IOBC/WPRS

Working Group “Integrated Protection of Stored Products”

Proceedings of the Meeting

at

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20-23 August, 2007

Editors
Shlomo Navarro, Cornel Adler and Dr. Lise Stengård Hansen

IOBC/WPRS Bulletin
The content of the contributions is in the responsibility of the authors
Introduction

The decision to hold the IOBC WPRS (OILB SROP) Working Group on Integrated Protection of Stored Products in Poland was taken during the previous Conference held in Prague in 2005 when Dr. Danuta Sosnowska and Dr. Pawel Olejarski, expressed their consent for cooperation on behalf of the Institute of Plant Protection in Poznan. The excellent facilities of the Institute of Plant Protection in Poznan with the conference rooms, accommodation at IOR and the restaurant, all created an excellent infrastructure for the IOBC Conference venue which was held during August 20-23 2007. The Poznan Conference Local Organizing Committee under the guidance of Dr. Danuta Sosnowska was consistent, persistent and worked without compromises to make the Poznan meeting an excellent gathering. The program, the lectures, the social evening, and the field trip to the beautiful Castle of Kornik, combined with a visit to one of the most famous Polish brewery factories, allowed participants to obtain useful information on the area. All left excellent impressions on the participants about Poland in general and Poznan in particular.

I would like also to thank Ms Aleksandra Obrępska-Stępłowska, Ms Renata Wojciechowska, and Ms Zaneta Fiedler for their devoted and dedicated work by assisting in the registration and many other preparations of the conference, so nicely, so sensitively and so gracefully.

At the end of the Poznan Conference the IOBC members convened to decide on the following issues:

1) Voting for the candidate country for the next venue. Greece was elected of the five candidate countries. However, because of a previous commitment of the WG during the conference in Prague, priority was given to Italy. The next venue is therefore planned to be held in Italy, at the University of Molise in Campobasso, during summer 2009, in cooperation with Prof. Pasquale Trematerra.

2) Election of a new convenor to replace Prof. Shlomo Navarro who led the group since 2001. Among four candidates Dr. Christos Athanassiou, was elected to lead the group.

3) The timely publication of the book of proceedings. Participants agreed that the papers should be submitted on time. The possibility of publishing the book before the next conference was also considered.

4) Prof. Shlomo Navarro, in cooperation with Dr. Cornel Adler and Dr. Lise Stengård Hansen, were requested to edit the current Poznan Conference Proceedings.

The editing of this book of proceedings was carried out with the editorial assistance of Dr. Samuel Angel, and the late Dr. Jonathan Donahaye former researchers at the Israel Agricultural Research Organization who were extremely helpful in guiding me in the editing process of the papers. Unfortunately, Dr. Donahaye tragically deceased in January 2008. Dr. Donahaye largely contributed to the stored products entomology community in general and to the IOBC in particular. He will be remembered as one of the leading world wide stored product entomologist and with his many publications on post-harvest technologies.

I would also like to thank my colleagues Dr. Cornel Adler, and Dr. Lise Stengård Hansen, for their contribution in the editing process, and Dr. Horst Bathon for his relentless support.

I wish continuing success to Dr. Christos Athanassiou in his endeavour as the new convenor of our Working Group.

Shlomo Navarro
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July, 2008
Introduction by the New Convenor

The IOBC/WPRS (OILB/SROP) Working Group on “Integrated Protection of Stored Products” met under the aegis of the Institute of Plant Protection in Poznan, Poland, in the conference facilities of the Institute during August 20-23 2007. Delegates from 25 counties attended. There were 69 participants with a total of 55 contributions (39 oral and 16 posters). It was the most numerous participation (in number of delegates and countries), in comparison with all the previous Integrated Protection of Stored Products Working Group meetings. This fact clearly indicates the incontestable success of this meeting, and the continuous increase of global interest in the topics examined by this Working Group. These interests particularly include biological control and integrated pest management, plus also stored-product pest ecology and biology, chemical control, chemical ecology, monitoring, urban entomology, risk assessment and aspects of futurology and legislation.

The phase out of methyl bromide and other chemicals, traditionally used for stored-product protection, the evolution of new control techniques and the consumer’s demand for food safety and residue-free food makes integrated protection a necessity. The dramatic and abrupt increase in price of food during recent years, may lead to a powerful socio-economic transformation, constituting an urgent need for a timely change in the development and practice of food protection and safety technologies. All of these aspects were doubtless reflected by the participating scientists during the meeting. These themes constitute continuous challenges for the scientists of the Working Group on “Integrated Protection of Stored Products”. Being the only Working Group on this subject, it is not restricted to the West Palaearctic Regional Section (WPRS), but has gradually evolved to become a Global Group, as indicated by the fact that more than one-third of the participating countries were not in the geographic area of WPRS.

The Working Group was initially convened by Prof. Giorgio Dominichini who led the group in Firenze in 1992, in Milano in 1993, in Prague in 1994, and in Firenze, in 1996. Dr. Cornel Adler, led the Group at the following three meetings, in Zurich in 1997, in Berlin in 1999 and in Lisbon in 2001, followed by Prof. Shlomo Navarro, at the next three meetings, in Kusadasi in 2003, in Prague in 2005 and in Poznan in 2007. During the Meeting in Poznan, it was a great honor for me to be elected as the next convenor of the Working Group. I would like to thank my three predecessors for their excellent work during the last 16 years, which has resulted in the establishment of a very prestigious Group in its field.

Many thanks are due to Prof. Shlomo Navarro, Dr. Cornel Adler and Dr. Lise Stengård Hansen, for the editing of the current Proceedings. Thanks also to Dr. Horst Bathon for his valuable contribution to this publication.

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Session 1: Overviews and trends on stored product protection
Implementation of methyl bromide alternatives in Poland

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Abstract: Methyl bromide (MB), a powerful ozone-depleting substance, was phased-out in Poland in 2005. MB had been widely used in Poland, and it cannot be replaced by one alternative, but various methods and means have to be used. The following MB alternatives are currently used or may soon be used in Poland: (a) phosphine, (b) contact insecticides, (c) heat, (d) high pressure and CO2, and (e) Integrated Pest Management (IPM). Future perspectives include: ECO2FUME, PH3 mixtures with CO2 or N2, sulfuryl fluoride, irradiation and cooling to sub zero temperatures.

Key words: stored product pests, methyl bromide, alternatives to methyl bromide, phosphine, heat treatment, controlled atmosphere, IPM

Introduction

Methyl bromide (MB) is a powerful ozone depleter, and in 1992 it was added to the list of ozone-depleting substances controlled by the Montreal Protocol, an international agreement aimed at protecting the earth’s ozone layer. In 1997, governments around the world established a global phase-out schedule for MB. According to this agreement, industrialized countries, including Poland, phased-out MB in 2005.

In Poland, MB had been widely used for fumigation of (a) durable commodities, (b) perishable products, and (c) structures and transport vehicles. This effective gaseous pesticide cannot be replaced by one alternative, but various methods and means have to be used. Of them, a special method and/or technique should be chosen or several different alternatives have to be combined for each specific situation. To help our fumigators, the GEF funded the project “Total Sector Methyl Bromide Phase Out in Countries with Economies in Transition”.

This project has been implemented by UNEP together with UNDP. The project comprised training sessions conducted by the company “BM Seminar” (Hude, Germany) and the delivery of equipment and material for post-harvest pest prevention, monitoring and control by UNDP. Results of the project program enabled former MB users to apply MB alternatives in an efficient, safe and economic way so that the countries (Bulgaria, Latvia, Lithuania and Poland) participating in the project will soon be ready to completely phase out MB in the post-harvest sector.

Review of MB alternatives and the current status of their implementation

The following MB alternatives are currently being used or may soon be used in Poland: (a) phosphine, (b) contact insecticides, (c) heat, (d) high pressure and CO2, and (e) Integrated Pest Management (IPM).

Phosphine

The most important alternative to MB is phosphine (PH3) from solid phosphides. The advantages of this fumigant are the following: (a) effective against a broad range of pests,
(b) good penetration into stored products, (c) disperses well in enclosed spaces, (d) no negative effects on products, including the germination of seeds (Bond 1984, Mueller 1998). Various phosphide products generating phosphine are registered in Poland, and are applied using the following methods: (a) fumigation under tarps, (b) fumigation in gas-tight chambers, (c) fumigation in containers, and (d) fumigation “in transit”. To shorten the exposure time, magnesium phosphide is used as it releases PH$_3$ faster than other phosphides, and works well at lower temperatures, e.g. at 5°C. Fumigation chambers and containers allow simplified use of PH$_3$ and do not require sophisticated application techniques. In general, phosphine from solids is accepted by the Polish fumigators as a MB alternative.

New technologies for application of solid phosphides were presented to fumigators during the project courses: (a) Speedbox, (b) dispenser technique, (c) phosphine recirculation J-system, and (d) phosphine generator.

Some Speedboxes are already in use in Poland, and soon they will be widely used as plates with magnesium phosphide needed for the machine are registered. Handling and maintenance of the Speedbox is simple, and the gas concentration required for killing the pests is reached in a short time. Application of phosphine tablets or pellets in silo bins using dispensers is more economic and safer than the traditional methods. The course participants accepted this technique. Phosphine recirculation system (J-System) has been installed in a silo of the ZZZ Company in Zamość, Poland, and it is already used in fumigation treatments of stored grain.

Effective exposure periods to PH$_3$ are typically 5 to 15 days, depending on the temperature, target species and developmental stages of pests. From a practical point of view, this is the main disadvantage of phosphine, when time is a critical logistical issue at the company level. However, use of PH$_3$ supplied as a gas from the PH$_3$ Generator may allow a reduction in treatment times. However, phosphide granules to be used in the generator are not yet registered in Poland.

A practical demonstration of the PH$_3$ Generator was performed in Port of Szczecin in 2007. Use of PH$_3$ supplied as a gas from the PH$_3$ Generator may allow a reduction in treatment times.

**Contact insecticides**

Contact insecticides are registered and used in Poland on stored grain and in storage structures and food production plants. These insecticides are applied directly to grain during handling on grain conveyors and elevators, or sprayed onto the surface of bag stacks, walls and floors of empty structures and transport vehicles. Empty spaces of structures are sometimes treated by “fogging” to control flying insects.

The main groups of active ingredients are pyrethroid and organophosphate compounds. Insect growth regulators are not yet registered in Poland, and they are relatively expensive. Contact insecticides combined with other pest control methods are considered by the course participants as MB alternatives for specific situations.

**Heat treatment** (HT)

HT is a new method for Polish fumigators. Within the Project, several practical demonstrations of the method were performed so far in Bulgaria, Lithuania and Poland, and all of them were completed with full success.

In the Flour Mill “Polskie Młyny”, Bialoleka, Poland, the heat treatment started in the afternoon of Tuesday, September 5, 2006. A total of 26 heaters were distributed in the four floors to be treated (8 in the cellar, 8 in the first, and 5 each in the second and third floors). The number of heaters had been determined based on the volume of the rooms to be treated (about 8,000 m$^3$ in total). Additionally, 9 ground fans (2 in the cellar, 3 in the first and 2 in the
second and 2 in third floors) were placed. Power was distributed via 2 big distributors in the first floor and a distribution board in the second floor. On the following morning the heaters were connected to the power supply and switched on. After 24 hours 4 heaters were shut down to avoid overheating of the power generator used for the treatment. The initial temperature (18°C) was unusually low as the mill has not been operational since October, 2005. Temperatures increased following a linear function to 40-42°C for 30 hours. At that time, objects deposited in the cellar such as used motors and stacked empty flour bags were saturated with energy and temperatures increased much more steeply to the target temperature of 54-55°C. Treatment continued until the morning of Friday, September 8, when the desired effect was achieved. Heaters were switched off at 11:00 o’clock in the morning.

During the trial, participants themselves monitored temperatures with infrared thermometers and the success of the treatment with a bio-test consisting of vials containing live confused flour beetles (*Tribolium confusum*). All course participants were assured that HT is an efficient method, and they found that temperatures ranging from 50 to 55°C held for 10-12 hours kill all of the stages of pests in a mill. Participants of the course are now certain that: (a) HT is highly flexible, (b) HT is easy to perform, (c) HT controls the pests efficiently.

**CO₂ under high pressure**
The insecticidal properties of CO₂ at atmospheric pressure are well known (Banks et al. 1991). Nevertheless, CO₂ fumigation at atmospheric pressure has been used much less than PH₃ and MB. The reasons were that CO₂ is slower-acting and more expensive than PH₃ (Mueller 1998). To address these problems, high pressure chambers that hold 20-30 bars of pressure and 100% carbon dioxide are now being used in several countries (Prozell et al. 1997). Sets of high pressure fumigation chambers are under construction in Bialystok and Lublin, Poland, and they will be in service in 2007 and 2009, respectively.

**CA and vacuum**
A practical demonstration of vacuum fumigation was presented to the course participants at a seed company in Poland. The participants accepted this method enthusiastically as (a) the used system is transportable, (b) simple to set up and practical where electricity is available, (c) environmentally friendly, (d) safe to the operators, (e) no need for ventilation time, (f) same exposure time or shorter than phosphine, and (g) product quality is better preserved than in normal atmosphere (Navarro et al. 1984, Varnava et al. 1994). The Volcani cubes used for the demonstration are now operating at the CNOS Company, Ozarow, Poland.

**Integrated Pest Management (IPM)**
IPM is a process that combines as many control measures as possible to reduce the pest population in a way which is efficient, economical and safe to the environment and humans (Mallis 2004). The principles of IPM were several times underlined during the GEF courses to ensure the fumigators that IPM uses the combination of two or more methods of insect control, and that all available methods should be always combined: sanitary, mechanical, biological, physical and chemical methods. Discussions with fumigators have proved that implementation of IPM into practice is not an easy process. The most important constraints are the following: (a) customers do not know the IPM theory, (b) they should be educated on the topics of IPM philosophy, and (c) all employees at all levels of a food plant should be involved in the development of the IPM program. All fumigators are now certain that IPM is the best alternative to MB.
**Future Perspectives**

ECO\textsubscript{2}FUME could be a good alternative to MB if gaseous PH\textsubscript{3} becomes available. The technique combines of the following methods: low concentration of PH\textsubscript{3} (up to 100 ppm only) + heat (32-37\textdegree C) + increased concentration of CO\textsubscript{2} (3-5\%) for 24-36 hours in gas-tight structures (Mueller 1998). It could be a good example of the practical implementation of the IPM idea.

The PH\textsubscript{3} mixture with CO\textsubscript{2} or N\textsubscript{2} as propellant can be released at lower temperatures, and doses of fumigant can be precisely administered. However, lack of registration in most countries also limits the application of other gaseous phosphine formulations. Gas formulations of PH\textsubscript{3} are not registered in Poland.

During the GEF courses, sulfuryl fluoride was presented in detail. Since then ?, we have made an effort to obtain registration of ProFume (sulfuryl fluoride) in Poland, but the producer is still not interested in registration (niche products, small economic market?). Fumigators in other countries that use sulfuryl fluoride for their fumigations report that the results of the fumigations are perfect. In other words, they do not need MB any more.

Irradiation with gamma rays from Co\textsuperscript{60} or with accelerated electrons from machine sources is used commercially for control of bacterial or fungal contamination. The dosages necessary for this purpose are usually higher than those required for control of insect pests (Ignatowicz 1999). Lack of irradiation facilities in Poland, however, is the major limitation of this method.

Cooling to sub zero temperatures (-10 to -18\textdegree C) is one of the most promising methods of disinfestation of raw materials, replacing methyl bromide treatment. However, this method requires much of time for the treatment (Brokerhof et al. 1992). It is most effective when combined with a brief exposure to low pressure that causes insects to leave the commodity. Further disadvantages of this method include: (a) long exposure may affect quality; (b) needs for uniformity of application; (c) no residual activity; (d) high energy costs.

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**References**


Restrictions to the use of fumigants and opportunities for substitution with botanicals and modified atmospheres

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Abstract: Concerns over the adverse effects of fumigant residues in food and the environment have led regulatory agencies to take actions by imposing strict limitations on fumigant registration. Of the long list of fumigants two decades ago, very few remain today. MB has a relatively quick killing effect on insects, but - because of its contribution to stratospheric ozone depletion - has been phased out in developed countries since 2005, and in developing countries phase out will take place by 2015. In contrast, phosphine remains popular, even though insects have developed resistance to it. These restrictions on the use of fumigants have posed new global challenges to the food industry, and have resulted in efforts to register new fumigants, and in the development of new technologies as alternative control methods.

Among the newly considered fumigants are sulfuryl fluoride, carbonyl sulphide, propylene oxide, methyl iodide, ozone, ethyl formate, and hydrogen cyanide. Sulfuryl fluoride seems to emerge as a promising candidate fumigant for disinfecting stored food commodities, food-processing facilities and as a quarantine fumigant. Other registered fumigants suffer from the limitation that they may be useful for treating a particular type of commodity or for application in a specific situation only. The potential use of volatiles of botanical origin shows promise but requires both commercial scale trials and registration procedure before they can be employed in practice. Among the new gaseous application technologies that have successfully replaced fumigants are the manipulation of modified atmospheres (MAs) alone or at high temperatures, and high pressure carbon dioxide that needs to be further explored for specific applications. A recent development is the use of MAs in a low-pressure environment. These niche applications of MAs that have resulted in very promising application treatments with market acceptability, should serve as models for global challenges for new application methods.

Key words: fumigation, methyl bromide alternatives, phosphine, gaseous treatments, botanicals, modified atmospheres.

Introduction

Increased public concern over the adverse effects of pesticide residues in food and the environment has led to the partial substitution by alternative control methods. Therefore, non-chemical and environmentally and user-friendly methods of pest control in the post harvest sector are becoming increasingly important. It is worth noting that of the 16 fumigants listed in common use some 22 years ago by Bond (1984), only very few remain today. Most of these fumigants have been withdrawn or discontinued on the grounds of environmental safety, cost, carcinogenicity and other factors. Methyl bromide (MB) has been phased out in developed countries since 2005 and will be phased out in developing countries by 2015, because of its contribution to stratospheric ozone depletion (UNEP 2002). Although there are exemptions for quarantine and pre-shipment purposes, as well as the possibility to apply for exemptions where no alternative exists, the applicant has to demonstrate that every effort is
being made to research alternative treatments. In contrast; phosphine remains popular, particularly in developing countries, because it is easier to apply than MB. However, many insects have developed resistance to phosphine over the last decade (Cao et al. 2003; Savvidou et al. 2003).

Food industries and particularly exporters are dependent on fumigation as a quick and effective tool for insect pest control in food commodities. Following on the WTO and Free Trade Policies, trade traffic of foodstuffs across the world has been considerably increased. Consequently, fumigation for disinfecting stored food commodities has been playing a significant role. Some developed countries have adopted the approach of zero tolerance of insect pests in food commodities. On the other hand, the fumigation technology that is required to obtain this zero infestation has been facing threats/constraints because of regulatory implementation and the development of resistance (Arthur and Rogers 2003).

The aim of the present paper is to elucidate the existing restrictions in view of the new global challenges to the use of gaseous treatments in stored products. These challenges derive from the increased demand of competitive markets for quality in food commodities free from pest and pesticide contaminants on the one hand, and the need to find and the cost involved in adopting alternative control measures on the other. They have resulted in efforts to register new fumigants in several countries, and in the development of new technologies as alternative control methods.

**The gaseous treatments**

The gaseous treatments may be categorized into three groups; a) residue-leaving fumigants that are synthetically produced volatile chemicals; b) volatile essential oils of botanical origin; and c) non-residual modified atmospheres (MAs).

**a) Fumigants and their current status:**
The chemical treatments discussed in this presentation are categorized under structural treatments and commodity fumigations. Among the synthetic chemical treatments, the list today is limited to MB, phosphine, sulfuryl fluoride, propylene oxide, carbonyl sulphide, ethyl formate, hydrogen cyanide, carbon disulphide, methyl iodide, ozone, and carbon dioxide.

**The current most commonly used fumigants**

*Methyl bromide*

One of the main features that make MB a commercially desirable fumigant is its speed of action. In addition, MB has a number of additional desirable features including its recognition by quarantine authorities, and its broad registration for use; it also has good penetration ability, and the commodity airs rapidly after exposure. When considering alternatives, the above properties need to be viewed against a background of MB as a highly toxic, odorless gas with substantial ozone-depleting potential and adverse effects on a number of durables, particularly loss of viability, quality changes, taint and residues.

MB plays an important role in pest control in durable and perishable commodities and particularly in quarantine treatments. The Montreal Protocol, an international treaty developed to protect the earth from the detrimental effects of ozone depletion and signed by 175 countries, is now phasing out ozone depleting compounds including MB on a worldwide basis. Accordingly, legislative changes have been made in different countries to control the use of MB, which has an average ozone depleting potential of 0.4. The ban on MB currently exempts quarantine and pre-shipment (QPS) treatments, emergency uses and certain critical
uses where no alternatives have yet been developed (TEAP 2000). However, these exemptions will be reviewed periodically in international meetings and they might not continue forever. In the absence of suitable alternatives, this loss of MB as a fumigant could seriously affect the protection of stored and exported food commodities from pest organisms. To combat this situation, one approach has been to accept the use of MB where no alternatives exist, but, after fumigation, to absorb the gas for recycling or to destroy it instead of releasing it to the atmosphere. There has been some limited implementation of recovery and recycling for MB, mainly in North America and Europe. Recovery and recycling systems are generally complex and expensive to install compared with the cost of the fumigation facility itself. These systems would also require a level of technical competence not normally found at fumigation facilities. Therefore, examples of recovery and recycle in current commercial use are few.

**Phosphine**

Phosphine is available in solid preparations of aluminium or magnesium phosphide and in cylinders containing carbon dioxide ECO2 FUME® or nitrogen FRISIN®. Lately, on-site phosphine generators that can release the fumigant up to the rate of 5 kg h⁻¹ are available in some countries (Argentina, Chile, China and USA). Metal phosphide formulations with slow or altered rates of phosphine release have been developed and tested in Australia (Waterford and Asher, 2000) and India (Rajendran, 2001). Improved application techniques such as the “Closed Loop System” in the USA, SIROFLO® and SIROCIRC® in Australia and PHYTO EXPLO® in Europe have been developed for application in different storage situations. Insect resistance is a serious concern that threatens the continued effective use of phosphine. Phosphine fumigation protocols have been revised in different countries to tackle the problem of insect resistance to the fumigant. Two major restrictions of phosphine are that it requires several days of exposure to achieve the same level of control as that of MB, and that it corrodes copper and its alloys and therefore electrical and electronic items need protection from exposure to the fumigant. Phosphine also reacts to certain metallic salts, which are contained in sensitive items such as photographic film and some inorganic pigments.

**Newly considered fumigants:**

**Sulfuryl fluoride**

Sulfuryl fluoride has been used as a structural fumigant for dry wood termite control for the past 45 years, but it also has potential applications in disinfecting flour mills and food factories (Bell et al. 1999). Although it can be used effectively for insect pest control in dry tree nuts and food grain, data are scarce on the effect of sulfuryl fluoride on quality of the treated commodity and persistence of residues. The fumigant is more penetrative into treated commodities than MB. Insect eggs are the most tolerant stage for sulfuryl fluoride. The relative egg tolerance can be overcome by increasing the exposure period and by raising the treatment temperature (Bell et al. 1999). Sulfuryl fluoride has been registered and used as a structural fumigant in Germany, Sweden and the USA. Sulfuryl fluoride is available under the trade name “Vikane” containing 99.8%sulfuryl fluoride and 0.2%inert materials. Apart from the USA, China has been producing sulfuryl fluoride (trade name “Xunmiejin”) since 1983 (Guogan et al. 1999). Also, sulfuryl fluoride can be applied under reduced pressure so that the exposure period can be drastically reduced (Zettler and Arthur, 2000). The fumigant was noted as highly toxic to diapausing larvae of the codling moth, *Cydia pomonella* in stored walnuts (Zettler et al. 1999). Sulfuryl fluoride is now registered under the new trade name “ProFume®” for the protection of stored food commodities (Schneider et al. 2003). ProFume® is registered in the US to allow virtually all mills and food processing facilities to test, adapt and consider adoption as an alternative to MB. Additionally, registration coverage in EC
countries for numerous milling and food processing applications is broad, and increasing (TEAP 2006).

**Propylene oxide**

Propylene oxide (PPO) is a colorless and flammable liquid, and is used as a food emulsifier, surfactant, cosmetics and starch modifier. Under normal temperature and pressure PPO has a relatively low boiling point (35°C) and a noticeable ether odor (Weast et al. 1986). It is a safe fumigant for use on food; it is registered and used in the USA as a sterilant for commodities such as dry and shelled walnuts, spices, cocoa powder and nutmeats (Griffith 1999). A disadvantage of PPO is that it is flammable at from 3 to 37% in air, and therefore, to avoid flammability it should be applied under low pressure or in CO₂-enriched atmospheres. Griffith (1999), in preliminary tests on some stored product pests, indicated that PPO has insecticidal properties under vacuum conditions as a fumigant. Navarro et al. (2004) studied the relative effectiveness of PPO alone, and in combination with low pressure or CO₂.

**Carbonyl sulphide**

Carbonyl sulfide (COS) is naturally present at low levels in food grains, vegetables (*Brassica* spp.) and cheese. Research work on carbonyl sulfide in Australia, Germany and the USA reveal that the egg stage is highly tolerant to the fumigant. Reports from Australia indicate that the fumigant does not affect the quality of wheat, and germination is not affected. However, investigations on carbonyl sulfide carried out in China showed contradictory results. Xianchang et al. (1999) reported that carbonyl sulfide affects germination of cereals except sorghum and barley, and imparts off-odour. Milled rice after treatment of paddy rice with carbonyl sulfide at the above dosages had an undesirable odour. Change in colour was also observed in fumigated soybeans. Zettler et al. (1999) also noticed an off-odour during the first 24 h of aeration in walnuts that were fumigated with carbonyl sulfide. It is suspected that hydrogen sulfide present in the supplied product, as an impurity, is partly responsible for the off-odour problem (Desmarchelier 1998).

**Ethyl formate**

Ethyl formate is known as a solvent and is used as a flavoring agent in the food industry. It is naturally present in certain fruits, wine and honey. In India, extensive laboratory tests against insect pests of food commodities and field trials on bagged cereals, spices, pulses, dry fruits and oilcakes have been carried out on the fumigant (Muthu et al. 1984). Currently ethyl formate is being used for the protection of dried fruits in Australia. It has been found suitable for in-package treatment of dried fruits. Studies in Australia indicate that, unlike phosphine, ethyl formate is rapidly toxic to storage insects including psocids (Annis and Graver 2000).

**Hydrogen cyanide**

Hydrogen cyanide (HCN) is currently registered only in India, New Zealand and with severe restrictions in Germany. HCN was one of the first fumigants to be used extensively under "modern" conditions. Its use for treating trees under tents against scale insects was developed in California in 1886 (Wogulum 1949). The high dermal toxicity of the gas makes it hazardous to applicators. HCN is one of the most toxic of insect fumigants; it is very soluble in water. HCN may be employed for fumigating many dry foodstuffs, grains, and seeds. Although HCN is strongly sorbed by many materials, this action is usually reversible when they are dry, and, given time, all the fumigant vapours are desorbed. With many foodstuffs, little, if any, chemical reaction occurs, and there is no detectable permanent residue. Because of the high degree of sorption at atmospheric pressure, HCN does not penetrate well through the bulk of some commodities.
Carbon disulphide
Carbon disulfide (CS₂), an old fumigant, is used at the farm level in some parts of Australia and to a limited extent in China (TEAP 2000). The major advantage of carbon disulfide is its small effect on seed germination. However, residues of carbon disulfide persist in treated commodities for a longer period than that of other fumigants (Haritos et al. 1999). The reduction in baking quality of wheat treated with this fumigant was shown by Calderon et al. (1970). Some of the limitations of the fumigant include high flammability, long exposure period, persistence in the treated commodity, lack of residue limits set by Codex Alimentarius and high human toxicity.

Methyl iodide
Methyl iodide has been patented as a pre-plant soil fumigant for control of a broad range of organisms including nematodes, fungi, and weeds (Grech et al. 1996) and the patent has subsequently been expanded to include structural fumigation against termites and wood rotting fungi (Ohr et al. 1998). Methyl iodide's potential as a fumigant for postharvest pest control has been known for more than 68 years (Lindgren, 1938). However, economic considerations at that time precluded its development in favor of the less-expensive MB. Methyl iodide was found to be very effective as a space fumigant (Shaaya et al. 2003; Zettler et al. 1999), being most toxic to eggs and least toxic to adults of Tribolium confusum (Tebbets et al. 1986). Yokoyama et al. (1987) showed that methyl iodide could prove valuable as a quarantine treatment for Carpocapsa pomonella in fresh fruits and as a rapid commodity disinfestation treatment of 24h or less. The fact that the US Environmental Protection Agency has listed methyl iodide as a possible human carcinogen could preclude registration in the US, particularly in California where it is listed as a compound known to cause cancer (EPA, 1998).

Cyanogen
Cyanogen (C₂N₂) is a colorless gas with almond like odor and was patented as a new fumigant effective against insects and microorganisms (Yong and Trang 2003). It is highly toxic to stored product insects and is fast acting. It has a good penetration through the grain mass and it desorbs quickly. It is phytotoxic and affects germination of treated seeds. But, it has potential for space and flour/rice mill fumigations and disinfestations. Yong and Trang (2003) compared the contrasting characteristics of cyanogen, MB and phosphine as fumigants.

Ozone
Ozone, a known sterilant, can be used as an insect control agent in food commodities at levels less than 45 ppm. Ozone is readily generated from atmospheric oxygen and is safe to the environment when used for fumigation. However it is highly unstable and breaks down to molecular oxygen quickly. A major disadvantage with ozone is its corrosive property towards most of the metals (Mason et al. 1999). Active research is going on to exploit ozone as a potential quarantine treatment for controlling stored-product pests (Hollingsworth and Armstrong 2005).

b) Volatile essential oils of botanical origin:
The application of botanical extracts as fumigants in the protection of stored products from insect attack is in its infancy (Cox 2002). There have been many plant extracts tested for their fumigant toxicity effect on stored product insects. Pascual-Villalobos (2003) studied the insecticidal effects of a group of plant essential oils (caraway, coriander, sweet basil, and garland chrysanthemum) against the damaging legume and cereal storage pests. Essential oils containing monoterpenoides were noted to be toxic to some stored product insects and
comparable to MB (Isman, 2000; Shaaya et al. 2003; Tunc et al. 2000; Weaver and Subramanyam 2000). The tested volatile plant extracts have the characteristics of essential oils with a typical aromatic scent from the plant from which they were extracted. Therefore, because of their aromatic nature, plant extracts may be applied in empty premises or to commodities such as seeds where the scent of the volatile essential oil would not present a restriction after the treatment. Most studies on volatile plant extracts have shown their efficacy in empty fumigation chambers. Due to their strong absorption, their application in bulk stored commodities is associated with poor penetration ability into the deep layers. Large scale applications that demonstrate the penetration capacity of these volatile oils are lacking in the literature. A major delaying factor to the use of these oils is that such alternatives of plant origin require toxicological and safety data for registration for use as fumigants.

c) Non-residual gaseous treatments, modified atmospheres (MAs):
The objective of MA treatments is to attain a composition of atmospheric gases rich in CO₂ and low in O₂, or a combination of these two gases at normal or altered atmospheric pressure within the treatment enclosure, for the exposure time necessary to control the storage pests and preserve the quality of the commodity. Terms used in reference to MA storage for the control of storage insect pests or for the preservation of food have appeared in the literature as CA, as sealed storage, or atmospheres used at high or low pressures to define the same method of treatment but using different means.

New application technologies that have successfully replaced fumigants

Cereal grain preservation
The initial research carried out during recent decades was concentrated first on the possible application of the MA technology to cereal grains (Adler et al. 2000; Banks and Annis 1990; Navarro 2006).

Tree nuts and dried fruits preservation
The possibility of applying MAs to control insects in dried fruits and tree nuts has been reviewed by Soderstrom and Brandl (1990). The influence of low O₂ or high CO₂ atmospheres as alternatives to fumigation of dried fruits has also been investigated by Soderstrom, and Brandl (1984); and Tarr et al. (1994). Ferizli and Emekci (2000) applied CO₂ for treating dried figs in a gastight flexible storage unit loaded with 2.5 tonnes of dried figs in perforated plastic boxes. These conditions resulted in complete mortality of both insects and mites. Full scale commercial application of organic raisins is being applied in California since 1984 (Navarro 2000).

Application of MAs at elevated temperatures
The influence of temperature on the length of time necessary to obtain good control with MAs is as important as with conventional fumigants. Navarro and Calderon (1980) compared the effect of temperature on the exposure time required to produce the mortality of adults of three storage insects in MAs. Donahaye et al. (1994) reported on responses of larval, pupal, and adult stages of two nitidulid beetles exposed to simulated burner-gas concentrations at three temperatures of 26, 30, and 35°C. Soderstrom et al. (1992) examined the influence of temperature over the range of 38–42°C on the effects of hypoxia and hypercarbia on Tribolium castaneum adults. Their results clearly indicate that raised temperatures could be used to reduce treatment duration. Navarro et al. (2003) showed the strong influence of temperatures of 35°, 40°, and 45°C on mortality of all four development stages of Ephestia cautella when the insects were exposed to CO₂ concentrations varying from 60 to 90% in air. Bell and Conyers (2002) investigated the potential to kill pests using MAs at raised
temperatures to increase their speed of action. These works led to the application of MAs at elevated temperatures by ECO2 in Holland with the objective to reduce the exposure times commercially to control pests in imported tobacco products, cocoa beans, rice, cereals, grains, nuts, peanuts, pulses, seeds and spices, as well as furniture and artifacts.

**Vacuum treatment and V-HF technology**

In a low-pressure environment, there is a close correlation between the partial pressure of the remaining O2 and the rate of kill. Until recently, this treatment could only be carried out in specially constructed rigid and expensive vacuum chambers. A practical solution has been proposed named the vacuum hermetic fumigation (V-HF) process that uses flexible liners (Finkelman et al. 2003). To achieve this, sufficiently low pressures (25-50 mmHg absolute pressure) can be obtained (using a commercial vacuum pump) and maintained for indefinite periods. This technology is currently in use at commercial level for pest treatment of organic soybeans and flours in Israel.

**High pressure carbon dioxide treatment (HPCT)**

Carbon dioxide still remains slower-acting and more expensive than phosphine or MB. CO2 treatments can be significantly shortened to exposure times that may be measured in hours using increased pressure (10-37 bar) applied in specially designed metal chambers that withstand the high pressures. Prozell et al. (1997) exposed cocoa beans, hazel nuts and tobacco to a quick disinestation process of exposure to carbon dioxide under pressure of 20-40 bars. Because of the high initial capital investment, these high-pressure chamber treatments are practical for high value products such as spices, nuts, medicinal herbs and other special commodities. A number of countries have adopted the use of this technology; among them are Germany and Turkey.

**Modified Atmosphere Packaging (MAP)**

Modified Atmosphere Packaging (MAP) is a technique used for prolonging the shelf-life period of fresh or minimally processed foods. In this preservation technique the air surrounding the food in the package is modified to a composition of low oxygen or a combination of low oxygen and high carbon dioxide. The interest in modified atmosphere packaging (MAP) has grown due consumer demand. This has led to advances in the design and manufacturing of polymeric films suitable for MAP. Under MA, products such as bakery goods, and dried foods are packaged. The effects of storage temperature and packaging atmosphere (air and N2) on the quality of almonds were studied by Garcia-Pascual et al. (2003). Guidelines for using modified atmospheres in packaged food, with special emphasis on microbiological and nutritional aspects, have been published by the Council of Europe (Anonymous 1999).

**Museum artifacts**

The possibilities of controlling pests in artifacts using inert gases were reported by Reichmuth et al. (1991, 1993). Museums throughout the world face the challenge of finding non-toxic methods to control insect pests. Recently several publications focus on practical rather than theoretical issues in the use of oxygen-free environments, presenting a detailed, hands-on guide to the use of oxygen-free environments in the eradication of museum insect pests (Maekawa and Elert 2002; Selwitz and Maekawa 1998).

As interest in modified atmospheres for food preservation peaked, conservation scientists began to study how this technology could be adapted to museum needs (Selwitz and Maekawa 1998). Although a carbon dioxide atmosphere had been favored for the preservation of foodstuffs, conservators saw more advantages in using nitrogen with low oxygen concentrations to treat museum artifacts, collectively termed "cultural property" because
anoxia provides a higher degree of inertness and is easier to establish for small-scale operations. The technology of MA has received the specific terminology of "anoxia" for the treatment of museum-artifacts, libraries, and among the manufacturers and suppliers of material and equipment for the use of nitrogen.

**Fresh storage of fruits and vegetables**
Fresh fruits and vegetables may be shipped or stored in controlled atmospheres. This topic is covered in depth in the book of Calderon and Barkai-Golan (1990) and in a more recently published chapter by Ben-Yehoshua et al. (2005).

**Narcissus bulbs treatments**
The large narcissus fly *Merodon eques* F. attacks narcissus bulbs and also bulbs of other geophytes. This species is a quarantine pest where complete mortality is required prior to export from Israel. Fumigation with MB has been used to eliminate narcissus fly infestation in flower bulbs due to its rapid killing time (4 h). However, MB is also known for its phytotoxic effect on the bulbs and its use is discouraged even for quarantine purposes in developed countries.

In experimental procedures, Donahaye et al. (1997) found that there was an extremely rapid depletion of O₂ within the sealed gastight enclosures in which the narcissus bulbs were placed for fumigation, due to the respiration of the newly harvested bulbs. This procedure also revealed the significant anoxia achieved within less than 20 hours (less than 0.1% O₂ and about 15% CO₂) during treatment at 28°C to 30°C and the possibility of using it alone as a control measure. The possibility of obtaining a bio-generated modified atmosphere utilizing the bulb respiration alone was adopted by Israeli farmers as a practical solution using specially designed flexible treatment chambers (Navarro and Donahaye 2005; Navarro et al. 2006). This MA method has been successfully applied by the narcissus bulb growers in Israel and fully replaced the use of MB since 2003.

**Conclusions**
No fumigant that has a broad spectrum of action like MB, and is inexpensive like phosphine, is presently available. Although there is no doubt that fumigation technology is extremely important for the protection of stored products, many demands are required from potential alternative fumigants, from the sensitivity and lack of resistance of target pests to requirements for registration of new fumigants and re-registration to maintain the use of old fumigants. However, there is increasing public concern over the adverse effects of pesticide residues in food and the environment. Existing gaseous alternatives to MB and phosphine suffer from the limitation that they may be useful for treating a particular type of commodity or for application in a specific situation only. Sulfuryl fluoride seems to emerge as a promising candidate fumigant for disinfesting stored food commodities, food-processing facilities and as a quarantine fumigant. Other fumigants are suitable to specific uses, such as propylene oxide for dry and shelled walnuts, spices, cocoa powder and nutmeats, ethyl formate can be suitable for dried fruits, carbon disulfide for seed materials, and carbonyl sulfide for grains. Plant extract essential oils and other volatiles of plant origin will need large scale demonstration of their penetration capacity, in addition to toxicological and safety data for registration for use as fumigants. The only gaseous treatment that retains the special capacity of fumigation for in-situ treatment of stored commodities, as well as offering a similar diversity of application technologies, is the MA method. MAs offer an alternative that is sustainable, safe, and environmentally benign to the use of conventional residue-producing chemical fumigants. The application of MA in several fields of application has already
received recognition and successfully replaced MB and phosphine. The major fields of application of MAs are: grain and pulses stored in bulk, protection of organic products, use of anoxia for museum artifacts, and libraries, and use of MAP for the food packaging industry. Newly developed MA methods for niche applications, have resulted in very promising applications such as for the treatment of seeds, narcissus bulbs, cocoa beans, dried fruits and nuts. The global challenges in stored products are the development of new and safer gaseous treatments, and new application methods of MAs that are commercially feasible.

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References


**Anobium punctatum** (Coleoptera, Anobiidae), a new pest of books in Israel

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**Abstract:** In 2004, *Anobium punctatum* was found to have caused a very serious infestation of books stored in one of the largest libraries in Israel containing approximately 5 million books. It was estimated that around 7% of the books were infested or suspected of being infested and required treatment. The damage caused is characteristic to the lifestyle of *A. punctatum*. This species is distributed in temperate climates, is common in Europe and North America and is considered to be the main pest of dry wood and dry wood products. It was recorded in Israel in the past from imported wood infested with larvae and in 1995. Several books were found infested with this species in one of the religious libraries in Israel. However, the damage was negligible and the population did not become established nor did it spread to other libraries in Israel. It is assumed that the dryness and high temperatures in Israel prevent the egg-laying activity of the adult beetles in nature. It is assumed that in the case of the severe library infestation mentioned above, the first infestation foci were of books brought from abroad. The level of infestation and damage and the long period of development indicated that the infestation had developed over many years. Two control operations are necessary in order to exterminate the beetles: fogging of the library to kill the adults and treatment of the books to kill the larvae and pupae. Three methods were considered: fumigation using methyl bromide; anoxia or freezing to -30°C, the last of which has already been implemented.

**Keywords:** *Anobium punctatum*, pests, library, books, Israel

**Introduction**

Beetles of the genus *Lyctus*, are known in Israel as pests of dry and processed wood. *Lyctus* was also the only beetle known in Israel as a pest of books which were printed and bound many years ago from paper and cardboard produced from wood and bound with starch glue (Wilamowski and Schnur, 2002). Many such books are very specialized and irreplaceable, and are valued due to their scientific and national importance. Since the 1960s, books have been produced using a greater proportion of synthetic materials and the component of wood is much smaller. Therefore such books are unsuitable for the development of the young stages of *Lyctus*. The female *Lyctus* lays eggs into the books. The larvae digest only the starch component of the books therefore the damage is mainly in the binding of the books which are glued with starch glue.

Apart from the common infestation of books with *Lyctus*, Halperin & Español (1978) reported that between 1948 and 1977, seven cases of specimens of *Gastrallus pubens* (Anobiidae) were found among insect collections in Israel, all originating from books. This species is known to feed on paper and bookbindings and is a pest in libraries (Español, 1963). The distribution of *G. pubens* includes arid areas of the Eastern Mediterranean and North Africa and especially the European coasts of the Mediterranean (Halperin & Español, 1978), but is not known to live and develop in Israel. There is a single report of *G. pubens* found on a
Moringa tree in Israel near the Dead Sea in 1977, but apart from this there have been no other reports of *G. pubens* in Israel in the wild (Halperin & Español, 1978). Therefore, it is assumed that the specimens they found in books were introduced with infested books from abroad and that these insect specimens infected the above book collections in Israel.

Calderon & Donahaye (1964) reported books in Tel Aviv infested with the species *Anobium punctatum* (deG.), in 1962. A number of books were found to be infested with the same species in a religious library in Israel in 1995. In both these cases, the damage was insignificant and there is no evidence that this species became established in Israel. According to Calderon & Donahaye (1964), Bytinsky-Salz (1966) and Halperin (1989) specimens of *Anobium punctatum* were brought to Israel mainly with old furniture from Europe. The distribution of this species is in countries with a temperate climate in Europe and South America (Robinson, 1990). Bytinsky-Salz (1966) maintained that *A. punctatum* cannot establish itself in Israel because of the high temperatures and dryness. However, as stated above, in 2004, the presence of *Anobium punctatum* was noticed in the Jewish National and University Library, the largest library in Israel and the damage to many of the books was enormous. This article represents the first known report of a large infestation of *A. punctatum* in books.

**Materials and methods**

In the more than 5 million book Jewish National University Library located in Jerusalem, infested books were found on three different floors containing the library book stacks. The total area of the bookstacks is 7703 m³ and contains 53,167 bookshelves, each 120 cm in width (Fig. 1). Extremely rare and valuable books are stored separately in a special, safe room, and these books were not infested. The temperature in the book stack halls is kept at between 24-26°C. There is no control of the relative humidity which varies between 30% and 35%.

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Fig. 1. View of book stacks in University Library.
In the three main halls there were many books that were printed as long ago as the 16th century and some are rare and irreplaceable copies. They deal with history, Judaism, Christianity, Islam and many other subjects.

Survey and checking: 1) A first survey was carried out by the library staff which initially identified the infestation and realized that the infestation was large and widespread. 2) An additional survey involved an entomologist (A.W.) and many specimens of adult beetles were collected from the bookshelves and some young stages were collected from inside books. 3) The identification of the specimens was done at the Entomology Laboratory, Ministry of Health, with the help of Dr. J. Donahaye of the Agricultural Research Organization, Bet Dagan, Israel. Some specimens were sent to the Smithsonian Institute in Washington D.C. by Prof. Shalom Appelbaum for confirmation of the identification. 4) A well-planned survey was made by Prof. S. Navarro in order to estimate the extent of the infestation and the damage. The survey was an essential and formed the basis for deciding on the most efficient treatment to eradicate the infestation and to save the books. 10 trained and guided librarians took part in the survey assisted by four entomologists. These survey personnel checked large sample areas on the three floors. The level of infestation and damage was estimated and a suitable treatment was decided upon.

Results

Identification of the pests: All the adult beetles which were collected belonged to the species *Anobium punctatum*, the common furniture beetle. The larvae and pupae of this species were found inside the books. Small numbers of live larvae and exuviae of the beetle *Anthrenus verbasci* (Dermestidae) were also found, some of them inside books and others associated with leather bindings. The infestation of *A. verbasci* was small and was not the main cause of the damage to the books. There were also a very small number of exuviae of *Attagenus* sp. (Dermestidae) amongst the books.

Description of the damage: The damage caused to the books is characterized by the larval activity of *A. punctatum* inside the books. The adults do not cause any direct damage. They live only 2-3 weeks and do not feed during this time, but only mate and lay eggs for the next generation (Simpson, 1994). This is the reason that a great number of dead adults were found on the book shelves and amongst the books.

In the infested books there were many holes and tunnels in which larvae and pupae were found (Fig. 2) and there were signs of gnawing in the pages. In the book bindings there were many characteristic emergence holes of the adults after the development of the young stages inside the books. These emergence holes were one of the signs of the infestation. There was an enormous amount of book-dust in the infested books and on the shelves, which is a result of the larval activity (Fig. 3). The book-dust was another sign of the infestation and the amount of book-dust indicated a serious infestation. The larva of *A. punctatum* is able to digest cellulose in the paper. It also gnaws the binding in which the starch glue is utilized as food. This often causes the disintegration of the binding (Fig. 4).

Extent of infestation and damage: Only books that were printed until the end of the 1950s were infested while the newer books were undamaged. The damage to a large number of books was more or less small and the books could still be used and read. A significant number of additional books suffered severe damage and required restoration before they could be used. A small number of books were completely destroyed and could not be restored for use (Fig. 5).
The estimation is that out of the 5 million books in the library, 7.3% of the books, i.e., 220,000, were infested or suspected to have an infestation and these books required control treatment in order to exterminate the pest (Navarro, 2004).

Fig. 2. Tunnels and holes in one of the infested books. Fig. 3. Book-dust from an infested book.

Fig. 4. Disintegration of book binding of infested book. Fig. 5. A very seriously damaged book.

**Discussion**

*Anobium punctatum* is known as a common pest of dry wood and its products in Europe and South America, but is not recorded in the literature as a common pest of books (Halperin and Español, 1978; Robinson, 1990). Several books were brought to Israel from abroad infested with *Anobium punctatum* several decades ago (Calderon and Donahaye, 1964) and other books were discovered in 1995 (Wilamowski, unpublished data). In the literature we found only one record of a book infested with *A. punctatum* (Simpson, 1994). The infestation found in the Israeli library is the first record of very serious damage caused by *A. punctatum* to books in libraries.

The survey carried out estimated that almost 220,000 books were infested or were suspected of infestation. A part of these damaged books did not necessarily have an active insects or larvae during the time of the survey, but could have been infested some years
previously. It is very hard to determine whether some books were still being actively infested, since the eggs and larvae, mainly the young stages, which hide in the tunnels, holes and binding, are very difficult and sometimes impossible to detect. Therefore the estimation of infestation which was made included books which may not have been actively infested at that time. It is very important to determine the numbers of books needing control operations in order to efficiently control and exterminate the pests from the library.

Even if a great number of the books are only suspected active infestation, it is still the highest level of infestation found in libraries in the world. In Yale University, 37,000 books were infested or suspected to be infested with *Gastrallus* sp. and were treated (Nesheim, 1984). Around 100 books were found to be infested with *Gastrallus pubens* in the University of Berkeley, and 1660 books were treated (Boal, 1990). *G. pubens* apparently reached the USA with infested books from Italy.

A very small number of live larval specimens, and a larger number of exuviae of *Anthrenus verbascii* were found in the library. This dermestid, like other Dermestidae is known to feed on dead bodies and exuviae of all stages of other insects, including those of the family Anobiidae. Generally, specimens of *A. verbascii* are found in the tunnels and holes created in wood by *A. punctatum* and they are considered as secondary pests (Hinton, 1945). *A. verbasci* is very common in Israel causing damage to a great variety of products and organic matter. It was not found in Israel as a pest to wood or books. A few specimens of exuviae of another dermestid, *Attagenus* sp., were also found in the library and this genus is also known to feed on other insects. Therefore the infestation of these two species in the library was secondary and was not responsible for causing damage to the books. In the infested Yale library some specimens of Dermestidae were also found (Nesheim, 1984).

Books infested with *A. punctatum* were brought to Israel from abroad in the past and it is assumed that the first infestation in the library was also caused by an infested book or books brought from abroad.

Larvae of *A. punctatum* finish their development and pupate within one year under optimal conditions in a European climate. Inside buildings, this development may take from 2 to 5 years (Hickin, 1953). In the infested library, suitable temperatures were found for the development of the larvae (24-26°C), but the relative humidity is very low: 30-35%. Under such microclimatic conditions, the development of the larvae is very long and slow and may continue for up to 3 years or more. This very long development period, the high population levels and enormous damage which was found indicates that the library had been infested for at least 15-20 years and perhaps more until it was discovered in 2004.

In order to control and to avoid the continuation of the damage, two operations were carried out: i) several foggings of the halls was carried out in order to kill the adults sitting on the shelves and books; and ii) freezing 220,000 books to -30°C to kill the young stages inside the books. Very important books which were especially valuable that were damaged to an extent in which they could not be used or read, are being restored in the Conservation department of the Library.

References


Potential of parasitic protozoans in biological control of stored product pests

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Abstract: The protozoans form a heterogenous group of single-celled eukariotic organisms of very diverse morphology, behavior and life cycle. Out of 15000 known protozoan species about 1200 are associated with insects and mites as symbionts, commensals and parasites. Several protozoans play important role in the natural control of stored products arthropods and may be considered for use in long-term biocontrol programs, attempts and introductions. Information useful for survey, identification and preservation of protozoans – recorded in living and dead stored product insects – will be provided. Such collected dead or infected specimens of insects or mites, should be submitted to reference laboratories for diagnosis and specialistic evaluation as to their potential use in biocontrol attempts.
Session 2: Biology and ecology of stored product pests
Description and putative function of the antennal sensilla of *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae)\(^1\)

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**Abstract:** The morphology of the antennal sensilla of both male and female *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) is described using Scanning Electron Microscopy complemented with Transmission Electron Microscopy. Five types of innervated sensilla as well as uninnervated microtrichia were found. These types are: sensilla trichodea; s. chaetica; s. basiconica; s. coeloconica; and s. placodea. No differences in shape, basic structure, and types of antennal sensilla were found between males and females. The types of sensilla of both sexes of *H. hebetor* were compared with what has been described in other parasitic Hymenoptera and their putative functions are discussed with reference to their morphology, distribution, ultrastructure and behavioural observations.

**Key words:** *Habrobracon hebetor*, antennal sensilla, SEM, TEM, behavioural observations.

\(^1\) Full text of this paper was submitted for publication after the conference, at the time of preparing the book of proceedings the citation of the article in press was:  
**Plodia interpunctella** (Hübner) mating suppression with an emulsive pheromone preparation – laboratory experiments

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**Abstract:** Three experiments were conducted in unventilated laboratory rooms (125 m³ - first (I) experiment, and 168 m³ – second (II) and third (III) exp.). Walls of the rooms, except ceilings and floors, were sprayed with the pheromone emulsive formulation of \((Z^9, E^{12})\)-tetradeca-9,12-dien-1-ol acetate (Z9E12-14Ac). Amounts of Z9E12-14Ac sprayed on the walls were: 32 mg/80 m² (I exp.) and 24 mg/120 m² (II and III exp.). The residual amounts of the pheromone remaining on the walls were checked after each 7 days. Each experiment consisted of two 7-day tests and a 7-day interval between them. An attractant for **P. interpunctella** female (consisting of nuts, chocolate and a mat of silken webbing produced by larvae) was exposed in the room, and 20 to 50 pairs of virgin 2-3 day adults of **P. interpunctella** were released at the beginning of each test. After 7 days the attractant was collected and kept in a rearing room to breed adults from the eggs laid on them. Control tests were performed in the same manner but without using the pheromone. The bred **P. interpunctella** moths were counted. Reductions of the moth populations were 95-75 % in comparison with the control tests.

**Key Words:** *Plodia interpunctella*, Lepidoptera, Pyralidae, stored-product, mating disruption

**Introduction**

Investigations of **Plodia interpunctella** (Hübner) mating disruption using synthetic sex pheromone, have more than 30-year history. The main component of pheromone blend released by females – \((Z^9, E^{12})\)-tetradeca-9,12-dien-1-ol acetate (ZETA)- is common to several stored-product pest species, e.g.: **Cadra cautella** (Walker) and **Ephestia kühniella** (Zeller). This compound was identified by Brady et al. and by Kuwahara et al. and published in 1971. Since then three additional components were identified: \((Z^9, E^{12})\)-tetradeca-9,12-dien-1-ol, \((Z^9, E^{12})\)-tetradeca-9,12-dien-1-al, \((Z^9)\)-tetradec-9-yn-1-ol acetate. Sonner and Whitmer (1977) investigated **P. interpunctella** population growth in the presence of synthetic sex pheromone composed of ZETA (70 %), \((Z^9, Z^{12})\)-tetradeca-9,12-dien-1-ol acetate (15 %), antioxidant and other impurities. The pheromone substantially reduced the growth rates of the population, but only at low population densities of these insects. Tests were conducted in rooms of 6.1 x 6.1 x 2.4 m. The rooms held 18 pheromone dispensers each releasing 1 mg of pheromone a day, which gave an average dose of ca. 0.2 mg/m³. At population densities of 0.1 or less insect/m² of surface, the pheromone effectively limited population growth. But at population densities of 0.3 or more insect /m², the release of synthetic pheromone had little impact on populations. Ryne at al. (2001) conducted their investigations in 2.5 x 2.5 x 2.5 m passively ventilated cubicles. Population densities were 10 and 30 insects. The synthetic pheromone (of one- or four-components) was emitted from MSTRS dispensers set giving off 0.075, 0.75 and 3.75 mg per spray of the main component every 15 min. The disruption effect
was measured by the presence of spermatophores in female’s bursa copulatrix. No statistical
differences in mating disruption effect was observed between treatments, however mating was
interrupted up to 93% by using high emission rate of pheromone. In the opinion of the authors
complexity of the environment and lightening affect mating disruption in the presence of
synthetic sex pheromone, because complexity of environment affects moth aggregation. At
close distances males and females can recognize each other by visual and acoustic cues.

Our aim was to investigate whether there is a possibility of camouflaging the
*P. interpunctella* female’s pheromone plume and to control mating by using ZETA emulsive
preparation for spraying on the walls of a store room.

**Methods and materials**

The efficiency of ZETA emulsive preparation was investigated in a laboratory rooms. Results
of pheromone preparation application were measured as the quantity reduction of *P.
interpunctella* moths bred from the eggs laid on an attractant as compared to those bred in the
control without the attractant. The best level for attractant placing was stated before
beginning of the experiments.

Three experiments were performed. Each of the experiments lasted 3 weeks and
consisted of two 7-day tests and one 7-day interval between them. The best attractant placing
level investigation (which partly served also as a control test for the first experiment) and the
first of the experiments were conducted in 10m x 5m x 2.5 m room. The second experiment
was conducted in two 8m x 7m x 3m rooms. Thus control tests (without the pheromone) were
conducted at the same time. The third experiment was conducted in a similar manner. All
rooms were not ventilated.

All walls and furniture of the experimental rooms were covered with polyethylene foil
sheets sprayed with pheromone emulsive preparation. The ceilings and the floors were not
covered. The pheromone emulsive preparation was not sprayed immediately on the painted
concrete walls because it would be impossible to determine amount of pheromone remaining
on the walls, so it was sprayed on polyethylene sheets. The sheets were stuck to the walls with
a glue tape. Small control foil sheets were hung in the experimental rooms and served to
determine the amount of pheromone remaining on the walls.

After covering the walls with the sprayed foil sheets three control sheets were hung, the
attractant was placed in the room and the determined number of *P. interpunctella* virgin moth
pairs was released the same day. After 7 days the first of the control sheets was taken away
and the attractant was collected and kept in a rearing room in order to breed adults. After 7-
days interval the second control sheet was taken away, a new attractant was placed in the
room and the determined number of *P. interpunctella* virgin moth pairs was released. The
control tests were conducted simultaneously.

**ZETA** – (Z9,E12)-tetradeca-9,12-dien-1-ol acetate (purity of 93 %) was manufactured by
Bedoukian Research, Inc.

**Emulsive preparation** of ZETA was made in Institute of Industrial Organic Chemistry in
Warsaw.

**Insects** – *P. interpunctella* moths were taken from culture maintained at the Department of
Applied Entomology, Agricultural University in Warsaw. Larvae were reared on artificial diet
consisting of 1000 g crushed nuts, 250 g wheat germs and 50 g brewer’s yeast. Insects were
segregated by sex during the pupal stage. Emerged males and females were kept in separate
chambers at 27±1°C and 70±5% r.h., with a 12-h light-dark cycles. Released moths were 2-3
day adult.
**Attractant** – The attractant consisted of nuts, chocolate and mats of silken webbing produced by *P. interpunctella* larvae. During each experiment 0.25 kg of the attractant was exposed on a flat dish, covered with corrugated paper sheets, placed at the level of 100 cm above the floor. After 7-day exposure the attractant was collected and kept in a rearing room equipped with controlled at 27±1°C and 70±5 % r.h. to breed adults from the eggs which were laid.

**Preparing of the foils** – Sheets of the 10 m² polyethylene (35 g/m²) were arranged horizontally and sprayed uniformly with water emulsion containing ZETA. After drying, the sheets were folded, enveloped with aluminium foil and stored for 2-3 days at 5 °C until the beginning of the experiment. In experiment I the foils were sprayed with 0.4 mg of ZETA on each m² (32 mg at the sum). In experiments II and III the sheets were sprayed with 0.2 mg ZETA on each m² (24 mg at the sum).

**Control sheets** – The small (0.25 m²) control sheets were sprayed with exactly determined amounts of the emulsive pheromone preparation: 0.1 mg in experiment I and 0.05 mg in experiments II and III. At the beginning of each experiment three control sheets were hung at level of 2 m along the medium line of the room. Every 7 days one of them was taken down and extracted with hexane. The extract was concentrated to 1 mL volume and the amount of extracted ZETA was determined by GC analysis.

**Additional conditions of the experiments**
The investigations were conducted during May – September 2006. The medium temperature of the rooms changed from 25°C at the beginning to 21°C at the end of the experiments. The rooms were lighted (without sunshine access) through the windows covered with foils. The numbers of released moth pairs were as described in Table 2.

**Results**
The results of the best attractant placing level investigation are showed in Table 1. Twenty pairs of *P. interpunctella* moths were released in this experiment.

Table 1. Quantities of *P. interpunctella* adults bred from eggs laid on the attractant placed on different levels.

