejskal V (2020). Frass produced by the primary pest *Rhyzopertha dominica* supports the population growth of the secondary stored product pests *Oryzaephilus surinamensis*, *Tribolium castaneum*, and *T. confusum*. *Bulletin of Entomological Research* 1–7. https://doi.org/10.1017/S0007485320000425

Received: 14 April 2020
Revised: 11 June 2020
Accepted: 15 June 2020

Keywords:
Bostrichidae; diet suitability; frass; interspecific interactions; Silvanidae; Tenebrionidae

Author for correspondence:
V. Stejskal, Email: stejskal@vurv.cz

© The Author(s), 2020. Published by Cambridge University Press

CAMBRIDGE UNIVERSITY PRESS

Frass produced by the primary pest *Rhyzopertha dominica* supports the population growth of the secondary stored product pests *Oryzaephilus surinamensis*, *Tribolium castaneum*, and *T. confusum*

J.A. Shah[1,2], T. Vendl[1], R. Aulicky[1] and V. Stejskal[1,2]

[1]Crop Research Institute, Drnovska 507/73, Prague 6-Ruzyne, CZ-16106, Czech Republic and [2]Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague 6-Suchdol, CZ-16500, Czech Republic

Abstract

Primary pests such as *Rhyzoperta dominica* may increase the contents of dockage, dust, and frass in grain mass. Although it has been suggested that frass can affect the population growth of stored product pests and ecological interactions among primary and secondary pests in stored grain, this has not been validated experimentally. Therefore, this work experimentally tested the hypothesis that *R. dominica* wheat frass may support population increases in secondary pests such as *Tribolium confusum*, *T. castaneum*, and *Oryzaephilus surinamensis* for the first time. The effect of frass on secondary pest performance was compared with the effects of various physical qualities of wheat grain (i.e., intact grain kernels, grain fragments, flour, grain + frass) and an artificially enriched control diet (milled wheat kernels, oat flakes, and yeast). The results showed that the clean intact grain kernels did not support the population growth of any tested species, and the nutrient-rich control diet provided the best support. Frass was a significantly better food medium for *O. surinamensis* and *T. castaneum* than flour or cracked grain, while *T. confusum* performed equally well on flour and frass. Our results showed that in terms of food quality and suitability for the tested species, frass occupied an intermediate position between the optimized breeding diet and simple uniform cereal diets such as cracked grain or flour. The results suggest that (i) the wheat frass of primary pest *R. dominica* is a riskier food source for the development of the tested secondary pests than intact or cracked wheat grain or flour; (ii) frass has the potential to positively influence interspecific interactions between *R. dominica* and the tested secondary pests; and (iii) wheat grain should be cleaned if increases in *R. dominica* populations and/or accumulated frass are detected.

Introduction

Storage pests can potentially cause extensive economic damage to stored grain (Mahroof and Hagstrum, 2012) and contamination from fragments and allergenic cereal dusts (Farant and Moore, 1978; Trematerra *et al.*, 2011; Hubert *et al.*, 2018). The actually realized pest potential is determined by the interplay between the biological abilities of each species and currently existing biotic and abiotic environmental factors. In a regulated warehouse environment, the relevant abiotic factors, such as temperature and humidity, are relatively limited and stable (Maier *et al.*, 1996; Stejskal *et al.*, 2019). Biotic interactions are much more complex and dynamic (Arbogast and Mullen, 1988) since they usually involve multiple relationships among arthropods, microorganisms, and different types of stored commodities (Crombie, 1941; Sinha, 1969; LeCato, 1975; Sinha and Sinha, 1990; Dukic *et al.*, 2016; Fleurat-Lessard, 2017; Rumbos *et al.*, 2019). Complexity is also increased by diverse arthropod reactions to chemical changes in grain caused by previous grain infestations (e.g., Stewart-Jones *et al.*, 2004; Trematerra, *et al.*, 2007; Stewart-Jones *et al.*, 2009) and the effects associated with the gut enzymes and microbiome composition of arthropods (e.g., Osipitan *et al.*, 2011; Hubert *et al.*, 2016; Naseri *et al.*, 2017).

With the advent of integrated and holistic approaches for commodity protection (Jayas *et al.*, 1995; White, 1995; Arbogast and Throne, 1997), there has been significant renewed interest in interspecific interactions. Negative interactions, such as predation, parasitism, and competition, are a particular focus of research (Lukas *et al.*, 2007; Athanassiou *et al.*, 2017; Quellhorst *et al.*, 2020). However, interspecific interactions are not necessarily negative. For example, Nansen *et al.* (2004) reported a positive commensal relationship between *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst). They did not illuminate the causes of this association, but one of the common explanations for positive interactions among stored-product insects is that substrate colonization by internally feeding species can disintegrate hard seeds, making

Downloaded from https://www.cambridge.org/core. Vyzkumny Ustav Rostlinne Vyroby, on 03 Aug 2020 at 10:47:28, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007485320000425

cereal food available to another species (e.g., Arbogast and Mullen, 1988). It has been shown that decreasing proportions of sound grain to dockage, cereal dust and flour can significantly affect the survival, multiplication, and interspecific interactions of a number of species (McGregor, 1964; LeCato, 1975; Sinha, 1975; Locatelli *et al.*, 2008). Internally feeding pests may contribute profoundly to the increase in dockage and dust contents in grain mass since they produce fine small dusty particles known as frass. Specifically, the feeding of bostrichid beetles such as *R. dominica* and *Prostephanus truncatus* (Horn) may result in large amounts of frass in an infested commodity (Stewart-Jones *et al.*, 2004; Stewart-Jones *et al.*, 2009; Edde, 2012; Kavallieratos *et al.*, 2017; Nyabako *et al.*, 2020). It was recently demonstrated that externally feeding pests such as *T. castaneum* (Bekon and Fleurat-Lessard, 1992; Arthur *et al.*, 2019) and *Trogoderma granarium* Everts (Kavallieratos *et al.*, 2017) may also produce some amount of frass. Not only insect species and its developmental stage (i.e. larva/adult) but also the type of food can influence the amount, form, and composition of the resulting faecal/frass materials (Weiss, 2006; Kavallieratos *et al.*, 2017). Earlier resources claimed (e.g., Potter, 1935) that frass of *R. dominica* mainly consists of food material that is chewed off but not eaten. It is currently known that frass contains not only the original non-ingested food material (grain) itself, but also faeces and additional components such as microorganisms and various chemical compounds (Breese, 1960; Osipitan *et al.*, 2011; Edde, 2012; Boiocchi *et al.*, 2017). Several previously published works (e.g., Bekon and Fleurat-Lessard, 1992; Kavallieratos *et al.*, 2017) listed frass of primary colonizers among the factors positively influencing colonization and population performance of secondary colonizers. However, these works were not designed to distinguish the positive effects of the frass from the positive effect of physical disintegration of the sound grain. Thus, the specific effects of frass of *R. dominica* and other primary colonizers on the development of other primary or secondary stored product species have remained incompletely understood. Therefore, the goal of this work was to separately evaluate the impacts of the frass produced *R. dominica* on three species of externally feeding pests: *Oryzaephilus surinamensis* (L.), *Tribolium confusum* (Jacquelin du Val), and *T. castaneum*. The selected species are important pests in grain stores in the Czech Republic (Stejskal *et al.*, 2014, 2015) as well in many other countries (e.g., Hagstrum and Subramanyam, 2006; Trematerra and Throne, 2012). The hypothesis was that frass is a more supportive food for each of the tested species of externally feeding pests than simple cereal substrates (irrespective of their physical state, such as whole grain, broken kernels, or flour). The reason for our hypothesis was that previous studies have indicated (Breese, 1960; Osipitan *et al.*, 2011; Edde, 2012; Boiocchi *et al.*, 2017) that in addition to undigested cereal starch particles, frass contains other chemical and microbial components. To test our hypotheses, we specifically compared the effects of frass on secondary pest (i.e. *T. castaneum*, *T. confusum*, and *O. surinamensis*) performance with the effects of various physical qualities of grain (intact grain, grain fragments, flour, grain + frass) and artificially enriched control diets (milled wheat kernels, oat flakes, and yeast).

Materials and methods

Beetle rearing

Tribolium castaneum, *T. confusum* (Coleoptera: Tenebrionidae), *O. surinamensis* (Coleoptera: Silvanidae) and *R. dominica* (Coleoptera: Bostrichidae) used in the study came from the laboratory cultures reared for more than 20 generations under close-to-optimal conditions (i.e., sensitive to insecticides and natural environmental stresses) at the Crop Research Institute in Prague, Czech Republic. The strain of *O. surinamensis* was kept in thermochambers (Aviko, Czech Republic) at 27 (±0.5) °C with 75 (±5) % relative humidity (r. h.). The food was a mixture of powdered wheat (80%), oat flakes (16%), germ (3%), and yeast (1%). Both *Tribolium* species were also kept in thermochambers (Aviko, Czech Republic) at 26 (±0.5) °C and 75 (±5) % r. h. The rearing boxes contained a mixture of milled wheat kernels, oat flakes, and yeast (10:10:2), and the substrate was covered with moistened filter paper. The rearing conditions of *R. dominica* were identical as described in Kucerova and Stejskal (2008).

Substrate preparation

Five types of substrates were tested in the experiment. They were whole undamaged wheat grains, cracked wheat grains, milled wheat grains (flour), frass (originating from the chewing activity of *R. dominica* on wheat grains), and a mixture of the frass and whole grains. In addition to these substrates, a control diet was used. In both *Tribolium* species, the control diet was composed of a mixture of milled wheat kernels, oat flakes, and yeast (10:10:2). The diet of *O. surinamensis* contained whole wheat kernels, oat flakes, and a mixture of milled wheat kernels, oat flakes, and yeast (10:10:2). All substrates were frozen for at least 10 days prior to the experiment to exclude all unwanted organisms. Cracked grains were milled using a centrifugal mill (ZM 200, Retsch) with a 10 mm sieve size, and the cracked kernels were sieved with a 2 mm-pore size sieve. The flour was bought from a shop. The frass originated from a laboratory culture of *R. dominica* reared on wheat grains. It was obtained by sieving the rearing medium using a 0.5 mm-pore size sieve using automatic elaborate shaking and vibratory sieving equipment (vibratory sieve shaker AS 200, Retsch, Germany). The ratio of whole wheat and frass in the mixed substrate was 1:1. Seven days before the experiment, all substrates were placed in a Lock & Lock plastic box ($180 \times 110 \times 110$ mm^3) with a saturated solution of NaCl (75%) to ensure uniform relative humidity. The substrate moisture content was measured using a moisture metre (Agromatic, Farmer Tronic, Denmark); the measured moisture content was 15.7%.

Study design

To study the population growth of the tested species (i.e., *T. castaneum*, *T. confusum*, and *O. surinamensis*), 10 grams (weighed on an Adventurer Pro analytical scale, Ohaus, USA) of a particular substrate was placed in a 100 ml plastic vial with a perforated cap covered with nylon mesh. The top of the inner walls of the cup were treated with synthetic fluoropolymer lubricant powder (polytetrafluoroethylene - Fluon) (Sigma-Aldrich Co., St. Louis, USA) to prevent beetles from escaping. Ten unsexed adult beetles were introduced into each cup and kept in a climatic chamber at 27 (± 0.5)°C and 75 (± 5)% r.h. in randomized positions. After 10 days, the cup contents were spilled out on Petri dishes, and all beetles were carefully removed by hand. For the determination of development time, the vials were checked for the presence of adults every other day (starting on the 33rd day). To prevent the disruption of beetle development, the vials were checked visually, and the substrate was not sieved (thus, especially at low adult densities, the method may not precisely reflect the real

Downloaded from https://www.cambridge.org/core. Vyzkumny Ustav Rostlinne Vyroby, on 03 Aug 2020 at 10:47:28, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007485320000425

Table 1. Population growth and development time (means ± SEs) of *Oryzaephilus surinamensis* on various substrates in comparison with the control diet.

Substrate	Larvae	Pupae	Adults	Total number of individuals	Control: experimental substrate ratio[a]	Development time (days)
Whole grains	0.0 ± 0.0 C	0.0 ± 0.0 B	0.4 ± 0.2 D	0.4 ± 0.2 D	131.1	n.a.
Cracked grains	0.8 ± 0.4 C	0.4 ± 0.2 B	17.2 ± 1.8 C	18.4 ± 1.9 C	9.5	41.0 ± 3.7
Flour	0.0 ± 0.0 C	0.0 ± 0.0 B	15.8 ± 3.2 C	15.8 ± 3.2 C	10.9	40.6 ± 2.4
Frass	11.6 ± 1.6 B	0.0 ± 0.0 B	48.0 ± 5.9 AB	59.6 ± 5.4 B	3.0	37.0 ± 2.1
Whole grains + frass	9.0 ± 3.6 B	0.0 ± 0.0 B	30.2 ± 5.8 BC	39.2 ± 3.3 B	4.6	35.4 ± 0.75
Control	98.0 ± 9.4 A	2.4 ± 0.7 A	82.2 ± 18.9 AB	182.6 ± 18.9 A	1	33.8 ± 0.49
F	81.3	10.5	62.8	167.5		2.0
p	< 0.001	< 0.001	< 0.001	< 0.001		0.13

Within each column (except for the control: experimental substrate ratio column), means followed by the same letter are not significantly different; in cases with no letters, no significant differences exist; Tukey's HSD post hoc test at 0.05, in all cases df = 5, 24.
[a]The ratio was computed as (total number of individuals in control substrate + 1)/(total number of individuals in experimental substrate + 1).

development time). The development time for each vial was recorded as the time at which at least one individual adult was present. After 8 weeks, the cups were open, and the numbers of larvae, pupae, and adults were counted under an Olympus SZX10 binocular microscope. Five replicates per species/substrate were performed.

Statistics

The effects of the substrate on the population size and development time were tested by one-way analysis of variance followed by Tukey's HSD post hoc test, where the substrate type was used as the factor. Because the data regarding the numbers of individuals (tested by the Kolmogorov–Smirnov test) were non-normally distributed, the data were $\log(x+1)$ transformed to ensure normality. For simplicity, untransformed means and standard errors are reported. The analyses were carried out using Statistica 12.0 (StatSoft Inc., 2010).

Results

Oryzaephilus surinamensis

The population growth of *O. surinamensis* on various substrates is summarized in table 1. Apparently, this species was able to thrive on frass produced by *R. dominica*; there were no significant differences in the numbers of larvae or adults or the total number of individuals developing in frass alone or in a mixture of frass and whole grains. However, the total number of individuals in frass alone and in a mixture of frass and whole grains were 3x and 4.6x less than in a control substrate (table 1). Moreover, population growth on substrates containing frass was higher than that on flour and cracked grains. In contrast, the species was not able to develop on undamaged grains. There was a trend of decreasing development time with increasing suitability of the substrate (as identified by the population growth on the respective substrate, see table 1), although the differences were not statistically significant.

Tribolium castaneum

The population growth of *T. castaneum* on various substrates is summarized in table 2. In general, the results were similar to those for the above species. Substrates containing frass (i.e., frass alone and frass + grains) proved to be suitable for the development of *T. castaneum* (there were no significant differences from the control diet – the population growth was only 1.3x and 1.8x less than in the control diet for both experimental substrates). All developmental stages (i.e., larvae, pupae, and adults) were present in the two substrates in comparable numbers. Population growth in frass alone was also greater than in flour and cracked grains. In undamaged grains, the species was not able to develop. The development times showed a similar pattern to those of *O. surinamensis* (although, again, the differences were not statistically significant). The development time was probably greatest in the presence of cracked grains, so only larvae and no adults were present in this substrate.

Tribolium confusum

The population growth of *T. confusum* on various substrates is summarized in table 3. Similar to both of the other species, *T. confusum* was able to thrive on frass, but in this case, population growth was not higher in frass than in flour and cracked grains. In contrast to the other two species, the performance of *T. confusum* on whole grains + frass was slightly (albeit nonsignificantly) better than that on frass alone. Generally, there were similar ratios of number of individuals in four experimental substrates (whole grains + frass, frass alone, flour, cracked grains) compared with control diet (ranging from 2.6 to 5.6). In undamaged grains, the species was not able to develop. The development time in both frass substrates and flour was similar but was slightly (although not significantly) shorter than that in cracked grains.

Discussion

The presence of broken kernels and frass is reported to affect the behaviour, interaction, and population growth of secondary pests (LeCato, 1975; Sinha, 1975; Arbogast and Mullen, 1988; Beckett and Evans, 1994; Kavallieratos *et al.*, 2017). Although there are available data on the effects of frass and dust as behavioural modifiers since they may contain kairomones (Stewart-Jones *et al.*, 2009), there have been no data showing how frass can affect the populations and speed of development of any secondary pest species. This work, therefore, constitutes the first comparison of the

Downloaded from https://www.cambridge.org/core. Vyzkumny Ustav Rostlinne Vyroby, on 03 Aug 2020 at 10:47:28, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007485320000425

Table 2. Population growth and development time (means ± SEs) of *Tribolium castaneum* on various substrates in comparison with the control diet.

Substrate	Larvae	Pupae	Adults	Total number of individuals	Control: experimental substrate ratio[a]	Development time (days)
Whole grains	0.0 ± 0.0 C	0.0 ± 0.0 C	0.0 ± 0.0 C	0.0 ± 0.0 D	133.2	n.a.
Cracked grains	23.2 ± 3.1 A	0.0 ± 0.0 C	0.4 ± 0.2 C	23.6 ± 3.0 BC	5.4	n.a.
Flour	3.6 ± 2.9 BC	4.0 ± 2.7 BC	4.4 ± 2.9 BC	12.0 ± 5.5 C	10.2	52.0 ± 1.0
Frass	49.4 ± 17.5 A	38.2 ± 12.8 A	12.2 ± 8.4 ABC	99.8 ± 10.2 A	1.3	47.7 ± 1.7
Whole grains + frass	23.0 ± 6.8 A	15.4 ± 6.9 ABC	33.8 ± 14.6 AB	72.2 ± 9.0 AB	1.8	48.6 ± 2.0
Control	25.4 ± 12.1 AB	40.0 ± 15.9 AB	66.8 ± 33.2 A	132.2 ± 16.2 A	1	45.8 ± 2.4
F	9.9	8.2	6.1	45.0		1.3
p	<0.001	<0.001	<0.001	<0.001		0.3

Within each column (except for the control:experimental substrate ratio column), means followed by the same letter are not significantly different; in cases with no letters, no significant differences exist; Tukey's HSD post hoc test at 0.05, in all cases df = 5, 24.
[a]The ratio was computed as (total number of individuals in control substrate + 1)/(total number of individuals in experimental substrate + 1).

Table 3. Population growth and development time (means ± SEs) of *Tribolium confusum* on various substrates in comparison with the control diet.

Substrate	Larvae	Pupae	Adults	Total number of individuals	Control: experimental substrate ratio[a]	Development time (days)
Whole grains	0.0 ± 0.0 B	0.0 ± 0.0 B	0.0 ± 0.0 D	0.0 ± 0.0 C	193	n.a.
Cracked grains	28.0 ± 8.5A	0.0 ± 0.0 B	5.6 ± 1.7 C	33.6 ± 9.8 B	5.6	53.4 ± 1.3 B
Flour	14.6 ± 11.9 AB	0.0 ± 0.0 B	41.0 ± 12.0 B	55.6 ± 10.5 B	3.4	49.4 ± 2.4 AB
Frass	3.0 ± 1.3 AB	0.0 ± 0.0 B	49.8 ± 14.8 B	52.8 ± 14.6 B	3.6	49.0 ± 1.4 AB
Whole grains + frass	1.8 ± 0.7 AB	0.4 ± 0.4 B	70.2 ± 6.6 AB	72.4 ± 7.6 AB	2.6	46.0 ± 1.2 AB
Control	15.6 ± 9.3 AB	18.2 ± 10.1 A	158.2 ± 26.5 A	192.0 ± 32.2 A	1	42.8 ± 3.0 A
F	2.9	4.6	41.9	36.6		4.0
p	0.036	0.005	< 0.001	< 0.001		0.016

Within each column (except for the control: experimental substrate ratio column), means followed by the same letter are not significantly different; Tukey's HSD post hoc test at 0.05, in all cases df = 5, 24.
[a]The ratio was computed as (total number of individuals in control substrate + 1)/(total number of individuals in experimental substrate + 1).

separately analysed effects of frass with the effects of cereal substrates (whole kernels, cracked kernels, and wheat flour) on the speed of development and productivity (performance) of three secondary pests, *O. surinamensis, T. castaneum,* and *T. confusum*. There was no (*T. castaneum* and *T. confusum*) or minimal (*O. surinamensis*) development of the tested pest species on whole undamaged seeds. Conversely, all tested species (with varying productivity) multiplied on seed fragments, flour, frass, and the mixed control diet. These results confirmed the results of other authors showing that the physical structure of cereal substrates plays an important role in the biology of secondary storage pests. For instance, several studies (Fraenkel and Blewett, 1943; Turney, 1957; Fleming, 1998; Beckel *et al.*, 2007) have found that *O. surinamensis* is mostly unable to develop on whole cereal (e.g., wheat, corn, rice) seeds, while crushed seeds and flour allow population growth in this species. Beckel *et al.* (2007; Skourti *et al.*, 2020) compared different flour milling grades (i.e., 1–20) and found that wheat grain milled at grade 20 yielded the largest number of progeny of *O. surinamensis*. Several works claim that *T. castaneum* can survive and multiply on whole seeds (Fraenkel and Blewett, 1943; Daniels, 1956), but other studies do not support these conclusions (White, 1982). Therefore, Li and Arbogast (1991) ranked cereal substrates in terms of their suitability for *T. castaneum* population development as follows: flour>cracked seeds>whole intact seeds. It appears from published works that a finer physical structure provides a more suitable medium for the development of *Tribolium* spp. and *Oryzaephilus* spp. Astuti *et al.* (2020) found that the particle size variation and protein content of flour products affected the survivorship and development time of *T. castaneum*. Fardisi *et al.* (2019) claimed that flour particle size affected the suitability of the microclimate for *T. castaneum* development. Frass, with its fine particle size, is closest to flour, semolina, and environmental dust, which are substrates upon which secondary pests multiply well (e.g., Locatelli *et al.*, 2017). Therefore, one partial explanation for the beneficial effect of frass on pest development may be its fine physical structure.

On the other hand, even the favourable physical decomposition of grain and other cereal substrates does not inherently result in the most appropriate dietary composition for *Tribolium* spp. or for *Oryzaephilus* spp. (LeCato and McCray, 1973; Shafique *et al.*, 2006; Astuti *et al.*, 2020). Notably, the presence of germ in the cereal diet is important. Armstrong and Howe (1963) and White

Downloaded from https://www.cambridge.org/core. Vyzkumny Ustav Rostlinne Vyroby, on 03 Aug 2020 at 10:47:28, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007485320000425

(1982) observed low survival of *O. surinamensis* when the insects fed only the endosperm of grain without the germ. Locatelli *et al.* (2017) found that *T. confusum* could also multiply on very fine grain silo environmental dust that had been supplemented with microscopic fragments of dietary insects and other organic and inorganic compounds. In our experiments, all three species of pests (*T. confusum*, *T. castaneum*, *O. surinamensis*) showed the highest productivity and development rates by far on the optimized laboratory control diet rather than on flour or frass. Astuti *et al.* (2020) stressed that in addition to protein and particle size variation, other predictors (such as water, ash, phenol, and riboflavin contents) also affect the development of *T. castaneum*. This observation is in concordance with earlier findings by Sokoloff *et al.* (1966) showing that the development of *T. castaneum* requires not only protein but also other nutrients, such as minerals and vitamins. Our results obtained with the optimized diet and the work of other authors (Fraenkel and Blewett, 1943; LeCato and McCray, 1973; Beckel *et al.*, 2007; Locatelli *et al.*, 2017) show that most secondary pests exhibit a higher performance on a richer diet than on carbohydrate-based cereals alone (i.e., a diet containing additional components such as proteins, lipids, vitamins, yeasts, etc.). In our experiments, frass was found to be a significantly better food medium than flour or cracked grain for *O. surinamensis* and *T. castaneum*, while *T. confusum* performed equally well on flour and frass. Our results showed that in terms of food quality and suitability for the tested species, wheat frass occupied an intermediate position between the optimal diet mixture and simple uniform cereal diets, such as cracked wheat grain or flour. Our biotests thus also indirectly indicated that *R. dominica* wheat frass is not just cereal dust containing waste and ingestible faecal particles but is instead a rich potential diet for secondary pests. According to the limited relevant published data, *R. dominica* frass consists not only of digested faecal parts but also of undigested food particles containing high nitrogen levels, digestive proteins, and the excreted microbiome (Breese, 1960; Edde, 2012). There are no available data on the protein and fat contents of frass produced by *R. dominica*. However, there are data on the frass composition produced by flies fed dried distiller grains (Yildirim-Aksoy *et al.*, 2020): the larvae produce frass with high protein (216 g/kg) and fat contents (60 g/kg). Stewart-Jones *et al.* (2009) studied the chemical composition of frass produced by stored insects and identified triglycerides and five free fatty acids as the most abundant chemicals in frass. They also found that *Sitophilus* spp. did not change the composition of free acids, while *R. dominica* feeding increased their contents by four–sixfold in frass. The results obtained by Osipitan *et al.* (2011) revealed that frass produced by the *P. truncatus* contained ten species of bacteria and six species of fungi. A recent analysis (Boiocchi *et al.*, 2017) showed that frass of *R. dominica* and another tested storage pests was enriched by an extensive microbiome (including bacteria – *Enterobacteriaceae*, *Pseudomonadacae* and *Bacillaceae*; yeasts – *Candida*; and other fungi – *Saccharomycetales*, *Pleosporaceae*, and *Nectriaceae*).

In conclusion, this work is an experimental proof that wheat frass produced by *R. dominica* may support the survival and population growth of secondary pests such as *T. confusum*, *T. castaneum*, and *O. surinamensis*. For these species, it may represent an even better diet than plain wheat grains in any physical form (whole grain, cracked grain, and flour). Our results thus imply several practical consequences for pest risk assessment and management in stored grain. First, the presence of frass in stored grain has the potential to affect interspecific interactions and may provide a hypothetical explanation for the previous finding reported by Nansen *et al.* (2004) that the presence of *R. dominica* has a positive effect on *T. castaneum* populations. It should be mentioned that the recent observations revealed certain beneficial interactions not only between primary and secondary colonisers but also between primary colonizers such as *P. truncatus*, *R. dominica*, and *Sitophilus* spp. (Athanassiou *et al.*, 2017; Kavallieratos *et al.*, 2017; Quellhorst *et al.*, 2020). Frass was also listed among the possible supportive interspecific ecological mechanisms among populations of primary colonisers (Kavallieratos *et al.*, 2017). We therefore suggest that additional experiments should be done to illuminate the exact effects of frass on various species of primary colonizers; e.g. in a similar way as demonstrated in this study. Second, our results imply the necessity of grain cleaning during storage when infested by *R. dominica* (Flinn *et al.*, 1992; Morrison *et al.*, 2019). The correlation of primary pests with the intensity of negative effects on food (i.e., injury and damage to kernels (IDK), contamination by insect fragments) may be low due to their accumulation over time (Stejskal, 2000; Hagstrum and Subramanyam, 2006). Thus, the presence of dust and frass should be checked regularly even in the absence of primary pests, since frass may accumulate from previous infestations and may support current populations of secondary pests. Finally, strategies for the improvement of the diet of stored pests during their cultivation are also sought for experimental purposes (e.g., Beckel *et al.*, 2007), and frass can be a candidate component of enhanced diets for secondary feeding pests.

Acknowledgements. The work was partially funded by projects RO018 and VH20182021038. Shah J.A. was supported by a scholarship provided by the Ministry of Education of the Czech Republic via CZU. The authors thank the reviewers for their help to improve the previous versions of the MS.

References

Arbogast RT and Mullen MA (1988) Insect succession in a stored corn ecosystem in southeast Georgia. *Annals of the Entomological Society of America* **81**, 899–912.

Arbogast RT and Throne JE (1997) Insect infestation of farm stored maize in South Carolina towards characterization of a habitat. *Journal of Stored Products Research* **33**, 187–198.

Armstrong MT and Howe RW (1963) The saw-toothed grain beetle (*Oryzaephilus surinamensis*) in home grown grain. *Journal of Agricultural Engineering Research* **8**, 256–261.

Arthur FH, Starkus LA, Gerken AR and Campbell JF (2019) Growth and development of *Tribolium castaneum* (Herbst) on rice flour and brown rice as affected by time and temperature. *Journal of Stored Products Research* **83**, 73–77.

Astuti LP, Rizali A, Firnanda R and Widjayanti T (2020) Physical and chemical properties of flour products affect the development of *Tribolium castaneum*. *Journal of Stored Products Research* **86**, 101555.

Athanassiou CG, Kavallieratos NG and Campbell JF (2017) Competition of three species of *Sitophilus* on rice and maize. *Plos One* **12**, e0173377.

Beckel HDS, Lorini I and Lazzari SNM (2007) Rearing method of *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) on various wheat granulometry. *Revista Brasileira de Entomologia* **51**, 501–505.

Beckett SJ and Evans DE (1994) The demography of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) on kibbled wheat. *Journal of Stored Products Research* **30**, 121–137.

Bekon AK and Fleurat-Lessard F (1992) Assessment of dry matter loss and frass production in cereal grain due to successive attack by *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). *Insect Science Application* **13**, 129–136.

Downloaded from https://www.cambridge.org/core. Vyzkumny Ustav Rostlinne Vyroby, on 03 Aug 2020 at 10:47:28, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007485320000425

Boiocchi F, Porcellato D, Limonta L, Picozzi C, Vigentini I, Locatelli D and Foschino R (2017) Insect frass in stored cereal products as a potential source of *Lactobacillus sanfranciscensis* for sourdough ecosystem. *Journal of Applied Microbiology* **123**, 944–955.

Breese MH (1960) The infestibility of stored paddy by *Sitophilus sasakii* (Tak.) and *Rhyzopertha dominica* (F.). *Bulletin of Entomological Research* **51**, 599–630.

Crombie AC (1941) On oviposition, olfactory conditioning and host selection in *Rhizopertha dominica* Fab. (Insecta, Coleoptera). *Journal of Experimental Biology* **18**, 62–78.

Daniels NE (1956) Damage and reproduction by the flour beetles, *Tribolium confusum* and *T. castaneum*, in wheat at three moisture contents. *Journal of Economic Entomology* **49**, 244–247.

Dukic N, Radonjic A, Levic J, Spasic R, Kljajic P and Andric G (2016) The effects of population densities and diet on *Tribolium castaneum* (Herbst) life parameters. *Journal of Stored Product Research* **69**, 7–13.

Edde PA (2012) A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. *Journal of Stored Product Research* **48**, 1–18.

Farant J and Moore CF (1978) Dust exposure in the Canadian grain industry. *American Industrial Hygiene Association Journal* **39**, 177–193.

Fardisi M, Mason LJ, Ileleji KE and Richmond DS (2019) Effect of chemical and physical properties of dried distillers grains with solubles (DDGS) on *Tribolium castaneum* (Herbst) development. *Journal of Stored Product Research* **80**, 57–64.

Fleming DA (1998) The influence of wheat kernel damage upon the development and productivity of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). *Journal of Stored Products Research* **24**, 233–236.

Fleurat-Lessard F (2017) Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins – An update. *Journal of Stored Products Research Volume* **71**, 22–40.

Flinn P, McGaughey WH and Burkholder WE (1992) *Effects of Fine Material on Insect Infestation in Fine Materials in Grain*. Wooster: North Central Regional Research Publication, Ohio State University. vol. 332, pp. 24–30.

Fraenkel G and Blewett M (1943) The natural foods and the food requirements of several species of stored products insects. *Transactions of the Royal Entomological Society of London* **93**, 457–490.

Hagstrum DW and Subramanyam B (2006) *Fundamentals of Stored Product Entomology*. St. Paul, Minnesota: American Association of Cereal Chemists, p. 323.

Hubert J, Stejskal V, Nesvorna M, Aulicky R, Kopecky J and Erban T (2016) Differences in the bacterial community of laboratory and wild populations of the predatory mite *Cheyletus eruditus* (Acarina: Cheyletidae) and bacteria transmission from its prey *Acarus siro* (Acari: Acaridae). *Journal of Economic Entomology* **109**, 1450–1457.

Hubert J, Stejskal V, Athanassiou CG and Throne J (2018) Health hazards associated with arthropod infestation of stored products. *Annual Review of Entomology* **63**, 553–573.

Jayas DS, White NDG and Muir WE (1995) *Stored Grain Ecosystems*. New York: Marcel Dekker Inc., p. 757.

Kavallieratos NG, Athanassiou CG, Guedes RNC, Drempela JD and Boukouvala MC (2017) Invader competition with local competitors: displacement or co-existence among the invasive khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), and two other major stored-grain beetles? *Frontiers in Plant Science* **8**, 1837.

Kucerova Z and Stejskal V (2008) Differences in egg morphology of the stored-grain pests *Rhyzopertha dominica* and *Prostephanus truncatus* (Coleoptera: Bostrichidae). *Journal of Stored Products Research* **44**, 103–105.

LeCato GL (1975) Interactions among four species of stored product insects in corn: a multifactorial study. *Annals of the Entomological Society of America* **68**, 677–679.

LeCato GL and McCray TL (1973) Multiplication of *Oryzaephilus* spp. and *Tribolium* spp. on 20 natural product diets. *Environmental Entomology* **2**, 176–179.

Li L and Arbogast RT (1991) The effect of grain breakage on fecundity, development, survival, and population increase in maize of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal of Stored Product Research* **27**, 87–94.

Locatelli DP, Limonta L and Stampini M (2008) Effect of particle size of soft wheat flour on the development of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Journal of Stored Products Research* **44**, 269–272.

Locatelli DP, Savoldelli S, Girgenti P, Lucchini GA and Limonta L (2017) Can environmental dust from silo area allow the development of stored product insects? *Journal of Stored Product Research* **71**, 41–46.

Lukas J, Stejskal V, Jarosik V, Hubert J and Zdarkova E (2007) Differential natural performance of four *Cheyletus* predatory mite species in Czech grain stores. *Journal of Stored Products Research* **43**, 97–102.

Mahroof RM and Hagstrum DW (2012) Biology, behavior, and ecology of insects in processed commodities. In Hagstrum DW, Phillips TW and Cuperus G (eds), *Stored Product Protection*. Manhattan, KS, USA: Kansas State University, pp. 33–44.

Maier DE, Adams WH, Throne JE and Mason LJ (1996) Temperature management of the maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), in three locations in the United States. *Journal of Stored Product Research* **32**, 255–273.

McGregor HE (1964) Preference of *Tribolium castaneum* for wheat containing various percentages of dockage. *Journal of Economic Entomology* **57**, 511–513.

Morrison WR, Bruce A, Wilkins RV, Albin CE and Arthur FH (2019) Sanitation improves stored product insect pest management. *Insects* **10**, 77.

Nansen C, Phillips TW and Palmer MW (2004) Analysis of the insect community in stored-maize facility. *Ecological Research* **19**, 197–207.

Naseri B, Borzoui E, Majd S and Mansouri SM (2017) Influence of different food commodities on life history, feeding efficiency, and digestive enzymatic activity of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology* **110**, 2263–2268.

Nyabako T, Mvumi BM, Stathers T, Mlambo S and Mubayiwa M (2020) Predicting *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) populations and associated grain damage in smallholder farmers' maize stores: a machine learning approach. *Journal of Stored Products Research* **87**, 101592.

Osipitan AA, Akintokun K, Odeyemi S and Bankole SO (2011) Evaluation of damage of some food commodities by larger grain borer – *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and microbial composition of frass induced by the insect. *Archives of Phytopathology and Plant Protection* **44**, 537–546.

Potter C (1935) The biology and distribution of *Rhyzopertha dominica* (Fab.). *Transactions of the Royal Entomological Society of London* **83**, 449–482.

Quellhorst H, Athanassiou CG, Bruce A, Scully ED and Morrison IIIWR (2020) Temperature-mediated competition between the invasive larger grain borer (Coleoptera: Bostrichidae) and the cosmopolitan maize weevil (Coleoptera: Curculionidae). *Environmental Entomology* **49**, 255–264.

Rumbos CI, Pantazis I and Athanassiou CG (2019) Population growth of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) on various commodities. *Journal of Economic Entomology* **113**, 1001–1007.