<table>
<thead>
<tr>
<th>Level [cm]</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity of bred adults</td>
<td>65</td>
<td>256</td>
<td>147</td>
<td>458</td>
</tr>
</tbody>
</table>

The results of the experiments – amounts of ZETA extracted from the control sheets, quantities of *P. interpunctella* bred adults and resulting calculated mating suppression – are showed in Table 2. The tests of experiment I were conducted without control tests, but we can qualify results of these tests as probably high mating suppression, referring them to the results of the best attractant placing level investigation.

**Discussion**
Usually results of tests concerning investigations of *P. interpunctella* (and not only) mating disruption effects, are referred to as amounts of emitted ZETA per m³ of room volume and per day or other time unit, but our rooms were unventilated, so emitted ZETA vapors
remained inside of them. Doubtless some amount of ZETA penetrated into the floor. We could neglect this amount and use average concentration of ZETA as independent variable, but ZETA vapor density is very high comparing to the density of air, so ZETA concentration was highest near the walls and on the floor and diminished in the direction of the ceiling and of the room center. Diffusion of ZETA and only small convective air motions were insufficient to equalize ZETA concentration, so average concentration space would be very limited and located at a low level of the room. Several calling females were seen near the attractant. Remaining moths were situated on the walls – not aggregated – usually above level of 150 cm. Because of these reasons we decided to use average emission intensity (EI - mg/m²-day) as our independent variable.

Table 2. Conditions and results of pheromone preparation application.

<table>
<thead>
<tr>
<th>Exp. No</th>
<th>Test No</th>
<th>Numbers of released moth pairs</th>
<th>Time from start of the Experiment [day]</th>
<th>Amounts of ZETA on the control sheets [mg]</th>
<th>Quantities of the P. interpunctella bred adults</th>
<th>Mating Suppression [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>I</td>
<td>20</td>
<td>0</td>
<td>0.1</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>30</td>
<td>7</td>
<td>0.007</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>I</td>
<td>20</td>
<td>0</td>
<td>0.05</td>
<td>20</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>20</td>
<td>7</td>
<td>0.0025</td>
<td>65</td>
<td>452</td>
</tr>
<tr>
<td>III</td>
<td>I</td>
<td>50</td>
<td>0</td>
<td>0.05</td>
<td>51</td>
<td>732</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>50</td>
<td>7</td>
<td>0.023</td>
<td>176</td>
<td>698</td>
</tr>
</tbody>
</table>

Table 3. Effects of P. interpunctella mating disruption referred to first 2-day average emission intensity and insect population density.

<table>
<thead>
<tr>
<th>Exp. No</th>
<th>Test or break</th>
<th>7-day average EI [mg/m²-day]</th>
<th>first 2-day average EI [mg/m²-day]</th>
<th>Insect population density [insects/m²]</th>
<th>Mating suppression [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Test I</td>
<td>0.053</td>
<td>0.2&gt;EI&gt;0.053</td>
<td>0.5</td>
<td>Prob. high</td>
</tr>
<tr>
<td></td>
<td>Break</td>
<td>0.017</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Test II</td>
<td>0.017</td>
<td>0.017</td>
<td>0.75</td>
<td>Prob. high</td>
</tr>
<tr>
<td>II</td>
<td>Test I</td>
<td>0.027</td>
<td>0.1&gt;EI&gt;0.027</td>
<td>0.33</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Break</td>
<td>0.007</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Test II</td>
<td>0.007</td>
<td>0.007</td>
<td>0.33</td>
<td>85</td>
</tr>
<tr>
<td>III</td>
<td>Test I</td>
<td>0.015</td>
<td>0.1&gt;EI&gt;0.015</td>
<td>0.83</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Break</td>
<td>0.0091</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Test II</td>
<td>0.0028</td>
<td>0.091&gt;EI&gt;0.028</td>
<td>0.83</td>
<td>75</td>
</tr>
</tbody>
</table>

Average EI can be calculated for each week of experiment conduction, but the most important were two first day of each test. During these days EI was higher than during all
week, but lower than 0.2 mg/m²/day (I test of) or 0.1 mg/m²/day (I tests of II and III exp.). For every II test of each experiment first 2-day average EI had a higher value than 7-day average EI, but not higher than the 7-day break average EI. Results of this calculation are found in Table 3.

The minimum average EI of ZETA, sufficient to reach mating suppression of more than 90 %, can be found in the compartment of 0.015-0.1 mg/m²/day, when population density is of 0.83 insect/m². Below this minimum EI value, high mating suppression can be achieved for lower _P. interpunctella_ population densities.

**Acknowledgements**

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**References**


Attraction of *Sitophilus zeamais* Motschulsky to different types of cereal pasta

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Abstract: Pasta factories use flour, a raw material obtained principally from hard wheat but also from other cereals (e.g. barley, corn, kamut, rice, spelt) which come directly from the mill. Flours are the main source of reproduction and diffusion of pests because of the structure of the mill, the possible use of infested cereals and large quantity of dust always present. As a result *Ephestia kuehniella* Z., *Lasioderma serricorne* (F.), *Oryzaephilus* spp., *Plodia interpunctella* (Hb.), *Rhyzopertha dominica* (F.), *Sitophilus* spp., *Stegobium paniceum* (L.), and *Tribolium* spp. present in warehouses, silos and in mills are carried into pasta factories where they can multiply. In many Italian pasta factories new technics and Integrated Pest Management have been suggested for the prevention and control of pests. Despite these provisions, the problem of pest attacks by *Lasioderma*, *Plodia*, *Rhyzopertha*, *Sitophilus* and *Stegobium* in pasta factories, from packaging to the consumer, remains unsolved because of specific aspects of used packaging and the negligence in warehouses and stores in addition to the long average shelf-life of the product. In that context, in our study, we compared attraction of *Sitophilus zeamais* Motschulsky to 8 different types of Italian cereal pasta produced with: barley, buckweath, durum wheat, 5 cereals (a mixture of durum wheat, barley, spelt, oat, and rye), kamut, corn, rice, and spelt. The results obtained in olfactometer tests demonstrated that *S. zeamais* adults revealed preferences in decreasing order for pasta realized with corn, buckweath, durum wheat, rice, barley, kamut, spelt, and 5 cereals.

Keywords: cereal pasta, different types, *Sitophilus zeamais*, olfactometer preferences

Introduction

General aspects

Every continent produces pasta, ranging from a minimum of 0.4% in Australia to a maximum of 38% in the EU. In north America pasta production is 18.2%; in central-South America; 22.4%, in Africa 4.4% and in Asia 2.1%. The world pasta production last year was approximately 11,400,000 tons; Italy is the top producer with 28.8%, followed by USA with 17.5%, then Brazil, Turkey and Russia.

In Italy global pasta production reached 3,224,646 tons, 3,106,246 tons of which consisted of dried pasta and 118,400 tons of industrial fresh pasta (UNIPI, 2006). As for domestic consumption (1,555,900 tons), annual per capita consumption was 28 kg, consisting mostly of dried pasta (26 kg), whereas the consumption of fresh pasta (2 kg) is gradually increasing. Sweden and Greece follow Italy with about 10-9 kg per capita per year. In the Americas, Venezuela is at the top, but the USA follows closely.

Exports (1,730,050 tons) accounted for 53% of production and there have been signs of slight growth. In 2006, 65% of exports were destined to the 25 member EU countries where
Germany is the most important market. As far as non EU markets are concerned, there were good performances in Japan, Russia and the new markets of China and India.

In Italy 80% of pasta consumed belongs to seven types (spaghetti, penne, rigatoni, macaroni, fusilli, farfalle, bucatini). Globally short cut pasta types are preferred, but spaghetti remains the classic type. The most popular type of pasta consumed is clearly the traditional dry pasta, but other kinds, such as fresh pasta – even if in reduced volumes in respect to dry pasta – show some increase and represent an important value segment. The situation of egg pasta is quite different in Europe. It accounts for less than 10% of total pasta consumption in Italy, Greece and Sweden, about 15% in France and more than 45% in Germany, Austria and Switzerland.

The raw material used for pasta production over the globe is not the same. In some north European countries no official proclamations exist while in other countries, such as Italy, durum wheat is the only raw material allowed. In some countries both durum and soft wheat are permitted. In the Americas, on the basis of the guidelines, the situation is quite different. In USA the Food and Drug Administration considers durum wheat to be the best raw material for pasta, but soft wheat is also allowed. In Canada and Panama only durum semolina is used. In Venezuela durum wheat represents the favourite raw material, while the opposite situation exists in Brazil where soft wheat flour is used exclusively.

**Development of new pasta**

In Europe, generally greater attention is paid to the health aspects of food, as well as for socializing, for new products, for ethnic cuisine, but also more or less prevailing local cultural practices. On this basis, new opportunities could come from convenience food, food with perceived health benefits, functional food, low fat foods and also premium quality and organic food.

Opportunities and innovations for pasta should consider habits and traditions of different countries; for example in Italy the use is strictly linked to basic needs other than nutritional consideration, while in USA the nutritional aspect prevails.

The development of new products with an increased nutritional value is now a reality in various markets, but owing to different cultural factors the penetration of these new products is not the same all over the world. A clear example is whole wheat or enriched pasta. The penetration of these new products in Europe at present is 1-2% as compared to the pasta global market, while in USA where more attention is paid to the nutritional values, it represents 10%. In Europe the only exception is Sweden where this kind of product has reached about 20% of penetration in the pasta market in the last three years.

The desire to increase the nutritional value of food and also to face and find solutions to the diffusion of various allergies and diseases such as the coeliac disease have also helped the development of the new types of pasta as for example pasta made from "kamut", pasta made of farro and gluten-free pasta particularly those without gliadin. The raw materials used for the latter are corn, quinoa, cassava, rice and potato. Coeliac disease is a digestive illness of the human system. People who suffer from that illness cannot tolerate gluten because their immune system reacts to it damaging the villi.

- **Barley pasta.** One of the earliest cultivated cereals in the world (*Hordeum vulgare* L.), is now gaining renewed interest particularly for the production of functional food, mainly because of its β-glucan, tocopherol and trocotrietol content.

- **Buckwheat pasta.** Common buckwheat (*Fagopyrum* spp.) is traditionally used for pasta products, for blended bread and for different types of other flour foods. In some countries of Central and Eastern Europe buckwheat groats are widely used. They may be rich in
retrograted starch and could thus be very suitable for diabetic patients and in the prevention of colon cancer. Buckwheat products are known as an important source of antioxidative substances, trace elements and dietary fibre. Buckwheat proteins have a high biological value, but relatively low true digestibility. Buckwheat has no gluten, so it is safe for patients with coeliac disease. Buckwheat has a high content of rutin and other polyphenols. Tartary Buckwheat, grown in China has a higher content of rutin than common buckwheat.

- **Corn pasta.** To produce good pasta from corn (*Zea mays* L.), it is necessary to have a thorough knowledge of the physico-chemical and rheological properties of the flour since the flour is deprived of gluten present in wheat. During the process of pastification a controlled hydrothermal treatment is applied to the dough to provoke the formation of a network similar to that of gluten. Without this network, it is impossible to extrude the dough to maintain the desired form that will endure during the following phase of the process up to the cooking of the dry pasta.

- **Durum wheat pasta** is the traditional Italian pasta [with *Triticum durum* (Desf.).]

- **5 cereals pasta** is pasta prepared with a mixture of durum wheat, barley, spelt, oat, and rye.

- **Kamut pasta.** Kamut [*Triticum turgidum turanicum* (Jakubz.)] is an ancient wheat cultivated thousand years ago. This wheat possesses a rich taste similar to butter, easily digestible, and it has higher protein content than hard wheat. In comparison to the normal wheat (*Triticum aestivum* L.), it contains higher mineral values such as magnesium and zinc. Besides it contains the natural antioxidant selenium and 30% more of vitamin E. People who suffer allergies from normal wheat and hard wheat, could employ kamut as a substitute.

- **Rice pasta.** Rice pasta (from *Oryza sativa* L.) also known as rice vermicelli (beehoon, bihun, maifun, bahn hoi, sen mee) is largely consumed in Asian countries in the home and restaurants. Lately, it is consumed in Europe as an alternative to the wheat pasta thanks to its high digestibility, neutral taste and the absence of gluten. As is the case with corn, the dough of this raw material must undergo hydrothermal treatment during the process of pastification.

- **Spelt pasta.** Spelt or farro (*Triticum spelta* L.) was the first grain cultivated by farmers in 5000 B.C. The Romans called it "farrum" and it was discovered in Mesopotamia. The grain has a higher protein and fibre content as compared to normal wheat. It also possesses a high content of B vitamins, particularly riboflavin (vitamins B2), necessary for the production of energy inside the cells as well as the reduction of the risks against arteriosclerosis. It is also a good source of niacin that has a multitude of benefits for the reduction of the cardiovascular risks. Many people sensitive to gluten have included this pasta in their diets.

- **Low carbohydrates pasta.** To fight obesity and to be able to maintain a good form-weight, a diet based on a smaller consumption of the carbohydrates has been developed. This diet consists in higher consumption of products rich in proteins, fibres, and fat and the reduction or elimination of the food rich in carbohydrates. For these types of pasta, the raw materials that are commonly employed are the by-products of soy (*Glycine max*) such as defatted flours, soy protein concentrates and isolates, wheat gluten, whey, eggs, bean and pulse flours alimentary fibres together with a small quantity of hard wheat or durum semolina.
**Pest problems**
Pasta factories, as any other food industry, can be infested by insects, leading to negative economic and commercial consequences (Süss and Locatelli, 1996, 2002; Riudavets et al., 2002, 2004; Barros et al., 2003; Stejskal et al., 2004; Trematerra and Süss, 2006, 2007).

As indicated above, pasta factories use flour, a raw material obtained generally from hard wheat, which comes directly from the mill. Because of the structure of the building, the possible use of infested cereals and the large quantity of dust always present, flour is the main source of reproduction and diffusion of pests. Consequently, the same pests [Ephestia kuehniella Z., Plodia interpunctella (Hb.), Cryptolestes spp., Gnathocerus cornutus (F.), Lasioderma serricorne (F.), Oryzaephilus spp., Rhyzopertha dominica (F.), Sitophilus spp., Stegobium paniceum (L.), Tribolium spp.] present in warehouses, silos and mills are carried into the pasta factories where they can multiply.

Sometimes the pasta factory and mill may constitute an industrial unity where the above-mentioned species can also fly and penetrate into the processing and packaging departments if the simplest prevention standards reported in the HACCP procedures are lacking.

Moreover, during the summer period some of these pests are able to multiply outside the industrial facilities, hidden under semolina encrustations or on rejected products (e.g. E. kuehniella, P. interpunctella, R. dominica, Sitophilus spp., Tribolium spp., Lasioderma and Stegobium).

The first barrier to pest infestation is the use of closed doors and windows. Packaging departments must be kept clean and placed under surveillance to prevent infestations. Infestations (mainly larvae of P. interpunctella and adults of R. dominica, Sitophilus and Tribolium) can occur during the storage process in industries, warehouses, general stores and retail shops already colonized by insects originating from other products.

The shelf-life of pasta is particularly long (in general 2 years, but also up to 3 years). During this period, insects can penetrate into the packages and reproduce many generations. They often enter through the micro-holes that have been made to remove air during packaging. The product can be attacked by P. interpunctella (larvae), R. dominica and Sitophilus spp. (adults), L. serricorne and S. paniceum (adults) when it is placed on the shelf near infested rice and other foodstuffs.

In a survey conducted over a period of several years, it was observed that complaints and returns by customers were due to attacks generally occurring after the product left the factory. Inspections carried out in all of Italy, in shops where infested alimentary pastas were bought (general stores, small and big department stores). Usually the stores were neglected and the shelves were dusty, with infestations in progress.

In recent years, new methods of protecting the production cycle have been introduced for the prevention and control of pests (monitoring, inspection, filth test, etc). Despite these preventive measures, the problem of pest attacks in pasta factories from packaging to the consumer remains unsolved. This is due to certain methods used in packaging and to the negligence of warehouses and stores. In addition the long average shelf-life of the product can lead to insect infestations on the shelf.

**Materials and methods**

**Pasta types**
In this work we compared attraction of adults of *Sitophilus zeamais* Motschulsky to 8 different types of commercial Italian cereal pasta [barley pasta, buckwheat pasta, corn pasta,
durum wheat pasta, 5 cereals pasta (a mixture of durum wheat, barley, spelt, oat, and rye), kamut pasta, rice pasta, and spelt pasta] (Fig. 1, Table 1).

**Insect rearing**

The adults of *S. zeamais* used in the olfactometer tests were taken from cultures that were kept in the laboratory in whole maize at 27±1°C and 65±5% relative humidity (r.h.) and continuous darkness.

![Image of pasta and adults](image)

**Fig. 1. Different types of commercial cereal pasta; adults of *S. zeamais* infesting pasta.**

<table>
<thead>
<tr>
<th>Pasta Type</th>
<th>Proteins grams</th>
<th>Carbohydrates grams</th>
<th>Fats grams</th>
<th>Cooking time minutes</th>
<th>Kcal 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>11.6</td>
<td>59.3</td>
<td>1.9</td>
<td>7</td>
<td>300</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>7.8</td>
<td>75.4</td>
<td>2.2</td>
<td>5-7</td>
<td>353</td>
</tr>
<tr>
<td>Corn</td>
<td>5.7</td>
<td>78.0</td>
<td>1.2</td>
<td>8</td>
<td>346</td>
</tr>
<tr>
<td>Durum wheat</td>
<td>14.0</td>
<td>70.2</td>
<td>1.5</td>
<td>12</td>
<td>350</td>
</tr>
<tr>
<td>5 cereals</td>
<td>13.0</td>
<td>68.2</td>
<td>1.5</td>
<td>11</td>
<td>338</td>
</tr>
<tr>
<td>Kamut</td>
<td>10.5</td>
<td>74.7</td>
<td>0.9</td>
<td>10-11</td>
<td>349</td>
</tr>
<tr>
<td>Rice</td>
<td>1.2</td>
<td>85.4</td>
<td>0.7</td>
<td>6-8</td>
<td>353</td>
</tr>
<tr>
<td>Spelt</td>
<td>13.8</td>
<td>69.9</td>
<td>2.0</td>
<td>10-11</td>
<td>353</td>
</tr>
</tbody>
</table>

**Olfactometric tests**

The tests were carried out in a cylindrical arena of plexi-glass (80 cm diam. x 40 cm high) for olfactometer assays. Nine modified Flit-Track M² trap-devices (Trécé Inc, USA) (choice tests) were placed in the arena.
In each trial 100 adults of *S. zeamais* of mixed sex and age were released at the center of the arena (Figure 2). The number of trapped insects was checked 15 h after their introduction in the arena; teflon paint was used to prevent maize weevil escape from the traps and from the arena.

Fourteen replicates were performed, using a total of 1400 insects. In order to measure the different attractiveness of each pasta type, 20 gr of pasta were used as bait for each trap. In all trials trap positions were rotated and trap contents were renewed after each replication.

The tests were conducted under controlled conditions set at $27\pm1^\circ C$, $65\pm5\%$ relative humidity (r.h.) and continuous darkness.

![Fig. 2. Schematic representation of circular arena used in the experiments (R= insect-releasing point, T-D= trap device).](image)

**Data analysis**

The data were submitted to the calculation of Kendall’s W association coefficient and to a classic one-way ANOVA analysis. A multiple regression analysis was also performed, with the aim to discovering any distinctive pasta features which might influence insects choices.

**Results and discussion**

We obtained 14 different experimental results, each in the form of frequencies (number of insects preferring a certain type of pasta). We considered this kind of data as a series of K different classifications of N objects (the 8 different types of pasta, plus the void trap).

As a consequence, the first step of the analysis was the calculation of Kendall’s W statistic, which furnished a global measure of the association among several different classifications. Fourteen ranks, one for each experimental trial, were derived by assigning a value of 1 to the pasta preferred in a given trial; 2 to the quality with the second higher frequency, and so on. The index varies between 0 (no association) and 1 (perfect association, i.e. the ranks are all identical).

In one case, the resulting value was $W=0.5327$. This is quite a high value, indicating that the choices among the trials had been similar; in other words, the insects tended to show the same choices in any trial.
In order to verify if the differences among the final results could be considered expressions of real preference, a classical one-way ANOVA analysis was performed on the nine groups formed by the different pasta types in the 14 experimental trials. The results are reported in Table 3. The extremely low p-value indicates that the between groups means are very unlikely to be equal.

Table 2. Distribution of insects in the olfactometric tests.

<table>
<thead>
<tr>
<th>PASTA TYPE</th>
<th>Insects trapped N.</th>
<th>Mean</th>
<th>Variance</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>250</td>
<td>17.8570</td>
<td>48.4400</td>
<td>6.9599</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>215</td>
<td>15.3570</td>
<td>71.7860</td>
<td>8.4726</td>
</tr>
<tr>
<td>Durum wheat</td>
<td>209</td>
<td>14.9290</td>
<td>24.2250</td>
<td>4.9219</td>
</tr>
<tr>
<td>Rice</td>
<td>195</td>
<td>13.9290</td>
<td>34.5330</td>
<td>5.8765</td>
</tr>
<tr>
<td>Barley</td>
<td>117</td>
<td>8.3571</td>
<td>21.9400</td>
<td>4.6840</td>
</tr>
<tr>
<td>Kamut</td>
<td>113</td>
<td>8.0714</td>
<td>25.7640</td>
<td>5.0758</td>
</tr>
<tr>
<td>Spelt</td>
<td>88</td>
<td>6.2857</td>
<td>9.6044</td>
<td>3.0991</td>
</tr>
<tr>
<td>5 cereals</td>
<td>81</td>
<td>5.7857</td>
<td>24.1810</td>
<td>4.9175</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>2.7857</td>
<td>3.5659</td>
<td>1.8884</td>
</tr>
</tbody>
</table>

Table 3. One-way ANOVA analysis.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>Sum of squares</th>
<th>D.f.</th>
<th>Mean Squares</th>
<th>F statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within groups</td>
<td>3064.97</td>
<td>8</td>
<td>383.12</td>
<td>13.06</td>
<td>2.515e-13</td>
</tr>
<tr>
<td>Between groups</td>
<td>3432.50</td>
<td>117</td>
<td>29.338</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6497.47</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Altogether the results obtained in olfactometer tests demonstrated that S. zeamais adults revealed preferences in decreasing order for pasta produced with corn (19.13%), buckwheat (16.45%), durum wheat (15.99%), rice (14.92%), barley (8.95%), kamut (8.65%), spelt (6.73%), and 5 cereals (6.20%).

In an attempt to identify the main factors influencing the choice of S. zeamais, the total preferences were considered as the dependent variable in the context of a multiple regression analysis. The 5 explanatory variables put into the model referred to the characteristics of the pasta: energetic value (Kcal/100 g of pasta), proteins (g/100 g of pasta), carbohydrates (g/100 g of pasta), fats (g/100 g of pasta), and cooking time (min).

The method employed was a stepwise regression, with the calculation of the adjusted R-square in order to stop the procedure.

The results showed that none of the variables considered, significantly influences insects choices. This means that the principal characteristics of pasta can’t be considered as “appealing” for infesting insects. On the other hand, adults of maize weevil show to be able to respond selectively to semiochemical signals coming from pasta.

The percentages of S. zeamais infestations on the various types of new pasta investigated were statistically different. Corn pasta seems to be more attractive than the buckwheat pasta,
traditional durum wheat pasta and rice pasta. On the other hand, barley pasta, kamut pasta, spelt pasta, and 5 cereals pasta are less attractive for adults of the maize weevil.

As has been mentioned above, other largely diffused raw materials different from durum wheat can be successfully employed for the production of pasta. Pasta produced with these materials offers nutritional benefits and in solution to the allergies problem to wheat and intolerance to the gluten. The organoleptic properties, including the quantity of solids released in cooking water, are much more comparable to the traditional pasta.

Warehouse managers and shopkeepers must be involved in the processing cycle by encouraging frequent visits of inspectors and the distribution of guides explaining the problems and their possible solutions. Only by controlling the entire processing cycle, from the purchase of raw material to the distribution of the finished product, will it be possible to reduce the risk of infestation.

References


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Bacterial flora of *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) from several tobacco stores in Turkey

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Abstract: In the present study, bacterial flora of *Lasioderma serricorne* from tobacco stores in eight localities in Turkey were studied. Totally, 15 different bacteria were isolated and identified on the basis of fatty acid methyl ester (FAMEs) analysis and carbon utilization profile by using Microbial Identification and Biolog Microplate Systems. Most isolated bacteria were from the genus *Bacillus*. Nine of 15 identified bacteria were from the genus *Bacillus*. Identified bacteria are *Bacillus cereus*, *B. megaterium*, *B. thuringiensis*, *B. subtilis*, *B. pumilus*, *B. atrophaeus*, *B. badius*, *B. clausii*, *B. parabrevis*, *Brevibacterium liquefaciens*, *Brevibacillus parabrevis*, *Micrococcus luteus*, *Pseudomonas syringae*, *Staphylococcus gallinarum* and *Salmonella typhimurium*. *B. cereus*, *B. megaterium* and *B. thuringiensis* were most common found bacteria in the investigated localities. *B. megaterium* were isolated from six of the eight investigated tobacco stores, *B. thuringiensis* from five and *B. cereus* from 4 stores.

Key words: cigarette beetle, *Lasioderma serricorne*, bacterial flora, biological control

Introduction

The cigarette beetle *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) is one of the most widespread and destructive pests of stored tobacco. It is also the major insect pest of stored tobacco in Turkey. Damage to tobacco is caused by the insect larvae that eat the stored leaf and contaminate the product with excreta and body oils (Kaelin et al. 1999). For many years, insect pests in stored products have been controlled by the direct application of residual insecticides and fumigants (Benezet, 1989). These methods brought several problems to human and animals. Fortunately, most bacteria capable of causing disease in insects do not harm animals or plants. This is one of the most important factors encouraging the use of bacterial pathogens as control agents. Increasing interest in developing environmentally safe pest control methods has inspired scientists to study the potential of bacterial pathogens for controlling harmful insect. Scientists have therefore recently tried to find more effective and safe bacterial agents against plant pests (Yaman, 2003).

There have been several studies on the cigarette beetle, but only a few of those on the bacterial pathogens of *L. serricorne* (Thompson and Fletcher, 1972; Kaelin et al. 1994, 1999; Tsuchiya et al., 2002). In this study, bacterial flora of *L. serricorne* from several tobacco stores in Turkey was studied.
Material and methods

Collection of insects
In the present study several tobacco stores from different regions of Turkey; İstanbul, İzmir, Aydın, Hatay, Bafra, Samsun, Akçaabat and Tokat were selected to provide insect samples for bacterial isolations.

Isolation of bacterial isolates
Ten dead *L. serricorne* adults were used for isolation for each sampled locality. Following macroscopic examination, dead adults were distinguished and surface sterilized in 70% alcohol and then washed in sterile water three times (Lipa and Wiland, 1972; Poinar, 1978; Yaman et al. 2005). The adults were homogenized in nutrient broth by using a glass tissue grinder. 100µl of the suspension were applied directly on nutrient agar plates. The plates were incubated at 28°C to 37°C for 2-3 days. After the incubation period the plates were examined and bacterial colonies were selected. Selected colonies were purified by sub-culturing on plates. Bacterial strains were maintained for long-term storage in nutrient broth with 15% glycerol at -86°C for further tests.

Identification of bacterial isolates
All of the isolated bacterial strains were identified based on fatty acid profiles determined by using the Microbial Identification System (Hewlett-Packard 6890A, Polo Alto, CA) with TSBA (Tripticose Soy Broth Agar) database in the Sherlock Microbial Identification System software package (MIDI, Microbial ID. Inc., Newark, DE) and carbon substrate utilization fingerprints analyzed by the Biology GN and GP database with Microlog software in Biolog Microplac system (Biolog Inc., Hayward, CA) at the Department of Plant Protection in Erzurum. The isolates were stored at the Department of Plant Protection, Faculty of Agriculture, Atatürk University.

Results and discussion

Although there are many biological control studies on *L. serricorne*, studies connected to bacterial flora of this pest are limited. In this extensive study, we isolated 15 different bacteria. Eleven spore-forming and four nonspore-forming were isolated and identified. Nine of 15 identified bacteria were from the genus *Bacillus*. Identified bacteria are *Bacillus cereus*, *B. megaterium*, *B. thuringiensis*, *B. subtilis*, *B. pumilus*, *B. atrophaeus*, *B. badius*, *B. claussii*, *B. parabrevis*, *Brevibacterium liquefaciens*, *Brevibacillus parabrevis*, *Micrococcus luteus*, *Pseudomonas syringae*, *Staphylococcus gallinarum* and *Salmonella typhimurium*. *B. cereus*, *B. thuringiensis* and *B. megaterium* were the most common bacteria in *L. serricorne*. *B. cereus* was isolated from *L. serricorne* by Thompson and Fletcher (1972) for the first time. They found that the cigarette betle was effectively controlled by *B. cereus* and *B. thuringiensis* var. *thuringiensis* and LD₅₀ was 4.29 x 10⁶ spores per gram of medium for *B. cereus*. Kaelin et al. (1994) isolated *B. thuringiensis* was isolated from dried tobacco residues and dead tobacco beetles collected in a large number of locations worldwide. Kaelin et al. (1999) isolated three *B. thuringiensis* (Bt) strains from stored tobacco residues. They characterized these isolates and tested them for their insecticidal activity against larvae of the cigarette betle. They found that the molecular
genetic analysis of the three isolates showed high homology to *B. thuringiensis* subsp. *tenebrionis*. Insect responses to spore/crystal suspensions from all bacterial strains were in the range of 60±80% mortality after 7 days. These results inspire us with hope that some of our isolates should be very effective against *L. serricorne*. Further researches will be directed to determine insecticidal effects of isolated bacteria on the cigarette beetle to find more effective agent against this pest.

Table 1- Bacteria isolated from *L. serricorne* from several tobacco stores in Turkey.

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>Sampled Tobacco Stores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Istanbul</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Bacillus thuringiensis</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus pumilus</em></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus atrophaeus</em></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus radius</em></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus claussii</em></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus parabrevis</em></td>
<td></td>
</tr>
<tr>
<td><em>Brevibacterium liquefaciens</em></td>
<td></td>
</tr>
<tr>
<td><em>Brevibacillus parabrevis</em></td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas syringae</em></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus gallinarum</em></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td></td>
</tr>
</tbody>
</table>

References


Lipa, J.J. 1975: An Outline of Insect Pathology. – Published for the U.S. Department of Agriculture and the National Science Foundation, Washington D.C., by the Foreign Scientific, Technical and Economic Information Warsaw, Poland.


Studies on the feeding, reproduction and development of *Cheletomorpha lepidopterorum* (Schaw) (Prostigmata: Cheyletidae) on various food sources

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Abstract: *Cheletomorpha lepidopterorum* as a biological control agent used against different mite pests was reared individually on immature stages of different mite diets belonging to suborder Astigmata (*Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *Rhizoglyphus echinopus* and *Caloglyphus betae*) at different temperatures (20, 25 and 30°C) and (70 – 80 % R.H.). It was noticed that the predator female passed through two ny mphal stages (protonymph and deutonymph), while males have only one nymphal stage. The predator mite developed faster when reared at 30°C than 20°C. When four astigmatid mites were compared as food, *Cheletomorpha lepidopterorum* showed a higher fertility and lived longer on *T. putrescentiae* as food than on other diets. Cannibalism was usually noticed when the preys were absent or scarce.

Key words: *Cheletomorpha lepidopterorum*, biology, astigmatid mites

Introduction

The dramatrical increase in world population requires an efficient modern human food supply, animal production industry and the manufacture of good quality feeds and food. Hence, great attention has been paid to increase of food products. Stored products and grains are liable to attack not only by insects but also by mites which cause direct injury. Contamination with mites makes sources of food undesirable and undigestable. Harmful mites damage the grain by hollowing out the germ, and can cause serious livestock respiratory ailments (Wraith et al., 1979; Cuthbert et al., 1980), and affect their growth (Wilkin and Thind, 1984). Furthermore, mites can spread the toxigenic fungus *Aspergillus flavus*. There is a link between contaminated maize and sterile grains, (Franzolin et al., 1999). Due to the importance of food grains as a source of food and in order to eliminate the usage of chemicals in controlling pests, previous and the present researchers have been directed their efforts towards the discovery of predaceous insects and mites, evaluating their feeding capacity, prey range and alternative and preferred food substances.

The family Cheyletidae (Acari: Prostigmata) includes many species which are known for their wide predatory habitats (Zaher, 1986). The majority of these species appear to feed on small insects and mites inhabiting leaves, litter, dry or damp humus and straw or animal droppings and on the soil surface. In Egypt, mites belonging to the family Cheyletidae were first recorded by El-Badry and Zaher (1960). They recorded two species, *Cheletogenes ornatus* and *Eutogenes frator* on fruit trees, associated with acarine pests and scale insects. They also found *Cheyletus malaccensis* Oudemans associated with other mites such as *Caloglyphus rhizoglyphoides* Zachvatkin. Sinha (1968) noticed a species of *Cheyletus* associated with acarid mites in stored food stuff, especially *Tyrophagus putrescentiae* (Schrank). In the U.S.A. Delfinado and Khaingfields (1976) surveyed and listed eight mite...
species of Cheyletidae associated with different stored grains granaries. Gene and Ozar (1986) collected cheyletid mites *Cheyletus malaccensis* and *Cheletomorpha lepidopterorum* predators of *T. putrescentiae*. The first species also preyed on beetles of the tenebrionid genus *Tribolium*. In Greece, Eliopoulos and Papadoulis (2001) collected five species of cheyletid mites from stored products. Of these, *C. lepidopterorum* was collected from hay, wheat, cotton seed, trefoil, litter and maize. The prey preference of a predator may be affected not only by the characteristics of a prey item as food, but also by the microenvironment or architecture produced by a prey species (Furuichi *et al*., 2005). The literature on mite pests-predators interaction in stored products is scarce. Therefore, the principle aim of the present investigation was the effect of different stored mite pests on the biological aspects of the predatory cheyletid mite, *Cheletomorpha lepidopterorum* (Schaw) at different temperatures (20, 25 and 30°C) and 70 – 80 % R.H.

**Materials and methods**

**Extraction and identification of mites**

Mites were extracted by using a modified Tullgren funnel kept for about 24 hours under a 60-watt electric lamp. Collected mites were put in Nesbitt’s clearing agent, then mounted on glass slide using Hoyer’s medium for examination. Identification of mounted species followed a review given by Hughes (1961), Attiah (1969), Krantz (1978), and Zaher (1986).

**Predator main culture**

In order to establish the main culture, the predatory mite, *Cheletomorpha lepidopterorum* collected from soybean straw and established in laboratory for three successive generations on astigmatid mite, *Tyrophagus putrescentiae*. The latter were placed in plastic cells (closed round, 2.5 cm in diameter) with a layer of mixture of plaster of Paris and Charcoal (9 : 1) on the bottom to depth of 0.5 cm. Water drops were added when needed. For individual rearing, newly deposited eggs were transferred individually to a new rearing plastic cell. Each newly developed larva was supplied with prey. Devoured larvae were replaced daily until maturity. Emerged females were allowed to mate with males and monitored for oviposition. All biological aspects were recorded twice daily during the predator’s development and daily during the female oviposition period. Other necessary data dealing with the predator’s biology, fecundity and other biological aspects were continuously recorded. The necessary data were subjected to one way analysis of variance (ANOVA.) The means were compared by Duncan’s multiple range test (Duncan, 1955).

**Food sources**

*Tyrophagus putrescentiae* (Acaridae), *Lepidoglyphus destructor* (Schrank) (Lepidophoridae), *Rhizoglyphus echinopus* (Fum. & Rob.) (Acaridae) and *Caloglyphus betae* Attiah (Acaridae) were used as prey for rearing the predator, *C. lepidopterorum*, collected from some stored product at El-Menofia Governorate. The first and fourth preys were collected separately from wheat bran, but the second and third mite pests were isolated from soybean straw. The isolated mite pests were maintained and mass reared by feeding on yeast granules in the laboratory at 25°C and 80 % R.H. The devoured individuals were counted and replaced by new ones.

**Chemical analysis**

**Fraction of amino acids:** Amino acids were extracted from tested samples according to Shade *et al.* (2002) as follows: 20 ml gm of each sample was taken and soaked separately in 75 % ethanol (100 ml). After 24 hr the samples were ground and filtered. The residue was washed with a few ml of 75 % ethanol and the volume was made up to 100 ml. Separation of
amino acids was accomplished with an ODS. 15 amino acids were examined using an HPLC system (hp 1050) with a UV detector (5 µm x 250 mm) column at 254 nm. The mobile phase consisted of two eluents; acetonitrile / tetrahydrofuran (90 / 10 v / v.) and tetrahydrofuran / water (5 / 95 V/V.). 32 % of solution one and 68 % of the second solution with 0.3 ml acetic acid and pH adjusted to 5.15 with 1 M NaOH. The flow rate was 1.5 ml/min. The column temperature was 60°C with an injection volume of 10 µl (Gertz, 1990).

**Total soluble sugars and reducing and non-reducing sugars:** Total soluble and reducing sugars were determined calorimetrically as described by Thomas and Dutcher (1924) using the modified picric acid method. Fifteen ml of sample were taken and then incubated for 15 days at 25±2°C. Each treatment was plunged immediately into 95 % boiling ethanol for 10 minutes in order to kill the living tissues. The samples were then refluxed for 10 – 12 hrs in a Soxhlet unit using 75 % ethanol. The dried residue was re-dissolved in 6 ml of isopropyl alcohol 50 % and used to determine sugars content.

**Reagents used**

A) **Picrate-picric:** The picrate-picric reagent was prepared by adding thirty six grams of picric acid to 500 ml of 1% solution of sodium hydroxide and 400 ml of hot distilled water. The mixture was shaken occasionally until the picric acid was dissolved, then cooled and diluted to one litter.

B) **Sodium carbonate:** Sodium carbonate reagent was prepared by dissolving twenty grams of sodium carbonate in 100 ml of distilled water.

**Determination of total soluble sugars:** For total soluble sugars estimation, approximately 0.8 ml of each sample extract was placed in a test tube containing 5 ml of distilled water and 4 ml of picrate / picric reagent. Then mixture was boiled for 10 min in a water-bath. After cooling, one ml of sodium carbonate reagent was added and the mixture was reboiled for 10 min. After cooling, the tubes were diluted to 50 ml with distilled water. The developed colour was measured using a spectrophotometer at 540 nm.

**Determination of reducing sugars:** The previously mentioned technique was also applied except that picrate-picric reagent and sodium carbonate were added together at the same time and boiled for only 10 minutes. The reducing sugars content was calculated using a glucose standard curve at the same previous wave length.

**Determination of non-reducing sugar contents:** Non-reducing sugars content was calculated as the difference between the total and soluble reducing sugars.

**Glucose content:** Extraction of glucose done for 10 ml gm tissue per sample was homogenised with acetonitril / water (76 / 24 v/v). The extract was filtered through a Whatman filter paper and micro filter 0.45 um partitioned three times with ethyl alcohol and stored in vials. HPLC analysis was used to determine glucose in the extracts. Analysis of glucose was performed on a model (hp 1050) HPLC equipped with UV detector. Separations and determinations were performed on APS column (4.6 x 200 mm). The mobile phase was the same as the one which used in extraction. UV detector was 192 nm and 2 ml / min flow rate according to Gertz, 1990.

**Results and discussion**

**Behaviour**

Field observation showed that the predatory mite *Cheletomorpha lepidopterorum* was usually found around their prey individuals. When touching the prey, it quickly moved backward to
attack it. The predator seized firmly the prey with the aid of its raptorial palps, then inserts its chelicerae in any part of the body and sucked its contents. The life history of the predator pass through one larval and two nymphal stages for female and one nymphal stage for male before reaching adulthood. The young larvae are colourless, the orange colour begins to appear at the end of the larval period, becoming more intense at each succeeding stage. The first pairs of legs are abnormally long and each leg terminates in a small claw and is occasionally used in walking. Before proceeding to the ensuing stage, active immature individuals usually enter a resting or quiescent stage.

**Mating**
The mating process is necessary for *C. lepidopterorum* individual production. Laboratory observation showed the adult tended to mate immediately after emergence. Just before mating, the male becomes more active by running around the female, and then it manipulated itself underneath the female, bending its opithosomal region upward and forward to meet that of female. Copulation usually lasted about 5 minutes.

**Hatching**
As incubation proceeds, the embryo grows and limits itself to any of the egg stages, then a longitudinal slit occurs medially and hatching larva crawls from the egg shell.

**Moulting**
Prior to moulting, the immature stage of *C. lepidopterorum* enters into a quiescent period during which it stops feeding and movement. It stretches its chelicerae, palps backwardly along the sides of the body. Immediately before moulting a dorsal transverse rapture occurs between the propodosoma and hysterosoma. The mite tries to disengage itself from the old skin by twisting movements and subsequently withdraws the forelegs and the anterior part of the body from the old skin. Afterwards, it crawls forward trying to get ride of the posterior part of the exuviae. The colour of the newly emerged individual is usually orange, then following feeding, gradually becomes darker.

**Incubation period**
The temperature showed a noticeable effect on the embryonic development of *C. lepidopterorum* as shown from Table 1. A temperature of 30ºC induced a shorter incubation period, while 20ºC resulted in the longest period for the predator eggs to develop, giving rise to females and males. The predator incubation period took the longest time when the mites fed on *T. putrescentiae* at 20ºC for female (4.08 days). It took 2.68 days for the predator eggs give rise to males when feeding on the astigmatid mite *C. betae* at 30ºC. Statistical analysis of the data showed that the L.S.D. at 0.05 level was 0.027 for diets and 0.0237 for temperature effect.

**Life cycle**
Food substances significantly influenced the life cycle of the predatory mite, *C. lepidopterorum* (Table 1). The longest period of female and male life cycle was recorded when the predator fed on *T. putrescentiae* where it lasted 15.06, 12.95 and 11.05 days respectively for females at 20, 25 and 30 ºC. It changed to 13.0, 12.04 and 9.97 days for males on the same prey under the same conditions. On the other hand, the shortest life cycle of the predator was observed when fed on *C. betae* (10.91, 10.35 and 9.45 days) for females and (10.0, 9.48 and 8.01 days) for males when fed at 20, 25 and 30 ºC, respectively. El-Naggar et al., (2006) reported that, the female life cycle of *C. lepidopterorum* lasted 16.37, 14.42 and 12.3 days on *T. putrescentiae* immatures at 15, 25 and 35 ºC, respectively. With regard to males, the temperature 35ºC accelerated the life cycle (9.48 days) when compared with 15 and 25ºC (12.87 and 11.31 days, respectively).
**Longevity**

Table 1 shows the longevity of females and males of *C. lepidopterorum* under different temperatures, for each diet separately. Under the present experimental conditions, longevity of females and males was maximal at 20°C and reduced at higher temperature. During this period, the female lived longer at 20°C (37.7 days) on *T. putrescentiae* when compared with 30°C (18.7 days) on *C. betae*. The longest period for the male was also on *T. putrescentiae* at 20°C (34.6 days). It was shorter and reached 15.0 days on *C. betae* at 30°C. These results showed that the higher temperature and the astigmatid mite *C. betae* shortened the predator longevity. The results suggested that the poor quality of this diet as food source required for the predator may be due to changes caused by the composition of the tested diets. Similar results were obtained by El-Naggar et al., (2006) who decided that a high temperature shortened the life cycle and adult longevity of the predator *C. lepidopterorum* when fed on immature stages of *T. putrescentiae*.

**Table 1. Biological aspects of the predatory mite *Cheletomorpha lepidopterorum* when fed on different diets at different temperatures.**

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Biological aspect</th>
<th><em>T. putrescentiae</em></th>
<th>Lepidoglyphus destructor</th>
<th>Rhizoglyphus echnipus</th>
<th>Caloglyphus betae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation period / day</td>
<td>♀️ 4.08 ± 0.06</td>
<td>3.88 ± 0.06</td>
<td>3.56 ± 0.05</td>
<td>3.25 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>♂️ 3.8 ± 0.04</td>
<td>3.54 ± 0.06</td>
<td>3.23 ± 0.07</td>
<td>3.2 ± 0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Life cycle / day</td>
<td>♀️ 15.06 ± 0.5</td>
<td>13.07 ± 0.38</td>
<td>12.27 ± 0.5</td>
<td>10.91 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>♂️ 13.0 ± 0.14</td>
<td>11.67 ± 0.08</td>
<td>10.0 ± 0.09</td>
<td>10.0 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Longevity / day</td>
<td>♀️ 37.7 ± 0.8</td>
<td>35.0 ± 0.67</td>
<td>30.1 ± 1.45</td>
<td>28.0 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>♂️ 34.6 ± 1.5</td>
<td>31.9 ± 1.60</td>
<td>29.9 ± 0.9</td>
<td>27.0 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>20 ºC</td>
<td>Incubation period / day</td>
<td>♀️ 3.81 ± 0.06</td>
<td>3.59 ± 0.05</td>
<td>3.31 ± 0.06</td>
<td>2.99 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>♂️ 3.5 ± 0.06</td>
<td>3.28 ± 0.06</td>
<td>2.98 ± 0.08</td>
<td>2.93 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Life cycle / day</td>
<td>♀️ 12.95 ± 0.23</td>
<td>11.96 ± 0.13</td>
<td>11.42 ± 0.17</td>
<td>10.35 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>♂️ 12.04 ± 0.15</td>
<td>10.97 ± 0.19</td>
<td>10.47 ± 0.48</td>
<td>9.48 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Longevity / day</td>
<td>♀️ 31.8 ± 0.79</td>
<td>29.9 ± 1.73</td>
<td>28.0 ± 0.67</td>
<td>26.0 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>♂️ 30.0 ± 1.05</td>
<td>28.0 ± 0.67</td>
<td>25.0 ± 0.67</td>
<td>22.9 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>25 ºC</td>
<td>Incubation period / day</td>
<td>♀️ 3.6 ± 0.05</td>
<td>3.02 ± 0.12</td>
<td>2.98 ± 0.08</td>
<td>2.89 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>♂️ 3.25 ± 0.07</td>
<td>3.0 ± 0.05</td>
<td>2.8 ± 0.07</td>
<td>2.68 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Life cycle / day</td>
<td>♀️ 10.01 ± 0.1</td>
<td>10.4 ± 0.20</td>
<td>9.95 ± 0.17</td>
<td>9.45 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>♂️ 9.97 ± 0.09</td>
<td>9.0 ± 0.10</td>
<td>8.5 ± 0.04</td>
<td>8.01 ± 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Longevity / day</td>
<td>♀️ 25.9 ± 0.99</td>
<td>25.9 ± 0.99</td>
<td>20.9 ± 0.74</td>
<td>18.7 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>♂️ 23.0 ± 1.63</td>
<td>23.0 ± 1.63</td>
<td>17.7 ± 0.82</td>
<td>15.0 ± 0.67</td>
<td></td>
</tr>
</tbody>
</table>

± S. D.L.S.D at 0.05 level for incubation period = 0.027 for diets effect = 0.0237 for temperature effect

**Fecundity**

The eggs of *C. lepidopterorum* are laid in isolated clusters and are attached to a substrate by a few strands of silk issuing from the mouth. The female makes no attempts to protect the eggs. Under the conditions used in these experiments, the number of *C. lepidopterorum* eggs differed depending on whether the mites fed on *T. putrescentiae* or on other diets, Table (2). However, the ratios of eggs hatchability were not significantly affected by the effect of diets and / or temperature. As shown by the data, the female of the predator deposited the highest rate of oviposition on *T. putrescentiae* (104.1 eggs) at 20°C, with hatchability 96.6 %. On the other hand, the lowest number of *C. lepidopterorum* deposited eggs was recorded at 30°C on *C. betae* (40.0 eggs) with hatchability 96.7 %. Statistical analysis of data showed that L.S.D. at 0.05 level = 0.3115 and 0.2697 for effect of diets and temperature on the predator...
fecundity, respectively. Similar results were obtained by El-Naggar et al. (2006). The authors demonstrated that the temperature induced a considerable effect on the predatory mite *C. lepidopterorum* fecundity, since the female laid a total average of 85.32, 80.27 and 70.35 eggs at 15, 25 and 30°C, respectively when fed on the immature stages of *T. putrescentiae*.

**Preoviposition, oviposition and postoviposition periods**

A general glance to the data in Table (2), revealed that *Tyrophagus putrescentiae* was the most favourable food for the predatory mite, *C. lepidopterorum* where it increased the predator oviposition period at 20°C (33.17 days). The least favourable prey was recorded for *C. betae* at 30°C (13.49 days). Also, from the same table, it can be seen for all tested preys, the oviposition period of the predator increased at low temperature and decreased by increasing the temperature. However, it clear from the results that the preoviposition and postoviposition periods of the predatory mite were not affected with the different preys at the tested temperatures. The preoviposition period was around 1.3 days but the postoviposition period was around 3.6 days. El-Enany et al. (1992) noted that feeding *C. lepidopterorum* on immature *Tyrophagus putrescentiae* did not show an obvious response with change of temperature from 24 to 30°C.

### Table 2. Egg numbers laid by *C. lepidopterorum* adult female and adult female longevity when fed on different diets at different temperatures.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Temp.</th>
<th>Eggs numbers</th>
<th>Hatchability %</th>
<th>Female longevity / day</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. putrescentiae</em></td>
<td>20 °C</td>
<td>104.1 ± 2.60</td>
<td>96.6 ± 0.97</td>
<td>1.11 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>25 °C</td>
<td>90.4 ± 0.70</td>
<td>95.6 ± 0.70</td>
<td>1.32 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>30 °C</td>
<td>50.1 ± 0.99</td>
<td>90.5 ± 0.97</td>
<td>1.65 ± 0.28</td>
</tr>
<tr>
<td>Lepidoglyphus</td>
<td>20 °C</td>
<td>95.0 ± 1.5</td>
<td>94.8 ± 0.63</td>
<td>1.12 ± 0.31</td>
</tr>
<tr>
<td>destructor</td>
<td>25 °C</td>
<td>77.4 ± 0.84</td>
<td>95.0 ± 1.05</td>
<td>1.28 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>30 °C</td>
<td>51.5 ± 1.18</td>
<td>96.1 ± 1.29</td>
<td>1.55 ± 0.22</td>
</tr>
<tr>
<td>Rhizoglyphus</td>
<td>20 °C</td>
<td>84.1 ± 0.99</td>
<td>96.2 ± 0.79</td>
<td>1.02 ± 0.18</td>
</tr>
<tr>
<td>echinopus</td>
<td>25 °C</td>
<td>70.1 ± 0.99</td>
<td>95.7 ± 0.82</td>
<td>1.25 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>30 °C</td>
<td>44.6 ± 0.84</td>
<td>95.6 ± 0.84</td>
<td>1.50 ± 0.23</td>
</tr>
<tr>
<td>Caloglyphus</td>
<td>20 °C</td>
<td>80.0 ± 1.05</td>
<td>94.0 ± 1.3</td>
<td>1.20 ± 0.24</td>
</tr>
<tr>
<td>betae</td>
<td>25 °C</td>
<td>64.5 ± 1.18</td>
<td>96.6 ± 1.3</td>
<td>1.31 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>30 °C</td>
<td>40.0 ± 1.05</td>
<td>96.7 ± 0.95</td>
<td>1.45 ± 0.30</td>
</tr>
</tbody>
</table>

**Food consumption**

During adulthood, female individuals of *C. lepidopterorum* consumed greater numbers of prey than males on each of the four tested foods, Table (3). The greatest numbers of the tested prey individuals consumed by the predator were significantly decreased by increasing the tested temperature for both sexes on different diets. The best introduced prey was *T. putrescentiae* where the number of individuals consumed was 85.2, 80.0 and 75.0 for predator females at 20, 25 and 30 °C, respectively. While those recorded for males were 36.0, 30.1 and 26.8 prey the immature under similar conditions. On the contrary, *Caloglyphus betae* was regarded as the lowest favourable prey for predator life. The highest number of consumed prey was 70.0, 65.0 and 60.0 individuals for predatory females changed to 27.9, 24.9 and 20.8 prey respectively for males at the same temperatures as mentioned previously. The female
immature stages of the predator consumed a total average of 10.2, 9.8, 9.6 and 9.0 prey individuals at 20 °C on *T. putrescentiae*, *L. destructor*, *R. echinopus* and *C. betae*, respectively. The male immature stages consumed a total average of 6.2, 5.42, 5.20 and 5.0 individuals of the same order of preys diets mentioned previously at 20 °C. However, at 30°C, the number of devoured preys was 9.4, 8.5, 8.3 and 8.0 for immature females and 7.0, 5.56, 5.50 and 5.30 for males. From obtained results, it was noticed that male young individuals proved to be less efficient predators than female (Table 3). The results of this study were quite similar to those of El-Naggar *et al.* (2006) who noticed that the average predator adult female of *C. lepidopterorum* devoured 90.26, 87.62 & 76.27 and 26.02, 24.11 and 19.67 preys of *T. putrescentiae* at 15, 25 and 30°C for females and males, respectively.

**Table 3. Food consumption of *C. lepidopterorum* when fed on different diets at different temperatures.**

<table>
<thead>
<tr>
<th>Temp. Biological aspect</th>
<th><em>Tyrophagus putrescentiae</em></th>
<th><em>Lepidoglyphus destructor</em></th>
<th><em>Rhizoglyphus echinopus</em></th>
<th><em>Caloglyphus betae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂</td>
<td>♂</td>
<td>♂</td>
<td>♂</td>
</tr>
<tr>
<td>20 °C Adults ♂</td>
<td>36.0 ± 1.49</td>
<td>10.2 ± 1.11</td>
<td>6.2 ± 1.2</td>
<td>90.2 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C Adults ♂</td>
<td>80.0 ± 0.65</td>
<td>70.0 ± 0.7</td>
<td>80.0 ± 0.65</td>
<td>79.9 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 °C Adults ♂</td>
<td>75.0 ± 0.9</td>
<td>65.0 ± 0.66</td>
<td>75.0 ± 0.9</td>
<td>75.0 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26.8 ± 0.79</td>
<td>24.7 ± 1.06</td>
<td>26.8 ± 0.79</td>
<td>26.8 ± 0.79</td>
</tr>
<tr>
<td>25 °C Immatures ♂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 °C Immatures ♂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Similarly, Taha *et al.* (1988) studied the biology of two cheyletid predators *Cheyletus malyensis* and *C. lepidopterorum* on immature and eggs of *Caloglyphus rhizoglyphoides* at 25°C and 70 % R.H. Both predators had limited prey abundance. Development reproduction and predacious efficiency of the 2 predators were also studied. They added that the females of the predators tended to live longer than males. Youssef *et al.* (1982) reported that the cheyletid mite, *Cheyletus malaccensis* attacked a number of *T. putrescentiae* to a greater degree than on book lice and house fly eggs and first instar larvae. However the later the stage of development of the prey stage the greater was the predatory fecundity. Also, from this study, Cannibalism was usually noticed when the prey was absent or scarce. This phenomenon was also recorded by El-Duweini (1978) where the mites *Cheletomorpha caucasica* Rolgin, *C. eckerti* Summers and Price, *Acaropsellina docta*, *Cheyletus malaccensis* (Cheyletidae) fed on the eggs of their own species in the absence of prey.

**Total reducing and non-reducing sugar contents**

The data in Table 4 revealed that *T. putrescentiae* had the highest amount of reducing sugar (0.209 mg/g fresh weigh) followed by *L. destructor, R. echinopus, C. betae* and *C. lepidopterorum* (0.179, 0.164, 0.158 and 0.150 mg/g fresh weigh, respectively. On the other hand, the results demonstrated that the predator *C. lepidopterorum* had the greatest amount and contained 0.136 mg/g fresh weight, of the non-reducing sugar while the astigmatid mites (diets) had relatively similar contents. Generally, *T. putrescentiae* was the prey containing the
highest total sugar 0.228 mg/g fresh weight. The lowest was *C. betae* with 0.171 mg/g fresh weight.

Table 4. Total reducing and non-reducing sugar contents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sugar contents (mg / fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total sugar</td>
</tr>
<tr>
<td><em>C. lepidopterorum</em></td>
<td>0.286</td>
</tr>
<tr>
<td><em>T. putrescentiae</em></td>
<td>0.228</td>
</tr>
<tr>
<td><em>L. destructor</em></td>
<td>0.196</td>
</tr>
<tr>
<td><em>R. echinopus</em></td>
<td>0.178</td>
</tr>
<tr>
<td><em>C. betae</em></td>
<td><strong>0.171</strong></td>
</tr>
</tbody>
</table>

**Relative concentration of glucose content**

The present experiments have demonstrated that the mite, *C. lepidopterorum* had the highest relative concentration of glucose content (93.16%) (Table 5). By contrast, the mite *C. betae* had the lowest glucose content (51.59%).

Table 5. Relative concentration of glucose content (%).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative concentration of glucose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. lepidopterorum</em></td>
<td>93.16</td>
</tr>
<tr>
<td><em>T. putrescentiae</em></td>
<td>65.10</td>
</tr>
<tr>
<td><em>L. destructor</em></td>
<td>61.12</td>
</tr>
<tr>
<td><em>R. echinopus</em></td>
<td>52.18</td>
</tr>
<tr>
<td><em>C. betae</em></td>
<td>51.59</td>
</tr>
</tbody>
</table>

**Relative concentration and fraction of free amino acids contents**

As shown in Table 6, the commonest amino acids found in the predaceous mite and the tested preys were Aspartic, Glycine and Cysteine. On the other hand, Therionine, Leucine and Hydroxy-prolin were the lowest amino acid contents present in the tested organisms. The highest relative concentration and fraction of amino acids contents was Aspartic acid where it was 21.22, 29.71, 38.10, 34.10 and 21.60 in the cheyletid mite, *R. echinopus*, *L. destructor*, *T. putrescentiae* and *C. betae*, respectively. Statistical significant deviations existed between the amino acids estimated in the different tested targets. From the data, it can be seen that, *T. putrescentiae* contained the highest contents of total sugar and high relative concentration of glucose. This may be the reason why *T. putrescentiae* was the best prey for predatory mite feeding. The results are in agreement with those obtained by Yassin (2006) who recorded that collembola proved to be the most suitable prey for feeding the cunaxid mite *Cunaxa caprolus* (Berlese) because it contained the highest total sugar and a high relative concentration of glucose.. From the results, it can be concluded that the cheletid mite *C. lepidopterorum* may be considered as potential biological control agent of the harmful stored product mites. The mite appeared more efficient because of its higher fecundity and long survival when fed on the acarid mite, *T. putrescentiae*. 
Table 6. Relative concentration and fraction of free amino acids contents.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Rt.</th>
<th>C. lepidopterorum</th>
<th>R. echinopus</th>
<th>L. destructor</th>
<th>T. putrescentiae</th>
<th>C. betae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>1.33</td>
<td>31.2</td>
<td>4.60</td>
<td>–</td>
<td>11.70</td>
<td>–</td>
</tr>
<tr>
<td>Aspartic</td>
<td>1.10</td>
<td>21.22</td>
<td>29.71</td>
<td>38.10</td>
<td>34.10</td>
<td>21.62</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.61</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.99</td>
<td>–</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.45</td>
<td>1.44</td>
<td>4.22</td>
<td>3.11</td>
<td>4.64</td>
<td>2.62</td>
</tr>
<tr>
<td>Valine</td>
<td>3.09</td>
<td>0.010</td>
<td>–</td>
<td>0.41</td>
<td>0.148</td>
<td>0.019</td>
</tr>
<tr>
<td>Leucine</td>
<td>4.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.210</td>
<td>0.180</td>
</tr>
<tr>
<td>Proline</td>
<td>1.8</td>
<td>7.10</td>
<td>10.0</td>
<td>–</td>
<td>2.07</td>
<td>1.66</td>
</tr>
<tr>
<td>Glutamic</td>
<td>1.40</td>
<td>3.10</td>
<td>3.41</td>
<td>5.70</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.2</td>
<td>2.00</td>
<td>0.016</td>
<td>0.018</td>
<td>0.028</td>
<td>0.009</td>
</tr>
<tr>
<td>Cysteine</td>
<td>4.30</td>
<td>0.180</td>
<td>0.100</td>
<td>0.060</td>
<td>5.11</td>
<td>3.22</td>
</tr>
<tr>
<td>Cystine</td>
<td>4.90</td>
<td>3.07</td>
<td>–</td>
<td>–</td>
<td>0.66</td>
<td>0.10</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.41</td>
<td>–</td>
<td>–</td>
<td>0.026</td>
<td>9.82</td>
<td>7.62</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>8.32</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>12.00</td>
<td>9.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.0</td>
<td>–</td>
<td>–</td>
<td>7.11</td>
<td>4.11</td>
<td>3.62</td>
</tr>
<tr>
<td>Agrinine</td>
<td>1.80</td>
<td>2.86</td>
<td>3.91</td>
<td>3.00</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

References


Zaher, M.A. 1986. Survey and ecological studies on phytophagous, predaceous and soil mites in Egypt. – PL. 480 programme, U.S.A. project No. EG.ARS.
Suitability of species as food for mould mite *Tyrophagus putrescentiae* (Selm)

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**Abstract:** In the present investigations the effect of food – four species of aromatic plants – on the length of the mould mite (*T. putrescentiae*) generation development period, the mortality rate during growth and fecundity and longevity of the mites were studied. Thus the demographic parameters have been calculated for comparisons of populations of particular mites depending on kind of food. The data obtained on aromatic plants are relatively low as compared with control (yeast). The development of a whole generation was observed on all five tested plant species. Average time of development of the mites was always longer on aromatic plants than in control. Mortality during development on *Ocinum basilicum*, *Levisticum officinale*, *Laurus nobilis* and *Origanum vulgare* was significantly higher than in control. The highest mortality of juvenile stage, 96.0%, was observed on *Origanum vulgare*. Average fecundity of the mould mite was significantly lower on tested food than on the control. Fecundity of females on *Anethum graveolens* was 4 times lower (76,63 eggs per females), and on *Origanum vulgare* 21 times lower (16,5 eggs per females) than on yeast (353 eggs per females). The longevity of females of the mould mite on tested herbs was comparable with control. Reproduction parameters obtained on tested aromatic plants indicate their high suitability as diets for studied mites. In comparing the intrinsic rate of natural increase (rm) it is seen that on tested food the population of the mould mite increased much less than in control. The highest values of reproduction parameters were observed for *T. putrescentiae* only on *Anethum graveolens* (rm= 0,815). Among the five aromatic plants intrinsic rate of natural increase on *Origanum vulgare* and *Ocinum basilicum* was negative (rm= -9.604 and rm = - 4.803 respectively). The net reproduction rate (R0) on each combination it was several times than in control. The results obtained seem to state that all five tested aromatic plants make the possibility of development of the mould mite, and the most attractive showed to be *Anethum graveolens*.

**Key words:** Tyrophagus putrescentiae, Ocinum basilicum, Levisticum officinale, Laurus nobilis, Origanum vulgare, mite development
Session 3:
Insect detection, monitoring, trapping, pheromones and mating disruption
Comparison of methods for sampling Psocids in stored wheat

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Abstract: Psocids are an emerging problem in stored grain and in grain processing facilities in the United States. We compared several methods for sampling psocids in wheat stored in steel bins – grain trier samples, cardboard refuges on the surface of the grain and near the bin hatch, and automated sampling using the StorMax Insector system. The psocid species found were Liposcelis entomophila in 2005 and L. decolor in 2006. The numbers of psocids in cardboard refuges on the wheat surface were low immediately after bins were filled in July 2005, peaked in October, dropped to almost zero in December as temperatures decreased during winter, and then remained at low levels until the study was ended in April 2006. In 2006, the number of psocids in surface refuges increased gradually from August to mid-October, and remained at this level until the study ended in early November. The number of psocids in cardboard refuges and in Insector probes was indicative of the number of psocids in grain samples in both years. The results indicated that cardboard refuges or Insectors may provide an efficient method for sampling psocids in bins of wheat, and that psocid populations can increase quickly to high levels during storage even though they are low early in the storage period.

Keywords: psocids, sampling, trapping, wheat, Liposcelis decolor, Liposcelis entomophila

Introduction

Psocids are pests in grain storages, grain processing facilities, and product warehouses (Rees and Walker, 1990). They can damage stored grain (Kucerova, 2002) and can cause health problems (Sidik et al., 1986). Most psocid pests of stored products are in the genus Liposcelis (Liposcelididae). The species Liposcelis bostrychophila Badonnel – the most studied stored product psocid pest – has a worldwide distribution (Lienhard and Smithers, 2002) and is commonly found in households, granaries, and warehouses (Broadhead, 1954; New, 1971; Turner, 1994). The psocid species known to infest grain in North America (Sinha, 1988; Mockford, 1993; Lienhard and Smithers, 2002) are Lepinotus reticulatus, Lip. bostrychophila, Lip. brunnea, Lip. corrodens, Lip. decolor, Lip. entomophila, Lip. paeta, and Lip. rugosa; all except Lip. rugosa have been reported in the United States. Prior to 1990, psocids were not considered serious pests of stored products, but, in some countries such as Australia, they have become the most frequently encountered storage pests (Rees, 2003).

Many studies have been conducted on management of psocid pests of stored products (e.g., Leong and Ho, 1994; Ho and Winks, 1995; Santoso et al., 1996; Wang et al., 1999a; Ding et al., 2002), but few detailed studies have been conducted on their biology (Fahy, 1971; Khalafalla, 1990; Wang et al., 1999b; Wang et al., 2000; Wang et al., 2001). Wang et al. (1999b, 2000) conducted life history studies on L. bostrychophila and developed predictive models. Sinha (1988) determined temperospatial distribution of psocids in stored wheat in Canada. The objective of our study was to determine whether the numbers of psocids in wheat samples could be predicted from numbers of psocids in corrugated cardboard refuges placed on the hatch of the bin or the surface of the grain or from manual or automated electronic
counts from StorMax Insector probe traps (OPI Systems, Calgary, Alberta, Canada) (Shuman et al., 2005). Insector probe traps are similar to standard probe traps, such as the WB Probe II Trap (Trécé, Inc., Adair, OK, USA), but insects falling into the Insectors are counted automatically as they pass through two intersecting infrared beams. Manual Insector counts are similar to catches that would be observed using the standard probe traps without automatic counting. Trier samples are considered to be the best estimate of the true population density of psocids in the grain, but their use poses worker safety hazards and is laborious. Therefore, we wished to determine which trapping method best correlates with the trier samples.

**Materials and methods**

Two steel bins (4.72-m diameter by 3.35-m high at the eaves) were filled with newly harvested hard red winter wheat in July 2005, and insects in the wheat were sampled biweekly from August 2005 through March 2006. Each bin was filled with 1,200 bushels (32.6 metric tonnes) of wheat to a depth of 2.4 m. A 1.2-m open-ended trier was used to take 1-m-deep grain samples. These samples were taken from the bin center and in the north, south, east, and west directions at 0.15 and 0.76 m from the bin wall (Fig. 1). Grain samples were sieved using a US Standard #10 sieve (2-mm openings) to remove psocids.

![Diagram of sampling locations](image)

Fig. 1. Location of four grain samples (denoted by numbers) taken around an Insector probe trap (denoted by letters) located in each cardinal direction and in the center of the bin.

One week prior to taking grain samples with the trier, twenty refuges made from 8.9- by 12.7-cm pieces of corrugated cardboard were randomly placed on the surface of the grain in each bin and three refuges were placed on the hatch, and these were removed just before grain samples were taken. Psocids were removed from the refuges by knocking them out of the cardboard into a white enamel pan. Also one week prior to taking the grain samples, we placed an Insector probe in the center of each sampling location (indicated by directional letters in Fig. 1). The five probes were pushed into the grain with the top of the probe one foot below the grain surface, and the probes were removed just before grain samples were taken. We counted psocids in the probe tips and collected electronic counts. The wheat was fumigated in June 2006, and then reused for sampling from July through November 2006.

Data were analyzed to determine whether the numbers of psocids in corrugated cardboard refuges placed on the hatch of the bin or the surface of the grain or from manual or automated electronic counts from Insectors were correlated with numbers in grain samples using the General Linear Models procedure of SAS (SAS Institute, 2001).
Results

The only psocid species found in the bins were *Lip. entomophila* in 2005 and *Lip. decolor* in 2006. A total of 115,059 and 130,222 psocids were collected in 2005 (Fig. 2) and 2006 (Fig. 3), respectively. The numbers of psocids collected in the two bins were very similar for a given trapping method within each year. The Insectors caught more psocids than the other methods (77,502 and 117,201 in the two bins in 2005 and 2006, respectively), with the fewest psocids found in grain samples (547 and 408 in 2005 and 2006, respectively) and intermediate numbers in the refuges (33,615 and 7,687 in surface refuges and 3,395 and 4,926 in hatch refuges in 2005 and 2006, respectively). Seasonal abundance was similar using the different methods, except that no psocids were found in the hatch refuges during winter; this was not surprising because the hatch is probably at the same temperature as the outside air, while the grain is warmer than ambient air during winter.

![Graph showing psocid collection](image)

Fig. 2. *Liposcelis entomophila* collected in grain samples, refuges, and Insectors in 2005. The totals in the individual graphs are the cumulative number collected from August to March.
Fig. 3. *Liposcelis decolor* collected in grain samples, refuges, and Insectors in 2006. The totals in the individual graphs are the cumulative number collected from August to November.

The number of psocids in grain samples was best predicted by manual Insector counts in both years (Figs. 4 and 5). However, electronic Insector counts and cardboard refuges also gave reasonable estimates of numbers of insects in grain samples (Table 1).

Table 1. Summary of correlations between numbers of psocids collected using different sampling methods with numbers in grain trier samples.

<table>
<thead>
<tr>
<th>Sampling method</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatch refuges</td>
<td>0.50</td>
<td>0.68</td>
</tr>
<tr>
<td>Surface refuges</td>
<td>0.49</td>
<td>0.54</td>
</tr>
<tr>
<td>Manual Insector counts</td>
<td>0.68</td>
<td>0.88</td>
</tr>
<tr>
<td>Electronic Insector counts</td>
<td>0.63</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Electronic Insector counts gave a good estimate of actual number of psocids in the probe traps (Fig. 6). In 2005, there were many missing electronic counts because numbers of psocids in Insectors often exceeded the data storage capabilities of the system; electronic counts overestimated actual numbers of psocids in the probe traps when catches were high. In 2006, there were no missing counts, and electronic counts underestimated actual numbers of psocids in the probe traps when catches were high.

Fig. 4. Correlations between numbers of Liposcelis entomophila found using different sampling methods and in grain samples in 2005.

Discussion

Although manual Insector counts provided the best estimates of numbers of psocids in grain trier samples, entering the bins to service the traps is labor intensive, requires moderately expensive equipment, poses worker safety issues, and the manual counting of thousands of psocids is laborious. Electronic Insector counts also provide good estimates of numbers of psocids in grain samples and the process is automated so no counting or bin entry is required, but the equipment is relatively expensive. The Insector system provides the additional
advantage of sampling beetles in the grain, while the refuges sample only psocids. Hatch refuges also provide good estimates of numbers of psocids in grain samples, require climbing to the hatch during warmer periods of the year, require a moderate level of counting psocids, and have no equipment costs. Thus, if one does not want to spend the money for the Insector system, the hatch refuges provide a low cost alternative for sampling psocids.

Fig. 5. Correlations between numbers of *Liposcelis decolor* found using different sampling methods and in grain samples in 2006.

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Fig. 6. Correlations between numbers of manual and electronic Insector counts.

References


Evaluating treatment efficacy in commercial food facilities: Insights gained from small-scale simulated warehouse experiments

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Abstract: Although critical to a successful IPM program, it is challenging to evaluate treatment efficacy in commercial food facilities because of the inability to obtain absolute estimates of insect population levels. These populations are spatially fragmented and occupy cryptic habitats, such as equipment, packages, and the structure of buildings that cannot be identified and sampled for practical and economical reasons. This leads to a number of important questions including (1) what impact are treatments such as surface, crack and crevice, or aerosol applications having on pest population dynamics, (2) how does application method impact suppression of established infestations and reduction of new infestations, and (3) how well do pheromone traps that indirectly sample from dispersing individuals represent the absolute population density and subsequent changes as a result of the treatment. While these questions cannot be accurately addressed in commercial facilities, they can be explored in small-scale simulated warehouses where resource amounts and distribution, and initial pest density can be controlled and refugia can be directly sampled to estimate absolute population levels. Using the red flour beetle, Tribolium castaneum (Herbst), as a model organism and replicated small sheds with shelving units containing hidden resource patches of flour as simulated warehouses, we have begun addressing the above questions. Results of one of these experiments are presented and the potential impact on how management programs should be implemented and evaluated in commercial food facilities is discussed.

Key words: red flour beetle, Tribolium castaneum, integrated pest management, population dynamics, pheromone trapping

Introduction

Evaluation of treatment effectiveness is fundamental to developing effective integrated pest management programs for the food industry. For stored-product pest management in food processing and storage facilities such as mills, processing plants, warehouses and retail stores there are a number of unique challenges that make it difficult to determine the impact of pest management tactics in the field and to scale results up from the laboratory to the field. First, the true pest population density and spatial distribution within a facility cannot be accurately determined, so it is difficult to evaluate changes in population levels after treatment. We have only incomplete and/or indirectly measured estimates of population levels obtained by methods such as visual inspection and pheromone trapping, and the relationships between these measures and the true population density are not clear. Visual inspection is limited in that a large, but unknown, proportion of a pest population is likely to be in areas that are not known or cannot be readily inspected. Pheromone trapping primarily is capturing dispersing individuals but the relationship between population density and dispersal may not be linear.
Additionally, insects can disperse considerable distances, even from outside of structures, so a result trap captures may not be proportional to actual population density inside a structure (Campbell and Arbogast 2004). Second, because of this pattern of pest distribution the pest management tactics are typically applied to a facility in a manner where there is considerable variation in the level of exposure within the pest population, ranging from no exposure to a lethal exposure level. The portion of the population exposed will depend on the type of treatment, method of application, and the population structure of the pest. Third, most operational commercial facilities will have multiple tactics occurring at the same time (e.g., sanitation, movement of products) that impact pest populations and pest population levels often exhibit temporal variability independent of treatments, thus it is difficult to isolate the impact of a single tactic.

Thus, while it is relatively easy to evaluate the impact of treatment with a pesticide, pathogen or natural enemy in the laboratory, in real world situations the spatial complexity of the landscape and the distribution of the pests needs to be taken into account. For example, if we want to evaluate the effectiveness of a pesticide application in a warehouse, we can determine the lethal dose for a given species and life stage on a particular type of substrate in the laboratory, but in the warehouse not all individuals are being exposed to the same level of the pesticide, if they are exposed at all. Thus, it becomes important to determine (1) the spatial distribution of the pesticide in the facility, (2) what proportion of the pest population is exposed to the treatment, and (3) what are the long term consequences of this level of contact between treatment and pest population. Level of exposure of the pest to the pesticide depends on the type of compound, the application method, the area treated, persistence of the compound, interaction of compound with different substrates, penetration of the compound into areas occupied by pests, and movement by the pest through treated areas. Proportion of the population exposed depends on the spatial distribution of the compound as well as the spatial distribution, behavioral patterns such as immigration and emigration and dispersal distances, and demography of the pest population. Given that in food facilities the majority of the population is hidden and most management tactics with the exception of fumigation and heat have limited ability to penetrate into these hidden areas, some portion of the population is likely to be escaping lethal exposure to treatment. In addition to these issues, immigration and recolonization by individuals from untreated areas may occur. As a result the long term impacts of these different management tactics can be difficult to evaluate or predict.

This leads to a number of important questions including (1) what impact are treatments such as surface, crack and crevice, or aerosol applications having on pest population dynamics, (2) how does application method impact suppression of established infestations and reduction of new infestations, and (3) how well do pheromone traps that indirectly sample from dispersing individuals represent the absolute population density and subsequent changes due to treatment. Intermediate scale experiments that reproduce some of the spatial patterns of pest distribution and pesticide application can be a useful approach to addressing these questions (e.g., Toews et al., 2005ab). Using sheds as pilot scale warehouses, the spatial complexity of commercial facilities can be simulated, locations of resource patches for insects can be controlled and directly sampled, initial pest population conditions and levels of immigration and emigration can be controlled, long term population impacts can be evaluated, environmental conditions can be regulated and controls and replications can be performed. Results from one specific experiment will be presented here as an illustration of the approach. In this experiment, pesticide was applied around foci of red flour beetle, *Tribolium castaneum* (Herbst), infestation hidden in refugia under a shelf and the impact of treatment on the initial population and on spread into uninfested patches under other shelves was evaluated.
Materials and methods

Pilot-scale warehouses
Studies were conducted in five climate-controlled sheds that were set up to be pilot scale warehouses (Toews et al. 2005ab). The sheds measured 2.8 m wide by 5.9 m long by ≥2 m tall. Before start of experiments, the sheds interiors were lined and sealed to reduce or eliminate insect immigration and emigration, and the interior linings were replaced between replicates to reduce residual pesticide cross-contamination. Each shed was provisioned with three custom-made shelving units 46 cm from the walls to provide insect refugia (Toews et al. 2005a). Shelving units, measuring 53 cm wide by 119 cm long by 12 cm tall, were designed to be easily moved to permit easy access to the food patches underneath during sampling. Temperature in the sheds was maintained between 25 and 27°C. Humidity was not controlled, but mean weekly values ranged from 45 to 55% RH. Experiments were run under 24 hr light conditions with light intensity of 42.0±2.6 lx at ground level.

Initiation of Experiments
Sheds were prepared by applying insecticide, infesting refugia with *T. castaneum*, and installing pheromone baited traps. Two sheds were randomly assigned as controls (water treated) and the remaining three sheds were insecticide treated. After removing the shelves to avoid contamination, insecticide was applied in a band around the outline of the shelves in the treatment-designated pilot-scale warehouses, while distilled water was similarly applied in the control-designated warehouses. In the experiment described here, the insecticide used was the synthetic pyrethroid *β*-cyfluthrin (Tempo® Ultra WP, Bayer Corp., Kansas City MO, USA) applied at the highest labeled concentration of 0.05%. Twenty-four h after application of insecticide, shelving units were returned into each warehouse and four food patches (50 g each of previously frozen white flour on pieces of 125 mm filter paper) were situated under each shelf (Fig. 1). For shelves assigned as being infested, we added equal numbers of eggs, small larvae, large larvae, pupae, and adults to the flour patches. In this experiment, only the north shelf in each shed was infested with 168 individuals of each life stage evenly distributed among the four patches under the shelf. Insects were allowed to disperse for 24 h before pitfall traps (Dome trap, Trécé Inc., Adair OK, USA), baited with *T. castaneum* pheromone and food oil were positioned in each corner of the shed. Pheromone lures and food oil were replaced every 6 wks during the study.

Monitoring treatment efficacy
The monitoring program used three different measures of efficacy and was initiated one wk after trap placement and continued weekly for the duration of the experiment (15 wk in this experiment). First, dead adults found on the floors outside the refugia were collected and counted. Second, pheromone traps were examined and larvae and adults captured during the trapping interval were counted and removed. Pheromone traps were positioned in the corners of the pilot-scale warehouse to simulate a condition where the true source of the insect population was not known. Finally, each flour patch was subsampled with the removed flour replaced with fresh flour (~2.5 g samples of flour from each food patch). Newly acquired samples were brought to the laboratory, weighed, sieved through a #60 U.S. standard testing sieve to remove insects, and the number of larvae, pupae and adults present determined (in this experiment number of eggs was not counted). Number of individuals per g of flour was then calculated.
Experimental design and analysis

Experimental treatments were designed as a completely randomized design with repeated measures. Response variables included the number of dead adults per warehouse, mean number of larvae and adults captured in pitfall traps, and mean number of larvae, pupae, and adults recovered in flour samples and were analyzed for insecticide treatment and sampling date effects using PROC MIXED (SAS Institute 1999). To normalize variances, a log transformation (Zar 1999) was performed on counts of dead adults and counts of adults and larvae collected from pitfall traps, but actual means and standard error were presented in all figures. All response variables were presented in the figures by interaction because one of the objectives was to compare trends suggested by traps with direct samples. If the interaction test was not significant (α = 0.1 for interactions), independent tests were conducted for differences between insecticide treatments and among treatment dates (α = 0.05). The slice option of the LSMEANS statement was used to investigate differences between insecticide treatments while controlling the effect of date when there was evidence of interactions.

Several simple correlation parameters were also examined to better understand relationships among variables by sampling week and insecticide treatment. Pearson product-moment correlation was determined for mean adult captures in traps and for mean adults recovered in food patches under all shelves and for adults recovered under all shelves and mean number of dead adults per pilot-scale warehouse. Correlations were calculated using PROC CORR (SAS Institute 1999).

Results

There were obvious treatment differences observed for dead adults and insects captured in the pitfall traps. For dead adult T. castaneum recovered on the floors, there was a significant insecticide treatment by sampling date interaction (F_{14, 42} = 2.19, P = 0.03) (Fig. 2). The plot of dead insects in the control treatment was flat relative to the fluctuating level of dead insects in the cyfluthrin-treated warehouses. In the pheromone and food oil baited pitfall traps, more adult T. castaneum were captured in the control treatment than in the insecticide treatment (Fig. 3). A significant insecticide treatment by sampling date interaction was detected for
adults captured in pitfall traps (F \(_{14, 42} = 3.0, P < 0.01\)) and at most time points significantly more adults were captured in the control compared to insecticide treated shed. Fewer larvae than adults were captured in traps, and the insecticide treatment by sampling date interaction was not significant (F \(_{14, 42} = 1.6, P = 0.11\)) (Fig. 3). Tests for differences between treatments (F \(_{1, 3} = 12.4, P = 0.04\)) and among sampling dates (F \(_{14, 42} = 3.7, P < 0.01\)) for captures of T. castaneum larvae in traps were significant.

Fig. 2. Mean ± SEM dead adults recovered weekly from pilot scale warehouses. Asterisks (*) indicate significant differences between the control and cyfluthrin treatments by date (protected lsmeans test, P< 0.05).

Although treatment effects were detected using the indirect sampling methods of pheromone trapping and counting of dead adults, no significant impact on the T. castaneum population within the food refuges was detected. Analyses of individuals recovered from direct sampling of the food refuges from the initially infested north shelves indicated no differences between the treatment and control (Fig. 4). There were no two-way interactions for larvae (F \(_{14, 42} = 0.5, P = 0.95\)), pupae (F \(_{14, 42} = 1.6, P = 0.12\)), or adults (F \(_{14, 42} = 1.3, P = 0.23\)). Similarly, there were no differences between the insecticide treatments for larvae (F \(_{1, 3} = 0.1, P = 0.84\)), pupae (F \(_{1, 3} = 0.1, P = 0.81\)), or adults (F \(_{1, 3} = 1.0, P = 0.38\)). However, because of fluctuations in population levels in refugia there were differences among sampling dates for number of larvae (F \(_{14, 42} = 6.4, P < 0.01\)), pupae (F \(_{14, 42} = 5.5, P < 0.01\)), and adults (F \(_{14, 42} = 4.5, P < 0.01\)). An initial peak in larvae occurred five wk into the study, which was followed by a similar peak in pupae two wk later. The adult population did not peak for three wks after the peak in pupae (Fig. 4).

Analyses of individuals recovered from direct sampling of refugia in the initially uninfested (south and east) shelves showed that cyfluthrin treatments suppressed insect infestation by immatures, relative to the control, for 6 wk. After this time period, the population of larvae was not different regardless of treatment (Fig. 5). Although there was a significant interaction between insecticide treatment and sampling date for larvae (F \(_{14, 42} = 1.7, P = 0.09\)), this statistic was not significant for pupae (F \(_{14, 42} = 1.6, P = 0.12\)) or adults (F \(_{14, 42} = 1.0, P = 0.46\)). Tests for differences between the control and cyfluthrin treatment were not significant for pupae (F \(_{1, 3} = 4.78, P = 0.11\)), but were significant for adults (F \(_{1, 3} = 35.5, P < 0.01\)). Obvious differences among sampling dates were detected for pupae (F \(_{14, 42} = 3.6, P < 0.01\)) and adults (F \(_{14, 42} = 8.8, P < 0.01\)).
A significant correlation was observed between adults captured in traps and adults recovered in food patches under all shelves for the control treatment ($r = 0.70, P < 0.01, n = 15$), but not the cyfluthrin treated replications ($r = 0.46, P = 0.09, n = 15$). In the control treatment, there was no correlation between adults recovered in food patches under all shelves and dead adults found on the floor ($r = 0.37, P = 0.18, n = 15$), but the correlation was significant for the same variables in the cyfluthrin treated warehouses ($r = 0.64, P = 0.01, n = 15, n = 15$).

Discussion

One objective in these experiments was to evaluate how accurately pheromone trapping represented the actual population in hidden refugia. This could be done by comparing T. castaneum population trends suggested by trap captures with direct estimates of the population in untreated and cyfluthrin-treated pilot-scale warehouses. The plot of adult captures in pitfall traps strongly suggested that the population was increasing in the control replications, but there was no suggestion of population increase in the cyfluthrin treated replications. However, the plot of adults recovered in the direct samples showed that insect populations were increasing at similar rates, regardless of insecticide treatment, starting five wk after the study commenced. Subsequent analyses proved that the number of adults captured in the traps was only correlated with the number of adults recovered in the food patches under the control treatment. These findings show that while under control conditions pheromone traps provided a good representation of the true pest population, application of pesticides could impact trap captures in a manner that could provide an inaccurate representation of the true pest population. Clearly, different insecticide compounds and
application methods could result in different relationships, but these results do indicate the difficulty in interpreting the results of pheromone monitoring programs in commercial food facilities where multiple pesticides might be used.

Death of adults before arrival at the pheromone traps is the most logical explanation for the discrepancy in adults captured in traps between the cyfluthrin-treated and control pilot-scale warehouses. Little is known about the time required for death following cyfluthrin exposure by contact. If the insects do not succumb immediately to the toxicant, it is also possible that stressed individuals may be less attracted to the pheromone-baited traps. The plot of dead insects removed from each warehouse on a weekly basis confirmed that the overall insect population in the cyfluthrin treated warehouses was indeed increasing, as suggested by the direct sampling in the food patches. It also shows that the cyfluthrin residues were not dissipating rapidly, which is logical since there was no exposure to extreme heat,
ultra-violet radiation, or hydrolysis on a porous surface. Interestingly, the plots of adults captured in the control replications and dead insects recovered in the cyfluthrin treated warehouses are virtually mirror images of each other.

![Graph showing mean ± SEM larvae, pupae, and adults per g flour recovered in food patches under the south and east shelves only. Asterisks (*) indicate significant differences between the control and cyfluthrin treatments by date (protected lsmeans test, P< 0.05).](image)

Fig. 5. Mean ± SEM larvae (A), pupae (B), and adults (C), per g flour recovered in food patches under the south and east shelves only. Asterisks (*) indicate significant differences between the control and cyfluthrin treatments by date (protected lsmeans test, P< 0.05).

Another objective of this experiment was to test the hypothesis that treatment with cyfluthrin in a banding pattern could reduce or prevent successful emigration from infested patches and colonization of uninfested patches. There are two pieces of evidence to refute this hypothesis. First, adults were captured in pitfall traps during every sampling interval of the experiment. The only way for insects to crawl into the traps (other than flight) would be to pass through the band of insecticide encircling the north shelves, where the insects were initially released. Second, the uninfested food patches (located under the south and east shelves) contained small numbers of larvae starting at the second week of the study. Interestingly, the initially uninfested food patches stayed relatively clean for about 6 wk; about 1 generation at these temperatures. After this period, the insect populations were similar
to those observed in the initially infested food patches. Although a considerable number of adults were killed in the insecticide treatment it was not enough to impact the overall population trends, suggesting that primarily adults were exposed to a lethal dose of the pesticide and the population was able to compensate for these lost adults. Different insect densities or distributions may respond differently to this treatment and this is an area for further exploration.

Use of pilot scale warehouses to simulate some of the spatial complexity of food facilities enables treatment efficacy to be evaluated in a more realistic manner than can be accomplished in a laboratory bioassay, but also enables the true impact to be tracked which would not be possible in a commercial food facility. Results of the specific experiment reported here, show that pheromone trapping may not always reliably reflect the true impact of a treatment, that cyfluthrin not applied directly to hidden refugia had little impact on the hidden populations of *T. castaneum*, and that having to cross a surface treated with cyfluthrin insecticide reduced the establishment of new populations in uninfested patches, for approximately one generation only. However, the six-wk window does suggest that this strategy deserves further investigation. Perhaps, this window could be expanded if the populations in other parts of the mill were suppressed using additional tactics. It needs to be emphasized that these findings at this point only apply to the specific set of conditions tested here, and how broadly applicable these patterns are needs to be further evaluated. Specifically, other factors that need to be tested include, but not limited to, other pesticides, application methods and treatment intervals, different *T. castaneum* densities and distributions, and increased distances between shelves.

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**References**


The use of sex pheromone traps for cigarette beetle as a tool for IPM in a cigarette factory in Cape Verde islands

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Abstract: Lasioderma serricorne (F.) (Coleoptera: Anobiidae) is the most serious insect threat to stored tobacco and cigarettes on the Cape Verde islands. A monitoring programme using sex pheromone traps for the cigarette beetle was initiated to detect sources of infestation and to assess the risk of tobacco damage. Trials were conducted to obtain estimates of the mean density of L. serricorne and to analyse its spatial pattern. The manager of the cigarette factory used the trap records to assess risk and implemented an empirical action threshold of 10 insects/week/trap. The variability in the trap catches indicated an aggregated pattern and negative binomial distribution fitted the data more accurately. From the results obtained a sequential sampling plan was developed for classifying the pest status of L. serricorne based on complete counts from insects caught in the pheromone traps. Two sequential sampling techniques were used: the sequential probability ratio test (SPRT), as the adult population followed the binomial negative distribution, and Iwao’s confidence interval method. It was shown that, using SPRT or Iwao’s methods, managers can make decisions using six and 13 traps, respectively, with a minimum risk of incorrect assessment. After five years of using this strategy, the relative population density of cigarette beetle has decreased below the level at which it is considered serious.

Key words: pheromone trap, Lasioderma serricorne, stored tobacco, sequential probability ratio test, Iwao’s confidence interval method

Introduction

The cigarette beetle, Lasioderma serricorne (F.) (Coleoptera: Anobiidae), is the most serious insect threat to stored tobacco (U.S.D.A. 1972; Ryan 1996). Damage to tobacco and cigarettes is mainly caused by the larvae that feed on the product and may contaminate it with their excreta. In addition, when exposed to insects or to their remains, the smokers and workers may have allergic responses (Almeida 1956; Ryan 1996; Bellas 1999).

Pheromone traps provide an easy, efficient and sensitive way to detect insect pests, in stores and facilities, and managers can use this information to locate infestations and make management decisions. For both, the synthetic sex pheromones traps for L. serricorne are perceived by users to be ‘very strong’ attractants because some insects are usually trapped if there is an infestation in the target facility. Thus, these pheromones are judged to be biologically very effective for detection and monitoring purposes (Phillips 1997). Pheromone traps have been used to monitor the density and spatial pattern of cigarette beetles to provide an early warning of pest incidence and to identify sources of infestations.

For management decisions, information about a population should be collected in the shortest amount of time, at the lowest possible cost, yet with a high level of reliability. Sequential sampling plans are useful for many insect pests because they minimize sampling
effort at a pre-selected level of precision. In sequential sampling, managers can specify error rates to minimize the risks of deciding to control the pest when it is not needed and the risk of not controlling the pest when control measures are in fact warranted, and give an exact measure so that with extremely high and extremely low populations very few sampling units need to be observed and the expenditure of time and cost is minimal. Sequential sampling plans have been little developed for stored-product insects (Southwood 1978; Subramanyam and Hagstrum 1996).

In 1997, the managers of the cigarette factory of Cape Verde Islands were faced with high infestations of cigarette beetle and some cigarette packs were rejected at that time. A monitoring program was implemented in September 1998 using sex pheromone traps for cigarette beetle, to help the managers in decision-making. Moreover trials were carried out until mid February 2001 to estimate the relative density and spatial pattern of the pest populations. From results obtained a sequential sampling plan was developed for classifying the pest status of *L. serricorne* based on complete counts from insects caught in the pheromone traps. In 2003, more data were collected in order to evaluate the efficacy of the sampling program and compare the relative density and spatial pattern of the cigarette beetle from previous experiments.

**Material and methods**

**Experimental sites**
The experiments were carried out from 28 September 1998 to 6 February 2001 (Experiment I) and from 1 January 2003 until mid September 2003 (Experiment II), in the cigarette factory as stores related (two tobacco stores and a cigarette store), in S. Vincent Island, in Cape Verde.

The temperature and relative humidity were recorded from a weather station at 1 km from the factory and stores, every week from September 1998 to September 1999, and every ten days during 2000, 2001 and 2003.

**Pheromones traps**
Mini Delta pheromone traps (AgriSense, UK) were used to study the relative density and spatial pattern of *L. serricorne*. The traps and lures were changed every six weeks and the number of trapped adult cigarette beetles was recorded weekly. Nine Mini Delta traps were used until July 2000 and due to the introduction of highly infested tobacco in the two tobacco stores, the number of traps increased to one more trap in each place: one in the factory, one in each tobacco store and one in the cigarette store, which contained packs of cigarettes. A total of 13 Mini Delta traps were used from that date until mid September 2003.

**Relative population density and spatial pattern**
Relative density and spatial pattern were determined using the sex pheromone trap as the sampling unit. For spatial pattern of *L. serricorne* adult males, Poisson and negative binomial distributions were analyzed for the set of 110 samples collected from September 1998 to January 2001. To determine the number of classes to use for fitting the distributions, the rule used was that the expected frequencies should be more than one and below this number they were cut off and pooled. Because of the low number of traps and the high number of cigarette beetles caught from 1998 to 2001, the more conservative values were used. For calculating the parameter *k* of the negative binomial distribution, the method of maximum likelihood was used. These mathematical distributions are used to describe random and aggregated distributions, respectively, and to allow the development of sequential sampling plans for classifying pest status (Ludwig & Reynolds 1988; Krebs 1989; Davis 1994). Since the data sets contained less than 30 sampling units, Iwao’s patchiness or mean crowding regression
was also used to analyze the relationship between Lloyd’s mean crowding index and mean density (Lloyd 1967; Iwao 1968; Southwood 1978; Krebs 1989; Davis 1994) for the set of the 110 samples collected from September 1998 to January 2001, and for the 37 samples collected during 2003.

**Sequential sampling plans for classifying Lasioderma serricorne status**

Two techniques were used: the sequential probability ratio test (SPRT) and the Iwao's confidence interval. These techniques for developing sequential sampling plans to classify pest status are fully described by several authors (Southwood 1978; Boivin and Vincent 1987; Krebs 1989; Davis 1994; Subramanyam and Hagstrum 1996).

The SPRT can be used for populations that follow the Poisson and the negative binomial distributions and two hypotheses were tested:

\[ H_0: \mu_0 \leq 5 \text{ cigarette beetles} \]
\[ H_1: \mu_1 \geq 10 \text{ cigarette beetles} \]

The upper decision line, or upper stop line, was calculated based on the action threshold of 10 insects/trap/week and the lower decision line or lower stop line, was calculated from a percentage of the threshold used, of 5 insects/trap/week. It was assumed the same specific error rates, \( \alpha \) and \( \beta \), of 0.05 operated in both cases.

Another sequential sampling plan was developed using Iwao's method. Although SPRT has two class limits and two error rates, Iwao's method has only one class limit, critical density, and tests the hypothesis that the density estimate from a set of traps is equal to the critical density against the alternative hypothesis that the set of traps is not equal to the critical density:

\[ H_0: \mu_0 = 10 \text{ cigarette beetles} \]
\[ H_1: \mu_1 \neq 10 \text{ cigarette beetles} \]

This method tests the error rate, of accepting the alternative hypothesis when the null hypothesis is true (type I) (Subramanyam and Hagstrum 1996).

**Results and discussion**

**Environmental conditions**

The environmental conditions are shown in Fig. 1. From September 1998 to February 2001, the mean temperature was 23.9±0.2ºC and ranged from 19.7ºC (last week of March 1999) to 28.3ºC (first week of October 1998). The mean relative humidity was 69.6±0.5% and varied from 55.5% r.h. (third week of March 2000) to 79.5% r.h. (third week of September 1999). During the 37 weeks of 2003 the mean temperature was 24.3±0.3ºC and varied from 20.9ºC (mid February) to 28.0ºC (in the beginning of September). The mean relative humidity was 69±0.8% and ranged from 59.5% r.h. (mid January) to 76.0% r.h. (mid of July). Larval activity of the cigarette beetle ceases when temperatures fall between 19.5ºC and 15.5ºC, and the larva hibernates at slightly lower temperatures (Runner 1919). The environmental conditions were favourable for pest development and that hibernation might not have occurred.

**Experiment I - Relative population density and spatial pattern**

The relative density of adult males of cigarette beetles, in the tobacco stores, in the factory and in the cigarettes are presented in Fig. 2. Several major peaks occurred during December 1998 (42 insects/trap/week) and the second week of February 1999 (26 insects/trap/week). In May and June 2000, the number of cigarette beetles caught increased to reach a maximum 62.5 insects/trap/week during the last week of July, due to introduction of highly infested tobacco into the tobacco stores. In the factory, the three major peaks occurred in mid
December 1998 and in the first week of January 1999 (17.3 insects/trap/week) and in the last week of August 2000 (32 insects/trap/week). In the cigarette store the highest trap catches occurred during the first week of trials (October 1998) with 48 insects/trap/week, in the first week of April 1999 with 20 insects/trap/week, and in the last week of January 2000, with 25 insects/trap/week.

Although environmental conditions in the tropical regions may be more favourable to cigarette beetle development, the higher temperature variability registered in temperate regions may have a more stimulating effect in increasing the intrinsic rate of cigarette beetle populations (Odum 2000).

With the introduction of the monitoring program the manager could detect the source of infestation and established an empirical threshold level, of 10 insects/trap/week. This threshold was used to apply control actions at the appropriate time, as fumigation and cleaning techniques.

Regarding dispersion, the trap catches can provide important information on the spatial pattern of cigarette beetle adults, which is largely determined by their behavior. The observed counts were compared with the expected frequencies predict by Poisson and negative binomial distributions. Between 1998 and 2001, from 110 samples taken, 94 fitted the negative binomial and 19 fitted the Poisson distribution. It can be assumed from this that the spatial pattern of adult cigarette beetles fitted mainly the negative binomial distribution (Carvalho et al. 2006).

Table 1 shows Iwao’s patchiness regression estimates for cigarette beetles captured in the pheromone traps. From the 110 data sets (1998-2001), the linear regression model fitted almost all data well ($r^2=0.66$). The slope-$\beta$ suggested a strong tendency of aggregation in the dispersion pattern related to density ($\beta=2.1$) and the intercept-$\alpha$ ($\alpha=0.15; P=0.90$) indicates that the basic component of the population is an individual.
Experiment I – Sequential sampling plans
Sequential Probability Ratio Test
The spatial pattern of \( L. \) serricorne adults is described by a negative binomial distribution with \( k_c = 0.8517 \) (Carvalho et al. 2006). Population means specified as the lower and upper limits are, respectively, 5 insects/trap/week and 10 insects/trap/week, and error limits are \( \alpha = \beta = 0.05 \), \( p_0 = H_0/k_c = 5.87 \) and \( p_1 = H_1/k_c = 11.74 \). The slope of the sequential lines is \( b = 6.96 \); the interception \( h_0 = -38.9694 \) for the lower stop line; and the interception \( h_1 = +38.97 \) for the upper stop line where \( A = B = 2.94 \). The stop line equations for sequential sampling are \((n\) is the number of sampling units observed\) (Carvalho et al. 2007):

Lower stop line: \( Y_o = b_o + h_o = 6.9616n - 38.9695 \)
Upper stop line: \( Y_i = b_o + h_i = 6.9616n + 38.9695 \)

![Graph](image)

Fig. 2. Relative density of \( L. \) serricorne adults’ in the factory and stores and threshold level.

Table 1. Iwao’s patchiness regression estimates for \( Lasioderma serricorne \) adults’ associated to cigarette facility and stores related sampled with pheromone traps.

<table>
<thead>
<tr>
<th>Data</th>
<th>( n^a )</th>
<th>( \alpha \pm EP_\alpha )</th>
<th>( t = \alpha / EP_\alpha )</th>
<th>( \beta \pm EP_\beta )</th>
<th>( t = (\beta - 1) / EP_\beta )</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 1998 to 2001</td>
<td>110</td>
<td>0.15±1.23</td>
<td>0.12 (p=0.90)</td>
<td>2.11±0.144</td>
<td>7.67 (p=0)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

\( ^a \) number of pairs \( x \rightarrow \bar{x} \) used in the regression

The use of these formulae, for binomial negative data, is shown in Table 2. The lower stop line crossed the \( x \) axis at \( n=6 \): which means that no decision should be taken until the running total is obtained from a minimum of six pheromone traps, with a probability of accepting \( H_0 \) when \( H_1 \) passes the 0.05 level. From SPRT, managers can make decisions using less than 54% of the 13 traps used, with a minimum risk of 0.05 of incorrect assessment. It is advisable to place these traps in a grid pattern in order to cover all the available space
especially near walls where the traps caught significantly more insects (Carvalho and Mexia 2003).

Table 2. SPRT method - sequential sampling decision lines for monitoring *Lasioderma serricorne* adults in a cigarette factory, relative to the threshold of 10 insects/trap/week.

<table>
<thead>
<tr>
<th>Number of traps</th>
<th>Total accumulate of insects caught</th>
<th>Lower stop line</th>
<th>Upper stop line</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>Examine at least six traps</td>
<td></td>
<td>101</td>
</tr>
<tr>
<td>3</td>
<td>for a decision</td>
<td></td>
<td>115</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Continue sampling</td>
<td>129</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>143</td>
</tr>
<tr>
<td>6</td>
<td>Below lower stop line, no action</td>
<td></td>
<td>157</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>3</td>
<td>171</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>10</td>
<td>185</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>17</td>
<td>199</td>
</tr>
</tbody>
</table>

**Iwao’s confidence interval**

The value of Student’s *t*, for an infinite number of degrees of freedom and at 0.05 probability level is 1.96. Based on this regression, the upper and lower decision lines were calculated as: 

\[ U_i = n\mu_0 + 1.96A \quad \text{and} \quad U_o = n\mu_0 - 1.96A, \]

respectively, where *n* is the number of traps observed, \( \mu_0 \) is the critical pest density and 

\[ A = \sqrt{n[\mu_0(\alpha + 1) + (\beta - 1)\mu_0^2]}. \]

\( \alpha \) and \( \beta \) are the y-intercept and slope estimates.

The upper and lower stop lines are shown in Table 3. To ensure a representative sample, the decision is usually not applied until a minimum traps are observed. The lower stop line crossed x axis at *n*=13: which means no decision until the running total of catches is obtained from a minimum of 13 pheromone traps. Based on Iwao’s results, the manager decided to maintain the number of traps.

Table 3. Iwao's confidence interval method - sequential sampling decisions lines for monitoring *Lasioderma serricorne* adults, in the cigarette factory, relative to the threshold of 10 insects/trap/week.

<table>
<thead>
<tr>
<th>Number of traps</th>
<th>Total number of insects caught</th>
<th>Lower stop line</th>
<th>Upper stop line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Examine at least 13 traps</td>
<td>45</td>
<td>129</td>
</tr>
<tr>
<td>5</td>
<td>for a decision</td>
<td>101</td>
<td>211</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>227</td>
<td>242</td>
</tr>
<tr>
<td>11</td>
<td>Below lower stop line, no action</td>
<td>257</td>
<td>272</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>257</td>
<td>272</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>257</td>
<td>272</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>257</td>
<td>272</td>
</tr>
</tbody>
</table>

Above upper stop line action is needed.
Experiment II – Relative population density and spatial pattern
The relative population density of the cigarette beetle during 2003 was lower than the empirical threshold of 10 insects/trap/week adopted by the cigarette factory managers. From the 37 weeks sampled, no trap catches were registered during 13 weeks in the tobacco stores and during 15 weeks in the cigarette store (Fig. 3).

After five years of using this strategy, the relative population density of cigarette beetle was decreased below the level at which it is considered noxious.

Table 4 shows Iwao’s patchiness regression estimates for cigarette beetles captured in the pheromone traps in 2003. This linear regression model fitted better ($r^2 = 0.78$) the data obtained in 2003. The slope-β suggested a stronger tendency of aggregation ($\beta = 3.3$) and the intercept-α ($\alpha = -0.9; P=0$) indicates a tendency for repulsion between individuals.

![Graph showing relative density of L. serricorne adults' in the factory and stores, in 2003, and threshold level.](image)

Table 4. Iwao’s patchiness regression estimates for *Lasioderma serricorne* adults’ associated to cigarette facility and stores related sampled with pheromone traps.

<table>
<thead>
<tr>
<th>Data</th>
<th>α ± EPα</th>
<th>$t = \alpha / EP_\alpha$</th>
<th>β ± EPβ</th>
<th>$t = (\beta - 1) / EP_\beta$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>-0.90±0.17</td>
<td>-5.24 (p=0)</td>
<td>3.34±0.30</td>
<td>7.89 (p=0)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

* number of pairs $\hat{x} - \bar{x}$ used in the regression

Acknowledgements
The authors would like to thank the company manager Dr. Jorge Benchimol Duarte and Dr. Filomena Santos, for allowing the use of the tobacco facilities, where these trials were conducted, and the production staff who assisted in the collection of data.

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References


Monitoring insect populations by using adhesive surfaces of different colours in a dried fig warehouse in Southern Greece

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Abstract. Adhesive surfaces were suspended above the floor of a fig warehouse in the region of Kalamata, Southern Greece, which is the most important region for dried fig production in Greece. The surfaces of these traps were of five different colours: black, green, blue, white and yellow. Half the number was baited with TDA, the male attractant of several stored-product Pyralidae, while the rest remained unbaited. Monitoring was performed at weekly intervals from May 2002 until September 2002, by examining the adhesive surfaces for captured individuals and replacing them with new traps. Twenty-three insect taxa were found during the entire monitoring period. The most abundant species was Plodia interpunctella (Lepidoptera: Pyralidae) followed by the parasitoids Habrobracon hebetor (Hymenoptera: Braconidae) and Cephalonomia sp. (Hymenoptera: Bethylidae). These three species represented >85 % of the total number of individuals counted. Captures of P. interpunctella were continuously high, especially during summer months, and exceeded 30 adults/trap in early July. On the other hand, the numbers of parasitoids notably increased only during September. For P. interpunctella and H. hebetor, the colour of the sticky surface had no effect on the number of captured individuals. In contrast, more Cephalonomia sp. adults were captured on yellow than on black traps. More P. interpunctella adults were captured on traps far from the windows, in comparison with traps that were close to the windows, but this trend was evident only in the case of pheromone-baited traps. For both parasitoids, more adults were found on traps that were placed close to the windows. The separation of the sticky area into sub-areas, indicated that most P. interpunctella males were found in the area above the pheromonic lure, while no specific trend was noted on the unbaited traps. For H. hebetor, most captures were recorded at the lowest part of the trap, while both parasitoids avoided the upper trap part. A noticeable proportion of the captured H. hebetor individuals were recorded at the marginal outlines (edges) of the sticky surfaces.

Key words: stored figs, TDA, stored-fig pests, pheromone-baited traps, Plodia interpunctella, Habrobracon hebetor, Cephalonomia sp.

Introduction

Dried fig production in Greece reaches 8000 t/year, located chiefly in two regions: Peloponese and Evoia. Like other durable products during their storage, dried figs are infested by numerous pests that can cause heavy damages (Buchelos, 1985; Eliopoulos and Athanassiou, 2005). However, several insect parasitoids are likely to exist in dried fig stores seems to be common in other Greek dried fruit stores as well, such as currant and sultanas storerooms (Eliopoulos et al., 2002a; Athanassiou and Eliopoulos, 2003). In other commodities, such as cereals, these species are already used as biocontrol agents but there is still inadequate information in the case of dried figs.

Monitoring of insect pests and their natural enemies in storerooms is essential in order to estimate insect population and draw-up a judicious control strategy. During the last decades, traps also capture parasitoid individuals that are baited with pheromones of some pests, used
for this purpose. Nevertheless, trap characteristics often determine the capture rate, and this parameter should be further evaluated. For instance, in a previous study, Eliopoulos and Athanassiou (2005) noted that for some species, the trap colour and the trapping location was determinative for capture rate. Hence, unless these parameters are taken into account, any pheromone-based monitoring protocol may be inaccurate. In the present study, we monitored insect populations by means of aerial sticky surfaces in a dried fig storage in Peloponese, Southern Greece, in order to: a) assess insect composition and population densities and: b) to examine the influence of certain characteristics of the traps on capture capacity.

Materials and methods

The experiment was conducted in Kalamata (Peloponese, Southern Greece), which is the major fig producing region of Greece. An horizontal (flat) warehouse, which is part of a series of nine similar warehouses (approx. 500 m²), was used for this study. At the western walls of the storeroom were two closed windows. The room contained dried figs harvested in 2001. During May 2002, 10 traps were suspended in the store room. These traps were rectangular cardboard surfaces 27 x 8 cm, covered with Tanglefoot (Buchelos and Levinson 1993). In half of the traps, a capsule containing 1 mg of TDA, (Z,E,-9,12-tetradecadien-1-yl acetate), the male attractant of several Pyralid stored-product moth species (Levinson and Buchelos, 1981) was placed, at the center of the trap (intersection of the diagonals). The remaining half of the traps used were unbaited. Moreover, the 10 traps were of different colours: 2 white, 2 black, 2 green, 2 yellow and 2 blue (one baited and one unbaited for each case). The traps were hung from a wire, at a height of approx. 1.5 m above the floor. The distance between two adjacent traps was >2 m. The traps were removed and replaced with new ones at weekly intervals till September 2002.

Then the traps were taken to the laboratory for counting and indentification of the captured individuals. For most of the Lepidopteran species, the captured adults were classified as males or females, based on genitalia examination. The same was performed for the parasitic wasp H. hebetor, by examining the last abdominal segment. During each trap-replacement date, the traps were rotated clockwise in order to minimize the influence of individual trapping locations (Buchelos and Levinson, 1993).

The trapping surfaces were divided into 15 areas of 3 x 5 cm each (Fig. 1), in order to estimate the within trap distribution of the captured individuals. Also, the number of captured individuals at the four edges (figural outlines) of the trapping surfaces was recorded. The other variables measured were trapping location, indicating the distance from the windows - where the more illuminated areas of the warehouse were situated, and with presence/absence of pheromone.

![Fig. 1. Segments of each trapping surface (each letter represents one segment).](image-url)
For the most abundant species the data were submitted to ANOVA with colour, presence of pheromone and trapping location (distance from the windows) as main effects. Separate one-way ANOVA was carried out in the case of insect presence among the different segments. Means were separated by the Tukey-Kramer HSD test.

Results

Species found and population fluctuation
A total number of 4705 individuals were found during the monitoring period, classified into 24 taxa, belonging to 4 orders (Table 1). Most of the species found belonged to the order Coleoptera (12 species). Among the rest of the species, 4 were known stored-product Pyralidae moths (Lepidoptera), and some species of parasitic wasps (Hymenoptera) were also found as well as scavenger Diptera. More than half (54%) of the individuals captured were *P. interpunctella* adult males. Also, approx. 20% of the total number of captured adults were males and females of *H. hebetor* (15 and 5% of the total, for males and females, respectively). Moreover, 12% of the individuals captured belonged to another parasitic wasp species, *Cephalonomia* sp., while a number (2.5% of the total) of other wasps, belonging to the genus *Habrobracon*, were not identified up to species level. Finally, approx. 2.5% of the total were *P. interpunctella* females.

Concerning the seasonal occurrence of the *P. interpunctella* males, the highest number of adults was found on the pheromone-baited traps, between June and September, while on the unbaited traps the highest number of adults was found during early August (Fig. 2, 3). Captures of *P. interpunctella* females were low during the entire monitoring period. For *H. hebetor* and *Cephalonomia* sp., the highest captures were recorded during September (Figs. 4-6).

![Fig. 2. Seasonal occurrence of *P. interpunctella* males in baited (A) and unbaited (B) traps.](image-url)
Fig. 3. Seasonal occurrence of *P. interpunctella* females in baited (A) and unbaited (B) traps.

Fig. 4. Seasonal occurrence of *H. hebetor* males in baited (A) and unbaited (B) traps.
Influence of pheromone, colour and location

For *P. interpunctella* males, as expected, the presence of pheromone significantly increased captures, while the distance from the windows (illuminated areas) significantly increased captures, only in baited traps. On the other hand, the colour of the trapping surface did not affect captures. For *P. interpunctella* females, more adults were found in the baited traps, but trap colour and location did not affect captures (Tables 1-4).

In the case of *H. hebetor*, for both sexes, pheromone, trap-colour or location had no effect on adult captures. One the other hand, for *C. tarsalis*, significantly more adults were found on yellow than on black traps (Tables 1-4). In addition, for the combined trap data (both baited and unbaited traps) the greater distance from the windows produced decreased captures.

Within trap distribution

In the case of *P. interpunctella* males, the distribution among the different trap segments differed between baited and unbaited traps (Fig. 7). Hence, in the baited traps, the highest number of males was found on the segments D, E and F, right above the pheromonic source, while the lowest number was found at the highest (A, B, C) and the lowest (M, N, O) segments. In the unbaited traps, the lowest number of males was found at the highest segments (A, B, C). For females, there were no significant differences among segments, while no adults were found at the highest segments (A, B, C) in the unbaited traps (Fig. 8). In the case of *H. hebetor*, for both sexes, more adults were generally found on the lowest trap segments, in comparison with the highest ones (Figs 9, 10). Similarly, the lowest number of *Cephalonomia* sp. was found on the highest three segments (A, B, C). (Fig. 11).
Fig. 6. Seasonal occurrence of *Cephalonomia* sp. adults in baited (A) and unbaited (B) traps.

Fig. 7. Mean number of *P. interpunctella* males/segment (+SE) in baited (A) and unbaited (B) traps (within each diagram, means followed by the same letter are not significantly different; HSD test at 0.05; each segment is represented by a letter, for segment description see Fig. 1).

**The edge effect**

For *P. interpunctella*, a ≥94% of the total number of adults was found at the main trap part, with the exception of females in the baited traps where 19% of the adults was found at the...
trap edges (Table 5). From the two parasitoid species, H. hebetor indicated a stronger edge effect than Cephalonomia sp., especially in the unbaited traps where ≥21% of the total number of the captured adults was found at the trap edges.

Fig. 8. Mean number of P. interpunctella females/segment (+SE) in baited (A) and unbaited (B) traps (within each diagram, means followed by the same letter are not significantly different; HSD test at 0.05; each segment is represented by a letter, for segment description see Fig. 1).

Fig. 9. Mean number of H. hebetor males/segment (+SE) in baited (A) and unbaited (B) traps (within each diagram, means followed by the same letter are not significantly different; HSD test at 0.05; each segment is represented by a letter, for segment description see Fig. 1).
Fig. 10. Mean number of *H. hebetor* females/segment (+SE) in baited (A) and unbaited (B) traps (within each diagram, means followed by the same letter are not significantly different; HSD test at 0.05; each segment is represented by a letter, for segment description see Fig. 1).

Fig. 11. Mean number of *Cephalonomia* sp. adults/segment (+SE) in baited (A) and unbaited (B) traps (within each diagram, means followed by the same letter are not significantly different; HSD test at 0.05; each segment is represented by a letter, for segment description see Fig. 1).

**Discussion**

Rectangular aerial exposed sticky surfaces, baited or unbaited, have been used with success for monitoring of insect populations, especially moths, in several stored-product commodities
(Buchelos, 1980; Levinson and Buchelos, 1981; Buchelos and Levinson, 1985; 1993; Athanassiou et al., 2002; Eliopoulos et al., 2002b; Athanassiou and Eliopoulos, 2003; Eliopoulos and Athanassiou, 2005). However, most data concern stored-product pest species, very few data are available for the seasonal occurrence of parasitoids, despite the fact that these species often occur in high numbers, and seem to play an important role especially in the case of dried fig facilities (Eliopoulos et al., 2002a; Eliopoulos and Athanassiou, 2005). The present results indicate that captures of the parasitoids represented more than one third of the total number of the captured individuals. For *H. hebetor*, a parasitoid of moth larvae, a considerable proportion of the total number of adults was found late in the season (September), indicating that wasps follow their hosts’ densities. Similar trends were also recorded for *Cephalonomia* sp., a parasitoid of beetle species. However, in the present study, the beetle numbers were relatively low in comparison with *P. interpunctella* and the two aforementioned wasp species. Apparently, this is due to the fact that aerial sticky traps were used and not floor or perforated traps which are more suitable for beetle detection (Athanassiou and Buchelos, 2001; Athanassiou and Eliopoulos, 2003). Nevertheless, there are beetle species that seem to have a strong flying activity, such as *Carpophilus* spp. Eliopoulos and Athanassiou (2005) in a fig storeroom found considerable numbers of *C. hemipterus* caught in aerial sticky traps. On the other hand, aerial traps seem to be the best tool for the detection of parasitic wasps (Eliopoulos et al., 2002b).

Table 1. Taxa found, number of individuals and % of the total number of traps on which each taxon was recorded.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number of % of traps</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plodia interpunctella</em> (Hübner) ♂ (Lep., Pyralidae)</td>
<td>2541 90.6</td>
</tr>
<tr>
<td><em>Habrobracon hebetor</em> (Say) ♂ (Hym., Braconidae)</td>
<td>680 46.9</td>
</tr>
<tr>
<td><em>Cephalonomia</em> sp. (Hym., Bethylidae)</td>
<td>566 65.0</td>
</tr>
<tr>
<td><em>Habrobracon hebetor</em> (Say) ♀ (Hym., Braconidae)</td>
<td>247 36.9</td>
</tr>
<tr>
<td><em>Habrobracon</em> spp. (Hym., Braconidae)</td>
<td>120 33.8</td>
</tr>
<tr>
<td><em>Plodia interpunctella</em> (Hübner) ♀ (Lep., Pyralidae)</td>
<td>119 33.1</td>
</tr>
<tr>
<td>Cecidomyiidae (Diptera)</td>
<td>74 30.0</td>
</tr>
<tr>
<td>Other Diptera</td>
<td>62 16.2</td>
</tr>
<tr>
<td><em>Carpophilus hemipterus</em> (L.) (Col., Nitidulidae)</td>
<td>59 8.7</td>
</tr>
<tr>
<td>Scatopidae (Diptera)</td>
<td>59 10.6</td>
</tr>
<tr>
<td><em>Holepyris sylvanidis</em> (Brèthes) (Hym., Bethylidae)</td>
<td>31 10.0</td>
</tr>
<tr>
<td><em>Cryptolestes ferrugineus</em> (Stephens) (Col., Cucujidae)</td>
<td>23 11.2</td>
</tr>
<tr>
<td><em>Ephestia elutella</em> (Hübner) (Lep., Pyralidae)</td>
<td>20 7.5</td>
</tr>
<tr>
<td><em>Ephestia cautella</em> (Walker) ♂ (Lep., Pyralidae)</td>
<td>16 6.3</td>
</tr>
<tr>
<td><em>Tribolium castaneum</em> (Herbst) (Col., Tenebrionidae)</td>
<td>16 9.4</td>
</tr>
<tr>
<td><em>Carpophilus dimittatus</em> (F.) (Col., Nitidulidae)</td>
<td>13 5.6</td>
</tr>
<tr>
<td><em>Venturia canescens</em> (Graven.) (Hym. Ichneumonidae)</td>
<td>12 6.2</td>
</tr>
<tr>
<td><em>Ephestia figulilella</em> (Gregson) (Lep., Pyralidae)</td>
<td>11 3.1</td>
</tr>
<tr>
<td><em>Tribolium confusum</em> J. Du Val (Col., Tenebrionidae)</td>
<td>8 3.7</td>
</tr>
<tr>
<td><em>Carpophilus obsoletus</em> (Erichson) (Col., Nitidulidae)</td>
<td>6 1.9</td>
</tr>
<tr>
<td><em>Cryptephagus</em> sp. (Col., Cryptophagidae)</td>
<td>6 1.9</td>
</tr>
<tr>
<td><em>Oryzaephilus surinamensis</em> (L.) (Col., Silvanidae)</td>
<td>5 3.1</td>
</tr>
<tr>
<td><em>Ephestia cautella</em> (Walker) ♀ (Lep., Pyralidae)</td>
<td>3 1.9</td>
</tr>
<tr>
<td>Trogoderma sp. (Col., Dermestidae)</td>
<td>3 1.3</td>
</tr>
<tr>
<td><em>Cryptolestes pusillus</em> (Schoenherr) (Col., Cucujidae)</td>
<td>1 0.6</td>
</tr>
<tr>
<td><em>Ahasverus advena</em> (Waltl) (Col., Silvanidae)</td>
<td>1 0.6</td>
</tr>
<tr>
<td>Lasioderma sp. (Col., Anobiidae)</td>
<td>1 0.6</td>
</tr>
</tbody>
</table>
Table 2. ANOVA parameters for main effects and interactions for the most abundant species (df: 4, 1 and 4 for colour, pheromone and location, respectively, total df: 159).