Skourti A, Kavallieratos NG and Papanikolaou NE (2020) Suitability of semolina, cracked wheat and cracked maize as feeding commodities for *Tribolium castaneum* (Herbst; Coleoptera: Tenebrionidae). *Insects* **11**, 99.

Shafique M, Ahmad M and Chaudry MA (2006) Feeding preference and development of *Tribolium castaneum* (Herbst.) in wheat products. *Pakistan Journal of Zoology* **38**, 27–31.

Sinha RN (1969) Reproduction of stored-grain insects on varieties of wheat, oats, and barley. *Journal of Economic Entomology* **62**, 1011–1015.

Sinha RN (1975) Effect of dockage in the infestation of wheat by some stored-product insects. *Journal of Economic Entomology* **68**, 699–703.

Sinha AK and Sinha KK (1990) Insect pests, *Aspergillus flavus* and aflatoxin contamination in stored wheat: a survey at North Bihar (India). *Journal of Stored Product Research* **26**, 223–226.

Sokoloff A, Franklin IR, Overton LF and Ho FK (1966) Comparative studies with *Tribolium* (Coleoptera, Tenebrionidiae) - I: productivity of *T. castaneum* (Herbst) and *T. confusum* Duv. On several commercial-available diets. *Journal of Stored Product Research* **1**, 295–311.

Statistica, 12.0 (2010) StatSoft, Inc., Tulsa, OK, USA.

Stejskal V (2000) Cumulative and actual population size of stored-product pests: effect on initial numbers and rate of increase. *Plant Protection Science* **36**, 65–68.

Downloaded from https://www.cambridge.org/core. Vyzkumny Ustav Rostlinne Vyroby, on 03 Aug 2020 at 10:47:28, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007485320000425

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

Stejskal V, Hubert J, Aulicky R and Kucerova Z (2015) Overview of present and past and pest-associated risks in stored food and feed products: European perspective. *Journal of Stored Products Research* **64**, 122–132.

Stejskal V, Vendl T, Li Z and Aulicky R (2019) Minimal thermal requirements for development and activity of stored product and food industry pests (Acari, Coleoptera, Psocoptera, Diptera and Blatodea): a review. *Insects* **10**, 149.

Stewart-Jones A, Hodges RJ, Birkinshaw LA and Hall DR (2004) Response of *Teretrius nigrescens* toward the dust and frass of its prey, *Prostephanus truncatus*. *Journal of Chemical Ecology* **30**, 1628–1646.

Stewart-Jones A, Stirrup TJ, Hodges RJ, Farman DI and Hall DR (2009) Analysis of free fatty acids in food substrates and in the dust and frass of stored-product pests: potential for species discrimination? *Journal of Stored Products Research* **45**, 119–124.

Trematerra P and Throne J (2012) Insect and mite pests of durum wheat. In Sissions M, Abecassis J, Marchylo B and Carcea M (eds), *Durum Wheat Chemistry and Technology*. St. Paul, MN, USA: AACC International Inc., pp. 73–83.

Trematerra P, Valente A, Athanassiou CG and Kavallieratos NG (2007) Kernel-kernel interactions and behavioural responses of the adults maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Applied Entomology and Zoology* **42**, 129–135.

Trematerra P, Stejskal V and Hubert J (2011) The monitoring of semolina contamination by insect fragments using the light filth method in an Italian mill. *Food Control* **22**, 1021–1026.

Turney HA (1957) Some effects of cracked grain on the reproduction of the saw-toothed grain beetle. *Journal of Kansas Entomological Society* **30**, 6–8.

Weiss MR (2006) Defecation behavior and ecology of insects. *Annual Review of Entomology* **51**, 635–661.

White GG (1982) The effect of grain damage on development in wheat of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research* **18**, 1151–1119.

White NDG (1995) A multidisciplinary approach to stored grain. *Journal of Stored Products Research* **28**, 1271–1237.

Yildirim-Aksoy M, Eljack R and Beck BH (2020) Nutritional value of frass from black soldier fly larvae, *Hermetia illucens*, in a channel catfish, *Ictalurus punctatus*, diet. *Aquaculture Nutrition* **00**, 1–8.

Downloaded from https://www.cambridge.org/core. Vyzkumny Ustav Rostlinne Vyroby, on 03 Aug 2020 at 10:47:28, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007485320000425

Journal of Stored Products Research 99 (2022) 102022

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Effect of grain excavation damages by *Sitophilus granarius* on the efficacy of grain protectant insecticides against *Cryptolestes ferrugineus* and *Tribolium castaneum*

Tomas Vendl [a,*], Jawad Ali Shah [b], Radek Aulicky [a], Vaclav Stejskal [a,b]

[a] Crop Research Institute, Drnovska 507/73, Prague 6-Ruzyne, CZ-16106, Czech Republic

[b] Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague 6-Suchdol, CZ-16500, Czech Republic

ARTICLEINFO

Keywords:
Stored grain Grain injury
Pest beetles
Grain protectants
Insecticide

ABSTRACT

Internally feeding primary pests produce dusty frass that is known to support the development of externally developing beetles. Apart from frass, primary pests create semi-opened cavities in kernels that provide an opportunity for hiding and feeding of secondary pests, increase grain surface area and decrease weight of excavated kernels. It is unknown whether the excavated kernels may affect the efficacy of grain protectants and the development of secondary pests. Therefore, this study explored insecticide droplet distribution on the surface of excavated kernels by *Sitophilus granarius.* Further, it evaluated the effect of grain mixtures - differing in various proportions of sound and excavated kernels without frass - on the development and efficacy of two different doses of grain protectant insecticides (pirimiphos-methyl and deltamethrin) in sensitive strains of *Cryptolestes ferrugineus* and *Tribolium castaneum.* It was found - using dyed insecticide spray - that half of kernels did not receive droplets inside the excavations. Bioassays revealed that (i) the adult survival and progeny production of *C. ferrugineus* and *T. castaneum* were recorded only on mixtures with a high proportion of excavated kernels; (ii) the higher dose of pirimiphos-methyl and deltamethrin led to 100% mortality in both pests under all conditions; and (iii) the lower dose enabled survival on grain mixtures with an increased ratio of excavated to sound kernels in both chemicals and secondary pest species. The work first showed that a high proportion of damaged kernels in a grain batch/sample can support the development of *C. ferrugineus* and *T. castaneum* even without the presence of frass and that excavated kernels may cause pest survival in the case of a lower concentration of grain protectants.

1. Introduction

Stored product arthropod pests cause weight losses of commodities and deterioration of quality (Egwuatu, 1987; Hagstrum and Phillips, 2017). The physical injury of grains in the form of internal excavations of kernels by internally feeding pests is used by food producers and traders as an IDK (insect-damaged kernels) grain quality index (Stejskal et al., 2020a). Apart from economic losses, storage arthropods cause deterioration and contamination of stored commodities and processed food that is of medical/hygienic importance (Hubert et al., 2011; 2018).

The management of these pest-related risks requires an effective combination of proactive and reactive targeted approaches. Grain protectant sprays are both proactive and reactive measures (Arthur and Subramanyam, 2012; Stejskal et al., 2021). Kljaji´c and Peri´c (2009) concluded from their experiments that wrong choice of insecticide grain protectant and its inadequate application might in practice result in poor treatment efficiency. Since the efficacy of various grain protectants may differ in various species and stages of stored product arthropods (Arthur, 1996), proper selection may be complicated by multispecies infestation of a single store or commodity batch. Athanassiou et al. (2009) found that grain protectant efficacy on secondary feeding insects may be affected by diet, i.e., various kinds of sound crop grain, such as rice, wheat, or maize. In addition, interspecific interactions may have an impact on the development of various pest species populations and insecticide performance in both negative and positive ways. Interspecific interactions of storage pests are now a subject of renewed research interest (Athanassiou et al., 2014; Kavallieratos et al., 2017; Sakka and Athanassiou, 2018). Interspecific population effects may be based not only on direct interactions but also on indirect ecological interactions that include changes in

* Corresponding author.

E-mail address: vendl@vurv.cz (T. Vendl).

https://doi.org/10.1016/j.jspr.2022.102022

Received 4 July 2022; Received in revised form 17 August 2022; Accepted 25 August 2022 0022-474X/© 2022 Elsevier Ltd. All rights reserved.

microclimatic conditions (e.g., changes in humidity or temperature) and physical changes/integrity of substrate. Kernels damaged by insect feeding may release attractive volatile odour compounds and affect pest behaviour and feeding (Trematerra et al., 2000, 2013; Đuki´c et al., 2018; Giunti et al., 2018). More importantly, the presence of grain damage together with frass, caused by primary coleopteran pests (e.g., *Rhyzopertha dominica, Trogoderma granarium*), can support the development of some secondary insect pest species (Kavallieratos et al., 2017; Shah et al., 2021; Lampiri et al., 2022). The effect of grain damage may likely be less pronounced in primary pests than in secondary species (Gvozdenac et al., 2018). The only exception among the secondary pests are the psocids that are able to directly attack sound or intact kernels (Gautam et al., 2013). It remains to be determined whether the excavated kernel itself (with unconsumed parts of endosperm and germ) can create proper microhabitats for secondary pests, providing physical protection/shelter and access to the food source remaining inside the damaged seed.

From the perspective of the chemical control of storage pests, it is also poorly understood whether the physical disintegration of grain by pests might influence the efficacy of insecticides. It is unclear if the internal space of the kernel cavities remains free of deposits of grain protectants, and if spray droplets are able to enter kernel excavations during grain spray treatment. There are also missing data on the effect of kernel excavations caused by primary pests on the efficacy of grain protectants on secondary pests. Currently, most of the tests of grain protectants are almost exclusively conducted on sound kernels (European and Mediterranean Plant Protection Organization, 2004a,b; Rumbos et al., 2013; Arthur and Morrison, 2020). For example, Rumbos et al. (2018) stated that untreated, clean and infestation-free durum wheat varieties with very little dockage were used in their tests. However, this may not always reflect the real storage conditions where the uncleaned stored grain may contain various levels of dockage, foreign materials, grain dust, frass, grain fragments, cracked grain and various degrees of kernel damage by primary pests (Golebiowska, 1969; Flinn et al., 1992; Stejskal and Kucerova, 1996; Stejskal et al., 2007; Alonso-Amelot and Avila-Núnez, 2011˜ ; Trematerra and Throne, 2012; Morrison et al., 2019; Skourti et al., 2020; Jian 2022; Jian and Zhang, 2022). Egwuatu (1987) documented that in some developing countries, the average damage caused by primary storage pests may be over 80%. However, because primary pests such as *R. dominica* or *Sitophilus* spp. strongly tend to aggregate (Plarre, 1996), the proportion of damaged kernels in the infested spots may be several magnitudes higher than the average number of damaged kernels in the entire grain mass (Mehta et al., 2021).

This work therefore addressed some of the above unsolved problems regarding the effect of kernel excavation by internally feeding pests on the development of some externally feeding pests and the efficacy of selected grain protectants. We specifically tested the following two hypotheses. *Hypothesis 1:* There exists a mixture of sound to excavated (damaged) kernels by *S. granarius* that enables population development of at least one species of the two secondary Coleoptera storage pests tested (*Cryptolestes ferrugineus* and *Tribolium castaneum*). *Hypothesis 2:* Any presence of excavated kernels decreases the efficacy of the regular or lower doses of grain protectant (based on either deltamethrine or pirimiphos-methyl) to the secondary storage pests *C. ferrugineus* and *T. castaneum*. To test both hypotheses, laboratory bioassay studies were conducted to explore the effect of seven mixtures of sound and damaged excavated kernels (when frass and dust were excluded by sieving) on the population development and efficacy of two doses of grain protectant insecticides (pirimiphos-methyl and deltamethrin) in sensitive strains of *C. ferrugineus* and *T. castaneum*. We included standard and lower doses to test the effect since dosage underestimation may occur due to mistakes, locally/temporally uneven spray application and natural decay of insecticide residues (LaHue, 1977; Kljaji´c and Peri´c, 2009). With a low water volume spray rate, it is likely that there is uneven deposition when grain is treated (Arthur, 2019). The additional aim of our work was to analyse the distribution of protectant droplets on damaged kernels to determine the proportion of damaged kernels containing dyed insecticide droplets inside their cavities.

2. Materials and methods

The experiments presented here were arranged in the following workflow. Initially, the effect of various proportions of excavated-to- sound kernels in a sample on the thousand kernel weight (TKW) parameter was measured, and the insecticide dosage used in the subsequent experiment was adjusted accordingly. Then, an application of dyed insecticide was performed on grain damaged (in a defined way) by *S. granarius* to analyse the microdistribution of spray deposits on the kernel surface and to estimate the percent of damaged kernels containing droplets inside the kernel excavations. Finally, bioassay experiments included laboratory experimental validation of the effect of various proportions of damaged-to-sound kernels in each sample on the survival and development of two secondary pest species and on the efficacy of two doses of two insecticides.

2.1. Experimental species

Laboratory cultures of *Sitophilus granarius*, *Cryptolestes ferrugineus* and *Tribolium castaneum* were reared at the Crop Research Institute (CRI - Prague, Czech Republic). *Cryptolestes ferrugineus* and *T. castaneum* were reared for more than 15 generations without contact with insecticide. They originated from small organic farms; thus, we assumed that both beetle strains were insecticide-sensitive. Both species were reared on a mixture of milled wheat kernels, oat flakes, and yeast (10:10:2), covered by moistened filter paper. Both species were kept in thermo-chambers
(Aviko Praha, Czech Republic) at 27 (±0.5) °C with 75 (±5) % relative humidity (r. h.). *Sitophilus granarius* was reared at 25 °C on cleaned wheat kernels that were dried and remoistened to 15% before application.

2.2. Grain samples and TKW

The effect of grain damage on grain weight was analysed using a thousand kernel weight (TKW) parameter. A required number of either sound or excavated kernels was counted using a Contador seed counter (Pfeuffer GmBH, Germany), and these excavated and sound (intact) kernels were subsequently mixed to obtain a desirable sound: excavated (S:E) kernel ratio. The S:E kernel ratios were 0:100, 5:95, 25:75, 50:50, 75:25, 95:5, and 100:0. For each of the seven S:E ratios, eight samples of 1000 kernels were prepared and weighed on an analytical scale (Adventurer Pro, Ohaus, USA).

2.3. Distribution of insecticide droplets on treated grain kernels

Adult mortality and larval development (and therefore insecticide efficacy) tightly depend upon whether excavations of the kernels provide refuge for the beetles. It is thus crucial to determine if insecticide spraying can deliver the insecticide into the excavation or not. Some kernels may be oriented with the excavation down (and thus not exposed to the spraying) (Stejskal et al., 2020a), or the excavation may be too small (yet still allowing beetles to enter) and thus the insecticide misses it (Fig. 1). For that reason, we sought to determine the insecticide distribution on excavated kernels, specifically the proportion of kernels with insecticide inside the excavations. To estimate (and visualize) the coverage of kernels by insecticide droplets, we sprayed the excavated kernels with coloured water with deltamethrin. The spraying procedure and number of sprayed grains were the same as in the experimental setup (see Section 2.4). One gram of purple food colour concentrated

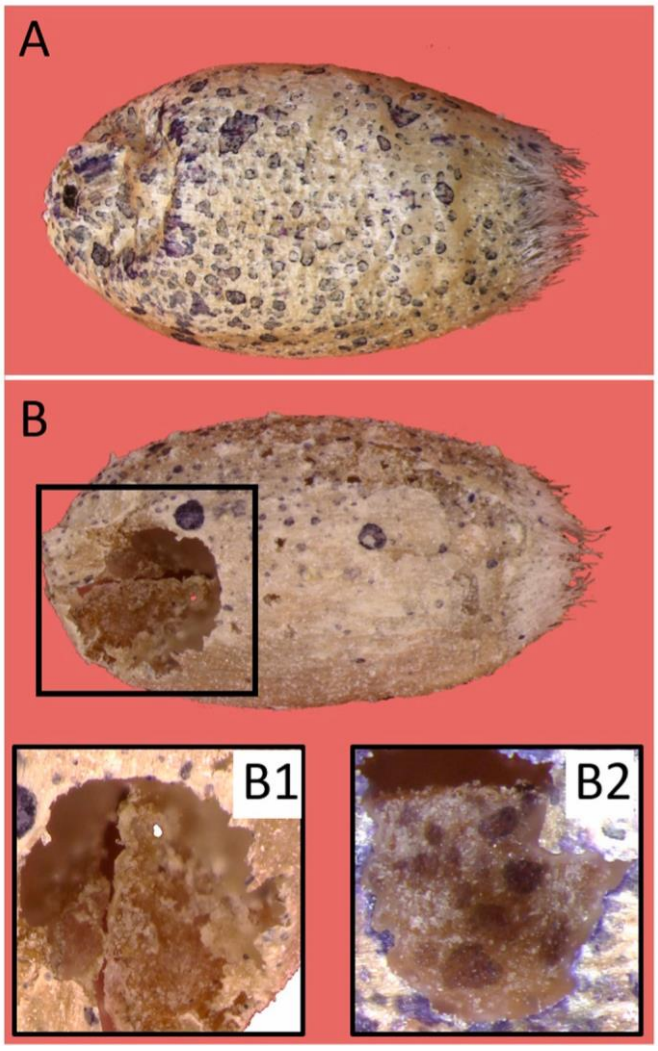


Fig. 1. Examples of excavated kernels with the excavation on the bottom (i.e., insecticide-unexposed) side (A) and with the excavation on the insecticide- exposed side. From these, the excavation without (B1) and with (B2) coloured insecticide droplets inside are shown.

paste (Knightsbridge PME Ltd., Enfield, UK) was diluted in 250 mL water with a labelled dose of deltamethrin. After spraying, the kernels were left on filter paper for 24 h for the water to dry after which they were mixed, and 10 samples consisting of 20 kernels were taken. The individual kernels were examined under an Olympus SZX10 stereomicroscope. Based on our previous work (Stejskal et al., 2020), we assumed that the positions of the droplets with insecticide on the kernel may affect the insecticide's efficacy. Thus, we sought to determine if the kernel excavation hole was positioned at the top of the kernel, and if so, if it resulted in a droplet entering the hole.

2.4. Grain protectants and experimental treatment

To test the effect of kernel excavation on the efficacy of grain protectants, the substrate was treated with commercial formulations of commonly used pesticides for grain protection. They included (i) K- Obiol®EC25 (Bayer, Leverkusen, Germany), containing pyrethroid deltamethrin (25 g/l) + piperonyl butoxide (225 g/l), and (ii) Actellic®50 EC (Syngenta, Guildford, Surrey, UK), containing organophosphate pirimiphos-methyl (500 g/l). Because we found that excavated kernels have half the weight of the sound kernels (Fig. 2), which can have possible consequences in an underestimation of the insecticide

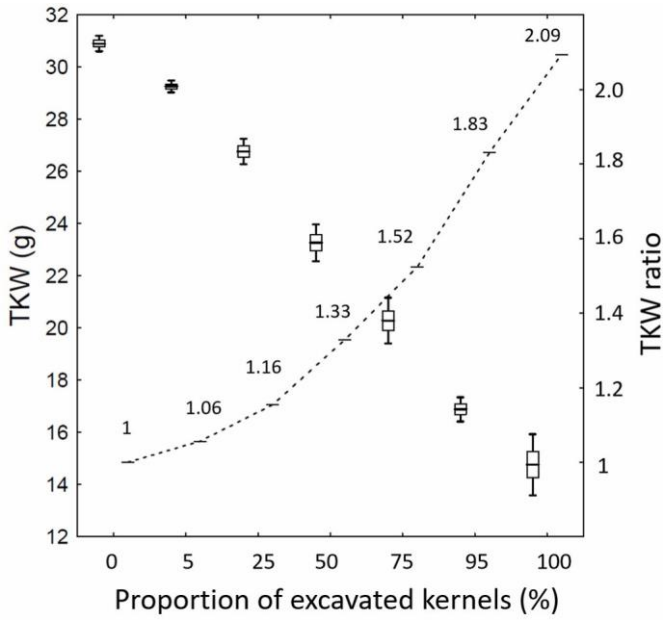


Fig. 2. Relationship between average TKW (thousand kernel weight) and seven mixtures of sound (S) and excavated (E) wheat kernels in defined ratios. TKW decreased (and its variability increased) with the increasing portion of the *Sitophilus granarius* excavated kernels and decreasing sound kernels.

dose used (in an extreme case by half) in practice, we used the label dose as well as half the label dose of both insecticides. Thus, K-Obiol®EC25 was tested at 5 and 10 ml per ton of grains, and Actellic®50 EC was tested at 4 and 8 ml per ton of grains. Because of the different weights of sound and excavated kernels, volume was used instead of weight. It was found that 1 kg of sound kernels ≈0.00136 m^3. Both insecticides were applied as water solutions. They were sprayed by hand using a micro- sprayer (Nuohua, Taizhou, China – container 60 ml) on the surface of 0.00136 m^3 kernels spread out on filter paper. Because the label amount of water dilutant needed to treat 0.00136 m^3 of grains was too small to cover the grains evenly, we followed the method suggested by Arthur et al. (2020) in such cases: we mixed the label amount of insecticide with an increased volume (i.e., 10 mL) of water. The control grain mixture was sprayed with water only.

2.5. Preparation of mixtures of sound and excavated kernels

The wheat used in the study was a mixture of undetermined cultivars purchased from a grain organic store (Czech Republic) in 2019. To prepare excavated (i.e., damaged by *S. granarius*) kernels used in various ratios as an experimental grain mixture, the kernels were desiccated, remoistened to 15% and placed in 5 L glass jars with 100 *S. granarius* adult individuals. The jars were kept in thermo-chambers in the dark at 26 °C. Every three months, new grain was added to replenish feeding substrate. After 12 months, which represents approximately four beetle generations, the kernels were sieved off using standard laboratory sieves with a mesh size of 2 mm to remove frass, hulls and other impurities. Further, the kernels were passed through a Contador seed counter (Pfeuffer GmBH, Germany) and only compact kernels were selected. Subsequently, three 10 g kernel samples were examined, which proved that nearly 100% of the kernels were internally damaged (i.e., either contained exit holes from *S. granarius* leaving the kernel or were extensively penetrated by beetle feeding; both types of damage are further referred to as kernel excavation irrespective of their mode of origin). Thus, all the sieved material was considered 100% damaged and was used as the damaged kernel substrate.

To test the effect of kernel excavation on survival and progeny production (as defined in the context of testing grain protectants by Huang and Subramanyam, 2005) of *T. castaneum* and *C. ferrugineus* and on grain

protectant efficacy, a total of seven mixtures of sound (S) (= intact) and excavated (E) wheat kernels were used as breeding substrates. These were S:E ratios of 0:100, 5:95, 25:75, 50:50, 75:25, 95:5, and 100:0. Because we found that sound and excavated kernels differ in weight (Fig. 2), we did not use weight as a unit, but used volume instead. We tested the ability of the beetles to survive and reproduce (progeny production) on a grain mixture consisting of various proportions of sound and excavated kernels, as well as the efficacy of two residual insecticides on the mixtures. An optimal diet consisting of a mixture of milled wheat kernels, oat flakes, and yeast in a ratio of 10:10:2 was used as a control substrate. Thirty millilitres of the prepared mixtures were placed in a 100 ml plastic vial with a perforated cap covered by nylon mesh. The top of the inner walls of the vials were treated with synthetic fluoropolymer lubricant powder (polytetrafluoroethylene) (Sigma–Aldrich Co., St. Louis, USA) to prevent insects from escaping. Ten unsexed adults of *T. castaneum* or *C. ferrugineus* were introduced into each vial and kept in a climatic chamber (POL-EKO APARATURA, Poland) at 26 (±0.5) °C. Because of the possibility of evaporation of deltamethrin and pirimiphos-methyl from grain mass and the resulting possible effect as gaseous insecticide (Ileke and Bulus, 2012), each of the substrate treatments and untreated substrates was placed in a separate climatic chamber. After 6 weeks, the vials were opened, the substrate was sieved, and the numbers of live/dead adults, larvae and pupae were counted under an Olympus SZX10 binocular microscope. For each substrate and species, eight replicates were performed.

2.6. Statistics

Differences in adult mortality, progeny production and TKW among substrates with different proportions of excavated kernels were tested with one-way ANOVA, followed by Tukey's HSD post hoc test. This was carried out for each pest species separately. Differences in the efficacy of insecticides depending on the dose and proportion of excavated kernels were tested with a three-way factorial ANOVA with insecticide type, insecticide dose and grain mixture as factors. Because of the violation of normal distribution (tested by Kolmogorov–Smirnov test), the data on mortality and larval development were subjected to log(x+1) transformation before analysis. For simplicity, untransformed means and standard errors are reported in the text and tables. The analyses were conducted with Statistica 12.0 (Statistica, 2010).

3. Results

3.1. Thousand kernel weight (TKW) and mixtures of sound and S. granarius excavated kernels

There was a clear effect of the proportion of excavated kernels in seven mixtures of sound and excavated kernel ratios on TKW ($F_{6, 49} = 459.9$; $p < 0.05$). The more excavated kernels there were in the mixture, the lower was the TKW (Fig. 2). The TKW decreased almost linearly, and its variability increased with the gradually increasing portion of the *S. granarius* excavated kernels and the decreasing sound kernels. It was quantified that in the 100% mixture of excavated kernels, the TKW was more than twofold lower in comparison with a 100% sound grain (14.8 ± 0.50 and 30.9 ± 0.13 g, respectively). Grains with 5% damaged kernels had approximately 6% lower TKW than sound kernels (TKW ratio); grains with 25% damaged kernels had approximately 16% lower TKW (Fig. 2). For grain treatment protectants, this implies (since label dosage concerns the volume of insecticide per weight unit of grain) that mixtures with a higher portion of excavated kernels may contain up to double the number of kernels per identical grain weight.

3.2. Distribution of insecticide droplets on the kernel surface and inside grain excavations

The proportion of kernels with dyed insecticide droplets both on the surface and inside the grain excavations was estimated. Three basic patterns of droplet micro-distribution on the surface of excavated kernels are visualized in Fig. 1. It was found that from a set of 200 examined kernels, 49.5 ± 2.9% of all excavated kernels were without insecticide inside the excavations. Of the total number, 43.0 ± 2.6% kernels were prevented from possibly receiving any single droplet inside the excavation cavity because the excavation hole was facing downwards (Fig. 1A) during the spray treatment. From the remaining (57.0 ± 2.6%) kernels that had excavation holes that were facing upwards during the spray treatment (Fig. 1B), the vast majority (i.e., 86.0 ± 1.8%) contained the insecticide droplet inside the excavation (Fig. 1B1), and only 14.0 ± 1.8% of those excavations were without the insecticide droplets (Fig. 1B2).

3.3. Development of C. ferrugineus and T. castaneum on mixtures of sound and excavated kernels

The survival and progeny production of *T. castaneum* and *C. ferrugineus* were affected by the number of excavated kernels in a respective mixture of sound and excavated kernels. Both species thrived best on grain mixtures consisting of only excavated kernels, although the number of progeny was lower than that in the control diet (Tables 1 and 2 for *T. castaneum* and *C. ferrugineus*, respectively). Generally, an increasing proportion of excavated kernels to sound kernels increased the number of progeny (Fig. 3). Apparently, excavated kernels represent suitable breeding substrates for both secondary pest species, and their presence in grain mass may support their population growth. Thus, Hypothesis 1 was not rejected.

In both species, on grain mixtures with 25% or less of excavated kernels, there was no or only negligible progeny production, so these ratios of excavated and sound kernels did not allow the populations to

Table 1
Survival and progeny production (means ± SEs) of *Tribolium castaneum* on wheat grain mixtures with different proportions of excavated and sound kernels. Within each column, means followed by the same letter are not significantly different; Tukey's HSD post hoc test at 0.05.

Substrate	Proportion of excavated kernels (%)	Live adults	Dead adults	Larvae	Pupae	Total number of progeny
Optimal diet (Control)	N/A	10.0 ± 0.0^{a}	0.0 ± 0.0^{a}	194.3 ± 3.7^{a}	6.9 ± 1.3^{a}	201.1 ± 3.8^{a}
Mixture of sound and excavated kernels	100	10.0 ± 0.0^{a}	0.0 ± 0.0^{a}	118.8 ± 6.8^{ab}	0.25 ± 0.16^{b}	119 ± 6.9^{ab}
	95	10.0 ± 0.0^{a}	0.0 ± 0.0^{a}	86.0 ± 3.6^{b}	0.1 ± 0.13^{b}	86.1 ± 3.5^{b}
	75	10.0 ± 0.0^{a}	0.0 ± 0.0^{a}	38.25 ± 5.8^{c}	0.0 ± 0.0^{b}	38.25 ± 5.8^{c}
	50	7.4 ± 0.68^{a}	2.6 ± 0.68^{b}	5.7 ± 1.57^{d}	0.0 ± 0.0^{b}	5.7 ± 1.57^{d}
	25	1.5 ± 0.82^{b}	8.5 ± 0.82^{c}	0.75 ± 0.53^{e}	0.0 ± 0.0^{b}	0.75 ± 0.53^{e}
	5	0.0 ± 0.0^{c}	10.0 ± 0.0^{c}	0.0 ± 0.0^{e}	0.0 ± 0.0^{b}	0.0 ± 0.0^{e}
	0	0.0 ± 0.0^{c}	10.0 ± 0.0^{c}	0.0 ± 0.0^{e}	0.0 ± 0.0^{b}	0.0 ± 0.0^{e}
		$F_{7,56}$ = 109.0 $p <$ 0.001	$F_{7,56}$ = 158.4 $p <$ 0.001	$F_{7,56}$ = 274.5 $p <$ 0.001	$F_{7,56}$ = 63.4 $p <$ 0.001	$F_{7,56}$ = 276.2 $p <$ 0.001

Table 2
Survival and progeny production (means ± SEs) of *Cryptolestes ferrugineus* on wheat grain mixtures with different proportions of excavated and sound kernels. Within each column, means followed by the same letter are not significantly different; Tukey's HSD post hoc test at 0.05.

Substrate	Proportion of excavated kernels (%)	Live adults	Dead adults	Larvae	Pupae	Total number of progeny
Optimal diet (Control)	N/A	10.0 ± 0.0[a]	0.0 ± 0.0[a]	42 ± 3.2[a]	8.0 ± 1.9[a]	50.4 ± 4.0[a]
Mixture of sound and excavated kernels	100	10.0 ± 0.0[a]	0.0 ± 0.0[a]	10.9 ± 1.4[b]	0.0 ± 0.0[b]	10.9 ± 1.4[b]
	95	8.6 ± 0.50[a]	1.4 ± 0.50[a]	9.0 ± 1.8[b]	0.0 ± 0.0[b]	9.0 ± 1.8[b]
	75	8.5 ± 0.27[a]	1.5 ± 0.27[a]	6.8 ± 1.3[b]	0.0 ± 0.0[b]	6.8 ± 1.3[b]
	50	7.6 ± 0.63[a]	2.4 ± 0.63[a]	3.3 ± 0.49[c]	0.0 ± 0.0[b]	3.3 ± 0.49[c]
	25	3.25 ± 1.13[b]	6.75 ± 1.13[b]	0.5 ± 0.27 [d]	0.0 ± 0.0[b]	0.5 ± 0.27 [d]
	5	0.0 ± 0.0[c]	10.0 ± 0.0[b]	0.0 ± 0.0 [d]	0.0 ± 0.0[b]	0.0 ± 0.0 [d]
	0	0.0 ± 0.0[c]	10.0 ± 0.0[b]	0.0 ± 0.0 [d]	0.0 ± 0.0[b]	0.0 ± 0.0 [d]
		$F_{7,56}$ = 60.6 p < 0.001	$F_{7,56}$ = 20.8 p < 0.001	$F_{7,56}$ = 117.9 p < 0.001	$F_{7,56}$ = 280.4 p < 0.001	$F_{7,56}$ = 125.4 p < 0.001

grow. Thresholds of the proportion of excavated to sound kernels permitting a stable population (i.e., the number of progeny of the 10 introduced adults was ≥10) estimated from quadratic equations were 53.9% and 98.6% for *T. castaneum* and *C. ferrugineus,* respectively. On mixtures with a low proportion of excavated kernels, there was also high adult mortality (Tables 1 and 2).

3.4. Efficacy of formulations with pirimiphos-methyl and deltamethrin in mixtures of sound and excavated kernels

One hundred percent efficacy was found for the tested label dosages of both commercial formulations with pirimiphos-methyl (Actellic®50 EC) and deltamethrin (K-Obiol®EC25) irrespective of species and tested ratio mixtures of sound to excavated kernels. However, using a half-label dosage of both protectants was less efficient and permitted adult survival and even progeny production of both tested species (*T. castaneum* and *C. ferrugineus*) on the mixtures with a higher proportion of excavated than sound grains (Tables 3–6). Thus, Hypothesis 2 was rejected for the recommended label dosage of both insecticides but was not rejected for the half label dosage. There were differences between formulations of insecticides: deltamethrin (K-Obiol) was more efficient than pirimiphos- methyl (Actellic) ($F_{1,196}$ = 268.5, $p < 0.05$ for *T. castaneum* and $F_{1,196}$ = 24.3, $p < 0.05$ for *C. ferrugineus*). In the case of a half dose of pirimiphos- methyl formulation, there were live larvae of both species present in 100% and 95% excavated mixtures, while in the case of deltamethrin, there were only a few larvae in the 100% excavated mixture (in all cases, there were significantly fewer progeny than in the untreated substrates; ANOVA: $p < 0.05$; Tables 3–6). In addition to the live larvae, we observed many dead larvae (not counted) of lower instars in the mixtures in which live adult beetles were present. Apparently, the beetles were capable of finishing part of the reproduction cycle (from eggs to young larvae) in the treated mixtures of grain with large proportions of excavated kernels, but young larvae died soon after hatching. Live adults were present in grain mixtures with 50% and more excavated kernels, but only in mixtures treated with a half label dose; in mixtures treated with a label dose, there was 100% adult mortality in all mixtures.

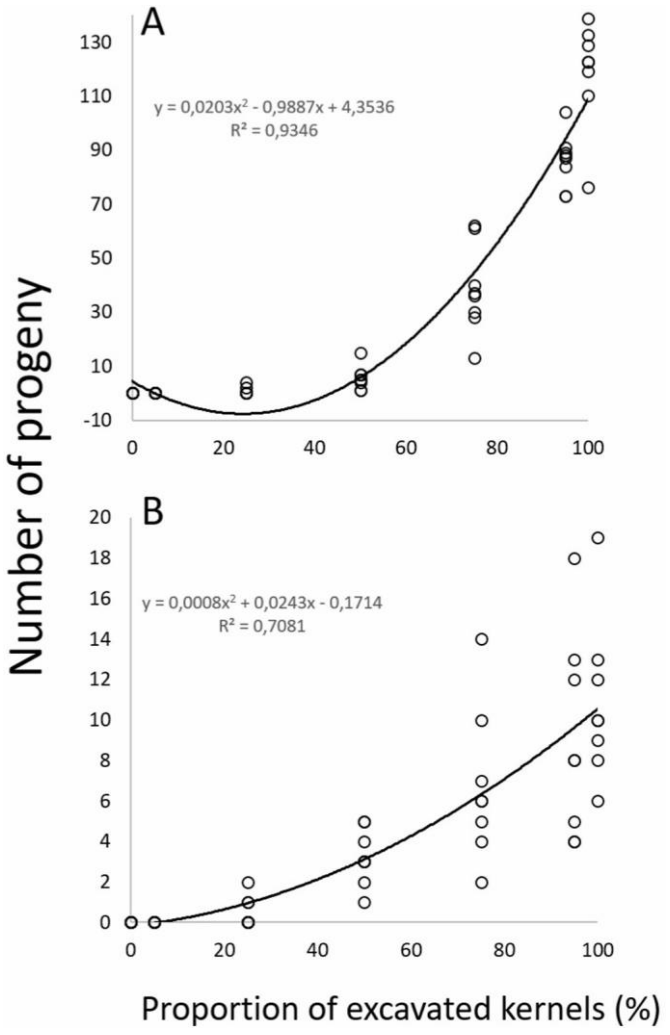


Fig. 3. Regression plots depicting the relation between the proportion of excavated kernels and progeny production in (A) *Tribolium castaneum* and (B) *Cryptolestes ferrugineus.*

4. Discussion

This work evaluated, to our knowledge for the first time, the effect of the presence of various proportions of damaged (=excavated by primary pest species) kernels vs. sound kernels on the efficacy of any type of grain protectant insecticide based on pirimiphos-methyl and deltamethrin. In addition, it also evaluated the survival and progeny production abilities of two secondary pests (*C. ferrugineus* and *T. castaneum*) depending on the percentage of damaged kernels per grain weight unit.