<table>
<thead>
<tr>
<th>Source</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. interpunctella ♂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour (or the trap)</td>
<td>0.70</td>
<td>0.5926</td>
</tr>
<tr>
<td>Pheromone (presence/absence)</td>
<td>175.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Location (distance from the window)</td>
<td>3.08</td>
<td>0.0185</td>
</tr>
<tr>
<td>Colour X Pheromone</td>
<td>0.71</td>
<td>0.5813</td>
</tr>
<tr>
<td>Colour X Distance</td>
<td>1.01</td>
<td>0.4512</td>
</tr>
<tr>
<td>Pheromone X Distance</td>
<td>2.80</td>
<td>0.0283</td>
</tr>
<tr>
<td>P. interpunctella ♀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour (or the trap)</td>
<td>0.57</td>
<td>0.6833</td>
</tr>
<tr>
<td>Pheromone (presence/absence)</td>
<td>6.56</td>
<td>0.0116</td>
</tr>
<tr>
<td>Location (distance from the window)</td>
<td>0.70</td>
<td>0.5895</td>
</tr>
<tr>
<td>Colour X Pheromone</td>
<td>0.96</td>
<td>0.4277</td>
</tr>
<tr>
<td>Colour X Distance</td>
<td>1.32</td>
<td>0.1898</td>
</tr>
<tr>
<td>Pheromone X Distance</td>
<td>0.56</td>
<td>0.6854</td>
</tr>
<tr>
<td>H. hebetor ♂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour (or the trap)</td>
<td>0.89</td>
<td>0.4705</td>
</tr>
<tr>
<td>Pheromone (presence/absence)</td>
<td>1.26</td>
<td>0.2628</td>
</tr>
<tr>
<td>Location (distance from the window)</td>
<td>0.58</td>
<td>0.6776</td>
</tr>
<tr>
<td>Colour X Pheromone</td>
<td>1.12</td>
<td>0.3470</td>
</tr>
<tr>
<td>Colour X Distance</td>
<td>0.75</td>
<td>0.7334</td>
</tr>
<tr>
<td>Pheromone X Distance</td>
<td>0.21</td>
<td>0.9324</td>
</tr>
<tr>
<td>H. hebetor ♀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour (or the trap)</td>
<td>1.00</td>
<td>0.4101</td>
</tr>
<tr>
<td>Pheromone (presence/absence)</td>
<td>0.62</td>
<td>0.4305</td>
</tr>
<tr>
<td>Location (distance from the window)</td>
<td>0.49</td>
<td>0.7404</td>
</tr>
<tr>
<td>Colour X Pheromone</td>
<td>0.28</td>
<td>0.8860</td>
</tr>
<tr>
<td>Colour X Distance</td>
<td>1.15</td>
<td>0.3132</td>
</tr>
<tr>
<td>Pheromone X Distance</td>
<td>0.46</td>
<td>0.7613</td>
</tr>
<tr>
<td>Cephalonomia sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour (or the trap)</td>
<td>3.35</td>
<td>0.0121</td>
</tr>
<tr>
<td>Pheromone (presence/absence)</td>
<td>0.55</td>
<td>0.4572</td>
</tr>
<tr>
<td>Location (distance from the window)</td>
<td>3.39</td>
<td>0.0112</td>
</tr>
<tr>
<td>Colour X Pheromone</td>
<td>1.53</td>
<td>0.1970</td>
</tr>
<tr>
<td>Colour X Distance</td>
<td>1.53</td>
<td>0.0966</td>
</tr>
<tr>
<td>Pheromone X Distance</td>
<td>0.15</td>
<td>0.9600</td>
</tr>
</tbody>
</table>

TDA is a pheromone that acts as a male attractant for several Pyralid species. Surprisingly, we observed higher captures of P. interpunctella females in the TDA-baited traps in comparison to the unbaited traps. This may be attributed to the presence of high number of males in the baited sticky surface, which may have some activity in attracting females as well. Further experimentation is required to clarify this hypothesis.

The colour of the sticky surface, as a visual stimulus, may play a key role in the detection of some species. In our study, the colour of the sticky surface, at least for the colours examined here, does not affect captures of either P. interpunctella or H. hebetor. However, for Cephalonomia sp., yellow traps were more effective in adult attraction than black ones, indicating that for this species visual stimuli play a key role in adults’ response. Eliopoulos
and Athanassiou (2003) found also that traps with brighter colours were more attractive than darker traps for the parasitic wasp *Cephalonomia tarsalis* (Ashmead). Schöller and Prozell (2003) by using different colours of funnel traps found that white/yellow funnels were more attractive to *H. hebetor* adults than green ones.

**Table 3.** Number of adults/trap in trap surfaces of different colours, for the most abundant species (within each row, means followed by the same letter are not significantly different; HSD test at 0.05, absence of letters indicates no significant differences).

<table>
<thead>
<tr>
<th>Species</th>
<th>Colour of the trapping surface</th>
<th>Baited traps</th>
<th>Unbaited traps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black</td>
<td>yellow</td>
<td>Blue</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♂</td>
<td>30.9 ± 4.7</td>
<td>24.3 ± 3.8</td>
<td>24.4 ± 4.0</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♀</td>
<td>1.7 ± 0.7</td>
<td>1.3 ± 0.4</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td><em>H. hebetor</em>♂</td>
<td>3.3 ± 1.2</td>
<td>6.7 ± 3.0</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td><em>H. hebetor</em>♀</td>
<td>0.9 ± 0.4</td>
<td>1.6 ± 0.7</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td><em>Cephalonomia</em> sp.</td>
<td>1.2 ± 0.3a</td>
<td>6.9 ± 2.6b</td>
<td>2.0 ± 0.6ab</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>yellow</td>
<td>blue</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♂</td>
<td>5.8 ± 1.6</td>
<td>1.9 ± 0.5</td>
<td>3.8 ± 0.9</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♀</td>
<td>0.5 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td><em>H. hebetor</em>♂</td>
<td>2.6 ± 1.8</td>
<td>4.0 ± 1.3</td>
<td>2.6 ± 1.4</td>
</tr>
<tr>
<td><em>H. hebetor</em>♀</td>
<td>1.0 ± 0.8</td>
<td>1.5 ± 0.7</td>
<td>1.3 ± 0.7</td>
</tr>
<tr>
<td><em>Cephalonomia</em> sp.</td>
<td>1.3 ± 0.5</td>
<td>2.7 ± 1.0</td>
<td>2.4 ± 0.8</td>
</tr>
</tbody>
</table>

**Table 4.** Number of adults/trap in traps located at different distances from the windows of the storeroom, for the most abundant species (within each row, means followed by the same letter are not significantly different; HSD test at 0.05, absence of letters indicates no significant differences).

<table>
<thead>
<tr>
<th>Species</th>
<th>Distance from the windows</th>
<th>Baited traps</th>
<th>Unbaited traps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 m</td>
<td>4 m</td>
<td>6 m</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♂</td>
<td>23.1 ± 2.6a</td>
<td>21.7 ± 3.7a</td>
<td>28.3 ± 4.6ab</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♀</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.6</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td><em>H. hebetor</em>♂</td>
<td>4.1 ± 1.4</td>
<td>4.6 ± 2.6</td>
<td>3.6 ± 1.4</td>
</tr>
<tr>
<td><em>H. hebetor</em>♀</td>
<td>1.4 ± 0.6</td>
<td>1.6 ± 0.5</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td><em>Cephalonomia</em> sp.</td>
<td>6.1 ± 2.1</td>
<td>2.8 ± 0.8</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>2 m</td>
<td>4 m</td>
<td>6 m</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♂</td>
<td>4.8 ± 2.2</td>
<td>2.3 ± 0.6</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♀</td>
<td>0.3 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td><em>H. hebetor</em>♂</td>
<td>4.9 ± 3.8</td>
<td>8.4 ± 6.1</td>
<td>5.6 ± 2.4</td>
</tr>
<tr>
<td><em>H. hebetor</em>♀</td>
<td>1.4 ± 1.0</td>
<td>2.4 ± 1.2</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td><em>Cephalonomia</em> sp.</td>
<td>7.3 ± 3.1</td>
<td>4.2 ± 2.0</td>
<td>3.3 ± 1.4</td>
</tr>
</tbody>
</table>

Differences in levels of illumination among trapping locations seem also a factor that may play some role in trapping performance. This was expressed more vigorously in the case of *P. interpunctella* males, but only for baited traps. Hence, according to the current results, traps that were placed in less illuminated areas had higher numbers of males; this could be
attributed to the nocturnal activity of Pyralid moths. In contrast, although no significant differences were noted in most cases, especially for *Cephalonomia* spp, more adult wasps were found close to the windows. This observation stands in agreement with the report by Eliopoulos and Athanassiou (2005) where both *H. hebetor* and *C. tarsalis* were found in high numbers in illuminated areas. Hence, illumination is an important factor for the above species, but higher captures at these locations should be considered as a direct consequence of this characteristic, rather than a higher activity of wasps at these locations. Moreover, apart from illumination, this behavioural trend could be related to outdoor access stimuli, since these species are also found outdoors.

Table 5. Percent of individuals captured on the edges (figural outlines) or at the main trap part, for the most abundant species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Edges (%)</th>
<th>Internal trap part (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baited traps</td>
<td>Unbaited traps</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♂</td>
<td>94</td>
<td>95</td>
</tr>
<tr>
<td><em>P. interpunctella</em>♀</td>
<td>81</td>
<td>79</td>
</tr>
<tr>
<td><em>H. hebetor</em>♂</td>
<td>81</td>
<td>77</td>
</tr>
<tr>
<td><em>H. hebetor</em>♀</td>
<td>86</td>
<td>77</td>
</tr>
<tr>
<td><em>Cephalonomia</em> sp.</td>
<td>88</td>
<td>93</td>
</tr>
</tbody>
</table>

Within trap distribution is another factor that, despite the fact that it plays an important role, has not been investigated in detail so far. Athanassiou et al. (2002) by using similar adhesive traps to monitor adult male activity of *E. kuehniella* in a warehouse with stored grain, found that an increased number of males were captured at the lower part of the sticky surface. This could be due to the fact that most adults occur in the product mass, right under the traps. For *P. interpunctella* males, the present data indicated that the most “attractive” part of the sticky surface was the segments right above the pheromone, which may suggest that males fly slightly upwards where they reach the pheromonic source. On the other hand, distribution within the trapping surface was rather random in other cases examined, but generally very few adults were found at the upper trap parts. This may be related to the presence of wire at this trap part, from which the traps were hung, since this wire “occupies” a portion of the surface and may have a certain repelling activity. The high parasitoid numbers found at the lowest trap segments, may be related, apart from behavioural preference, to the highest moth presence at higher segments (lower saturation at the lower segments).

Quartey and Coaker (1992) reported a strong «edge» preference for *E. cautella*. Athanassiou et al. (2002), using similar traps, also reported that, for *E. kuehniella* males, such behavior is expressed more vigorously in unbaited than in TDA-baited strips. This characteristic is considered as a part of the male mate-finding behaviour (Levinson and Hoppe, 1983). Eliopoulos and Athanassiou (2005) also noted a strong «edge» preference for *C. hemipterus*. In the present work, a similar preference was noted for parasitoids, and also
for *P. interpunctella* females in the unbaited traps, which provides an additional indication that female behavior is different between baited and unbaited traps.

Multi-species detection is more important in storage facilities than in the case of detection in outdoor species, because in these facilities all species (including parasitoids) can be found in the final product (food), even if they are not related directly to the product. Nevertheless, most studies usually focus on 1-2 species, despite the fact that seasonal and spatial trends usually are directly related with species coexistence. Also, some trap parts may be more ‘attractive’ than others, or some species are likely to be found in specific trap parts. Finally, trapping location seems to be one of the most crucial factors in insect detection in storage facilities, since considerable variation occur among location (trap-trap variance) (Subramanyam and Hagstrum, 1995).

References


Monitoring mill moth (*Ephestia kuehniella* Zeller) by pheromone traps in Belarus

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**Abstract:** The prevalence, biology and phenology of the Mediterranean flour moth or mill moth *Ephestia kuehniella* Zeller in flour mills and bakeries of Belarus were given. The dynamics of development in the closed heated rooms and influence of ecological factors on these changes are described, the use of pheromone traps for monitoring mill moths were given. Results of the researches for 2004 – 2006 on efficiency of the new synthesized samples of working substances for catching the pest under production conditions at different densities of insect populations were discussed. The most effective concentrations that allow carrying out monitoring and identifying mill moth occurrence, dependence between catching in the pheromone traps and their density on volume unit of a room is revealed. The catching of mill moths using synthetic sex pheromones, kayromones and new attractants in milling rooms, unpacked storage of flour storage rooms and bakeries is given. The results will allow to establish beforehand density of the Lepidoptera populations for the further drawing up of their activity forecast, damage and the application of various protection methods according to the pest number. The successful use of pheromones for mass trapping in grain processing enterprises of Belarus was demonstrated.

**Key words:** mill moth, pests, monitoring, grain processing, flour-grinding enterprises, bakeries, flour-mills, pheromones, kayromones, traps, catching efficiency

**Introduction**

The mill moth (*Ephestia kuehniella* Zeller) is the predominant storage *Lepidopteran* in the Belarussian flour-milling enterprises and bakeries. It is widely distributed in countries of temperate climate; however in heated rooms it is distributed everywhere [1,2,3].

*Ephestia kuehniella* is the dominating pest in Belarussian mills and bakeries. During the day it usually sits quietly on ceilings and on walls and begins to fly only as twilight approaches. At room temperature it lives for about 2 weeks. Life expectancy increases considerably under conditions where moths have access to water. Egg lay occurs singly or in small groups at random with an average of up to 200 eggs per moth. The maximum amount of eggs laid is about 420. Egg lay occurs at temperatures from 13 to 33°C. At the minimal temperature embryonic development proceeds for 21 days, and at optimum (26°C) - 4 days. The larva, after hatching from the egg goes in search of food. At 17°C the larva finishes its development in 128 days, at 20°C in 55 – 70 days, and at 30°C in 29 days. After termination of feeding the larva builds a cocoon, to which adhere small particles of food, and it passes the stages of transformation to the adult stage inside it. In a mild climate the general duration of development is about three months. At temperatures of 10–20°C there may be four generations per year. Under the Belarussian climatic conditions it can develop 2 – 3 generations per year [1,4]. However, in heated rooms 6-10 generations per year may develop. Larvae over-winter in the pupal cocoons. The larvae spin silken webbing. All stages are
relatively tolerant to low temperatures and even at -10°C they can survive a one-day exposure. The lower temperature threshold for development is 11°C [1,3].

Larvae of these moths weave plentiful amounts of webbing which leads to clogging of the machinery and contamination of storages in factories, and in this connection, constant cleaning of equipment is required by the work-staff. For many years, *E. kuehniella* was controlled in factories by means of fumigation with methyl bromide one or two times per year. However, methyl bromide has been forbidden because of its destructive influence on ozone. Now, cleaning of rooms together with the expensive use of contact pesticides (for example, synthetic pyrethroids) is the sole means of control. Parasitoids, which suppress populations of *E. kuehniella* in bakeries and grain processing enterprises are known [5]. The fluctuations in Mediterranean flour moth populations in Belarus still remain unexplored. However, it is possible to obtain exact data on their presence in factories (flour-mills and bakeries) and their changes in population size, using pheromone-sticky traps that attract *Phycitid* males. The purpose of this research was to study the attraction by the trap SGP and SK, developed by SGC «Bioorganic Chemistry Institute» NAS of Belarus and to establish the most effective density for allocation of the pheromone-sticky traps.

**Materials and methods**

Studies in mill moth number fluctuations were carried out over 4 years in bakeries of Minsk and in the mill complex in Lida, Belarus, with the use of a synthetic sex pheromone, (SGP and S) that attracts *Phycitid* males. The pheromone was synthesized in the SSC «Bioorganic chemistry institute» NAS of Belarus. During the years 2004 to 2006 the attraction of the synthetic sex pheromone "Mirron", was investigated, whose active ingredient is 2-acylcyclohexane-1,3-dion modified in a lateral circuit and a cyclic part. The attraction to the mill moth by SGP "Mirron", was compared with attraction by SGP "Kyunemon" a derivative from 2-acylcyclohexane-1,3-dion, and a control trap (without pheromone). In 2005 studies on traps densities in shops of the flour-mill complex in Lida were also carried out. Kayromone attraction was also studied using synthetic substances provided by SSI «Bioorganic chemistry institute» NAS of Belarus These simulation materials are based on the fatty acids from grain cereals which are attractive to male and female adults

Atrakon – These are traps made from laminated paper "Tetrapack" in the size 29x48 cm with replaceable sticky plates made from gofferplast with a trap surface area of 400 cm² on which Pestifix had been applied They were used to catch Mediterranean flour moths. The sticky plates have an effective duration of 4 – 5 weeks, depending on the density of population of catch [6]. Attention was paid to maintenance of the constant size and the form of face apertures, which influence traps catching efficiency. The gelatinous sticky plates were placed on the bottom, which were filled, in 7 – 14 days. Traps were refreshed by capsules with attractant and this was carried out before their application in the well aired rooms. Capsules were inserted with the help of tweezers. Traps were hung out at temperatures above 11°C and the distribution rate was 1 trap per 150-700 m³. For mill moth detection they were numbered and hung out at a height of about 1.5 m, randomized, but at a distance of not less than 10 m from each other. In a mill complex in Lida during 2004 and 2005 12 such traps were hung up. In bakeries of Minsk during 2005 to 2007 5–8 pieces were hung in shops of unpacked storage of flour.

Trapping of SGP "Mirron" was compared with control traps (without pheromone) to evaluate average catching efficiency. Pest numbers were detected by visual counting of moths caught on the sticky plates, with their subsequent identification in the laboratory.
Results and discussion

For the period of research in shops the minimum temperature was +18ºC in winter, and it rose to 30ºC in the summer and higher. Therefore, flour moth development in bakeries was observed all the year round. The studies established that as a result of seasonal changes, the counts of moth numbers in pheromone-glutinous traps between July, 7 2004 and July, 9 2005 showed, that during the winter the numbers of moths were low. The greatest numbers of moths were caught in traps from July till September and this was shown as the period of their mass flight (Fig. 1). Therefore, various samples of the synthetic sex pheromones and kayromone attraction materials were investigated during the period 2004 – 2007.

![Fig. 1. Flight dynamics of the mill moth on pheromone-sticky traps (Minsk bakeries).](image)

Table 1. Attraction of the Mediterranean flour moth moth (Ephestia kuehniella) to SGP (Lida flour-mill complex).

<table>
<thead>
<tr>
<th>Variables</th>
<th>SGP content</th>
<th>Lepidoptera number, insects/trap per day</th>
<th>Total insects/trap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10.08.</td>
<td>17.08.</td>
</tr>
<tr>
<td>2004 Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP «Kyunemon»</td>
<td>1 mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP «Mirron»</td>
<td>1 mg</td>
<td>0.2</td>
<td>0.65</td>
</tr>
<tr>
<td>2005 Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP «Kyunemon»</td>
<td>1 mg</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>SGP «Mirron»</td>
<td>1 mg</td>
<td>2.89</td>
<td>2.78</td>
</tr>
</tbody>
</table>

As a result of mill moth synthetic sex pheromones attraction studies in a flour-milling complex in Lida, it was established, that catches with the synthetic sex pheromone "Mirron" trapped between 4 and 14 moths per week. It was noted, that in August, moth flight activity was less intensive than in September. In 2005, for the period till September, 9 the number of
moths caught, in a trap baited with the synthetic sex pheromone "Mirron", was 646 individuals making an average of 0.54 individuals per trap per day, whereas in traps without pheromone only one individual was recorded (Table 1). After two years of research it may be concluded that pheromone "Mirron" is highly efficient in trapping *E. kuehniella*.

As a result of studies of attraction of 5 samples synthetic sex pheromones №27, №28, №29, №30 and synthetic sex pheromone "Mirron" in bakeries of Minsk during 2006, and in shops of unpacked storage of flour it was established, that alongside with the synthetic sex pheromone "Mirron" a high attraction activity was also shown by the synthetic sex pheromone №30 which caught 193 mill moths over the period of investigation. The composition №28 showed unstable catching efficiency over the study period, only one individual was caught (Table 2).

As a result of the influence of trap density on synthetic sex pheromone "Mirron", catching efficiency was much higher where traps were more densely located (1 trap per 150 m³). In 2005, it was shown that at a concentration of flour moth attractant of 1 mg, the trap density of 150 m³ produced a 10 times higher catch than a trap density of 1 trap per 700 m³ (Table 3).

Table 2. Attraction mill moth (*Ephestia kuehniella*) by the SGP (Minsk bakeries, 2006).

<table>
<thead>
<tr>
<th>Variant</th>
<th>SGP content</th>
<th>moths / trap per day</th>
<th>Total insects / trap.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>07.07.</td>
<td>13.07.</td>
</tr>
<tr>
<td><strong>Bakery №1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SSP «Mirron»</td>
<td>1 mg</td>
<td>11.0</td>
<td>5.0</td>
</tr>
<tr>
<td>SGP №28</td>
<td>3 mg</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>SGP №29</td>
<td>3 mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP №30</td>
<td>3 mg</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td><strong>Bakery №6</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP «Mirron»</td>
<td>1 mg</td>
<td>3.6</td>
<td>2.0</td>
</tr>
<tr>
<td>SGP №28</td>
<td>3 mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP №29</td>
<td>3 mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP №30</td>
<td>3 mg</td>
<td>2.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 3. Accommodation density influence on attraction of traps with SGP "Mirron" (Lida mill complex rooms, 2005).

<table>
<thead>
<tr>
<th>Variant</th>
<th>SGP content</th>
<th>moths / trap per day</th>
<th>Total insects / trap.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>06.09.</td>
<td>19.09.</td>
</tr>
<tr>
<td>1 trap per 150 m³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP «Mirron»</td>
<td>1 mg</td>
<td>3.98</td>
<td>3.87</td>
</tr>
<tr>
<td>1 trap per 700 m³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGP «Mirron»</td>
<td>1 mg</td>
<td>1.72</td>
<td>1.76</td>
</tr>
</tbody>
</table>
During 2005 tests with a new synthetic karyomones were carried out in bakeries of Minsk. At a density of traps of 1 trap per 200 m$^3$ using the synthetic karyomones of 1 mg the trapping efficiency was up to 3 moths per trap per day. In the first week of the survey 0.2 individuals per day were caught. In bakery #6, the number of pests was very low, during the survey period, only 1 moth was caught (Table 4). The study also showed that during recent years in the Belarus bakeries and grain processing enterprises, the Mediterranean flour moth (*Ephestia kuehniella*) has become widely distributed in comparison with other pests. The flour moth causes serious damage to stored grain and its processed products. It is established, that the pest develops in heated rooms all-the-year-round. The comparative evaluation of the attraction of various synthetic sex pheromones using pheromone traps help was carried out. As a result of this study it was revealed that the most effective compound is the synthetic sex pheromone "Mirron" (active ingredient: 2-acylcyclohexane-1,3-dion modified in a lateral circuit and a cyclic part, at a concentration of 1 mg on a dispenser. This is now included in the list of preparations recommended for use in pest detection in factories and grain processing enterprises in Belarus. It was shown that to estimate the number of moths and their flight dynamics, the optimum density of pheromone-sticky traps should be 1 trap per 150 m$^3$. The data obtained has shown that the technology of application of the synthetic sex pheromone "Mirron" for mass catching and definition of terms of occurrence, is the most ecologically safe and effective measure for reduction in the number of flour moths and the damage they cause.

Table 4. Attraction mill moth (*Ephestia kuehniella*) by the karyomone (Minsk bakeries, 2005).

<table>
<thead>
<tr>
<th>Variant</th>
<th>SSP content</th>
<th>11.08.</th>
<th>17.08.</th>
<th>28.08.</th>
<th>04.09.</th>
<th>11.09.</th>
<th>18.09.</th>
<th>25.09.</th>
<th>average</th>
<th>Total insects / trap.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakery #1</td>
<td>Control</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>New SK</td>
<td>3 mg</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
<td>17</td>
</tr>
<tr>
<td>Bakery #6</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>New SK</td>
<td>3 mg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
<td>1</td>
</tr>
</tbody>
</table>

References

Potential of near infrared spectroscopy (NIRS) technology to discriminate between infestations of stored product pests in rice

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Abstract. Contamination of cereal grains with insect pests is a commercial issue relevant to decision-making on the possible use of a commodity for food, feed or other industrial uses. Both the level of infestation and also the species of stored product pest and in which developmental stage it is found could be of practical importance. Sets of rice grain samples infested by eggs, larvae (0-100 individuals per 100 g) or adults prior to emergence (0-10 individuals per 100 g) of *Sitophilus oryzae*, *Rhyzopertha dominica* or *Sitotroga cerealella* were prepared with the aim of studying the potential of the NIRS technique to detect the level of infestation and/or to discriminate between insect species or stages of development. Preliminary data showed that it was possible to group egg, larval or adult infested rice samples within the *R. dominica* lot. Also, by PCA of spectral data a discrimination of *S. oryzae* from *R. dominica* adults inside kernels was obtained whilst this was not the case for larvae of *R. dominica* versus *S. cerealella*. To predict the level of *R. dominica* egg infestation in the grain, it was necessary to fit an equation with the second derivative of the log 1/R data to get 85% of variability. These results should be confirmed in further studies.

Key words: *Sitophilus oryzae*, *Rhyzopertha dominica*, *Sitotroga cerealella*, near-infrared spectroscopy, internal grain infestation

Introduction

The rice weevil (*Sitophilus oryzae* L.), the lesser grain borer (*Rhyzopertha dominica* Fabricius) and the Angoumois grain moth (*Sitotroga cerealella* Olivier) are primary pests that can damage intact grain kernels.

A rapid method for detection and identification of insects could be useful in making pest management decisions. The available techniques for detection (staining, density separations, biochemical tests, acoustic sensors, X-ray etc.) are time consuming and only applicable to some insect species. Therefore a better method is needed.

Near-infrared spectroscopy (NIRS) is a procedure that can rapidly detect and measure the chemical composition of biological materials. For a good review on applications of NIRS in entomology, see Throne *et al.* (2002). Authors have reported on the use of NIRS to determine the level of external and internal infestation in bulk wheat samples (Ridgway and Chambers, 1996), to detect the presence of hidden insect larvae in single grains (Dowell *et al*., 1998) or for taxonomic purposes of stored grain beetles (Dowell *et al*., 1999).

NIRS is a fast technique, non destructive and can be automated. However its practical application for inspection of stored grain is not yet resolved. According to the literature, if single kernels are scanned it remains too slow, whereas in bulk samples it is not accurate for low infestations or for eggs and/or young larvae.

Near-infrared transmittance (NIT) is currently used by commercial elevators in Denmark and Sweden for rapid analysis of grain protein and moisture content (Hansen *et al*., 2002) and also it is being studied to detect insects and mites.
Internal infestations are difficult to detect because the insects hide inside the grain. However, the detection of this type of contamination is relevant, to prevent losses during the milling process. The aim of our study was to assess the potential of the NIRS technique to discriminate among pest species and stages of development (qualitative analysis) and to predict the level of infestation (quantitative analysis) in contaminated rice bulk samples.

**Materials and methods**

**Rice samples**
A set of rice samples infested with eggs, larvae (0-100 individuals per 100 g) or adults prior to emergence (0-10 individuals per 100 g) of *Sitophilus oryzae*, *Rhyzopertha dominica* or *Sitotroga cerealella* were prepared. The series of samples were made up by adding 0, 1, 2, etc. infested rice grains in samples (of 68 g for adults or 21 g in the case of larvae or egg infestations) of uninfested rice. Two replications were made for the lots infested with adults and 4 replications for the ones infested with eggs or larvae.

Infested grains with insects at the desired stage of development were obtained from synchronized cultures of the 3 pests and before mixing they were frozen to kill the insects and prevent any further development of the pest before the samples were scanned.

**NIRS technique**
Rice samples (prepared as described above) were placed for analysis in a holder equipped with a quartz glass. Their spectra were registered in the range from 400 to 2500 nm, at 2 nm intervals, using a NIRS Systems Model 6500 spectrophotometer (Foss-NI Systems, Inc., Silver Spring, MD, USA). Spectra of the samples were obtained as an average of 32 scans at each wavelength (run time of 1 min. and 50 seconds.) which meant an output of 1050 data as log (1/R), where R is reflectance. This process was repeated for all samples including all insect pests species and all stages of development. Data outputs were saved in separate files.

**Qualitative analysis**
Original data (log 1/R) were transformed to the first or second spectral derivative and modified with the SNV-DeTrending algorithm (Barnes et al., 1989). Then a Principal Component Analysis (PCA) was performed on the registered spectra, which reduced to 10 or 20 the number of independent variables that explained most of the original variability found in the samples. In this way, each sample is represented by a dot, the Mahalanobis \( H \leq 3 \) distance was used to establish the relative position of the samples with respect to the whole population. Such results were represented as two Principal Component (PC) graphs.

**Quantitative analysis**
Analysis was conducted with rice samples infested with eggs of *R. dominica*. Modified partial least squares (PLSm) regression was performed to set up a calibration equation that was then cross-validated. The predictive capacity of the model was developed by the coefficient of determination \( r^2 \) and the ratio of the standard deviation (SD) to the standard error of the population both from the cross validation (SECV).

**Results and discussion**

**Qualitative analysis**
It was possible to group egg, larvae or adult infested rice samples within the *R. dominica* lot. In Fig. 1, we can see that the plot of PC1 and PC3 discriminate between the rice samples infested with eggs and those infested with larvae or adults of the lesser grain borer.
It was also possible to separate larvae from adult infested samples by performing a new PCA eliminating the variability due to the egg infested samples (Fig. 2).

Also, by PCA of spectral data a discrimination between *S. oryzae* and *R. dominica* adults inside kernels was obtained (Fig. 3) whilst this was not the case for larvae of *R. dominica* versus *S. cerealella* (Fig. 4).

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**Fig. 1.** Principal Components (PC1 and PC3) Plot of rice samples infested with *R. dominica* eggs versus larvae and adults.

**Fig. 2.** Principal Components (PC2 and PC3) Plot of rice samples infested with *R. dominica* larvae versus adults.

**Fig. 3.** Principal Component (PC1 and PC3) Plot of rice samples infested with *S. oryzae* versus *R. dominica* adults.
Rice grains infested with *S. oryzae* produce higher numbers of broken kernels, as compared with *R. dominica*, during the milling process so the separation of both pest species as can be seen in Figure 3 is of practical importance.

**Quantitative analysis.** The calibration equation was restricted to the case of rice samples infested with eggs of *R. dominica*. The equation obtained from the second spectral derivative gave a better predictive potential than that obtained from the first derivative. $R^2$ from cross-validation indicated a high predictive potential, explaining 85% of the variability and an SD/SECV=2.66 which indicates, according to Williams and Sobering (1996), that this equation could be useful for sampling.

Dowell *et al.* (1998) scanned single kernels by using NIRS with a single kernel characterization system and were able to differentiate uninfested from infested kernels with larvae. Although, according to Throne *et al.* (2002), the method was unable to detect eggs or first instar larvae in single kernel scans. Also, Hansen *et al.* (2002) reported that in bulk wheat samples infested with less than 20 eggs per 100 g, the predictive values obtained from NIT were not related to the real infestation level of *Sitophilus granarius*. Our samples contained just a few infested grains within a bulk of uninfested rice grains (0-100 eggs or larvae per 100 g of grain) but our results were more promising.

In summary, our results show that the NIRS technique has potential: to discriminate between stages of development in bulk rice samples infested with *R. dominica*, to discriminate between infestations of *S. oryzae* and *R. dominica* adults prior to emergence from the grain and also to predict the level of *R. dominica* egg infestation in a sample. Nevertheless these preliminary results should be confirmed in further studies.

**Acknowledgments**

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**References**


Monitoring of insect populations in a pasta factory and related facilities in Greece

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Abstract. Trapping and sampling was conducted, in order to indicate insect populations in a pasta factory in Greece. Also, samplings in packaged pasta products were carried out, to indicate the potential presence of insects in the final product. For this purpose, Dome traps were placed in several areas of the factory and inspected for captured insect individuals, from May until December 2005. The most abundant insect species was found to be Tribolium confusum Jacquelin du Val (Coleoptera: Tenebrionidae), followed by Sitophilus oryzae (L.) (Coleoptera: Curculionidae) and Cryptolestes spp. (Coleoptera: Cucujidae). For T. confusum, most adults were found at the areas where pasta is processed, while S. oryzae was mainly found at the mill area, especially at the points that raw grains enter the mill and at the wheat silos. Nevertheless, for all species, extremely few individuals were found at the storage areas of the final products, or in the areas where the product is packed, indicating that insect activity is low in these areas, and that infestation after processing and during initial storage is less likely in the factory’s storerooms. Approx. 4500 packaged pasta products were taken between 2005 and 2006, placed in incubators at 27°C and 70° % r.h., and examined for insect presence. No insects were found in these samples, which considered as an additional indication that the product is pest-free and any infestation occurs through invasion of insects that exist in the storerooms where the product is transferred (super markets, large storerooms etc.). For this purpose, traps were placed at specific super markets and large storerooms, which contained the specific products, during the same interval, as above. Trap captures were considerably higher in these areas in comparison with the respective figures in the factory area, especially in the case of S. oryzae, which was by far the most abundant species. Also, many of the species found in these areas, such as Rhizopertha dominica (F.) (Coleoptera: Bostrychidae) and Lasioderma serricorne (F.) (Coleoptera: Anobiidae) were not found in the traps in the factory areas. These data indicate that, in the majority of cases, infestation is more likely to occur at the end-point areas of the products through entrance of insects in the packaged products, rather than through insects that exist in the product before packaging.

Key words: stored pasta, Sitophilus oryzae, Tribolium confusum, wheat, semolina, food safety, stored-food pests

Introduction

Insect presence in the packaged amylaceous products is not only a matter of quantitative loss and qualitative degradation, but, above all, has a specific ‘‘psychological’’ impact on consumers. ‘‘Zero’’ insect tolerance to food may not be feasible from a practical point of view, but it is required by the regulations in many countries of the developed world, while in other cases, there are specific thresholds for the presence of insect fragments in food (Perez-Mendoza et al., 2003; Hansen et al., 2004). Several studies have examined and analysed the
insect presence in several types of storage facilities, such as floor mills, retail stores, feed mills, raw grain stores, botanical warehouses etc. (Arbogast et al., 1998, 2000, 2002; Trematerra and Sciarretta, 2004; Athanassiou et al., 2005; Trematerra and Gentile, 2007; Carvalho et al., 2007). Precision Integrated Pest Management (IPM) programs in stored-product facilities require accurate monitoring of insect populations in order to indicate their spatio-temporal distribution. In this effort, monitoring is not only used for insect detection, but also for estimation of their population dynamics and locations of infestation, in order to apply targeted (focal) control measures, which can be insecticidal applications or non-chemical methods (sanitation etc.) (Arbogast et al., 1998; Trematerra and Gentile, 2007).

The problem is more complicated in the case of facilities that are involved in the entire food production procedure, from raw material to the final packaged food. One example is the pasta industry, which contains at the same facilities a) raw grain which is stored in bins and related storerooms, b) processed amylaceous products that are produced by milling the raw grain, such as semolina and c) the final product, such as pasta and related products, which is packaged and stored at adjacent facilities initially, before distribution to the purchasers. Insects can infest the product at several points of this procedure, but insect presence in the final product is the critical issue. Buchelos (1995) lists four types of insect infestations, based on the point that insects enter in the aforementioned procedure a) primary infestation, where raw grain is infested from the field, just before harvest, b) secondary infestation, where infestation-free product is placed in facilities with infested product, c) cross infestation, where insects infest the products through processing machinery, means of transportation or packaging material and d) invasion, where insects invade the product from holes and openings in the packages. Hence, when a monitoring strategy is planned, one of the most crucial aims is to indicate the type or the types of infestation that prevail in the target facility. In the present work, we monitored insect populations in a pasta factory in Greece, in order to estimate species composition, insect populations and within-facility distribution of insects.

Materials and methods

The monitoring was carried out between May and December 2005, in a pasta factory in Greece of approx. 60000 m². A total number of 67 Dome traps (Trécé Inc., USA), was placed at several locations of the entire facility [17 traps in the wheat bins (one out of each bin), 15 traps in the semolina mill (10 on the ground floor, 5 on the other floors), 4 traps in the bins of pasta making area, 7 traps at the end of the production lines (1 on each line), 3 traps in the smashing machine area, 3 traps in the semolina bins, 2 traps in the packaging materials, 1 trap in the compressor, and 15 traps at the storeroom where the final packaged product is stored]. In addition, 75 traps were placed in selected storerooms with the final product, as well as in three big supermarkets. The traps were inspected at weekly intervals. The traps were placed on the shelves, but also in storerooms with pasta and rice.

Apart from trapping, samples of final packaged product were taken and placed in incubator chambers in order to indicate potential insect infestations. A total of 4500 samples were taken from the daily production lines of the factory, at a ratio of 1 package per 10000 packages produced. Based on previous extensive samplings, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) was by far the main insect species found in the final packaged pasta products.

Results

A total of 433 beetle adults were found during the entire monitoring period, in the area of the factory (Table 1). The most abundant species was the confused flour beetle, *Tribolium*
confusum (Coleoptera: Tenebrionidae), followed by S. oryzae. These two species corresponded to approx. 51 and 30 % of the total number of individuals found.

Low numbers were generally found for T. confusum, and captures did not exceed 0.5 adults/trap (Fig. 1). The highest numbers were recorded early in the monitoring season (May) and October. From the total number of trap inspections, 83 % were found to contain adults of T. confusum. In addition, this species was found in approx. one half of the trapping locations. The majority of the T. confusum individuals was found at the mill (chiefly on the ground floor) and in the pasta-making area (Fig. 2). On the other hand, very low adult numbers were found in the other facilities.

Table 1. Number of adult beetles (Coleoptera) found in the pasta facility during the monitoring period.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitophilus oryzae (L.) (Curculionidae)</td>
<td>131</td>
</tr>
<tr>
<td>Tribolium confusum Du Val (Tenebrionidae)</td>
<td>221</td>
</tr>
<tr>
<td>Stegobium paniceum (F.) (Anobiidae)</td>
<td>1</td>
</tr>
<tr>
<td>Cryptolestes spp. (Cucujidae)</td>
<td>78</td>
</tr>
<tr>
<td>Oryzaephilus spp. (Silvanidae)</td>
<td>2</td>
</tr>
</tbody>
</table>

Fig. 1. Adults/trap (y-axis) for T. confusum in the pasta factory during the monitoring period.

In the case of S. oryzae, low numbers were found during the entire monitoring period, since its presence did not exceed 0.35 adults/trap (Fig. 3). As above, the highest numbers were recorded during May and fall. From the total number of trap inspections, S. oryzae adults were found in less than 10 %, and only in 14 trapping locations. Generally, most adults of this species were found at different locations in comparison with T. confusum, while more than 85 % of the total were found in only 6 trapping locations. Most weevils were found in the wheat bins, and also on the ground floor of the mill, where wheat exists (Fig. 4). On the other hand, extremely few weevils were found in the facilities in which the final product was stored.

In the facilities where the final product is stored, after their removal from the pasta factory (super markets etc.), the numbers of insects found was notably higher in comparison
with the above figures (Fig. 5). The most abundant species was *S. oryzae*, while presence of *T. confusum* was low, despite the fact that this species was the most abundant in the factory facilities. More than 10 taxa were found, while most of them had not been recorded in the traps that were located in the factory. Generally, *S. oryzae* adults were found in more than 90% of the traps in these facilities. For *S. oryzae*, the number of adults/trap in these facilities were 8.3, 5.3 and 2.3 in the shelves, the pasta store area and the rice store area, respectively, while the corresponding numbers for *T. confusum* were 1.2, 0.1 and 0.1.

![Fig. 2. Mean number of *T. confusum* adults/trap (y-axis) on each type of facility (A: mill, B: pasta-making area, C: end of the production lines, D: facility with packaging material, E: facility with final product).](image1)

![Fig. 3. Adults/trap (y-axis) for *S. oryzae* in the pasta factory during the monitoring period.](image2)

Of the 4500 packages which were examined from May 2005 until June 2006, approx. 50% of the products examined were spaghetti, but there were other types as well (in total more than 10 types). No insects were found after the incubation treatment. It should be noted, that a percentage of these products were kept for additional periods in the laboratory, but even then, no insects emerged.
Fig. 4. Mean number of *S. oryzae* adults/trap (y-axis) on each type of facility (A: mill, B: pasta-making area, C: end of the production lines, D: facility with packaging material, E: facility with final product).

Fig. 5. Number of adults/trap (y-axis) for insects caught in supermarkets and related areas.

**Discussion**

Although *S. oryzae* was almost exclusively the insect species found in packaged pasta in a specific facility, *T. confusum* was the most numerous species in the area of the pasta factory. Females of *S. oryzae* oviposit in the internal part of the kernel, and (apodous) larvae develop inside the kernel (Aitken, 1975). Hence, *S. oryzae* cannot develop in milled (processed) wheat products such as flour and semolina. In contrast, *T. confusum* is more prone to be found in flour and semolina, since it cannot easily infest sound grain kernels (Aitken, 1975). For both species, the insect numbers can be considered as extremely low in the factory facilities. However, at the other facilities, *S. oryzae* captures were high, which can be considered as a clear indication that this species has a notable presence in these facilities. An additional indication consists of the presence of other species, which did not exist in the factory. Finally, one more indication is the fact that no insects were found in the packaged products after the incubation. It should be also noted that, during pasta-making, the grain is exposed to smashing, sieving, entoloter etc. while at the next stage the product is exposed to >50 °C for
>5 h (according to the type of the product), drying and high atmospheres. All these characteristics, in conjunction with the insecticidal application and the sanitation measures, can lead us to the conclusion that it is likely that most infestation occur after the removal from the final product from the pasta factory facilities.

Trematerra and Gentile (2007) examined the spatiotemporal distribution in a semolina mill in Italy and also found that the most abundant species were *T. confusum* and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Adults of these species were found in large numbers of trapping locations. These findings are in accordance with the observations recorded in the present study, while in the case of *S. oryzae* the distribution among locations was rather limited. The targeted applications with insecticides (e.g. aerosols) at specific locations with high numbers of insects is one of the solutions suggested (Campbell et al., 2002; Trematerra and Sciaretta, 2004). Apart from increased efficacy, the cost of these applications is lower, in comparison with treating larger areas with fumigants. Indirectly, although the aim of the present work was to monitor insect populations, the fact that the monitoring procedure was not restricted only in the factory area provided the cues for the insect presence in the final product. Generally, despite the fact that the presence of insects is very common in these areas, very few data exist for insects that exist in super market and related facilities. However, when insects are found in a given package, their presence is directly related to the manufacturer, despite the fact that the infestation is likely to occur in other areas. In response, the manufacturer usually increases the insecticidal use, while the solution is at other critical points, such as sanitation, packaging, short storage duration etc. Moreover, taken into account that *S. oryzae* is the main species that is associated with pasta, the type of wheat processing (heat, entoleter etc.) may substantially reduce weevil presence, even in the case of existing populations in the raw grain. Riudavets et al. (2007) classifies the rice weevil in the category of invaders, which are the insect species that cannot penetrate the package, but enter through existing holes. Hence, the reduction of the number and the size of the holes, maybe a solution to the above problem.

After precision monitoring, the next target is an IPM approach, the application of focal applications, in order to reduce of pesticide use. Moreover, these applications should be combined with other, non chemical measures, such as insect-proof packaging and sanitation. These actions should be taken not only at the production point, but also during transportation and final storage.

Acknowledgments

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References


New funnel pheromone trap for monitoring of moths in dusty places

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Abstract: A new funnel trap “Panko” that can deflect the dust and allow the use of a glue strip as the entrapment mechanism was compared with the funnel pheromone trap of “AgriSense”. The numbers of stored product moths trapped by the funnel pheromone trap “Panko” were similar or higher than those for the funnel pheromone trap “AgriSense”. Efficacy of the “Panko” trap in dusty areas seems to be higher than that of the “AgriSense” trap. The funnel pheromone trap “Panko” should be recommended for moth monitoring in dusty premises.

Key words: Stored product moths, pheromone trap, dusty premises

Introduction

No one type of trap is best to use in a pest monitoring program in food industry plants. It is important to match the specific trap to the environmental conditions in each particular situation, e.g. dusty vs. non-dusty area. Too much dust can cause sticky trap to be ineffective. Dusty warehouses and flour mills offer challenges for conventional sticky glue traps (Mueller 2004). Therefore, we developed a new funnel trap “Panko” (Fig. 1A) that can deflect the dust and allow use of glue strip as the entrapment mechanism (PPA 2006).

Fig. 1. A new funnel trap “Panko” (A) and the funnel pheromone trap “AgriSense” (B).

The aim of the study was to compare the efficacy of two pheromone traps: the new funnel pheromone trap “Panko” and widely used funnel pheromone trap “AgriSense” (Fig. 1B).
Material and methods

Experiments on the efficacy of pheromone traps were performed in a laboratory chamber and under field conditions. Pheromone traps were placed 1.5-2 m above the floor, and within 4-5 m apart. After recording the catch data, the trap placement was changed. Number of trapped insects was recorded each 5-7 days.

Adults of the Mediterranean flour moth, *Ephestia kühniella* Zeller, were released into the laboratory chamber (99.5 m³), and the releases were repeated after each data recording. Conditions during the experiment: were temperature of 24-27°C during the day, and ca. 21°C at night; 25-40% R.H. and illumination similar to the outside light.

Trials in bakery 1 and 2 were limited to the storerooms (86 m³ each), in which flour was stored. Conditions during the experiment were: temperature 20-24°C, and 50% R.H.

Trials in the grocery shop were limited to a storeroom (80.6 m³) with food products. Conditions during the experiment were: temperature 20-25°C, and 50% R.H.

Results and discussion

In the laboratory chamber, the number of the Mediterranean flour moths trapped in the funnel traps “Panko” and funnel pheromone traps “AgriSense” were similar. No statistical differences were found (Fig. 2).

![Figure 2](image)

**Fig. 2.** Number of moths trapped by the funnel traps “Panko” and “AgriSense” (darker column) in a laboratory chamber.

In the bakery 1, the numbers of the Mediterranean flour moths trapped by “Panko” traps and “AgriSense” traps were similar. However, the numbers of moths (*E. kuehniella*) trapped in the bakery 2 by funnel pheromone traps "Panko" and funnel pheromone traps “AgriSense” were different (Fig. 3). The significant statistical difference found was (ANOVA; $F_{1.16}=5.810$, $p<0.05$).
In the grocery shop, the number of the Indian meal moths (*Plodia interpunctella* Hübner) trapped by “Panko” traps was higher (ANOVA; $F_{1,17}=61.14$, $p<0.05$) than that for the “AgriSense” traps (Fig. 4).

It was concluded that the number of stored product moths trapped by the funnel pheromone traps “Panko” was similar or higher than that for the funnel pheromone traps “AgriSense”. Efficacy of the “Panko” trap in dusty areas appears to be higher than that of the “AgriSense” trap.

The funnel pheromone trap “Panko” should be recommended for moth monitoring in dusty premises, such as flour mills and warehouses.
References


Session 4:
Biological control
Population dynamics of the natural enemies of stored product pests in cereal and dried fruit companies

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Abstract: The population dynamics of stored product pests and their naturally occurring parasitoids were assessed for several months in different companies which stored rice and dried fruits. Pests and parasitoids found in the different companies were identified and their levels assessed. Three different traps types were used to capture the species of interest: pheromone traps, yellow sticky traps and light traps. Monthly samples of the different products were also taken randomly. Presence of natural enemies was observed in every company and each company had different species and amounts of natural enemies and pests. The results showed that the traps used had different capture levels. Light traps had higher captures per trap than yellow traps in relation to natural enemies. Pheromone trap turned out to be very efficient for capturing Lepidopteran pests. Finding a naturally existing population of natural enemies which could be able to control the pest efficiently is essential, for application as an alternative to pesticides. The present results, though preliminary, show the possibility of instauration of biocontrol based on the naturally occurring populations of natural enemies, as they seem to adapt to the host cycle.

Key words: cereals, dried fruits, stored product pests, natural enemies, traps, population dynamics.

Introduction

Many different pests affect food processing factories, due to the storage of goods. The need of preventing the pest from entering the product from manufacture till packaging and during storage necessitates determining the possible pests attacking the products and the natural enemies which could control these pests. Pasta, flour, pet food, rice and dried fruit companies need to keep their storage and processing facilities free of possible pest infestations. Sanitation practices are required to avoid the establishment of insect pests (Arthur et al 2005). In some studies the application of control methods together with biocontrol has resulted in increased effectiveness (Flinn 1998). Another way of helping to control pest populations is to discover whether the companies have naturally occurring parasitoids. To develop this idea into an alternative, efficient pest management tool, it is necessary to have a better understanding of the pest population dynamics (Campbell & Arbogast 2004). Important factors affecting population cycles of pests and above all of parasitoids are temperature (Arthur et al 2005, Flinn 1998, Flinn & Hagstrum 2002, Menon et al 2001) and chemical control treatments (Toews et al 2006, Campbell & Arbogast 2004). To this end many sampling methods have been described and applied to monitor and identify pests and natural enemies. There are many antecedents on the use of insect traps (Campbell & Arbogast 2004, Hagstrum et al 1998, Roesly et al 2003, Toews et al 2006) and insect attractants (Campbell & Arbogast 2004, Roesly et al 2003, Toews et al 2006). In the latter cases, lures used were specific for certain groups of insects.

The study of natural enemies as parasitoids of specific pests in certain industries for biocontrol is not new (Flinn 1998, Flinn & Hagstrum 2001, Flinn & Hagstrum 2002, Menon
et al 2001). In the present study we tried to establish a comparison between companies of different areas storing different products (Lucas et al, 2002). The objectives were as follows: use of various monitoring techniques (traps and sample collection); assessment of the levels of pests and natural enemies in the different factories; comparing the insect species present in the different companies to see if there exists specialized beneficial fauna; follow pest and natural enemies’ life cycles and check effects of treatments and temperature on them.

Material and methods

Study site
Monthly sampling was carried out in nine different factories, grouping them in categories depending on the product they worked with: Pasta, Flour, Pet food, Rice, Organic flour and Dried Fruit. Within each category one company was chosen for describing the abundance of pests and parasitoids and their life cycles, and for assessing temperature, treatment effects and trap efficiencies. Each company was chosen on the basis of abundance and interest of the species captured. Samplings started in February in companies number 1, 2 and 4, and in April for the rest of companies. Sampling in companies number 5, 6 and 7 was started afterwards since these were found later on.

Within the organic flour category the selected company was number 1. This company was a three storey factory (600m² per floor approximately) located in the periphery of an industrial area, bordered by a small forest. No chemical fumigation was carried out as insect control technique, as the company was managed according to principles of organic farming and processing. Within the pet food category the selected company was number 3. This was a five storey mill located in an industrial area. In this company four areas were sampled, these being the raw material tower, production tower, package room and warehouse. Weekly treatments with natural pyrethrins were carried out only in the above mentioned areas.

Within the dried fruits category the chosen company was number 4. This was a three storey factory (3000m², having around 1200 m² in the first storey and two more storeys of around 800 m² each) located in an industrial area. The only chemical treatment since February was a single fumigation with cipermethrin performed in June. Within the rice category the chosen company was number 8. This factory was located in an industrial area. Treatments performed were recorded.

Insect traps
Insects inside the factories were monitored by using pheromone traps (product names are mentioned below, can be deleted here Killgerm S.A., C/ De L’Enginy 9, Viladecans, Barcelona, Spain), yellow sticky traps (Sanidad Agrícola Econex S.L. Murcia, Spain), two models of light traps, one being Extertronic 2002 (Electronica Escuder, Benicarló, Castellon, Spain) and the other model was Flytrap commercial 2x15 (Disnordic S.L., Terrassa, Barcelona, Spain) and by taking samples of products. Only inside sampling was carried out.

Pheromone traps (Killgerm S.A., Barcelona, Spain) were 11.5cm diameter circular traps, 20cm tall, black and white striped. One specific Plodia/Ephestia pheromone lure (Agrisense BCS Ltd, Mid Glamorgan, UK) was placed in the centre of each trap with a plastic rope. Pheromone traps were placed at least 1.5m above ground, and the specific lures were replaced monthly. Trap number varied among companies, company number 3 and 4 having four pheromone traps, whilst company number 1 had three and company number 8 had two pheromone traps.

Yellow sticky traps (Sanidad Agrícola Econex S.L. Murcia, Spain) 10 cm², were hung no higher than 2m above the ground. In all the companies, attempts were made to situate the trap within an air current, and in an illuminated area. Traps were cleaned if few insects had been
captured in that month, but were never kept for longer than two months. Company number 1 and 8 had just one yellow trap, whilst company number 3 and 4 had three yellow traps.

Light traps (Electronica Escuder, Benicarló, Castellon, Spain & Disnordic S.L., Terrassa, Barcelona, Spain) were hung higher than 2m in nearly all locations. Every month the trap tray was collected, contents emptied in a bag for later laboratory analysis. As some combined the light source of attraction with yellow sticky plates, the sticky surfaces had to be replaced. Companies number 1, 4 and 8 had three light traps and company number 3 had none.

Samples of approximately 1 kilogram were collected in the different companies. In company number 1 samples only from occasionally infested material were collected, whereas in company number 3 different areas were continuously sampled, this being the basement, and two levels in the mill tower. In company number 4 the samples were obtained from two mezzanines in the facilities which were likely to have some kind of infestation due to dust accumulation. Finally in company number 8 the six samples were obtained from the rice, when available and collected when arriving at the company and before applying any treatment to it.

**Results and discussion**

Insect species associated with pet food industries: in the selected pet food company number 3, eleven species of stored product pests and natural enemies were captured (Table 1). Out of the total number of insects captured the percentage of Coleoptera was the highest (66.36%), followed by Lepidoptera (28.27%) and Hymenoptera (5.37%) (Table 1). Among the Lepidoptera captured *Ephestia kuehniella* was the most abundant, perhaps due to the presence of drier food (Ryne *et al.*, 2002). Referring to Hymenoptera, the natural enemy captured mostly was the Ichneumonid *Venturia canescens*, which is probably due to the abundance of Lepidopteran hosts present, these being the different *Ephestia* species and *Plodia interpunctella* (Table 1). In respect to coleopterans, the highest captures corresponded to *Tribolium confusum*. Population dynamics of the main species were similar; all groups of insects had a light increase in June, perhaps due to warmer temperatures, except for the insects captured in samples, like *Tribolium confusum* or *Plodia interpunctella* which decreased somewhat. All traps captured high numbers of insects from the samples (n=5376, 68.72%). The pheromone traps captured n= 1960 insects (25.05%) and the smallest number of captures were found on yellow traps (n=488, 6.24%). No light traps were used in this company (Table 3). Weekly treatments with natural pyrethrins were carried out in the factory facilities. Even though no decrease of captures was noticed after treatments in the traps, it was noticeable in the samples. Perhaps the reduction in captures could be explained by the accumulated effect of weekly treatments, but this didn’t occur for all species.

As regards insect species associated with organic flour industries: in the selected organic flour company number 1, twelve species of stored-product pests and natural enemies were captured (Table 1). The percentage of Coleoptera captured was the lowest (11.5%) and Lepidoptera was the highest (68.28%). Hymenoptera represented 20.22% of the total captures (Table 2). The most abundant coleopteran species was *Sitophilus oryzae* (Table 1). Population dynamics of Hymenoptera had a peak in May, which then decreased gradually. The different families of Coleoptera had varied behaviours. All had high populations between May and June Later, some experienced decreases while others increased a little. All traps captured high numbers of insects. The biggest amount were in the pheromone traps (n=426, 62%), followed by the light traps (n= 165, 24%), samples (n= 63, 9.17%) and the smallest captures on yellow traps (n= 33, 4.8 %) (Table 3). No treatments were applied in this company, as it is organically managed. The difference in the amount of natural enemies found here in
comparison to the ones in the rest of companies is noticeable (Table 1); therefore with organic management biocontrol can occur naturally (Fig. 1).

Table 1. Total number of insect species captured in the different companies during four months.

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Species</th>
<th>Company 1 Ecological Mill</th>
<th>Company 3 Mill</th>
<th>Company 4 Dried Fruits</th>
<th>Company 8 Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidoptera</td>
<td>Pyralidae</td>
<td>Plodia interpunctella</td>
<td>389</td>
<td>8</td>
<td>378</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ephestia kuehniella</td>
<td>76</td>
<td>2144</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ephestia elutella</td>
<td>3</td>
<td>56</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ephestia cautella</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>Ichneumonidae</td>
<td>Ichneumonidae</td>
<td>4</td>
<td>4</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Venturia canescens</td>
<td>40</td>
<td>400</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Braconidae</td>
<td>Braconidae</td>
<td>Habrobracon spp</td>
<td>79</td>
<td>0</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Pteromalida</td>
<td>Pteromalida</td>
<td></td>
<td>14</td>
<td>16</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Coleoptera</td>
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<td>Tribolium confusum</td>
<td>0</td>
<td>5180</td>
<td>43</td>
<td>1</td>
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<tr>
<td></td>
<td>Bostrichidae</td>
<td>Rhyzoperta dominica</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>7</td>
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<tr>
<td></td>
<td>Laemophloeidae</td>
<td>Cryptolestes ferrugineus</td>
<td>0</td>
<td>4</td>
<td>10</td>
<td>0</td>
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<tr>
<td></td>
<td>Curculionidae</td>
<td>Sitophilus oryzae</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Anobiidae</td>
<td>Lasioderma serricorne</td>
<td>7</td>
<td>8</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Silvanidae</td>
<td>Oryzaephilus surinamensis</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTALS</td>
<td></td>
<td></td>
<td>687</td>
<td>7824</td>
<td>480</td>
<td>118</td>
</tr>
</tbody>
</table>

Table 2. Percentage of captures belonging to each group out of the total captured. Natural enemies refer to the different groups of Hymenoptera.

<table>
<thead>
<tr>
<th></th>
<th>Company 1</th>
<th>Company 3</th>
<th>Company 4</th>
<th>Company 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidopteran pests</td>
<td>68.28</td>
<td>28.26</td>
<td>79.78</td>
<td>27.95</td>
</tr>
<tr>
<td>Natural enemies</td>
<td>20.22</td>
<td>5.37</td>
<td>8.96</td>
<td>13.56</td>
</tr>
<tr>
<td>Coleopteran pests</td>
<td>11.49</td>
<td>66.36</td>
<td>11.25</td>
<td>58.48</td>
</tr>
</tbody>
</table>

In relation to insect species associated with dried fruit industries: In the selected company number 4, ten species of stored product pests and natural enemies were captured (Table 1). Coleoptera captures were the lowest (11.25%) and Lepidoptera were the highest (79.78%). Hymenoptera captures just represented 8.96% of the total (Table 2). The most
abundant Coleoptera species was *Tribolium confusum* (Table 1). Population dynamics of Hymenoptera increased from May to June (Fig. 2), after that they decreased. The different families of Coleoptera had a small increase from May to June, after which some of them decreased and just *Lasioderma serricorne* (Coleoptera Anobiidae) kept increasing. All traps captured high numbers of insects, with the largest amount of insects in the pheromone traps (n=350, 72.92%), followed by light traps (n= 73, 15.21%), samples (n= 33, 6.87%) and yellow traps (n= 24, 5%) (Table 3). Treatments performed in company number 4 were recorded (Fig. 2).

Table 3. Trap efficiency: pest and natural enemies’ percentage of captures for each trap.

<table>
<thead>
<tr>
<th></th>
<th>Company 1</th>
<th>Company 3</th>
<th>Company 4</th>
<th>Company 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pheromone trap</td>
<td>62.01</td>
<td>25.05</td>
<td>72.92</td>
<td>19.48</td>
</tr>
<tr>
<td>Light trap</td>
<td>24.02</td>
<td>–</td>
<td>15.21</td>
<td>26.26</td>
</tr>
<tr>
<td>Yellow trap</td>
<td>4.08</td>
<td>6.24</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Samples</td>
<td>9.16</td>
<td>68.70</td>
<td>6.87</td>
<td>54.24</td>
</tr>
</tbody>
</table>

Fig. 1. Populations dynamics of *Plodia interpunctella* (grey bars) and *Ephestia* spp. (black bars) versus natural enemies (Ichneumonidae and Braconidae) (line) in company number 1. No chemical treatments are applied as the company is ecologically managed.

Fig. 2. Population dynamics of *Plodia interpunctella* (patterned bars) and *Ephestia* spp. (dotted bars) versus natural enemies (Ichneumonidae and Braconidae) (line) in company number 4.
Referring to insect species associated with rice industries: in the selected company number 8, eleven species of stored product pests and natural enemies were captured (Table 1). The percentage of Coleoptera captured was the highest (58.48%). Lepidopteran captures reached 27.95% and Hymenopteran captures represented a 13.56% of the total (Table 2). The most abundant coleopteran species was *Sitophilus oryzae* (Table 1). Population dynamics of Hymenoptera had a peak from May to June, after that they decreased gradually. The different families of Coleoptera had high populations between May and June. Later some decreased while some others increased. Therefore no clear pattern was noticed. All traps captured some insects, the lowest amount being in the pheromone traps (n=23, 19.48%), followed by light traps (n= 31, 26.26%) and samples having the highest captures (n= 64, 54.24%) (Table 3). Fortnightly treatments with natural pyrethrins and chlorpyriphos were carried out in company number 8.

One of the objectives was to determine the best sampling method for capturing natural enemies and pests. The results obtained in the different companies showed that the traps used had variable capture levels. Light traps had higher captures per trap than yellow traps in relation to natural enemies. Pheromone trap turned out to be very efficient for capturing Lepidopteran pests. In other studies trap detection was found to be better than sample detection (Hagstrum et al., 1998). In the present study the effectiveness of the samples was very variable, in some companies high numbers of insects were detected, while in other companies the sampling method did not work efficiently.

The specific natural enemies and pests present in the different companies were assessed. Presence of natural enemies was observed in every company and each company had different species and amounts of natural enemies (Table 1). This could be explained by association to the pests (hosts) present in the company. The organic flour and pet food companies had the highest levels of natural enemies. Pest infestation in the pet food company was very high at some stages of the study. These results show the possibility of instauration of biocontrol based on the naturally occurring population of natural enemies, as they adapt to the host cycle. Pests were found to be quite specific for each company. For instance company number 8 had *Sitophilus oryzae* as its main pest, which is explained by the grain stored there (Tables 1 and 2). Similarly the flour companies had high numbers of flour moths *Plodia interpunctella* and *Ephestia kuehniella* (Table 1, Table 2). Levels of pest and natural enemies before and after treatments were not noticeably different, maybe due to adaptation to the chemicals used, due to the wrong frequency of treatments or maybe owing to creation of insect refuges in certain places within the facilities, where the treatments do not reach properly.

**Acknowledgements**

Many thanks to the department of Environmental Horticulture (IRTA Recerca i Tecnologia Agroalimentaries, Cabrils, Spain) for the weather data handed over. Thanks to the Education and Science Ministry, INIA (PROJECT RTA 2005-00068-00-00), for the financial help.

**References**


The biology of *Lariophagus distinguendus* a natural antagonist of stored grain beetles – film-presentation

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**Abstract:** The parasitic wasp *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) parasitizes larvae and pupae of several beetle species, which develop inside seeds and grains. Its major host-species, the granary weevil *Sitophilus granarius* is a threatening pest of stored grain worldwide. Thus *Lariophagus distinguendus* possesses special adaptations to find and parasitise beetle-larvae inside grains, which give it a high potential in biological control. The film shows at first how a female of *S. granarius* chews a hole through the outer coating of a wheat kernel and then turns round to oviposit a single egg into the grain. The offspring develops inside the kernel, pupates and finally gnaws an exit hole through which it emerges. The second part of the film deals with the natural enemy *L. distinguendus*. It shows the characteristic courtship behaviour, which is initiated by a female sex pheromone. Further sequences present the parasitisation behaviour. Upon contact with an *S. granarius* infested grain, females of *L. distinguendus* continuously drum with their antennae on the grain’s surface until a suitable oviposition site is detected. This site is investigated with the abdominal tip and the ovipositor is inserted into the grain. During this process secretions, which are excreted along the ovipositor form a feeding tube that is used by the wasp to take up host hemolymph. After host feeding, the ovipositor is reinserted through the tube and an egg is released. A few days later a wasp larva hatches from the egg and develops inside the grain into an adult wasp, thereby feeding from the host larvae. Then the young adult emerges through the grain coat. Beside macroscopical pictures of the life cycles of beetles and wasps the film will give insight into the hidden procedures of parasitisation and development, which take place inside the grains.

**Key words:** *Lariophagus distinguendus, Sitophilus granarius*, host feeding, macroscopical pictures
Biological control of *Anobium punctatum* in infested books, using the parasitoid *Lariophagus distinguendus* – preliminary results

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Abstract: A severe infestation of thousands of books by *Anobium punctatum* was discovered in an Israeli library. Initial successful treatment was carried out, involving fogging the library space and freezing the books at -30°C. Other known methods for treating books (anoxia or methyl bromide), involve removal from the library and treatment is very problematic. BIP Company from Berlin suggested checking the possibility of biological control of the infested books using the parasitoid wasp, *Lariophagus distinguendus* while the books remained on the library shelves. Research has proven that the above mentioned wasp has the ability to identify and parasitize host larvae in closed and limited spaces such as grains of rice or pulses, and to reduce the populations of stored pests like *Sitophilus granarius* or Bruchidae. During each of three months, wasp pupae supplied by BIP were released in a closed library room in which infested books were concentrated in an open carton exposed to the wasps. Infested control books were placed in material-covered cartons. 46% of the *Anobium* larvae among the exposed books were found dead. 86% of the larvae were found live in the control, covered box. In parallel, *Sitophilus granarius* larvae in wheat kernels, in four gauze packages with a mesh width of 1.5 mm, were placed amongst cartons of uninfested books for 5 days and exposed to the wasps. There was no emergence of adult *S. granarius*. However, emergence of wasps, were found which had developed in the host larvae. These results indicate the ability of the wasps to identify the host and to parasitize them, either between or inside the books. These trials show the potential for *Lariophagus distinguendus* to serve as a biological control agent in books on shelves. More experiments are necessary to make this potential practical.

Key words: *Anobium punctatum*, *Lariophagus distinguendus*, biological control, libraries, books

Introduction

A very severe infestation of the Anobiid *Anobium punctatum* (Degeer) in books was detected in 2004 in the Jewish National and University Library in Jerusalem. A survey estimated that 220,000 books were infested or suspected of being infested and damaged to various degrees. It was necessary to carry out a control operation on the books in order to avoid the continuation of the damage and to exterminate the pests. Two types of treatment were carried out: 1) two fogging treatments of the three main book stack halls. The first fogging was carried out using natural pyrethrum in order to kill the individual beetles outside the books and on the shelves. Three months later, a second fogging was carried out using permethrin. The aim was to kill all adults which may have emerged from books after the first fogging. The decrease in the level of adult populations to the minimum possible was to prevent additional egg laying and to eliminate the pest population in the library. 2) All the infested and suspected books were frozen to -30°C in order to kill the young stages of the insects developing inside
the books. The freezing treatment was particularly problematic, requiring a suitable freezing room, special packaging of the books in boxes and the necessity to transport the boxes to the freezing room outside the library and then to return them. The treatment took 7-8 days and the number of books which could be treated at any one time was very limited. The whole process was time consuming, very expensive and has other disadvantages which may damage the books. The freezing of 220,000 books took more than a year and cost over 400,000 $US. Other control methods such as anoxia and the use of methyl bromide also require the removal of the books to a treatment room, involve similar technical problems and require a long period of time.

Sabine Prozell of BIP Company, Berlin, raised the idea of using biological control of the young stages of *A. punctatum* as a possible method to eliminate the pest which live and develop inside books, without the need to remove the books from the library. It was suggested to try the wasp *Lariophagus distinguendus* (Förster) as a potential biological control agent. *L. distinguendus* is a small (1-2mm), black wasp with a world-wide distribution including Israel (Gerling, 1989). This wasp parasitizes stored product beetles which cause damage to grains, such as *Sitophilus oryzae* (Gonen and Kugler, 1970), *Rhizopertha dominica* (Kaschef, 1959a), other species of the family Bruchidae (Bellows, 1985), and *Stegobium paniceum* (Kaschef, 1956; Champ, 1966), which, like *A. punctatum*, belongs to the family Anobiidae.

Some aspects of the biology of *L. distinguendus* while parasitizing *Sitophilus oryzae* were studied by Gonen and Kugler (1970). This wasp is able to identify the larvae of *Sitophilus* inside a limited space, such as grains of wheat or rice. The wasp stings and immobilizes the larva using its ovipositor, then lays an egg inside the larva. Therefore, it was assumed that this wasp may have the ability to identify the larvae of *A. punctatum* inside the books and to parasitize them. The studies by Reppchen at al. (2002, 2003), Steidle and Schöller (2002) and others in recent years proved that this wasp has the potential to reduce the population of stored product beetles which cause damage to grains. A practical use of biological control using *L. distinguendus* supplied by BIP has already being applied (Niedermayer and Steidle, 2008). Successful biological control using *L. distinguendus* was carried out on a 16th century wooden altar in a historic German cathedral infested with wood eating larvae (Duke, 2005)

A preliminary experiment was conducted to check the use of *L. distinguendus* for the biological control of the infested books. The main goal was to check the ability of this wasp to identify the larvae inside the books and to parasitize them.

**Materials and methods**

**Experiment room**

The experiment was conducted in a sealed library room (5 x 2 m) in which many books are stored on shelves. About 600 books, brought from outside the library were stored in cartons on the floor in the above room. Some of these books were found to be infested, while those on the shelves showed no signs of infestation. In this room no chemical control treatments had been carried out that would endanger the parasitoid. The temperature in this room was 24-25°C and the humidity was 40%.

**Plan and organization of experiments**

The ability of the wasps to identify and parasitize the larvae in the books was checked in two ways:

i) Packages containing *S. granarius* larvae in wheat grains were exposed to the parasitoid. Plastic gauze packages with a mesh width of 1.5 mm containing wheat grains, inside which *Sitophilus granarius* larvae were developing, were kept in plastic boxes with...
openings, supplied by BIP. One such box was put inside each of four out of ten cartons containing uninfested books, in order to check the ability of the wasps to identify the *Sitophilus* larvae among the books and to determine the percentage of parasitism. These 4 boxes of *S. granarius* were exposed to the parasitoid for five days, in two series, 2 months apart. After 5 days exposure, the boxes were sent to BIP laboratories to check for parasitism. The boxes of *Sitophilus* larvae were incubated at 26°C and 70% humidity. The number of wasps emerging and adult *Sitophilus* were counted. Two additional boxes containing *Sitophilus* larvae were put in another room with the same environmental conditions but unexposed to wasps and acted as controls.

ii) Books infested with *A. punctatum* were exposed to the parasitoid. Among the books in the cartons, 16 books were found to be infested in which at least one live larva was found as well as signs of an active infestation (book dust holes and tunnels). These infested books were divided into two groups: nine books in a carton (R) were exposed to the wasps. The remaining 7 books in a carton (R8), the control, were covered and closed with canvas material which did not allow the wasps to penetrate.

The ten cartons of uninfested books, four of which contained *Sitophilus* boxes, were arranged in two rows on the floor. The two cartons of books infested with *A. punctatum* were placed between these two rows (see Fig. 1).

![Fig. 1. Arrangement of book cartons, 4 of them with *Sitophilus* boxes among the books, and the 10 wasp packages on the floor.](image)

**Release of wasps**

Ten packages containing wasp pupae, also supplied by BIP, were placed in the room, near the books cartons in 3 series, for 3 consecutive months. The parasitoid packages of plastic gauze had a mesh width of 1.5 mm and each contained around 30 female wasps, in the pupal stage, inside wheat grains. Adult wasps were observed to emerge almost immediately after placing them near the cartons.

**Results and discussion**

A number of studies have already proved the ability of *L. distinguendus* to identify and to parasitize larvae of *Sitophilus* species and to reduce the population level of this pest. This
wasp is attracted to grains and food stuff in which the host larvae are developing. The host is located by odour (Kaschef, 1959b). In our experiment, in which S. granarius larvae were placed among the books, they were identified and parasitized and no S. granarius adults emerged. However, emergence of adult wasps was found in all boxes, as shown in Table 1. This result indicates that this parasitoid is attracted and was able to parasitize a potential host not only to food stuffs but also in books.

Table 1. Results of S. granarius parasitism in the boxes among the books.

<table>
<thead>
<tr>
<th>Date</th>
<th>Carton No.</th>
<th>No. L. distinguendus emerged</th>
<th>No. S. granarius emerged</th>
</tr>
</thead>
<tbody>
<tr>
<td>04.09.2006</td>
<td>1</td>
<td>59</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>0</td>
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<tr>
<td></td>
<td>3</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>29.10.2006</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

The control unexposed packages of S. granarius showed normal development of the larvae, and adult beetles emerged as in the usual beetle cultures of this species in BIP laboratories.

The results of the second experiment, shown in Table 2, indicate that L. distinguendus can also identify and parasitize Anobium punctatum larvae inside books.

Table 2. The activity of the wasps in books infested with Anobium punctatum larvae.

<table>
<thead>
<tr>
<th></th>
<th>Live larvae</th>
<th>Dead larvae</th>
<th>% mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed books</td>
<td>7</td>
<td>6</td>
<td>46.0</td>
</tr>
<tr>
<td>Control books</td>
<td>6</td>
<td>1</td>
<td>14.2</td>
</tr>
</tbody>
</table>

Among the books exposed to L. distinguendus, the percentage mortality of Anobium punctatum larvae was over three times higher than that of the control books.

Gonen and Kugler (1970) found that the female wasps prefer to attack older larvae of Sitophilus oryzae and only 28% of the younger larvae were attacked. The fact that in this study only 46% of the exposed A. punctatum larvae were killed may not indicate the inability of the wasps to significantly reduce the pest population. The ability of the wasps to reduce the pest population is conditional on the emergence of new generations of wasps from the parasitized larvae, which would attack the remaining A. punctatum larvae. It is worth emphasizing that the life cycle of A. punctatum, under optimal conditions, takes one year or more. The life cycle of the wasp from egg to adult is 16 to 23 days. Therefore there is a real possibility that L. distinguendus may serve as an efficient biological agent against A. punctatum.

This preliminary experiment conducted at the University and National Library is the first to check the ability of the wasp to act successfully in a large library and to exterminate the pest in situ without the need to invest major resources to remove the books from the library. If
this method succeeds in reducing the level of *A. punctatum* larvae below a damaging threshold, it may serve as a model for archives and museums. It should be noted that at the IOBC conference on integrated protection of stored products in Poznan, Poland, (2007) five different lectures were given dealing with biological control by *L. distinguendus*. It appears that using *L. distinguendus* has the best chance to serve as the control agent of pests in a variety of foodstuffs, libraries and so on.

The above experiment showed that a wasp *Lariophagus distinguendus* had the ability to identify and parasitize *A. punctatum*, both between and inside books. There is thus a potential for *Lariophagus distinguendus* to serve as a biological control agent in books on the shelves. More experiments are necessary to prove this potential practical.

References


Virulence of isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin to adults of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)

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Abstract: Bioassays with conidia of twenty isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin have been tested on adults of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Newly emerged insects were treated indirectly with conidial suspensions (1x10⁶ conidia/ml) of the isolates. Lethal effect of the isolates was evaluated as percentages of cumulative daily mortality due to mycoses. Virulence of each fungal isolate was estimated by values of the median lethal time (LT₅₀), calculated by probit analysis. Fourteen of the tested isolates caused a mycosis with rapid lethal effect. The first dead insects were noted on the second day. Mortality of *A. obtectus* adults in variants treated with conidial suspensions of the isolates 412 and 414 of *B. bassiana* increased from 7.5% and 1.25% on the second day to over 75% on the fifth day and up to 100% on the sixth day. Isolates 225, 229 and 343 caused the lowest lethal effect – 49.17%, 39.17% and 53.75%, respectively, on the 10th day. The isolates 412, 414 and 417 had the highest virulence to *A. obtectus* adults. The average values of the median lethal time (LT₅₀) of the isolates were 3.426, 3.776 and 3.832 days, respectively. The values of LT₅₀ calculated at significance level p < 0.05 varied within narrow confidence intervals from 3.119 to 3.773, from 3.549 to 4.018 and from 3.556 to 4.129 days. Significant difference between the virulence of the three isolates couldn’t be found. The isolates 225 and 229 of *B. bassiana* had the lowest virulence. Their values of LT₅₀ varied within the confidence intervals from 13.080 to 18.920 days, with an average value of 15.730 days and from 11.620 to 15.400 days with an average value of 13.790 days, respectively.

Key words: entomopathogenic fungi, *Beauveria bassiana*, *Acanthoscelides obtectus*, lethal effect, median lethal time (LT₅₀), virulence

Introduction

The bean weevil (*Acanthoscelides obtectus* Say, Coleoptera: Bruchidae) is one of the most important pests of the common bean – *Phaseolus vulgaris* L. The larvae injure the beans in the field but the greatest damage is caused by them on the stored seeds. Control of the stored product insects is conducted mainly with insecticides for direct treatment or fumigation. The insecticides are more or less toxic, sometimes cause resistance of the insect populations and the treated products are not pesticide-free.

That is why alternative strategies for stored pest control have been developed. Panda and Khush (1995) and Throne et al. (2000) (according Throne and Lord, 2004) noted that host plant resistance was generally considered to be compatible with other management strategies, which combined action can be key components of an integrated pest management program. The integrated strategies also included application of bioagents – parasitoids, entomopathogens (Cox and Wilkin, 1998; Arthur, 1966; Schöller, 1998). The virulence of strains of
entomopathogenic fungal species has been investigated for control of stored product pests from order Coleoptera (Smith et al., 1998; Rice and Cogburn, 1999). In a technology for protecting stored beans in El Salvador Parada and Serrano (1998) included treatment with Beauveria bassiana (Bals.) Vuillemin at a dosage of 0.04% or conidial concentration higher than 1x10^{10} per g. Padin et al. (2002) conducted experiments with Tribolium castaneum, Sitophilus oryzae and A. obtectus in stored durum wheat and beans treated with B. bassiana and reported a reduction in insect infestation and in grain losses. Agona et al. (2005) included conidial treatment with water suspension of B. bassiana in an integrated pest management against the bean weevil in the field. Dal Bello et al. (2006) conducted experiments with B. bassiana and diatomaceous earth (DE) for control of A. obtectus and S. oryzae and concluded that the median lethal time with the DE-dry fungus for the first one was significantly lower than those with an aqueous fungal suspension. Treatment with B. bassiana showed middle to high level of control with different stored product insects including the maize weevil – Sitophilus zeamais Motschulsky (Hidalgo et al., 1998), the cowpea weevil – Callosobruchus maculatus F. (Staneva and Draganova, 2000; Cherry et al., 2005), the rice weevil – S. oryzae L. (Dal Bello et al., 2001; Padin et al., 2002), the larger grain borer – Prostephanus truncatus Horn. (Meikle et al., 2001), the sawtoothed grain beetle – Oryzaephilus surinamensis L. (Throne and Lord, 2004).

The aim of the study was to estimate the virulence of isolates of entomopathogenic fungus B. bassiana to adults of A. obtectus.

**Materials and methods**

Twenty B. bassiana isolates were isolated in pure cultures from dead arthropods belonging to eleven species collected from natural populations of the pests from different regions in Bulgaria. Strains were stored in the Collection of entomopathogenic fungi in the Department of Biological and Integrated Pest Control (Plant Protection Institute, Kostinbrod, Bulgaria). The identity and origin (initial hosts) of the isolates are shown in Table 1.

<table>
<thead>
<tr>
<th>Isolate of B. bassiana</th>
<th>Species of the initial host</th>
<th>Stage of the initial host</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>Tetranychus urticae Koch. (Acari: Tetranychidae)</td>
<td>imago</td>
</tr>
<tr>
<td>339</td>
<td>Aphis pomi De Geer (Homoptera: Aphididae)</td>
<td>larvae</td>
</tr>
<tr>
<td>208</td>
<td>Eurygaster integriceps Put. (Heteroptera: Scutelleridae)</td>
<td>imago</td>
</tr>
<tr>
<td>311, 312</td>
<td>Agriopis bajaria (Denis &amp; Schiff.) (Lepidoptera: Geometridae)</td>
<td>larvae</td>
</tr>
<tr>
<td>414</td>
<td>Operophtera brumata (L.) (Lepidoptera: Geometridae)</td>
<td>larvae</td>
</tr>
<tr>
<td>412</td>
<td>Cydia pomonella L. (Lepidoptera: Tortricidae)</td>
<td>larvae</td>
</tr>
<tr>
<td>417</td>
<td>Hedya nubiferana (Haw.) (Lepidoptera: Tortricidae)</td>
<td>larvae</td>
</tr>
<tr>
<td>373</td>
<td>Yponomeuta malinella Zell. (Lepidoptera: Yponomeutidae)</td>
<td>larvae</td>
</tr>
<tr>
<td>343</td>
<td>Dociostaurus maroccanus Thunbg. (Orthoptera: Acrididae)</td>
<td>larvae</td>
</tr>
<tr>
<td>256</td>
<td>Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae)</td>
<td>larvae</td>
</tr>
<tr>
<td>282</td>
<td>Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae)</td>
<td>imago</td>
</tr>
<tr>
<td>225, 226, 227, 228, 229, 232, 233, 255</td>
<td>Phytodecta fornicata Brugg. (Coleoptera: Chrysomelidae)</td>
<td>imago</td>
</tr>
</tbody>
</table>

Table 1. Origin of the isolates of Beauveria bassiana (Bals.) Vuill. applied in bioassays.
The isolates were cultured on SDA (Sabouraud dextrose agar) in tubes and incubated at 25 ± 1°C for 15 days. Conidia from fifteen-day-old cultures of each fungal isolate were washed down by water. The conidia concentrations were determined by haemocytometer and suspensions were diluted to 1x10^6 conidia/ml.

The adults of *A. obtectus* used in bioassays were from laboratory population reared on seeds of *P. vulgaris* at temperature 25 ± 1°C and 70 ± 1% RH. Newly emerged adults were treated indirectly (Draganova & Staneva, 1990). Insects were placed for 24 h in Petri dishes with filter paper discs (100 mm in diameter) on which by 1 ml of conidial suspensions (1x10^6 conidia/ml) had been dropped previously. After contamination with fungal conidia insects were moved to clean Petri dishes. Experiments were carried out in three replicates with 40 insects per replicate at temperature 25 ± 1°C and 70 ± 1% RH in a climate chamber. Insects in control variants were treated with water. Insect mortality was noted daily during 15 days. Dead insects were placed into a humid chamber for fungal pathogen exhibition expressed as produced hyphal growth. Results of the bioassays were evaluated as percentages of cumulative daily mortality due to mycoses, corrected according to Abbott (1925). Virulence of each *B. bassiana* isolate was estimated by values of the median lethal time (LT_{50}), calculated by probit analysis (Finney, 1971).

![Graph showing the lethal effect of Beauvaria bassiana (Bals.) Vuill. isolates to adults of Acanthoscelides obtectus Say for the first 7 days.](image)

**Results and discussion**

The results of the bioassays with isolates of *B. bassiana* applied to adults of *A. obtectus* were exhibited in Fig. 1 and Table 2.
The isolates 339, 412 and 340 of *B. bassiana* had the highest initial effect to the treated adults of *A. obtectus* (Fig. 1). Calculated cumulative mortality on the second day was 8.75% ± 2.80, 7.50% ± 2.51 and 6.25% ± 1.36, respectively. It could be explained with the lethal effect of produced toxic metabolites by the fungal isolate on the susceptible part of the treated insect population.

According to Roberts (1981) species from the genus *Beauveria* release toxic metabolites (beauvericin, beauverolides, bassianolide, etc.) as a step of development of the mycosis that preceds the insect’s death.

Later the isolate 339 of *B. bassiana* showed less lethal effect in comparison with the other tested isolates. On the 10th day 55% ± 2.80 cumulative mortality was found in the variant treated with conidial suspensions of the isolate.

Fourteen of the tested twenty isolates caused a mycosis with rapid lethal effect (Fig. 1). The first dead insects were noted on the second day. Mortality of *A. obtectus* adults in variants treated with conidial suspensions of the isolates 412 and 414 of *B. bassiana* increased from 7.5% and 1.25% on the second day to over 75% on the fifth day and up to 100% on the sixth day. On the 7th day the isolates 225, 229, 343, 282 and 339 had a lethal effect below 30%. Three of these isolates (229, 225 and 343) caused the lowest lethal effect on the 10th day – 39.17%, 49.17% and 53.75%, respectively.

### Table 2. Virulence of isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. to adults of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae).

<table>
<thead>
<tr>
<th>Isolate, species</th>
<th>Median lethal time LT&lt;sub&gt;50&lt;/sub&gt; (days)</th>
<th>Regression coefficient (b ± S&lt;sub&gt;b&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average values <em>a</em></td>
<td>Fiducial limits from to</td>
</tr>
<tr>
<td>208 Beauveria bassiana</td>
<td>4.945&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.857 to 5.035</td>
</tr>
<tr>
<td>225 Beauveria bassiana</td>
<td>15.730&lt;sup&gt;j&lt;/sup&gt;</td>
<td>13.080 to 18.920</td>
</tr>
<tr>
<td>226 Beauveria bassiana</td>
<td>4.729&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.615 to 4.844</td>
</tr>
<tr>
<td>227 Beauveria bassiana</td>
<td>5.286&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.140 to 5.439</td>
</tr>
<tr>
<td>228 Beauveria bassiana</td>
<td>4.236&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.147 to 4.326</td>
</tr>
<tr>
<td>229 Beauveria bassiana</td>
<td>13.790&lt;sup&gt;j&lt;/sup&gt;</td>
<td>11.620 to 15.400</td>
</tr>
<tr>
<td>232 Beauveria bassiana</td>
<td>4.399&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.259 to 4.544</td>
</tr>
<tr>
<td>233 Beauveria bassiana</td>
<td>4.157&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.011 to 4.307</td>
</tr>
<tr>
<td>255 Beauveria bassiana</td>
<td>4.797&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.721 to 4.875</td>
</tr>
<tr>
<td>256 Beauveria bassiana</td>
<td>3.879&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.834 to 3.925</td>
</tr>
<tr>
<td>282 Beauveria bassiana</td>
<td>8.551&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.305 to 8.804</td>
</tr>
<tr>
<td>311 Beauveria bassiana</td>
<td>5.624&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4.903 to 6.545</td>
</tr>
<tr>
<td>312 Beauveria bassiana</td>
<td>4.517&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.303 to 4.742</td>
</tr>
<tr>
<td>339 Beauveria bassiana</td>
<td>10.860&lt;sup&gt;g&lt;/sup&gt;</td>
<td>8.818 to 13.400</td>
</tr>
<tr>
<td>340 Beauveria bassiana</td>
<td>4.785&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.784 to 4.786</td>
</tr>
<tr>
<td>343 Beauveria bassiana</td>
<td>11.140&lt;sup&gt;g&lt;/sup&gt;</td>
<td>10.490 to 11.820</td>
</tr>
<tr>
<td>373 Beauveria bassiana</td>
<td>7.101&lt;sup&gt;g&lt;/sup&gt;</td>
<td>6.790 to 7.425</td>
</tr>
<tr>
<td>412 Beauveria bassiana</td>
<td>3.426&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.119 to 3.763</td>
</tr>
<tr>
<td>414 Beauveria bassiana</td>
<td>3.776&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.549 to 4.018</td>
</tr>
<tr>
<td>417 Beauveria bassiana</td>
<td>3.832&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.556 to 4.129</td>
</tr>
</tbody>
</table>

*<sup>a</sup>p-level < 0.05
*<sup>b</sup>Different letters indicate significant difference between virulence of the isolates
The isolates 412, 414 and 417 of *B. bassiana* had the highest virulence to *A. obtectus* adults (Table 2). The average values of the median lethal time of the isolates were 3.426, 3.776 and 3.832 days, respectively. The values of LT₅₀ calculated at significance level p < 0.05 varied within narrow confidence intervals from 3.119 to 3.773, from 3.549 to 4.018 and from 3.556 to 4.129 days, respectively. The confidence intervals overlapped over 30%, so significant differences between the virulence of the isolates 412, 414 and 417 of *B. bassiana* could not be found.

The isolate 256 of *B. bassiana* was highly virulent as well, with values of the median lethal time in limits from 3.834 to 3.925 days and an average value 3.879 days (Table 2). Significant differences (p < 0.05) between the virulence of the isolates 412 and 256 of *B. bassiana* were found as the fiducial limits of the values of LT₅₀ didn’t overlap. The difference between the virulence of isolates 256, 414 and 417 was not significant.

Comparison between isolates with relatively low virulence (339, 343, 229 and 225) estimated according to the values of the median lethal time didn’t show significant difference between isolates 339 and 343 and between isolates 229 and 225 (Table 2). Significant difference has been proved at p level < 0.05 between isolate 339 and other two isolates - 229 and 225 and between isolate 343 and the isolates 229 and 225. The calculated LT₅₀ – values for them varied within confidence intervals from 8.818 to 13.400 days (isolate 339), from 11.620 to 15.400 days (isolate 229), from 13.080 to 18.920 day (isolate 225) and from 10.490 to 11.820 days (isolate 343).

The results of the experiments showed that the most virulent isolates of *B. bassiana* caused high mortality to bean weevil adults in a short time. They are considered to be harmless to humans and the environment, which makes them appropriate for IPM strategies against *A. obtectus* on stored seeds.

Conclusions

1. Fourteen of the tested twenty isolates of *B. bassiana* caused mycosis with rapid lethal effect on adults of *A. obtectus*.
2. The isolates 412, 414 and 417 of *B. bassiana* had the highest virulence to *A. obtectus*. The values of LT₅₀ calculated at significance level p < 0.05 varied within narrow confidence intervals from 3.119 to 3.773, from 3.549 to 4.018 and from 3.556 to 4.129 days, respectively. Significant difference between the virulence of the three isolates couldn’t be found.
3. The isolates 225 and 229 of *B. bassiana* had the lowest virulence. Their values of LT₅₀ varied within the confidence intervals from 13.080 to 18.920 days with an average value 15.730 days and from 11.620 to 15.400 days with an average value 13.790 days, respectively.

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Experiences with beneficial insects for pest control in storage buildings and processing units

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Abstract: In a three year project, a practical approach for the control of stored-product pest insects in storage buildings and processing units is developed using the laboratory-mass-reared parasitoids and predators Trichogramma evanescens, Habrobracon hebetor, Lariophagus distinguendus and Anisopteromalus calandrae. T. evanescens and H. hebetor are mainly applied against Plodia interpunctella, but also against Ephesia kuehniella, E. elutella and E. cautella in mills, silos and bakeries as well as in storage buildings with big bags. L. distinguendus and A. calandrae are mainly applied against Stegobium paniceum in processing units. The evaluation of the pest development within the storage units was carried out by a comprehensive insect trap survey and a comparison was drawn to previous years where conventional pest control was still used. After the first year of experience a positive conclusion can be drawn. Four out of eight trials did not need any additional chemical treatments and three further trials needed only local chemical treatments based on water formulated compounds. However, limitations regarding the successful introduction of beneficials such as insufficient cleaning or constructional deficits became obvious. Further trials during this and next year will be carried out to confirm the experiences of the first year.

Key words: beneficial insects, Trichogramma evanescens, Habrobracon hebetor, Lariophagus distinguendus, Anisopteromalus calandrae, mill, silo cells, bakery

Introduction

Beneficial insects have a big potential to reduce pest problems in stored products. However, up to now chemical treatments have been favoured because of their knock down effect, their large range of target pests as well as high efficacy. The revision of the biocide list in the EU leading to a ban of many active ingredients will however imply new opportunities for the introduction of beneficial insects. Furthermore, pest control in organic stored products is not satisfactory up to now and the use of beneficial insects for indoor application could become an important tool in the future.

Desinfecta AG and Andermatt Biocontrol AG have been implementing, investigating and evaluating the production and release of beneficial insects, respectively in collaboration with FiBL in a three years project (2006-2008).

The goal of the project is to develop introduction strategies for four beneficials in storage buildings and processing units under consideration of economic aspects.
Materials and methods

In the trials, the following beneficial insects are applied: *Trichogramma evanescens*, *Habrobracon hebetor*, *Lariophagus distinguendus* and *Anisopteromalus calandrae*. The application rates of the beneficials are listed in Table 1.

In the first year (2006) eight locations were chosen for the trials. Six locations had moth problems (*Ephestia* spp., *Plodia interpunctella*), two locations were infested with *Stegobium paniceum*. Three out of the six locations with moth problems consisted mainly of silo cells for cereals or processed grains, two locations were storage rooms for nuts and coffee beans, respectively and one location was a bakery (Table 2).

Table 1: Application rates of the beneficial insects in relation to the location type.

<table>
<thead>
<tr>
<th>Beneficial insect</th>
<th>Empty room</th>
<th>Processing unit</th>
<th>Silo</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichogramma evanescens</em></td>
<td>20/m²</td>
<td>40/m²</td>
<td>160/m²</td>
</tr>
<tr>
<td><em>Habrobracon hebetor</em></td>
<td>0.5/m²</td>
<td>1/m²</td>
<td>2/m²</td>
</tr>
<tr>
<td><em>Lariophagus distinguendus</em></td>
<td>0.5/m²</td>
<td>1/m²</td>
<td>2/m²</td>
</tr>
<tr>
<td><em>Anisopteromalus calandrae</em></td>
<td>0.5/m²</td>
<td>1/m²</td>
<td>2/m²</td>
</tr>
</tbody>
</table>

In the case of the trial with *S. paniceum*, one location was a pasta packing unit and one location was an old mill containing office rooms.

All locations had a history of pest control with chemical insecticides for many years. Due to the lack of comparable units the trials had to be carried out without a comparison to an untreated control. Instead, the results of 2006 were compared with the situation in the previous year (2005) where still conventional pest control was used. The evaluation of the pest development within the storage units was carried out by a comprehensive insect trap survey. Table 2 gives an overview of the different trial locations and in Table 3 the amount, frequency and repetition of the beneficial release in 2006 is reported.

Table 2: Locations of the trials with beneficial insects.

<table>
<thead>
<tr>
<th>Location</th>
<th>Type</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Silo cells and upper space</td>
<td>150 m², 22 grain silo cells, height 20 m</td>
</tr>
<tr>
<td>2</td>
<td>Silo cells and upper space</td>
<td>1200 m², 100 grain silo cells, height 50 m. Only 30 cells were treated with beneficials</td>
</tr>
<tr>
<td>3</td>
<td>Mill including silo cells</td>
<td>Mill + 6 grain silo cells (steel) + 4 grain silo cells (concrete) + 2 flour silo units</td>
</tr>
<tr>
<td>4</td>
<td>Bakery with flour storehouse</td>
<td>Flour storehouse 200 m² + bakery 80 m²</td>
</tr>
<tr>
<td>5</td>
<td>Nut storehouse</td>
<td>600 m²</td>
</tr>
<tr>
<td>6</td>
<td>Coffee beans storehouse</td>
<td>2400 m²</td>
</tr>
</tbody>
</table>
| 7        | Pasta packing unit          | Packing unit 11’000 m³  
Silo 10’000 m³                                    |
| 8        | Office rooms in an old mill | 70 m²                                              |
Results and discussion

Location 1
The initial infestation by *P. interpunctella* was localized in two silo cells probably due to the existing regulations which were not suitable for allowing time for sufficient cleaning. In comparison to the previous year where 4 applications of pesticides were necessary, no chemical treatment was applied besides the beneficials in 2006. However, the moth trap catches were higher in 2006 than in 2005. From July onwards *H. hebetor* was applied in addition to *T. evanescens* because larvae of *P. interpunctella* were detected.

In the future, the introduction of the beneficials should start later because of the low temperature at the beginning (12°C) which seems to be a limiting factor for the activity of *T. evanescens*.

<table>
<thead>
<tr>
<th>Location</th>
<th>Pest</th>
<th>Beneficial</th>
<th>Total amount</th>
<th>Frequency (repetition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>P. interpunctella</em></td>
<td><em>T. evanescens</em> <em>H. hebetor</em></td>
<td>1'410'000 1'100</td>
<td>12x (2 w) 5x (2 w)</td>
</tr>
<tr>
<td>2</td>
<td><em>P. interpunctella</em></td>
<td><em>T. evanescens</em> <em>H. hebetor</em></td>
<td>3'750'000 1'750</td>
<td>11x (2 w) 7x (2 w)</td>
</tr>
<tr>
<td>3</td>
<td><em>E. kuehniella</em> <em>P. interpunctella</em></td>
<td><em>T. evanescens</em> <em>H. hebetor</em></td>
<td>3'840'000 13'750</td>
<td>13x (2 w) 6x (2 w)</td>
</tr>
<tr>
<td>4</td>
<td><em>P. interpunctella</em></td>
<td><em>T. evanescens</em></td>
<td>1'320'000</td>
<td>11x (2 w)</td>
</tr>
<tr>
<td>5</td>
<td><em>P. interpunctella</em></td>
<td><em>T. evanescens</em></td>
<td>560'000</td>
<td>14x (2 w)</td>
</tr>
<tr>
<td>6</td>
<td><em>E. elutella</em> <em>E. cautella</em></td>
<td><em>T. evanescens</em> <em>H. hebetor</em></td>
<td>1'600'000 25'000</td>
<td>4x (2 w) 4x (2 w)</td>
</tr>
<tr>
<td>7</td>
<td><em>S. paniceum</em> <em>A. calandrae</em></td>
<td></td>
<td>24'000</td>
<td>12x (4 w)</td>
</tr>
<tr>
<td>8</td>
<td><em>S. paniceum</em> <em>A. calandrae</em></td>
<td></td>
<td>1'500</td>
<td>3x (4 w)</td>
</tr>
</tbody>
</table>

Location 2
In 2005, fumigation was necessary, whereas none was applied in 2006 where the introduction of the beneficials took place. The level of trap catches was lower in 2006 than in 2005. In this trial it became evident, that coverage of the silo cells is of great importance and should take place to avoid reinfestation with moths. Reinfestation actually was the cause of earlier spot treatments with chemicals.

Location 3
Several spot treatments were necessary during the trial. The trial was finally stopped in mid-September because the moth infestation exceeded the acceptable level in some of the silo cells and in the mill. The condition for a successful result using beneficials was not present due to inappropriate separation of the flow of goods, a leak in the building, and the development of *P. interpunctella* because of loads of dust. Moreover, in the steel silo the temperatures were too high for a successful introduction of beneficials.

Location 4
In comparison to 2005, where a chemical treatment was necessary in November, no additional treatment was applied in 2006. Both rooms had fewer moths in the monitoring traps compared to the previous year. Cleaning of the rooms was improved during the trial period, which also contributed to the good result.
Location 5
In contrast to 2005, no additional treatment was necessary in 2006 except for a punctual spot application in November due to infested nuts brought to the storehouse at that time. In the beginning a combined application of *T. evanescens* and *H. hebetor* was planned, but *H. hebetor* was left out in the end because of the absence of moth larvae. In total fewer moth catches were recorded in 2006 than 2005.

Location 6
The introduction of *T. evanescens* and *H. hebetor* had to be interrupted and several chemical treatments were necessary to reduce the problems with *E. cautella* and *E. elutella*. Two major reasons were responsible for the failure of the introduction strategy in the storehouse: i) there has not yet been much knowledge on the efficacy of the introduced beneficials against the pest insects and ii) moreover, there was a continuous supply of infested goods.

Location 7
The trial with introductions of *A. calandrae* had already started in 2005 and continued in 2006. No chemical treatments were necessary, whereas periodical chemical treatments were necessary previously.

Location 8
For several years the office rooms had been treated with dichlorvos. After the introduction of *A. calandrae* in 2006 for the first time there was no further need to apply chemicals. No infestation was recorded at the end of the trial.

Conclusions

No additional chemical treatments were necessary in four out of the eight locations in which beneficials were introduced. In three further trials only spot applications of insecticides were necessary. In many cases the buildings were not ideal for the introduction of beneficials providing too many hiding places for the pests and hindering from a sufficient cleaning. But, also in such cases the introduction of beneficials can be successful. Only two cases (Locations 3 and 6) had to be considered inappropriate for the introduction of beneficials.

Acknowledgements

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References


Lariophagus distinguendus Förster (Hym.: Pteromalidae): development on Sitophilus granarius L. (Col.: Curculionidae) at low temperatures

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Abstract: The granary weevil Sitophilus granarius is an important pest in grain stores throughout temperate regions. The larval parasitoid Lariophagus distinguendus is a potential candidate for biological control of this pest. In cooler climates the successful use of this parasitoid depends on its ability to survive low temperatures during winter. Investigations have been carried out at the DPIL to determine development rates of L. distinguendus at temperatures down to 15°C. Furthermore, an investigation was set up to simulate conditions in a grain store during winter. Grain infested with S. granarius and L. distinguendus was gradually cooled to a target temperature of 5°C and maintained there for 15 weeks. The grain was then gradually transferred to room temperature and emergence of S. granarius and L. distinguendus was registered. Preliminary results of these investigations are presented.

Key words: grain, Sitophilus granarius, Lariophagus distinguendus, biocontrol, temperature

Introduction

The granary weevil Sitophilus granarius L. (Col.: Curculionidae) is an important pest in grain stores in temperate regions throughout the world (Rees 1996). Much research effort has been put into finding an efficient biological control method for the important weevil pests of stored grain. In Europe, an EU funded Working Group (WG 4) of a COST Action (no. 842) was active during 2001-2005 and focussed on biological control in stored products (Hansen and Wakefield 2007; proceedings available at http://cost842.csl.gov.uk/). WG 4 concluded that preventative application of biocontrol against weevils in bulk commodities is one of three situations that hold the greatest potential for widespread application in Europe (Hansen 2007a).

The larval parasitoid Lariophagus distinguendus Förster ((Hym.: Pteromalidae) has been suggested as a candidate for biological control of Sitophilus spp. in grain stores (Ryoo et al. 1991, Steidle 1998, Steidle and Schöller 2002, Hansen and Steenberg 2002, Lucas and Riudavets 2002, Reppchen et al. 2002). In a laboratory study using units containing 9 kg wheat infested with S. granarius, L. distinguendus exerted a suppression level of >99.9%, which was higher than in units with another parasitoid species, Anisopteromalus calandrae (Howard), and much higher than in units with a surface treatment of the entomopathogenic fungus Beauveria bassiana (Bals.) Vuillemin (Ascomyceta: Hypocreales) (Hansen and Steenberg 2007). This study was conducted at room temperature.

In Northern Europe, grain temperatures are reduced by aeration with cool ambient air to a target temperature of 5°C as soon as possible after harvest to prevent biological deterioration of the grain. Temperatures in grain stores in Denmark can go below 15°C by the
end of September. Thus, to be interesting for practical application, the parasitoid must be active at temperatures below 20°C, so they can establish in the first generation of larvae that are deposited in the grain shortly after harvest. Very little information can be found on the biology of *L. distinguendus* at low temperatures, i.e. below 20°C. Ryoo et al. (1991) found that very few specimens developed at 18°C. Charnow et al. (1981) reported that eggs are produced at 18°C. These sporadic results were insufficient to estimate the potential of using this parasitoid species in Danish grain stores, and an investigation was initiated. Hansen (2007b) investigated the development and reproduction of *L. distinguendus* at 16, 18 and 20°C and found that this parasitoid is able to develop and reproduce at 16°C. The intrinsic rate of natural increase, $r_m$, was 0.0182, 0.0222 and 0.0792 d$^{-1}$ at 16, 18 and 20°C, respectively. In comparison, a rough estimate of the $r_m$ of the host, *S. granarius*, is 0.016 and 0.040 d$^{-1}$ at 17 and 21°C, respectively, i.e. lower than the parasitoid (Andersen 1963). In addition, at 16 and 18°C, almost as many hosts, and at 20°C half as many hosts, are killed by parasitoid-induced mortality, e.g. host-feeding and unsuccessful parasitism (Pawson et al. 1987), as by successful parasitism (Hansen 2007b).

The results of these investigations are encouraging as they suggest that it may be possible to establish *L. distinguendus* in grain stores in Denmark successfully during the first few months when grain temperatures are above 15°C. An investigation was then conducted to determine the survival of *L. distinguendus* during a simulated winter: the parasitoid was established in infested grain which was then gradually cooled to 5°C and maintained at that temperature for 15 weeks. Parasitoid survival was determined after returning the grain to room temperature. Preliminary results of the investigation are presented.

**Materials and methods**

Plastic containers with ventilated lids and bottoms of 1 L capacity containing 800 g of wheat (*Triticum aestivum* L.), were used. Adults *S. granarius*, 36 per kg grain, were added and the grain was incubated at 20°C and 70% RH. After 3 and 5 weeks, adults of *L. distinguendus* at a rate of 36 per kg grain were added. A similar number of units were set up as untreated controls, i.e. with *S. granarius* alone. Six weeks after start the cooling process was initiated, the units were exposed to a temperature decrease of 5°C, incubated for 4 weeks and moved to the next temperature step. Thus after 8 weeks the units were at 5°C and were then incubated at that temperature for 15 weeks. The temperature was then gradually increased to room temperature, and the final samples were taken after 4 weeks’ incubation at 20°C.

Five units were taken out for sampling at each temperature change, i.e. eight times. At each sampling 1440 kernels from each unit were isolated and incubated for 9 weeks and the emergence of weevils or parasitoids was registered.

**Results and discussion**

Both *S. granarius* and *L. distinguendus* were able to survive through the cold period. In the units with *S. granarius* alone, the initial infestation level before the cold period was 8%. Some decrease in the infestation was seen during the cold period, but at the final examination the infestation level was approximately the same, 7.1%.

The initial infestation level (kernels with either *S. granarius* or *L. distinguendus* species, as kernels with parasitoids are initially infested with *S. granarius*) in the units with *L. distinguendus* was lower than in the untreated units: 3.7%, and the degree of parasitisation was 32%. At the final examination the infestation level was 3.6%, with 35% parasitized.
The numbers of insects in the units in both test lines before and after the cold period were surprisingly similar, suggesting that the population of both weevils and parasitoids were relatively unaffected by the low temperatures.

Thus it seems that the parasitoids would survive during winter in grain stores in Northern Europe and show the same level of activity after the grain temperature increases in spring. This makes it reasonable to assume that they will be able to exert control of the pest weevils during a full storage season, i.e. from harvest and until spring, provided that they are introduced in sufficient numbers and at the right time.

Acknowledgements

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References


Development of a mass rearing device for the use of _Lariophagus distinguendus_ (Förster) against _Sitophilus granarius_ L. in stored grain

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Abstract: One of the major pests of stored products in Europe is the granary weevil _Sitophilus granarius_ L. To control this pest, _Lariophagus distinguendus_ (Förster) can be used. It showed good results in various laboratory experiments and is commercially available in different European countries. Nevertheless the existing system of shipment and release has also some disadvantages that should be improved. Therefore, the aim of the present study is to develop a mass rearing device which can be placed in grain stores to obtain a continuous release of the wasps throughout the season. So far, the studies revealed the following results:

- Experiments under (extreme) climate conditions of grain stores showed that the parasitisation ability of _L. distinguendus_ during cold months depends on the minimum temperature and on the maximum temperature in warmer months. Mean daily temperatures are less important. Thus, the use of _L. distinguendus_ might not only be constrained by cold temperatures during winter, but also by high temperatures in summer.
- In release experiments in empty storages _L. distinguendus_ females were able to locate hosts over a maximum distance of up to 5 m. Thus, distances between mass rearing devices should not be larger than 10 m.
- _L. distinguendus_ is able to recognize and parasitise the alternative host _Acanthoscelides obtectus_ in beans. Thus, _A. obtectus_ can be used as host for the rearing of the wasps in mass rearing devices in grains instead of granary weevils, which would not be accepted by grain store owners.

Key words: _Lariophagus distinguendus, Sitophilus granarius, Acanthoscelides obtectus_, stored grain, mass rearing device

Introduction

Arthropod pests are a threat for stored products causing post-harvest losses between 9 – 14 % worldwide (Oerke, 1994). One of the major pests in Europe is the granary weevil _Sitophilus granarius_ (Coleoptera: Curculionidae). It lays eggs inside grain kernels where the larvae develop by eating up the grain from the inside. To control _S. granarius_, the wasp _Lariophagus distinguendus_ (Hymenoptera: Pteromalidae) is currently used in different European countries. However, the current system of shipment and release of the wasps has some disadvantages. First, grain store owners have to order and release wasps at least four times a year (Schöller and Prozell, 2006) which is considered to be very laborious. Due to the lack of appropriate monitoring, often applicators are not able to determine the release time and the amount of wasps necessary. Furthermore, wasps are shipped as adults and are at least 2-3 days old when they arrive and are released. By that time many wasps have already passed their fecundity peak, which is during the first days of their lives.

As an alternative to the currently used procedure, the present study aims at developing a mass rearing device comparable to a system already in use for the control of the chestnut leaf-
miner moth (Kehrli et al. 2005). This device consists of a box containing parasitic wasps and their hosts to continuously rear the wasps. From these boxes wasps will enter into the storage through a filter system with an appropriate mash size. With this system, there will be only one “application” per year, no releases will be necessary and the wasps can parasitise right after hatching. However, for this mass rearing device to work it has to be ensured that wasps are able to develop under the climate conditions of a storage. Furthermore, they have to disperse from the mass rearing device over some distance to the silos or bins containing the grain or to locate residual populations of their hosts. Finally, due to safety reasons and concerns by potential applicators granary weevils can not be used as hosts in the rearing devices. Therefore, wasps have to develop on alternative hosts that do not present a threat to the stored grains. In this study, the bean weevil *Acanthoscelides obtectus* shall be used as alternative hosts, because it only develops on beans. Thus, the present study addresses the following aspects which are important for the development of the rearing box.

- The parasitisation ability of wasps under climate conditions of storages
- The range of *L. distinguendus* in empty storages
- The ability of *L. distinguendus* to recognize and parasitize the alternative host *Acanthoscelides obtectus* in beans.

**Material and methods**

**Insect rearing**

The strain of *L. distinguendus* used in the experiments was obtained from cultures maintained in the laboratory of the Institute of Zoology of The University of Hohenheim. The culture was kept at 25 ± 2 °C, 50 ± 5 % relative humidity and a photoperiod of 16:8 h on wheat infested with *S. granarius*. The culture of *S. granarius* was reared under the same conditions as *L. distinguendus*. The bean weevil *A. obtectus* was reared on the cowpea *Vigna unguiculata* L. and the runner bean *Phaseolus vulgaris* L. var. vulgaris. The culture was kept at 28 ± 1 °C, 85 ± 5 % relative humidity. Larval stages used in the experiments appeared after 21- 28 days.

**Climate conditions and parasitisation**

To investigate the parasitisation ability of *L. distinguendus* under natural climate conditions, climate data was measured in different empty storages in long term recordings. Data loggers (PCE-HT 110) where placed in the middle of the storages at a hight of ± 1m above the ground. Analyses were conducted with the PCE-HT110 Software.

To test the parasitisation ability of *L. distinguendus* under natural conditions, Petri dishes containing 10 g of grain infested with *S. granarius* were placed in the storages. One male and one female wasp were added. After one week the wasps were removed and the grain was incubated at 25 ± 2 °C, 50 ± 5 % relative humidity in the lab. After 3 weeks the emerged offspring was counted.

The combination of temperature and parasitisation data was analysed with a Generalized Linear Model (GLZ) analysis using Statistica 6.0 (Statsoft).

**Dispersal in empty storages**

The range of *L. distinguendus* in empty storages was studied by releasing 10 female wasps per week. Open Petri dishes containing 6 g of grain infested with the granary weevil *S. granarius* were offered in distances of 1, 5, 10 and 15 m to the releasing point. After one week the infested grain was removed from the storage and incubated at 25 ± 3 °C, 50 ± 10 % for 3 weeks. Emerging offspring was counted.
The alternative host *A. obtectus*

To test the host recognition ability of *L. distinguendus* on beans infested with *A. obtectus*, a two-choice-test was performed. The test device consists of an elevated 60 mm Petri dish with a hole in the centre for a test tube. A bean infested with *A. obtectus* and an uninfested bean was placed on opposite sides in the Petri dish. A female wasp was added in an open test tube in the middle of the Petri dish. The behaviour of the wasp was observed for 600 s and recorded with the observation program “The Observer”. The two different bean types were tested separately.

**Results and discussion**

**Climate conditions and parasitisation**

From April 2006 to July 2007 extreme temperatures in empty storages were observed. Minimum temperatures of -5.2 °C in the winter time and maximum temperatures of 45.5°C in the summer time could be measured. Temperature changes of up to 20°C occurred during very few days. Preliminary analyses of the impact of temperature on parasitisation show that the parasitisation during the cooler months mainly depends on the minimum temperature. In warmer months parasitisation depends on the maximum temperature. The mean temperature does not play a role for the parasitisation. At mean weekly temperatures below 5°C and above 40°C no or only little parasitisation could be observed (Fig. 1). Thus, low minimum and high maximum temperatures in poorly insulated storages might prevent *L. distinguendus* from parasitizing. Furthermore, these extreme temperatures will probably have an impact on the survival of wasps emerging out of the mass hatching device.

![Graph showing minimum and maximum temperatures during the year in correlation with the number of offspring of *L. distinguendus* in empty storages.](image)

Dispersal in empty storages

Results from over 60 releases in empty storages, during 2006 and 2007, show that *L. distinguendus* was able to locate infested grain up to a maximum distance of 5 m from the release point. In 27.4% of all cases wasps were able to reach the grain and parasitize (Fig. 2). Thus, distances between mass rearing devices should not be larger than 10 m. Furthermore, these results indicate that *L. distinguendus* was not able to control residual populations of pest beetles in empty storages, unless it was distributed at 10 m distances.

**The alternative host *A. obtectus***

Results of the two-choice-test show that *L. distinguendus* was able to distinguish between beans infested with larvae of *A. obtectus* and uninfested beans. The wasps showed...
significantly longer drumming and drilling behaviour (host recognition behaviour) on infested beans than on uninfested ones (Figures 3 and 4). Even though *L. distinguendus* showed drumming and drilling behaviour on infested beans it is still not shown that parasitisation takes place. Further experiments need to be done to see whether development of the wasp on beans was possible or not.

Fig. 2. Dispersal of *L. distinguendus* in empty storages, frequency of parasitisation events measured with *S. granarius* infested wheat in distances between 1 to 15 m (n = 60).

Fig. 3. Duration of drumming (mean ± standard deviation) of female *L. distinguendus* on infested (hatched bars) and uninfested (empty bars) cowpeas and runner beans, * - *p* < 0.05, ** - *p* < 0.01 (Wilcoxon-matched Pairs Test, n = 25).

Fig. 4. Duration of drilling (mean ± standard deviation) of female *L. distinguendus* on infested (hatched bars) and uninfested (empty bars) cowpeas and runner beans, * - *p* < 0.05, ** - *p* < 0.01 (Wilcoxon-matched Pairs Test, n = 25).
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References


Studies on storage, release and host finding of Trichogramma evanescens to control Ephestia kuehniella

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Abstract: The Mediterranean flour moth (MFM), Ephestia kuehniella Zeller (Lep.: Pyralidae), is a serious pest of flour and feed mills. Parasitoids from the genus Trichogramma are of interest for control of pyralid moths in flourmills. The objective of the present study was to evaluate the efficacy of inundative releases, the host-finding ability and feeding method of Trichogramma evanescens. Controlled releases of T. evanescens were made in metal boxes. Wheat flour in small sacks was placed the metal boxes and then 10 female and 10 male adults of E. kuehniella and about 1500 parasitized eggs were simultaneously placed in metal boxes three times each until the host adults died. Despite a reduction of E. kuehniella population compared with non-release control population, the parasitoid was not able to suppress the population to economic threshold level in infested boxes. When T. evanescens adults were stored at 4ºC for 7 days and at 10ºC for 15 days, storage had no significant effect on parasitisation, and adult and female emergence compared with untreated control, but stored at 4ºC for 11 days showed significant reduction in emergence. Host finding capacity of the wasp was also tested in U-shaped plates (100, 120 and 200 cm) using E. kuehniella eggs as host. The release distance did not influence the parasitism of the wasp (from release point to the host eggs) and it effectively parasitized as far as the distance of 200 cm. In another test we also compared conventional feeding method to glass micropipette; the females parasitized significantly higher numbers of hosts when honey was offered in a glass micropipette than the conventional.

Key words: Trichogramma evanescens, Ephestia kuehniella, storage, release, host finding

Introduction

Moth species of the genus Ephestia, especially Ephestia kuehniella Zeller, are serious pests in cereal-based food processing facilities in Turkey (Anonymous, 1995). Augmentative biological control by using Trichogramma species offers a promising new approach for managing stored-product moths. The studies with Trichogramma species have demonstrated marked reductions in moth captures and infestations in bulk wheat, Triticum aestivum L., storage (Schöller et al. 1996); and bakeries (Prozell and Schöller 1998, Steidle et al. 2001) as well as in warehouses and retail stores (Prozell et al. 1996). For such species, the biology of the host and the environmental conditions specific to its habitat may have affected the parasitoids’ dispersal capacity. The location of new hosts is probably an obligatory component of their life cycle (Vinson 1998). One of the most important questions regarding the use of Trichogramma parasitoids for inundative release against E. kuehniella is how many to release on each occasion.