4.1. Kernel excavations and TKW

The application of insecticides according to labels is based on the correct determination of the dose according to the weight of the grain being treated. Damage caused by internally feeding pests leads to a decrease in the weight of individual kernels and dramatically increases grain volume and its surface relative to its weight. For example, Campbell and Sinha (1976) documented experimentally that developing from egg to pupa, *S. granarius* causes 60% weight loss on a single kernel, while *R. dominica* causes 17% weight loss. Thus, infestation by various pests may have a possible impact on the correct dosage of grain protectants under both experimental and practical conditions. For that reason, it is crucial to accurately characterize the degree of grain damage. Alonso-Amelot and Avila-Núnez (2011) ˜ stressed that there are at

Table 3
Survival and progeny production (means ± SEs) of *Tribolium castaneum* on wheat grain mixtures with different proportions of excavated and sound kernels treated with two doses of deltamethrin (K-Obiol). * symbolizes cases that are different from untreated substrate (ANOVA: $p < 0.05$).

Proportion of excavated kernels (%)	Dose	Live adults	Dead adults	Larvae	Pupae
100	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	10.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.4*	0.0 ± 0.0
95	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0*	0.0 ± 0.0
75	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	8.9 ± 0.3*	1.1 ± 0.3*	0.0 ± 0.0*	0.0 ± 0.0
50	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	6.2 ± 0.5	3.8 ± 0.5	0.0 ± 0.0*	0.0 ± 0.0
25	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 4
Survival and progeny production (means ± SEs) of *Tribolium castaneum* on wheat grain mixtures with different proportions of excavated and sound kernels treated with two doses of pirimiphos-methyl (Actellic). * symbolizes cases that are different from untreated substrate (ANOVA: $p < 0.05$).

Proportion of excavated kernels (%)	Dose	Live adults	Dead adults	Larvae	Pupae
100	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	8.4 ± 0.6*	1.6 ± 0.6*	19.4 ± 2.2*	0.0 ± 0.0
95	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	8.4 ± 0.6*	1.6 ± 0.6*	13.4 ± 2.2*	0.0 ± 0.0
75	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	7.8 ± 0.5*	2.2 ± 0.5*	0.5 ± 0.3*	0.0 ± 0.0
50	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	3.4 ± 0.8*	6.6 ± 0.8*	0.0 ± 0.0*	0.0 ± 0.0
25	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 5
Survival and progeny production (means ± SEs) of *Cryptolestes ferrugineus* on wheat grain mixtures with different proportions of excavated and sound kernels treated with two doses of deltamethrin (K-Obiol). * symbolizes cases that are different from untreated substrate (ANOVA: $p < 0.05$).

Proportion of excavated kernels (%)	Dose	Live adults	Dead adults	Larvae	Pupae
100	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	8.6 ± 0.5*	1.4 ± 0.5*	0.5 ± 0.3*	0.0 ± 0.0
95	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	8.6 ± 0.5	1.4 ± 0.5	0.0*	0.0 ± 0.0
75	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	7.4 ± 0.7	2.6 ± 0.7	0.0*	0.0 ± 0.0
50	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	4.6 ± 0.7*	5.4 ± 0.7*	0.0*	0.0 ± 0.0
25	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0
5	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 6
Survival and progeny production (means ± SEs) of *Cryptolestes ferrugineus* on substrates with different proportions of excavated and sound kernels treated with two doses of pirimiphos-methyl (Actellic). * symbolizes cases that are different from untreated substrate (ANOVA: $p < 0.05$).

Proportion of excavated kernels (%)	Dose	Live adults	Dead adults	Larvae	Pupae
100	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	9.0 ± 0.4*	1.0 ± 0.4*	1.6 ± 0.6*	0.0 ± 0.0
95	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	7.5 ± 0.8	2.5 ± 0.8	1.6 ± 0.4*	0.0 ± 0.0
75	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	7.3 ± 0.7	2.7 ± 0.7	0.0*	0.0 ± 0.0
50	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0
	1/2 label	6.6 ± 0.9	3.4 ± 0.9	0.0*	0.0 ± 0.0
25	Label	0.0 ± 0.0*	10.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	1.4 ± 0.7	8.6 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
5	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0	Label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1/2 label	0.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

least seven ways to accurately assess the degree of damage to stored cereal grain. Since thousand kernel weight (TKW) is the most routinely used parameter in practice, it was also selected for our experiments. In our experiments, we determined that different mixtures of *S. granarius* excavated kernels and sound kernels can differ significantly in TKW. In the simulated

extreme case, i.e., samples with 100% healthy grains versus samples in which all grains had internal excavation caused by *S. granarius,* there was up to a twofold difference in TKW for both types of samples. This part of our work has shown that if the dose of grain protectants is not compensated for in terms of TKW when applied to lots with damaged grains (which are lighter than healthy grains and therefore there are many more per unit weight of grain), then it may affect the dosage per number of seeds. Weight grain damage may be an issue in some developing countries. For example, Alonso-Amelot and Avila-Núnez (2011) ˜ estimated the average losses caused by infestation of *Sitophilus oryzae* in wheat after 90 days of exposure; the weight losses ranged from 5.9 to 9.6%, and 10–18% kernels were damaged. However, problems with local presence of highly damaged kernels may also occur in developed countries since insects and losses in the entire batch of commodities are not uniformly distributed. They are much higher in spots of pest aggregation (Plarre, 1996; Mehta et al., 2021). In addition, highly infested and damaged kernels can come with imported commodities (Stejskal et al., 2020b). *4.2. Distribution of droplets on excavated kernels*

One of the important factors of grain protectant efficacy is their spatial distribution in the grain mass. In the last decade, a number of researchers have addressed the effect of partial treatment of cereals with different types of insecticidal protectants on their efficacy. Daglish et al. (2018) warned that the uneven distribution of pesticide grain protectants might lead to the occurrence of zones within the grain bulks that are underdosed or even untreated, which allow insect infestation. Although some authors (Minett and Williams, 1971; Subramanyam et al., 2014) found that complete control of storage pests can be achieved if only a certain proportion of the kernels receive chemical treatment, several other authors claimed regular insecticide distribution throughout the commodity mass is a necessity (Kavallieratos et al., 2015; Arthur, 2019; Scully et al., 2021). For example, the results obtained for a deltamethrin protectant showed that long exposure times and treatment of an entire rice mass may be necessary to completely control *T. castaneum* (Kavallieratos et al., 2015).

The aim of our work was not to study insecticide coverage differences among different parts of the seed mass (macro-distribution). However, we currently focused our research interests on nonuniformities in the micro-distribution of dyed spray insecticide droplets on kernel surfaces (particularly in excavations) damaged by the primary pest *S. granarius*. From our experiments, it was apparent that when static grain samples were sprayed from above (top-down), relatively little proportion of the treated kernels (approximately 50%) received insecticide into their excavations. The main reason was that a high proportion of kernels are always oriented with the excavation or exit hole down (Stejskal et al., 2020a), and they inherently cannot be exposed to top-down spraying. This uneven micro-distribution of the insecticide may theoretically provide a chance for individuals of secondary pests to actively hide inside the excavated seeds and thus avoid the treated surface parts of the grain.

Hoy et al. (1998) stressed that insects have been frequently confronted in nature with situations where toxins and repellents are not distributed spatially uniform on host plants. Thus, they may evolve adaptations that allow them to avoid toxic parts of host plants. A prerequisite for effective avoidance of toxins by arthropods are their morphological climbing adaptations (Vendl et al., 2019) as well as behavioural adaptations such as increased motility and activity (Jian, 2019). It was laboratory proved the ability of storage Coleoptera pests to avoid pyrethroid insecticide residues applied on stored grain (Velez´ et al., 2017) and protective nets (Morrison et al., 2018). Behavioural avoidance may occur at either entire population or individual level (Morales et al., 2013). Published works thus indicate that there is a risk that storage pests may actively avoid treated parts of the grain even under field conditions. Since our experiment addresses only the micro-distribution of the insecticide itself on the kernel surface, further detailed behavioural experiments are needed to confirm whether the altered behaviour (i.e., avoidance of kernel surface covered with toxic droplets and feeding inside untreated kernel cavities) of secondary pests can be observed in interaction with treated grain kernels.

4.3. Kernel excavation and secondary pest development

Grain dockage (impurities, moulds, grain dust, grain fragments, and frass; Jian, 2022) may support populations of so-called secondary externally feeding pests (Flinn et al., 1992; Shah et al., 2021). This work first showed that wheat kernels damaged (excavated) by the primary internally feeding pest *S. granarius* can support the development of externally feeding secondary pests *C. ferrugineus* and *T. castaneum*, even without the presence of frass and other dockage or foreign materials. Under laboratory conditions, Campbell and Sinha (1976) estimated that when insects were allowed to develop from egg to pupa, *S. granarius* caused 60% and *R. dominica* caused 17% weight loss in a single wheat kernel. This indicates that even after the complete development of internally feeding pests, some unconsumed parts of endosperm and germ remain inside the kernel cavities. However, our experiments found that the population develops only in mixtures with a higher proportion of damaged kernels. This result thus indicates that the secondary pests required multiple damaged kernels or grain dockage to support their successful breeding in the particular area/aggregation of the infested grain. Further work is needed to elucidate the effect of the combined presence of excavated kernels with frass (Skourti et al., 2020) and some other organic impurities, such as dockage and foreign materials (Jian, 2022), on the development of secondary pests and their interactions with grain protectants.

In contrast to the work of some other authors (e.g., Rumbos et al., 2018), in our work, no survival or progeny production of either tested secondary pest species or populations was found on completely clean and sound wheat kernels at the tested humidity and temperature. The reasons for the different survival and progeny production of various secondary pests on sound grain should be addressed in more detail in subsequent works. In our view, this issue may have implications for the general methodology of testing grain protectants (Arthur and Morrison, 2020; European and Mediterranean Plant Protection Organization, 2004a, b). Most likely, more extensive characterization of the grain kernels used, apart from crop grain botanical species variety (Athanassiou et al., 2009), should be provided in grain protectant studies. The studies should include - for the treated and untreated (control) variants – the defined level of biological/physical damage of kernels and percent of dockage or foreign materials as defined by Jian and Zhang (2022).

4.4. Kernel excavations and insecticide efficacy

Chemical interventions in grain stores include preharvest treatment of empty grain stores with residual surface sprays and application of grain protectants (Arthur and Subramanyam, 2012; Stejskal et al., 2021). Despite cleaning efforts, there are always some damaged grain residues that may host high numbers of pest populations of primary and secondary pests (Kucerova et al., 2003; Arthur et al., 2006; Tilley et al., 2014, 2017), and thus, they may contain a high proportion of heavily damaged and excavated kernels. This may be one of the reasons why it was documented that the efficacy of preharvest chemical treatment of empty stores may be negatively affected by improper sanitation (Morrison et al., 2019). For example, Tilley et al. (2014) found that cyfluthrin application as a residual insecticide to grain elevator boots did not provide full control of *C. ferrugineus, S. oryzae*, and *R. dominica*. As far as insecticide grain protectants are concerned, most experimental laboratory or semi-field works are conducted on sound grain, free from any storage pest damage (Arthur, 1996; Kavallieratos et al., 2015). There are also field experimental works that describe the differential potential of various types of grain chemical protectants to prevent or decrease seed damage and injury (Giga et al., 1991; Huang and Subramanyam, 2005). However, none of the above or other published papers contain information on the effect of damaged kernels or impurities on the effectiveness of the grain protectant itself. Thus, our work documents for the first time that the presence of excavated kernels

can negatively affect the efficacy of grain protectants. We found that the application of grain protectant at a full dose did not result in survival or progeny production in sensitive laboratory populations of *C. ferrugineus* and *T. castaneum*. However, when the dose was halved in our experiments, survival and progeny production occurred on treated grain mixtures that contained a higher proportion of kernels with excavation. This may have implications for the efficacy of the products in practice. Pest interaction with a lower than labelled rate can occur not only due to technologically poor or erroneous application (e.g., poor dilution, miscalculated grain weight, uneven application, insecticide drift into the environment, etc.) but also over time with each correct application, when pesticide residues in the grain naturally degrade (weeks/months) (Noble et al., 1982). Thus, our work indicates that if pest invasion of damaged grain patches occurs several weeks or months after treatment with grain protectants, when there is already 50% degradation of insecticide residues, then some secondary pest species may survive or even reproduce.

5. Conclusion remarks

This work found that completely clean and sound wheat kernels may – under conditions in our experiments – prevent survival and progeny production in the tested species. The work indicates that the current label dosage of both grain protectants seems to be robust enough that the presence of excavated grain does not decrease the efficacy of the tested formulations on insecticide-sensitive populations/strains of both tested species. However, the tested combination of lower than label doses and a high proportion of excavated kernels was a reason for decreased efficacy even in insecticide-sensitive strains of *C. ferrugineus* and *T. castaneum*. The effect of kernel excavation in the presence of frass on the efficacy of grain protectants remains to be further explored. To make more general conclusions, a similar consequent study is needed on another primary (*Prostephanus* sp., *Rhyzopertha* sp. or *Sitophilus* spp.) and secondary pest species from the taxa Psocoptera, Acari, Coleoptera, and Lepidoptera. The presence of resistant populations (Baliota et al., 2022) in interaction with damaged grain may also profoundly change the insecticide properties of grain protectants; therefore, this interaction should also be explored experimentally in the future.

From a practical point of view, this work is of particular importance for the refinement of laboratory test methodologies for testing grain protectants and implies some conclusions for their application in field conditions. The work points out the importance of the preparation of treated and control grain samples in terms of defining the content of broken seeds or kernels damaged by pests. This qualitative parameter can influence the survival of some secondary pests in both control and treated samples. Furthermore, the work indicates the importance of good storage practices in terms of cleanliness and quality of stored grain. A high proportion of unclean and broken grain - in particular excavated kernels - can negatively affect the effectiveness of applied grain protectants, especially if the residue content of the active ingredient is reduced below the level prescribed in product labels and instructions.

Author statement

Tomas Vendl: Writing- Original draft preparation, Writing- Reviewing and Editing, Data curation, Visualization, **Jawad Ali Shah:** Writing- Original draft preparation, Data curation, **Radek Aulicky:**

Writing- Original draft preparation, Resources, Funding acquisition, **Vaclav Stejskal:** Writing- Original draft preparation, Writing- Reviewing and Editing, Conceptualization, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The work was supported by the Ministry of Agriculture Czech Republic, grant number VZ-RO0418. The language proofreading was partially funded by the Ministry of Education, Youth, and Sports "Strengthening strategic management of science and research in the CRI" project grant number CZ.02.2.69/0.0/0.0/18_054/0014700. We express our thanks to Dagmar Janovska for access to automatic grain ´ quality evaluation equipment and two anonymous reviewers for their helpful comments that improved the manuscript.

References

Alonso-Amelot, M.E., Avila-Núnez, J.L., 2011. Comparison of seven methods for stored˜ cereal losses to insects for their application in rural conditions. J. Stored Prod. Res. 47, 82–87.
Arthur, F.H., 1996. Grain protectants: current status and prospects for the future. J. Stored Prod. Res. 32, 293–302.
Arthur, F.H., 2019. Residual efficacy of a deltamethrin emulsifiable concentrate formulation against *Rhyzopertha dominica* (F.) and *Sitotroga cerealella* (Oliver) after partial treatment of brown rice. Insects 10, 95.
Arthur, F.H., Subramanyam, Bh, 2012. Chemical control in stored products. In: Hagstrum, D.W., Phillips, T.W., Cuperus, G. (Eds.), Stored Product Protection. Kansas State University, Manhattan, KS, pp. 95–100.
Arthur, F., Morrison, W.R., 2020. Methodology for assessing progeny production and grain damage on commodities treated with insecticides. Agronomy 10, 804.
Arthur, F.H., Hagstrum, D.W., Flinn, P.W., Reed, C.R., Phillips, T.W., 2006. Insect populations in grain residues associated with commercial Kansas grain elevators. J. Stored Prod. Res. 42, 226–239.
Arthur, F.H., Athanassiou, C.G., Morrison III, W.R., 2020. Mobility of stored product beetles after exposure to a combination insecticide containing deltamethrin, methoprene, and a piperonyl butoxide synergist depends on species, concentration, and exposure time. Insects 11, 151.
Athanassiou, C.G., Arthur, F.H., Throne, J.E., 2009. Efficacy of grain protectants against four psocid species on maize, rice and wheat. Pest Manag. Sci. 65, 1140–1146.
Athanassiou, C.G., Kavallieratos, N.G., Throne, J.E., Nakas, C.T., 2014. Competition among species of stored-product psocids (Psocoptera) in stored grain. PLoS One 9, e102867.
Baliota, G.V., Lampiri, E., Batzogianni, E.N., Athanassiou, C.G., 2022. Insecticidal effect of four insecticides for the control of different populations of three stored-product beetle species. Insects 13, 325.
Campbell, A., Sinha, R.N., 1976. Damage of wheat by feeding of some stored product beetles. J. Econ. Entomol. 69, 11–13.
Daglish, G.J., Nayak, M.K., Arthur, F.H., Athanassiou, C.G., 2018. Insect pest management in stored grain. In: Athanassiou, HeidelbergC.G., Arthur, F.H. (Eds.), Recent Advances in Stored Product Protection. Springer-Verlag GmbH, Berlin, Germany, pp. 45–64.
Đuki´c, N.J., Radonji´c, A.B., Andri´c, G.G., Kljaji´c, P.J., Pra˘zi´c-Goli´c, M.P., 2018. Attractiveness of infested and uninfested whole wheat grain and coarse wheat meal odors to coleopteran storage insect pests. Food And Feed Res. 45, 113–118.
European and Mediterranean Plant Protection Organization, 2004a. Admixture of plant protection products to stored plant products to control insects and mites, PP 1/203 (1). In: EPPO Standards PP1, , second ed.vol. 3, pp. 217–219 Efficacy evaluation of insecticides and acaricides. Paris, France.
European and Mediterranean Plant Protection Organization, 2004b. Laboratory testing of plant protection products against insect and mite pests of stored plant products, PP 1/204(1). In: EPPO Standards PP1, , second ed.vol. 3, pp. 220–223 Efficacy evaluation of insecticides and acaricides. Paris, France.
Egwuatu, R.I., 1987. Current status of conventional insecticides in the management of stored product insect pests in the tropics. Int. J. Trop. Insect Sci. 8, 695–701.
Flinn, P.W., McGaughey, W.H., Burkholder, W.E., 1992. Effects of fine material on insect infestation: a review. In: North Central Regional Research Pub. 332. OARDC Special Circ. 141. Ohio Agricultural Research and Development Center. The Ohio State University, Wooster, OH, pp. 24–30.
Gautam, S.G., Opit, G.P., Giles, K.L., Adam, B., 2013. Weight loss and germination failure caused by psocids in different wheat varieties. J. Econ. Entomol. 106, 491–498.
Giga, D.P., Mutemerewa, S., Moyo, G., Neeley, D., 1991. Assessment and control of losses caused by insect pests in small farmers' stores in Zimbabwe. Crop Protect. 10, 287–292.
Giunti, G., Palmeri, V., Algeri, G.M., Campolo, O., 2018. VOC emissions influence intra- and interspecific interactions among stored-product Coleoptera in paddy rice. Sci. Rep. 8, 1–9.

Golebiowska, Z., 1969. The feeding and fecundity of *Sitophilus granarius* (L.), *Sitophilus orvzae* (L.) and *Rhyzopertha dominica* (F.) in wheat grain. J. Stored Prod. Res. 5, 143–155.

Gvozdenac, S.M., Prvulovi´c, D.M., Radovanovi´c, M.N., Ovuka, J.S., Mikli˘c, V.J., A˘canski, J.M., Tanaskovi´c, S.T., Vukajlovi´c, F.N., 2018. Life history of *Plodia interpunctella* Hübner on sunflower seeds: effects of seed qualitative traits and the initial seed damage. J. Stored Prod. Res. 79, 89–97.

Hagstrum, D.W., Phillips, T.W., 2017. Evolution of stored-product entomology: protecting the world food supply. Annu. Rev. Entomol. 62, 379–397.

Hoy, C.W., Head, G.P., Hall, F.R., 1998. Spatial heterogeneity and insect adaptation to toxins. Annu. Rev. Entomol. 43, 571–594.

Huang, F., Subramanyam, B., 2005. Management of five stored-product insects in wheat with pirimiphos-methyl and pirimiphos-methyl plus synergized pyrethrins. Pest Manag. Sci. 61, 356–362.

Hubert, J., Erban, T., Nesvorna, M., Stejskal, V., 2011. Emerging risk of infestation and contamination of dried fruits by mites in the Czech Republic. Food Addit. Contam.: Part A 28, 1129–1135.

Hubert, J., Stejskal, V., Athanassiou, C.G., Throne, J.E., 2018. Health hazards associated with arthropod infestation of stored products. Annu. Rev. Entomol. 63, 553–573.

Ileke, K.D., Bulus, D.S., 2012. Evaluation of contact toxicity and fumigant effect of some medicinal plant and pirimiphos methyl powders against cowpea bruchid, *Callosobruchus maculatus* (Fab.)[Coleoptera: Chrysomelidae] in stored cowpea seeds. J. Agric. Sci. 4, 279–284.

Jian, F., 2019. Influences of stored product insect movements on integrated pest management decisions. Insects 10, 100.

Jian, F., 2022. A review of distribution and segregation mechanisms of dockage and foreign materials in on-farm grain silos for central spout loading. KONA Powder Part. J. 39, 100–109.

Jian, F., Zhang, Q., 2022. Regression models for distributions of dockage and foreign materials in grain silos filled by central spouts. J. Stored Prod. Res. 95, 101901.

Kavallieratos, N.G., Athanassiou, C.G., Arthur, F.H., 2015. Efficacy of deltamethrin against stored-product beetles at short exposure intervals or on a partially treated rice mass. J. Econ. Entomol. 108, 1416–1421.

Kavallieratos, N.G., Athanassiou, C.G., Guedes, R.N., Drempela, J.D., Boukouvala, M.C., 2017. Invader competition with local competitors: displacement or coexistence among the invasive khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), and two other major stored-grain beetles? Front. Plant Sci. 8, 1837.

Kljaji´c, P., Peri´c, I., 2009. Residual effects of deltamethrin and malathion on different populations of *Sitophilus granarius* (L.) on treated wheat grains. J. Stored Prod. Res. 45, 45–48.

Kucerova, Z., Aulicky, R., Stejskal, V., 2003. Accumulation of pest-arthropods in grain residues found in an empty store. J. Plant Dis. Prot. 110, 499–504.

LaHue, D.W., 1977. Pirimiphos-methyl: gradient of effective doses on hard winter wheat against attack of four species of adult insects. J. Econ. Entomol. 70, 295–297.

Lampiri, E., Baliota, G.V., Morrison, W.R., Domingue, M.J., Athanassiou, C.G., 2022. Comparative population growth of the khapra beetle (Coleoptera: Dermestidae) and the warehouse beetle (Coleoptera: Dermestidae) on wheat and rice. J. Econ. Entomol. 115, 344–352.

Mehta, Vkucer, Kumar, S., Jayaram, C.S., 2021. Damage potential, effect on germination, and development of *Sitophilus oryzae* (Coleoptera: Curculionidae) on wheat grains in Northwestern Himalayas. J. Insect Sci. 21, 8.

Minett, W., Williams, P., 1971. Influence of malathion distribution on the protection of wheat grain against insect infestation. J. Stored Prod. Res. 7, 233–242.

Morales, J.A., Cardoso, D.G., Della Lucia, T.M.C., Guedes, R.N.C., 2013. Weevil x insecticide: does 'personality'matter? PLoS One 8, e67283.

Morrison III, W.R., Wilkins, R.V., Gerken, A.R., Scheff, D.S., Zhu, K.Y., Arthur, F.H., Campbell, J.F., 2018. Mobility of adult *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) after exposure to long-lasting insecticide-incorporated netting. J. Econ. Entomol. 111, 2443–2453.

Morrison III, W.R., Bruce, A., Wilkins, R.V., Albin, C.E., Arthur, F.H., 2019. Sanitation improves stored product insect pest management. Insects 10, 77.

Noble, R.M., Hamilton, D.J., Osborne, W.J., 1982. Stability of pyrethroids on wheat in storage. Pestic. Sci. 13, 246–252.

Plarre, R., 1996. Three-dimensional distribution of *Sitophilus granarius* (L.)(Coleoptera: Curculionidae) in wheat influenced by the synthetic aggregation pheromone. J. Stored Prod. Res. 32, 275–283.

Rumbos, C.I., Dutton, A.C., Athanassiou, C.G., 2013. Comparison of two pirimiphos- methyl formulations against major stored-product insect species. J. Stored Prod. Res. 55, 106–115.

Rumbos, C.I., Dutton, A.C., Tsiropoulos, N.G., Athanassiou, C.G., 2018. Persistence and residual toxicity of two pirimiphos-methyl formulations on wheat against three stored-product pests. J. Stored Prod. Res. 76, 14–21.

Sakka, M.K., Athanassiou, C.G., 2018. Competition of three stored-product bostrychids on different temperatures and commodities. J. Stored Prod. Res. 79, 34–39.

Scully, E.D., Gerken, A.R., Fifield, A., Nguyen, V., Van Pelt, N., Arthur, F.H., 2021. Impacts of Storicide II on internal feeders of Brown rice. J. Stored Prod. Res. 90, 101758.

Shah, J.A., Vendl, T., Aulicky, R., Stejskal, V., 2021. Frass produced by the primary pest *Rhyzopertha dominica* supports the population growth of the secondary stored product pests *Oryzaephilus surinamensis, Tribolium castaneum*, and *T. confusum*. Bull. Entomol. Res. 111, 153–159.

Skourti, A., Kavallieratos, N.G., Papanikolaou, N.E., 2020. Suitability of semolina, cracked wheat and cracked maize as feeding commodities for *Tribolium castaneum* (Herbst; Coleoptera: Tenebrionidae). Insects 11, 99.

Subramanyam, B., Boina, D.R., Sehgal, B., Lazzari, F., 2014. Efficacy of partial treatment of wheat with spinosad against *Rhyzopertha dominica* (F.) adults. J. Stored Prod. Res. 59, 197–203.

Stejskal, V., Kucerova, Z., 1996. The effect of grain size on the biology of *Sitophilus granarius* L.(Col., Curculionidae). I. Oviposition, distribution of eggs and adult emergence. J. Appl. Entomol. 120, 143–146.

Stejskal, V., Kosina, P., Holesovska, P., 2007. Injury capability of pests to stored legumes in Namibia. Integrat. Protect. Stored Prod. IOBC WPRS Bull. 30, 193–196.

Stejskal, V., Vendl, T., Li, Z., Aulicky, R., 2020a. Efficacy of visual evaluation of insect- damaged kernels of malting barley by *Sitophilus granarius* from various observation perspectives. J. Stored Prod. Res. 89, 101711.

Stejskal, V., Vendl, T., Kolar, V., Li, Z., Aulicky, R., 2020b. First population quantification of the infestation of legumes by stored-product bruchids imported in freight containers into Europe. Bull. Insectol. 73, 233–239.

Stejskal, V., Vendl, T., Aulicky, R., Athanassiou, C., 2021. Synthetic and natural insecticides: Gas, liquid, gel and solid formulations for stored-product and food- industry pest control. Insects 12, 590.

Tilley, D.R., Subramanyam, B., Casada, M.E., Arthur, F.H., 2014. Stored-grain insect population commingling densities in wheat and corn from pilot-scale bucket elevator boots. J. Stored Prod. Res. 59, 1–8.

Tilley, D.R., Casada, M.E., Subramanyam, B., Arthur, F.H., 2017. Temporal changes in stored-product insect populations associated with boot, pit, and load-out areas of grain elevators and feed mills. J. Stored Prod. Res. 73, 62–73.

Trematerra, P., Throne, J., 2012. Insect and mite pests of durum wheat. In: Sissons, M., Abecassis, J., Marchylo, B., Carcea, M. (Eds.), Durum Wheat, Chemistry and Technology, second ed. A.A.C.C. Press, St. Paul, Minnesota, p. 73e83.

Trematerra, P., Sciarreta, A., Tamasi, E., 2000. Behavioural responses of *Oryzaephilus surinamensis, Tribolium castaneum* and *Tribolium confusum* to naturally and artificially damaged durum wheat kernels. Entomol. Exp. Appl. 94, 195–200.

Trematerra, P., Ianiro, R., Athanassiou, C.G., Kavallieratos, N.G., 2013. Behavioral responses of *Sitophilus zeamais* Motschulsky adults to conditioned grain kernels. J. Stored Prod. Res. 53, 77–81.

V´elez, M., Barbosa, W.F., Quintero, J., Chediak, M., Guedes, R.N.C., 2017. Deltamethrin- and spinosad-mediated survival, activity and avoidance of the grain weevils *Sitophilus granarius* and *S. zeamais*. J. Stored Prod. Res. 74, 56–65.

Vendl, T., Stejskal, V., Aulicky, R., 2019. Comparative tarsal morphology of two secondary stored product beetle pests, *Oryzaephilus surinamensis* (L.) and *Cryptolestes ferrugineus* (Stephens), that vary in their climbing ability on smooth surfaces. J. Stored Prod. Res. 82, 116–122.

gels

MDPI

Review

Gel Carriers for Plant Extracts and Synthetic Pesticides in Rodent and Arthropod Pest Control: An Overview

Jawad Ali Shah [1], Tomas Vendl [2,*], Radek Aulicky [2], Marcela Frankova [2] and Vaclav Stejskal [1,2,*]

[1] Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 16500 Prague, Czech Republic
[2] Crop Research Institute, Drnovska 507/73, 16106 Prague, Czech Republic
* Correspondence: vendl@vurv.cz (T.V.); stejskal@vurv.cz (V.S.); Tel.: +42-02-3302-2360 (T.V.); +42-02-3302-2217 (V.S.)

Abstract: Insecticides and rodenticides form the basis of integrated pest management systems worldwide. As pest resistance continues to increase and entire groups of chemical active ingredients are restricted or banned, manufacturers are looking for new options for more effective formulations and safer application methods for the remaining pesticide ingredients. In addition to new technological adaptations of mainstream formulations in the form of sprays, fumigants, and dusts, the use of gel formulations is becoming increasingly explored and employed. This article summarizes information on the current and potential use of gel (including hydrogel) and paste formulations against harmful arthropods or rodents in specific branches of pest management in the agricultural, food, stored product, structural wood, urban, medical, and public health areas. Due to the worldwide high interest in natural substances, part of the review was devoted to the use of gels for the formulation of pesticide substances of botanical origin, such as essential or edible oils. Gels as emerging formulation of so called "smart insecticides" based on molecular iRNA disruptors are discussed.

Keywords: hydrogels; polymers; nanotechnology; insecticides; rodenticides; formulations; essential oils; plant extracts; integrated pest management; vector control

check for updates

Citation: Shah, J.A.; Vendl, T.; Aulicky, R.; Frankova, M.; Stejskal, V. Gel Carriers for Plant Extracts and Synthetic Pesticides in Rodent and Arthropod Pest Control: An Overview. *Gels* **2022**, *8*, 522. https://doi.org/10.3390/gels8080522

Academic Editors: Yulong Zhang and Hui-Juan Xu

Received: 12 July 2022
Accepted: 16 August 2022
Published: 20 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Soft gels as material consisting of two or more components, one of which is a liquid, found applications in wide range of products such as foods, agrochemicals, pharmaceuticals, cosmetics, soft-optical devices, art conservation preparations, paints, adhesives, etc. [1]. Gels were also proposed as basis of technologies for the removal of environmental pollutants [2] or of sensors employed in various environmental and biological applications [3]. Various soft gels and pastes have also been used for some types of traditional insecticide [4,5] and rodenticide formulations [6]. In recent decades, especially with the advent of advances in encapsulation and nanotechnologies [4,7–9], researchers focused their attention on pesticide gel application to new areas of protection of human resources, health, and environment against harmful and invasive organisms. Pesticide gels are thus beginning to be a complement, or in some cases an important environmentally safer alternative, to traditional pesticide formulations.

Traditional types of pesticide preparations include solid, gas, liquid, and gel formulations [10]. Each pesticide's active ingredient must be suitably formulated using a proper carrier, synergists, additives, and other formulation components. Optimized commercial formulations not only prolong the residual effect and increase the biological efficacy of pesticides on pests but also increase their safety to humans, non-target organisms, and the natural environment. Since pest resistance to pesticides continues to increase and entire groups of chemical active ingredients are restricted or banned, manufacturers are looking for new options for more effective formulations and safer application methods for the remaining pesticide ingredients. Thus, advanced application techniques [11] and

formulations of insecticides and rodenticides based on polymers, nanotechnology, and encapsulation of active ingredients are gradually coming to the forefront of pesticide manufacturers' concerns [12–18]. According to Tay et al. [4], the most frequently used polymeric encapsulated wall materials include gelatin, gum arabic, starch, sugar, ethyl cellulose, carboxymethyl cellulose, paraffin, polyvinyl alcohol, polyethylene, polypropylene, polystyrene, polyacrylamide, polyethers, polyesters, polyamides, polyureas, polybutadiene, polyisoprene, polysiloxanes, polyurethanes, epoxy resins, and inorganic silicates. Among other perspective preparations, gels and hydrogels are formulations that are becoming increasingly scientifically explored and employed in practice during the past few decades. To our knowledge, there is no general inventory summarizing the usage of pesticide gels for various areas and branches of pest control. Since insecticide aerogels were extensively covered by many studies and reviews [19], they are not included in the study. This review article summarizes information on gel, hydrogels in particular, and paste formulations against harmful arthropods and rodents. This is not intended to be a systematic review of the field or of the chemical characterization of pesticide gels. Instead, it aims to generally summarize the main areas of current use of gels against harmful organisms and to present potential future applications in various areas of pest control.

2. Overview of Pest Control Areas Regarding Usage Gel Formulations

Protective chemicals designed and registered to control harmful organisms are generally called pesticides. In European Union (EU) legislation, pesticides are divided into biocides and plant protection products. The active ingredients of pesticides include a large, diverse set of chemical groups and can be either natural or synthetic in origin [10,20]. Currently, the most extensive research is conducted on the substances and their carriers (oils, algae gels), which are of natural origin and are thus often easily biodegradable. For example, edible or essential oils [11,21] are used as natural pesticides and repellents. In terms of target animal pests, the most important categories of pesticides are insecticides (including acaricides), which are designed to control harmful arthropods (i.e., insects and mites), and rodenticides, which are designed to control rodents or other harmful vertebrates. Insecticides and rodenticides are used globally in various spheres of human society. They are widely used along the food production chain, from primary agricultural production to commodity storage and food production. Pesticides are an important element of national and international phytoquarantine and environmental protection against invasive organisms. Another important area of their use is the control of pests of medical importance. Here, pesticides are an important tool to ensure public health.

Based on the openly published literature records, this review recognized main areas for which pesticide gels were suggested. Their inventory is summarized in Figure 1, and the structure of the review is arranged accordingly. Due to the worldwide high interest in natural substances, a separate chapter is devoted to the use of gels for the formulation of pesticide substances of botanical origin, such as essential or edible oils. The review also recognized gels as emerging formulations—so-called "smart insecticides"—based on molecular iRNA disruptors.

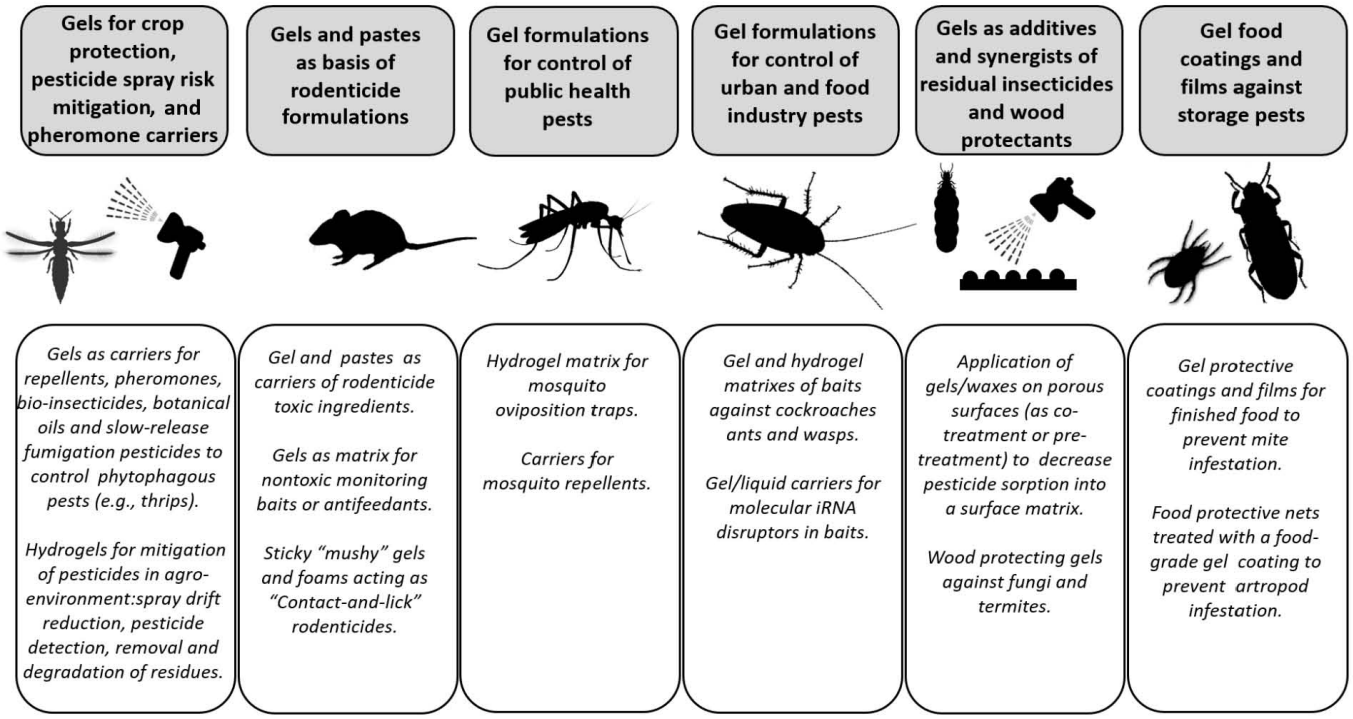


Figure 1. Graphical illustration of usage of gels—including hydrogels—in various areas of pest control and mitigation of pesticide environmental impacts.

3. Gels in Plant Protection and Pesticide Sprays Risk Mitigation

Gel-based formulations, and in particular highly sorptive hydrogels, are being increasingly explored to ensure environment safety and sustainable agricultural production. Information on the research or application of gels and hydrogels in practice can be found in several dedicated reviews on this topic [22–26]. According to the reviews, gels and hydrogels have much broader applications in agriculture than only plant protection and pest control. However, regarding topics related to plant protection, two broad areas of research and application of gels have been identified during preparation of this review. The first area was the role of hydrogels as carriers of direct pest control agents and as indirect mediators which increase plant health. The second area was the mitigation of pesticide negative side effects, such as water or soil contamination by pesticides residues.

3.1. Gels as Carriers for Bioinsecticides and Slow-Release Pesticides or Pheromones in Crop Protection

Gel preparations are important as means of both direct and indirect plant protection. The indirect protection is based on providing a controlled supply of moisture and/or nutrients. Additionally, gels serve as a supportive means of improving overall plant/seed resilience and health [27,28]. Healthy and unstressed plants and seeds are more tolerant/resistant to pest infestation or disease infection. Hydrogel-based products are considered soil conditioners and yield enhancers because of their ability to increase the water holding capacity of the soil substrate and improve soil texture [29]. They do not only increase soil substratum water capacity and improve the soil structure but are also capable of releasing the retained water and nutrients over an extended period of time [30]. Regarding the use of gels and hydrogels as supporting forms of irrigation to improve the physiological state of plants, critical papers showing both the general limitations of their biological activity and the specific inhibitory effect of different soil conditions can be found [31,32].

As direct means of protection, gels can serve as carriers of pesticides and biocontrol agents within the framework of the so-called integrated pest management (IPM) sys-

tem. Gels, as carriers of direct control agents, have unique properties, especially in terms of the so-called controlled long-term release of active pesticide substances [24,33–36] or pheromones [37]. For example, controlled release of chlorpyrifos [6] and carbaryl beads [5] using hydrogels was described. Importantly, the authors claimed that the PA-CA hydrogel possessed biocompatibility with *Escherichia coli*, thereby also positively addressing a biosafety issue [38]. In terms of various higher taxa groups of target pest organisms, highly sorptive pesticide hydrogels have been formulated not only as insecticides but also as fungicides [39–41] and herbicides [42–45]. Apart from pesticide synthetic compounds, hydrogels were suggested as a formulation matrix for natural compounds [46] and bioagents [47]. The natural-based formulations may be potentially employed in situations in which biocontrol is required, such as in some types of organic farming systems. Ropek and Kulikowski [47] elaborated a hydrogel-based biopreparation containing entomopathogenic fungus *Beauveria bassiana*. Although some authors state that high temperatures have rather negative effects on the efficacy of hydrogel-based insecticides [4], Ropek and Kulikowski [47] reported that the *B. bassiana* gel preparation acted faster at temperatures of 25 and 30 °C than at low temperatures.

Although interesting hydrogel application systems have been proposed for the control of agricultural pests, the application of many of them remains at the research or finished technical solution stage. One of the frequent reasons for the difficulty in transferring research results into practice is the prohibitively high cost of international pesticide registrations.

3.2. Hydrogels for Mitigation of Pesticides in Agroenvironment (Spray Drift Reduction, Pesticide Detection, Removal and Degradation of Residues)

Gels were suggested not only for pesticide efficacy enhancement and prolonged degradation time of pesticide residues after their application in field conditions, but also for mitigation of pesticide negative effects associated with plant protection [48]. Hydrogels were designed as pesticide application formulations to reduce insecticide spray drift during spraying under field conditions. Song et al. [49] explored possibilities of reduction of pesticide spray drift using folate/Zn^{2+} supramolecular hydrogels. These authors designed novel organic solvent-free hydrogel showing biocompatibility and biodegradability. The primary method by which the hydrogels reduced drift was by increasing droplet size, which was caused by the three-dimensional network of the internal structure of the gel. In our view, such a line of research and development points in the direction of new strategies for spray drift reduction in terms of pesticide formulation. Thus, it expands the possibilities of using supramolecular hydrogels in agriculture. In addition, it has been revealed that preparation based on hydrogels can contribute to the targeted degradation of pesticides in contaminated soil. For example, Yang et al. [50] found that superabsorbent hydrogel coating increased degradation and decreased the formation bound residues, such as carbendazim, in soil. Several procedures were explored in terms of hydrogel capacity for removal of various pesticides from the contaminated water. The incentive for such studies was to develop practical procedures and formulations of hydrogels as preparations for the decontamination of water from insecticides. The results achieved by Aouada et al. [51] suggested that PAAm-MC hydrogels are potentially viable absorbents for removal of paraquat pesticide from aqueous solution and cleaning water contaminated with dyes, heavy metals, and other pesticides. Alammar et al. [52] constructed neonicotinoid-scavenging nanocomposite hydrogels. Therefore, Gosset et al. [53] developed sensors based on encapsulated algae for pesticide detection in water. Recently, a new concept of a smart MOF-on-MOF hydrogel serving for visual detection of pesticide residues to enable effective removal of pesticides from contaminated environment was proposed [54].

4. Rodenticide Gels and Pastes

Rodents are serious agricultural, storage, food industry, public health, and veterinary pests. They are highly damaging to agricultural crops [55] and contaminate environment with feces containing pathogens [56,57]. Designing the appropriate bait formulation for the

target rodent species is difficult in terms of attractiveness, palatability, and safety. Active ingredients of rodenticides are acute or chronic substances [56]. Chronic anticoagulants accumulate in the body of poisoned rodents [58,59], and thus, secondary poisoning may occur after consumption by predators. Therefore, effective and safe rodent control and monitoring is a challenge. New works on innovative rodenticide formulations associated with gels (that are often called rodenticide "soft baits"), polymers, and encapsulated active compounds [22] point the way towards further development to effectively manage rodents and mitigate the negative effects of rodenticides [14].

4.1. "Contact-and-Lick" Rodenticide Sticky Gels and Foams

The concept of using contact rodenticides, which involves the rodent soiling itself with a contact toxic substance, was developed historically long ago. In the 1950s, several historical formulations of greasy and sticky poisonous pastes, saps, and greasy sticky material (called "mushy mass") were developed and used for rodent crop pests, especially common voles (*Microtus arvalis*) [60] in Europe. Due to high labor demands, agricultural contact adhesive rodenticides are rarely used these days. However, the concept of soft adhesive toxic coat contaminants (foam with anticoagulants) has recently been refreshed for the control of synanthropic rodents, such as Norway rats (*Rattus norvegicus*), roof rats (*R. rattus*), and house mice (*Mus musculus*). A contact rodenticide formulation in the form of a foam (Racumin Foam—Bayer, Germany) with coumatetralyl as the active ingredient is currently registered in a number of EU countries and elsewhere. Although this product is used in practice, no freely available peer-reviewed scientific study on its efficacy has been published to our knowledge.

4.2. Toxic Bait Gels and Pastes (Soft Baits)

Wilson [61] described a brief historical development of bait formulations in his paper titled "Evolution of rodenticides". In the historical timeline, the "oldest" solid baits were based on poisoned grain. The latest rodenticide generation of bait formulations was identified by Wilson [61] as soft baits, which are based on gels and pastes. Cornwell [22] was one of the first who extensively tested encapsulated forms of various rodenticide active ingredients formulated with ethyl cellulose, gelatin, gelatin/gum arabic, gelatin/carrageen, polyester wax, and polawax. Currently, soft baits for rodents are commonly produced and used. Pastes and gels are applied to target sites using various handheld injection press devices or are preprepared by the manufacturer in small packages for direct application; the latter formulations are known as ready-to-use rodenticide sachets. There are a number of historical and recent scientific studies that demonstrate the attractiveness, palatability, and efficacy of different formulations of soft baits on house mice, roof rats, and Norway rats under different environmental conditions [62–65]. Soft baits have been even evaluated for controlling rodents outside buildings and for environmental protection against invasive pests (e.g., the Polynesian rat, *R. exulans*) [66].

4.3. Nontoxic Monitoring Gel Baits and Antifeedants

Gel soft baits can be used not only for direct rodent control but also as a suitable matrix for the formulation of repellents and antifeedants or as attractants for rodent monitoring using nontoxic baits. Rodent repellents and antifeedants, such as alginate-based microcapsules containing eucalyptus oil, are still mostly in the research stage [67]. Nevertheless, nontoxic soft baits for rodent monitoring are already being produced and used in practice. Their advantage is that they do not need to be registered as pesticides in most states. There have been published results of lab and field tests on different formulations of commercial soft nontoxic baits for monitoring synanthropic house mice and roof rats in indoor environments and agricultural orchards [65,68–70].

5. Gels for Risk Mitigation of Mosquitoes

Mosquitoes are among the most important vectors of pathogens which are the causative agents of serious diseases such as malaria [71]. Mosquito-risk-reduction strategies include, among others, either methods that prevent mosquitoes from coming into contact with humans or methods that reduce the size of mosquito populations. In particular, repellents serve as a personal protective shield (applied to skin, clothing, or netting over beds) against mosquito contacts with humans. There are a number of detailed published reviews regarding insecticidal repellents [72,73] covering different formulations, such as lotions, sprays, gels, creams [74], and polymers [75]. Therefore, in this much more general review, we limited ourselves to providing a few examples regarding the development of hydrogel mosquito repellent formulations. For example, Milutinović et al. [76] demonstrated that the hydrogel formulation based on polyacrylic acid containing 5% DEET (*w*/*w*) could serve as a suitable vehicle for repellent preparations containing DEET, and Pinto et al. [77] characterized a new repellent formulation based on nanostructured hydrogels. Delong et al. [78] screened various natural extracts in order to develop a new hydrogel formulation (pHEMA hydrogels with pendant triazinyl-β-cyclodextrin) containing repellents of plant origin, which may provide a suitable, eco-friendly approach to achieving mosquito bite protection. They found that methyl salicylate possessed an optimal stability, and thus, it achieved a longer duration of protection with higher repellent activity. Kumar et al. [79] showed the promising repellent activity and delayed release of citronella-oil-microsponge-loaded hydrogel. Most recently, Rogeiro et al. [80] developed nanoparticle mosquito repellent based on a slow-release system composed of zein nanoparticles containing the encapsulated active agents icaridin and geraniol incorporated in a cellulose gel matrix.

Elimination of mosquito breeding sites, application of pesticides and bioagents, egg and adult trapping, and other measures are among the methods of direct control of mosquito population density. As important tools for mosquito-borne malaria control in the outdoor environment, both synthetic and natural substances are finding application, whether as repellents or as population density reduction agents [81–83]. Recently, Mapossa et al. [84] reviewed new various controlled-release formulations for mosquito control, such as polymer microcapsules, polymer microporous formulations, polymer micelles, nanoemulsions, solid lipid nanoparticles, liposomes, and cyclodextrins. In addition to traditional gels, new hydrogels are among the formulations that already have or are finding new applications for mosquito control. In addition to attractant or repellent formulations, they may be employed as carriers of larvicide formulations [85] or in mosquito ovitraps [86–88]. Friuli et al. [88] validated a new recipe of hydrogels to imitate the natural oviposition site's conditions in order to incorporate them inside "lure-and-kill" ovitraps as a biomimetic oviposition substrate. The study compared oviposition between standard substrates (absorbing paper/masonite) and a hydrogel composition panel under labor field conditions. The tests showed that a 2-hydroxyethylcellulose (HEC)-based physical hydrogel was five times more attractive than the control in a laboratory oviposition assay. Under field conditions, the same hydrogel substrate was more efficient than a standard masonite ovitrap, with a four-times-longer activity duration.

6. Gels Baits for Control of Ants, Cockroaches and Other Urban Pests

Insecticide gel baits have the potential to greatly reduce the amount of spray insecticides needed for pest control. Ready-to-use forms of gel and paste baits are delivered to the destination using pressurized propellant-based containers, gun-type applicators with removable injection tips, or by baits in tamper-resistant box stations [89]. Compared to residual spray formulations, baits are relatively less toxic, odorless, and may be applied in minute amounts to areas where residual spray is not permissible [90]. However, it should be noted that the bait efficacy is tightly dependent on pest movement activity, which is highly influenced by temperature. Thus, a lower temperature threshold for insect movement imposes a limit on bait efficacy [91]. The current baits mainly contain relatively nonrepellent, slow-acting compounds [90,92–94]. Most recently, the development of bait

technologies for liquid baits with liposomes as carriers of dsRNA has been suggested by several research teams [95,96]. In recent decades, a new generation of attractive (synthetic or natural) baits or gel-based baits, frequently hydrophilic, has been established. Microencapsulated oil bait was constructed for ant control [97]. Novel usage of various types of hydrogels (superabsorbent polymer–SAP [98]) was explored as bait matrix for the risk management of various urban pests [4]. Regarding taxonomical pest spectra, the hydrogel baits were evaluated for control of ants [99–101], wasps [4,102], and cockroaches [98]. Hydrogel-based baits have been most extensively evaluated as baits for control of a serious medical and urban pest, the Argentine ant, *Linepithema humile* [101,103,104]. Oladipupo et al. [98] stressed that hydrogels, used as a bait-delivery option, require a relatively small amount of insecticide active ingredient. Hydrogels are capable of absorbing water from a moist substrate, which compensates for water loss through surface evaporation. Hydrogels can be rehydrated via irrigation or rainfall, and the rehydration process allows the hydrogels to attract urban pests again [4]. The tested gel groups included synthetic polyacrylate hydrogels [99] or biodegradable hydrogels of natural origin (alginate hydrogels) [4,100,105]. For example, Tay et al. [100] developed an alginate hydrogel to deliver aqueous bait for pest ant management. The storage of natural and synthetic hydrogel baits hydrated with sucrose solution may be complicated by the fact that they must be stored in a refrigerator to prevent fermentation [4]. Further and more detailed information on various aspects of hydrogel usage and technological development can be found in an extensive review by Tay et al. [4].

7. Gels as Additives of Residual Insecticides and Wood Protectants

Food industry facilities and empty grain stores are treated with insecticidal surface residual sprays [10]. The activity and persistence of insecticide deposits largely depend on the particular composition, texture, and porosity of the treated surface. Steel, ceramic, or painted surfaces do not absorb liquids, whereas wood and brickwork have high sorption capacities for many liquid insecticide formulations. To avoid sorption into a porous surface matrix, cotreatment or pretreatment of a surface by means of protective coatings has been developed in the past. For example, Hewlett [106] experimentally documented that pre-treatment of cement with various gelatins greatly prolonged the toxic lifetime of insecticide films. Parkin and Hewlet [107] found that coating bricks with starch paste and water glass increased the activity of DDT and pyrethrins against some species of storage Coleoptera. Some petroleum oil films were highly toxic to tested beetle species (*Sitophilus* sp.) when applied to cement pretreated with gelatin [108]. Tyler and Rowlands [109] mixed carboxymethyl as a protective cotreatment with malathion sprays; it resulted in markedly improved persistence of the residual film on an alkaline cement substrate. Gudrups et al. [110] found that pyrethroid insecticide (permethrin) applied to concrete with a wax polish coating provided control of some storage Coleoptera species for 14 days. The idea of gel co/pretreatment may be an inspiration for technological improvements of plant-based insecticide formulations regarding their field efficacy.

The surface of structural/construction wood (timber) is required to be efficiently treated or impregnated by protective pesticides with long-term stability and activity. In this regards, Obounou-Akong et al. [111] tested hydrogels that fill the tracheid cell walls and lumens, limiting the leachability of boron salts when the wood is humidified. According to these authors, hydrogels appear to be valuable additives for improving boron fixation in wood and developing waterborne wood preservation formulations against harmful organisms, such as fungi and termites.

8. Gel Coatings for Finished Food Protection against Mites

In addition to transport purposes, the packaging of agricultural commodities and prepared foods serves mainly to protect them from contamination [112,113] and other negative environmental influences. Packaging is an important element of protection against pests. Various types of packages differ in the resilience to penetration or invasion by various

pest species [114–117]. Vacuum packaging or packaging filled with inert gases can then provide increased food protection [118–120]. The real challenge for effective pest protection is unpackaged food. Particularly problematic are those types of food (dried fish, ham, cheese, salami and uncooked, cured, dried, and smoked or unsmoked meat products) that are stored for long periods of time or have to be technologically ripened in conditions that allow them to be attacked by pests. To control these pests, various formulations of nonresidual fumigants and modified atmospheres or residual liquid pesticides dips or films were tested and/or used in practice [121]. Since residues of most pesticides—including acaricides [122]—are not legally acceptable or refused by customers, as alternatives, food-grade protective gel coatings (films) or nets with gels possessing acaricide properties have been proposed [123–126]. Any type of food coating must not affect the sensory properties of dry-cured hams but should allow water permeability [127]. This limits the number of potential types of chemical gels that can be used for these purposes. One of the potential candidate compounds seems to be propylene glycol. This type of gel is completely miscible with water and many organic solvents and is used in cosmetic and pharmaceutical formulations. Laboratory test by Zhao et al. [125] revealed that food matrix coated with xanthan gum + 20% propylene glycol and carrageenan/propylene glycol alginate + 10% propylene glycol effectively controls mite populations. The researchers also tested various combinations of mechanical packaging in the form of nets with gel-based chemical protectants. For example, Campbell et al. [127] showed that ham nets treated with a food-grade coating of 1% propylene glycol alginate + 1% carrageenan + 40% propylene glycol delivered protective effect not only against acaroid pests but also against some mold/fungi species.

9. Gel Formulations with Natural Plant Extracts as Pesticides and Repellents

Recently, plant extracts (e.g., essential and edible oils) have gained popularity. Their use is offered in many areas of pest control. Edible and essential oils have been tested as botanical insecticides against a wide range of pests that attack both stored commodities and standing crops [128,129]. From an ecological and partly toxicological point of view, plant oils are valued because they are biodegradable and thus do not tend to accumulate in the environmental chains. However, this welcome chemical property is associated with the problem of their short bioactivity after application [130]. For that reason, it is important to develop formulations that ensure greater stability and controlled release of the natural substances. Mechanisms of improving essential oil stability include various encapsulation techniques of natural substances by formulating them as nano- and microemulsions [131], microspheres [132], and nanoparticles [133]. The use of highly sorptive hydrogels may also provide higher and longer activity of essential oils applied as pesticides and repellents [46,98,134].

9.1. Hydrogels as Matrix for Essential Oils Based Baits or Fumigation Pesticides

Essential oils (EOs) formulated in hydrogels may be used as natural-based pesticides (acting as contact pesticides, fumigants, or baits) for the direct control of pests. For example, Gharbi and Tay [46] conducted a series of fumigation assays to assess the vulnerability of two species of pest thrips (*Frankliniella occidentalis* and *F. insularis*) to fumigation with EOs released from hydrogel formulation. The tested EOs included either (R)-linalool, (S)-linalool, racemic linalool, or a binary mixture of (R)-linalool with one of twelve other essential oils. It was found that the least saturated hydrogels conditioned in essential oils were the most effective, and both species of thrips were more sensitive to (R)-linalool than to (S)-linalool; *F. occidentalis* was significantly more resistant to all treatments than *F. insularis*. The study demonstrates that essential oils in combination with hydrogels are a promising alternative to conventional insecticides for thrips control.

Cockroach pesticide baits—based on locally available natural compounds—have been also explored [135]. The use of EOs on urban pests has been investigated in the USA [94], particularly on cockroaches. Oladipupo et al. [98] tested how hydrogels prolong bioavail-

ability of various essential oil components (limonene, carvacrol, and β-thujaplicin,) to control German cockroach (*Blattella germanica*). The study revealed that limonene, carvacrol, and β-thujaplicin in SAP gels show promising potential to reduce adult male survival/longevity, suppress egg hatchability and female fecundity, and delay the interoothecal period.

9.2. Hydrogels Used as Carriers for Natural (EOs) Repellents

Although the repellent effects of essential oils have long been recognized, their use in an unformulated state may not always be suitable. One of the means of prolonging essential oils' efficacy and making them easy to apply is their incorporation into a polymer or gel matrix. Historically, most research on the application of incorporated essential oils in gelatinous (alginate) matrices, often in combination with the oil encapsulation, has been aimed at mosquito control [136,137] and some other human ectoparasites [138]. These formulations are suitable for fabric or textile impregnation, and they proved to have high wash durability [139]. Oydele et al. [140] prepared ointment and cream formulations of lemongrass oil as mosquito repellent. Apart from mosquitos, there are few research or practical uses of gel formulations with essential oils against stored product pests. Abd-El-Bar and Fawki [141] used gelatin capsules as carriers of three essential oils and tested their insecticidal activity against the storage beetle pest *Acanthoscelides obtectus*.

A special case of the use of essential oils is their incorporation into a glue or adhesive for food packaging production [142]. Before stiffening, the glue is viscous, and after packaging is finished, the glue poses a repellent effect against stored product pests. Repellent systems based on essential oils in gel matrices are also promising in sustainable agriculture [143]. Picard et al. [144] incorporated oils of *Thymus vulgaris* and *Satureja montana* into polymer alginate matrices and found that the polymer repelled western flower thrips, *F. occidentalis*, for 4 days. Recently, de Oliveira et al. [134] used hydrogel-based repellent systems containing botanical compounds against two agricultural pests, the whitefly *Bemisia tabaci* and the spider mite *Tetranychus urticae*.

As already mentioned above, essential oils as repellents were also suggested for the risk management of rodents [67].

10. Outlooks and Perspectives

The pesticide industry is currently facing several major challenges. Among the most important challenges are loss of active ingredients due to consumer perception and changing society needs and increasing pest resistance to multiple active pesticide ingredients. The decreasing number of active substances is to some extent compensated by the development of new pesticide formulations with increased bioactivity or more efficient transport of the active substance to the sites of action. New or innovative gel formulations may be one of the options—in addition to traditional pesticide formulations—to address the above problems and challenges. For example, the presented review shows that various gel and hydrogel carriers of insecticides as well as biological control agents (micro-organisms) have already been proposed and constructed for control of several pest species.

What can be expected of the use of gel formulations in the near future? One possible criterion that can help to estimate the future trend of development of gel pesticide formulations is a bibliometric analysis comparing pesticide gels with other formulations. Figure 2 compares publications excerpted from the WoS database regarding gel vs. other types of pesticide formulations such as solid, gas, liquid pesticide formulations. This figure clearly shows that research on non-gel formulations is orders of magnitude more numerous. This indicates that research and practical applications of gels as pesticides form—although they play a key role in certain areas—only a narrow niche and thus still await their wider development and finalization into practical applications. In our opinion, the exploitation of this potential can be expected in the areas mentioned in the following paragraphs.

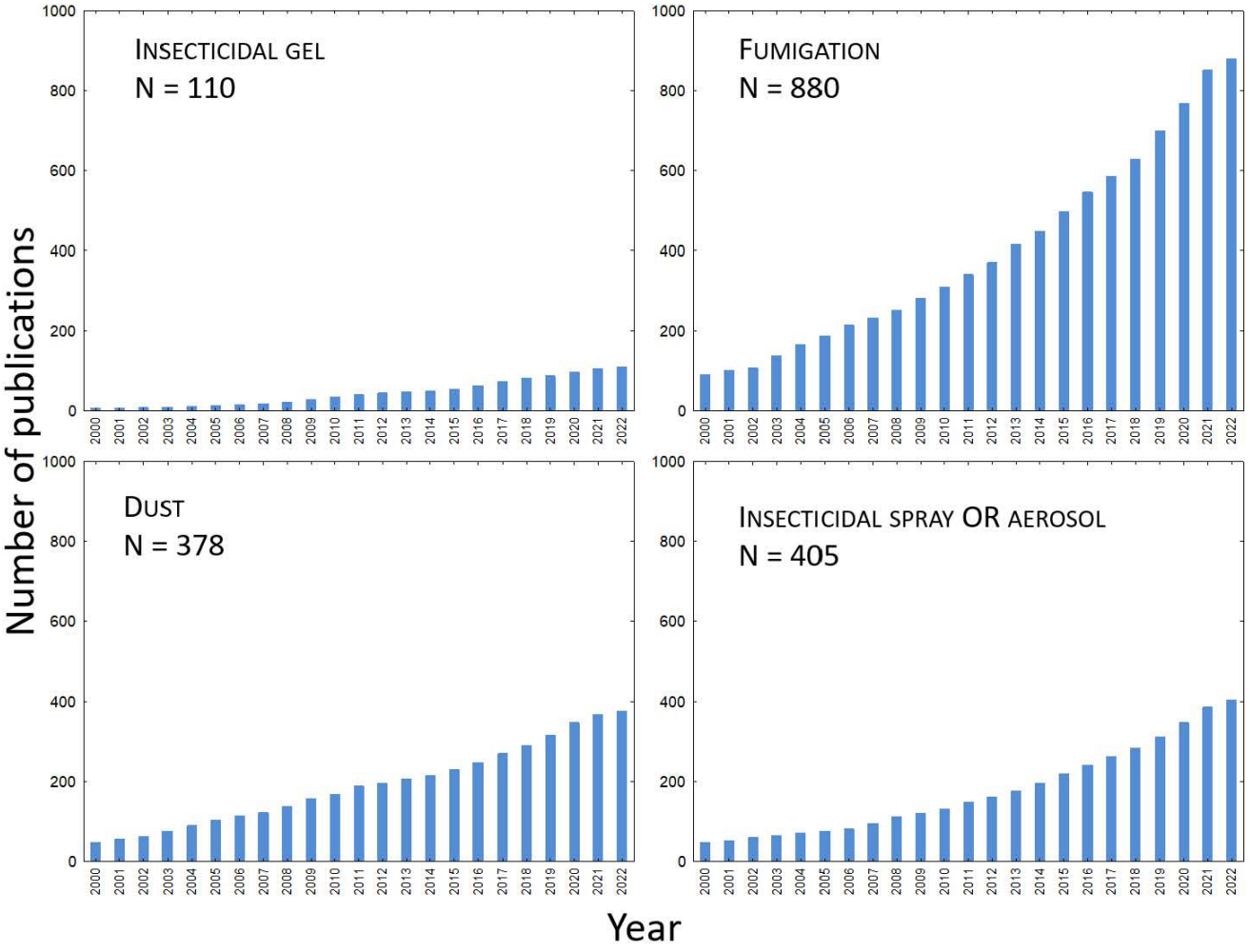


Figure 2. Comparison of cumulative number of WoS peer-reviewed scientific publications from 2000 to the present on the topic of gels and other pesticide formulations (solid dusts, gas-fumigants, liquid sprays) regarding their use for pest control.

Without much speculation and large predictive uncertainties, further development of pesticide gel formulations can be expected in those areas in which they are already in use and have proven themselves. This is particularly the case in the areas of the design of innovative or new types of gel insecticide and soft rodenticide baits or mosquito repellent formulations. Innovative controlled-release gel formulations of traditional insecticides, such as carbamates [5] or organophosphates [6], have also been designed for the direct control of field agricultural pests. However, it is questionable what application of these types of gel formulations will find practical use due to increasingly stringent registration measures against these and possibly other groups of neurotoxic pesticide compounds. Thus, a more optimistic outlook for agricultural pest control seems to be in the development of gel formulations as carriers of microbial bio-agents [47], natural botanical insecticides (plant extracts, essentials oils) [46], natural repellents, and gel-based lures for the controlled release [132] of pheromones applied as pheromone mating disruptors [37,145,146] within the framework of IPM strategy. Gels and other polymers (especially biopolymers) then also have good prospects as carriers of environmentally acceptable impregnating agents (e.g., borates) for structural/construction wood protection [111,147–149].

Currently, in urban insects, gel baits are generally the preferred pesticide formulations, compared to insecticide sprays and dusts, due to their instrumentally undemanding application methods, potential for secondary transmission, low acute toxicity, minimal nontarget effects, and low environmental contamination [150]. The most intensive devel-

opment can be expected in the field of insecticidal baits, which will include hydrogels as carriers. [4]. Combinations of gel insecticide baits with controlled release of pheromones seems to also be promising approach. For example, it has been shown for ant control that the addition of a synthetic trail pheromone ((Z)-9-hexadecenal) to hydrogel baits could further increase bait efficacy [151] before the hydrogels lose too much moisture [152]. In addition, species-specific pheromones can also further improve target specificity of the bait.

Of the new developments in rodent control [153], the most obvious trend is that rodenticide manufacturers and users are increasingly favoring the development and use of soft rodenticide baits [22]. This is due to their ease of application, attractiveness, and palatability. Soft types of baits may gain further market space, as the registration of many types of drinkable anticoagulant baits has not been extended in the EU and other countries. Most importantly, the new generation of soft gel and paste bait formulations may reduce the risks of uncontrolled bait transport by rodents from bait stations and their uncontrolled environmental contamination. Gels and pastes have potential as they represent suitable formulations from a food industry perspective since they can be specifically constructed for allergen-free operations. In addition, from the available scientific documentation, it appears that soft rodenticide baits are less susceptible to infestation and spoilage by storage arthropods [154]. Species-specific RNAi-based agents, for which gels and pastes can be used as carriers, are considered a future alternative to toxic baits [155].

Currently, arthropod resistance to insecticides is becoming a serious problem worldwide [156–159]. In the field of pest management, molecular methods are mainly used for the diagnosis of harmful organisms [160], while as methods for direct pest control these new approaches—with the exception of GMOs—have only a limited sphere of application. However, resistance to traditional active ingredients may provide an opportunity to develop molecular methods and formulations to control pests, including various forms of nanoformulations [161] and polymers and the use of gels. Although the results are still in the research stage, they do not look pessimistic. For example, a substantially new generation of bait technologies for liquid baits with liposomes as carriers of dsRNA has been suggested by several research teams [95,96]. Specifically, a new method was developed [95] which uses liposome vesicles (so-called dsRNA lipoplexes) as carriers of dsRNA molecules. Laboratory tests showed that the protected molecules (lipoplexes), formulated as liquid or gel baits, may trigger lethal RNA interference in the cockroach digestive system. Oral-delivery-mediated RNAi was first used to silence the LeVgR gene in *Liposcelis entomophila* (Enderlein) [96]. The VgR gene may thus become an important potential target for disrupting insect reproduction for pest management through the oral delivery of dsRNA. Owing to these new approaches, there is a real chance that in the future, such types of technologies could become the basis for "smart" pest control products delivered via gel baits.

11. Conclusions

Various gel carriers of rodenticides and insecticides, as well as biological control agents (microorganisms), have been proposed for the control of many pest species. Traditional granular formulations for the control and/or monitoring of rodents have recently started to be replaced by paste and gel formulations. Various gel bait formulations have been successfully used for several decades to control public health and urban pests, such as cockroaches and ants; new hydrogel-based formulations are mainly in the research and development stage. Profound advances have been made in the use of gel formulations as repellents in the management of the medical risks of mosquitoes as vectors of pathogens such as malaria. Despite research effort, few if any practical formulations of traditional neurotoxic insecticides seem to be available and registered for the control of agricultural and forest pests. However, gel nanoformulations were constructed for the slow release of pheromones for behavioral manipulation of horticultural pests. The potential of gels for application of plant extracts (e.g., essential oils, EOs) in agriculture is being newly explored; hydrogels were suggested as an emerging technology for controlled fumigation release of EOs for plant pest control in greenhouses, e.g., resistant plant pests. Promising results have

been obtained for the protection of foodstuffs such as dried ham by harmful mites using gel formulations. Current research shows that gel formulations would find significant application as carriers of so-called "smart insecticides", which are based on molecular disruptors of iRNA.

Author Contributions: J.A.S., writing—original draft preparation, writing—review and editing; T.V., conceptualization, writing—original draft preparation, writing—review and editing; R.A., writing—original draft preparation, writing—review and editing, funding acquisition; M.F., writing—original draft preparation, writing—review and editing; V.S., conceptualization, writing—original draft preparation, writing—review and editing, supervision, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: The study was funded by the Ministry of Agriculture Czech Republic, grant number VZ-RO0418 (V.S., R.A., T.V., M.F.—CRI) and by the NAZV agency of the Ministry of Agriculture Czech Republic, grant number QK21010064 (V.S., R.A., T.V.—CRI). J.A.S. was supported by the Czech University of Life sciences Prague.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. This review reports the results of research only. The review includes historical, current, and future perspectives at the worldwide scale. Therefore, it should be stressed that many of the listed pesticides, active ingredients, and/or application procedures may not be legal or registered at the moment of MS preparation/publishing (current or future) or in a certain geographical area. Mentions of trade names or commercial products in this publication are solely for the purpose of providing specific information and do not imply recommendation, endorsement (or even instruction for their use) by authors or institutions (Crop Research Institute Prague—CRI). Some of the mentioned terminology, terms, notions, and concepts may be used by various scientific communities (and therefore also in this review) in a different way than they are used or defined by a particular legislation or specific pesticide registration requirements.