Trichogramma species vary greatly in their searching behaviour, host preference and response to environmental conditions. Consequently, they vary in their suitability for use in
biological control (Hassan, 1989). *Trichogramma* longevity is known to be also influenced by presence of food (Yu et al., 1984; Lim, 1986; Hohmann et al., 1989; Leatemia et al., 1995). The provision of supplemental food appears to be the most practical and economical means of promoting longevity in the field and thus reducing the frequency of releases in a *Trichogramma* augmentation program. Development of storage techniques for biocontrol agents is considered of greatest importance to provide flexibility and efficiency in mass production (Leopold, 1998).

The objective of the study was to investigate the possibility of enhancing parasitism levels of flour moth eggs by storage, augmentative releases and feeding method of *T. evanescens*.

**Materials and methods**

**Rearing of *Ephestia kuehniella***

Mediterranean flour moth, *Ephestia kuehniella* adults were reared in a mixture consisting of one kg wheat flour, 50 g yeast and 30 g wheat germs. Throughout the rearing, cultures were kept in a rearing room, at 27±1°C, 70±5 % R.H., and 14:10 L:D.

**Rearing of *Trichogramma evanescens***

The wasp *T. evanescens* was reared on eggs of flour moth and kept in test tubes at 24±1°C, 70–80% RH, 14:10 h L: D. To obtain eggs for the tests, large numbers of 1-to 2-day-old adults of *E. kuehniella* were collected from stock cultures and placed in plastic jars with screen bottoms. Eggs that fell through the screen were collected the following days, sifted to remove insect parts and frass, and placed in Petri dish. Strips of lightweight cardboard (2.5 by 4 cm) were brushed with gum arabica. Eggs were sprinkled on these cards and placed in tubes along with adult *T. evanescens*. Individual *T. evanescens* females were prepared for the tests by isolating them in small tubes. To do this, adults were scattered from rearing tubes on a white paper and captured by placing test tubes (180 mm in length and 18 mm in diameter), open end down, over them. When the parasites walked up in the tube towards a source of light, their sex could be easily determined under a binocular, and an egg card was placed in the tube (Wührer and Hassan, 1993). After 24 hours, egg cards were removed from the tubes and incubated under controlled conditions. Parasitised eggs, hatched or not, are characterized by a black coloration after 3–4 days of incubation at 24°C.

**Storage experiments**

Newly emerged adults and the prepupae of *T. evanescens* were placed in environmental chamber under a storage temperature of 4°C and 10°C up to 20 days in full darkness, respectively. Following each period of storage, the wasps were transferred to room temperature. The host eggs were counted and equal numbers (50±5) were sprinkled on egg-cards which were placed in tubes along with adult *T. evanescens*. Each test was replicated 5 times. All females were fed with honey, had mated and had no previous contact with host eggs (i.e. were inexperienced). The lid of the glass tube was covered tightly with synthetic cloth to prevent the wasp from escaping. After 24 hours, the wasps were removed from the egg cards which were then incubated under controlled conditions. Parasitisation, adult emergence, and sex ratio were evaluated. Females that died during the experiment were excluded from consideration.

**Releasing experiments**

The potential of the parasitoid *T. evanescens* to control flour moths was evaluated in laboratory tests by releasing different densities of parasitized *E. kuehniella* eggs. Metal boxes (50x50x80 cm) were used in release experiments. Performance of the released wasps was
monitored using egg cards. Parasitized egg cards by *T. evanescens* were placed in the boxes. Egg cards were used on the 0-3 days of trial (day 0 = day of release). Unparasitized host eggs were removed. After 24 h exposure in the boxes, the control cards were returned to the rearing room and held at 24°C for 5 days. Bulk wheat flour was used as rearing media and then 10 female and 10 male adults of *E. kuehniella* and about 1500 parasitized eggs were simultaneously released in a metal box in three times until the host adults died. In the second experiment, small wheat flour bags were placed in the metal boxes together with *E. kuehniella* adults mentioned above. Parasitisation, adult emergence, and sex ratio were recorded.

**Host finding experiments**

For the measurement of host finding, *T. evanescens* females were 1-day-old, mated, fed, and had no prior oviposition experience. They were reared on *E. kuehniella* eggs mentioned above. Egg cards (50±5 eggs per card) were placed in u-shaped metal plates of 100, 140 and 200 cm long at 20 cm intervals. The upper surfaces of the plates were covered by stretch film to prevent the wasps from escaping. Each treatment was replicated two times. Six females were placed at one end of the plate and the tests were ended when all females had died. Host encounters were measured by the number of eggs parasitized.

For testing of *T. evanescens*’ penetration ability in bulk wheat flour, equal numbers of host eggs (100±5) were sprinkled on cards. These egg cards were placed in the bulk wheat flour in depth of 0, 6 and 9 mm in flour in boxes. Each treatment was replicated four times. Ten females were placed in the each box and the wasps were monitored daily until all females had died. Host encounters were measured by the number of eggs parasitized.

**Feeding experiments**

A laboratory study was conducted to determine the effect of different food sources on the parasitisation of *Trichogramma evanescens*. Newly eclosed female wasps were provisioned with honey from one of three sources: in a capillary glass tube (Ø 1 mm x 10 mm in length), in a plastic pipe (Ø 2 mm x 10 mm in length) and a drop of honey on egg card (conventional). For each feeding methods compared different dilutions of honey as food sources (1/3). All tests were done in glass Petri dishes that contained *E. kuehniella* eggs distributed on lightweight cardboard (2.5 by 4 cm). The cardboards were glued with gum Arabica and 150±5 host eggs were sprinkled on it and placed in Petri dishes (Ø 90 mm) together. The honey was filled in capillary glass tube, and the top end of the tube was covered with parafilm. The tube was glued on the egg-card, which was placed vertically. The same procedure was applied to plastic pipes and the bottom end plugged with sponge to allow slow drainage of honey. Honey was dripped onto egg cards with a toothpick to provide honey droplets no larger than 2 mm in diameter. Wasps were monitored daily until all females had died. Each treatment was replicated five times and parasitisation was evaluated.

**Statistical analysis**

Data were subjected to analysis of variance (ANOVA), for determination of differences between means. The data were transformed to square root before statistical analysis was performed, when significant differences occurred, Tukey-HSD was applied for determining least significance difference of means. Normalized data are presented in tables and figures (SPSS, 1999).

**Results and discussion**

**Storage experiments**

When *T. evanescens* stored at 4°C for 7 days, storage had no significant effect on its parasitization, adult, female and male emergence compared with untreated control (Fig. 1), but,
when if stored at 4°C for 11 days, these values showed significant reduction (F=48,208, df=2, P<0.001; F=36,88, df=2, P<0.001; F=11,562, df=2, P<0.001 and F=6,083, df=2, P=0.006, respectively).

![Parasitisation, adult and female emergence ratios of T. evanescens adults stored at 4°C for 7 and 11 days. Means followed by the same letter are not significantly different at 5% level of confidence by an analysis of variance and Tukey-HSD test.](image)

Fig. 1. Parasitisation, adult and female emergence ratios of *T. evanescens* adults stored at 4°C for 7 and 11 days. Means followed by the same letter are not significantly different at 5% level of confidence by an analysis of variance and Tukey-HSD test.

Data obtained from newly emerged *T. evanescens* stored at 10°C for 10 days are given Fig. 2. when *T. evanescens* stored at 10°C for 5 and 10 days, there was no significant effect on its parasitisation, adult, female and male emergence, but adult, female and male emergence were significantly reduced with increasing storage time up to 15 when compared with untreated control (F=394,662, df=4, P< 0.001; F=107,968, df=4, P<0.001; F=10,260, df=4, P<0.001 and F=3,342, df=4, P=0.030, respectively). No parasitisation and adult emergence occurred after 20 days storage.

When the prepupal stage of *T. evanescens* is stored at 10°C for up to 15 days, they parasitized more hosts than the untreated control (Fig. 3), but adult, female and male emergence were not significantly changed with increasing storage time when compared with untreated control. On the other hand, storage of prepupal stage for 20 days was not significantly decreased (F=293,253, df=4, P<0.001; F=31,584, df=4, P<0.001; F=13,308, df=4, P<0.001 and F=4,483, df=4, P=0.010, respectively) and no parasitization and adult emergence occurred after 20 days.

The length of time wasps are held in cold storage conditions seems to be the most important factor influencing their quality. Storage of *T. evanescens* adults at low temperature could be useful for inundative biological control strategies.

**Releasing experiments**

Parasitized *T. evanescens* eggs were simultaneously released together with 10 females and 10 males into the metal boxes. As can be seen Table 1, when *T. evanescens* was released on bagged wheat flour in small sacks and bulk wheat flour, the *E. kuehniella* population was suppressed by 37-60% compared to the untreated control.

Thus, adult *E. kuehniella* populations in the treatment bins were suppressed 50% compared with the controls for both storing methods. This study showed that augmentative parasitoid releases in stored wheat flour greatly decreased the number of moth.
Fig. 2. Parasitisation, adult and female emergence ratios of *T. evanescens* adults stored at 10ºC for up to 20 days. Means followed by the same letter are not significantly different at 5% levels of confidence by an analysis of variance and Tukey-HSD test.

This study showed that augmentative parasitoid releases in stored wheat greatly decreased the damage of moth and in wheat flour.

**Host finding experiments**

The number of hosts attacked did not change with increasing distance from the starting point (Fig. 5). *T. evanescens* females were able to find hosts up to 200 cm horizontal distance from the starting point, which was the longest distance tested. The dispersion pattern of the parasitoid among the hosts was the same regardless of host distance: On the other hand, female and male emergences were significantly changed with increasing distance. The shorter distance (100 cm) has less female emergence, but more male emergence. On the other hand, adult emergence not changed regarding host distance from start point.

The vertical dispersion pattern of the parasitoid in bulk wheat flour was interesting and the wasp penetrated up to 9 mm (Fig. 6). The parasitisation ratios at 3 and 6 mm in depth were 63.64% and 76.10% compared to untreated control.
Table 1. *E. kuehniella* density in boxes in which the parasitoid *T. evanescens* was released on bagged wheat flour and bulk wheat flour.

<table>
<thead>
<tr>
<th>Release</th>
<th>Parasitized egg</th>
<th>Eclosion holes</th>
<th>E. kuehniella adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Bulk wheat</td>
<td>Control</td>
<td>-</td>
<td>161</td>
</tr>
<tr>
<td>1</td>
<td>1852</td>
<td>1741</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>1674</td>
<td>1569</td>
<td>65</td>
</tr>
<tr>
<td>Bagged flour</td>
<td>Control</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td>1</td>
<td>4262</td>
<td>3310</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>4488</td>
<td>3576</td>
<td>40</td>
</tr>
</tbody>
</table>

Fig. 4. Suppression of *E. kuehniella* population when *T. evanescens* was released on bagged wheat flour and bulk wheat.

Fig. 5. Host finding of *T. evanescens* placed in u-shaped metal plates in different lengths (100, 140 and 200 cm).
**Feeding experiments**

*T. evanescens* females parasitized more host eggs when honey was offered in glass micropipette than conventional (a small smear of honey was applied on the eggs cards) and in rubber pipe ($F = 16.525$, df=2 $P < 0.01$) (Fig. 7). *T. evanescens* provided with honey in glass micropipette exhibited greater parasitisation than those provided both in plastic pipe and conventional methods. On the other hand the parasitisation of *T. evanescens* provisioned both with in plastic pipe and conventional was approximately equal. Our results show that provisioning *T. evanescens* with honey in glass micropipette caused greater parasitisation than the other methods.

According to literature the availability of honey as an adult food source dramatically increases the longevity of *Trichogramma* species. Supplementary foods can increase the longevity of *T. platneri* by as much as 11 fold, but they must be accessible on a daily basis to the searching parasitoids (Hagen, 1986; Waage et al., 1985).

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**Fig. 6.** Penetration of *T. evanescens* placed in bulk wheat flour in different depths (0, 6 and 9 mm).

**Fig. 7.** Parasitisation of *T. evanescens* when honey was offered in glass micropipette, plastic pipe, and conventional methods. Means followed by the same letter are not significantly different at the 5% levels of confidence by an analysis of variance and Tukey THS multiple range test.
Conclusions

− *T. evanescens* adult can be stored for 10 days.
− Prepupal stage of *T. evanescens* stored up to 15 days parasitized as much as untreated control
− *E. kuehniella* population was suppressed by 50% compared to untreated control.
− *T. evanescens* females were able to find hosts up to 200 cm horizontal distance from the start.
− The vertical dispersion of the parasitoid was up to 9 mm in bulk wheat flour.
− Females *T. evanescens* parasitized a significantly higher number of hosts when honey was offered in glass micropipette

References


Factors affecting the attachment of conidia of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) to different body parts of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) adults

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Abstract: Laboratory experiments were carried out to assess the effect of temperature and relative humidity (r.h.) on the attachment of conidia of the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) to the elytra, femur, tibia, tarsus, sternite VI and sternite VII of adult *T. confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). In addition, the attachment was measured on different grain commodities treated with *M. anisopliae* dry conidia. The commodities tested were barley, maize, rice and wheat. In a first treatment, adults of *T. confusum* were exposed on wheat treated with *M. anisopliae* conidia at three temperatures (20, 25 and 30°C) and two r.h. levels, 55 and 75 % r.h.. Dead adults were removed from the treated substrate after 7 days of exposure and stored at 0°C. In a second trial the treated grain was maintained at 25°C and 75 % r.h. for 7 days, stored at 0°C. In both cases the attachment of conidia was evaluated by counting the number of conidia, using a scanning electron microscope (SEM). The tarsus and tibia had the lowest numbers of attached conidia compared to the other parts of the body, for all combinations tested. The highest numbers of attached conidia were found on sternites VI and VII, especially on sternite VI, where significantly more conidia were found at 20 and 25°C and 55 % r.h. Significantly higher numbers of attached conidia were found on the femur at 30°C than at 20°C at both r.h. levels. Wheat had significantly higher numbers of attached conidia compared to those on barley or rice. The present results indicate that conidial attachment of *M. anisopliae* to the cuticule of *T. confusum* adults is affected by temperature, r.h. and commodity.

Key words: *Metarhizium anisopliae*, *Tribolium confusum*, conidia, attachment

Introduction

*Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) is a serious pest of stored products worldwide. It can feed and develop in several foodstuffs and it is an important pest of cereal products (Daniels, 1956; Howe, 1960; Aitken, 1975; Buchelos and Athanassiou, 1993, 1998). Although it develops best on broken grain kernels or processed grain products, particularly wheat flour, this species is also able to infest whole kernels (Aitken, 1975). Previous studies have shown that *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) can be used with success against several stored-product insect species (Batta, 2004, 2005; Kavallieratos et al., 2006; Michalaki et al., 2006). This fungus is a good candidate for evaluation of pathogenicity in laboratory bioassays, as it produces large numbers of conidia, which can easily be harvested and used for mass conidia production.
Michalaki et al. (2006) found that mortality of *T. confusum* larvae after exposure to *M. anisopliae* varied according to temperature and relative humidity (r.h.) levels, and was also dependent on the type of commodity treated. Studies have shown that conidial germination and viability of several species of entomopathogenic fungi is affected by several abiotic factors, such as temperature and r.h. (Hedgecock et al., 1995; Moore et al., 1996; Moore and Higgins, 1997; Hong et al., 1998; Huafeng et al., 1998; Luz and Fargues, 1999). However, very little is known with regard to the factors that affect conidial attachment to the cuticle of stored-product insects, despite the fact that attachment seems to play a key role in the fungal virulence (Lord, 2001; Akbar et al., 2004).

In the present study we examined the effect of temperature and r.h. in different grains on the attachment of conidia of the entomopathogenic fungus *M. anisopliae* to specific areas of cuticle of *T. confusum* adults. Furthermore, we measured attachment of conidia to different grain commodities treated with *M. anisopliae* dry conidia. In addition, we evaluated the potential relationship of *M. anisopliae* conidia attachment to the mortality of *T. confusum* adults under different temperature and r.h. regimes and on different grains.

**Materials and methods**

**Test insect**

*Tribolium confusum* adults were taken from a culture kept on wheat flour plus 5 % brewers yeast (by weight) at 28 ± 1 °C and 65 ± 5 % r.h.. The culture has been kept for more than 5 years at the Laboratory of Agricultural Entomology, Department of Entomology and Agricultural Zoology at the Benaki Phytopathological Institute. All individuals used in the tests were < 2 weeks old.

**Grains**

Untreated, clean wheat (var. Mexa), whole (raw) barley (var. Persephone), paddy rice (Thaibonnet) and maize (Dias), were used in the tests. The moisture content of the four commodities, was determined by a Dickey – John moisture meter (Dickey-John Multigrain CAC II, Dickey-John Co, USA), and they ranged between 10.9 and 11.5 %. Before starting the tests, the grains were left for 7 d at appropriate conditions to equilibrate with the required r.h. levels (see below).

**Fungal formulations**

The *M. anisopliae* isolate used in the tests was strain Meta 1, obtained by Y.A. Batta [Laboratory of Plant Protection, Department of Plant Production and Protection, Faculty of Agriculture, An-Najah National University, P.O. Box 425 (Tulkarm), West Bank, Palestine, Via Israel]. This isolate was first obtained from an infected individual of *Harpalus caliginosus* (F.) (Coleoptera: Carabidae) (Batta, 2003). The fungus was subcultured on plates with oat meal agar (O3506, Sigma, Munich, Germany) for mass production of the fungal conidia. During conidial production, plates were incubated at 20 ± 1 °C and 16 h illumination per day. After 14 days of incubation, the fungal conidia were collected by scraping the conidial layers formed on the plate surface using a sterilized scalpel. The conidia were added to 100 ml sterile distilled water, stirred and filtered through muslin.

A formulation containing fungal conidia and a dust carrier (1:4 o w/w) was prepared according to Batta (2004) and Kavallieratos et al. (2006). Harvested conidia were thoroughly mixed with the carrier in screw-capped bottles. The concentration of fungal conidia in the conidial suspension was determined using a haemocytometer (Precicolor, HBG, Giessen-Luetzellinden, Germany). A single concentration of the formulations was used containing 8 x 10^{10} conidia/g (Michalaki et al., 2006; Kavallieratos et al., 2006).
Grain treatment
For the treatment of each commodity, batches of 1 kg were prepared. The fungal formulation was applied at the rate of 1 g/kg of product, corresponding to $8 \times 10^{10}$ conidia/kg of grain. Each batch was placed in a glass jar (15 cm in diameter, 35 cm in height) and shaken manually for approx. 5 min to achieve an equal distribution of the dust on the entire grain mass. For each grain, there was an additional batch which was untreated and served as a control.

SEM counts
For the evaluation of attachment of conidia to different body parts of *T. confusum*, 30 adults were exposed on 30 g of wheat treated with *M. anisopliae* conidia. Assessments were made at three temperatures (20, 25 and 30°C) and two r.h. levels, 55 and 75 %. Dead adults were removed from the treated substrate after 7 days of exposure and stored at 0°C. Thirty six specimens (6 for each temperature-r.h. combination) were sputter coated with gold and examined using a Philips XL-20 Scanning Electron Microscope at a x 1800 magnification. Three of the six specimens for each combination were placed ventrally and three dorsally on aluminium stubs in order to count the conidia on the elytra (Fig. 1), the femur and tibia (Fig. 2), the tarsus (Fig. 3), sternite VI (Fig. 4) and sternite VII (Fig. 4). Thus, the counts were based on 3 specimens from each combination. For the evaluation of the attachment of conidia to different grains, the treated grains were left at 25°C and 75 % r.h. for 7 days and were then stored at 0°C. Twenty seeds of each treated commodity were sputter coated with gold and examined as above.

Fig. 1. Part of the elytra of *T. confusum* after 7 days of exposure on wheat treated with $8 \times 10^{10}$ conidia of *M. anisopliae* /kg of grain at 25 °C and 55 % r.h.

Bioassays
Four samples of 30 g each, were taken from the treated wheat. Each sample was placed in a small cylindrical glass vial to which 30 *T. confusum* adults were introduced. Each vial (7.5 cm diameter and 12.5 cm height), was enclosed apart from a hole 1.5 cm in diameter at the top and covered with muslin to provide sufficient ventilation. Dead adults were counted after 7 d of exposure. The tests were conducted at 25°C and 55 % r.h. Furthermore, for the other
commodities from each grain-temperature-r.h. combination, four samples of 30 g each were taken. Each sample was placed in a small cylindrical glass vial where 30 T. confusum adults were introduced, as above. Dead adults were counted after 7 d of exposure. The tests were conducted at three temperatures, 20, 25 and 30°C and two r.h. levels, 55 and 75 %. Each experiment was repeated three times (4x3 vials for each combination). The desired r.h. levels were obtained by using saturated salt solutions as recommended by Greenspan (1977).

Fig. 2. Tibia of T. confusum after 7 days of exposure on wheat treated with 8 x 10¹⁰ conidia of M. anisopliae /kg of grain at 25°C and 55 % r.h.

Fig. 3. Tarsus of T. confusum after 7 days of exposure in wheat treated with 8 x 10¹⁰ conidia of M. anisopliae conidia /kg of grain at 25°C and 55 % r.h.
Data analysis
The data for the attachment of conidia to different body parts were analyzed using the GLM procedure of SAS (SAS Institute, 1995) with the number of conidia as the response variable and the part of the body, temperature and r.h. as main effects. For the mortality experiments, the data were analyzed as above, using the GLM analysis of variance, with the insect mortality as the response variable and the temperature and r.h. as main effects. The control mortality was corrected as recommended by Abbott (1925), but in most cases, control mortality was low (<5 %). For the attachment of conidia to grains, the data were analyzes using GLM analysis of variance. The relatively conservative Tukey HSD test was chosen for the post-hoc comparison,

Fig. 4. Sternites VI, VII of *T. confusum* after 7 days of exposure in wheat treated with $8 \times 10^{10}$ conidia of *M. anisopliae* /kg of grain at 25°C and 55 % r.h.

Results

Attachment of *M. anisopliae* conidia to different body parts
The majority of main effects and associated interactions were significant at $P < 0.01$ (Table 1). The tarsus and tibia had the lowest numbers of attached conidia, compared to the other parts of the body, in all combinations tested. The highest numbers of attached conidia were found on sternites VI and VII, especially on sternite VI, where significantly more conidia were found at 20, 25°C and 55 % r.h. (Table 2). Significantly higher numbers of attached conidia were found on the femur at 30°C than at 20°C at both r.h. levels.

Attachment of *M. anisopliae* conidia in relation to commodity
Significant differences were noted in attachment of conidia to wheat, barley, paddy rice and maize ($F = 8.0, df = 3, 396; P < 0.01$) (Fig. 5). A significantly higher number of attached conidia was observed on wheat compared to barley or rice (Fig. 5).

Mortality of *T. confusum* adults
The main effect and associated interaction were significant at $P < 0.01$ (Table 3). On wheat treated with conidia of *M. anisopliae* significant differences in the mortality of *T. confusum*
were noted among the three temperatures at 55 % r.h. \( (F = 60.9, df = 2, 33; P < 0.01) \) and 75 % r.h. \( (F = 23.4, df = 2, 33; P < 0.01) \) (Fig. 6A, B). Thus, at 55 % r.h. significantly more adults were dead after 7 days of exposure at 25°C than at 20 or 30°C (Fig. 6A). In contrast, at 75 % r.h. mortality increased with temperature. At 30°C significantly more adults were dead than 20 or 25°C (Fig. 6B). Comparison of the different grains treated with conidia of *M. anisopliae* showed that significantly more adults were dead on wheat than on barley, paddy rice or maize \( (F = 29.1, df = 3, 46; P < 0.01) \) (Fig. 7).

Table 1. ANOVA parameters for conidia attachment on *T. confusum* adults (df total = 215).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part of body</td>
<td>5</td>
<td>51.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temperature</td>
<td>2</td>
<td>9.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>r.h.</td>
<td>1</td>
<td>1.2</td>
<td>0.27</td>
</tr>
<tr>
<td>x Temperature</td>
<td>10</td>
<td>410.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body part x r.h.</td>
<td>5</td>
<td>415.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temperature x r.h.</td>
<td>2</td>
<td>115.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 2. Mean number (±SE) of attached conidia on different body parts of *T. confusum* adults exposed for 7 d on wheat treated with 8 x 10¹⁰ conidia of *M. anisopliae* /kg of wheat at three temperatures and two r.h. levels (within each temperature, means followed by the same lower case letter are not significantly different, in all cases df = 5, 30; within each body part means followed by the same upper case letter are not significantly different; elytra, sternite VI, sternite VII df = 2, 6, femur, tibia, tarsus, df = 2, 24, Tukey-Kramer HSD test at \( P = 0.05 \)).

<table>
<thead>
<tr>
<th>r.h.</th>
<th>55 %</th>
<th>75 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>20°C</td>
<td>25°C</td>
</tr>
<tr>
<td>Body part</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Elytra</td>
<td>3.6±1.5bA</td>
<td>3.7±0.3bcdA</td>
</tr>
<tr>
<td>Femur</td>
<td>7.0±1.2bB</td>
<td>4.8±0.7bB</td>
</tr>
<tr>
<td>Tibia</td>
<td>2.7±0.8bA</td>
<td>1.9±0.6cdA</td>
</tr>
<tr>
<td>Tarsus</td>
<td>1.2±0.4bA</td>
<td>1.2±0.5dA</td>
</tr>
<tr>
<td>Sternite VI</td>
<td>21.3±7.4aA</td>
<td>15.0±1.2aA</td>
</tr>
<tr>
<td>Sternite VII</td>
<td>7.3±2.9bA</td>
<td>5.0±0.0bcA</td>
</tr>
<tr>
<td>F</td>
<td>11.1</td>
<td>31.9</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01 &lt;0.01 &lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. ANOVA parameters for mortality levels of *T. confusum* adults (df total = 71).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>2</td>
<td>51.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>r.h.</td>
<td>1</td>
<td>106.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temperature x r.h.</td>
<td>2</td>
<td>52.3</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Discussion**
The conidia of entomopathogenic fungi adhere to the cuticle, and germinate, before penetrating through the cuticle, eventually leading to the death of the exposed insects. Lord (2001) first reported that conidia of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) can be used with success against stored-product insect pests in conjunction with desiccant materials, since both substances act on the cuticle. However, the author did not find a significant increase in the number of conidia that attached to the cuticle when desiccant dusts were present. Moore et al. (1996) found that the conidial viability of *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycotina: Hyphomycetes) was affected by temperature and that high temperatures had an adverse effect on viability. Lord (2005) and Athanassiou and Steenberg (2007) also found that *B. bassiana* was more effective at moderate temperatures, against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), respectively.

![Barley Maize Rice Wheat Commodity Mean number of attached conidia](image)

**Fig. 5.** Mean number (+SE) of attached *M. anisopliae* conidia on four types of grain treated with 8 x $10^{10}$ conidia of *M. anisopliae* /kg of grain after 7 d of exposure at 25 °C and 55 % r.h. (df = 3, 396; means followed by the same letter are not significantly different, Tukey-Kramer HSD test at $P = 0.05$).

In light of the present findings, *M. anisopliae* conidia have different degrees of attachment to different body parts of *T. confusum* adults. Although, the sternites had the highest conidial number, conidia were found on all the body parts examined, indicating that the 7 d interval was sufficient for a satisfactory contact of the conidia with the insect in the treated substrate. The highest number of conidia attached to the sternites can be partially attributed to the larger area that these parts cover. However, other wide areas were also examined, such as the elytra, with significantly lower attachment. We assume that the structure of a given area may be responsible for these variations; for instance, elytra are thicker than sternites. On the other hand, the attached conidia to the tarsi and the other leg parts may be partially removed through walking, a factor that may have a smaller effect for sternites. Nevertheless, the results might have been different if other species had been examined, since a different mobility behaviour would have been involved.
Fig. 6. Mean mortality (+SE) of *T. confusum* adults after 7 d of exposure on wheat treated with $8 \times 10^{10}$ conidia of *M. anisopliae* /kg of grain at 3 temperatures and: A: 55 % r.h., B: 75 % r.h. (means followed by the same letter are not significantly different).

Fig. 7. Mean mortality (+SE) of *T. confusum* adults after 7 d of exposure on four types of grain treated with $8 \times 10^{10}$ conidia of *M. anisopliae* /kg of grain at 25 °C and 55 % r.h. (means followed by the same letter are not significantly different).

It is well established that the effectiveness of several entomopathogenic fungi is affected by temperature and moisture/humidity. Lord (2005) found that *B. bassiana* was more effective at 55 than at 75 % r.h. against *R. dominica*. Our results for *M. anisopliae* and *T. confusum* support this observation. Also, Michalaki et al. (2006) found that the same *M. anisopliae* isolate as the one used in the current study was generally more effective at 25 °C than at 20 or 30 °C against *T. confusum* larvae. In the present study temperature and r.h. affected attachment, but there was no consistent trend, indicating that other factors may contribute to these variations. Moreover, it was evident that under the same temperature and r.h. regimes, a good correlation was not obtained between *T. confusum* mortality and the level of attachment. It would appear that insect mortality is not a direct consequence of the level of attachment, and other factors, such as the germination and penetration of conidia into the haemocel may contribute to a larger degree.

One other parameter not examined in detail to date, seems to play an important role that is the influence of commodity. Conidia would also be expected to be attached to the grain kernels. Hence, the conidial retention ability of the target substrate may have a direct effect on
insect mortality. Kavallieratos et al. (2005) found that the degree of retention of diatomaceous earth (DE) particles varied among eight different grains, and this had an effect on mortality of *R. dominica* adults. Based on the present measurements, there were more attached conidia in wheat than in barley or rice. Again, no direct relation between attachment and adult mortality was noted, despite the fact that more adults were dead on wheat, since mortality was also high on rice.

Microbiological control in stored product commodities can be a safe alternative to traditional pesticides, but many issues need to be further evaluated before wider applications. In the case of fungi, their mode of action has not been examined in detail, and additional experimentation is needed on this aspect. The current study on the investigation of the cuticular attachment was a first step towards this effort in understanding the virulence mechanisms of entomopathogenic fungi against stored-product insect species.

**Acknowledgments**

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Efficacy of submultiples doses of *Bacillus thuringiensis* compounds against the Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae)

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**Abstract:** The efficacy of submultiples doses of some *Bacillus thuringiensis* compounds against the Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) was investigated in the laboratory. The products that have been tested were: Agree WP (*B. thuringiensis* subsp. *kurstaki* / subsp. *aizawai*, Thuricide WP (*B.t. subsp. kurstaki*), Xentari WG (*B.t. subsp. aizawai*) and BMP 123 WP (*B.t. encapsulated d-entotoxin*). Six trials (and six repetitions in each trial) of each product were carried out in laboratory conditions (temperature: 26±1°C, relative humidity: 60±2% and photoperiod: 16h light / 8h dark). Six doses (recommended, 1/2, 1/4, 1/8, 1/16 and 1/32 of the recommended dose) of each compound were mixed with the diet of the pest. The experiment was conducted with second instar larvae. Three days after the treatment, the mortality of *E. kuehniella* larvae in the recommended dose for Agree, Thuricide, Xentari and BMP was 97, 72, 34 and 42% respectively, and seven days after 100, 99, 79 and 82 % respectively. In the lowest dose, three days after the treatment the mortality was 33, 21, 14 and 8% and seven days after 54, 32, 19 and 12%, respectively.

**Key words:** *Bacillus thuringiensis, Ephestia kuehniella*, encapsulated d-entotoxin, larvae mortality
Enterococcus mundtii, a pathogenic bacterium to Ephestia kuehniella Zeller

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Abstract: Ephestia kuehniella Zeller is a worldwide pest, particularly of stored grain, fruit and nuts. The insect is responsible for not only direct pest of stored products but also allergy for some people. In the present study, we isolated a nonspore-forming, pathogenic bacterium from morbid larvae of E. kuehniella. This bacterium was characterized as Enterococcus mundtii by Microbial Identification and Biolog Systems. Laboratory experiments carried out to determine the insecticidal activity showed that the infectivity of this bacterium reached to 98% on third instar larvae of E. kuehniella on sixth day of the experiment. The larvae infected with the bacterium showed lack of appetite, diarrhea and failed to coordinate movement. It is first recorded non-spore forming bacteria showing such a pathogenicity to E. kuehniella.

Key words: Ephestia kuehniella, Enterococcus mundtii, pathogenic bacterium
Effect of gamma radiation and cold storage on emergence and life time adults *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) parasitizing larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae).

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**Abstract:** Laboratory studies were conducted on the effect of gamma radiation and cold storage on emergence and life time of adults of *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) parasitizing larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Adults of *V. canescens* emerged from *E. kuehniella* and irradiated with a dose of 0.2 or 0.4 kGy were as fertile as the adults that emerged from untreated hosts (control). Body mass of parasitoid adults emerged from moth larvae irradiated with a dose of 0.2 or 0.4 kGy of gamma radiation was similar to that of the adult wasps emerged from non-irradiated larvae. Storage period of irradiated and parasitized moth larvae at low temperatures (5°C) did not inhibit the development of parasitoids, but increased their mortality rates, when they were exposed to ambient temperature (25°C). Fertility of emerged adults of *V. canescens* was not affected by the period of storage at a low temperature. Longevity of parasitoid adults that emerged from 0.2 or 0.4 kGy irradiated host larvae was similar to those parasitoids that emerged from untreated hosts. These finding indicate that *E. kuehniella* larvae irradiated with low gamma radiation doses may be used for the mass-culture of *V. canescens* parasitoids.

**Key words:** *Venturia canescens*, parasitoid, *Ephestia kuehniella*, gamma radiation, mass culture, quality control, cold storage.

**Introduction**

Most of parasitoids attacking stored-product insect pests are from the order Hymenoptera. One of them, *Venturia canescens* (Gravenhorst), is a cosmopolitan, solitary wasp (ichneumonid endoparasitoid, thelytokous strain), usually encountered in flour mills and flour stores and grain products (Carlson 1979). It attacks final instar larvae of *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Ephestia* species, and the tineid moth, *Nemapogon granella* (L.) (*V. canescens* is koinobiont endoparasitoid). By artificially increasing their density, repeated releases of mass-reared natural enemies can reduce the pest population below the economic injury level.

Biological control of stored-product pests requires the development of economically efficient techniques for mass-rearing, storage, transportation, and application to their natural enemies. The use of nuclear techniques is seen as a possible way around some of these problems. However, little work has been devoted to this area of research (Roth et al. 1991, Morgan et al. 1986).

Larvae of the Mediterranean flour moth, *Ephestia kuehniella* (Zeller), are suitable hosts for *V. canescens*. Thus, *V. canescens* could be effectively used for the population suppression of stored product moths. Irradiation of lepidopteran larvae with low doses prevents their
further development. Host larvae irradiated with doses from 0.1 kGy to 0.5 kGy remain alive for several weeks (Celmer-Warda 2004) if the irradiated larvae could be stored for several weeks without a decline in their suitability as hosts to *V. canescens*. Irradiated host larvae, parasitized by *V. canescens* stored-product moths could be a final product for sale. Moreover, exploration for parasitoids in flour mills and stores of flour and grain products could be carried out easier since the irradiation treatment may be applied to prevent eclosion of fertile adult pests without decreasing suitability of these larvae as hosts for the parasitoids. Irradiated pest larvae may be exposed at surveyed commodity stores without releasing the pest.

The main objective of the present study was to elaborate the utility of the irradiation techniques in order to improve the production and release of parasitoids in high quality. The aim of releasing mass-produced natural enemies is to control a pest. In this context, quality control should determine whether the natural enemy is still in condition to properly control the pest. The present study compares parameters that are relatively easy to determine in the laboratory (emergence, longevity, fecundity adult size and oviposition behaviour of wasps). In addition, the suitability of irradiated larvae of the Mediterranean flour moth, *E. kuehniella*, as host for *V. canescens* parasitoids was determined.

**Materials and methods**

**Test insects**

The Mediterranean flour moths used in the experiments were obtained from laboratory colonies maintained in darkness at 27±1°C and 70±5% R.H. Moth larvae were kept in a mixture consisting of wheat flour (1 kg), yeast (5g) and wheat germs (30g). *V. canescens* (thelytokous strain that produces only females) were obtained from laboratory colonies and reared on *E. kuehniella* larvae as host.

**Acceptance and suitability of irradiated hosts.**

Groups of last instar larvae of *E. kuehniella* (the “wandering” phase) were selected and placed into jars (100 larvae per jar) together with some food, and irradiated with a dose of 0.2 or 0.4 kGy of gamma radiation at a dose rate ca. 30 Gy/min. The absorbed dose was measured using a Fricke dosimeter. After the irradiation treatment, moth larvae (100 larvae per treatment, in 8 replications) were placed into little sacks (5 x 5cm) made from gauze (25 larvae per sack) and provided with food (wheat germs). These sacks were placed into a glass cabin (25 x 25 x 30cm) containing one-day old parasitoids (about 50 *V. canescens* wasps). After 24 hours, the larvae were removed from the parasitoids’ cabin, released from the sacks, transferred into 130 ml dishes with food (dried wheat germs) and were kept in a rearing room controlat 27±1°C and 70±5% R.H. Parasitoid emergence was then determined.

**Storage experiments**

Groups of last instar larvae of *E. kuehniella* (the “wandering” phase) were selected, placed into jars (100 larvae per jar) with food, and irradiated with a dose of 0.2 or 0.4 kGy of gamma radiation at a dose rate ca. 30 Gy/min. The absorbed dose was measured using a Fricke dosimeter. After the irradiation treatment, moth larvae (100 larvae per treatment, in 8 replications) were placed inside little sacks (5 x 5cm) made from gauze (25 larvae per sack) and provided with food (wheat germs). These sacks were placed into a glass cabin (25 x 25 x 30cm) with one-day old parasitoids (about 50 *V. canescens* wasps). After 24 hours, the larvae were removed from the parasitoids’ cabin, released from the sacks, transferred into 130 ml dishes with food (dried wheat germs) and were kept in a rearing room controlat 27±1°C and 70±5% R.H. for 2, 4 and 6 weeks. After this period the larvae were kept in a rearing room control at 27±1°C and 70±5% R.H. Parasitoid emergence was then determined.
**Body mass**
One-day old adults wasps which emerged from moth larvae that were stored and without storage at a low temperature for 2 and 4 weeks, were killed using CO₂, and then weighed using a sensitive balance (Sartorius Supermicro, d = 0.0001 mg). One hundred parasitoids were weighed for each treatment (0.2, 0.4 kGy and control).

**Longevity**
Cohorts of twenty one-day adults wasps which emerged from moth larvae that were stored and without storage at a low temperature for 2 and 4 weeks, were placed into a glass cabin (25 x 25 x 30cm) with honey as food source. The number of dead parasitoids was recorded daily until all of the parasitoids in the cage had died.

**Oviposition behaviour of wasps from irradiated/cold stored hosts**
Samples of 10 wasp adults which emerged from irradiated and non irradiated host larvae (after 2 and 4 weeks in cold storage and without storage) were placed into glass cabins provided with gauze sacks containing ca. 100 larvae of *E. kuehniella* each. The oviposition time and number of ovipositing wasps were observed, recorded and compared to the control group.

**Fecundity of *V. canescens***
Adult parasitoids that emerged from irradiated and non irradiated host (stored 2, 4 weeks in a cold storage and without storage) were introduced in to glass box (about 50 wasps per treatment) with non-irradiated moth larvae (100 larvae per treatment). After 24 hours the larvae were removed from the cabin, released from the sacks and transferred into 130 ml dishes with food (dried wheat germs), and kept at a temperature of 25±1°C. Fecundity of adult parasitoids that emerged from irradiated and cool stored in in the cold larvae was compared to the control.

**Statistical analysis**
Irradiated for which host larvae and ,were compared by Kruskall-Wallis test. Data collected for the number of *V. canescens* adults which emerged from irradiated larvae and offspring of *V. canescens* that were obtained from irradiated larvae, were analyzed using a two factor analysis of variance (ANOVA, with dose of radiation and storage time, as sources of variation (SPSS 10.). All data were transformed to square root before statistical analysis was performed. When significant differences occurred the Tukey-HSD test was applied as a means of separation.

**Results**

**Acceptance and suitability of irradiated hosts**
Adults of *Venturia canescens* more often parasitized irradiated larvae of the Mediterranean flour moth than the control larvae. The lowest rate of parasitism was noted for the control larvae, indicating the preference of the parasitoid for irradiated larvae. Adult emergence of *V. canescens* were affected by dose of irradiation (F=117,33; df=3; P<0,05). The number of adult emergence of *V. canescens* obtained from larvae irradiated with dose 0,2 kGy was significantly higher then in other groups (Fig. 1).

**Storage experiments**
When the host larvae wereand untreated control had irradiated with gamma radiation and were stored at low temperature (5°C), adult emergence of *V. canescens* were affected by storage period (F=459.608, df=3, P<0,001) (Table 1). The two-way ANOVA analysis showed that adult emergence of the wasp was decreased with increased increase in the storage period.
The highest mean adult emergence was recorded at 0 week storage, followed in decreasing order, by 2, 4, and 6 weeks storage. According to the results adult emergence of *V. canescens* was also influenced by gamma radiation doses (\( F=32,174 \) df=2 \( P<0.001 \)) (Fig. 1). There was interaction between gamma radiation doses and storage period regarding adult emergence (\( F=4.752, \) df=6, \( P<0.001 \)) (Table 1). The number of adult emergence of *V. canescens* obtained from non stored larvae was significantly higher than the stored larvae. Only a few parasitoids completed their development following 6 weeks of cold storage.

![Fig. 1. Mean (±SD) number *V. canescens* adults emerged from moth larvae irradiated with a dose of 0.2 kGy or 0.4 kGy of gamma radiation and untreated (control) and then cool stored for 0, 2, 4, and 6 weeks.](image)

**Table 1. Two-way ANOVA results comparing the effects of each radiation dose and storage time treatments for adult emergence from cold stored host larvae after irradiation**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>df</th>
<th>Mean square (treatment)</th>
<th>Mean square (error)</th>
<th>( F )</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>2.84</td>
<td>16,348</td>
<td>8,174</td>
<td>32,174</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Storage</td>
<td>3.84</td>
<td>350,297</td>
<td>116,766</td>
<td>459,60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Storage x Dose</td>
<td>6.84</td>
<td>7,244</td>
<td>1,207</td>
<td>4,752</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Body mass**

Body mass of *V. canescens* adults which emerged from host larvae irradiated with a dose of 0.2 or 0.4 kGy was very similar (0.2 kGy =3.3854 mg, SE= 0.3421; 0.4 kGy=3.3862 mg, SE= 0.3423) to that of the adult wasps emerged from non-irradiated larvae (3.4358 mg, SE= 0.3472).

With the increase of the period of storage at low temperature, body mass of tested parasitoids decreased. After 2 weeks of storage body mass of emerged adults were similar to non storage (0 kGy (control)=3,3594 mg, SE=0,3477; 0,2 kGy= 3,3158, SE=0,3266; 0,4kGy= 3,2976, SE=0,3421). After 4 weeks of storage body mass decreased significantlygreatly (0 kGy (control)=3,3063 mg, SE=0,3843; 0,2 kGy= 3,3286, SE=0,3539; 0,4kGy= 3,2611, SE=0,3438).
**Longevity**

Longevity of *V. canescens* adults which emerged from host larvae irradiated with a dose of 0.2 or 0.4 kGy was similar to that of the adults that emerged from untreated hosts (control). Time of mortality did not differ between treatments. In all treatments, parasitoid adults were dead on the 40th day after their emergence from host larvae. However, mortality rates of parasitoid adults that emerged from non-irradiated larvae were relatively lower than those which developed in irradiated larvae (Fig. 2).

![Graph showing longevity of V. canescens adults](image)

**Fig. 2.** Longevity of *V. canescens* adults that emerged from irradiated and non-irradiated (control) larvae of the Mediterranean flour moth, *E. kuehniella*.

With the increase of the period of storage at low temperature longevity of tested parasitoids decreased. Adults of *V. canescens* which emerged from larvae stored 2 weeks were dead on the 35th day after their emergence from host larvae. After 20 days, the number of parasitoids decreased by 50% (Fig. 3).

After 4 weeks of storage longevity of parasitoids decrease more then after 2 weeks of storage. Mortality on the order of 50% was observed after 15 days from the start of the experiment. Adult parasitoids were dead after 30 days in all treatments (Fig. 4).

**Oviposition behaviour of wasps from irradiated/cold stored hosts**

It was observed that *V. canescens* females, that emerged from 0.2 kGy or 0.4 kGy irradiated larvae and then cold stored for 2 and 4 weeks, were attracted by host larvae, and parasitoids of all combination treatments readily oviposited (Table 2).

With the increase of the period of storage at low temperature reactions of tested parasitoids decreased. After 2 weeks of storage parasitoids found hosting at the same time as non storage. But after 4 weeks, this time was prolonged significantly (Table 2).

**Fecundity of V. canescens**

Data obtained for offspring from cold stored host larvae after irradiation are given in Figure 3. Results indicated that storage period significantly affected the adult emergence of offspring. The two way ANOVA analysis showed that in all treatments adult emergence from offspring was decreased with increased period of increase in the storage (F=358,290 df=3 P<0.001) (Fig. 5). The highest mean adult emergence was recorded at 0 week storage, followed in
gradually decreasing order, by 2, 4, and 6 weeks storage. The adult emergence of *V. canescens* offspring was also influenced by gamma radiation doses ($F=551.402$, $df=2$, $P<0.001$). The number of progeny of wasps which emerged from larvae irradiated with 0.4 kGy and stored at a low temperature was comparatively less than both the irradiated with 0.2 kGy and the untreated control. There was an interaction between gamma radiation doses and storage period regarding adult emergence of offspring ($F=101.146$, $df=6$, $P<0.001$) (Table 3). The number of adult emergence of *V. canescens* obtained from non stored larvae was significantly higher than the stored larvae, in all treatments.

![Graph showing longevity of V. canescens adults](image)

**Fig. 3.** Longevity of *V. canescens* adults that emerged from irradiated and non-irradiated (control) larvae of the Mediterranean flour moth, *E. kuehniella* (after 2 weeks of storage at low temperature).

**Discussion**

Biological control in commodity storage has some unique advantages. Release of the biological agents (e.g., parasitoids) within storage structures where they are protected from vagaries of the weather has a big advantage. Biological agents leave no harmful chemical residues on the commodities. Most are harmless to humans and can be applied by relatively unskilled workers. An additional long-term advantage is that stored-product pests are not known to develop resistance to their parasites. Biological control agents for storage pests usually are small to very small and therefore inconspicuous, have short life cycles, and have high reproductive potentials. They usually respond in a density-dependent fashion to host abundance, and populations can be self-perpetuating.

Many predators and parasitoids can be cold stored for only a short time. It is necessary, therefore, to have good storage methods suitable for planning mass-production and because of the difficulty of accurately predicting demand from clients (van Lenteren 2003).

Thus, the aim of this study was to assess suitability and acceptability of irradiated host larvae of stored product moths by a parasitoid, *V. canescens*. The following criteria for quality control of mass-reared parasitoids were adopted: (a) fertility of *V. canescens* adults which emerge from irradiated moth larvae; (b) longevity of *V. canescens* adults which emerge from irradiated moth larvae; and (c) weight (in mg) of *V. canescens* adults which emerge from irradiated moth larvae as compared to adults developed in non-irradiated host larvae.
Fig. 4. Longevity of *V. canescens* adults that emerged from irradiated and non-irradiated (control) larvae of the Mediterranean flour moth, *E. kuehniella* (after 4 weeks of storage at low temperature).

Fig. 5. Mean number of offspring of *V. canescens* adults emerged from moth larvae irradiated with a dose of 0.2 kGy or 0.4 kGy of gamma radiation and cool stored for 2, 4, and 6 weeks as compared to the control (wasps emerged from non-irradiated hosts).

No significant differences were found between the parasitization rates and parasitoid emergence between the control and irradiated host larvae, but there was a difference in the number of parasitoids which emerged from moth larvae stored in cold temperature for 4 and 6 weeks. The highest mean adult emergence was recorded at 0 week storage, followed in decreasing order, by 2, 4, and 6 weeks storage.

Few standard methods have been developed for quality control tests, and even fewer have been accepted and generally put into use for the popular *Trichogramma* species, reared on a large scale in different areas of the word (Laing & Bigler 1991). Storage of natural enemies assures their availability in sufficient number at the time of release. Therefore, the development of storage techniques for biological control agents is considered of utmost importance to provide flexibility and efficiency in mass production, to synchronize a desired
stage of development for peak release and make available standardized stocks for use in research (Greenberg et al., 1996; Leopold, 1998).

Table 2. Oviposition activity of *V. canescens* parasitoids that emerged from moth larvae irradiated with a dose of 0.2 kGy or 0.4 kGy of gamma radiation and cool stored for 2 and 4 weeks as compared to the control

<table>
<thead>
<tr>
<th>Storage time at low temperature</th>
<th>Dose (kGy)</th>
<th>Number of ovipositing parasitoids</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>after 2 minutes</td>
<td>after 5 minutes</td>
<td>after 10 minutes</td>
<td></td>
</tr>
<tr>
<td>0 weeks</td>
<td>0.0</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>0.0</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>0.0</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Two-way ANOVA results comparing the effects of each radiation dose and storage time treatments for offspring from cold stored host larvae after irradiation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>df</th>
<th>Mean square (treatment)</th>
<th>Mean square (error)</th>
<th>F</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>2,84</td>
<td>26,601</td>
<td>13,301</td>
<td>551,402</td>
<td>&lt;0,001</td>
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<tr>
<td>Storage</td>
<td>3,84</td>
<td>25,928</td>
<td>8,643</td>
<td>358,290</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Storage x Dose</td>
<td>6,84</td>
<td>14,639</td>
<td>2,440</td>
<td>101,146</td>
<td>&lt;0,001</td>
</tr>
</tbody>
</table>

However, none of these methods are known for *V. canescens*, a parasitoid of moth larvae and common pest of stored products. We assumed that quality control test for *V. canescens* may be based upon the fertility, longevity and weight of wasps obtained from host larvae.

Longevity of *V. canescens* adults emerged from irradiated larvae stored 2 weeks in cold place was not affected. The quality of the adults was greatly affected by low temperatures. The rate of survival of adult from stored larvae decreased with prolonged storage and this value is much lower than the IOBC standard 80% (van Lenteren, 1994). Therefore, to improve the rate of survival, the authors recommend the reduction of storage time during production. Results obtained in this study indicate that *V. canescens* adults can be stored for shorter periods without much loss of parasitoid performance. The length of storage time in the cold seems to be the most important factor influencing the quality of reared wasps. Other storage techniques use previous diapause induction (Greenberg et al., 1996). To possibly further improve storage of *V. canescens*, additional research with previous diapause induction should be conducted to ascertain the effect of storage temperatures.

The possibility of storing beneficial insects for extended periods has been studied by several authors. Burgio and Nicoli (1994) were able to store *Diglyphus isaea* in the diapausing stage at low temperature for 2 months, and they found that during this period mortality of predators was not increased, and their fecundity was not affected.

More detailed studies are needed to improve quality of mass-cultured parasitoids from irradiated and cold stored larvae of the Mediterranean meal moth.
Acknowledgement

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References


Session 5:
Phytochemicals
Repellent activity and persistence of the essential oils from *Carum copticum* and *Vitex pseudo-negundo* on *Tribolium castaneum*

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**Abstract:** The protection of stored agricultural products against insects is carried out mostly with chemical insecticides. These insecticides have harmful effects on the environment. The current study showed that plant extracts and essential oils may have potential as safe alternative compounds to currently used insecticides. Essential oils are volatile and can act like fumigants offering the prospect for use in stored-product protection. Some also have the ability to repel insects. The objective of the present study was to test the possible properties of essential oil vapours of the medicinal plants, *Carum copticum* and *Vitex pseudo-negundo* against *Tribolium castaneum* (Herbst), and to elucidate their repellency effect and half-life time. The experiments were conducted at 27 ± 1°C, 60 ± 5% R.H. and in darkness. The essential oils were obtained from seeds of *C. copticum* and dry leaves of *V. pseudo-negundo*, subjected to hydrodistillation using a modified Clevenger-type apparatus. Results showed that *V. pseudo-negundo* causes a greater degree of repellency to *T. castaneum*. The highest concentration (3 µl/ml acetone) of the *C. copticum* and *V. pseudo-negundo* oils cause 87.50 and 100% repellency on adult insects, respectively. The persistence or half-life time of the *C. copticum* (36.24 days) oil was higher than that of *V. pseudo-negundo* (2.98 days). The results demonstrated the efficacy of these two essential oils for use in organic food protection. They can prevent the infestation of the stored-product pests to the warehouses.

**Key words:** *Carum copticum*, *Vitex pseudo-negundo*, *Tribolium castaneum*, persistence, repellency

**Introduction**

Protection of agricultural stored products against insect pests is of utmost importance to secure a continuous and safe supply all over the world. Conventional treatments have been used for this purpose, but nowadays, other ecologically sound methods based on the use of natural compounds are needed for an integrated approach to pest management (Pemonge et al., 1997). Among methods used in integrated pest management, plants and their by products have played a significant role. Higher plants are a rich source of chemicals and novel insecticides (Arnason et al., 1989). A number of plant families are known to produce secondary metabolites having biological activity such as repellents, antifeedants and toxins (Philogene, 1981). The insecticidal activity of a large number of essential oils extracted from aromatic plants was evaluated on the main insect pests affecting stored products (Shakarami et al., 2004; Negahban & Moharramipour, 2007; Negahban et al., 2007a; Sahaf et al., 2007). These essential oils may be more rapidly degraded in the environment than synthetic compounds, and some have increased specificity that favors beneficial insects (Pillmoor et al., 1993).
Insects are important pests of stored seeds and may damage the seeds embryos, causing a decrease in germination. The red flour beetle, *Tribolium castaneum*, causes quantitative and qualitative damage to grain. Adults of *T. castaneum* are attracted by the stored products and oviposit in these products. Larvae feed on the products and damage them. If adults of these beetles can be prevented from entering into the products, the risks of product losses caused by beetles will be reduced. Research shows that insect repellent activity has been found in many plant species (Shakarami *et al.*, 2003; Shakarami *et al.*, 2005; Negahban *et al.*, 2006; Negahban *et al.*, 2007b). In this study, the repellency, toxicity and persistence of *Carum copticum* and *Vitex pseudo-negundo* essential oils were tested against adults of *Tribolium castaneum* in order to find the substances that can be utilized in the control of this pest.

**Material and methods**

**Insect cultures**

*Tribolium castaneum* was reared in plastic containers (20 cm height× 14 cm width× 8 cm height) containing wheat flour mixed with yeast (10:1, w/w). The cultures were maintained in the dark in growth chamber set at 27±1°C and 60±5% RH. Adult insects, 1-7 days old, were used. All experimental procedure was carried out under the same environmental conditions as the cultures.

**Plant materials**

Seeds of *Carum copticum* C. B. Clarke (Apiaceae) were collected in October 2004 from the field of Tarbiat Modares University, Tehran, Iran. Leaves of *Vitex pseudo-negundo* (Haussk) Hand, I. MZT (Verbenaceae) were collected in October 2005 from Sabzevar, Iran. The leaves of *V. pseudo-negundo* were dried naturally on laboratory benches at room temperature (23-27°C) for 6 days until it was crisp. The leaves and seeds were stored at -24°C until they were needed and then hydrodistilled to extract their essential oils.

**Extraction of essential oil**

Essential oils were extracted from the plant samples by hydrodistillation. Conditions of extraction were 20 g of seeds of *C. copticum* or 50 g of leaves *V. pseudo-negundo*; 600 ml distilled water, 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Extracted oil was stored in a refrigerator at 4°C.

**Repellency bioassay**

The experiment was conducted as described by Smith *et al.*, (1994) with some modifications. The repellency bioassay test chambers consisted of two plastic chambers (65 ml volume) joined to either side of a central main chamber of the same size by a small piece of plastic tube (2 cm long and 5 mm diameter). Test solutions were prepared by dissolving 0.2, 0.5, 1 and 3 µl of each essential oil in 1 ml acetone. Each solution was applied on 40 seeds of wheat, as uniformly as possible. In the control, the food was treated with acetone only. The treated and control seeds were air-dried under a fan for 10 min to evaporate the solvent completely, and put into the centre of treated and control chambers. Three replications were used for each concentration. Fifty unsexed adults (1-7 days old) of *T. castaneum* were released at the centre of the main chamber. The main chamber was covered by a lid, and the treated and control chambers were covered by screen and left in darkness. Observations on the number of beetles present in the treated and untreated chambers were recorded after 24 h. Percentage repellency (PR) values were computed using the formula of Talukder and Howse (1993, 1995) as follows: PR= 2(C-50), where C is the percentage of insects on the untreated chamber.

Analysis of variance (ANOVA) was used to determine the effect of essential oil concentrations on repellency. Following a significant ANOVA, differences amongst means were
established using Least Significant Difference (LSD) test at 5% level. Arcsine square-root transformation was performed on percentage repellency, but non-transformed data are presented in table.

**Mortality half-life determination**

The mortality half-life, as opposed to chemical half-life, involves determining the mortality caused by a pesticide over time (Stark & Wennergren, 1995). Determination of chemical half-life involves quantitation of actual residue levels over time. Our approach involved exposing 10 (1-7 days old) adults to glass vial (27 ml) treated with the essential oil at 926 µl per L air. Thereafter, new adults were introduced in vials every 2 days. For each time step (2 days), ten adults were removed after 24 h and the mortality was recorded 48 h later. The mortality half-life data for each experiment were analyzed by the method of Finney (1971), indicating the loss of essential oil activity over time.

**Results**

**Repellency**

The essential oil of *V. pseudo-negundo* caused more repellency on *T. castaneum* than *C. copticum*. At the highest concentration (3 µl) the repellency caused by *V. pseudo-negundo* (100%) was higher than *C. copticum* (87.50%) (Table 1). Also, the repellency increased with concentration in all cases.

<table>
<thead>
<tr>
<th>Concentration of essential oil (µl/ml acetone)</th>
<th>% repellency¹ (Mean±SE)</th>
<th>C. copticum</th>
<th>V. pseudo-negundo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>77.78±0.00ᵇ</td>
<td>90.88±2.11ᵇ</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>81.37±2.65ᵇ</td>
<td>93.55±0.00ᵇ</td>
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</tr>
<tr>
<td>1.0</td>
<td>84.18±1.39ᵇ</td>
<td>94.44±0.00ᵇ</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>87.50±0.00ᵇ</td>
<td>100.00±0.00ᵇ</td>
<td></td>
</tr>
</tbody>
</table>

¹Means in each column followed by the same letters do not differ significantly using Least Significant Difference (LSD) test at P<0.05.

**Half-life time of the essential oils**

The effects of *C. copticum* and *V. pseudo-negundo* oil on mortality of *T. castaneum* over time (mortality half-life) are presented in Fig. 1. The half-life time of *C. copticum* oil was longer than oil from *V. pseudo-negundo* 45 days after application of the *C. copticum* essential oil, no mortality was attained, but for *V. pseudo-negundo* this occurred after 14 days. Persistence or half-life time (LT₅₀) of *C. copticum* oil (36.24 days) was higher than oil from *V. pseudo-negundo* (2.98 days) (Table 2).

**Discussion**

Findings of this study showed that essential oils of *C. copticum* and *V. pseudo-negundo* have potent repellency on *T. castaneum*. So, the key finding of the research carried out is that sub-lethal doses of these essential oils repelled the insects. To the best of our knowledge, no studies have been reported previously concerning the activity of *C. copticum* (Apiaceae) and *V.*
pseudo-negundo (Verbenaceae) as a repellent against T. castaneum. However, Jacobson (1989) pointed out that the most promising botanical insect control agents are in the families of Annonaceae, Asteraceae, Canellaceae, Lamiaceae and Rutaceae.