References

1. Prathap, A.; Sureshan, K.M. Sugar-based organogelators for various applications. *Langmuir* **2019**, *35*, 6005–6014. [CrossRef] [PubMed]
2. Mondal, S.; Das, S.; Nandi, A.K. A review on recent advances in polymer and peptide hydrogels. *Soft Matter* **2020**, *16*, 1404–1454. [CrossRef] [PubMed]
3. Cao, X.; Gao, A.; Hou, J.-T.; Yi, T. Fluorescent supramolecular self-assembly gels and their application as sensors: A review. *Coord. Chem. Rev.* **2021**, *434*, 213792. [CrossRef]
4. Tay, J.W.; Choe, D.H.; Mulchandani, A.; Rust, M.K. Hydrogels: From controlled release to a new bait delivery for insect pest management. *J. Econ. Entomol.* **2020**, *113*, 2061–2068. [CrossRef] [PubMed]
5. Işıklan, N. Controlled release study of carbaryl insecticide from calcium alginate and nickel alginate hydrogel beads. *J. Appl. Polym. Sci.* **2007**, *105*, 718–725. [CrossRef]
6. Baloch, F.E.; Afzali, D.; Fathirad, F. Design of acrylic acid/nanoclay grafted polysaccharide hydrogels as superabsorbent for controlled release of chlorpyrifos. *Appl. Clay Sci.* **2021**, *211*, 106194. [CrossRef]
7. Nuruzzaman, M.; Rahman, M.M.; Liu, Y.J.; Naidu, R. Nanoencapsulation, nano-guard for pesticides: A new window for safe application. *J. Agric. Food Chem.* **2016**, *64*, 1447–1483. [CrossRef]
8. Sun, C.; Zeng, Z.; Cui, H.; Verheggen, F. Polymer-based nanoinsecticides: Current developments, environmental risks and future challenges. A review. *Biotechnol. Agron. Soc. Environ.* **2020**, *24*, 59–69. [CrossRef]
9. Athanassiou, C.G.; Kavallieratos, N.G.; Benelli, G.; Losic, D.; Usha Rani, P.; Desneux, N. Nanoparticles for pest control: Current status and future perspectives. *J. Pest. Sci.* **2018**, *91*, 1–15. [CrossRef]
10. Stejskal, V.; Vendl, T.; Aulicky, R.; Athanassiou, C. Synthetic and Natural Insecticides: Gas, Liquid, Gel and Solid Formulations for Stored-Product and Food-Industry Pest Control. *Insects* **2021**, *12*, 590. [CrossRef]
11. Campolo, O.; Giunti, G.; Russo, A.; Palmeri, V.; Zappalà, L. Essential oils in stored product insect pest control. *J. Food Qual.* **2018**, *2018*, 6906105. [CrossRef]
12. Nansen, C.; Hinson, B.; Davidson, D.; Vaughn, K.; Hosseini, A. Novel approaches to application and performance assessment of insecticide applications to crop leaves. *J. Econ. Entomol.* **2009**, *103*, 219–227. [CrossRef] [PubMed]
13. Meredith, A.N.; Harper, B.; Harper, S.L. The influence of size on the toxicity of an encapsulated pesticide: A comparison of micron-and nano-sized capsules. *Environ. Int.* **2016**, *86*, 68–74. [CrossRef] [PubMed]

14. Greaves, J.H.; Rowe, F.P.; Redfern, R.; Ayres, P. Microencapsulation of rodenticides. *Nature* **1968**, *219*, 402–403. [CrossRef] [PubMed]
15. Hohenberger, J.; Friesen, A.; Wieck, S.; Kümmerer, K. In search of the Holy Grail of Rodent control: Step-by-step implementation of safe and sustainable-by-design principles on the example of rodenticides. *Sustain. Chem. Pharm.* **2022**, *25*, 100602. [CrossRef]
16. Rumbos, C.I.; Dutton, A.C.; Athanassiou, C.G. Efficacy of two formulations of pirimiphos-methyl as surface treatment against *Sitophilus granarius*, *Rhyzopertha dominica*, and *Tribolium confusum*. *J. Pest Sci.* **2014**, *87*, 507–519. [CrossRef]
17. Marsh, R.E. Microencapsulation of rodenticides. *Proc. Vertebr. Pest Conf.* **1990**, *14*, 62–64.
18. Stejskal, V.; Aulicky, R.; Pekar, S. Brief exposure of *Blattella germanica* (Blattodea) to insecticides formulated in various microcapsule sizes and applied on porous and non-porous surfaces. *Pest Manag. Sci.* **2009**, *65*, 93–98. [CrossRef]
19. Ali, S.; Akram, W.; Sajjad, A.; Shakeel, Q.; Ullah, M.I. Aerogels as Pesticides. In *Aerogels II: Preparation, Properties and Applications*; Inamudin, Mobin, R., Ahamed, M.I., Altalhi, T., Eds.; Materials Research Forum LLC: Millersville, PA, USA, 2021; pp. 168–182.
20. Benelli, G.; Pavela, R. Beyond mosquitoes—Essential oil toxicity and repellency against bloodsucking insects. *Ind. Crops Prod.* **2018**, *117*, 382–392. [CrossRef]
21. Nerio, L.S.; Olivero-Verbel, J.; Stashenko, E. Repellent activity of essential oils: A review. *Bioresour. Technol.* **2010**, *101*, 372–378. [CrossRef]
22. Cornwell, P.B. Studies in microencapsulation of rodenticides. *Proc. Vertebr. Pest Conf.* **1970**, *4*, 83–97.
23. Guilherme, M.R.; Aouada, F.A.; Fajardo, A.R.; Martins, A.F.; Paulino, A.T.; Davi, M.F.T.; Rubira, A.F.; Muniz, E.C. Superabsorbent hydrogels based on polysaccharides for application in agriculture as soil conditioner and nutrient carrier: A review. *Eur. Polym. J.* **2015**, *72*, 365–385. [CrossRef]
24. Mishra, S.; Thombare, N.; Ali, M.; Swami, S. Applications of Biopolymeric gels in Agricultural Sector. In *Polymer Gels: Perspectives and Applications*; Thakur, V.K., Thakur, M.K., Voicu, S.I., Eds.; Springer: Singapore, 2018; pp. 185–228.
25. Singh, A.; Dhiman, N.; Kar, A.K.; Singh, D.; Purohit, M.P.; Ghosh, D.; Patnaik, S. Advances in controlled release pesticide formulations: Prospects to safer integrated pest management and sustainable agriculture. *J. Hazard. Mater.* **2020**, *385*, 121525. [CrossRef]
26. Campea, M.A.; Majcher, M.J.; Lofts, A.; Hoare, T. A review of design and fabrication methods for nanoparticle network hydrogels for biomedical, environmental, and industrial applications. *Adv. Funct. Mater.* **2021**, *31*, 2102355. [CrossRef]
27. Qu, B.; Luo, Y.C. Chitosan-based hydrogel beads: Preparations, modifications and applications in food and agriculture sectors—A review. *Int. J. Biol. Macromol.* **2020**, *152*, 437–448. [CrossRef] [PubMed]
28. Księżak, J. The influence of different doses of hydrogel on the quality of seeds and the yield of faba beans. *Pol. J. Agron.* **2018**, *33*, 8–15.
29. Sarvas, M.; Pavlenda, P.; Takacova, E. Effect of hydrogel application on survival and growth of pine seedlings in reclamations. *J. For. Sci.* **2007**, *53*, 204–209. [CrossRef]
30. Hayat, R.; Ali, S. Water absorption by synthetic polymer (Aquasorb) and its effect on soil properties and tomato yield. *Int. J. Agric. Biol.* **2004**, *6*, 998–1200.
31. Letey, J.; Clark, P.R.; Amrhein, C. Water-sorbing polymers do not conserve water. *Calif. Agric.* **1992**, *46*, 9–10. [CrossRef]
32. Fonteno, W.C.; Bilderback, T.E. Impact of hydrogel on physical properties of coarse-structured horticultural substrates. *J. Am. Soc. Hortic. Sci.* **1993**, *118*, 217–222. [CrossRef]
33. Michalik, R.; Wandzik, I. A mini-review on chitosan-based hydrogels with potential for sustainable agricultural applications. *Polymers* **2020**, *12*, 2425. [CrossRef] [PubMed]
34. Rudzinski, W.E.; Dave, A.M.; Vaishnav, U.H.; Kumbar, S.G.; Kulkarni, A.R.; Aminabhavi, T.M. Hydrogels as controlled release devices in agriculture. *Des. Monom. Polym.* **2002**, *5*, 39–65. [CrossRef]
35. Rudzinski, W.E.; Chipuk, T.; Dave, A.M.; Kumbar, S.G.; Aminabhavi, T.M. PH-sensitive acrylic-based copolymeric hydrogels for the controlled release of a pesticide and a micronutrient. *J. Appl. Polym. Sci.* **2003**, *87*, 394–403. [CrossRef]
36. Aouada, F.A.; de Moura, M.R.; Mattoso, L.H.C. Biodegradable Hydrogel as Delivery Vehicle for the Controlled Release of Pesticide. In *Pesticides-Formulations, Effects, Fate*; Stoytcheva, M., Ed.; InTech: London, UK, 2011; ISBN 978-953-307-532-7.
37. Bhagat, D.; Samanta, S.K.; Bhattacharyya, S. Efficient management of fruit pests by pheromone nanogels. *Sci. Rep.* **2013**, *3*, 1294. [CrossRef]
38. He, F.; Zhou, Q.; Wang, L.; Yu, G.; Li, J.; Feng, Y. Fabrication of a sustained release delivery system for pesticides using interpenetrating polyacrylamide/alginate/montmorillonite nanocomposite hydrogels. *Appl. Clay Sci.* **2019**, *183*, 105347. [CrossRef]
39. Xiang, Y.; Zhang, G.; Chen, C.; Liu, B.; Cai, D.; Wu, Z. Fabrication of a pH-responsively controlled-release pesticide using an attapulgite-based hydrogel. *ACS Sustain. Chem. Eng.* **2018**, *6*, 1192–1201. [CrossRef]
40. Singh, B.; Sharma, D.K.; Gupta, A. Controlled Release of thiram fungicide from starch-based hydrogels. *J. Environ. Sci. Health B* **2007**, *42*, 677–695. [CrossRef]
41. Singh, B.; Sharma, D.; Gupta, A. In vitro release dynamics of thiram fungicide from starch and poly(methacrylic acid)-based hydrogels. *J. Hazard. Mater.* **2008**, *154*, 278–286. [CrossRef]
42. Xu, C.; Cao, L.; Bilal, M.; Cao, C.; Zhao, P.; Zhang, H.; Huang, Q. Multifunctional manganese-based carboxymethyl chitosan hydrogels for pH-triggered pesticide release and enhanced fungicidal activity. *Carbohydr. Polym.* **2021**, *262*, 117933. [CrossRef]
43. Supare, K.; Mahanwar, P. Starch-chitosan hydrogels for the controlled-release of herbicide in agricultural applications: A study on the effect of the concentration of raw materials and crosslinkers. *J. Polym. Environ.* **2022**, *30*, 2448–2461. [CrossRef]

44. Rehab, A.; Akelah, A.; Issa, R.; D'Antone, S.; Solaro, R.; Chiellini, E. Controlled release of herbicides supported on polysaccharide based hydrogels. *J. Bioact. Compat. Polym.* **1991**, *6*, 52–63. [CrossRef]
45. Li, J.; Li, Y.; Dong, H. Controlled release of herbicide acetochlor from clay/carboxylmethylcellulose gel formulations. *J. Agric. Food Chem.* **2008**, *56*, 1336–1342. [CrossRef] [PubMed]
46. Gharbi, K.; Tay, J.W. Fumigant toxicity of essential oils against *Frankliniella occidentalis* and *F. insularis* (Thysanoptera: Thripidae) as affected by polymer release and adjuvants. *Insects* **2022**, *13*, 493. [CrossRef] [PubMed]
47. Ropek, D.; Kulikowski, E. Potential of hydrogel application for plant protection. *Ecol. Chem. Eng. A* **2009**, *16*, 1191–1198.
48. Galhardi, J.A.; de Oliveira, J.L.; Ghoshal, S.; Fraceto, L.F. Soil enzyme responses to polymeric nanopesticides: An ecological risk analysis approach to promote sustainable agriculture. *ACS Agric. Sci. Technol.* **2022**, *3*, 443–452. [CrossRef]
49. Song, Y.; Zhu, F.; Cao, C.; Cao, L.; Li, F.; Zhao, P.; Huang, Q. Reducing pesticide spraying drift by folate/Zn^{2+} supramolecular hydrogels. *Pest Manag. Sci.* **2021**, *77*, 5278–5285. [CrossRef]
50. Yang, Y.; Zhang, S.; Yang, J.; Bai, C.; Tang, S.; Ye, Q.; Wang, H. Superabsorbent hydrogels coating increased degradation and decreased bound residues formation of carbendazim in soil. *Sci. Total Environ.* **2018**, *630*, 1133–1142. [CrossRef]
51. Aouada, F.A.; Pan, Z.; Orts, W.J.; Mattoso, L.H. Removal of paraquat pesticide from aqueous solutions using a novel adsorbent material based on polyacrylamide and methylcellulose hydrogels. *J. Appl. Polym. Sci.* **2009**, *114*, 2139–2148. [CrossRef]
52. Alammar, A.; Park, S.-H.; Ibrahim, I.; Arun, D.; Holtzl, T.; Dumée, L.F.; Lim, H.N.; Szekely, G. Architecting neonicotinoid-scavenging nanocomposite hydrogels for environmental remediation. *Appl. Mater. Today* **2020**, *21*, 100878. [CrossRef]
53. Gosset, A.; Oestreicher, V.; Perullini, M.; Bilmes, S.A.; Jobbágy, M.; Dulhoste, S.; Bayard, R.; Durrieu, C. Optimization of sensors based on encapsulated algae for pesticide detection in water. *Anal. Methods* **2019**, *11*, 6193–6203. [CrossRef]
54. Jia, W.; Fan, R.; Zhang, J.; Zhu, K.; Gai, S.; Yin, Y.; Yang, Y. Smart MOF-on-MOF hydrogel as a simple rod-shaped core for visual detection and effective removal of pesticides. *Small* **2022**, *18*, 2201510. [CrossRef] [PubMed]
55. Aulicky, R.; Tkadlec, E.; Suchomel, J.; Frankova, M.; Heroldová, M.; Stejskal, V. Management of the common vole in the Czech lands: Historical and current perspectives. *Agronomy* **2022**, *12*, 1629. [CrossRef]
56. Buckle, A.P.; Smith, R. *Rodent Pests and Their Control*; CABI: Wallingford, UK, 2014.
57. Frankova, M.; Kaftanova, B.; Aulicky, R.; Rodl, P.; Frynta, D.; Stejskal, V. Temporal production of coloured faeces in wild roof rats (*Rattus rattus*) following consumption of fluorescent non-toxic bait and a comparison with wild *R. norvegicus* and *Mus musculus*. *J. Stored Prod. Res.* **2019**, *81*, 7–10. [CrossRef]
58. Frankova, M.; Stejskal, V.; Aulicky, R. Suppression of food intake by house mouse (*Mus musculus*) following ingestion of brodifacoum-based rodenticide bait. *Crop. Prot.* **2017**, *100*, 134–137. [CrossRef]
59. Frankova, M.; Stejskal, V.; Aulicky, R. Efficacy of rodenticide baits with decreased concentrations of brodifacoum: Validation of the impact of the new EU anticoagulant regulation. *Sci. Rep.* **2019**, *9*, 16779. [CrossRef]
60. Grulich, I. Boj proti hrabosi polnímu. In *Common Vole (Microtus arvalis)*, 1st ed.; Kratochvíl, J., Balát, F., Eds.; CAV Edition: Prague, Czech Republic, 1959; pp. 285–316. (In Czech)
61. Wilson, G. The Evolution of Rodenticides. Professional Pest Manager. 2018. Available online: https://professionalpestmanager.com/the-evolution-of-rodenticides/ (accessed on 8 July 2022).
62. Vuksa, M.; Djedovic, S.; Jokic, G.; Stojnic, B. Palatability and efficacy of RB soft bag formulated baits in controlling house mouse and Norway rat in mills and storage facilities of agricultural products. *J. Process. Energy Agric.* **2011**, *15*, 267–269.
63. Vukša, M.; Dedovic, S.; Jokic, G.; Stojnic, B. Palatability and efficacy of RB soft bag formulated baits in controlling house mouse and Norway rat in animal food blender facilities and pig farm. *Biotechnol. Anim. Husb.* **2011**, *27*, 1801–1810. [CrossRef]
64. Jordan, K.K.; Riegel, C.; Bauder, F.M.; Smith, P.L. Urban Field Efficacy of a New Cholecalciferol-based Soft Bait on Commensal Rats in New Orleans, Louisiana, USA. In Proceedings of the 28th Vertebrate Pest Conference, Rohnert Park, CA, USA, 26 February–1 March 2018; Woods, D.M., Ed.; Independently published: Davis, CA, USA, 2018; pp. 23–32.
65. Sked, S.; Abbar, S.; Cooper, R.; Corrigan, R.; Pan, X.; Ranabhat, S.; Wang, C. Monitoring and controlling house mouse, *Mus musculus domesticus*, Infestations in low-income multi-family dwellings. *Animals* **2021**, *11*, 648. [CrossRef]
66. Kappes, P.J.; Siers, S.R. Relative acceptance of brodifacoum pellets and soft bait sachets by Polynesian rats (*Rattus exulans*) on Wake Atoll. *Manag. Biol. Invasions* **2021**, *12*, 685–699. [CrossRef]
67. Sachdeva, S.; Singla, N. Antifeedant and repellent potential of alginate based microcapsules containing eucalyptus oil against house rat, *Rattus rattus*. *J. Entomol. Zool. Stud.* **2018**, *6*, 608–617.
68. Frynta, D.; Eliasova, B.; Frankova, M.; Aulicky, R.; Rodl, P.; Stejskal, V. Production of UV-light-detectable faeces from house mice (*Mus musculus domesticus*) after consumption of encapsulted fluorescent pigment in monitoring bait. *Pest Manag. Sci.* **2012**, *68*, 355–361. [CrossRef] [PubMed]
69. Frankova, M.; Kaftanova-Eliasova, B.; Rodl, P.; Aulicky, R.; Frynta, D.; Stejskal, V. Monitoring of *Rattus norvegicus* based on non-toxic bait containing encapsulated fluorescent dye: Laboratory and semi-field validation study. *J. Stored Prod. Res.* **2015**, *64*, 103–108. [CrossRef]
70. Wales, K.N.; Meinerz, R.; Baldwin, R.A. Assessing the Attractiveness of Three Baits for Roof Rats in California Citrus Orchards. *Agronomy* **2021**, *11*, 2417. [CrossRef]
71. World Health Organization. *World Malaria Report 2016*; World Health Organization: Geneva, Switzerland, 2016.
72. Tavares, M.; da Silva, M.R.M.; de Oliveira de Siqueira, L.B.; Rodrigues, R.A.S.; Bodjolle-d'Almeira, L.; dos Santos, E.P.; Ricci-Júnior, E. Trends in insect repellent formulations: A review. *Int. J. Pharm.* **2018**, *539*, 190–209. [CrossRef] [PubMed]

73. Peterson, C.; Coats, J. Insect repellents—Past, present and future. *Pestic. Outlook* **2001**, *12*, 154–158. [CrossRef]
74. Hazarika, H.; Krishnatreyya, H.; Tyagi, V.; Islam, J.; Gogoi, N.; Goyary, D.; Chattopadhyay, P.; Zaman, K. The fabrication and assessment of mosquito repellent cream for outdoor protection. *Sci. Rep.* **2022**, *12*, 2180. [CrossRef]
75. Barradas, T.N.; Senna, J.P.; Junior, E.R.; Mansur, C.R.E. Polymer-based drug delivery systems applied to insects repellents devices: A review. *Curr. Drug Deliv.* **2016**, *13*, 221–235. [CrossRef]
76. Milutinović, R.; Vuleta, G.; Milić, J.; Stajković, N. Assessment of efficiency of repellent formulations with N, N-diethyl-m-toluamide in laboratory conditions. *Int. J. Cosmet. Sci.* **1999**, *21*, 7–14. [CrossRef]
77. Pinto, I.C.; Cerqueira-Coutinho, C.S.; Santos, E.P.; Carmo, F.A.; Ricci-Junior, E. Development and characterization of repellent formulations based on nanostructured hydrogels. *Drug Dev. Ind. Pharm.* **2017**, *43*, 67–73. [CrossRef]
78. Delong, W.; Weibin, M.; Mingchen, J.; Zhonglin, Y.; Juntao, F.; Xing, Z. pHEMA hydrogels with pendant triazinyl-β-cyclodextrin as an efficient and recyclable reservoir for loading and release of plant-based mosquito repellents: A new aqueous mosquito repellent formulation. *RSC Adv.* **2016**, *6*, 27301–27312. [CrossRef]
79. Kumar, N.; Kumar, S.; Singh, S.P.; Rao, R. Enhanced protective potential of novel citronella essential oil microsponge hydrogel against *Anopheles stephensi* mosquito. *J. Asia Pac. Entomol.* **2021**, *24*, 61–69. [CrossRef]
80. Rogerio, C.B.; Abrantes, D.C.; De Oliveira, J.L.; de Araújo, D.R.; da Costa, T.G.; De Lima, R.; Fraceto, L.F. Cellulose hydrogels containing geraniol and icaridin encapsulated in zein nanoparticles for arbovirus control. *ACS Appl. Bio Mater.* **2022**, *5*, 1273–1283. [CrossRef] [PubMed]
81. Benelli, G.; Canale, A.; Conti, B. Eco-friendly control strategies against the asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae): Repellency and toxic activity of plant essential oils and extracts. *Pharmacologyonline* **2014**, *1*, 44–51.
82. Spinozzi, E.; Maggi, F.; Bonacucina, G.; Pavela, R.; Boukouvala, M.C.; Kavallieratos, N.G.; Canale, A.; Romano, D.; Desneux, N.; Wilke, A.B.B.; et al. Apiaceae essential oils and their constituents as insecticides against mosquitoes—A review. *Ind. Crops Prod.* **2021**, *171*, 113892. [CrossRef]
83. Pavoni, L.; Pavela, R.; Cespi, M.; Bonacucina, G.; Maggi, F.; Zeni, V.; Canale, A.; Lucchi, A.; Bruschi, F.; Benelli, G. Green micro- and nanoemulsions for managing parasites, vectors and pests. *Nanomaterials* **2019**, *9*, 1285. [CrossRef]
84. Mapossa, A.B.; Focke, W.W.; Tewo, R.K.; Androsch, R.; Kruger, T. Mosquito-repellent controlled-release formulations for fighting infectious diseases. *Malar. J.* **2021**, *20*, 165. [CrossRef] [PubMed]
85. Shin, J.; Seo, S.M.; Park, I.K.; Hyun, J. Larvicidal composite alginate hydrogel combined with a pickering emulsion of essential oil. *Carbohydr. Polym.* **2021**, *254*, 117381. [CrossRef]
86. Johnson, B.J.; Ritchie, S.A.; Fonseca, D.M. The state of the art of lethal oviposition trap-based mass interventions for arboviral control. *Insects* **2017**, *8*, 5. [CrossRef]
87. Friuli, M.; Cafarchia, C.; Lia, R.P.; Otranto, D.; Pombi, M.; Demitri, C. From tissue engineering to mosquitoes: Biopolymers as tools for developing a novel biomimetic approach to pest management/vector control. *Parasites Vectors* **2022**, *15*, 79. [CrossRef]
88. Friuli, M.; Cafarchia, C.; Cataldo, A.; Lia, R.P.; Otranto, D.; Pombi, M.; Demitri, C. Proof of concept of biopolymer based hydrogels as biomimetic oviposition substrate to develop tiger mosquitoes (*Aedes albopictus*) cost-effective lure and kill ovitraps. *Bioengineering* **2022**, *9*, 267. [CrossRef]
89. Rust, M.K.; Owens, J.M.; Reierson, D.A. *Understanding and Controlling the German Cockroach*; Oxford University Press: New York, NY, USA, 1995.
90. Tee, H.; Lee, C. Sustainable cockroach management using insecticidal baits: Formulations, behavioral responses and issues. In *Urban Insect Pests-Sustainable Management Strategies*; Dhang, P., Ed.; CAB International: Oxfordshire, UK; Boston, MA, USA, 2014; pp. 65–85.
91. Stejskal, V.; Vendl, T.; Li, Z.; Aulicky, R. Minimal thermal requirements for development and activity of stored product and food industry pests (Acari, Coleoptera, Lepidoptera, Psocoptera, Diptera and Blattodea): A review. *Insects* **2019**, *10*, 149. [CrossRef]
92. Appel, A.G. Laboratory and field performance of an indoxacarb bait against German cockroaches (Dictyoptera: Blattellidae). *J. Econ. Entomol.* **2003**, *96*, 863–870. [CrossRef] [PubMed]
93. Lovell, J.B. Amidinohydrazones—A new class of insecticides. In Proceedings of the 10th British Crop Protection Conference-Pests and Diseases, Brighton, UK, 19–22 November 1979; British Crop Protection Council: Cambridge, UK, 1979; pp. 575–582.
94. Oladipupo, S.O.; Hu, X.P.; Appel, A.G. Essential oils in urban insect management—A review. *J. Econ. Entomol.* **2022**, toac083. [CrossRef] [PubMed]
95. Huang, J.H.; Liu, Y.; Lin, Y.H.; Belles, X.; Lee, H.J. Practical use of RNA interference: Oral delivery of double-stranded RNA in liposome carriers for cockroaches. *J. Vis. Exp.* **2018**, *135*, e57385. [CrossRef]
96. Wang, S.; Miao, S.; Yang, B.; Wang, Z.; Liu, Q.; Wang, R.; Du, X.; Ren, Y.; Lu, Y. Initial characterization of the vitellogenin receptor from a Psocoptera insect: Function analysis and RNA interference in *Liposcelis entomophila* (Enderlein). *J. Stored Prod. Res.* **2021**, *92*, 101803. [CrossRef]
97. Markin, G.P.; OHill, S. Microencapsulated oil bait for control of the imported fire ant. *J. Econ. Entomol.* **1971**, *64*, 193–196. [CrossRef]
98. Oladipupo, S.O.; Hu, X.P.; Appel, A.G. Essential oil components in superabsorbent polymer gel modify reproduction of *Blattella germanica* (Blattodea: Ectobiidae). *J. Econ. Entomol.* **2020**, *113*, 2436–2447. [CrossRef]
99. Buczkowski, G.; Roper, E.; Chin, D. Polyacrylamide hydrogels: An effective tool for delivering liquid baits to pest ants (Hymenoptera: Formicidae). *J. Econ. Entomol.* **2014**, *107*, 748–757. [CrossRef]

100. Tay, J.W.; Hoddle, M.S.; Mulchandani, A.; Choe, D.H. Development of an alginate hydrogel to deliver aqueous bait for pest ant management. *Pest Manag. Sci.* **2017**, *73*, 2028–2038. [CrossRef]
101. Rust, M.K.; Soeprono, A.; Wright, S.; Greenberg, L.; Choe, D.-H.; Boser, C.L.; Cory, C.; Hanna, C. Laboratory and field evaluations of polyacrylamide hydrogel baits against Argentine ants (Hymenoptera: Formicidae). *J. Econ. Entomol.* **2015**, *108*, 1228–1236. [CrossRef]
102. Choe, D.H.; Campbell, K.; Hoddle, M.S.; Kabashima, J.; Dimson, M.; Rust, M.K. Evaluation of a hydrogel matrix for baiting western yellowjacket (Vespidae: Hymenoptera). *J. Econ. Entomol.* **2018**, *111*, 1799–1805. [CrossRef] [PubMed]
103. Boser, C.L.; Hanna, C.; Holway, D.A.; Faulkner, K.R.; Naughton, I.; Merrill, K.; Randall, J.M.; Cory, C.; Choe, D.H.; Morrison, S.A. Protocols for argentine ant eradication in conservation areas. *J. Appl. Entomol.* **2017**, *141*, 540–550. [CrossRef]
104. Merrill, K.C.; Boser, C.L.; Hanna, C.; Holway, D.A.; Naughton, I.; Choe, D.-H.; Rankin, E.E.W. Argentine ant (*Linepithema humile*, Mayr) eradication efforts on San Clemente Island, California, USA. *West. N. Am. Nat.* **2019**, *78*, 829. [CrossRef]
105. Klotz, J.H.; Shorey, H. *Low-Toxic Control of Argentine Ants Using Pheromone-Enhanced Liquid Baits*; California Department of Consumer Affairs: Sacramento, CA, USA, 2000; p. 35.
106. Hewlett, P.S. The formation of insecticidal films on building materials. *Ann. Appl. Biol.* **1948**, *35*, 228–232. [CrossRef]
107. Parkin, E.; Hewlett, S. The formation of insecticidal films on building materials. I. Preliminary experiments with films of pyrethrum and D.D.T. in a heavy oil. *Ann. Appl. Biol.* **1946**, *33*, 381. [CrossRef]
108. Hewlett, P.S. The toxicities of three petroleum oils to the grain weevils. *Ann. Appl. Biol.* **1947**, *34*, 575–585. [CrossRef] [PubMed]
109. Tyler, P.S.; Rowlands, D.G. Sodium carboxymethyl cellulose as a stabilizer for malathion formulations. *J. Stored Prod. Res.* **1967**, *3*, 109–115. [CrossRef]
110. Gudrups, I.; Harris, A.H.; Dales, M.J. Are residual insecticide applications to store surfaces worth using? In Proceedings of the 6th International Working Conference on Stored-Product Protection, Canberra, Australia, 17–23 April 1994; Highley, E., Wright, E.J., Banks, H.J., Champ, B.R., Eds.; CAB International: Wallingford, UK, 1994; pp. 785–789.
111. Obounou-Akong, F.; Gérardin, P.; Thévenon, M.F.; Gérardin-Charbonnier, C. Hydrogel-based boron salt formulations for wood preservation. *Wood Sci. Technol.* **2015**, *49*, 443–456. [CrossRef]
112. Krizkova-Kudlikova, I.; Stejskal, V.; Hubert, J. Comparison of detection methods for *Acarus siro* (Acari: Acaridida: Acarididae) contamination in grain. *J. Econ. Entomol.* **2007**, *100*, 1928–1937. [CrossRef]
113. Hubert, J.; Stejskal, V.; Athanassiou, C.G.; Throne, J.E. Health hazards associated with arthropod infestation of stored products. *Annu. Rev. Entomol.* **2018**, *63*, 553–573. [CrossRef]
114. Hubert, J.; Erban, T.; Nesvorna, M.; Stejskal, V. Emerging risk of infestation and contamination of dried fruits by mites in the Czech Republic. *Food Addit. Contam. Part A* **2011**, *28*, 1129–1135. [CrossRef] [PubMed]
115. Stejskal, V.; Bostlova, M.; Nesvorna, M.; Volek, V.; Dolezal, V.; Hubert, J. Comparison of the resistance of mono-and multilayer packaging films to stored-product insects in a laboratory test. *Food Control* **2017**, *73*, 566–573. [CrossRef]
116. Aulicky, R.; Vendl, T.; Stejskal, V. Evaluation of contamination of packages containing cereal-fruit bars by eggs of the pest Indian meal moth (*Plodia interpunctella*, Lepidoptera) due to perforations in their polypropylene foil packaging. *J. Food Sci. Technol.* **2019**, *56*, 3293–3299. [CrossRef]
117. Vendl, T.; Stejskal, V.; Kadlec, J.; Aulicky, R. New approach for evaluating the repellent activity of essential oils against storage pests using a miniaturized model of stored-commodity packaging and a wooden transport pallet. *Ind. Crops Prod.* **2021**, *172*, 114024. [CrossRef]
118. Riudavets, J.; Castane, C.; Alomar, O.; Pons, M.J.; Gabarra, R. Modified atmosphere packaging (MAP) as an alternative measure for controlling ten pests that attack processed food products. *J. Stored Prod. Res.* **2009**, *45*, 91–96. [CrossRef]
119. Kucerova, Z.; Kyhos, K.; Aulicky, R.; Stejskal, V. Low pressure treatment to control food-infesting pests (*Tribolium castaneum*, *Sitophilus granarius*) using a vacuum packing machine. *Czech J. Food Sci.* **2013**, *31*, 94–98. [CrossRef]
120. Kucerova, Z.; Kyhos, K.; Aulicky, R.; Lukas, J.; Stejskal, V. Laboratory experiments of vacuum treatment in combination with an O_2 absorber for the suppression of *Sitophilus granaries* infestations in stored grain samples. *Crop Prot.* **2014**, *61*, 79–83. [CrossRef]
121. Golob, P.; Cox, J.R.; Kilminster, K. Evaluation of insecticide dips as protectants of stored dried fish from dermestid beetle infestation. *J. Stored Prod. Res.* **1987**, *23*, 47–56. [CrossRef]
122. Stara, J.; Stejskal, V.; Nesvorna, M.; Plachy, J.; Hubert, J. Efficacy of selected pesticides against synanthropic mites under laboratory assay. *Pest. Manag. Sci.* **2011**, *67*, 446–457. [CrossRef]
123. Rogers, W.; Campbell, Y.L.; Zhang, X.; Shao, W.; White, S.; Phillips, T.W.; Schilling, M.W. The application of food grade short chain fatty acids to prevent infestation of *Tyrophagus putrescentiae* on dry cured ham and the effects on sensory properties. *J. Stored Prod. Res.* **2020**, *88*, 101684. [CrossRef]
124. Shao, W.; Campbell, Y.L.; Phillips, T.W.; Freeman, C.; Kundu, S.; Crist, C.A.; Williams, J.B.; Schilling, M.W. The application of chitosan in food-grade coatings to control *Tyrophagus putrescentiae* on dry-cured hams and the effects on sensory properties. *J. Stored Prod. Res.* **2021**, *94*, 101899. [CrossRef]
125. Zhao, Y.; Abbar, S.; Phillips, T.W.; Williams, J.B.; Smith, B.S.; Schilling, M.W. Developing food-grade coatings for dry-cured hams to protect against ham mite infestation. *Meat Sci.* **2016**, *113*, 73–79. [CrossRef] [PubMed]
126. Campbell, Y.; Shao, W.; Dinh, T.; To, K.; Rogers, W.; Zhang, X.; Phillips, T.; Schilling, W. Use of nets treated with food grade coatings on controlling mold growth and mite infestation in dry-cured ham aging facilities. *J. Stored Prod. Res.* **2020**, *89*, 101716. [CrossRef]