Fig. 1. Mortality of adult insects, Tribolium castaneum exposed to Carum copticum and Vitex pseudo-negundo essential oils at the initial concentration of 926 µl/l air over time. Exposure to oils was carried out in each time step of 2 days for 24 h.

Table 2. Half life (LT₅₀) of Carum copticum and Vitex pseudo-negundo essential oils against Tribolium castaneum.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>LT₅₀ (Day)</th>
<th>Slope±SE</th>
<th>Intercept±SE</th>
<th>Degree of freedom</th>
<th>Chi-square (χ²)</th>
</tr>
</thead>
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<tr>
<td>C. copticum</td>
<td>36.24 (27.69-61.71)</td>
<td>-1.75±0.43</td>
<td>2.73±0.59</td>
<td>4</td>
<td>1.62</td>
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<tr>
<td>V. pseudo-negundo</td>
<td>2.98 (1.63-4.70)</td>
<td>-1.23±0.33</td>
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</table>

The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility they have fumigant action that might be of great importance for stored product insects (Coats et al., 1991; Regnault-Roger & Hamraoui 1995; Lee et al., 1997; Ahn et al., 1998). So, the repellency of C. copticum and V. pseudo-negundo oils could be attributed to these components.

Results of this study and earlier studies indicate that some plant extract and essential oils might be useful for managing insects in enclosed spaces because of their fumigant action. Commercial success with these product based on well-known chemistry will likely provide an impetus for the development and commercialization of future pesticides based on essential oils with even greater potency (Isman, 2000). In overall, these plant materials confirmed their usefulness as potent insect-control agents.

References


Insecticidal activity of volatile monoterpenoids to *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) and *Cryptolestes pusillus* Schönherr (Coleoptera: Cucujidae)

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IMIDA, Estación Sericícola, c/ Mayor s/n, 30150 La Alberca, Murcia, Spain
*Corresponding author e-mail: mjesus.pascual@carm.es

Abstract: Botanicals have potential to be developed as low risk insecticides, following the consumer demands of safe and quality food. Plant essential oils are a source of volatile monoterpenoids which are being studied in our research group as alternative products to control stored rice pests. Laboratory bioassays were performed in 15 ml volume glass vials at 30°C to assess mortality after 24 h against *Sitophilus oryzae*, *Rhyzopertha dominica* and *Cryptolestes pusillus*. Doses causing 50 % mortality in insects ranged from 1.9 to 196 µg / ml of air volume and the decreasing order of sensitivity for test species was *C. pusillus*, *R. dominica* and *S. oryzae*. The most active products were estragole, camphor, linalool, carvone, eugenol and E-anethole with LC50 within the range 1.9-7.3 µg / ml and with some selectivity depending on the pests. Repellent effects of monoterpenoids were observed for linalool to *C. pusillus*.

Key words: stored rice pests, estragole, camphor, linalool, carvone, eugenol, E-anethole, repellency.

Introduction

Pests cause serious damage to stored grain and grain products and consequently quality losses in these products. The lesser grain borer (*Rhyzopertha dominica* Fabricius) and the rice weevil (*Sitophilus oryzae* L.) were the main damaging pests found in stored rice in Spain although *Cryptolestes pusillus* Schönherr is quite frequent (Pascual-Villalobos and Del Estal, 2004; Pascual-Villalobos et al., 2006).

Using plants to control insect damage in stored products is a common practice in traditional farm storage in developing countries. Recently there has been an interest in plant products as source of insecticides in all around the world, due to the environmental and toxicological side effects of many synthetic insecticides, therefore, new classes of insecticides derived from plants with a lower toxicity on mammals and a lesser persistence in the environment are being examined (Regnault-Roger et al., 1993; Don-Pedro, 1996a).

Many plant secondary metabolites have insecticidal activity against insects and the essential oils extracted from plants have been widely investigated for pest-control properties, including toxic (Don-Pedro, 1996b; Clemente et al., 2003), repellent (Pascual-Villalobos and Ballesta-Acosta, 2003), antifeedant, ovicidal, and other activities (Regnault-Roger and Hamraoui, 1994; Alvarez-Castellanos et al., 2001).

Bekele and Hassanali (2001) reported that the mixture of the main components of the essential oils are as effective as the essential oils, showing clearly that the toxic action of the oils results from the combined action of their major constituents.

Other studies on the toxic and repellent properties of terpenes are published in the literature by García et al., 2005; Pascual-Villalobos et al., 2004; Obeng-Ofori et al., 1998 or Ngoh et al., 1998.
This paper reports the results of the toxic volatile activity and repellent effects of monoterpenoids against *S. oryzae*, *R. dominica* and *C. pusillus*.

**Material and methods**

**Insect rearing**

*S. oryzae*, *R. dominica*, and *C. pusillus*, were maintained at 30° C and 51 % rh, in the dark. The food used was, whole rice grains for *Sitophilus oryzae* and *Rhyzopertha dominica* and wheat flour with 5 % yeast for *Cryptolestes pusillus*.

**Chemicals**

(-)-Linalool, Camphor, γ-Terpinene, p-Cymene, Geraniol, R-(-)-Carvone, S-(+)-Carvone, E-Anethole, (++;)-Limonene, Fenchone, Estragole, Eugenol, Geranial and Methyl Eugenol were obtained from Aldrich Co. (UK). These compounds were serially diluted with analytical grade acetone to appropriate concentrations. The doses of monoterpenoids used per vial in the bioassay of volatile toxicity ranged from 0.01 to 50 µl depending on insects.

For the repellence test, the dose of monoterpenoids on rice was 100 µl.

**Bioassays**

**Volatile toxicity:** Groups of 10 insects were placed inside 15 ml glass vials. The monoterpenoids were applied on Whatman filter paper discs (2 cm diameter) in 4 ml glass vials to avoid direct contact between monoterpenoids and the beetles. Serial dilutions (four concentrations for *Sitophilus oryzae* and *Rhyzopertha dominica* and three for *Cryptolestes pusillus*) were prepared in acetone to estimate LC 50. Three replications per dose were set up and the vials were incubated in the dark at 30° C and 51 % relative humidity. The mortality was observed after 24h.

**Repellence assays:** The monoterpenoids were applied to the rice (100 µl of the compound mixed in 1000 µl of acetone) and the solvent was allowed to evaporate for 2 hours at room temperature. Groups of 20 insects were added in the middle of a 250 ml cylindrical plastic container with 140 holes (3mm diam.) filled in with the treated rice. Each container was placed on a Petri dish to collect the insects which left the grain following the procedure described by Mohan and Fields (2002). The experiment was maintained at room temperature. Each treatment was replicated three times and the repellence (number of insects leaving the grain) was observed after 24h. A control was prepared the same way using acetone but without the application of the compound.

**Data analysis**

Data were corrected for mortality observed in the control and analyzed using the software POLO-PLUS for Probit analysis. The lethal concentrations, LC 50, were calculated with 95 % confidence limits. T-test was used to compare the number of insects leaving the grain in treated and untreated rice in the repellence bioassay.

**Results**

**Fumigant toxicity of monoterpenoids**

Toxicities of volatile monoterpenoids, such as, *E*-anethole, camphor, R-carvone, S-carvone, p-cymene, estragole, eugenol, fenchone, geraniol, limonene, linalool and γ-terpinene against *S. oryzae*, *R. dominica* and *C. pusillus*, are shown in Table 1, Table 2 and Table 3 respectively.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg l⁻¹)</th>
<th>Mortality (%)</th>
<th>LD₅₀ (mg l⁻¹) (95 FL)</th>
<th>LD₉₅ (mg l⁻¹) (95 FL)</th>
<th>Intercept ± SE</th>
<th>Slope ± SE</th>
<th>χ² (df)</th>
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<td>E-Anethole</td>
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<td>Camphor</td>
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<td>1.46 ± 0.198</td>
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<td>19.35</td>
<td>-0.85 ± 0.240</td>
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<td>p-Cymene</td>
<td>28.33</td>
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<td>123.77 (91.295-176.980)</td>
<td>379.09 (241.821-1058.111)</td>
<td>-7.08 ± 1.059</td>
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<td>921.28 (18.794-0.778) (0.778-9.963)</td>
<td>-4.00 ± 0.198</td>
<td>0.69 ± 0.161</td>
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<td>Fenchone</td>
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<td>25.59 (18.794 - 34.110)</td>
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<td>3.88 ± 0.630</td>
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<td>Geraniol</td>
<td>29.30</td>
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<td>151.29 (85.469-598.212)</td>
<td>2807.90 (664.74-0.44831.10³)</td>
<td>-2.83 ± 0.678</td>
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<td>706.78 (365.73-12347)</td>
<td>-6.77 ± 1.033</td>
<td>2.95 ± 0.450</td>
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<td>γ-Terpinene</td>
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<td>186.89 (125.721-459.369)</td>
<td>1081.30 (447.36-0.12554.10³)</td>
<td>-4.90 ± 1.112</td>
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</table>

Chi-square value for goodness of fit of data to the Probit model was not significant (P > 0.05) except for Camphor, γ-Terpinene, Geraniol, Fenchone and Eugenol.
Table 2. Insecticidal activity of volatile monoterpenoids to *Rhysoperta dominica* Fabricius (Coleoptera: Curculionidae) after 24h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg l⁻¹)</th>
<th>Mortality (%)</th>
<th>LD₅₀ (mg l⁻¹) (95 FL)</th>
<th>LD₉₀ (mg l⁻¹) (95 FL)</th>
<th>Intercept ± SE</th>
<th>Slope± SE</th>
<th>Χ² (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Anethole</td>
<td>6.65 0</td>
<td>6.58 50</td>
<td>32.93 73 (4.639-14.551)</td>
<td>65.86 100</td>
<td>-1.77 ± 0.365</td>
<td>1.86 ± 0.293</td>
<td>13.32 (10)</td>
</tr>
<tr>
<td>Camphor</td>
<td>23.21 6</td>
<td>32.49 33</td>
<td>46.42 70 (30.484 - 52.941)</td>
<td>92.85 100</td>
<td>-10.91 ± 1.872</td>
<td>6.89 ± 1.200</td>
<td>30.28 (10)</td>
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<td>R-Carvone</td>
<td>0.63 6</td>
<td>6.39 70</td>
<td>31.96 100 (1.410-6.665)</td>
<td>63.93 100</td>
<td>-1.18 ± 0.292</td>
<td>2.26 ± 0.355</td>
<td>21.99 (10)</td>
</tr>
<tr>
<td>S-Carvone</td>
<td>0.64 3</td>
<td>6.40 36</td>
<td>32.00 100 (2.414-13.712)</td>
<td>64.00 100</td>
<td>-2.08 ± 0.431</td>
<td>2.53 ± 0.425</td>
<td>28.48 (10)</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>28.33 0</td>
<td>57.33 36</td>
<td>114.66 93 (57.397-75.941)</td>
<td>286.66 100</td>
<td>-12.05 ± 2.180</td>
<td>6.62 ± 1.190</td>
<td>3.69 (10)</td>
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<td>Estragole</td>
<td>6.43 6</td>
<td>32.16 46</td>
<td>45.03 93 (11.277-33.444)</td>
<td>64.33 100</td>
<td>-4.68 ± 0.816</td>
<td>3.44 ± 0.533</td>
<td>27.72 (10)</td>
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<td>Eugenol</td>
<td>0.71 3</td>
<td>7.11 66</td>
<td>35.56 96 (1.968-17.662)</td>
<td>71.13 96</td>
<td>-1.19 ± 0.268</td>
<td>1.38 ± 0.213</td>
<td>26.81 (10)</td>
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<td>Fenchone</td>
<td>6.32 6</td>
<td>18.96 60</td>
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<td>63.20 100</td>
<td>-3.99 ± 0.674</td>
<td>3.12 ± 0.493</td>
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<td>Geraniol</td>
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<td>-1.71 ± 0.641</td>
<td>0.26 ± 0.232</td>
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<td>280.00 100</td>
<td>-20.48 ± 4.288</td>
<td>10.32 ± 2.169</td>
<td>19.36 (10)</td>
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<td>Linalool</td>
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<td>34.52 86</td>
<td>40.27 83 (26.832 - 34.787)</td>
<td>57.53 100</td>
<td>-20.07 ± 3.957</td>
<td>13.36 ± 2.592</td>
<td>22.56 (10)</td>
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<tr>
<td>γ-Terpinene</td>
<td>28.33 0</td>
<td>56.66 50</td>
<td>113.33 86 (52.051 - 76.254)</td>
<td>283.33 100</td>
<td>-9.42 ± 1.590</td>
<td>5.23 ± 0.874</td>
<td>10.94 (10)</td>
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</table>

Chi-square value for goodness of fit of data to the Probit model was not significant (P > 0.05) except for γ-Terpinene, p-Cymene and E-Anethole.
Table 3. Insecticidal activity of volatile monoterpenoids to Cryptolestes pusillus Schönherr (Coleoptera: Cucujidae) after 24h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose mg l$^{-1}$</th>
<th>Mortality (%)</th>
<th>$LD_{50}$ (mg l$^{-1}$) (95 FL)</th>
<th>$LD_{95}$ (mg l$^{-1}$) (95 FL)</th>
<th>Intercept $\pm$ SE</th>
<th>Slope $\pm$ SE</th>
<th>$\chi^2$ (df)</th>
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<tr>
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<td>46.42</td>
<td>3</td>
<td>3.71</td>
<td>23.20</td>
<td>-1.18 ± 0.369</td>
<td>2.07 ± 0.313</td>
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<tr>
<td>(1.859-6.640)</td>
<td>(12.574-54.650)</td>
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<td><strong>R-Carvone</strong></td>
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<td>4.75</td>
<td>9.31</td>
<td>-3.81 ± 0.843</td>
<td>5.63 ± 1.233</td>
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<tr>
<td>(3.587-6.509)</td>
<td>(6.705-36.025)</td>
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<td>-3.81 ± 0.843</td>
<td>5.63 ± 1.233</td>
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<td>(3.587-6.509)</td>
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<td>-5.14 ± 1.034</td>
<td>3.79 ± 0.699</td>
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<td>(13.201-32.074)</td>
<td>(41.398-177.887)</td>
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<td><strong>Estragole</strong></td>
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<td>32.16</td>
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<td>3.30</td>
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<td>-1.38 ± 0.362</td>
<td>2.66 ± 0.471</td>
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<tr>
<td><strong>Eugenol</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.71</td>
<td>4.26</td>
<td>7.11</td>
<td>3</td>
<td>9.51</td>
<td>22.95</td>
<td>-4.20 ± 0.851</td>
<td>4.30 ± 0.805</td>
</tr>
<tr>
<td>(7.149-11.695)</td>
<td>(17.130-46.563)</td>
<td></td>
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<tr>
<td><strong>Fenchone</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6.32</td>
<td>12.64</td>
<td>18.96</td>
<td>20</td>
<td>9.51</td>
<td>22.95</td>
<td>-4.20 ± 0.851</td>
<td>4.30 ± 0.805</td>
</tr>
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<td>(7.149-11.695)</td>
<td>(17.130-46.563)</td>
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</tr>
<tr>
<td><strong>Geraniol</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5.86</td>
<td>29.30</td>
<td>58.60</td>
<td>0</td>
<td>32.39</td>
<td>95.11</td>
<td>-5.31 ± 1.323</td>
<td>3.51 ± 0.835</td>
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<tr>
<td>(24.096-40.460)</td>
<td>(66.996-226.850)</td>
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</tr>
<tr>
<td><strong>Limonene</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5.60</td>
<td>56.00</td>
<td>84.00</td>
<td>3</td>
<td>33.90</td>
<td>147.11</td>
<td>-3.95 ± 0.829</td>
<td>2.58 ± 0.480</td>
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<tr>
<td>(10.917-59.451)</td>
<td>(78.372-1547.864)</td>
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<tr>
<td><strong>Linalool</strong></td>
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</tr>
<tr>
<td>0.57</td>
<td>2.89</td>
<td>5.78</td>
<td>0</td>
<td>4.09</td>
<td>7.37</td>
<td>-3.93 ± 0.806</td>
<td>6.43 ± 1.279</td>
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<tr>
<td>(3.529-4.734)</td>
<td>(6.017-11.148)</td>
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<tr>
<td><strong>γ-Terpinene</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5.66</td>
<td>28.33</td>
<td>56.66</td>
<td>0</td>
<td>–</td>
<td>-7.47 ± 2.345</td>
<td>5.31 ± 1.544</td>
<td>7.96 (7)</td>
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</tbody>
</table>

Chi-square value for goodness of fit to the Probit model was not significant (P > 0.05) for eugenol and limonene.

R-carvone ($LC_{50} = 1.90$ mg l$^{-1}$), S-carvone ($LC_{50} = 2.74$ mg l$^{-1}$), eugenol ($LC_{50} = 3.85$ mg l$^{-1}$) and linalool ($LC_{50} = 4.89$ mg l$^{-1}$) had potent volatile activity against the rice weevil whilst R-carvone ($LC_{50} = 3.32$ mg l$^{-1}$), S-carvone ($LC_{50} = 6.60$ mg l$^{-1}$), eugenol ($LC_{50} = 7.34$ mg l$^{-1}$) and E-anethole ($LC_{50} = 8.90$ mg l$^{-1}$) had similar action against the lesser grain borer.
Table 4. Repellency of monoterpenoids to stored rice pests, after 24 h. (n=3, and 20 insects per 250 ml volume of rice treated with 100 µl of test compound).

<table>
<thead>
<tr>
<th>Insect</th>
<th>Compound</th>
<th>Insects leaving the grain</th>
<th>T-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td><strong>Sitophilus oryzae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>1.3 ± 0.66</td>
<td>2.00</td>
<td>0.184</td>
<td>ns</td>
</tr>
<tr>
<td>Geraniol</td>
<td>6.6 ± 1.20</td>
<td>5.54</td>
<td>0.031</td>
<td>*</td>
</tr>
<tr>
<td>Camphor</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>E-Anethole</td>
<td>0.3 ± 0.33</td>
<td>1.00</td>
<td>0.423</td>
<td>ns</td>
</tr>
<tr>
<td>Fenchone</td>
<td>1.0 ± 0.57</td>
<td>1.73</td>
<td>0.225</td>
<td>ns</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>1.0 ± 0.57</td>
<td>1.73</td>
<td>0.225</td>
<td>ns</td>
</tr>
<tr>
<td>Estragole</td>
<td>0.3 ± 0.33</td>
<td>1.00</td>
<td>0.423</td>
<td>ns</td>
</tr>
<tr>
<td>S-Carvone</td>
<td>5.0 ± 1.52</td>
<td>3.27</td>
<td>0.082</td>
<td>ns</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Limonene</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Geranial</td>
<td>8.3 ± 1.20</td>
<td>6.93</td>
<td>0.020</td>
<td>*</td>
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<tr>
<td>Eugenol</td>
<td>4.0 ± 1.52</td>
<td>2.62</td>
<td>0.120</td>
<td>ns</td>
</tr>
<tr>
<td>Methyl Eugenol</td>
<td>0.6 ± 0.33</td>
<td>2.00</td>
<td>0.184</td>
<td>ns</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.00</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Rhyzoperta dominica</strong></td>
<td></td>
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</tr>
<tr>
<td>Linalool</td>
<td>0.6 ± 0.66</td>
<td>1.00</td>
<td>0.423</td>
<td>ns</td>
</tr>
<tr>
<td>Geraniol</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Camphor</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>E-Anethole</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Fenchone</td>
<td>3.0 ± 1.15</td>
<td>2.59</td>
<td>0.122</td>
<td>ns</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Estragole</td>
<td>2.3 ± 0.66</td>
<td>3.50</td>
<td>0.073</td>
<td>ns</td>
</tr>
<tr>
<td>S-Carvone</td>
<td>2.6 ± 0.66</td>
<td>4.00</td>
<td>0.057</td>
<td>ns</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Limonene</td>
<td>0.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Geranial</td>
<td>1.0 ± 0.00</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Eugenol</td>
<td>0.3 ± 0.33</td>
<td>1.00</td>
<td>0.423</td>
<td>ns</td>
</tr>
<tr>
<td>Methyl Eugenol</td>
<td>0.3 ± 0.33</td>
<td>1.00</td>
<td>0.423</td>
<td>ns</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.00</td>
<td></td>
<td></td>
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<tr>
<td><strong>Cryptolestes pusillus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>12.3 ± 1.20</td>
<td>6.80</td>
<td>0.021</td>
<td>*</td>
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<tr>
<td>Geraniol</td>
<td>4.0 ± 2.51</td>
<td>1.13</td>
<td>0.374</td>
<td>ns</td>
</tr>
<tr>
<td>Camphor</td>
<td>4.0 ± 0.57</td>
<td>5.19</td>
<td>0.035</td>
<td>*</td>
</tr>
<tr>
<td>E-Anethole</td>
<td>8.6 ± 1.33</td>
<td>8.69</td>
<td>0.013</td>
<td>*</td>
</tr>
<tr>
<td>Fenchone</td>
<td>7.3 ± 0.33</td>
<td>19.00</td>
<td>0.003</td>
<td>**</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>2.6 ± 1.20</td>
<td>1.14</td>
<td>0.370</td>
<td>ns</td>
</tr>
<tr>
<td>Estragole</td>
<td>8.3 ± 0.88</td>
<td>11.00</td>
<td>0.008</td>
<td>**</td>
</tr>
<tr>
<td>S-Carvone</td>
<td>11.0 ± 1.52</td>
<td>11.00</td>
<td>0.008</td>
<td>**</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>3.0 ± 0.00</td>
<td>5.00</td>
<td>0.038</td>
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<tr>
<td>Limonene</td>
<td>1.6 ± 0.88</td>
<td>3.46</td>
<td>0.074</td>
<td>ns</td>
</tr>
<tr>
<td>Geranial</td>
<td>10.3 ± 1.66</td>
<td>0.75</td>
<td>0.529</td>
<td>ns</td>
</tr>
<tr>
<td>Eugenol</td>
<td>8.3 ± 1.66</td>
<td>7.76</td>
<td>0.016</td>
<td>*</td>
</tr>
<tr>
<td>Methyl Eugenol</td>
<td>10.6 ± 1.20</td>
<td>3.35</td>
<td>0.079</td>
<td>ns</td>
</tr>
<tr>
<td>Control</td>
<td>1.0 ± 0.57</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*p < 0.05, **p <0.01, ns = non significant
As regards *C. pusillus*, estragole (LC$_{50}$ = 3.30 mg.l$^{-1}$), camphor (LC$_{50}$ = 3.71 mg.l$^{-1}$), linalool (LC$_{50}$ = 4.09 mg.l$^{-1}$) and S-carvone (LC$_{50}$ = 4.75 mg.l$^{-1}$) were the monoterpenoids with the highest activity.

**Repellency of monoterpenoids.**
The behaviour of the insects when exposed to treated rice is summarized in Tables 4 and 5. The repellency activity to *S. oryzae*, was produced by geranial (8.3 ± 1.20 insects left the grain, out of 20), followed by geraniol (6.6 ± 1.20) and S-carvone (5.0 ± 1.52), although in this latter one there was not a significant difference.

The bioassays show that there were no repellency effects to *R. dominica* (Table 4).

Monoterpenoids proved to be more repellent for *C. pusillus*, where linalool (12.3 ± 1.20), S-carvone (11.0 ± 1.52), methyl eugenol (10.6 ± 1.20) and geranial (10.3 ± 1.66), produced the highest number of insects leaving the treated grain. Therefore, mixtures of compounds between linalool and another monoterpenoids were tested for this test species.

Linalool:estragole, followed by linalool:fenchone and linalool:S-carvone at 50:50 doses turn out to be also repellent for *C. pusillus* (Table 5), but the response of the insects to the treated rice was not much different when compared with applications of linalool alone (Tables 4 and 5).

### Table 5. Repellency of mixtures of monoterpenoids to *Cryptolestes pusillus*, after 24 h. (n=3, and 20 insects per 250 ml volume of rice treated with 50 µl + 50 µl of test compounds).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Insects leaving the grain</th>
<th>T-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linalool : Geraniol</td>
<td>8.0 ± 0.00</td>
<td>15.50</td>
<td>0.005 **</td>
</tr>
<tr>
<td>Linalool : Camphor</td>
<td>6.3 ± 1.76</td>
<td>12.12</td>
<td>0.007 **</td>
</tr>
<tr>
<td>Linalool : E-Anethole</td>
<td>7.0 ± 1.73</td>
<td>2.63</td>
<td>0.119 ns</td>
</tr>
<tr>
<td>Linalool : Fenchone</td>
<td>12.0 ± 2.64</td>
<td>3.92</td>
<td>0.059 ns</td>
</tr>
<tr>
<td>Linalool : γ-Terpinene</td>
<td>5.0 ± 1.00</td>
<td>5.28</td>
<td>0.034 *</td>
</tr>
<tr>
<td>Linalool : Estragole</td>
<td>14.0 ± 1.52</td>
<td>2.62</td>
<td>0.120 *</td>
</tr>
<tr>
<td>Linalool : S-Carvone</td>
<td>11.6 ± 1.20</td>
<td>13.00</td>
<td>0.006 *</td>
</tr>
<tr>
<td>Linalool : p-Cymene</td>
<td>5.0 ± 0.57</td>
<td>6.04</td>
<td>0.026 *</td>
</tr>
<tr>
<td>Linalool : Limonene</td>
<td>7.0 ± 1.73</td>
<td>2.59</td>
<td>0.122 ns</td>
</tr>
<tr>
<td>Linalool : Geranial</td>
<td>11.3 ± 0.88</td>
<td>7.75</td>
<td>0.016 *</td>
</tr>
<tr>
<td>Linalool : Eugenol</td>
<td>11.0 ± 1.00</td>
<td>8.66</td>
<td>0.013 *</td>
</tr>
<tr>
<td>Linalool : Methyl Eugenol</td>
<td>10.6 ± 1.33</td>
<td>10.96</td>
<td>0.008 **</td>
</tr>
<tr>
<td>Control</td>
<td>1.0 ± 0.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05, **p <0.01, ns = non significant

**Discussion**

The effect of monoterpenoids on each insect depends on several parameters of the insects, like species susceptibility, the chemical and the mode of application. R-carvone, S-carvone, eugenol and linalool, had the greatest effects on the three pests. However, estragole and camphor were more active on *Cryptolestes pusillus*. All these compounds are alcohols or ketones (with oxygenated structures) so higher insecticidal activity could be related to the presence of functional groups with oxygen in a chemical structure.

Regnault-Roger and Hamraoui (1995) pointed out to the same conclusion: carvacrol, linalool, eugenol and terpineol (monoterpenoids with oxygenated structures) were the most toxic against *Acanthoscelides obtectus* (Say) (Coleoptera). Lee *et al.*, (2001) found menthione
and linalool as the most biologically active fumigant against *Sitophilus oryzae* and Kim and Ahn (2001), reported that estragole and fenchone were more potent adulticidal agents against *S. oryzae* than E-anethole. Obeng-Ofori and Reichmuth (1999), determined that fixed oils combined with either 1,8 cineole, eugenol or camphor were more effective against stored product beetles than oils used alone. Lwande *et al.*, (1999), reported that some compounds like nerol, geraniol, carvacrol, and β-ionone were the most repellent components against *R. appendiculatus* Neumann (Acari: Ixodidae).

Monoterpenoids as alternatives for pest management will have more potential if they do not produce damage in the environment or do not cause side effects and health risks to humans. The mixtures between monoterpenoids are worth to be studied in depth using different concentrations and other monoterpenoids or products to obtain synergistic effects. Also the mode of application on the stored products is of practical importance and should be developed. Finally the mode of action of monoterpenoids in the insect, e.g. if they inhibit acetylcholinesterase activity and if this is related to the toxicity found in these bioassays has to be better researched to gain basic knowledge for further applications.

**Acknowledgements**

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**References**


Toxicity and repellency of essential oils of *Lippia adoensis* from two agro-ecological zones in Cameroon to *Prostephanus truncatus* and two strains of *Sitophilus zeamais*

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Abstract: Essential oils extracted from the leaves of *Lippia adoensis*, harvested from two agro-ecological zones (Mbe and Dang)) in Cameroon were evaluated in the laboratory for toxicity and repellency to *Prostephanus truncatus* and Cameroonian and German strains of *Sitophilus zeamais*. The essential oils were analyzed by gas chromatography. Maize grains were coated with the oils at four rates (10, 20, 40, 80 µl/40 g) for the assessment of weevil mortality over an 8-day period. Repellency tests on filter papers included five dosages (0.5, 1, 3, 5 and 10 µl/half disc) of the essential oils. The three dominant constituents were thymol (50.9%), p-cymene (8.9%) and carvacrol (8.3%) for L. *adoensis* from Mbe (LAM), and geraniol (37.2%), linalool (27.7%) and cis, beta-farnesene (10.8%) for L. *adoensis* from Dang (LAD). The two types of *L. adoensis* were generally significantly toxic to *P. truncatus* and both strains of *S. zeamais*, although the action of LAD was slower than that of LAM. The 6-day LD50-values indicated LAD was more toxic to the German (12.3 (10.5-14.0) µl/40 g) compared with the Cameroonian strain (29.7 (17.3-54.2) µl/40 g) of *S. zeamais*, but both strains responded similarly to LAM (7.1 (4.8-9.0) and 6.6 (4.3-8.3) µl/40 g, respectively). Both essential oils were highly repellent to the weevils but much less so to *P. truncatus*. Although the essential oil from *L. adoensis* may provide effective control of *S. zeamais* and *P. truncatus* on maize, there is the need for testing the toxicity of the plant species from different agro-ecological zones to the beetles in different environments, if the plant is to be of value in the integrated management of *S. zeamais* in Africa.

Key words: *Lippia adoensis*, *Sitophilus zeamais*, essential oils, toxicity, strains

Introduction

Maize in storage is attacked by several important cosmopolitan pests such as the maize weevil, *Sitophilus zeamais* Motschulsky and the Angoumois grain moth, *Sitotroga cereallela* (Oliver) (Lepidoptera: Gelechiidae), and the less cosmopolitan larger grain borer, *Prostephanus truncatus* (Horn). Grain damage and losses of 33% and 30%, respectively, caused by *S. zeamais* have been reported in Cameroon, where maize constitutes the most important food crop (Kamga *et al.*, 1992; Nukenine *et al.*, 2002). In recent years, *P. truncatus* has acquired the status of a serious pest of stored maize in several countries stretching from the southern USA through Mexico, Central America, Panama and Columbia (Tigar *et al.*, 1994). This insect was accidentally introduced into Tanzania (Hodges *et al.*, 1983) and Togo (Krall, 1984) from were it rapidly spread to other parts of Africa and is inflicting heavy losses on maize and cassava (Hodges, 1994). Losses in maize caused by *P. truncatus* attack in Africa...
and Latin America varied from 9-45% (Hodges et al., 1983; Markham et al., 1991). Although yet to be reported in Cameroon, the beetle is devastating maize and cassava chips in neighbouring Nigeria.

Control of stored-product insect pests is primarily dependent upon continued applications of synthetic insecticides which are still the most effective for the protection of stored foods, feedstuffs and other agricultural commodities from insect infestation. Although effective, their repeated use for decades has disrupted natural biological control systems and led to outbreaks of stored-product insect pests, development of resistance to various types of insecticides, undesirable effects on humans and non-target organisms, and environmental concerns (Champ & Dyte, 1977; White & Leesch, 1995). Additionally, peasant farmers who produce most of the grains in Africa not only lack the skills to use synthetic insecticides, but also lack the means. Recognition of these detrimental consequences of over reliance on synthetic insecticides, led to the formulation of the concepts of integrated pest management (IPM). IPM is a sustainable approach, combining biological, cultural, physical, and chemical tools to regulate pest populations while minimizing economic, environmental, and human health risks, by using *inter alia* reduced-risk insecticides. Among potential reduced-risk pesticides are botanical insecticides, ie, substances based on plant extracts, or purified substances of plant origin. As part of an IPM strategy, phytochemicals could be implicated in pest prevention, early pest detection and pest control when they act as repellents, attractants in baits and toxicants, respectively (Adler et al., 2000). Essential oils from several plant species are reported to be toxic to insects (Sampson et al., 2005). Studies have demonstrated the efficacy of essential oils or their components in protecting grains against the attack of *Sitophilus* spp. *P. truncatus* (Obeng-Ofori et al., 1997; Lee et al., 2003; Tapondjou et al., 2002).

*Lippia adoensis* is a shrub or weed widely available in Cameroon and traditionally used for grain protection and mosquito repellence. Decoctions of dried leaves from *Lippia adoensis* Hochst. ex Walp (syn. *L. multiflora* Mold., *L. grandifolia* Martius and Shan, *L. schimperi* Walp) (Verbenaceae) are used in African folk medicine as remedies against many diseases such as malaria and hypertension, but also as drugs to treat indigestion, cough and fever (Kanko et al., 1999). The oil is used as spice and condiment as well as mouth disinfectant (Demissiew, 1993; Menut et al., 1995). Volatile oils of *L. adoensis* from Nigeria showed good contact and fumigant toxicity to *Callosobruchus maculatus* (F.), while the leaf powder was less toxic to the beetle (Gbolade and Adebayo, 1993; Adebayo and Gbolade, 1994). Significant repellency effect of the leaf essential oils from this plant in Kenya were reported against *S. zeamais* (Mwangi et al., 1992). The chemical composition of plant essential oils vary across environments. Essential oils from air-dried leaves of *L. adoensis* from different regions of Ivory Coast were characterized by four different major compositions of pure compounds: neral/geranial, 1,8-cineole, 1,8-cineole/nearl/geranial and linalool (Kanko et al., 1999). Different strains of the same insects species could manifest variations in biological characteristics, which could in turn lead to differential susceptibilities to insecticides. Baker (1988) showed differences in the development of four strains of *S. oryzae* on the same substratum.

The present study was therefore undertaken to determine the toxic and repellent effects of the essential oil of *L. adoensis* derived from leaves harvested in two agro-ecological zones (Dang and Mbe) of Cameroon to Cameroonian and German strains of *S. zeamais* as well as *P. truncatus*.
Materials and methods

Insects

*Sitophilus zeamais* and *P. truncatus* were reared on maize in a controlled temperature and humidity chamber (25 ± 1°C and 60 ± 5% r.h.) in darkness. Parent adults of *S. zeamais* were obtained from farmers’ stocks around Ngaoundere, Cameroon in April 2004 (Cameroonian strain) (CS). For the German strain (GS) of the insect and *P. truncatus*, the parent adults were obtained from laboratory stock cultures (more than 30 years old) at the Institute for Stored Product Protection, Berlin, Germany (German strain).

Collection and preparation of plant materials

The leaves of *L. adoensis* were collected in July 2004 (wet season) at Dang (LAD) and Mbe (LAM), located in the Vina Division, Adamawa province, Cameroon. Dang (Plateau) and Mbe (plain) are situated 15 km and 70 Km north of the capital city of the province, Ngaoundere, respectively. The site where the leaves were harvested had the following coordinates: altitude (1090 and 616 masl), latitude (7° 41’ and 7° 86’ N), Longitude (13° 52’ and 13° 60’ E) for Dang and Mbe, respectively. The agro-ecology at Dang is Sudano-Guinean savanna and at Mbe Sudano-Sahelian savanna. The Vina Division is characterized by two seasons - a dry season from November to March and a wet season spanning April to October. The identity of the plants was confirmed at the Cameroon National Herbarium in Yaoundé, where voucher samples were deposited. The leaves were dried at room temperature for seven days, and then crushed. The crushed leaves were stored in opaque containers inside a refrigerator at 4 °C. They were transported to Berlin, Germany within 10 days after crushing, where they were ground until the powder passed through a 0.20 mm sieve and then stored in a freezer at -18 °C until needed for extraction of the essential oils.

Extraction and characterization of essential oil

Seven hundred and 900 grams, respectively, of the crushed leaves of LAD and LAM were subjected to steam distillation for 4 hours. The oils collected were dried over anhydrous sodium sulphate and they yielded 1.2 (yellow, sweet smell) and 0.7% (orange, strong smell) (wt/wt) liquid for LAD and LAM, respectively, and were stored in a refrigerator until needed. Chemical analysis of the oil was achieved by GC-MC on a HP 5890 II gas chromatograph coupled to a HP 5972 mass selective spectrometer using DB wax fused silica capillary column.

Toxicity bioassay

Forty-gram-samples of disinfested maize were mixed with 10, 20, 40 and 80 ul solutions (in 1 ml acetone) of the essential oils from LAD and LAM in 250-ml glass bottles by tumbling for 10 minutes with a Rotatory Shaker (Multifix GmBH, Germany) to ensure even spread of the materials over the surface of the grains. Control for each set of treatments consisted of grain mixed with 1 ml acetone alone. The treated or control grains were kept for 20 min to allow the solvent to evaporate. Each treatment was repeated four times. A lot of 20 insects for *P. truncatus* and the German and Cameroonian strains of *S. zeamais* of mixed sexes, aged 1-2 weeks, were added into the jars containing the treated or untreated grains. Mortality was recorded 1, 2, 4, 6 and 8 days after infestation.

Repellency bioassay

The area preference test described by Mcdonald et al. (1970) was used to evaluate the repellent action of the essential oils of LAD and LAM against *P. truncatus* and the two strains of *S. zeamais*. Test arenas consisted of 7 cm Whatman no. 1 filter paper cut in half (19.25 cm²). Different test solutions were prepared by dissolving 0.5, 1, 3, 5 and 10 µl of the
essential oils in 1 ml acetone. Each solution was applied to a half filter paper disc as uniformly as possible with a pipette. The other filter paper halves were treated with acetone alone. Chemically treated and control half discs were air-dried for 10 min to evaporate the solvent completely. Full discs were subsequently remade by attaching treated halves to untreated halves with clear adhesive tape. Each remade filter paper disc was placed into 7 cm Petri dish and 20 unsexed adult insects of each species or strain were released separately at the centre of the filter paper disc under infrared light at 25 °C and 60% r.h. The Petri dishes were then covered and left under the same condition. Each treatment was repeated 5 times. The number of insects present on control (NC) and treated (NT) strip were recorded after 2 h exposure. Percent repellency (PR) values were computed as PR = [(NC-NT)/(NC+NT)]×100.

The mean repellency values of each essential oil was calculated and assigned to repellency classes (Juliana & Su, 1983) from 0 to V: class 0 (PR < 0.1%), class I (PR = 0.1-20%), class II (0.1-40%), class III (40.1-60%), class IV (60.1-80%), class V (80.1-100).

**Data analysis**
Probit analysis (Finney, 1971; SAS institute, 2003) was applied to determine lethal dosages causing 50% (LD_{50}) and 99% (LD_{99}) mortality of *P. truncatus* and the two strains of *S. zeamais* at 4-, 6- and 8-day exposure period. Abbott’s formula (Abbott, 1925) was used to correct for control mortality before probit analysis. Percent repellency data were analyzed using ANOVA after transforming them into arcsine values. Fisher’s Protected LSD test was applied for the separation of means.

**Results**

**Essential oil composition**
The five major constituents analyzed in the oils represented 84.6% and 78.4% for LAD and LAM, respectively. These constituents were geraniol (37.2%), linalool (27.7%), cis-β-farnesene (10.3%), β-caryophyllene (5.2%) and neral (3.7%) for the oil from LAD, and thymol (50.9%), cymol (8.9%), carvacrol (8.3%), β-caryophyllene (5.6%) and cis-β-farnesene (4.7%) for the LAM oil.

**Toxicity**
The effect of the essential oil from the leaves of *L. adoensis* on mortality of *S. zeamais* was dose-dependent, with mortality increasing over time and ascending concentrations, irrespective of insect strain, species or origin of the oils (Fig. 1). However, the action of LAD was slower than that of LAM. Control mortality was ≤5%. The maximum tested dosage of 80 µl/40g induced 100% mortality after 4 and 1 day respectively for LAD and LAM, regardless of insect strain. This dosage caused 100% mortality of *P. truncatus* within a day for LAM, but only 28% within 8 days for LAD. Within 8 days of exposure, the smallest dosage (10 µl/40g) of the LAD oil achieved 11%, 45% and 3% mortality respectively for the CS and GS of *S. zeamais* and *P. truncatus*. At the same dosage and time-point, LAM accomplished 74% (CS), 66% (GS) and 18% mortality of the beetles. Generally the LD_{50} and LD_{99} values of the LAD oil were higher for the CS than the GS of the weevil (Table 1), but the values were lower than those for *P. truncatus*. In contrast, the LD_{50} values of the LAM oil were similar for both strains of weevil, but higher for *P. truncatus*.

**Repellency**
Table 2 gives the repellency values for each of the test oils on the two strains of *S. zeamais* and *P. truncatus*. Overall, the essential oils evoked strong repellency in the two weevil strains but were only weakly repellent to *P. truncatus*. The repellent action of LAM to *P. truncatus* reduced with increasing dosages and tended to be attractive at dosages above 3 µl/half disc.
The repellent effect of the oil from LAD to GS of *S. zeamais* was higher than that from LAM. The oil from LAD was also more repellent to the GS compared with CS of *S. zeamais*.

![Fig. 1](image_url)

Fig. 1. Mortality of *Prostephanus truncatus* and Cameroonian and German strains of *Sitophilus zeamais* (mean ± S.E.) over 8 days when exposed to maize coated with different dosages of essential oils of *Lippia adoensis* harvested in Dang and Mbe, Cameroon at 25 °C and 60% r.h.
Table 1. Toxicity of essential oils from *Lippia adoensis* harvested in Dang and Mbe, Cameroon on maize grains to *Prostephanus truncatus* and Cameroonian and German strains of *Sitophilus zeamais* at 25 °C and 60% r.h.

<table>
<thead>
<tr>
<th>Insect</th>
<th>Dang</th>
<th>Mbe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD$_{50}$ (µl l/40 g)</td>
<td>LD$_{99}$ (µl l/40 g)</td>
</tr>
<tr>
<td></td>
<td>(95% FL)</td>
<td>(95% FL)</td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. zeamais</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cameroon</td>
<td>43.0 b</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Germany</td>
<td>(1.0-29.8)</td>
<td></td>
</tr>
<tr>
<td><em>P. truncatus</em></td>
<td>&gt;80</td>
<td>&gt;80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. zeamais</em></td>
<td>29.7</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Cameroon</td>
<td>(17.3-54.2)</td>
<td></td>
</tr>
<tr>
<td><em>S. zeamais</em></td>
<td>12.3</td>
<td>65.2</td>
</tr>
<tr>
<td>Germany</td>
<td>(10.5-14.0)</td>
<td>(50.3-96.2)</td>
</tr>
<tr>
<td><em>P. truncatus</em></td>
<td>&gt;80</td>
<td>&gt;80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. zeamais</em></td>
<td>24.4</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Cameroon</td>
<td>(15.9-37.3)</td>
<td></td>
</tr>
<tr>
<td><em>S. zeamais</em></td>
<td>11.9</td>
<td>53.2</td>
</tr>
<tr>
<td>Germany</td>
<td>(10.3-13.4)</td>
<td>(42.2-74.7)</td>
</tr>
<tr>
<td><em>P. truncatus</em></td>
<td>&gt;80</td>
<td>&gt;80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* are considered significantly different when 95% fiducial limits (FL) fail to overlap.

*b* Fiducial limits not calculated because of poor fit of model

**Discussion**

Our results show quantitative and qualitative differences in chemical composition and insecticidal activities of essentials oils of the same plant from different agro-ecological zones in Cameroon. Variations in the chemical composition of botanicals are common (Adler *et al.*, 2000). The major components of *L. adoensis* in Nigeria were Linalool (26.1%), geraniol (19.0%) and 1,8-cineole (17.4%) (Kasali *et al.*, 2004), which is different from those in the present study. Essential oils from air-dried leaves of *L. multiflora* from different regions of Ivory Coast were characterized by four compositions: neral/geranial, 1,8-cineole, 1,8-cineole/neral/geranial and linalool (Kanko *et al.*, 1999).

It has been recognized that some plant-derived insect-control agents could be developed into products suitable for IPM because they are selective to pests, have no or little harmful action against non-target organisms or the environment. They act in many ways on various types of pest complex and may be applied to the plant in the same way as other agricultural chemicals (Arnason *et al.*, 1989). Many plant extracts such as neem extract and essential oils are known to possess ovicidal, repellent and insecticidal activities against various stored product insects (Hill & Schoonhoven, 1981; Shaaya *et al.*, 1997). Results of the present study suggest that essential oils from *L. adoensis* could be used as an insecticide or repellent against...
adult *S. zeamais* on maize, since it caused significant mortality of the weevil, accomplishing 100% mortality within 1 or 4 days depending on the origin of the leaves and dosage. The oils from this plant could also be of value in the protection of maize grains against the infestation of *P. truncatus*. However, the susceptibility of the three insects to the two oils was different, which is likely due to the differential chemical composition of the oils. Sampson *et al.* (2005) stated that biological activity of essential oils is often affected by its chemical composition and interactions among the structural components. Even minor compounds can have a critical function due to coupled effects, additive action between chemical classes and synergy or antagonism.

Table 2. Mean percent repellency (PR) values for different dosages of essential oils and major compounds of *Lippia adoensis* from two agro-ecological zones against *Prostephanus truncatus* and two strains of *Sitophilus zeamais* in the choice arena at 25 °C and 60% r.h.

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Cameroonian strain of <em>Sitophilus zeamais</em></th>
<th>German strain of <em>Sitophilus zeamais</em></th>
<th><em>Prostephanus truncatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean percent repellency (PR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>F (P&gt;F)</td>
</tr>
<tr>
<td>0.5</td>
<td>50 ± 7.0 c</td>
<td>45 ± 11.9 b</td>
<td>30 ± 4.1 abc</td>
</tr>
<tr>
<td>1</td>
<td>73 ± 8.5 b</td>
<td>83 ± 8.5 a</td>
<td>38 ± 8.5 ab</td>
</tr>
<tr>
<td>3</td>
<td>88 ± 4.8 ab</td>
<td>98 ± 2.5 a</td>
<td>20 ± 7.1 bc</td>
</tr>
<tr>
<td>5</td>
<td>93 ± 4.8 a</td>
<td>98 ± 2.5 a</td>
<td>13 ± 4.8 c</td>
</tr>
<tr>
<td>10</td>
<td>87 ± 4.3 ab</td>
<td>100 ± 0 a</td>
<td>40 ± 7.1 a</td>
</tr>
<tr>
<td>F (P&gt;F)</td>
<td>7.9 (0.0013)</td>
<td>11.8 (0.0002)</td>
<td>3.2 (0.0439)</td>
</tr>
<tr>
<td>Mean</td>
<td>78 ± 4</td>
<td>85 ± 5</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Repellency class</td>
<td>IV</td>
<td>V</td>
<td>II</td>
</tr>
</tbody>
</table>

|Dosage | Lippia adoensis from Mbe | | | |
|--------|--------------------------|-------------------------------|---------------------------|
|        | Mean percent repellency (PR) |                         | F (P>F)                  |
|        | Mean |                          |                           |
| 0.5    | 23 ± 7.5 c                  | -23 ± 15.5 c                | 38 ± 2.5 a                |
| 1      | 50 ± 10.0 b                 | 20 ± 5.7 b                  | 33 ± 9.5 a                |
| 3      | 95 ± 2.9 a                  | 73 ± 8.5 a                  | 13 ± 6.3 a                |
| 5      | 95 ± 5.9 a                  | 83 ± 4.8 a                  | -25 ± 10.4 b              |
| 10     | 95 ± 2.9 a                  | 88 ± 7.5 a                  | -48 ± 13.8 b              |
| F (P>F)| 28.6 (0.0001)               | 26.8 (0.0001)               | 15.8 (0.0001)             |
| Mean   | 72 ± 7                       | 48 ± 10                      | 2 ± 8                     |
| Repellency class | IV                                      | III                        | I                        |

Mean of five replicates of 20 insects each. Values followed by different letter(s) are significantly different at the 5% probability level, Fisher’s Protected LSD test.

The difference in susceptibility and repellence response of the two strains of weevils and two species of beetles to LAD was not surprising, since geographic variation in susceptibility to insecticide is common (see Huang *et al.*, 2004). Additionally, the CS which was less susceptible is a field strain, while the GS has been in laboratory culture for over 30 years. These results are in agreement with the findings of Huang *et al.* (2004), in which field strains of three stored-product insects, *Cryptolestes ferrugineus*, *Tribolium castaneum* and *Plodia interpunctella*, were generally less susceptible to spinosad than the corresponding laboratory strains. Low genetic diversity due to inbreeding of laboratory strains could explain their increased susceptibility to the essential oil when compared with the field strain. Obeng Ofori
et al. (1997) showed that 1,8 cineole evoked strong repellency in S. granaries and S. zeamais but was moderately repellent to P. truncatus and T. castaneum. Taponjou et al. (2005) detected no significant variation in the repellent effect of the essential oils of Cupressus sempervirens and Eucalyptus saligna towards S. zeamais and T. confusum.

The baseline data presented here indicate that essential oils of L. adoensis could be effective in controlling S. zeamais and P. truncatus infestation on maize at dosages of 175 l/kg or higher depending on the location of the plant. Testing the toxicity of L. adoensis oils from all zones in Cameroon would help validate the value of the essential oil as a grain protectant against weevil attack in the country. The ongoing studies (data analysis) on the effect of the essential oil on progeny production and damage reduction for S. zeamais and P. truncatus would throw more light on its potential in stored product protection and IPM. Nonetheless, information on spectrum of insecticide activity, mammalian toxicity and persistence needs to be documented.

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References


Effectiveness of novel compounds for the control of stored product mites

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Abstract: Stored product mites are prevalent on post harvest cereals in the UK and their presence may result in direct damage to the grain, cause occupational allergies in workers and reduce palatability of cattle-feed and growth rate of pigs and poultry. Best practice for grain storage relies on the use of physical control methods such as drying and cooling but mites may still be present in the surface layers of grain due to absorption of moisture at the grain surface. Stored product mites have shown resistance to organophosphate insecticides and there is a need to develop alternative control methods. As part of a wider study to examine possible target sites for storage mite control agents, we examined four compounds with potential to interfere with the mite cuticle, either by disruption of chitin synthesis or the sclerotisation process. The compounds examined were flufenoxuron, lufenuron, kojic acid and nikkomycin Z. The compounds were incorporated in the mite diet and the effect on mortality of the flour mite, *Acarus siro*, and subsequent population growth was determined. Kojic acid and nikkomycin Z had no effect on mite population growth at the F2 generation. Lufenuron at 100mg/kg resulted in a significant decrease in numbers at the F2 generation (79% population inhibition). Flufenoxuron also resulted in significant decreases in population growth (> 99% population inhibition) at both 10mg/kg and 100mg/kg. Subsequent tests with lufenuron also showed a significant reduction in population growth at the F2 generation when tested against *Tyrophagus longior* at concentrations of 10mg/kg and 100 mg/kg and a reduction in population growth at the F2 generation at 100 mg/kg for *Lepidoglyphus destructor*. The effectiveness of chitin synthesis inhibitors appears to depend on the particular mode of action. The study has shown that disruption of cuticular processes would provide an effective mode of action for novel control agents. The potential of such compounds to act as effective control agents is discussed with regard to time for effect, application methods, cost and residues.

Key words: storage mites, *Acarus siro*, *Tyrophagus longior*, *Lepidoglyphus destructor*, chitin synthesis

Introduction

Stored product mites are common pests in stored cereals and oilseeds particularly in temperate countries with a maritime climate. In the UK storage mites have been found in 81% of commercial grain stores, 72% of farm stores and 89% of oilseed stores (Prickett, 1992, 1997). In addition to the direct damage to the grain or oilseed as a result of feeding (Newstead and Duvall, 1918; Solomon, 1946) their presence may also cause taint (Solomon, 1946; Freeman and Turtle, 1947). Effects on the nutritive value of animal feed due to mite presence have also been reported (Braude et al, 1980; Wilkin and Thind, 1983; Ždářková et al. 1992). There has been increasing concern in recent years with regard to the allergenicity of mites in terms of their ability to cause asthma and other allergic conditions and also due to occupational exposure (Armentia et al., 1997; Arruda and Naspitz, 1997; Bernd et al., 1996; Blainey et al., 1989; Cuthbert et al., 1979; Hallas and Iversen, 1996; Iversen et al., 1990; Revsbech and Andersen, 1987; Śpiewak et al., 2001; Tee, 1994; Van Der Heide et al., 1998; Van Hage-Hamsten et al., 1985, 1987, 1992; Vidal et al., 1997). In extreme cases ingestion of
mites has been shown to result in anaphylactic shock (Bernd et al., 2001; Blanco et al., 1997; Matsumoto et al., 1996; Sanchez-Borges et al., 1997). In the UK best practice for grain storage is detailed in the Home Grown Cereals Authority Grain Storage Guide (Anon, 2003). This promotes the use of cooling and drying to ensure safe storage of grain at conditions where stored product mites and insects are unable to reproduce. However, storage mites can still occur and reach high numbers in the surface layers of the grain due to absorption of moisture at the surface. Currently mites can be controlled using organophosphate insecticides such as pirimiphos methyl. However, resistance to such products has been shown (Starzewski, 1991) and there is increasing pressure to reduce the amounts of organophosphate pesticides used. Currently only phosphine fumigation is approved for treatment of oilseed in the UK, a commodity that is very susceptible to mite infestation. There is therefore a need for alternative products for control of storage mites. This area has recently been reviewed extensively (Collins, 2006). In this review the potential of substances including insect growth regulators, inert dusts, botanicals and pyrethroids was examined with regard to both efficacy and the ability to be incorporated in an integrated pest management (IPM) programme.

Although it is possible to screen a wide range of compounds for effectiveness against storage mites a greater understanding of the physiology and biochemistry of storage mites would be beneficial to adopt a targeted approach for effective and selective control. In comparison with stored product insects, little is known with regard to the physiology of storage mites. However, an obvious target for disruption is the mite cuticle owing to its role in osmoregulation, respiration and as an attachment for skeletal muscles. Mites form new cuticle in each of the nymphal stages. Chitin synthesis inhibitors have shown potential as effective control agents for storage mites but relatively few have been studied (Lipa and Chmielewski, 1976; Collins et al., 2001, 2003a, b). Chitin synthesis inhibitors have the potential to act on the reproduction of the adult mite in addition to affecting the juvenile stages. Studying chitin synthesis inhibitors with different modes of action could also provide information on processes connected with the cuticle of storage mites. This study has examined the effect of three chitin synthesis inhibitors and a compound with potential to affect sclerotization on the flour mite *Acarus siro* L. The most effective compound was also tested against two other storage mite species, *Tyrophagus longior* (Gervais) and *Lepidoglyphus destructor* (Schrank). The compounds chosen were flufenoxuron, nikkomyein Z, lufenuron and kojic acid. Flufenoxuron was included for comparative purposes having previously been shown to effectively control storage mites on wheat and oilseed (Collins et al., 2001, 2003a, b). Nikkomyein Z is an antibiotic produced by *Streptomyces tendae* and is believed to act in mites by competitively inhibiting chitin synthetase (Mothes and Seitz, 1982). It exhibits structural similarity to UDP-N-acetylglicosamin. Lufenuron is believed to cause degeneration of the epidermal cells needed for the synthesis of moulting fluid and chitin (Dean et al., 1998). The precise mode of action of flufenoxuron, known to be effective against mites, is not known but it is believed that benzoylureas may inhibit the action of a transporter responsible for conveying chitin precursor to chitin synthetase (Mitsui et al., 1985) or alternatively may inhibit cuticular protein synthesis (Auda et al., 1989). Kojic acid, a phenoloxidase inhibitor, which as such may interfere with sclerotisation, was also chosen as a candidate compound for acting on the cuticle.

**Materials and methods**

**Mites**

*Acarus siro* 9258/2, *T. longior* T101 and *L. destructor* G15 were used. Mites were reared on a standard diet of yeast and wholemeal flour (3:1). All mite cultures were maintained at 20°C
and 80% RH. In the case of A. siro the mites used were 4-8 day old virgin adults, obtained from isolated tritonymphs. Tritonymphs, together with a small amount of untreated food (<0.005 g), were placed singly in small lengths of glass tube plugged at each end with non-absorbent cotton wool. The emerging adults were retained in the tubes for 4-6 d and then sexed using distinctive features (Hughes, 1976). For tests with T. longior or L. destructor, adults were obtained from cultures of known age adults (4-10 days old) and were probably mated prior to use in the test.

**Preparation of test samples**

A known quantity of the test material (technical grade) was dissolved in an appropriate solvent (water for Nikkomycin Z, acetone for all other test materials) to give a stock solution which, when added to the food medium (yeast and wholemeal flour (3:1)) gave a concentration of 100 mg/kg. Serial dilutions were made from this stock solution to give three final concentrations of 1, 10 and 100 mg/kg. An appropriate volume of test solution was added to 20g of food. Additional solvent was added and the food mixture stirred to ensure even incorporation of the test material. Excess solvent was aired off in a fume cupboard and the food was covered with aluminium foil during this period to prevent contamination by mould. The food media treated with nikkomycin Z, using water as the carrier, was broken down to the original particle size using a pestle and mortar. Food media treated using acetone as the carrier solvent did not require this procedure. Treated media was conditioned at 20°C and 80% RH in the cell used for the bioassay for at least 24 h prior to introduction of the mites.

**Exposure**

Exposure of the mites was carried out in test cells similar to those used by Thind and Edwards (1990) or in the larger recovery cells described by Thind and Muggleton (1998). Each test cell contained 5 male and 5 female mites and approx. 0.01g of the appropriate medium. The cells were placed in desiccators over an aqueous solution of potassium hydroxide to provide an equilibrium relative humidity of 80%, and the desiccators were maintained in an incubator at 20°C. At 5-7 d intervals, the desiccator lids were removed for 10-15 minute to provide a change of air and prevent the build up of CO₂. Each cell was visually inspected under a binocular microscope at magnification of 10-50x for assessing quantity of available food in relation to mite numbers and, if necessary, additional medium (ca. 0.01g) was added.

**Assessment of egg production and total population**

The effect of the treatment was studied by confining five pairs of mites on treated or untreated media for a continuous period of 35 or 40 (nikkomycin Z only) days. After four days of confinement, the cells were examined under a binocular microscope at a magnification of 10-50x and the number of eggs in each cell was counted. Any newly-hatched larvae seen in the cells were included in the total number of eggs recorded. In addition adult mortality was noted. Mites were considered dead if they appeared shrivelled and/or wafer like and remained motionless for at least 2-3 minutes of continual observation. After 10 days cells were re-examined for egg hatch, larvae and protonymph development. After 20 days emergence of the F₁ generation was observed. Further incubation of confined mites for a total period of 35 or 40 days allowed assessment of the effect of the treated medium on the mite population up to the F₂ generation.

At the end of the test period all test cells were frozen (-18°C) to kill all the life stages. The mite numbers (nymphs and adults) on the treated and control food media were assessed. If mite numbers were low the contents of the cell were washed directly on to a filter paper held under suction in a 3-piece Hartley funnel. If mite numbers were high, numbers were assessed by washing out the contents of the cell with a 54:46 glycerol/water mixture into a measuring cylinder. The total volume was made up to 100 ml and the measuring cylinder was
covered with Parafilm and inverted several times to ensure an even distribution of the mites. A 10 ml portion of this solution was pipetted on to a filter paper held under suction in a 3-piece Hartley funnel. A solution of methylene blue in ethanol (0.03% w/v) was used to stain the food material on the filter paper enabling the mites to be seen more easily. The total number of adults and nymphs on each filter paper was recorded following examination under a binocular microscope. If the highest concentration of a test material had no detrimental effect on the number of mites at the F2 generation and observation indicated that mite numbers in the other cells were similar, the lower concentrations were not examined.

Mortality and number of eggs laid were compared using one-way ANOVA followed by Dunnett’s test. A square-root transformation was applied to the data for the number of mites at the F2 generation prior to comparison using one-way ANOVA followed by Dunnett’s test.