127. Campbell, Y.; Zhang, X.; Shao, W.; Williams, J.B.; Kim, T.; Goddard, J.; Abbar, S.; Phillips, T.; Schilling, M.W. Use of nets treated with food-grade coatings on dry-cured ham to control *Tyrophagus putrescentiae* infestations without impacting sensory properties. *J. Stored Prod. Res.* **2018**, *76*, 30–36. [CrossRef]
128. Palermo, D.; Giunti, G.; Laudani, F.; Palmeri, V.; Campolo, O. Essential oil-based nano-biopesticides: Formulation and bioactivity against the confused flour beetle *Tribolium confusum*. *Sustainability* **2021**, *13*, 9746. [CrossRef]
129. Nikolaou, P.; Marciniak, P.; Adamski, Z.; Ntalli, N. Controlling stored products' pests with plant secondary metabolites: A review. *Agriculture* **2021**, *11*, 879. [CrossRef]
130. Turek, C.; Stintzing, F.C. Stability of essential oils: A review. *Compr. Rev. Food Sci. Food Saf.* **2013**, *12*, 40–53. [CrossRef]
131. Giunti, G.; Laudani, F.; Lo Presti, E.; Bacchi, M.; Palmeri, V.; Campolo, O. Contact toxicity and ovideterrent activity of three essential oil-based nano-emulsions against the olive fruit fly *Bactrocera oleae*. *Horticulturae* **2022**, *8*, 240. [CrossRef]
132. Pascual-Villalobos, M.; Castañé, C.; Martín, F.; López, M.; Guirao, P.; Riudavets, J. (E)-Anethole microspheres as an alternative insecticide in funnel traps. *J. Stored Prod. Res.* **2021**, *93*, 101862. [CrossRef]
133. Camara, M.C.; Monteiro, R.A.; Carvalho, L.B.; Oliveira, J.L.; Fraceto, L.F. Enzyme stimuli-responsive nanoparticles for bioinsecticides: An emerging approach for uses in crop protection. *ACS Sustain. Chem. Eng.* **2021**, *9*, 106–112. [CrossRef]
134. De Oliveira, J.L.; Campos, E.V.; Camara, M.C.; Della Vechia, J.F.; de Matos, S.T.S.; de Andrade, D.J.; Goncalves, K.C.; Nascimento, J.D.; Polanczyk, R.A.; de Araujo, D.R.; et al. Hydrogels containing botanical repellents encapsulated in zein nanoparticles for crop protection. *ACS Appl. Nano Mater.* **2019**, *3*, 207–217. [CrossRef]
135. Kelany, Y.; Ibrahim, A.; Hegazy, M. Improving the efficiency of two local baits used for the control of the German cockroach, *Blattella germanica* (L.), (Dictyoptera: Blattellidae). *Int. J. Pharm. Biol. Sci.* **2017**, *12*, 59–66.
136. Teli, M.D.; Chavan, P.P. Application of gelatine based microcapsules containing mosquito repellent oils on cellulosic biopolymer. *J. Bionanosci.* **2016**, *10*, 390–395. [CrossRef]
137. Rana, M.; Singh, S.S.J.; Yadav, S. Effect of microencapsulated plant extracts on mosquito repellency. *J. Appl. Nat. Sci.* **2017**, *9*, 2127–2131. [CrossRef]
138. Lucia, A.; Toloza, A.C.; Guzmán, E.; Ortega, F.; Rubio, R.G. Novel polymeric micelles for insect pest control: Encapsulation of essential oil monoterpenes inside a triblock copolymer shell for head lice control. *PeerJ* **2017**, *5*, e3171. [CrossRef]
139. Kamari, A.; Yusoff, S.N.M.; Wong, S.T.S.; Fatimah, I. A mini review of materials used as improvers for insect and arthropod pest repellent textiles. *Curr. Appl. Sci. Technol.* **2022**, *22*, 18. [CrossRef]
140. Oyedele, A.O.; Gbolade, A.A.; Sosan, M.B.; Adewoyin, F.B.; Soyelu, O.L.; Orafidiya, O.O. Formulation of an effective mosquito-repellent topical product from lemongrass oil. *Phytomedicine* **2002**, *9*, 259–262. [CrossRef]
141. Abd El-Bar, M.; Fawki, S. Fumigant activity and chemical composition of three essential oils used in gelatin capsules for the control of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) in Egypt. *Afr. Entomol.* **2021**, *29*, 534–546. [CrossRef]
142. Navarro, S.; Zehavi, D.; Angel, S.; Finkelman, S. Natural nontoxic insect repellent packaging materials. In *Intelligent and Active Packaging for Fruits and Vegetables*; Wilson, C.L., Ed.; CRC Press: New York, NY, USA, 2007; pp. 201–236.
143. Milićević, Z.; Krnjajić, S.; Stević, M.; Ćirković, J.; Jelušić, A.; Pucarević, M.; Popović, T. Encapsulated clove bud essential oil: A new perspective as an eco-friendly biopesticide. *Agriculture* **2022**, *12*, 338. [CrossRef]
144. Picard, I.; Hollingsworth, G.R.; Salmieri, S.; Lacroix, M. Repellency of essential oils to *Frankliniella occidentalis* (Thysanoptera: Thripidae) as affected by type of oil and polymer release. *J. Econ. Entom.* **2012**, *105*, 1238–1247. [CrossRef]
145. Trematerra, P.; Athanassiou, C.; Stejskal, V.; Sciarretta, A.; Kavallieratos, N.; Palyvos, N. Large-scale mating disruption of *Ephestia* spp. and *Plodia interpunctella* in Czech Republic, Greece and Italy. *J. Appl. Entomol.* **2011**, *135*, 749–762. [CrossRef]
146. Ortiz, A.; Porras, A.; Marti, J.; Tudela, A.; Rodríguez-González, Á.; Sambado, P. Mating disruption of the olive moth *Prays oleae* (Bernard) in olive groves using aerosol dispensers. *Insects* **2021**, *12*, 1113. [CrossRef] [PubMed]
147. Mohareb, A.; Thévenon, M.-F.; Wozniak, E.; Gérardin, P. Effects of monoglycerides on leachability and efficacy of boron wood preservatives against decay and termites. *Int. Biodeter. Biodegr.* **2010**, *64*, 135–138. [CrossRef]
148. Patachia, S.; Croitoru, C. Biopolymers for wood preservation. In *Biopolymers and Biotech Admixtures for Eco-Efficient Construction Materials*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 305–332.
149. Alade, A.A.; Naghizadeh, Z.; Wessels, C.B.; Tyhoda, L. A review of the effects of wood preservative impregnation on adhesive bonding and joint performance. *J. Adhes. Sci. Technol.* **2021**, *36*, 1593–1617. [CrossRef]
150. Wang, C.; Lee, C.Y.; Rust, M.K. *Biology and Management of the German Cockroach*, 1st ed.; CABI: Wallingford, UK, 2021; pp. 1–304.
151. Welzel, K.F.; Choe, D.H. Development of a pheromone-assisted baiting technique for Argentine ants (Hymenoptera: Formicidae). *J. Econ. Entomol.* **2016**, *109*, 1303–1309. [CrossRef]
152. McCalla, K.A.; Tay, J.-W.; Mulchandani, A.; Choe, D.-H.; Hoddle, M.S. Biodegradable alginate hydrogel bait delivery system effectively controls high-density populations of Argentine ant in commercial citrus. *J. Pest Sci.* **2020**, *93*, 1031–1042. [CrossRef]
153. Campbell, K.J.; Beek, J.; Eason, C.T.; Glen, A.S.; Godwin, J.; Gould, F.; Holmes, N.D.; Howald, G.R.; Madden, F.M.; Ponder, J.B. The next generation of rodent eradications: Innovative technologies and tools to improve species specificity and increase their feasibility on islands. *Biol. Conserv.* **2015**, *185*, 47–58. [CrossRef]
154. Vendl, T.; Frankova, M.; Aulicky, R.; Stejskal, V. First record of the development of *Sitophilus oryzae* on two rodent bait formulations and literature overview of stored product arthropods infestations in rodent baits. *J. Stored Prod. Res.* **2020**, *86*, 101557. [CrossRef]
155. Horak, K.E. RNAi: Applications in vertebrate pest management. *Trends Biotechnol.* **2020**, *38*, 1200–1202. [CrossRef]

156. Brevik, K.; Schoville, S.; Mota-Sanchez, D.; Chena, Y. Pesticide durability and the evolution of resistance: A novel application of survival analysis. *Pest Manag. Sci.* **2018**, *74*, 1953–1963. [CrossRef]
157. Sanou, A.; Nelli, L.; Guelbéogo, W.M.; Cissé, F.; Tapsoba, M.; Ouédraogo, P.; Sagnon, N.; Ranson, H.; Matthiopoulos, J.; Ferguson, H.M. Insecticide resistance and behavioural adaptation as a response to long-lasting insecticidal net deployment in malaria vectors in the Cascades region of Burkina Faso. *Sci. Rep.* **2021**, *11*, 17569. [CrossRef] [PubMed]
158. Aulicky, R.; Stejskal, V.; Frydova, B.; Athanassiou, C.G. Susceptibility of two strains of the confused flour beetle (Coleoptera: Tenebrionidae) following phosphine structural mill fumigation: Effects of concentration, temperature, and flour deposits. *J. Econ. Entomol.* **2015**, *108*, 2823–2830. [CrossRef] [PubMed]
159. Aulicky, R.; Stejskal, V.; Frydova, B. Field validation of phosphine efficacy on the first recorded resistant strains of *Sitophilus granarius* and *Tribolium castaneum* from the Czech Republic. *J. Stored Prod. Res.* **2019**, *81*, 107–113. [CrossRef]
160. Yang, Q.; Kucerova, Z.; Li, Z.; Kalinović, I.; Stejskal, V.; Opit, G.; Cao, Y. Diagnosis of *Liposcelis entomophila* (Insecta: Psocodea: Liposcelididae) based on morphological characteristics and DNA barcodes. *J. Stored Prod. Res.* **2012**, *48*, 120–125. [CrossRef]
161. Chandrashekharaiah, M.; Kandakoor, S.B.; Gowda, G.B.; Kammar, V.; Chakravarthy, A.K. Nanomaterials: A review of their action and application in pest management and evolution of DNA-tagged particles. In *New Horizons in Insect Science: Towards Sustainable Pest Management*; Chakravarthy, A.K., Ed.; Springer: Berlin, Germany, 2015; pp. 113–126.

Hindawi
BioMed Research International
Volume 2022, Article ID 3443578, 10 pages
https://doi.org/10.1155/2022/3443578

Research Article

Biorational Control of *Callosobruchus maculatus* (Coleoptera: Buchidae) in Stored Grains with Botanical Extracts

Rasheed Akbar,[1] **Imtiaz Ali Khan**,[2] **Brekhna Faheem**,[3] **Rashid Azad**,[1] **Maid Zaman**,[1] **Rubab Altaf**,[1] **Amjad Usman**,[2] **Muhammad Fawad**,[4] **Abid Farid**,[1] **Ahmad Ur Rahman Saljoqi**,[5] **Asad Syed**,[6] **Ali H. Bahkali**,[6] **Abdallah M. Elgorban**,[6] **Jawad Ali Shah**,[7] **and Abdul Qayyum**[8]

[1]*Department of Entomology, The University of Haripur, Haripur 22620, Pakistan*
[2]*Department of Entomology, The University of Agriculture, Peshawar, Pakistan*
[3]*Department of Zoology, Abdul Wali Khan University, Mardan, Pakistan*
[4]*Department of Environmental Sciences, The University of Haripur, Haripur 22620, Pakistan*
[5]*Department of Plant Protection, The University of Agriculture, Peshawar, Pakistan*
[6]*Department of Botany and Microbiology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia*
[7]*Department of Plant Protection, Faculty of Agrobiology Food & Natural Resources, Czech University of life Sciences, Prague, Czech Republic*
[8]*Department of Agronomy, The University of Haripur, Haripur 22620, Pakistan*

Correspondence should be addressed to Rasheed Akbar; rasheed.akbar@uoh.edu.pk

Received 14 June 2022; Accepted 16 August 2022; Published 29 August 2022

Academic Editor: Hafiz Ishfaq Ahmad

Copyright © 2022 Rasheed Akbar et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Globally, around 2000 plant species are used against pest control. The utilization of botanicals is considered the most economic and biodegradable methods for the control of stored grains pests. Therefore, the current study was carried out to investigate the repellency potential of five botanicals against *Callosbruchus maculatus* F. in Haripur, Pakistan. The concentrations of *Azadirachta indica* L., *Nicotiana tabacum* L., *Melia azedarach* L., *Nicotiana rustica* L., and *Thuja orientalis* L. were, i.e., 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% in four replicates to establish contact effects. The data were recorded after 1, 2, 3, 6, 24, 48, 72, and 96 hours. The repellency effect of these plant species against *C. maculatus* were increased in both the time- and dose-dependent manner, and highest effect was observed at 72 h. In addition, the repellency effect was 91% for *A. indica* (class: V), 86% *M. azedarach*, 82%, *N. tabacum* (class: V), 79% *N. rustica* (class: IV), and 75% *T. orientalis* (class: IV) at 3% concentration against *C. maculatus*. Furthermore, following 96 hours' exposure to treatment the sensitivity response of insects decreases as the time interval increases, i.e., 86% *A. indica* (class: V) was followed by 71% *M. azedarach* (class: IV), 65% *N. tabacum* (class: IV), 61% *N. rustica* (class: IV), and *T. orientalis* 57% (class: III) repellency at highest concentration of 3%. The current study concluded that *A. indica* and *M. azedarach* can be incorporated for the management of *C. maculatus* and these plant species might be helpful in the productions of new biopesticides.

1. Introduction

The practice of using plant extracts as biopesticides or medicines is well known [1]. As many as 2000 plant species are in use globally in the control of insect pests. Local people adopt more economic and biodegradable method used as different plant part extracts as pesticides against stored products [2]. However, the effectiveness or use of biopesticide increases as pest management in field and stored product pests [3].

Among the stored products, insect pests, the Genus *Callosbruchus* causes annual losses to different stored

products including 30% in Mung bean, 20% in pigeon pea and 15% in chick pea [4]. About 2.5-3 million tons of stored grains are lost annually due to *C. maculatus* [5]. The Bruchid beetle, *C. maculatus* F. (Coleoptera: Bruchidae), is a cosmopolitan pest attack on economically important legumes such as mung bean, lentil, black gram, and cow peas [6].

This beetle damages the pulses both quantitatively and qualitatively which then become unfit for consumption [7]. *C. maculatus* breed from March to November and maximum damage is caused from February to August when all the developmental stages are present [8]. It is reported that farmer uses highly toxic insecticides to protect their stored commodities including mung bean. The use of chemical insecticides which have known side effects including handling hazards, toxic residues, and development of insecticide resistance [9]. Therefore, insecticides having toxic residues should be discouraged for the control of insect pests [10]. Due to injudicious use of insecticides, most of the stored product pests showed resistance against synthetic insecticides [11].

It is necessary to investigate alternative sources for the management of stored insect pests [12]. For the control of insect pests in storage, there is limited information regarding the utilization of plant products. Overuse of insecticides creates resistance in pest and has a harmful impact on the environment. Therefore, alternative strategies for the management of pests should be adopted [13]. The plant extracts not only environmental friendly but also social acceptable and easily available for local store keeper, farmers, and the people whose business is related with stored commodities. Keeping in view the importance of botanicals pesticides, the present studies were conducted with the aims to find out repellency response of *C. maculatus* against different plant extracts.

2. Material and Methods

The experiment was laid out in completely randomized design (CRD) with factorial arrangement having five treatments each with four replications. The leaves and fruits of five selected plants viz. *A. indica*, *M. azedarach*, *N. rustica*, *N. tabacum*, and *T. orientalis* were collected from different locations of district Swabi, Khyber-Pakhtunkhwa Pakistan (as shown in Table 1 and Figure 1).

2.1. Collection and Establishment of Stock Culture Insects. *C. maculatus* were collected from infested godowns at District Swabi. The collected *C. maculatus* were then brought to Entomological Laboratory, Department of Entomology, the University of Haripur, and released in a glass jar having mung bean as favorite food medium; the jars were covered with muslin cloth and kept in the lab at 30°C and 60 ± 5% RH [9].

2.2. Preparation of Plant Aqueous Extract. Six concentrations of all the selected 5 botanicals were prepared according to the methods adopted by [14]. Leaves and fruits were placed in distilled water for the duration of 48 hr. 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 g of each botanicals (different parts) were directly diluted in 50 ml of distilled water to make 0.5, 1, 1.5, 2 2.5 and 3% (*w*/*v*) solution. Each concentration was prepared separately [9].

2.3. Phytochemical Screening of Selected Plant Aqueous Extracts. The standard solution of 200 ml extracts was prepared by mixture of selected plant extract and distilled water [15]. The extracts were subjected for phytochemical for the following standard methods.

2.3.1. Extraction Procedure. Maceration: For maceration (for fluid extract), whole or coarsely powdered plant drug was kept in contact with the solvent in a stopper container for a defined period with frequent agitation until soluble matter is dissolved [16].

2.3.2. Tests for Alkaloid Wegener's tests. Extracts of the test plants were dissolved individually in dilute hydrochloric acid 1.5% and filtered with Whatman No. 1 filter paper by the treatment filtrates with few drops of iodine in 2 to 3 drops of potassium iodide. The presence of brown reddish precipitates that pointed out the presence of alkaloids in the samples [17].

2.3.3. Tests for Phenols. In ferric chloride test, for the screening of phenol plant aqueous extracts, the phenol plant aqueous extracts were treated with 3-4 drops of ferric chloride solution. The appearance of bluish black color indicated the presence of phenols [18].

2.3.4. Tests for Phytosterols: Salkowski's Test. The test plant aqueous extracts were treated with chloroform and filtered with Whatman no. 1 filter paper. Few drops of concentrated sulphuric acid were added and then vertexed it and allowed to stand for some time. The golden yellow color indicated the presence of phytosterol [18].

2.3.5. Tests for Diterpenes. To observe the presence of diterpenes, the plant aqueous extracts were treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicated the presence of diterpenes [18].

2.3.6. Tests for Saponins. For dilution about 2 ml of plant aqueous extracts were taken in test tube, in distilled water and vortexed it for 5 minutes. Foam produced and persisted for ten minutes indicated the presence of saponins [17].

2.3.7. Tests for Flavonoids. In the alkaline reagent test, for the presence of flavonoids, the plant aqueous extracts were treated with 2-3 drops of lead acetate solutions. The formation of intense yellow color, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids [19].

2.4. Bioassay of C. maculatus Adults. The repellency effect of tested botanicals used against the beetles was assessed by using the area preference method [9]. In bioassays, 6 concentrations viz. 0.5, 1, 1.5, 2, 2.5, and 3% of aqueous extracts were used. Whatman No.1 filter paper was equally divided into 2 halves (about 7.2 cm diameter). First half portion of each filter paper was treated with the extract by using

Table 1: List of plant species and plant parts used in the experiment with *C. maculatus* during 2021.

Sr. no.	Common name	Botanical name	Family	Part used
1	Neem	*Azadirachta indica*	Meliaceae	Seed
2	Bakion	*Melia azedarach*	Meliaceae	Fruit
3	White Patta	*Nicotiana rustica*	Solanaceae	Leave
4	Virginia tobacco	*Nicotiana tabacum*	Solanaceae	Leave
5	Chinese arborvitae	*Thuja orientalis*	Cupressaceae	Fruit

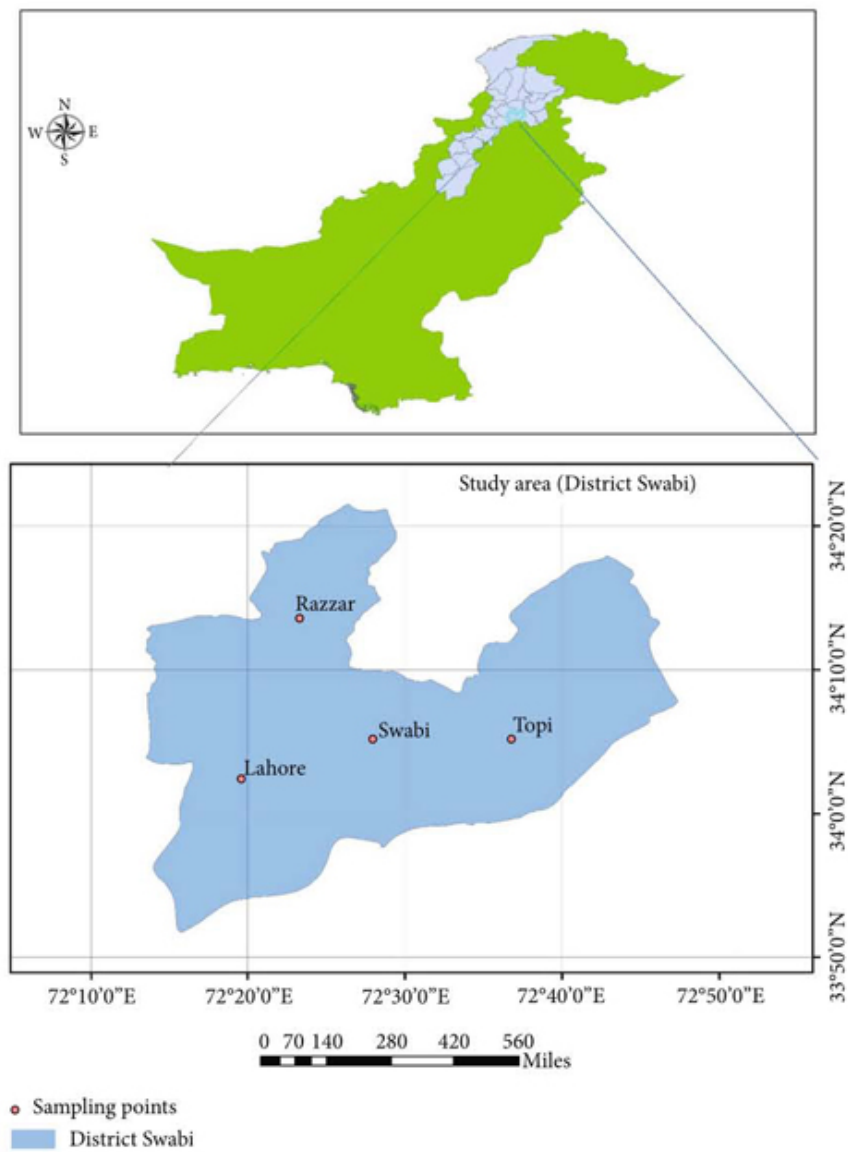


Figure 1: Location from which plant species collected during 2021.

micropipette, and the 2nd half portion of filter paper was treated with distilled water as a control. Each filter paper was air dried for about 30 minutes, till complete evaporation of solvent. The filter paper was then pasted length wise, edge wise with the help of masking tape and kept at the bottom of 16 cm diameter Petri dishes. Ten pairs of freshly emerged adult beetles (total of 20 per dish) were released at the center of the test arena in the Petri dishes and covered with muslin cloth and kept in an incubator at 27 ± 2°C and 65 ± 5% relative humidity. Total numbers of insects residing on treated and untreated portions of filter paper were counted after 1, 2, 3, 6, 24, 48, 72, and 96 hours, and percent repellency (PR) was calculated by using the formula adopted by [20]

$$\mathrm{PR} = \left[\frac{Nc - Nt}{Nc}\right] \times 100, \quad (1)$$

where Nc is the no. of insects counted in control and Nt is the no. of insects counted in treated.

Table 2: Repellency classes according to McDonald et al. [21].

Sr. no.	Class	&R1
1	0	>0.01-0.1
2	I	0.1-20
3	II	20.1-40
3	III	40.1-60
4	IV	60.1-80
5	V	80.1-100

1%R: percentage of repellency rate.

The botanicals were then categorized into different classes (as shown in Table 2), [21].

2.5. Statistical Analysis. The recorded data were subjected to analysis of variance (ANOVA) with two factors CRD (complete randomized design), and means were separated by using the least significant difference (LSD) test at 5% level of probability. Statistical analyses were carried out using STATISTIX 8.1 [22].

3. Results

3.1. Screening of Aqueous Extracts of Plants for Phytochemical Constituents. In this experiment, phytochemical constituents of five plant species were determined from their crude extracts (as shown in Table 3). It was clear from the results that all the phytochemical constituents were present in *M. azedarach*, with both phytosterol and phenol in moderate amount while the rest of phytochemicals were present in lower quantities. *A. indica* also exhibited all the phytochemicals in high quantities. Moreover, in *N. tabacum* all the phytochemicals were present, whereas diterpenes and phenols were present in high amount and the others in moderate quantities. In *N. rustica*, saponins were not present while, rest in low quantities. In *T. orientalis*, all the phytochemicals were present in low quantities.

3.2. Repellency. The settling response of *C. maculatus* was significantly ($P < 0.05$) affected by concentration. The adults of *C. maculatus* preferred the untreated arena (control) as compared with treated arena. The preference response of tested insects significantly declined with the increases in concentrations of extracts. The repellency of five different botanicals against *C. maculatus* were studied under controlled laboratory conditions, and result revealed different trends in different parameters which are explained as follows.

3.2.1. Mean Percent Repellency of C. maculatus after 1 h Exposure Period. After one hour of exposure, highest repellency of *C. maculatus* was observed with *A. indica* (53.75 ± 4.26) ($df = 5$, $P < 0.05$, $F = 34.38$) which show class III repellency, while the lowest was recorded with *T. orientalis* (32.25 ± 0.75) which show class II repellency. An increasing trend in repellency was observed with the increase in concentration of botanicals (Figure 2).

3.2.2. Mean Percent Repellency of C. maculatus after 2 h Exposure Period. The repellency of tested botanical against *C. maculatus* after two hours of exposure. *A. indica* showed the highest repellency (57.5 ± 3.22) against *C. maculatus*, and the lowest repellency was observed with *T. orientalis* (41.25 ± 3.75) ($df = 5$; $P < 0.05$; $F = 34.38$) (Figure 3).

3.2.3. Mean Percent Repellency of C. maculatus after 3 h Exposure Period. Result showed the highest repellency of *C. maculatus* against *A. indica* (60 ± 2.04) at 3% concentration, while the lowest repellency was observed against *T. orientalis* (51.5 ± 3.09) ($df = 5$; $P < 0.05$; $F = 49.98$) after the exposure period of 3 hours (Figure 4).

3.2.4. Mean Percent Repellency of C. maculatus after 6 h Exposure Period. Results described the repellency of tested botanical insecticides against *C. maculatus* ($df = 5$; $P < 0.05$; $F = 49.33$). At 3% concentration after 6 hours of exposure, highest repellency (class IV repellency) was recorded in *A. indica* (66 ± 3.7). In comparison, the lowest repellency (class III repellency) was observed in *T. orientalis* (51.25 ± 2.39) (Figure 5).

3.2.5. Mean Percent Repellency of C. maculatus after 24 h Exposure Period. After 24 hours of exposure at 3% concentration ($df = 5$; $P < 0.05$; $F = 54.07$), highest repellency was observed in *A. indica* (72.5 ± 2.5) and the lowest repellency was observed in *T. orientalis* (60.25 ± 2.25) (Figure 6).

3.2.6. Mean Percent Repellency of C. maculatus after 48 h Exposure Period. After 48 hours of exposure, the repellency effect of different concentration of selected plant extract presented (Figure 7) ($df = 5$; $P < 0.05$; $F = 109.38$). Results revealed the highest repellency (class V repellency) at 3% concentration in *A. indica* (85 ± 2.04) while the lowest repellency was observed in *T. orientalis* (70 ± 2.04) (class IV repellency).

3.2.7. Mean Percent Repellency of C. maculatus after 72 h Exposure Period. After 72 hours of exposure ($df = 5$; $P < 0.05$; $F = 104.80$), *A. indica* (91.25 ± 1.49) showed the highest repellency (class V repellency), while the lowest repellency (class III repellency) was recorded in *T. orientalis* (75 ± 3.53) at 3% concentration (Figure 8).

3.2.8. Mean Percent Repellency of C. maculatus after 96 h Exposure Period. After 96 hours ($df = 5$; $P < 0.05$; $F = 123.10$), the repellency effect of tested plant extract against *C. maculatus* was significantly presented. Decreasing trend in repellency was observed after 72 hours of exposures; however, the highest repellency (class V repellency) was observed in *A. indica* (86.25 ± 1.75) and lowest repellency (class III repellency) was observed in *T. orientalis* (57.5 ± 1.44) at 3% concentration (Figure 9).

4. Discussion

Entomologists and pest controllers around the world are using plant-based insecticides increasingly frequently, most likely as a result of public awareness of the risks connected with many chemical pesticides. However, the method of extraction, the section of the plant used, and the type of solvent employed for their extraction all directly or indirectly affect the efficiency of many botanical insecticides [23].

Table 3: Phytochemical composition of crude extracts of five plant species during 2021.

Plant species	Phytochemical constituents of five plant species					
	Alkaloids	Flavonoids	Saponins	Diterpenes	Phytosterol	Phenols
A. indica	+++	+++	++	++	+++	+++
N. tabacum	++	++	++	+++	++	+++
M. azedarach	+	+	+	+	++	++
N. rustica	+	+	—	+	+	+
T. orientalis	+	+	+	+	+	+

+++: highly present; ++: moderately present; +: low present; –: not present.

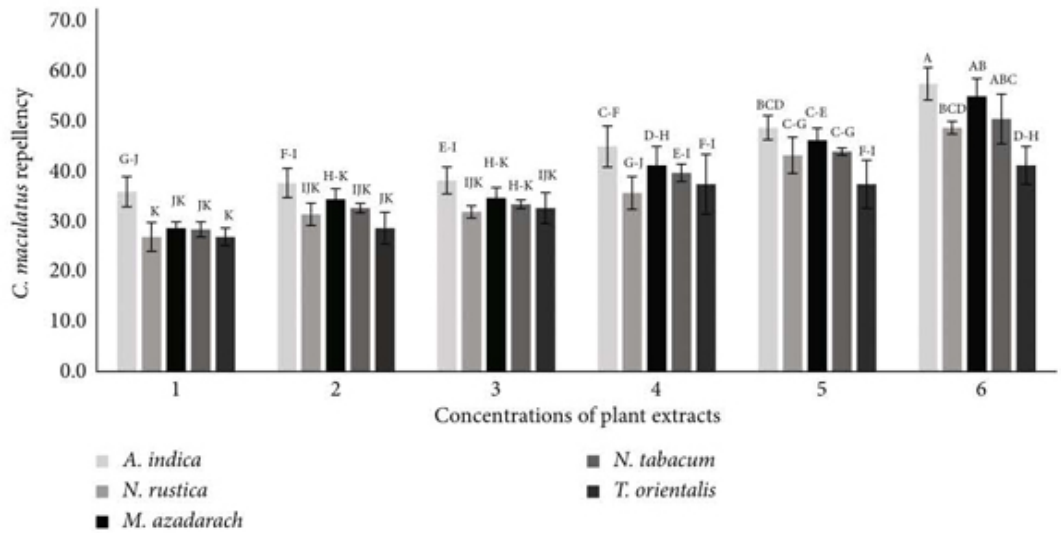


Figure 2: Mean percent repellency of *C. maculatus* after 1-hour exposure period treated with six different concentrations of crude extracts of five plant species.

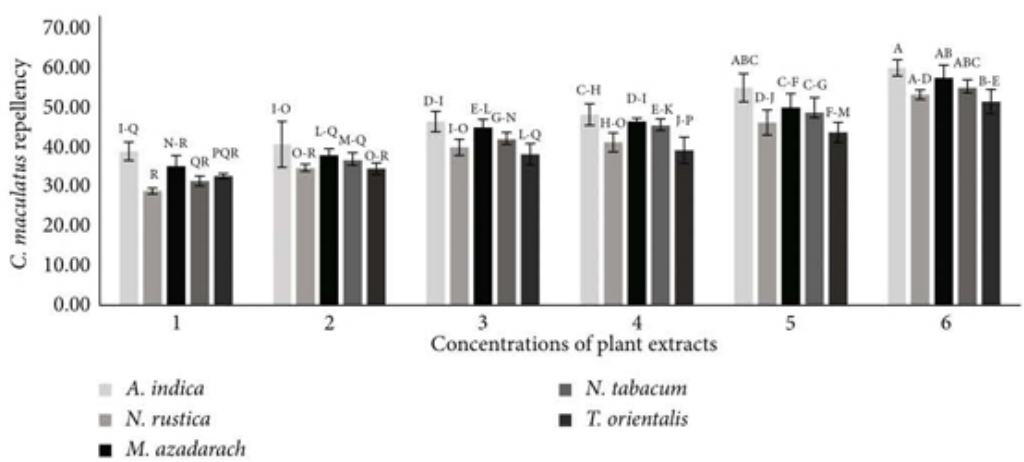


Figure 3: Mean percent repellency of *C. maculatus* after 2-hour exposure period treated with six different concentrations of crude extracts of five plant species.

Different solvents' polarities may result in variations in how well they extract the active ingredient found in botanicals. Present experiments based on phytochemicals in five plants species yielded variable results. All the phytochemicals were found in all the five plant species. Some earlier researchers have reported results similar to the current study, such as [24] reported that the aqueous extract of *N. tabacum* leaves tested positive for alkaloids, tannins, flavonoids, steroids, cardiac glycosides, essential oils, resins, and polypeptides. [25] stated that tobacco leaves contain nicotine, as we know that nicotine is an alkaloid which is the most biologically active component of tobacco. Alkaloids, being one of the largest group of phytochemicals in plants have pronounced effect on humans which have led to development of pain killer medication [26]. Moreover, these alkaloids have also been act as insect repellents as mentioned by [27]. According to [28], *A.*

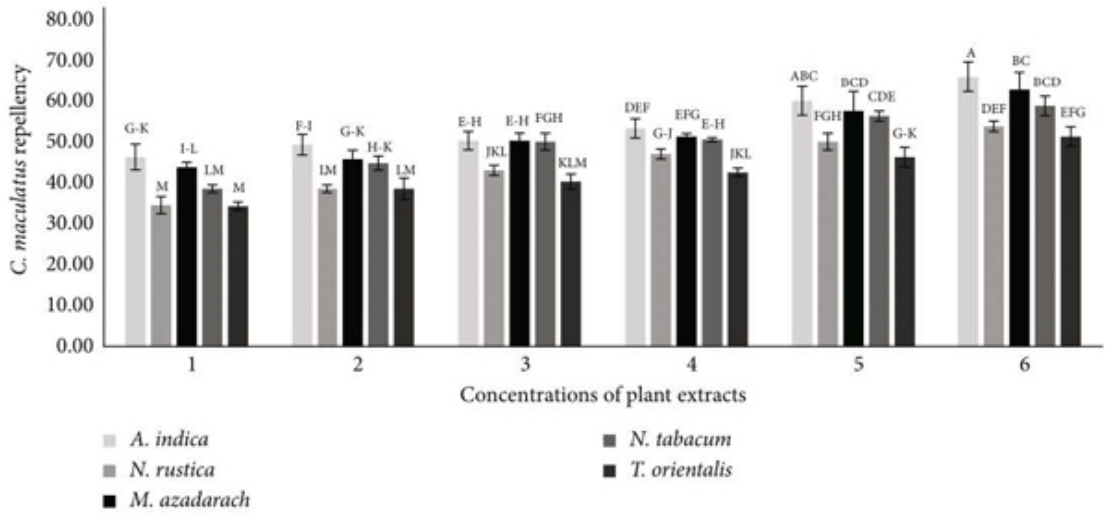


FIGURE 4: Mean percent repellency of *C. maculatus* after 3-hour exposure period treated with six different concentrations of crude extracts of five plant species.

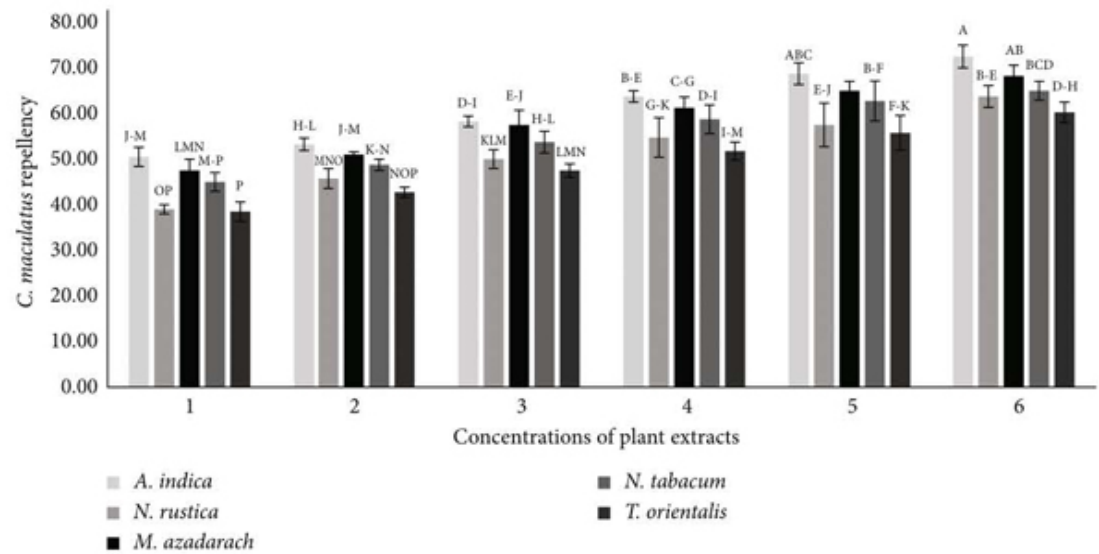


FIGURE 5: Mean percent repellency of *C. maculatus* after 6-hour exposure period treated with six different concentrations of crude extracts of five plant species.

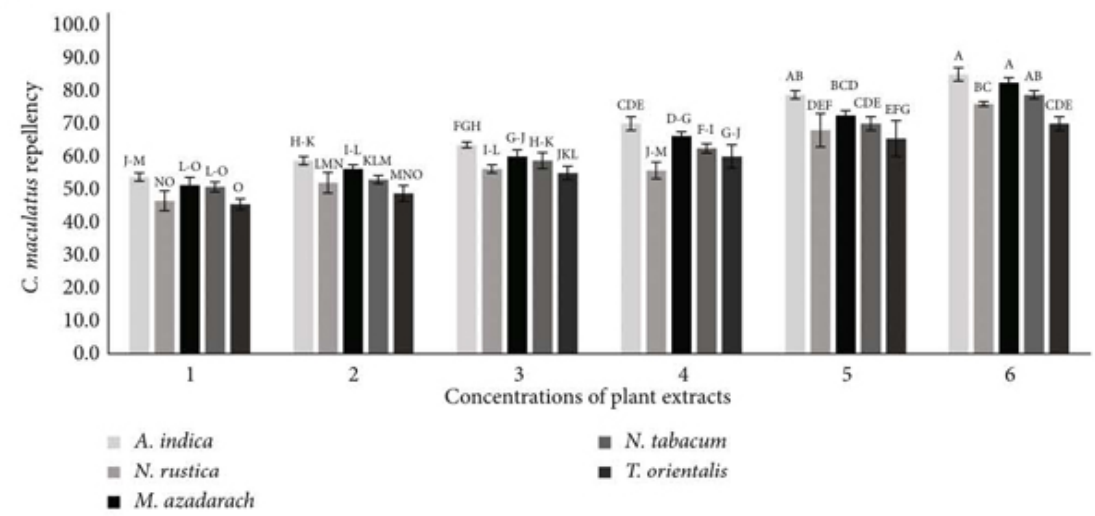


FIGURE 6: Mean percent repellency of *C. maculatus* after 24-hour exposure period treated with six different concentrations of crude extracts of five plant species.

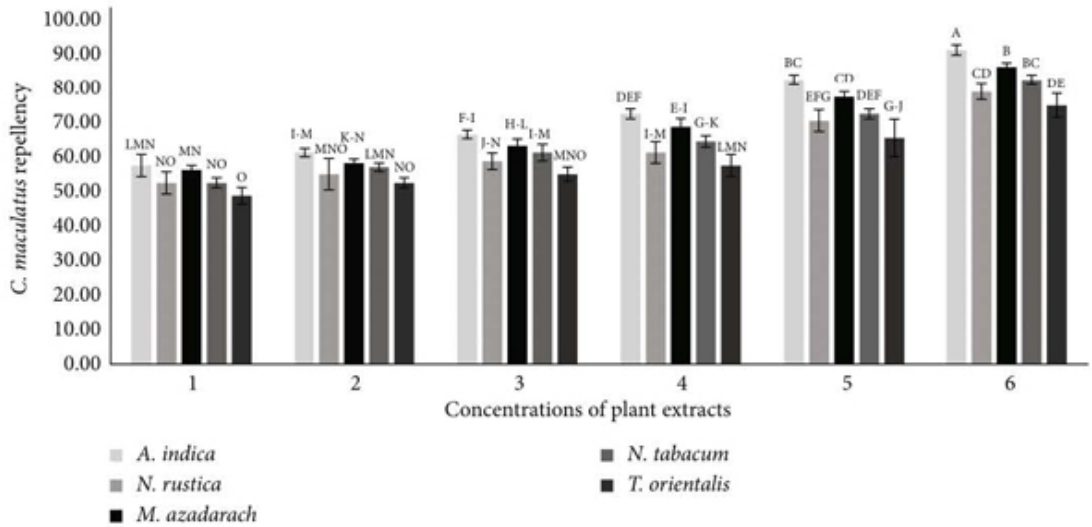


FIGURE 7: Mean percent repellency of *C. maculatus* after 48-hour exposure period treated with six different concentrations of crude extracts of five plant species.

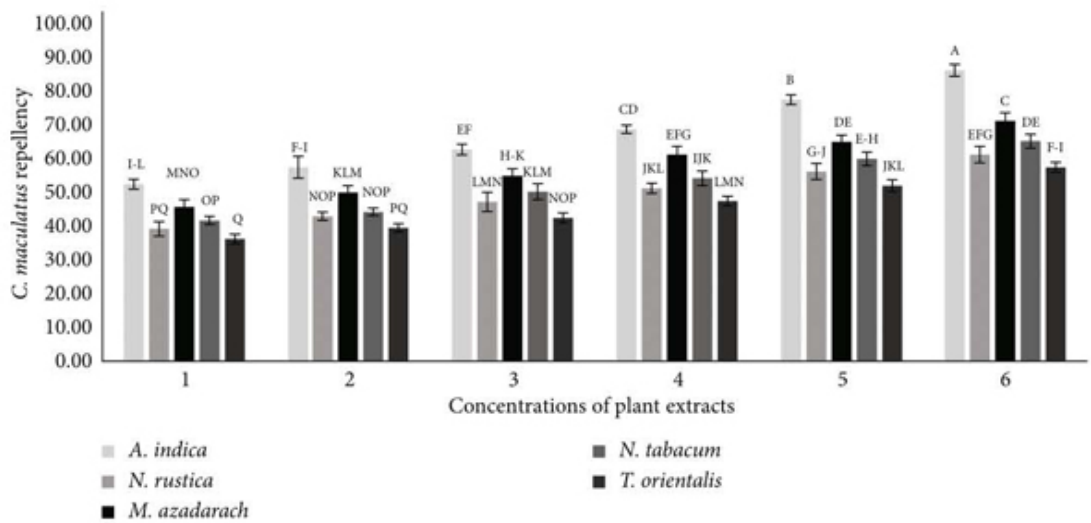


FIGURE 8: Mean percent repellency of *C. maculatus* after 72-hour exposure period treated with six different concentrations of crude extracts of five plant species.

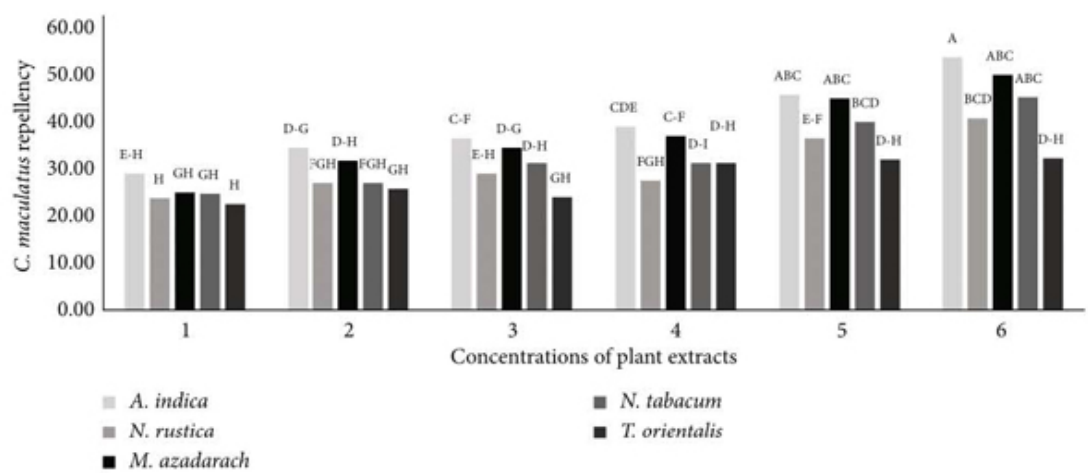


FIGURE 9: Mean percent repellency of *C. maculatus* after 96-hour exposure period treated with six different concentrations of crude extracts of five plant species.

indica crude extracts showed the presence of alkaloids, glycosides, flavonoids, saponins, tanins, and phenolic compounds. In the present research, crude extracts of *M. azedarach* indicated high presence of terpiniods, saponins, flavonoids, and phenols. [29] yielded results similar to our findings. According to [30], crude extracts of *M. azedarach* gave phenols, flavonoids, tannins, alkaloids, terpinoids, and saponins.

Outcomes of the present studies are consistent with [31] who also reported the highest repellency of *A. indica* against *T. castaneum*, with decreasing trend with the passage of time. Our results also agreed with some earlier researcher [32, 33] that *A. indica* repels insect and causes them to stop their feeding. Neem extracts contain azadirichtin and salannin that function as insect feeding deterrent. [34, 35] also reported the use of *A. indica* for the control different foliage pests. In case of *M. azedarach*, our results are in agreement with [36] who also reported that the repellency effect of *M. azedarach* decreases after the 72-hour exposure period. Research carried out worldwide during the last three decades have also shown significant repellency effect of the *M. azedarach* for the management of stored product pests [30]. Tobacco (*N. tabacum* and *N. rustica*) is traditionally known as a natural insecticide [37]. In our studies, we observed 82% repellency in *N. tabacum* and 76% in *N. rustica* against *C. maculatus* at 3% concentration. Our results are agreed with [38] who recorded similar repellency trend as in our study (increases repellency at increased concentration of plant extracts. This result also coincides with the findings of [39] who also reported the maximum repellency in *N. tabacum* at high concentration against *T. castaneum*. Nicotiana species contain nicotine which is an alkaloid act as a potent insecticide that bind the acetylcholine receptor and affect the nerve transmission that act as a feeding deterrent. In our study, among the tested botanicals, the lowest repellency was observed in *T. orientalis* against *C. maculatus*. Our results are in contradiction with the findings of [40] who observed high repellency (92%) in *T. orientalis* against *Tribolium confusum*. Difference in results might be due to the different plant parts used for the extraction that have different percentage compositions of the ingredients [41]. The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids [42]. Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions [43]. Due to their high volatility, they have fumigant activity that might be of importance for controlling stored product insects [44]. Studies carried out worldwide during last the three decades have significantly extended our knowledge on botanical pesticides. Many plant-derived natural products active against insect could be produced from locally available raw materials, perhaps in many cases right at the site of usage, so as to be relatively inexpensive [43]. In this study, pure liquid extract of *A. indica* and *M. azedarach* was effective at managing the population of *C. maculatus*. It may therefore be one of the alternative control options in our immediate environment. The natural phytochemicals from Plants have potential being ecofriendly can replace synthetic pesticides for the insect pests [45]. Nevertheless, despite their efficiency, the extracts have no negative impact on the stored pulses. Consequently, these plant extracts may be utilized to help reduce the number of *C. maculatus*.

5. Conclusion

It is reported that the utilization of phytochemicals is ecofriendly, socially acceptable, and economically feasible approach for the management and biocontrol of *C. maculatus*. Based on our result, it can be concluded that *A. indica* and *M. azedarach* at all concentration might serves as alternative to insecticides in rural areas of tropic and subtropic region. The promising and effective repellency of these botanicals suggesting that these botanicals as a potential candidate agent against the *C. maculatus* and can be recommended for their integration with other control strategies that will reduced environmental pollution and health hazards problems. Moreover, use of these plant extracts can open new avenue for the management of *C. maculatus*.

Data Availability

Data is included in the article.

Additional Points

Novelty of the Study. This laboratory work evaluated the anti-insect potential of local plant species from District Swabi and Haripur, Khyber Pakhtunkhwa of Pakistan, against destructive insect pest of stored grain, i.e., *Callosobruchus maculatus*. Bioassays revealed that water extracts of *A. indica*, *M. azedarach*, *N. rustica*, *N. tabacum*, and *T. orientalis* at various concentrations particularly at 3% and exhibited considerable repellency of insect pest individuals suggesting their biocidal potential against this insect pest.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors extend their appreciation to the Researchers supporting project number (RSP-2021/367), King Saud University, Riyadh, Saudi Arabia.

References

[1] W. R. Tavares, M. D. C. Barreto, and A. M. L. Seca, "Aqueous and ethanolic plant extracts as bio-insecticides— establishing a bridge between raw scientific data and practical reality," *Plants*, vol. 10, no. 5, pp. 920–929, 2021.

[2] K. D. Singh, A. J. Mobolade, R. Bharali, D. Sahoo, and Y. Rajashekar, "Main plant volatiles as stored grain pest management approach: a review," *Journal of Agriculture and Food Research*, vol. 4, article 100127, 2021.

[3] U. C. Arakere, S. Jagannath, S. Krishnamurthy, S. Chowdappa, and N. Konappa, "Microbial bio-pesticide as sustainable solution for management of pests: achievements and prospects," *Biopesticides*, vol. 2, pp. 183–200, 2022.

[4] S. Sekender, S. Sultana, T. Akter, and S. Begum, "Susceptibility of different stored pulses infested by pulse beetle,

Callosobruchus chinensis (Lin.)," *Dhaka University Journal of Biological Sciences*, vol. 29, no. 1, pp. 19–25, 2020.

[5] S. Gupta, S. D. Apte, G. Post, G. College, and M. P. Khargone, "Influence of storage containers on Callosobruchus maculatus (Fab.) infesting green gram," *Archives of Applied Science Research*, vol. 8, no. 8, pp. 21–23, 2016.

[6] W. Demis and E. Yenewa, "Review on major storage insect pests of cereals and pulses," *Asian Journal of Advances in Research*, vol. 1, no. 1, pp. 41–56, 2022.

[7] U. Zafar, D. I. Khan, M. Mamoon-Ur-Rashid, and M. Shah, "Entomotoxicity of plant powders against pulse beetle (Callosobruchus chinensis) on stored mung bean (Vigna radiata)," *Journal of Entomology and Zoology Studies*, vol. 6, no. 1, pp. 1637–1641, 2018.

[8] L. A. Tapondjou, C. Adler, H. Bouda, and D. A. Fontem, "Efficacy of powder and essential oil from Chenopodium ambrosioides leaves as post-harvest grain protectants against six-stored product beetles," *Journal of Stored Products Research*, vol. 38, no. 4, pp. 395–402, 2002.

[9] R. Akbar and I. A. Khan, "Toxicity of five plant extracts against Fallosobruchus maculatus Fab. (Coleoptera Bruchidae) a major insect pest of stored pulses," *Fresenius Environmental Bulletin*, vol. 30, no. 5, pp. 5098–5107, 2021.

[10] G. M. W. Lengai, J. W. Muthomi, and E. R. Mbega, "Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production," *Scientific African*, vol. 7, article e00239, 2020.

[11] M. Özkara, D. Akyil, and M. Konuk, "Pesticides, environmental pollution, and health," in *Environmental health risk-hazardous factors to living species*, p. 27, IntechOpen, 2016.

[12] F. Jian, "Influences of stored product insect movements on integrated pest management decisions," *Insects*, vol. 10, no. 4, p. 100, 2019.

[13] H. A. Raza, R. M. Amir, M. A. Idrees et al., "Residual impact of pesticides on environment and health of sugarcane farmers in Punjab with special reference to integrated pest management," *Journal Global Innovation Agriculture Social Science*, vol. 7, no. 2, pp. 79–84, 2019.

[14] G. Vijayalakshmi, K. Elango, E. A. P. Vasanthi et al., "Insecticidal, oviposition deterrent and antifeedant property of certain plant extracts against pulse beetle, Callosobruchus chinensis Linn. (Coleoptera: Bruchidae)," *Legume Research*, vol. 44, no. 11, pp. 1386–1391, 2021.

[15] M. Suleman, S. Nouren, S. M. Hassan et al., "Vitality and implication of natural products from viburnum grandiflorum: an eco-friendly approach," *Polish Journal of Environmental Studies*, vol. 27, no. 3, pp. 1407–1411, 2018.

[16] C. Y. Hamany Djande, L. A. Piater, P. A. Steenkamp, N. E. Madala, and I. A. Dubery, "Differential extraction of phytochemicals from the multipurpose tree, Moringa oleifera, using green extraction solvents," *South African Journal of Botany*, vol. 115, pp. 81–89, 2018.

[17] S. Snigdha, M. Ravish, G. Narayan, N. Manisha, K. Nilotpol, and D. Kishore, "Phytochemical analysis of papaya leaf extract: screening test," *EC Dental Science*, vol. 18, no. 3, pp. 485–490, 2019.

[18] R. P. Senthilkumar, V. Bhuvaneshwari, V. Malayaman, R. Ranjithkumar, and S. Sathiyavimal, "Phytochemical screening of aqueous leaf extract of Sida acuta Burm. F. and its antibacterial activity," *Journal of Emerging Technologies and Innovative Research*, vol. 5, no. 8, pp. 474–478, 2018.

[19] S. Nathenial, A. Fatima, R. Fatima et al., "Phytochemical study of acetone solvent extract of Coriander sativum," *Journal of Pharmacognosy and Phytochemistry*, vol. 8, no. 6, pp. 136–140, 2019.

[20] D. Obeng-Ofori, "Plant oils as grain protectants against infestations of Cryptolestes pusillus and Rhyzopertha dominica in stored grain," *Entomologia Experimentalis et Applicata*, vol. 77, no. 2, pp. 133–139, 1995.

[21] L. McDonald, R. Guy, and R. Speirs, "Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects," in *Marketing Research Repertory*, vol. 882, p. 183, Agriculture Reserve Service, US. Department of Agriculture, Washington, 1970.

[22] R. G. D. Steel, J. H. Torrie, and D. A. Dickey, "Principles and Procedures of Statistics," in *Principles and Procedures of Statistics*, McGraw-Hill, New York, 2nd edition, 1980.

[23] O. M. Obembe and O. C. Ogungbite, "Entomotoxic effect of tobacco seed extracted with different solvents against Callosobruchus maculatus infesting stored cowpea," *International Journal of Entomology Research*, vol. 1, no. 1, pp. 22–26, 2016.

[24] Y. Sharma, A. Kaur, R. Bhardwaj et al., "Preclinical assessment of stem of Nicotiana tabacum on excision wound model," *Bioorganic Chemistry*, vol. 109, article 104731, 2021.

[25] S. P. Kernodle, S. Webb, T. M. Steede, and R. S. Lewis, "Combined reduced expression of two gene families lowers nicotine content to ultra-low levels in cultivated tobacco," in *Plant Cell Reports*, Springer, 2022.

[26] T. Behl, G. Rocchetti, S. Chadha et al., "Phytochemicals from plant foods as potential source of antiviral agents: an overview," *Pharmaceuticals*, vol. 14, no. 4, 2021.

[27] R. Nirola, M. Megharaj, A. Subramanian et al., "Analysis of chromium status in the revegetated flora of a tannery waste site and microcosm studies using earthworm E. fetida," *Environmental Science and Pollution Research*, vol. 25, no. 6, pp. 5063–5070, 2018.

[28] R. Kumar, S. Sharma, and L. Devi, "Investigation of total phenolic, flavonoid contents and antioxidant activity from extracts of Azadirachta indica of Bundelkhand region," *International Journal of Life-Sciences Scientific Research*, vol. 4, no. 4, pp. 1925–1933, 2018.

[29] J. Iqbal, A. Qayyum, and S. Z. Mustafa, "Repellent effect of ethanol extracts of plant materials on Tribolium castaneum (Herbst) (Tenebrionidae: Coleoptera)," *Pakistan Journal of Zoology*, vol. 42, no. 1, pp. 81–86, 2010.

[30] I. Naimi, M. Zefzoufi, H. Bouamama, and T. B. M'hamed, "Chemical composition and repellent effects of powders and essential oils of Artemisia absinthium, Melia azedarach, Trigonella foenum-graecum, and Peganum harmala on Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae)," *Industrial Crops and Products*, vol. 182, article 114817, 2022.

[31] M. Berhe, S. Dugassa, S. Shimelis, and H. Tekie, "Repellence and larvicidal effects of some selected plant extracts against adult Anopheles arabiensis and Aedes aegypti larvae under laboratory conditions," *International Journal of Tropical Insect Science*, vol. 41, no. 4, pp. 2649–2656, 2021.

[32] R. C. Lima, M. F. Almeida, A. de Sousa Freitas et al., "Effect of the aqueous extract of neem leaves (Azadirachta indica A. Juss.) on the control of Costalimaita ferruginea (Fabricius, 1801)," *Annual Research & Review in Biology*, vol. 36, no. 9, pp. 31–37, 2021.

[33] S. E. Atawodi and J. C. Atawodi, "Azadirachta indica (neem): a plant of multiple biological and pharmacological activities," *Phytochemistry Reviews*, vol. 8, no. 3, pp. 601–620, 2009.

[34] P. M. Ngegba, G. Cui, M. Z. Khalid, and G. Zhong, "Use of botanical pesticides in agriculture as an alternative to synthetic pesticides," *Agriculture*, vol. 12, no. 5, 2022.

[35] F. Manzoor, G. Nasim, S. Saif, and S. Asma Malik, "Effect of ethanolic plant extracts on three storage grain pests of economic importance," *Pakistan Journal of Botany*, vol. 43, no. 6, pp. 2941–2946, 2011.

[36] N. A. Turi, S. Raziuddin, S. Shah, and S. Ali, "Estimation of heterosis for some important traits in mustard (Brassica juncea L.)," *Research Journal of Agriculture and Biological Sciences*, vol. 4, no. 1, pp. 6–10, 2006.

[37] S. M. Habib-ur-Rehman, Q. Mansoor-ul-Hasan, H. A. S. Ali, and M. Yasir, "Repellent potential of three medicinal plant extracts against Tribolium castaneum (Coleoptera: Tenebrionidae)," *Punjab University Journal of Zoology*, vol. 33, no. 2, pp. 121–126, 2018.

[38] M. Sagheer, K. Ali, M. U. Hasan, A. Rashid, U. Sagheer, and A. Alvi, "Repellent and toxicological impact of acetone extracts of Nicotiana tabacum, Pegnum hermala, Saussurea costus and Salsola baryosma against red flour beetle, Tribolium castaneum (Herbst)," *Pakistan Journal of Zoology*, vol. 45, no. 6, pp. 1735–1739, 2013.

[39] A. L. Souto, M. Sylvestre, E. D. Tölke, J. F. Tavares, J. M. Barbosa-Filho, and G. Cebrián-Torrejón, "Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: prospects, applications and challenges," *Molecules*, vol. 26, no. 16, p. 4835, 2021.

[40] M. Amoura, A. Benabdallah, S. Kilani-Morakchi, and C. Messaoud, "Fumigant and repellent potentials of Mentha pulegium L. and Citrus limon L. (Burm) essential oils against Tribolium confusum Duval. (Coleoptera: Tenebrionidae)," *Journal of the Entomological Research Society*, vol. 45, no. 1, pp. 73–80, 2021.

[41] S. Molapour, R. Shabkhiz, O. Askari, H. Shiri, A. Keramati, and V. Mahdavi, "Chemical components and insecticidal effects of Lavandula angustifolia and Origanum vulgare essential oils on the growth different stages of Habrobracon hebetor Say (Hymenoptera: Braconidae)," *Jordan Journal of Biological Sciences Short Communication*, vol. 13, no. 2, 2020.

[42] Y. Wang, L. T. Zhang, Y. X. Feng et al., "Comparative evaluation of the chemical composition and bioactivities of essential oils from four spice plants (Lauraceae) against stored-product insects," *Industrial Crops and Products*, vol. 140, article 111640, 2019.

[43] A. K. Chaudhari, V. K. Singh, A. Kedia, S. Das, and N. K. Dubey, "Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects," *Environmental Science and Pollution Research*, vol. 28, no. 15, pp. 18918–18940, 2021.

[44] O. Campolo, G. Giunti, A. Russo, V. Palmeri, and L. Zappalà, "Essential oils in stored product insect pest control," *Journal of Food Quality*, vol. 2018, Article ID 6906105, 18 pages, 2018.

[45] A. Ashraf, N. A. Qureshi, N. Shaheen et al., "Termiticidal and protozocidal potentials of eight tropical plant extracts evaluated against Odontotermes obesus rambur (Blattodea; termitidae) and Heterotermes indicola wasmann (blattodea; rhinotermitidae)," *Polish Journal of Environmental Studies*, vol. 29, no. 5, pp. 3493–3507, 2020.

5. Summary and discussion

An increase in the world population, climate change, and loss caused by insect pests to the grain during storage is a significant challenge for food security (Skendžić *et al.*, 2021). It is reported that insect pests in storage and field cause damages worth approximately $100 billion annually (Yallapa *et al.*, 2012).

In the past few decades, stored product pests have become more significant in Europe (Stejskal *et al.*, 2014). Chemical treatment and temperature modification have traditionally been the primary methods to manage storage pests (Toews *et al.*, 2006). Even if pesticides typically do not surpass the legally permitted Maximum contaminant levels, harmful pesticides are restricted because of today's society's sensitivity to pesticide traces in food. The only solution is discovering non-chemical alternates and combining those with an Integrated Pest Management strategy.

Based on the above issues, current pest management strategies try to integrate various techniques as an alternative to the synthetic pesticides to manage stored product pests. One of the main current challenges is to cope with resistance developed by stored product pests to existing pesticides. It is the need of the time to work on innovative ways and raise the understanding of integrated stored pest management techniques which are sustainable, effective, and environmentally friendly.

This doctoral dissertation is divided into three parts related to non-chemical pest control: a) using nitrogen hypoxic atmospheres/controlled atmospheres for the protection of durable commodities; b) impact of physically disturbed/destructed kernels on storage pests, biology, and control. c) utilizing botanical extracts/ essential oils for stored product pests. Basis for the work is five separate scientific articles.

5.1 Nitrogen hypoxic atmospheres

Many scholars have stated that there isn't sufficient scientific evidence on using nitrogen for their numerous real-world applications because of the difficulty of using controlled atmospheres to preserve agricultural products. This study assessed different hypoxic and anoxic nitrogen atmospheric settings for treating two important stored-product pests in a lab setting and outdoors in silos.

The goal of an N2 hypoxic atmosphere is to provide environments with high nitrogen concentrations and low or no oxygen levels that are fatal to the storage pests. Anoxic environments can be used for stored food pests employing various techniques or methods as an efficient and environmentally friendly pest management approach (Stejskal *et al.*, 2021; Navarro and Navarro, 2020; Carvalho *et al.*, 2012).

A controlled atmosphere not only prevents insect pests but also helps to preserve the quality of stored food (Ren *et al.*, 2012; Carvalho *et al.*, 2012). In several ways, inert gases' physical benefits vary from those of most other gaseous pesticides (Mitcham *et al.*, 2009; Zhou *et al.*, 2022; Liu, 2022). Inert gases, therefore, have an advantage over traditional insecticide-resistant pest populations and their ecological benefits. Examples include the latest demonstrations by Sakka *et al.*, (2020) and Agrafioti *et al.*, (2022) of the strong prospect of hypoxic environments to control populations of most phosphine-resistant insect species. Controlled atmospheres are also emerging in biosecurity and Phyto-quarantine management plans (Zhao *et al.*, 2021). To be effective, a stable supply of high-dose nitrogen must be maintained to control stored product pests completely (Navarro and Navarro, 2020).

The first article aims to find a better technique for controlling internally stored grain insects such as *S. granarius* and *C. chinensis*. Both species develop inside the grain and are significant pests of grains and legumes (Park *et al.*, 2003; Kalpna *et al.*, 2022) that are difficult to control with traditional fumigants and chemicals. Therefore, this study focused on the issue of maximizing the period of administration to hypoxia nitrogen settings for managing two significant storage pests in both lab and small-silos environments. A hypoxic nitrogen atmosphere with different concentrations of N2 gas from 95-100% was tested against all developmental phases of both species in laboratory and field conditions from 1-20 days of exposure. Because there are indices that CA work better in a laboratory but may not show the same results in the field (Jay, 1984), we tested whether the controlled atmosphere treatment is effective in both laboratory and field conditions. According to our lab results, *C. chinensis* and *S. granarius* adults represented the most sensitive developmental phases, similar to other results produced by different investigators for various stored grain insect pest species. For instance, Hashem *et al.*, (2022) discovered that mature bruchids *A. obtectus* were much more susceptible to the exposure of hypoxia/hypercapnia, which resulted in 100% fatality within three days of exposure. The results from our lab and field

experiments show that 99% nitrogen concentration is effective for all the developmental stages of both pests. Thus, we proved that nitrogen hypoxic environment is suitable for control of *S. granarius* and *C. chinensis*

5.2 Physically disturbed/destructed kernels impact storage pests, biology, and control.

In the second part of thesis, we for the first time, have experimented with how physically damaged grains by the primary pests, *Rhyzopertha dominica* and *Sitophilus granarius,* affect the growth and effectiveness of grain protectants against the secondary pests. Both the pests (*R. dominica* and *S. granarius*) are internal feeders and are very difficult to control through traditional pest management practices. A critical issue in the food industry is food protection during storage. It mostly depends on a comprehensive knowledge of the biology, habitat, and activity of various insect pests that attacked the stored grains. The results and outcome are further described in the below section.

The second article aims to identify the relationship between the frass produced by the primary pest *R. dominica* and how it affects the secondary pest's population growth of *Tribolium castaneum*, *T. confusum,* and *Oryzaephilus surinamensis*. All tested species are significant pests of stored grain in Czechia (Stejskal *et al.,* 2014). Such study was performed for the first time because there was no data available on the specific effect of the frass produced by primary pests on developing other secondary pests. The experiment was based on the hypothesis that basic cereal materials, regardless of their actual shapes, i.e., intact seed, cracked seeds, or powder, are less supporting foods than frass for all the tested species. Our findings support those of further writers, which indicated that the physical form of wheat substrates significantly influences the life cycle of secondary storage pests. According to Kavallieratos *et al.,* (2017), cracked grains and frass impact secondary pests' behavior, interactions, and birth rates.

Our results show that *O. suinamensis* and *T. castaneum* grow better on frass than on broken kernels or flour, while *T. confusm* grows equally on frass and flour. Our findings demonstrated that wheat frass, regarding the quality of food and compatibility for the studied organisms, fell in between the ideal diet combination and conventional regular grain diets, like broken cereal kernel or flour. Thus, our experiments also suggested that *R. dominica* frass provides a rich feed for secondary pests rather than just grain powder comprising feces and edible waste particles. Based on our findings, the following conclusions are drawn. 1) The presence of frass produced by the primary

insect positively helps the development of examined secondary insects than whole grain, broken kernel, or powder. 2) The stored grain must be kept clean from frass and *R. dominica* to discourage the growth and population of the secondary pests.

Article third is linked to the second experiment. It is clear from our experiment and Lampiri et al., (2022) that the frass and damaged grains produced by primary pests support the population growth of secondary pests. This study was performed for the first time to know how the primary insect damages caused to grains affect the growth of the secondary insects and the ability of the chosen kernel protectants. Apart from the frass and dust produced by internally feeding insects, it also makes holes and cavities in the grains, which provide shelter and food accession for secondary pests such as *Cryptolestes ferrugineus* and *Tribolium castaneum*. Moreover, the cavities may also influence the performance of the grain protectants (pirimiphos-methyl & deltamethrin) due to pits, holes, and various kinds of grains (Athanassiou et al., 2009).

Our findings revealed that a high ratio of damaged grains supports the growth of both secondary insects even without the frass's presence and will require a high dose of grain protectant to control the pests. The primary pests cause severe damage to the stored grains, especially in developing countries (Egwatu, 1987). The influence of cracked kernels or contaminants on the grain protectant's efficacy is not discussed in any published research. Our research thus provides the very foremost evidence that the existence of hollowed grains decreases the effectiveness of grain protecting insecticides. For susceptible lab cultures of both secondary pests, we discovered that the treatment of grain protective agents at the recommended level could ultimately control these insects. Furthermore, when we applied half of the recommended dose in our experiment, endurance and offspring exhibition took place on the sprayed seed mixture with a higher percentage of damaged grains. This might influence how effectively the products work in real-world situations.

Our research thus suggests that several secondary insect species might flourish or increase if pest infestation on damaged grain portions happens a few months after spraying since chemical breakdown has already reached 50%. On the other hand, the storage of completely clean and undamaged grains may prevent the infestation of the tested insects. The effect of grain protectants on the damaged grains in the presence of frass needs to be explored.

5.3 Botanical extracts as repellent.

The third part deals with of utilizing botanical extracts, gels, and essential oils for managing agricultural and stored grain pests because the pests have developed resistance to most synthetic pesticides. The plant's secondary metabolites, which comprise terpenoids and monoterpenoids, can be utilized as a substitute for synthetic pesticides to manage stored product pests (Wang *et al.,* 2019; Chaudhari *et al.,* 2021).

Article fourth (Review) aims to discover new ways of using chemicals and botanical extracts for the management of agriculture, stored food, medical, and public health-relevant pests in the form of gels that are environmentally friendly and safe to the non-target organism. Because other traditional types of pesticides such as synthetic liquid, dust, fumigants, and powder are dangerous for the pest and non-target organisms (humans, pets, livestock), the long residual effect remains active in the environment, causing a negative impact on health. The primary concern is using innovative formulation methods such as encapsulation, nanotechnology, and gels because pests are developing resistance to the traditional formulation types, due to which the whole group of active ingredients is banned. Suppose the usage of gels formulation is adequately utilized. In that case, it will be safe for the environment and non-target organisms and increase the efficacy and residual effect of the chemical against the selected pests. Gels have the unique properties of holding and releasing active ingredients for a long time (Mishra *et al.*, 2018). Gels can also monitor other stored product pests such as bait and antifeedant. Because phytochemicals are biodegradable and do not accommodate the environment, nowadays, more focus is on using botanical extracts to manage stored product pests in the limelight. But the main drawback of phytochemicals is their short residual effect after application (Turek and Stintzing, 2013). Applying botanical insecticide in gel form may increase stability and control the release of the active ingredient.

The chemical business is presently facing many significant obstacles. The biggest challenges are the lack of active compounds due to public opinion, shifting social needs, and growing insect resistance to various practical pesticide components. Creating novel pesticide formulations with higher biocompatibility or more effective delivery of the active chemical to the areas of action may offset the declining number of active compounds. Along with conventional chemical applications, novel gel compositions could be a solution to tackle the abovementioned issues and difficulties.

The fifth article is linked to the fourth. In this study, we practically applied five botanical extracts to verify their repellency against *C. maculatus*, one of the severe pests in storage pulses (Kalpna *et al.*, 2022). The spread of such pests and legumes over international borders is a significant cause of food losses in many regions (FAO). The primary source of protein in developing countries is legumes. According to the outcomes of this research, *Azadirachta indica* and *Melia azedarach* have the potential to control *C. maculatus*, so these phytochemicals may also be helpful in the development of innovative biopesticides. Our findings supported some prior researchers (Atawodi and Atawodi, 2009) who found that *A. indica* repels insects and stops them from feeding. International research has also demonstrated a considerable repellency efficacy of *M. azedarach* for treating stored grain pests (Naimi *et al.*, 2022). These phytochemicals can be combined with other control methods to lower ecological pollution and health risks because of their remarkable and strong repellent properties against *C. maculatus*. Additionally, employing these natural ingredients may provide a fresh approach to bruchids control. According to reports, using botanicals and biocides to control stored product pests is an eco-sustainable, publicly appropriate, and financially viable strategy. Botanical pesticides can be used as a replacement for synthetic pesticides and grain fumigants.

6. Conclusions

This doctoral dissertation consists of three sections.

The first section deals with utilizing a nitrogen-hypoxic environment to manage two important stored grain pests: *Sitophilus granarius* and *Callasobruchus chinensis*, which developed resistance to most pesticides. The results consist of lab and field experiments. The lab results showed that 10-20 days of treatment led to 100% suppression of all the developmental phases of *S. granarius* and C. chinensis with 99% and 100% nitrogen concentrations. In contrast, 95% nitrogen concentration was inadequate for the 100% control of both species at specified temperatures and humidity. In the lab tests, nitrogen-hypoxic 99% concentration was even more effective than 100% nitrogen concentration for the complete control of both species. According to model investigation in enclosed silos without significant temperature variations or direct sunlight warming effects, a targeted hypoxic concentration above 99% can be successfully attained in only one day of nitrogen beginning purification and retained steadily for the following 10-20 days. According to bio testing, both treatment schedules (Ten + One and Twenty + One days) completely controlled all developmental stages of both species in the predetermined environmental circumstances. The application of nitrogen-hypoxic conditions in small silos for very brief exposure times can thus be considered an effective strategy for eliminating *C. chinensis* and *S. granarius* species in tiny silos with a size comparable to a typical cargo container.

The second section involves discovering the association of intact grain-feeding activity of primary pests *Rhyzopertha dominica* and *Sitophilus granarius* with the biology of secondary pests (*Tribolium castaneum*, T. confusum, and *Oryzaephilus surinamensis*) and grain protectants against secondary pests (*Cryptolestes ferrugineus* and *T. castaneum*).

The lab results showed that all three tested insect species, *T. castaneum*, *T. confusum*, and *O. surinamensis*, could feed and multiply on the frass produced by *R. dominica* and could not feed on intact grain. In conclusion, our study provides scientific evidence that secondary pests, including *T. confusum*, *T. castaneum*, and *O. surinamensis*, may survive and multiply in populations of wheat frass generated by *R. dominica*. Thus, our findings suggest several functional implications for grain storage pest evaluation and control. Frass may assemble from earlier outbreaks and feed existing colonies of secondary pests; therefore, it is important to frequently check storages for dust and frass even when there are no primary pests.

According to the results of our grain protectant research, wheat kernels that are clean and sound may be able to stop the tested species from surviving and producing offspring under the setting of our studies. Our research showed that the present label dose from both grain protection insecticides was quite compelling, and the existence of damaged grain does not reduce the performance of the experimented doses against secondary pests. Furthermore, the evaluated mixture of smaller than labeled amounts and many damaged kernels was a factor in reduced efficacy.

The third section includes the utilization of botanical extracts for the control of *Callosobruchus maculatus.*

Extracts from five plant species and six different concentrations were used in this experiment for the repellency effect against the *C. maculatus*. The results demonstrated that *Azadirachta indica* and *Melia azedarach extracts* recorded the highest repellency effect. The repellency increases with the increase in dose. According to our findings, at any dosage, *A. indica* and *M. azedarach* extracts may replace pesticides used to control stored grain insects. In conclusion, we have covered all the hypotheses and achieved all the goals in this doctoral dissertation.

7. LIST OF USED LITERATURE

Abd El Ghany, A. M. (2012). Plant extracts as effective compounds pest control. *Saarbrucken, LAP Lambert Academic Publishing AG & Co KG*.

Abdel-Aziz, S. (2011). Control Strategies of Stored Product Pests. *Journal of Entomology*, *8*(2), 101-122.

Agrafioti, P., Kaloudis, E., & Athanassiou, C. G. (2022). Utilizing low oxygen to mitigate resistance of stored product insects to phosphine. *Journal of the Science of Food and Agriculture*, *102*(13), 6080-6087.

Ahmed, S. M. (1994). Neem (*Azadirachta Indica* A. Juss) a safer insecticide potentials and prospects. *Pest Management*, 1-3.

Ahmed, S. S., Naroz, M. H., & El-Mohandes, M. A. (2022). Use of modified atmospheres combined with phosphine in controlling stored date fruit pests, *Oryzaephilus surinamensis* and *Tribolium confusum*, and effect on the fruit chemical properties. *International Journal of Tropical Insect Science*, *42*(2), 1933-1941.

Aitken, A. D. (1975). Insect Travellers. Vol. 1. Coleoptera. *Technical Bulletin, Ministry of Agriculture, Fisheries and Food, Agricultural Development and Advisory Service, Pest Infestation Control Laboratory*, *31*.

Arora, G. L., & Singh, T. (1971). The biology of *Callosobruchus chinensis* (L.). *Research bulletin of the Panjab University, Science,* (21) 55-56.

Arthur, F. H. (2012). Aerosols and contact insecticides as alternatives to methyl bromide in flour mills, food production facilities, and food warehouses. *Journal of Pest Science*, *85*(3), 323-329.

Atawodi, S. E., & Atawodi, J. C. (2009). *Azadirachta indica* (neem): a plant of multiple biological and pharmacological activities. *Phytochemistry Reviews*, *8*(3), 601-620.

Atawodi, S. E., and Atawodi, J. C. (2009). *Azadirachta indica* (neem): a plant of multiple biological and pharmacological activities. *Phytochemistry Reviews*, *8*(3), 601-620.

Athanassiou, C. G., Arthur, F. H., & Throne, J. E. (2009). Efficacy of grain protectants against four psocid species on maize, rice, and wheat. *Pest Management Science*, 65, 1140–1146.

Athanassiou, C. G., Chiou, A., Rumbos, C. I., Sotiroudas, V., Sakka, M., Nikolidaki, E. K., Panagopoulou, E. A., Kouvelas, A., Katechaki, E., & Karathanos, V. T. (2017). Effect of nitrogen in combination with elevated temperatures on insects, microbes, and organoleptic characteristics of stored currants. *Journal of Pest Science*, *90*(2), 557-567.

Athanassiou, C. G., Kavallieratos, N. G., & Campbell, J. F. (2017). Competition of three species of *Sitophilus* on rice and maize. *PLoS One*, *12*(3), e0173377.
Athanassiou, C. G., Opit, G. P., & Throne, J. E. (2010). Influence of commodity type, percentage of cracked kernels, and wheat class on population growth of stored product psocids (Psocoptera: Liposcelidae). *Journal of Economic Entomology*, *103*(3), 985-990.

Aulicky, R., & Stejskal, V. (2015). Efficacy and limitations of phosphine "spot-fumigation" against five coleoptera species of stored product pests in wheat in a grain store-Short Note. *Plant Protection Science*, *51*(1), 33-38.

Aulicky, R., Kolar, V., Plachy, J., & Stejskal, V. (2016). Preliminary report on controlled nitrogen atmosphere in metal silo bin in the Czech Republic. *Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products*, CAF Canada: 329-332.

Aulicky, R., Kolar, V., Plachy, J., & Stejskal, V. (2017). Field efficacy of brief exposure of adults of six storage pests to nitrogen-controlled atmospheres. *Plant Protection Science*, *53*, 169-176.

Barratt, B. I. P., Moran, V. C., Bigler, F., & Van Lenteren, J. C. (2018). The status of biological control and recommendations for improving uptake for the future. *BioControl*, *63*(1), 155-167.

Begum, A. A., & Huda, M. (1974). The effect of food on the life history of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *Bangladesh Jornal of Zoology*, *2*(1), 71-76.

Bell, C. H. (2000). Fumigation in the 21st century. *Crop protection*, *19*(8-10), 563-569.

Birch, L. C. (1945). The influence of temperature, humidity, and density on the oviposition of the small strain of *Calandra oryzae* L. and *Rhyzopertha dominica* Fab. (Coleoptera). *Australian Journal of Experimental Biology & Medical Science*, *23*(3).

Birch, L. C. (1947). The oxygen consumption of the small strain of *Calandra oryzae* L. and *Rhyzopertha dominica* Fab. as affected by temperature and humidity. *Ecology*, *28*(1), 17-25.

Boeke, S. J., Baumgart, I. R., Van Loon, J. J. A., Van Huis, A., Dicke, M., & Kossou, D. K. (2004). Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against *Callosobruchus maculatus*. *Journal of Stored Products Research*, *40*(4), 423-438.

Bohinc, T., Horvat, A., Andrić, G., Golić, M. P., Kljajić, P., & Trdan, S. (2020). Natural versus synthetic zeolites for controlling the maize weevil (Sitophilus zeamais)–like Messi versus Ronaldo? *Journal of Stored Products Research*, *88*, 101639.

Boniecki, P., Koszela, K., Świerczyński, K., Skwarcz, J., Zaborowicz, M., & Przybył, J. (2020). Neural Visual Detection of Grain Weevil (*Sitophilus granarius* L.). *Agriculture*, *10*(1), 25.
Brader, B., Lee, R. C., Plarre, R., Burkholder, W., Kitto, G. B., Kao, C., ... & Denning, R. (2002). A comparison of screening methods for insect contamination in wheat. *Journal of Stored Products Research*, *38*(1), 75-86.

Buckland, P. C. (1981). The early dispersal of insect pests of stored products as indicated by archaeological records. *Journal of Stored Products Research*, *17*(1), 1-12.

Carvalho, M. O., Barbosa, A., Barros, G., Magro, A., Adler, C., Navarro, S., Riudavets, J., & Timlick, B. (2012). Quality preservation of stored rice using modified atmospheres in Portugal. *In Proceedings of the 9th International Conference on Controlled Atmospheres and Fumigation in Stored Products*, Antalya, Turkey, 15-19 October.

Carvalho, M. O., Pires, I., Barbosa, A., Barros, G., Riudavets, J., Garcia, A. C., Brites, C., & Navarro, S. (2012). The use of modified atmospheres to control *Sitophilus zeamais* and *Sitophilus oryzae* on stored rice in Portugal. *Journal of stored products research*, *50*, 49-56.

Casida, J. E. (2012). The greening of pesticide–environment interactions: some personal observations. *Environmental health perspectives*, *120*(4), 487-493.

Champ, B. R., & Dyte, C. E. (1976). *Report of the FAO global survey of pesticide susceptibility of stored grain pests*. FAO.

Chaudhari, A. K., Singh, V. K., Kedia, A., Das, S., & Dubey, N. K. (2021). Essential oils and their bioactive compounds as ecofriendly novel green pesticides for management of storage insect pests: prospects and retrospects. *Environmental Science and Pollution Research*, *28*(15), 18918 –18940.

Chavan, P. D., Yeshbir, S., & Singh, S. P. (1997). Growth and development of *Callosobruchus chinensis* on cowpea lines. *Indian journal of Entomology*, *59*(3), 304-310.

Chen, H. M., Liu, C. A., George Kuo, C., Chien, C. M., Sun, H. C., Huang, C. C., ... & Ku, H. M. (2007). Development of a molecular marker for a bruchid (*Callosobruchus chinensis* L.) resistance gene in mungbean. *Euphytica*, *157*(1), 113-122.

Coulson, S. J., & Bale, J. S. (1991). Anoxia induces rapid cold hardening in the housefly *Musca domestica* (Diptera: Muscidae). *Journal of Insect Physiology*, *37*(7), 497-501.

Dal Bello, G., Padin, S., Lastra, C. L., & Fabrizio, M. (2000). Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. *Journal of stored products research*, *37*(1), 77-84.

Dales, M. J. (1996). A review of plant materials used for controlling insect pests of stored products. *Natural research institute bullitien*, *65*, 10738.

Dayan, F. E., Cantrell, C. L., & Duke, S. O. (2009). Natural products in crop protection. *Bioorganic & medicinal chemistry*, *17*(12), 4022-4034.

Devi, D. A., & Mohandas, N. (1982). Relative efficacy of some antifeedants and deterrents against insect pests of stored paddy. *Entomon*, *7*(3), 261-264.

Doharey, R. B., Katiyar, R. N., & Singh, K. M. (1990). Eco-toxicological studies on pulse beetles infesting green gram. 4. Comparative efficacy of some edible oils for the protection of green gram (Vigna radiata (L.) Wilczek against pulse beetles, *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (F). *Bulletin of grain technology*, *28*(2), 116-119.

Donahaye, E. J., Navarro, S., Rindner, M., & Azrieli, A. (1996). The combined influence of temperature and modified atmospheres on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research*, *32*(3), 225-232.

Don-Pedro, K. N. (1985). Toxicity of some citrus peels to *Dermestes maculatus* Deg. and *Callosobruchus maculatus* (F). *Journal of Stored Products Research*, *21*(1), 31-34.

Dubey, N. K. (Ed.). (2011). *Natural products in plant pest management*. CABI.

Dubey, N. K., Kumar, A., Singh, P., & Shukla, R. (2010). Exploitation of natural compounds in eco-friendly management of plant pests. In *Recent developments in management of plant diseases* (pp. 181-198). Springer, Dordrecht.

Edward G. J., & Wilfred C. (1981). Weight loss and Mortality of three life stages of *Tribolium castaneum* (Herbst) when exposed to four modified atmospheres. *Journal of stored Product Research,* 17, 117-124.

Egwuatu, R. I. (1987). Current status of conventional insecticides in the management of stored product insect pests in the tropics. *International Journal of Tropical Insect Science, 8*(4-5-6), 695–701.

Eliopoulos, P. A. (2019). Life table parameters of the parasitoid *Cephalonomia tarsalis* (Hymenoptera: Bethylidae) and its host the saw-toothed grain beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). *Journal of Plant Protection Research*, *59*(4).

Estabrook, E. M., & Yoder, J. I. (1998). Plant-plant communications: rhizosphere signaling between parasitic angiosperms and their hosts. *Plant Physiology*, *116*(1), 1-7.

FAOSTAT. (2017). Food and Agriculture Organization of the United Nations. *FAOSTAT Database*. Rome, Italy.

Fields, P. G. (1992). The control of stored-product insects and mites with extreme temperatures. *Journal of Stored Products Research*, *28*(2), 89-118.

Fields, P. G., & White, N. D. (2002). Alternatives to methyl bromide treatment for stored product and quarantine insects. *Annual review of entomology*, *47*, 331.

Flinn, P. W., & Hagstrum, D. W. (1994). Field validation of a decision support system for farm-stored grain. In *Proc. Sixth International Working Conference on Stored-Product Protection* (pp. 921-4). Wallingford, UK: CAB International.

Flora, J. W., Byers, L. E., Plunkett, S. E., & Faustini, D. L. (2006). Residue formations of phosphorus hydride polymers and phosphorus oxyacids during phosphine gas fumigations of stored products. *Journal of agricultural and food chemistry*, *54*(1), 107-111.

Gautam, S. G., Opit, G. P., Giles, K. L., & Adam, B. (2013). Weight loss and germination failure caused by psocids in different wheat varieties. *Journal of economic entomology*, *106*(1), 491-498.

Gbaye, O. A., & Odeyemi, O. O. (2005). Evaluation of hypercarbia atmosphere on the mortality of dried-fruit beetle, *Carpophilus hemipterus* (Linnaeus). *Journal of Food, Agriculture and Environment*, 3(3&4), 43-46.

Ghanem, I., & Shamma, M. (2007). Effect of non-ionizing radiation (UVC) on the development of *Trogoderma granarium* Everts. *Journal of stored products research*, *43*(4), 362-366.

Giga, D. P., Mutemerewa, S., Moyo, G., & Neeley, D. (1991). Assessment and control of losses caused by insect pests in small farmers' stores in Zimbabwe. *Crop Protection*, *10*(4), 287-292.

Good, N. E. (1936). *The flour beetles of the genus Tribolium* (No. 1488-2016-123671).

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B., & Givskov, M. (2002). Food spoilage—interactions between food spoilage bacteria. *International journal of food microbiology*, *78*(1-2), 79-97.

Gustavsson, J., Cederberg, C., Sonesson, U., Van Otterdijk, R., & Meybeck, A. (2011). Global food losses and food waste.

Hagstrum, D. W., & Phillips, T. W. (2017). Evolution of stored-product entomology: protecting the world food supply. *Annual review of entomology*, *62*, 379-397.

Hagstrum, D. W., & Subramanyam, B. (2006). Fundamentals of Stored-Product Entomology; AACC International: St. *Paul, MN, USA*, 323.

Hagstrum, D. W., & Subramanyam, B. (2009). A Review of Stored-Product Entomology Information Sources. *American Entomologist, 55*(3), 174-183.

Hashem, M. Y., Ahmed, S. S., Naroz, M. H. (2022). Comparative effects of hypoxia, hypercapnia, and hypoxia/hypercapnia on the mortality and survival of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae). *Journal of Stored Product Research, 98*, 101988.

Highland, H. A. (1991). Protecting packages against insects. *Ecology and Management of Food-Industry Pests, FDA Technical Bulletin, 4*, 345-350.

Hochachka, P. W. (1991). Metabolic strategies of defense against hypoxia in animals. *Plant life under oxygen deprivation*. Edited by Jackson MB, Davies DD, Lambers H. The Hague: SPB Academic Publishing by; 23-29.

Huang, Y., Chen, S. X., & Ho, S. H. (2000). Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology*, *93*(2), 537-543.

Hubert, J., Nesvorna, M., & Stejskal, V. (2009). The efficacy of sieving, filth flotation and Tullgren heat extraction for detecting various developmental stages of *Tribolium castaneum* and *Ephestia kuehniella* in samples of wheat grain, flour, and semolina. *Journal of Stored Products Research*, *45*(4), 279-288.

Hubert, J., Stejskal, V., Athanassiou, C. G., & Throne, J. E. (2018). Health hazards associated with arthropod infestation of stored products. *Annual review of entomology*, *63*, 553-573.

Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, *51*, 45-66.
Jacobson, M. (1982). Plants, insects, and man—their interrelationships. *Economic Botany*, *36*(3), 346-354.

Jay, E. G. (1984). Imperfections in our current knowledge of insect biology as related to their responses to controlled atmospheres. *In Controlled atmosphere and fumigation in grain storages*, Ripp, B. E., Banks, H. J., Bond, E. J., Carlverley, D. J., Jay, E. G., Navarro, S., Eds.; Elsevier, Amsterdam, 493–508.

Jay, E. G., & Cuff, W. (1981). Weight loss and mortality of three life stages of *Tribolium castaneum* (Herbst) when exposed to four modified atmospheres. *Journal of Stored Products Research*, *17*(3), 117-124.

Jian, F., Jayas, D. S., & White, N. D. (2004). Movement and distribution of adult rusty grain beetle, *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae), in stored wheat in

response to different temperature gradients and insect densities. *Journal of economic entomology*, *97*(3), 1148-1158.

Jotwani, M. G., & Sircar, P. (1965). Neem seed as a protectant against stored grain pests infesting wheat seed. *Indian Journal of Entomology*, *27*(2), 160-164.

Kalpna, Hajam, YA., & Kumar, R. (2022). Management of stored grain pest with special reference to Callosobruchus maculatus, a major pest of cowpea: A review. *Heliyon, 8*(1), e08703.

Kavallieratos, N. G., Athanassiou C. G., Guedes, R. N. C., Drempela, J. D., & Boukouvala M. C. (2017). Invader competition with local competitors: displacement or co-existence among the invasive khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), and two others major stored grain beetles? *Frontiers in Plant Science 8*, 1837.

King, E. G., Hopper, K. R., & Powell, J. E. (1985). Analysis of systems for biological control of crop arthropod pests in the US by augmentation of predators and parasites.

Kiran, S., & Prakash, B. (2015). Assessment of toxicity, antifeedant activity, and biochemical responses in stored-grain insects exposed to lethal and sublethal doses of Gaultheria procumbens L. essential oil. *Journal of agricultural and food chemistry*, *63*(48), 10518-10524.

Kucerova, Z. (1999). Vulnerability of wheat varieties to stored product psocids. *Stored Product Protection, Proceedings of the 7th Inernational Working Confrence on Stored Product Protection Beijing, China*, 1251-1254.

Lampiri, E., Baliota, G. V., Morrison, W. R., Domingue, M. J., & Athanassiou, C. G. (2022). Comparative population growth of the khapra beetle (Coleoptera: Dermestidae) and the warehouse beetle (Coleoptera: Dermestidae) on wheat and rice. *Journal of Economic Entomology*, 115, 344-352.

Landolt, P. J., & Phillips, T. W. (1997). Host plant influences on sex pheromone behavior of phytophagous insects. *Annual review of entomology*, *42*(1), 371-391.
Lee Jr, R. E., Chen, C. P., & Denlinger, D. L. (1987). A rapid cold-hardening process in insects. *Science*, *238*(4832), 1415-1417.

Levinson, H. Z., & Kanaujia, K. R. (1981). Phagostimulatory responses and oviposition behaviour of *Sitophilus granarius* L. to newly harvested and stored wheat grains. *Zeitschrift für angewandte Entomologie*, *91*(1-5), 417-424.

Levinson, H. Z., Levinson, A., Ren, Z., & Mori, K. (1990). Comparative olfactory perception of the aggregation pheromones of *Sitophilus oryzae* (L.), *S. zeamais* (Motsch.) and *S. granarius* (L.), as well as the stereoisomers of these pheromones. *Journal of Applied Entomology*, *110*(1-5), 203-213.

Liu, Y. B. (2022). Effects of long-term low oxygen storage treatment on survival of rice weevil (*Sitophilus oryzae*) and confused flour beetle (*Tribolium confusum*). *Journal of Economic Entomology*, *115*(5), 1712-1718.

Lu, B., Ren, Y., Du, Y. Z., Fu, Y., & Gu, J. (2009). Effect of ozone on respiration of adult *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). *Journal of Insect Physiology*, *55*(10), 885-889.

Makarieva, A. M., Gorshkov, V. G., Li, B. L., & Chown, S. L. (2006). Size-and temperature-independence of minimum life-supporting metabolic rates. *Functional Ecology*, *20*(1), 83-96.

Matthews, P. G., & White, C. R. (2011). Discontinuous gas exchange in insects: is it all in their heads? *The American Naturalist*, *177*(1), 130-134.

Mayhew, T. J., & Phillips, T. W. (1994). Pheromone biology of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). In *Proceedings of the Sixth International Working Conference on Stored-Product Protection* (Vol. 1, pp. 541-544). CABI Press, Canberra, Australia.

Mishra, S., Thombare, N., Ali, M., & Swami, S. (2018). Applications of Biopolymeric gels in Agricultural Sector. *In Polymer Gels*: Perspectives and Applications; Thakur, V. K., Thakur, M. K., Voicu, S. I., Eds.; Springer: Singapore, 185–228.

Mitcham, E., Martin, T., & Zhou, S. (2006). The mode of action of insecticidal controlled atmospheres. *Bulletin of Entomological Research*, *96*(3), 213–222.

Moncini, L., Simone, G., Romi, M., Cai, G., Guerriero, G., Whittaker, A., Benedettelli, S., & Berni, R. (2020). Controlled nitrogen atmosphere for the preservation of functional molecules during silos storage: A case study using old Italian wheat cultivars. *Journal of Stored Products Research*, *88*, 101638.

Mowery, S. V., Mullen, M. A., Campbell, J. F., & Broce, A. B. (2002). Mechanisms underlying sawtoothed grain beetle (*Oryzaephilus surinamensis* [L.] (Coleoptera: Silvanidae) infestation of consumer food packaging materials. *Journal of Economic Entomology*, *95*(6), 1333-1336.

Munakata, K. (1977). Insect antifeedants of *Spodoptera litura* in plants. In: Host Plant Resistance to Pests, P. A. Hedin (Ed.) ACS Symposium Series, American Chemical Society, Washington, DC, USA; 62:185-96.

Naimi, I., Zefzoufi, M., Bouamama, H., & T. B. M'hamed, T. B. (2022). Chemical composition and repellent effects of powders and essential oils of *Artemisia absinthium*, *Melia azedarach*, *Trigonella foenum-graecum*, and *Peganum harmala* on *Tribolium*

castaneum (Herbst) (Coleoptera: Tenebrionidae). *Industrial Crops and Products*, *182*, 114817.
Navarro, S. (2006). Modified atmospheres for the control of stored-product insects and mites. *Insect management for food storage and processing*, 105-146.

Navarro, S., & Navarro, H. (2020). Advances in insect pest management in postharvest storage of cereals: Use of controlled atmosphere and temperature control. In Advances in Postharvest Management of Cereals and Grains; Maier, D.E., Ed.; Burleigh Dodds Science Publishing Limited: Cambridge, UK, p. 478.

Navarro, S., Athanassiou, C., Varnava, A., Vroom, N., Yiassoumis, D., Leandrou, I., & Hadjioannou, S. (2012). Control of stored grain insects by using nitrogen in large concrete silos in Cyprus. In *Proc. 9th Int. Conf. Controlled Atmosphere and Fumigation in Stored Products* (pp. 478-487).

Nayak, M. K., Daglish, G. J., Phillips, T. W., & Ebert, P. R. (2020). Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. *Annual Review of Entomology*, *65*, 333-350.

Niedermayer, S., & Steidle, J. L. (2013). The Hohenheimer Box–A new way to rear and release *Lariophagus distinguendus* to control stored product pest insects. *Biological Control*, *64*(3), 263-269.

Nilson, T. L., Sinclair, B. J., & Roberts, S. P. (2006). The effects of carbon dioxide anesthesia and anoxia on rapid cold-hardening and chill coma recovery in *Drosophila melanogaster*. *Journal of insect physiology*, *52*(10), 1027-1033.

Nopsa, H., Daglish, J. F., Hagstrum, G. J., Leslie, D. W., Phillips, J. F., Scoglio, T. W., Thomas-Sharma, C. S., & Garrett, K. A. (2015). Ecological networks in stored grain: Key postharvest nodes for emerging pests, pathogens, and mycotoxins. *BioScience*, *65*(10), 985-1002.

Obeng-Ofori, D., Reichmuth, C. H., Bekele, A. J., & Hassanali, A. (1998). Toxicity and protectant potential of camphor, a major component of essential oil of *Ocimum kilimandscharicum*, against four stored product beetles. *International Journal of pest management*, *44*(4), 203-209.

Owusu, E. O. (2000). Effect of some Ghanaian plant components on control of two stored-product insect pests of cereals. *Journal of Stored Products Research*, *37*(1), 85-91.

Pankey, J. H., Griffin, J. L., Leonard, B. R., Miller, D. K., Downer, R. G., & Costello, R. W. (2004). Glyphosate–insecticide combination effects on weed and insect control in cotton. *Weed Technology*, *18*(3), 698-703.

Park, I. K., Lee, S. G., Choi, D. H., Park, J. D., & Ahn, Y. J. (2003). Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against

Callosobruchus chinensis (L.) and *Sitophilus oryzae* (L.). *Journal of Stored products research*, *39*(4), 375-384.
Parkin, E. A. (1956). Stored product entomology (the assessment and reduction of losses caused by insects to stored foodstuffs). *Annual Review of Entomology*, *1*(1), 223-240.

Parr, M. J., Tran, B., Simmonds, M. S., Kite, G. C., & Credland, P. F. (1998). Influence of some fatty acids on oviposition by the bruchid beetle, *Callosobruchus maculatus*. *Journal of Chemical Ecology*, *24*(10), 1577-1593.

Parrella, M. P., Heinz, K. M., & Nunney, L. (1992). Biological control through augmentative releases of natural enemies: a strategy whose time has come. *American Entomologist*, *38*(3), 172-180.

Pascual-Villalobos, M. J., & Robledo, A. (1998). Screening for anti-insect activity in Mediterranean plants. *Industrial crops and products*, *8*(3), 183-194.

Pavela, R. (2005). Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. *Fitoterapia*, *76*(7-8), 691-696.

Pavela, R. (2016). History, presence, and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects–a review. *Plant Protection Science*, *52*(4), 229-241.

Pereira, J., & Wohlgemuth, R. (1982). Neem (*Azadirachta indica* A. Juss) of West African origin as a protectant of stored maize 1. *Zeitschrift für Angewandte Entomologie*, *94*(1-5), 208-214.

Phillips, T. W., & Throne, J. E. (2010). Biorational approaches to managing stored-product insects. *Annual review of entomology*, *55*(1), 375-397.

Pickett, J. A., Rasmussen, H. B., Woodcock, C. M., Matthes, M., & Napier, J. A. (2003). Plant stress signalling: understanding and exploiting plant–plant interactions. *Biochemical Society Transactions*, *31*(1), 123-127.

Pimentel, D. (1991). Ethanol fuels: Energy security, economics, and the environment. *Journal of agricultural and environmental ethics*, *4*(1), 1-13.

Prickett, A. J., Muggleton, J., & Llewellin, J. A. (1990). Insecticide resistance in populations of *Oryzaephilus surinamensis* and *Cryptolestes ferrugineus* from grain stores in England and Wales. In *Proceedings of the Brighton Crop Protection Conference, Pests and Diseases* (No. 3, pp. 1189-1194). British Crop Protection Council Publications.

Rajashekar, Y., Bakthavatsalam, N., & Shivanandappa, T. (2012). Botanicals as grain protectants. *Psyche*, *2012*.

Rajashekar, Y., Tonsing, N., Shantibala, T., & Manjunath, J. R. (2016). 2, 3-Dimethylmaleic anhydride (3, 4-Dimethyl-2, 5-furandione): A plant derived insecticidal molecule from Colocasia esculenta var. esculenta (L.) Schott. *Scientific reports*, *6*(1), 1-7. Rajendran, S., & Sriranjini, V. (2008). Plant products as fumigants for stored-product insect control. *Journal of stored products Research*, *44*(2), 126-135.

Rao, H. G., & Wilbur, D. A. (1972). Loss of wheat weight from feeding of lesser grain borer. *Journal of the Kansas Entomological Society*, 238-241.

Reichmuth, C. (2000). Biological control in stored product protection. *IOBC WPRS BULLETIN*, *23*(10), 11-24.

Ren, Y., Yang, C., Newman, J., Agarwal, M., Kostas, E., & Cheng, H. (2012). Nitrogen application offers for both control of insect and grain quality. *In Proceedings of the 9th International Conference on Controlled Atmospheres and Fumigation in Stored Products*,

Antalya, Turkey, 15-19 October. Navarro, S., Banks, J. H., Jayas, D., Bell, C. H., Noyes, R. T., Feizli, A. G., Emekci, M., Isikber, A. A., Alagusundaram, K., Eds. ARBER Professional Congress Services, Turkey, 471–477.

Riudavets, J. (2017). Biological control of stored products pests. *IOBC WPRS Bulletin*.

Sakka, M. K., Gatzali, F., Karathanos, V. T., & Athanassiou, C. G. (2020). Effect of nitrogen on phosphine-susceptible and-resistant populations of stored product insects. *Insects*, *11*(12), 885.

Schöller, M., Prozell, S., Al-Kirshi, A. G., & Reichmuth, C. (1997). Towards biological control as a major component of integrated pest management in stored product protection. *Journal of Stored Products Research*, *33*(1), 81-97.

Schwardt, H. H. (1933). Life history of the lesser grain borer. *Journal of the Kansas Entomological Society*, *6*(2), 61-66.

Singh, D., & Mehta, S. S. (2010). Menthol containing formulation inhibits adzuki bean beetle, *Callosobruchus chinensis* L. (Coleoptera; Bruchidae) population in pulse grain storage. *Journal of Biopesticides*, *3*(3), 596.

Sinha, K. K., & Sinha, A. K. (1992). Impact of stored grain pests on seed deterioration and aflatoxin contamination in maize. *Journal of Stored Products Research*, *28*(3), 211-219.

Skendžić, S., Zovko, M., Živković, IP., Lešić, V., & Lemić, D. (2021). The Impact of Climate Change on Agricultural Insect Pests. *Insects*, *12*(5), 440.

Smith, L. B. (1965). The intrinsic rate of natural increase of *Cryptolestes ferrugineus* (Stephens) (Coleoptera, Cucujidae). *Journal of Stored Products Research*, *1*(1), 35-49.

Smith, L. B. (1970). Effects of cold acclimation on supercooling and survival of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae), at subzero temperatures. *Canadian Journal of Zoology*, *48*(4), 853-858.

Sola, M. (2018). Approaches for the Biological Control of Stored Product Pests. Universitat Autònoma de Barcelona.

Steidle, J. L., Fischer, A., & Gantert, C. (2005). Do grains whisper for help? Evidence for herbivore-induced synomones in wheat grains. *Entomologia experimentalis et applicata*, *115*(1), 239-245.

Stejskal, V., & Hubert, J. (2008). Risk of occupational allergy to stored grain arthropods and false pest-risk perception in Czech grain stores. *Annals of Agricultural and Environmental Medicine*, *15*(1), 29-35.

Stejskal, V., & Kučerová, Z. (1996). The effect of grain size on the biology of *Sitophilus granarius* L. (Col., Curculionidae). I. Oviposition, distribution of eggs and adult emergence. *Journal of Applied Entomology*, *120*(1-5), 143-146.

Stejskal, V., Aulicky, R., & Kucerova, Z. (2014). A review of pest control strategies and damage potential of seed-infesting pests in the Czech stores. *Plant Protection Science*, *50*, 165- 173.

Stejskal, V., Vendl, T., Aulicky, R., & Athanassiou, C. (2021). Synthetic and natural insecticides: Gas, liquid, gel and solid formulations for stored-product and food-industry pest control. *Insects*, *12*(7), 590.

Talukder, F. A. (2006). Plant products as potential stored-product insect stored management agents-A mini review. *Emirates Journal of Food and Agriculture*, 17-32.

Talukder, F. A., & Howse, P. E. (1995). Evaluation of *Aphanamixis polystachya* as a source of repellents, antifeedants, toxicants and protectants in storage against *Tribolium castaneum* (Herbst). *Journal of Stored Products Research*, *31*(1), 55-61.

Talukder, F. A., Islam, M. S., Hossain, M. S., Rahman, M. A., & Alam, M. N. (2004). Toxicity effects of botanicals and synthetic insecticides on *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). *Bangladesh J. Environ. Sci*, *10*(2), 365-371.

Teshome, A., & Tefera, T. (2011). Potential application of entomopathogenic fungi in the management of maize weevil. In *10th African Crop Science Conference Proceedings, Maputo, Mozambique, 10-13 October 2011* (pp. 249-253). African Crop Science Society.

Tiwari, A., Kumar, M. L., & Saxena, R. C. (1995). Effect of Nicotiana tabacum on *Tribolium castaneum*. *International journal of pharmacognosy*, *33*(4), 348-350.

Tiwari, S. N. (1994). Efficacy of some plant products as grain protectants against *Rhyzopertha dominica* (F.) (Coleoptera; Bostrichidae). *International Journal of Pest Management*, *40*(1), 94-97.

Toews, M. D., Campbell J. F., & Arthur F. H. (2006). Temporal dynamics and response to fogging or fumigation of stored-product Coleoptera in a grain processing facility. *Journal of Stored Product Research*, *42*, 480-98.

Trematerra, P., & Gentile, P. (2010). Five years of mass trapping of *Ephestia kuehniella* Zeller: a component of IPM in a flour mill. *Journal of Applied Entomology*, *134*(2), 149-156.

Trematerra, P., Fontana, F., Mancini, M., & Sciarretta, A. (1999). Influence of intact and damaged cereal kernels on the behaviour of rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *Journal of Stored Products Research*, *35*(3), 265-276.

Trematerra, P., Sciarreta, A., & Tamasi, E. (2000). Behavioural responses of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum* to naturally and artificially damaged durum wheat kernels. *Entomologia Experimentalis et Applicata*, *94*(2), 195-200.

Trematerra, P., Stejskal, V., & Hubert, J. (2011). The monitoring of semolina contamination by insect fragments using the light filth method in an Italian mill. *Food Control*, *22*(7), 1021-1026.

Trematerra, P., Valente, A., Athanassiou, C. G., & Kavallieratos, N. G. (2007). Kernel-kernel interactions and behavioral responses of the adult's maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Applied Entomology and Zoology 42*, 129-135.

Tripathi, A. K., Prajapati, V., Aggarwal, K. K., Khanuja, S. P. S., & Kumar, S. (2000). Repellency and toxicity of oil from *Artemisia annua* to certain stored-product beetles. *Journal of economic entomology*, *93*(1), 43-47.

Turek, C., & Stintzing, F. C. (2013). Stability of essential oils: A review. *Comprehensive Reviews in Food Science and Food Safety*, *12*(1), 40-53.

Van Lenteren, J. C. (2012). The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, *57*(1), 1-20.

Vendl, T., Shah, J. A., Aulicky, R., & Stejskal, V. (2022). Effect of grain excavation damages by *Sitophilus granarius* on the efficacy of grain protectant insecticides against *Cryptolestes ferrugineus* and *Tribolium castaneum*. *Journal of Stored Products Research*, *99*, 102022.

Wakefield, M. E., Bryning, G. P., & Chambers, J. (2005). Progress towards a lure to attract three stored product weevils, *Sitophilus zeamais* Motschulsky, *S. oryzae* (L.) and *S.*

granarius (L.) (Coleoptera: Curculionidae). *Journal of Stored Products Research*, *41*(2), 145-161.

Wallbank, B. E., & Collins, P. J. (2003). Recent changes in resistance to grain protectants in eastern Australia. In *Proc. Aust. Postharvest Technical Conference "Stored Ggrain in Australia* (pp. 66-70).

Walter, V. E. (1990). Stored Product Pests. In Story, K. and Moreland, D. (eds) *Handbook of pest control.* p 526-529.

Wang, Y., Zhang, L. T., Feng, Y. X., Zhang, D., Guo, S. S., Pang, X., Geng, Z. F., Xi, C., & Du, S. S. (2019). Comparative evaluation of the chemical composition and bioactivities of essential oils from four spice plants (Lauraceae) against stored-product insects. *Industrial Crops and Products*, *140*, 111640.

Ware, G. W., & Whitacre, D. M. (2004). An introduction to insecticides. *The pesticide book*, *6*.

White, N. D., Fields, P. G., Demianyk, C. J., Timlick, B., & Jayas, D. S. (2011). Arthropods of stored cereals, oilseeds, and their products in Canada: artificial ecosystems on grasslands. *Arthropods of Canadian grasslands*, *2*, 267-289.

Wilkin, R. (2000). Options for new approaches to pest control in food. *IOBC WPRS BULLETIN*, *23*(10), 1-10.

Xie, Y. S., Fields, P. G., & Isman, M. B. (1995). Repellency and toxicity of azadirachtin and neem concentrates to three stored-product beetles. *Journal of economic entomology*, *88*(4), 1024-1031.

Yallapa, R., Nandagopal, B., & Thimmappa, S. (2012). Botanicals as Grain Protectants. *Psyche: A Journal of Entomology*, *2012*, 1-13.

Yan, Y., Williams, S. B., Baributsa, D., & Murdock, L. L. (2016). Hypoxia treatment of *Callosobruchus maculatus* females and its effects on reproductive output and development of progeny following exposure. *Insects*, *7*(2), 26.

Yocum, G. D., & Denlinger, D. L. (1994). Anoxia blocks thermotolerance and the induction of rapid cold hardening in the flesh fly, *Sarcophaga crassipalpis*. *Physiological Entomology*, *19*(2), 152-158.

Zettler, J. L., & Arthur, F. H. (2000). Chemical control of stored product insects with fumigants and residual treatments. *Crop Protection*, *19*(8-10), 577-582.

Zhao, Q. Y., Li, T. X., Song, Z. J., Sun, T., Liu, B., Han, X., Li, Z.-H., & Zhan, G. P. (2021). Combination of modified atmosphere and irradiations for the phytosanitary

disinfestation of *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Insects, 12*(5), 442.

Zhou, M., Zhang, X., Pan, B., Zhu, J., Qian, X., Li, X., Xu, K., Tang, B., & Li, C. (2022). The endogenous metabolic response of *Tribolium castaneum* under a high concentration of CO2. *Agriculture, 12*, 979.