**Results**

The effect of various chitin inhibitors was assessed initially on the flour mite (*A. siro*). The effect on mortality of the parental generation, ability to produce eggs and effect on fecundity up to the F2 generation was assessed for flufenoxuron, nikkomycin Z, lufenuron and kojic acid. Mites fed on the flufenoxuron treated diet did not show any difference in mortality of the parental generation. The number of eggs laid after four days of exposure to the treated diet was reduced significantly at the 10 and 100 mg/kg flufenoxuron concentrations in comparison to the number of eggs laid by mites fed on the control diet (ANOVA, P<0.05). The number of mites at the F2 generation was significantly reduced when mites were fed 10 or 100mg/kg flufenoxuron (ANOVA, P<0.05) (Table 1). This represented greater than 99% inhibition of population growth.

Table 1. The effect of flufenoxuron treated food on *Acarus siro* 9258/2. Number of eggs laid by and % mortality of parental generation and derived mean number of mites (adults and juveniles) at the F2 generation (95% confidence intervals in brackets).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality of parental generation after 7 days (%)</th>
<th>Number of eggs present after 4 days</th>
<th>Derived mean number of mites at F2 generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (acetone)</td>
<td>2.5 ± 2.5a</td>
<td>39.8 ± 4.6a</td>
<td>6760a (3637, 10845)</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>0a</td>
<td>30.8 ± 8.1a</td>
<td>7649a (6374, 9040)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>0a</td>
<td>13.0 ± 6.0b</td>
<td>37b (13, 73)</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>2.0 ± 2.0a</td>
<td>16.0 ± 2.8b</td>
<td>17b (10, 26)</td>
</tr>
</tbody>
</table>

In each column means followed by the same letter are not significantly different (P>0.05)

Mites fed on the Nikkomycin Z treated diet did not show any difference in mortality of the parental generation (Table 2). There were no differences in the number of eggs laid after four days of exposure to the treated diet in comparison with the number of eggs laid by mites fed on the control diet (ANOVA, P>0.05). The addition of 100 mg/kg nikkomycin Z showed no detrimental effects on the population of *A. siro* and, in fact, the number of mites present at the F2 generation was significantly greater (ANOVA, P<0.05) than for the control (Table 2).
Table 2. The effect of 100 mg/kg nikkomycin Z treated food on *Acarus siro* 9258/2. Number of eggs laid by and % mortality of parental generation and derived mean number of mites (adults and juveniles) at the F2 generation (95% confidence intervals in brackets).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality of parental generation after 7 days (%)</th>
<th>Number of eggs present after 4 days</th>
<th>Derived mean number of mites at F2 generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>6.0 ± 4.0a</td>
<td>100.4 ± 13.6a</td>
<td>2547a (1938, 3240)</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>7.5 ± 2.5a</td>
<td>90.0 ± 15.1a</td>
<td>4152b (3325, 5071)</td>
</tr>
</tbody>
</table>

In each column means followed by the same letter are not significantly different (P>0.05).

*Acarus siro* fed on diet containing 100 mg/kg lufenuron showed a greater mortality of the parental generation after 7 days exposure although this increase was not significantly different (ANOVA P>0.05) (Table 3). No differences were observed in the number of eggs laid by mites fed on the treated and control food. However, the addition of 100 mg/kg lufenuron did have a detrimental effect on the development of *A. siro* and there were significantly fewer mites present at the F2 generation at this concentration compared with the control (ANOVA, P<0.05) (Table 3). This represented a 79% inhibition of population growth. There was no significant difference in the number of mites present at the F2 generation for either of the two lower concentrations of lufenuron compared to the control.

Table 3. The effect of lufenuron treated food on *Acarus siro* 9258/2. Number of eggs laid by and % mortality of parental generation and derived mean number of mites (adults and juveniles) at the F2 generation (95% confidence intervals in brackets).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality of parental generation after 7 days (%)</th>
<th>Number of eggs present after 4 days</th>
<th>Derived mean number of mites at F2 generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (acetone)</td>
<td>4.0 ± 2.5a</td>
<td>67.4 ± 9.4a</td>
<td>4406a (1850, 8055)</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>5.0 ± 2.9a</td>
<td>90.0 ± 6.9a</td>
<td>5291a (4263, 6430)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>4.0 ± 2.4a</td>
<td>60.6 ± 5.0a</td>
<td>4642a (3356, 6135)</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>18.0 ± 7.3a</td>
<td>57.4 ± 18.0a</td>
<td>961b (215, 2241)</td>
</tr>
</tbody>
</table>

In each column means followed by the same letter are not significantly different (P>0.05)

*Acarus siro* fed on diet containing 100 mg/kg kojic acid showed a significantly greater mortality of the parental generation after 7 days exposure (ANOVA, P<0.05) (Table 4). The addition of up to 100 mg/kg kojic acid did not have an effect on the total number of mites present at the F2 generation (ANOVA, P>0.05) (Table 4).

The effect of lufenuron was investigated for an additional two storage mite species, *T. longior* and *L. destructor*. The addition of 10 and 100 mg/kg lufenuron resulted in a significant reduction in the total number of *T. longior* at the F2 generation (ANOVA, P<0.05) (Table 5). This effect was particularly apparent at 100 mg/kg with greater than 99% inhibition of population growth. A significant decrease in the population size of the F2 generation was
also observed for *L. destructor* fed on 100mg/kg lufenuron compared with *L. destructor* fed on the control diet (ANOVA, P<0.05) (Table 5), representing approximately 60% inhibition of population growth.

Table 4. The effect of 100mg/kg kojic acid treated food on *Acarus siro* 9258/2. Percentage mortality of parental generation and derived mean number of mites (adults and juveniles) at the F2 generation (95% confidence intervals in brackets).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality of parental generation after 7 days (%)</th>
<th>Derived mean number of mites at F2 generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (acetone)</td>
<td>2.0 ± 2.0a</td>
<td>3347a (2170, 4778)</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>12.0 ± 4.9b</td>
<td>3108a (1664, 4998)</td>
</tr>
</tbody>
</table>

In each column means followed by the same letter are not significantly different (P>0.05)

Table 5. The effect of lufenuron treated food on *Tyrophagus longior* T101 and *Lepidoglyphus destructor* G15. Derived mean number of mites (adults and juveniles) at the F2 generation (95% confidence intervals in brackets).

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Tyrophagus longior</em> T101 Derived mean number of mites at F2 generation</th>
<th><em>Lepidoglyphus destructor</em> Derived mean number of mites at F2 generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (acetone)</td>
<td>5159a (4341, 6048)</td>
<td>5126a (3227, 7464)</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>6076a (5250, 6960)</td>
<td>6446a (4288, 9043)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>3369b (1966, 5151)</td>
<td>4686a (1857, 8801)</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>7b (4, 11)</td>
<td>2070b (753, 4040)</td>
</tr>
</tbody>
</table>

In each column means followed by the same letter are not significantly different (P>0.05)

**Discussion**

The potential for growth regulators as a means of control for pestiferous insects has been widely examined. Far less data is available for the effects of these compounds on astigmatic mites. Mite cuticle consists of the procuticle, which is comprised of chitin and protein, the epicuticle, containing lipids, phenols and proteins and a lipid layer (Evans, 1992). This layer covers the cuticle acting as a waterproofing agent and has a vital role in osmoregulation. The mite cuticle is a good target for the development of novel compounds due to the role in respiration, osmoregulation and as an anchorage for skeletal muscles. In addition to examination for the use as potential novel control methods the use of insect growth regulators may provide a tool to investigate mite physiology and hence areas to target for specific control. Chitin synthesis inhibitors are only one of a number of growth regulators and were studied as a means of targeting the cuticular processes of storage mites. The materials tested were chosen for their potential to interfere with the mite cuticle either by disruption of chitin synthesis or the sclerotisation process. As chitin occurs only in arthropods and fungi the
mammalian toxicity of these compounds should be low and the target effects should be selective.

Flufenoxuron belongs to a class of compounds known as benzoylureas. It has previously been shown to suppress population growth of stored product mite species in wheat and oilseed at concentrations as low as 2 ppm (Collins et al., 2001; Collins, 2003a, b). In the current study significant reductions in the population were observed at concentrations of 10 and 100 mg/kg (10 and 100 ppm). The mode of action of flufenoxuron has been studied in insects and it is believed to act by impairing chitin synthesis (Clarke and Jewess, 1990) and reducing incorporation of chitin in the cuticle (Lee et al., 1990). Moulting is an important part of mite development and flufenoxuron may therefore act at this stage preventing further development. It was also observed that incorporation of flufenoxuron in the mite diet reduced the number of eggs laid by female *A. siro*. As adult mortality was not affected this cannot explain the reduced egg numbers observed.

Lufenuron, a compound that also belongs to the benzoylurea class, was also shown to reduce the population growth of *A. siro* but to a lesser extent than that observed with flufenoxuron. The reduction in the number of eggs laid observed when mites were fed on a diet containing the higher concentrations of flufenoxuron, was not seen with lufenuron. It is therefore likely that the greater reduction in population growth with flufenoxuron is due to the compound acting at multiple levels of mite development. Lufenuron interferes with polymerization and deposition of chitin. In cat flea larvae it was found to disrupt the formation of endocuticle and this was caused by degeneration of the epidermal cells needed for synthesis of moult fluid and chitin (Dean et al., 1998).

The potential for control by lufenuron of a further two storage mite species was also studied. Lufenuron, at a concentration of 100 mg/kg, was very effective against *T. longior*. Of the three mite species tested *L. destructor* was the species least affected by lufenuron at the concentrations used. Collins (2003b) in tests with flufenoxuron on oilseed rape also found differences in susceptibility between the *Acarus*, *Tyrophagus* and *Lepidoglyphus* genera. The most susceptible species was found to be *L. destructor* in contrast to the results in this study using lufenuron. This again highlights the potential different mode of action of these two compounds.

Nikkomycin Z had no detrimental effect on the development of *A. siro* and, in fact, there were a greater number of mites at the F2 generation than for the control. An increase in the population has been noted with some growth regulators when used against mites (Downing et al., 1990; Thind and Edwards, 1989) but the reason for this is not known. Nikkomycin Z has been shown to have a detrimental effect on the two-spotted spider mite, *Tetranychus urticae*. In this species it has been shown to disrupt cuticle, eggshell and yolk synthesis (Mothes and Seitz, 1982).

Kojic acid also had no detrimental effect on the population growth of *A. siro* at the concentrations used in this study. Kojic acid acts as a specific competitive inhibitor of phenoloxidase. Phenoloxidase in insects pays a role in host defence and wound healing in addition to the sclerotization process. Kojic acid therefore has the potential to interfere with immune response mechanisms involving melanization in addition to potential effects on sclerotization. Kojic acid is used widely as a food additive and does not present a concern for human health at levels normally found in food (Burdock et al., 2001).

The results from this study show that some chitin synthesis inhibitors would appear to provide an effective alternative to traditional pesticide treatments. The effectiveness of a chitin synthesis inhibitor appears to depend on the mode of action. Further studies could examine the effects of combining two of the chitin synthesis inhibitors with different modes of action, for example lufenuron and flufenoxuron, to determine if this results in improved
Further information on the mode of action of these compounds in storage mites would also be useful in the search for other novel compounds with similar effects. The use of these compounds in combination with other treatments should also be investigated. For example, kojic acid could reduce the immune response making storage mites more susceptible to pathogens. In addition to improving our understanding of the physiology of astigmatic mite cuticle (composition, biosynthesis, degradation, etc), an improved understanding of the functional aspects of the cuticle such as its role in osmoregulation and in defence against pathogens, would provide a means to combat mite infestations with tailored and targeted control measures.

The potential for use of chitin synthesis inhibitors in the storage environment remains to be seen. One of the main disadvantages is the time needed to effectively eradicate a population, this being much greater than for synthetic chemical insecticides. Products based on these types of compounds are already available commercially for control of insect and mite pests on a variety of crops. Therefore the compounds could be formulated and produced at a reasonable economic cost for the storage environment. However, careful consideration would need to be given to the application method and the effects of residues on cereals for human or animal consumption. Residue levels for flufenoxuron have recently been established for a variety of products (Anon, 2006). Some insect species have also developed resistance to growth regulators and effective resistance management strategies would need to be considered if such products were developed for use in this market. However, these compounds, perhaps in combination with other treatments could provide effective control of storage mites.

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Comparative fumigant toxicity of *Rosmarinus officinalis* and *Artemisia sieberi* against *Tribolium castaneum*

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**Abstract**: Essential oils of *Rosmarinus officinalis* L. (Lamiaceae) and *Artemisia sieberi* Besser (Asteraceae) collected from Tehran province were examined for their potent fumigant toxicity against a major stored-product beetle, *Tribolium castaneum* (Herbst). Dry ground leaves from each plant were subjected to hydrodistillation using a modified clevenger-type apparatus. The mortality of 1-3 day old adults of the insect increased with concentrations from 2.50 to 8.93 µl/l air for *R. officinalis* and 3.57 to 28.57 µl/l air for *A. sieberi*. The LC50 and LC95 values to the selected essential oils were 6.84 and 9.66 µl/l air for *R. officinalis*, and 19.40 and 60.89 µl/l air for *A. sieberi* respectively. The test results based on a 95% confidence interval indicated that *T. castaneum* was more sensitive to *R. officinalis* than *A. sieberi*. Fumigant effects of these essential oils were considered to warrant further research on their potential for commercial use against stored-product insects.

**Key words**: fumigation, essential oil, *Rosmarinus officinalis*, *Artemisia sieberi*, *Tribolium castaneum*

**Introduction**

The extended use of broad-spectrum insecticides has resulted in the development of resistant insect populations (Buglio & Wilkins, 2004). Naturally occurring substances are an alternative to conventional pesticides (Plimmer, 1993) and plant essential oils, have traditionally been used to kill or repel insects (Isman, 2000). Essential oils are effective against several insect species with varying potencies (Ho et al., 1995; Huang et al., 2000; Tunc et al., 2000; Negahban et al., 2006); acting as toxins, growth inhibitors, development disruptors, deterrent or repellents.

*Artemisia* and *Rosmarinus* species are some of these plants that may possess medicinal, insecticidal, repellent or antifeedent properties (Grainge & Ahmed, 1988; Jacobson, 1989; Negahban et al., 2007; Katerinopoulos et al., 2005; Momen et al., 2001). *Artemisia abrotanum* L., *Artemisia absinthium* L., *Artemisia vulgaris* L. and *R. officinalis* are used medicinally and hence of more commercial value (Evans, 2001; Fahim et al., 1999). *Artemisia vulgaris* has been reported to be repellent and toxic to *Tribolium castaneum* (Wang et al., 2006).

*Artemisia scoparia* is used as choleretic, anti-inflammatory and diuretic agent in the treatment of hepatitis (Hikino, 1985). *Artemisia sieberi* is used as a control agent against *Callosobruchus maculatus* and *Sitophilus oryzae* (Negahban et al., 2007). *R. officinalis* has hepato-protective and anti-mutagenic effects (Fahim et al., 1999) and repellent and oviposition-deterring activity on the spider mites (Momen et al., 2001; Miresmailli et al., 2006). In this study, two essential oils extracted from *A. sieberi* and *R. officinalis* were used to compare their toxicity against a major stored product insect, *T. castaneum*. 243
Material and methods

Plant
Plant material of *A. sieberi* and *R. officinalis* were collected at full flowering stage from Tehran in December 2006 and April 2007 respectively. The plants were dried naturally on laboratory benches at room temperature (24°C) for 7 days until crisp.

Extraction of essential oils
Fresh aerial parts were cut into small pieces and essential oils were extracted from the plant samples using a Clevenger-type apparatus where the plant material is subjected to hydrodistillation. Conditions of extraction were 50 g of air-dried samples; 1:10 plant material/water volume ratio, 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Extracted oil was stored in a refrigerator at 4°C.

Insect
*T. castaneum* was reared on wheat flour mixed with yeast (10:1, w/w) at 27±1°C and 65±5% r. h. Adult insect (1-3 days old) were used for fumigant toxicity tests.

Fumigant toxicity
Experiment was designed to assess 50% and 95% lethal dose. A series of dilution was prepared to evaluate mortality of insects after an initial dose setting experiment. To determine the fumigant toxicity of the *A. sieberi* and *R. officinalis* oils, filter paper (2 cm diameter) were impregnated with oil at a dose calculated to give the fumigant, 2.50 to 8.93 µl/l air for *R. officinalis* and 3.57 to 28.57 µl/l air for *A. sieberi*. Then the filter paper was attached to the under surface of the screw cap of a glass vial (volume 280 ml). The cap was screwed tightly on the vial containing 20 adults. Control insects were kept under the same conditions without any essential oil. Each dose was replicated five times. Insects in each bottle were exposed for 24 h to the essential oil and then transferred to a clean ventilated bottle. Thereafter the mortality was recorded for 48 h after ventilation. When no leg or antennal movements were observed, insects were considered dead. Insect mortality was corrected according to Abbott (1925). Probit analysis (Finney, 1971) was used to estimate LC$_{50}$ and LC$_{95}$ values.

Results
Experiments were conducted to determine whether the insecticidal activity of *A. sieberi* and *R. officinalis* oils against *T. castaneum* adults was attributable to fumigant action. In all cases, a strong difference in mortality was observed as oil concentration was increased. At 19.40 µl/l air *A. sieberi* oil caused about 50% mortality with a 24 h exposure and at 60.89 µl/l air, kills of *T. castaneum* reached 95%. For *R. officinalis* 50% and 95% mortality were observed at 6.84 and 9.66 µl/l air, respectively.

According to the 95% fiducial limits of LC$_{50}$ and LC$_{95}$ values *T. castaneum* was significantly more susceptible to *R. officinalis* than to *A. sieberi* (Table 1 and Fig. 1).

Discussion
In this experiment, two essential oils were tested by fumigant toxicity against 1-3 days old adults of *T. castaneum*. Essential oils constituents can penetrate into insects rapidly and interfere with their physiological functions (Lee *et al*., 2002). Due to their high volatility, the essential oils have fumigant and gaseous action and might be of importance for stored-product insects (Ahn *et al*., 1998). The essential oils of *A. sieberi* and *R. officinalis* were shown here to possess fumigant toxicity to *T. castaneum*. Also the insecticidal activity varied with plant
derived material and concentrations of the oil. The results showed high mortality caused by *R. officinalis* compared to *A. sieberi* against *T. castaneum*. Haralambos *et al.*, (2005) already reported the high susceptibility of insects to *R. officinalis*.

![Graph](image_url)

**Fig. 1.** Percentage mortality of adult *Tribolium castaneum* exposed to *Artemisia sieberi* and *Rosmarinus officinalis* after 48 h.

**Table 1.** Fumigant toxicity of *Artemisia sieberi* and *Rosmarinus officinalis* essential oils against *Tribolium castaneum*.

<table>
<thead>
<tr>
<th>Probit parameters</th>
<th><em>R. officinalis</em></th>
<th><em>A. sieberi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>160</td>
<td>350</td>
</tr>
<tr>
<td>Intercept ± SE</td>
<td>-9.176 ± 1.484</td>
<td>-4.265 ± 0.767</td>
</tr>
<tr>
<td>Slope ± SE</td>
<td>10.988 ± 1.801</td>
<td>3.312 ± 0.603</td>
</tr>
<tr>
<td>$\chi^2$ (df)</td>
<td>3.51 (2)</td>
<td>5.87 (5)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.1724</td>
<td>0.319</td>
</tr>
<tr>
<td>LC$_{50}$ (µl/l air) (95% fiducial limit)</td>
<td>6.84 (6.54-7.20)</td>
<td>19.40 (17.64-21.65)</td>
</tr>
<tr>
<td>LC$_{95}$ (µl/l air) (95% fiducial limit)</td>
<td>9.66 (8.74-11.62)</td>
<td>60.89 (44.05-119.16)</td>
</tr>
</tbody>
</table>

*Artemisia sieberi* oil has shown potent toxicity giving 90-100% mortality within 24 h exposure at 37 µl/l air for *C. maculatus* and *S. oryzae* (Negahban *et al.*, 2006). Miresmailli *et al.*, (2006) found rosemary to cause 95% mortality at 10.05-17.78 µl/l air after 24 h exposure against *Tetranychus urticae*. The rosemary oils in the current study may be accounted more toxic than Artemisia species reported by Negahban *et al.*, (2007). At the same concentrations, the slopes of the mortality curve from the *A. sieberi* were steep compared to *R. officinalis*. At 6.84 and 9.65 µl/l air of *R. officinalis* the mortality of *T. castaneum* was 50% and 95% after 24 h but *A. sieberi* showed only 7% and 15% respectively at the same dosage. There are more than 40 compounds in the Rosmary oil, mostly monoterpenes that may have caused a higher toxicity of the rosemary oils, in part this could be attributed to higher concentrations of 1,8-cineole and α-pinene (Miresmailli *et al.*, 2006). The two selected essential oils and their major constituents however were shown to warrant further research, including understanding of
sorption, adsorptions and residues on target grain and the influence of any residues on product acceptability, worker safety and mammalian consumption.

References


Fumigant toxicity of essential oil from *Tanacetum polycephalum* against *Tribolium castaneum* and *Callosobruchus maculatus*

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Abstract: In an attempt to find a natural and cheaper method for the control of stored product pests, fumigant toxicity of essential oil from *Tanacetum polycephalum* L. (Asteraceae), was tested against adults of two stored product insects, *Tribolium castaneum* (Herbst) and *Callosobruchus maculatus* (F.). Dry aerial parts of the plant were subjected to hydrodistillation using a modified cleveger-type apparatus. In this study, fumigant toxicity was tested against 1-7 day old adults of *T. castaneum* and *C. maculatus* with five replications at 25±1°C and 65±5% RH under a dark condition. LT₅₀ values calculated as the time to attain 50% mortality of tested insects during fumigation were determined at four different concentrations. For *T. castaneum*, LT₅₀ values ranged from 7.43 h for the lowest dose (32 µl/l air) to 5.2 h for the highest dose (483 µl/l air). The estimate of LT₅₀s for *C. maculatus* was decreased from 8.0 h for 32 µl/l air to 6.2 h for 483 µl/l air. Probit analysis showed that *C. maculatus* (LC₅₀ = 0.90 µl/l air) was more susceptible than *T. castaneum* (LC₅₀ = 10.68 µl/l air). It was found that plant essential oils particularly *T. Polycephalum* could be used either as a safe pesticide or a model for a new synthetic pesticide to control stored-product pests.

Key words: Fumigation, botanical insecticides, essential oil, *Tanacetum polycephalum*, *Tribolium castaneum*, *Callosobruchus maculatus*

Introduction

The red flour beetle, *Tribolium castaneum* and the pulse weevil, *Callosobruchus maculatus* are two of the most widespread and destructive primary insect pests of stored cereals and stored legumes, respectively. Control of these insect populations around the world is primarily dependent upon continued applications of organophosphorus and pyrethroid insecticides and the fumigants methyl bromide and phosphine. Methyl bromide was banned in 2005 in developed countries because it depletes ozone in the atmosphere (Butler & Rodriguez, 1996). Unfortunately, phosphine fumigation may become increasingly limited in use because resistance of stored-grain insects to phosphine has now been discovered in more than 45 countries (Bell & Wilson, 1995). These problems have highlighted the need for the development of selective insect-control alternatives with fumigant action. Many plant extracts and essential oils may be an alternative source of stored-product insect control agents (Desmarchelier, 1994; Shaaya *et al.*, 1997) because they constitute a rich source of bioactive chemicals. The present research was undertaken to investigate the fumigant toxicity of essential oil from *Tanacetum polycephalum* against adults of *T. castaneum* and adults of *C. maculatus*, two important stored-product insects in ASEAN countries (Semple, 1986).
Materials and methods

Insect cultures

*T. castaneum* and *C. maculatus* were reared on wheat flour mixed with yeast (10:1, w/w) and bean grains, respectively. Adult insects, 1-7 days old, were cultured in a controlled temperature and humidity chamber (25±1°C and 65±5% RH) in darkness. All experiments were carried out under the same environmental conditions.

Plant material and extraction of essential oil

Leaves of *T. polycephalum* were collected in September, 2006 from Qom province in Iran. The leaves were cut into small pieces, washed with distilled water and the volatile oil obtained by hydrodistillation (3h) using Clevenger type apparatus. The light-yellow oil (yield 1.5% w/w) thus obtained was dried over anhydrous sodium sulphate and stored at 4 °C.

Fumigant toxicity

To determine the fumigant toxicity of the *T. polycephalum* oil, filter papers (2 cm diameter) were impregnated with oil at a dose calculated to give the fumigant 32 to 483 µl/l air. The filter paper was then attached to the under surface of the screw cap of a 31 ml glass vial. The cap was screwed tightly on the vial containing ten adults (1-7 days old) of each species of insect separately. Each concentration and control was replicated five times. A bioassay was designed to determine median effective time to cause mortality of 50% of the test insects (LT50 value) at the same concentration of the oil. The mortality was assessed by direct observation of the insects every hour up to mortality. Time-mortality data for each experiment were analyzed by the method of Finney (1971) with time as the explanatory variable to derive estimated hours for 50% mortality (LT50). Estimates were compared using overlap of the 95% fiducial limits. Non-overlap at the 95% fiducial limits is equivalent to a test for significant differences. Another experiment was designed to assess 50% lethal doses. A series of dilutions was prepared to evaluate mortality of insects after an initial dose-setting experiment. Ten adults of *T. castaneum* and *C. maculatus* were put into 280 and 620 ml glass bottles with screw lids, which were dosed as described in the first experiment above, respectively. Concentrations of the oil tested on *T. castaneum* were 0, 7.14, 8.93, 10.71, 12.50 and 14.29 µl/l air. *C. maculatus* was evaluated at 0, 0.40, 0.81, 1.21, 1.29 and 1.61 µl/l air. Control insects were kept under the same conditions without any essential oil. Each dose was replicated five times. The insects were exposed to the essential oil vapour for 24h and the dead insects were counted after 48h. The mortality was determined as described in the previous experiment. Data obtained from the various dose-response bioassays were subjected to probit analysis (Finney, 1971) in order to estimate LC50 and LC95 values.

Results and discussion

In all cases, considerable differences in mortality of insects to essential oil vapour were observed with different concentrations and exposure times. The mortality was increased as concentrations of the oil and exposure time increased (Fig. 1). Both of the insects, *T. castaneum* and *C. maculatus* were killed within 12 h from commencement of exposure in all of the tested essential oil concentrations. Based on the fiducial limits of the LT50 values, the speed of the mortality of *T. castaneum* and *C. maculatus* was not significantly different at low concentrations. The estimate of LT50s for *T. castaneum* was decreased from 7.43 h for 32 µl/l air to 5.20 h for 483 µl/l air. With the *C. maculatus*, LT50 values were ranged from 8.00 h for the lowest dose to 6.18 h for the highest dose. However, the results of probit analysis showed
that *C. maculatus* (LC$_{50}$= 0.90 µl/l air, 95% FL: 0.78-1.01 µl/l air) was significantly more susceptible than *T. castaneum* (LC$_{50}$= 10.68 µl/l air, 95% FL: 9.92-11.50 µl/l air) (Table 1).

Fig. 1. Percent mortality of *Tribolium castaneum* and *Callosobruchus maculatus* exposed to essential oil of *Tanacetum polycephalum* for various periods of time. Vertical bars indicate standard error of mean.

Table 1. Fumigant toxicity of *Tanacetum polycephalum* essential oil against *Tribolium castaneum* and *Callosobruchus maculatus*.

<table>
<thead>
<tr>
<th>Insect species</th>
<th>LC$<em>{50}$$</em>{1,2}$</th>
<th>LC$<em>{95}$$</em>{1,2}$</th>
<th>Slope±SE</th>
<th>Degree of freedom</th>
<th>Chi square ($\chi^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. castaneum</em></td>
<td>10.68 (9.92-11.50)</td>
<td>17.86 (15.54-23.04)</td>
<td>7.35±1.90</td>
<td>3</td>
<td>0.70</td>
</tr>
<tr>
<td><em>C. maculatus</em></td>
<td>0.9 (0.78-1.01)</td>
<td>2.01 (1.73-2.94)</td>
<td>4.46±0.67</td>
<td>3</td>
<td>0.25</td>
</tr>
</tbody>
</table>

1Units LC$_{50}$ and LC$_{95}$= µl/l air, applied for 24 h at 25°C.
295% lower and upper fiducial limits are shown in parenthesis.

In this study, the essential oil of *T. polycephalum* demonstrated fumigant toxicity to *T. castaneum* and *C. maculatus*. Studies have not been reported previously concerning the activity of *Tanacetum polycephalum* as fumigant on insect pests. The insecticidal activity varied with insect species, concentrations of the oil and exposure time. The results showed higher mortality rates for *C. maculatus* than for *T. castaneum*. The fumigant activity of some essential oils has been evaluated against a number of stored product insects. The *T. polycephalum* oil described here appears to have greater fumigant toxicity than that of the oils from *Artemisia annua* L. (Tripathi et al., 2000), *Carum copticum* C. B. Clarke (Sahaf et al., 2007), *Artemisia aucheri* Boiss (Shakarami et al., 2003) and *Artemisia sieberi* Besser (Negahban et al., 2007). However, *Artemisia scoparia* Waldst and Kit may be more toxic than
our tested essential oil in this study (Negahban et al., 2006). There is a need to conduct further studies on other essential oils against stored-product insects in the presence of commodity load to establish their efficacy as fumigants.

References


The effect of *Mentha piperita* L. and *Geranium robertianum* L. on the course of population processes of the lesser grain borer *Rhyzopertha dominica* F. (Coleoptera, Bostrichidae)

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**Abstract:** The subject of the investigations was the lesser grain borer *Rhyzopertha dominica* F. The aim of the studies was to determine the effect of the peppermint (*Mentha piperita* L.) and Herb Robert (*Geranium robertianum* L.) on the population processes of *R. dominica*. The herbs were powdered and added to wheat – an optimal nutrient for this insect species – in the proportion of 0.5 g herbs to 40 g wheat. The experiments were conducted under laboratory conditions in an incubator, at a temperature of 28°C and at 60 ± 5% relative humidity (r. h.).

The results obtained allow us to state that the addition of Herb Robert to the wheat had a greater limiting effect on the lesser grain borer population than the addition of peppermint. Herb Robert kept the population at a very low level during the whole time of the investigations. It also, caused an increase in population mortality in comparison with the mortality of insects feeding on wheat only. Moreover, the addition of both Herb Robert and peppermint to the wheat substrate caused an increase in female mortality. Consequently, we deduced that the females are more sensitive to the effect of the chemical compounds in these plants. The compounds in the powdered Herb Robert demonstrated the properties of an antifeedant for this insect species.

**Key words:** *Rhyzopertha dominica* F., repellent, *Geranium robertianum* L., *Mentha piperita* L., population dynamics, sex ratio, mortality

**Introduction**

Investigations carried out on the ecology of storehouse pests are aimed at, among other things, in finding effective methods of reducing their populations. Because of this, apart from providing unsuitable conditions for the pests to reproduce themselves, environmentally friendly substances are now under more intensive investigation, to select those that have an adverse effect on these insect pests. Such substances may exist in certain species of plants. They are becoming of increasingly significant importance as a means of preventing infestation by pests of stored products. Many investigations have been carried out on repellents, attractants and antifeedants (Nawrot 1973, 1983, Nawrot, Czaplicki 1978, Jood et al. 1993, Ignatowicz, Wesolowska 1994, Collins et al. 2002, Nawrot 2002, Kłysi 2004, 2006, Koona and Njoya 2004). Plants contain many compounds which added to a stored commodity may change the behaviour of insects, influence their intake of food and cause disturbances in their development (Nawrot 1983).

The subject of this investigation was the lesser grain borer *Rhyzopertha dominica* F., a dangerous pest of stored grain. In this research, a hypothesis was postulated that the application of chemical substances contained in the powdered forms of Herb Robert (*Geranium robertianum* L.) and the peppermint (*Mentha piperita* L.) may have an inhibitory effect on the development, and also cause a decrease in the population numbers of *R. dominica.*
Materials and methods

The investigations presented here, were laboratory experiments that were conducted in a thermostatically controlled chamber at a temperature of 28°C and at 60 ± 5 % relative humidity (r. h.). Wheat was used as a substrate because of its known favourable habitat for the development of *R. dominica* (Kłyś 2006). Cultures of *R. dominica* on wheat alone served as controls for cultures to which Herb Robert and the peppermint were added. The herbs were applied in powdered form in the proportion of 0.5 g herbs to 40 g of wheat. All the experimental colonies were started with 40 single-age adult individuals at a sex ratio of 1:1. They were obtained according to the method devised by Ciesielska (1971, 1978). Using this method, it was possible to carry out the experiments over a long term. The cultures were set-up in glass vessels with a base area of 28 cm². The first assessment was made after 40 days, i.e. after an approximate period of *R. dominica* development from egg to imago under the given thermal and humidity conditions. Successive assessments were made every 30 days. The insects used for the experiments were obtained from general laboratory cultures that were held under the same conditions as the experimental cultures. Prior to the experiments, emerging adults were held for 15 days under laboratory conditions. Each of the variants were repeated six times. The effects of Herb Robert and the peppermint on the population processes of the lesser grain borer were assessed by analysing such parameters as: population number, mortality and the population’s sex ratio. The data were statistically analysed using the Chi-square test from Statistica v. 5.5 s. 999 –FGPJ – N4.

Results and discussion

The addition of powdered peppermint to wheat caused a decrease in *R. dominica* population up to the 220 th day of the experiments. After this period until the 310 th day, no inhibiting influence upon the population of *R. dominica* was found and from then on the population number was higher than in the control. On the other hand, the addition of Herb Robert to the wheat caused a significant decrease in the *R. dominica* population during the whole period of the experiments. Up to the 190 th day the population development was strongly inhibited (Fig. 1). Chi-square analysis revealed that differences between population size on the wheat substrate and the substrate of wheat with Herb Robert were highly significant from the 70 th day to the end of the experiments. However, between the wheat substrate and wheat with peppermint, differences in population size were only statistically significant from the 70 th to 130 th day and after the 190 th and 220 th days. Furthermore, the total population number in the peppermint substrate during the whole investigative period was comparable with the control culture, whereas in the substrate containing Herb Robert the population was more than three times lower than in the control culture.

The highest mortality rate was found in wheat with Herb Robert, and this was maintained during the whole period of the experiments. It was higher at each time interval in comparison with mortality rate of the control population bred on wheat only. A particularly high mortality rate was caused by Herb Robert at the time interval between the 40 th and 160 th day of the experiments. On the other hand, with the addition of peppermint in the food, no increase in the mortality rate was noted. The mortality was even lower than in the control culture (Fig. 2).

Much information can be found in the scientific literature concerning plants that reduce population growth of granary pests. Sharaby (1989) stated that powdered leaves of selected Myrtaceae guava *Psidium guajava* (L.), and eucalyptus *Eucalyptus globulus* (LABLL) considerably reduce the development of the rice weevil *Sitophilus oryzae* L. and the granary weevil *Sitophilus granarius* L.. It became apparent that the leaves of the guava were more
toxic for both species than the leaves of the eucalyptus, which, in turn, had a much stronger repellent effect on the two insect species.

Fig. 1. Comparison of population dynamics of *Rhyzopertha dominica* in different habitat and diet conditions.

Błażejewska and Cieślińska (1996) studied the influence of the dried fruit of coriander (*Coriandrum sativum*) and fennel (*Foeniculum vulgare*) on the development and fertility of the rice weevil. It was found that the fruit of the latter had a stronger influence than the former.

Recently Kłyś (2006) who studied the effects of the powdered herbs: sage (*Salvia officinalis* L.), wormwood (*Artemisia absinthium* L.), lavender (*Lavandula officinalis* L.) and peppermint (*Mentha piperita* L.) on the population processes of the saw-toothed grain beetle *Oryzaephilus surinamensis* L. came to the conclusion that peppermint and wormwood exert a particularly strong influence on this insect. All the plants used in the experiments caused a decrease in the number and an increase in the mortality of the populations. The author
obtained similar results in studies on *R. dominica* populations with the application of sage (Kłyś 2004).

In the scientific literature there are no specific data concerning the effects of natural plants on the sex ratio of storehouse pest populations. Some information on this subject is given in investigations carried out by Kłyś (2004, 2004). The author found some disturbances taking place in the sex ratio of a population influenced by the addition of applied plants. Similarly, it was found that the introduction of chemical substances contained in *G. robertianum* and *M. piperita* to the food generally caused an increased number of males in the population of live individuals. This is shown by an increase of the sex ratio above 1 (males:females) during most of the time intervals, in comparison with the control population (Fig. 3). Conversely, among the individuals dying out in the population, the sex ratio was lower than 1 from the beginning to the end of the experiments. This reveals a higher mortality rate of the females in the food substrate with the plant additives (Fig. 4).

![Graph](image_url)

**Fig. 3.** Comparison of sex ratios in *Rhyzopertha dominica* in different habitat and diet conditions (individuals live).

Banasik and Ignatowicz (1995) examined the repelling or attracting effect of powdered, selected plant species on the granary weevil and the rice weevil individuals. The authors showed some increase in repellent properties of the powders together according to their concentration in wheat grains. To deter these pests from infesting the stored product, they recommend using powders from the tansy (*Tanacetum vulgare* L.), sweet clover (*Melilotus officinalis* L.), yarrow (*Achillea millefolium* L.), and the purple deadnettle (*Lamium purpureum* L.). Similarly, Kłyś (in press) has shown a deterrent effect of powdered sage on *R. dominica* individuals.

Moreover, Ignatowicz and Wesolowska (1994) examined to what extent the substances existing in herbs from dill seeds *Anethum graveolens* L., chamomile *Matricaria chamomilla* L., and black elderberry *Sambucus nigra* L. flowers as well as from wormwood *Artemisia absinthium*, which were sprayed as water extracts on wheat grains, affect the behaviour and reproduction of the granary weevil. They came to the conclusion that the extracts from sage only do not show the repellent properties, but they do have a negative effect on reproduction of the granary weevil.
Koona and Njoya (2004) also used powdered leaves of *Lantana camara* L. and soybean oil as protectants of stored maize against infestation by *Sitophilus zemais* Motsch. They showed that there was significantly lower percentage damage when the maize grains were treated with either of the two natural products.

Mishra *et al.* (2007) studied the efficacy of application of vegetable seed oils as grain protectants against infestation by *Callosobruchus chinensis*. They evaluated the dissolved extracts of vegetable seed oils from bitter gourd (*Momordica charentia*), small bitter gourd (*Momordica dioica*), bottle gourd (*Lagenaria siceraria*) and ridge gourd (*Luffa acutangula*) as grain protectants against *C. chinensis* in stored legume-pulse grains. They found that all the seed oils were effective as grain protectants.

Similar investigations were carried out by Nawrot (1983) on plants of specific smell, such as: *Tanacetum vulgare*, *Artemisia absinthium*, *Ambrosia arthemisifolia*. He searched for chemical compounds with a deterrent effect on the adult beetles of *Tribolium confusum* Duv., and *Sitophilus granarius* L., and on the larvae of *Trogoderma granarium* Ev.

These results concerning the effects of powdered Herb Robert and peppermint plants on populations of *R. dominica* enable a deduction to be made that Herb Robert added to wheat more strongly reduces the development, and inhibits the population numbers of the lesser grain borer better than the peppermint. The chemical compounds contained in the powdered Herb Robert are antifeedants for this insect species. Under the influence of Herb Robert, the population mortality rate increases. Furthermore, the addition of these two herbs to wheat grains causes an increase in proportion of males in the population, as a result of a higher mortality rate of the females. It is suspected that females are more sensitive to the effect of the chemical compounds contained in these plants. Further investigations on the behaviour of Herb Robert may also elucidate our suspicion of the existence of chemical compounds inhibiting the feeding of insects and may help us to find effective methods of reducing the population numbers of *R. dominica*.

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**Fig. 4.** Comparison of sex ratios in *Rhyzopertha dominica* in different habitat and diet conditions (individuals dead).
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Effectiveness of bitterbarkomycin against *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in stored maize

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Abstract: The insecticidal effect of bitterbarkomycin (BBM), which is a sesquiterpene extract from the plant *Celastrus angulata* Maxim. (Celastrales: Celastraceae), was evaluated at the dose rates of 0.01, 0.05 and 0.1 ppm on stored maize, against adults of three stored-grain beetle species, the lesser grain borer, *Prostephanus truncatus* (Horn) (Bostrychidae), *Rhyzopertha dominica* (F.) (Bostrychidae), the larger grain borer, and the rice weevil, *Sitophilus oryzae* (L.) (Curculionidae). Mortality was measured on treated maize after 7, 14 and 21 d of exposure. From the species tested, *S. oryzae* was by far the least susceptible, since mortality did not exceed 13 % even after 21 d of exposure on maize treated with the highest BBM dose. At the same conditions, *R. dominica* mortality reached 100 % after 14 d of exposure while at 0.01 ppm of BBM, approx. 55 % of the exposed *R. dominica* adults were dead. In the case of *P. truncatus*, mortality, after 21 d, was 100 % even at the lowest dose rate. The present results indicate that BBM can be used with success to protect stored maize against *R. dominica* and *P. truncatus*, but higher dose rates and longer exposures are needed for *S. oryzae*.

Key words: *Prostephanus truncatus*, *Rhyzopertha dominica*, *Sitophilus oryzae*, bitterbarkomycin, botanicals, grain protectants.

Introduction

Grain protectants are residual contact insecticides that are applied directly on the grains to protect them against pests during storage (Arthur 1996). However, the development of resistance by several species, as well as the residues that can often found in food, make it necessary to develop of new, reduced-risk substances. Among these, some botanicals combine high insecticidal efficacy with low mammalian toxicity while at the same time are safe for the environment (Weaver and Subramanyam 2000).

Prakash and Rao (1997) record >200 plant species which have been evaluated against several stored product insect species. One of the most common plant species in this effort is the neem tree, *Azadirachta indica* A. Juss (Meliaceae), since there are >100 published works so far, for various stored-grain insect species. Also, many researchers have tested *Acorus calamus* L. (Acoraceae), *Vitex negundo* L. (Verbenaceae), *Arachis hypogaea* (Fabaceae) and *Cocos nucifera* L. (Arecaceae). However, in most of the cases the required dose rates of these plant derivatives are high; for instance, azadirachtin, the main toxic component of *A. indica*, should be applied at doses 50-200 ppm or even higher (Athanassiou et al. 2005). This fact
affects negatively the potential of a wider use of botanicals, over traditional grain protectants, since the latter are effective at doses <10 ppm and in many cases even <1 ppm. Hence, new plant extracts, that can be applied at low doses, comparable with those for grain protectants, should be evaluated.

The plant *Celastrus angulata* Maxim (Celastraceae) is indigenous to east countries of Asia and mainly in China. Pulverized skin root of this plant has been used in the past against insects (Jacobson and Crosby, 1971). Wang et al. (1991) isolated, from skin root of *C. angulata* the sesquiterpene polyol ester angulatin A, known as bitterbarkomycin (BBM), which has increased insecticidal activity. The authors tested with success BBM, at doses between 25 and 50 ppm, against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *Aphis gossypii* Glover (Hemiptera: Aphidoidea), *Pieris rapae* (L.) (Lepidoptera: Pieridae) and *Brevicoryne brassicae* L. (Hemiptera: Aphidoidea). Athanassiou et al. (2006) found that the combination of BBM with diatomaceous earth (DE) in a single formulation (DEBBM) at low doses was very effective against several stored-grain beetle species.

The term primary colonizers describe insects that can infest with ease sound kernels, and this infestation accommodates the infestation from other species, the secondary colonizers, which are unable to infest sound kernels. From the spectrum of stored-grain insect species, only few are classified in the category of primary colonizers, and these species are regarded extremely dangerous for grain commodities. Moreover, the immature stages of these species complete their development inside the kernel, and most of the time they are not exposed to toxic substances that are applied on the external kernel part. In this study, we evaluated the insecticidal effect of BBM against three species of primary pests, the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), the lesser grain borer, *Rhizopertha dominica* (F.) (Coleoptera: Bostrychidae) and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae).

**Materials and methods**

**Insects, commodity and formulation**

Adults of *P. truncatus*, *R. dominica* and *S. oryzae* were used in the tests. *P. truncatus* was reared on whole maize and the adults of *R. dominica* and *S. oryzae* on whole hard wheat, at 29±1°C, 70% RH and continuous darkness. All tests were conducted on untreated, clean maize. The BBM formulation was an EC formulation that contained 0.5% of a.i. (BBM).

**Bioassays**

The dose rates used were 0.01, 0.05 and 0.1 ppm of BMM. Lots of 720 g of maize were sprayed with 30 ml of water that contained the above-mentioned BMM doses, while an additional series of lots was spayed with water, and used as controls. From each lot, 4 samples, of 60 g each, were taken and placed in cylindrical glass vials (8 cm height, 6.5 cm diameter). Then, 30 adults of *P. truncatus* were introduced into each vial. The same procedure was repeated for adults of *R. dominica* and *S. oryzae*, by taking new samples from the maize lots. The vials were then placed in incubator chambers at 28 °C and 75% RH. Dead adults were counted after 7, 14, and 21 days of exposure in the treated and untreated commodity.

**Statistical analysis**

Mortality in the control vials was low (<5%), so no correction was considered necessary. The data were submitted to one-way ANOVA, separately for each species and exposure (in all cases df=2.9). For the comparison of the means, the Turkey-Kramer HSD test was used at 0.05.
Results

Mortality of *P. truncatus*

After 7 d of exposure, significant differences were noted between the 3 doses used (F=16.9; P=0.0009). At the two higher doses, mortality was 76 and 87 %, respectively, which was significantly higher than the mortality at the lowest dose. At the 14-d exposure, mortality at the two highest doses was 99 and 100 %, respectively, while at the low dose mortality did not exceed 73 % (F=14.7; P=0.0015). Similar trends were also recorded one week later (F=7.5; P=0.012) (Fig. 1).

![Fig. 1](image.png)

Fig. 1. Mortality of *P. truncans* adults (% ± SE) on maize treated with three doses of BBM, after 7, 14 and 21 d of exposure (within each exposure, means followed by the same letter are not significantly different; HSD test at 0.05).

Mortality of *R. dominica*

After 7 d of exposure, significant differences were noted among the three BBM doses (F=32.7; P<0.0001), but mortality did not exceed 56%. One and two weeks later, all adults were dead at the two highest doses, but at the lowest dose mortality was 38 and 56 %, respectively (for 14 d, F=56.3; P<0.0001, for 21 d, F=40.5; P<0.0001) (Fig. 2).

Mortality of *S. oryzae*

For this species, mortality was extremely low, and did not exceed 17 % for any of the cases examined. Moreover, there were no significant differences among doses (for 7 d, F=0.81; P=0.47, for 14 d, F=2.02; P=0.19, for 21 d, F=1.19; P<0.35) (Fig. 3).

Discussion

Athanassiou et al. (2006) found that DEBBM, an BBM enhanced DE formulation was very effective against *P. truncatus*, *R. dominica* and *S. oryzae*, at the application rate of 75 ppm of DEBBM. Also, Athanassiou and Korunic (2007) found that *R. dominica* and *S. oryzae* were
more susceptible to DEBBM than the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Nevertheless, the use of BBM alone has not been evaluated as a grain protectant so far. In the present study, BBM was effective against the two Bostrychids, *P. truncatus* and *R. dominica*, since complete (100%) mortality occurred with 0.05 ppm, which can be considered as an extremely low dose rate not only for botanicals but also for the majority of traditional grain protectants. This is particularly important, since both species are particularly tolerant to many insecticides. For instance, *R. dominica*, is resistant to many organophosphorous insecticides (Zettler and Cuperus 1990), many of which are very effective against most common stored-product pests. Similarly, *P. truncatus*, a key pest of stored maize in central Africa, is resistant to pyrethroids, which can easily control other maize pests, such as the maize weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). The high BBM efficacy recorded here, and the factors that affect this effect, should be further evaluated against these two species.

![Fig. 2. Mortality of *R. dominica* adults (% ± SE) on maize treated with three doses of BBM, after 7, 14 and 21 d of exposure (within each exposure, means followed by the same letter are not significantly different; HSD test at 0.05).](image)

In contrast with the two Bostrychids, BBM had no effect in *S. oryzae* adults. Also, although progeny data are not presented in this study, we observed relatively high numbers of *S. oryzae* offspring in the treated substrate, suggesting that BBM had no effect in suppressing progeny production. On the other hand, progeny production for *P. truncatus* and *R. dominica* was completely suppressed in the BBM-treated substrate. This may be attributed to the increased parental mortality, but also to some larvicidal effect, since both species oviposit at the external kernel part, and newly-hatched larvae enter the kernel, which means that they contact the treated substrate. In contrast, *S. oryzae* females oviposit at the internal kernel part, so larvae have no contact with the toxic agent. Higher BBM dose rates and longer exposure intervals are required to be evaluated against *S. oryzae*.

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The high dose rates that are required for botanicals in order to obtain a satisfactory level of insect control are the main drawbacks to their wider use. For instance, in the case of aza-
dirachtin, given that a mature neem tree produces approx. 5.5 kg of seed kernels (Koul et al. 1990), a number of neem trees would be needed for use in eg. 1000 t of wheat (Weaver and Subramanyam 2000). This fact makes, for the time being, the use of many botanicals impractical for large-scale applications. A formulation of high a.i. content could be a solution to this implication, but such a formulation may substantially increase the cost of the formulation. However, BBM is a botanical that seems to be effective at extremely low doses, at least for some species, and for this purpose, BBM is a potential candidate for further experimentation, under a wider range of conditions (species, climate conditions, commodities etc.). Although nowadays the use of botanical in stored commodities is limited, some botanicals are expected to play a certain role in the future, as a part of an ecologically compatible, IPM-based control strategy.

Fig. 3. Mortality of *S. oryzae* adults (% ± SE) on maize treated with three doses of BBM, after 7, 14 and 21 d of exposure (within each exposure, means followed by the same letter are not significantly different; HSD test at 0.05).

References


Insecticidal activity of essential oil from *Vitex agnus-castus* against *Callosobruchus maculatus*

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Abstract: To control stored product insect pests, studies are increasingly focused on plants with insecticidal activity. In this study, fumigant toxicity of essential oil from *Vitex agnus-castus* L. was assessed against *Callosobruchus maculatus* (F.). Dry aerial parts of the plant were subjected to hydrodistillation using a modified Clevenger-type apparatus. Fumigant toxicity was tested against 1-7 days old adults of *C. maculatus* with five replications at 25±1°C and 65±5% RH in dark condition. Mortality at 37 µl/l air reached 40% after 24 h exposure. While at 370 and 556 µl/l air mortality was about 50% after 12 h exposure. At the same concentrations, the mortality increased to more than 90% after 21h. At the highest concentration (740 µl/l air) 100% of mortality was observed after 18 h exposure. The LC50 value of the oil was estimated to be 219.46 µl/l air (95% fiducial limits: 147.32-346-73 µl/l air). These results indicated a strong activity of the oil on *C. maculatus*. So it is suggested that natural pesticides based on plant essential oils may represent alternative stored-product protectants whose time has come.

Key words: botanical insecticides, essential oil, *Vitex agnus-castus, Callosobruchus maculatus*

Introduction

Pulses form an important component in the diet of most of people. After harvest, these goods are usually stored for long periods. Stored grains can be destroyed by insects, fungi and vertebrate pests. Insect pests are often the most important because of the favourable climatic conditions for their development (Alzouma, 1990). The pulse weevil, *C. maculatus* is one of the most important insect pests of stored legumes. The use of synthetic insecticides is recognized as the conventional way to control these insect populations. These are the most effective treatments for the protection of stored food but, their repeated use for decades has disrupted biological control by natural enemies and led to outbreaks of other insect species and sometimes resulted in the development of resistance (Champ & Dyte, 1976; Subramanyam & Hagstrum, 1995; White & Leesch, 1995). Some plant secondary metabolites have important role in plant-insect interactions, and are commonly responsible for plant resistance to insects (Mann, 1987). The present study was conducted to determine the efficiency of the essential oil from *V. agnus-castus* as a fumigant in the management of *C. maculatus*.
Materials and methods

Insect cultures
*Callosobruchus maculatus* were reared on bean grains. Adult insects, 1-7 days old, were cultured in a controlled temperature and humidity chamber (25±1°C and 65±5% RH) in darkness. All experiments were carried out under the same environmental conditions.

Plant material and extraction of essential oil
Aerial parts of *Vitex agnus-castus* were collected in September, 2005 from Qum in Iran. The leaves were cut into small pieces, washed with distilled water and the volatile oil obtained by hydrodistillation (3h) using Clevenger’s apparatus. The light-yellow oil (yield 1.5%) thus obtained was dried over anhydrous sodium sulphate and stored at 4 °C.

Fumigant toxicity
To determine the fumigant toxicity of the *V. agnus-castus* oil, filter papers (Whatman No. 1, cut in 2 cm diameter) were impregnated with oil at doses calculated to give equivalent fumigant concentrations of 37 to 740 µl/l in air. The impregnated filter papers then were attached to the screw caps of glass vials with volumes of 27 ml. Caps were screwed tightly on the vials each of which contained ten adults (1-7 days old) of each species only one species of insect. Each concentration and control was replicated five times. Mortality was determined after 3, 6, 9, 12, 15, 18, 21 and 24 h from commencement of exposure. Percentage insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls (Abbott, 1925). Another experiment was designed to assess 50% and 95% lethal doses. A series of dilutions was prepared to evaluate mortality of insects after an initial dose setting experiment. Ten adults of *C. maculatus* were put into 620 ml glass bottles with screw lids, which were dosed as described in the first experiment above. Mortality of *C. maculatus* was evaluated at 32.26, 129.03, 258.06, 387.10 and 451.61 µl/l air. Control insects were kept under the same conditions without any essential oil. Each dose was replicated five times. The insects were exposed for 24h to the essential oil vapour and after 48h the dead insects were counted. The mortality was determined as described in previous experiment. Data obtained from the various dose-response bioassays were subjected to probit analysis (Finney, 1971) in order to estimate LC50 values. When no leg or antennal movements were observed, insects were considered dead.

Results and discussion

In all cases, considerable differences in mortality of insects to essential oil vapour were observed with different concentrations and times. The mortality increased with concentration from 37 to 740 µl/l air and with exposure time from 3 to 24h. Mortality at 37 µl/l air reached 40% after 24 h exposure, while at 370 and 556 µl/l air mortality was about 50% after 12 h exposure. At the same concentrations, the mortality increased to more than 90% after 21h. At the highest concentration (740 µl/l air) 100% of mortality was observed after 18 h exposure (Figure 1). The LC50 value of the oil was estimated to be 219.46 µl/l air (95% fiducial limits: 147.32-346-73 µl/l air) (Table1). Studies have not been reported previously concerning the activity of *V. agnus-castus* as a fumigant on insect pests. In this study, essential oils from *V. agnus-castus* showed potent toxicity to *C. maculatus*. In all cases, an increase in mortality was observed with increasing time of exposure and concentration. *Vitex agnus-castus* resulted in quick knock down followed by the death of the insect in a period of time as short as 24 h. The efficacy of *V. agnus-castus* oil described here seems to have more fumigant toxicity than that of some other
species. The essential oil from Labiatae species (ZP51) resulted in 85-100% mortality on four stored product insect species within 4 days of exposure at 70 µl/l air (Shaaya et al., 1997). However, the *V. agnus-castus* essential oil described here appears to have lower fumigant toxicity than that of the oils from *Carum copticum* C. B. Clarke (Sahaf et al., 2007), *Artemisia aucheri* Boiss (Shakarami et al., 2003), *Artemisia scoparia* Waldst and Kit (Negahban et al., 2006) and *Artemisia sieberti* Besser (Negahban et al., 2007). The essential oils that are most efficacious against pests are often the most phytotoxic. However, more studies are needed on this common plant to establish its potential as a control agent against stored grain pests.

![Graph showing percent mortality of *Callosobruchus maculatus* exposed to different concentrations of *Vitex agnus-castus* essential oil.](image)

**Fig. 1.** Percent mortality of *Callosobruchus maculatus* exposed to different concentrations of *Vitex agnus-castus* essential oil.

**Table 1.** Fumigant toxicity of *Vitex agnus-castus* essential oil against *Callosobruchus maculatus*.

<table>
<thead>
<tr>
<th>Insect species</th>
<th>LC50&lt;sup&gt;1,2&lt;/sup&gt; (µl/l)</th>
<th>LC95&lt;sup&gt;1,2&lt;/sup&gt; (µl/l)</th>
<th>Slope±SE</th>
<th>Degree of freedom</th>
<th>Chi square (χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. maculatus</em></td>
<td>219.46 (147.32-346.73)</td>
<td>11333.00 (3262-220851)</td>
<td>0.96±0.20</td>
<td>3</td>
<td>0.42</td>
</tr>
</tbody>
</table>

<sup>1</sup> Units LC<sub>50</sub> and LC<sub>95</sub> = µl/l air, applied for 24 h at 25°C.

<sup>2</sup> 95% lower and upper fiducial limits are shown in parenthesis.

**References**


Session 6:
Physical, chemical and other techniques for stored product protection
Susceptibility of life stages of *Tribolium confusum* du Val to gaseous ozone

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Abstract: In this study susceptibility of life stages *Tribolium confusum* du Val. to gaseous ozone was tested. The toxicity of gaseous ozone at high initial concentration of 19.4 mg/L for a 2-h exposure period against all life stages of *T. confusum* was studied. Susceptibility of all life stages of *T. confusum* to ozone fumigation for a 5h of exposure period in the presence of two kg of wheat was also tested. Toxicity data for empty space ozone treatments indicated a remarkable difference in susceptibility between life stages of *T. confusum*. Ozone treatment resulted in very low mortalities of the adults, pupae and eggs of *T. confusum*, ranging from 4.2 to 14.1 % while the only larvae stage had a high mortality (74%). Adults, eggs and pupae of *T. confusum* were the most tolerant to ozone treatment, while the larvae were easy to kill. For every half-hour flushed ozone fumigation for 5-h in the presence of commodity, there was a significant difference in the mortalities of adults, larvae, pupae and eggs of *T. confusum* placed on top and the bottom of two kg of wheat. These results indicated that gaseous ozone could present a problem of penetration into a commodity. Toxicity data for half-hour flushed ozone fumigation in the presence of commodity also indicated a remarkable difference in susceptibility between the life stages of *T. confusum*. The larvae placed on the bottom of two kg of wheat were easily killed, whereas eggs, pupae and adults of *T. confusum* were still tolerant. These findings indicate that ozone treatment resulted in a remarkable difference in susceptibility of the various life stages of *T. confusum*.

Key words: Gaseous ozone, *Tribolium confusum*, life stage, susceptibility, toxicity

Introduction

Ozone is a triatomic form of oxygen (O3) and is referred to as activated oxygen, allotropic oxygen or pure air. It is an unstable gas and its life span is about 20 minutes, depending on the temperature. Thus, it does not accumulate substantially without a continuous ozone generator (Peleg, 1976; Miller et al., 1978). At room temperature, ozone is nearly a colourless gas. Ozone has a pungent, characteristic odour described as similar to “fresh air after a thunderstorm” (Coke, 1993). It has a longer half-life in the gaseous state than in aqueous solution (Rice, 1986). Ozone in pure water rather quickly degrades to oxygen, and even more rapidly in an impure solution (Hill and Rice, 1982). Ozone is a blue gas at ordinary temperature but at concentrations at which it is normally produced the colour is not noticeable. Ozone can be generated by electrical charges in air and is currently used in the medical industry to disinfect microorganisms and viruses, as a means of reducing odour, and for removing taste, colour, and environmental pollutants in industrial applications (Kim et al., 1999).
Electrical generation of ozone eliminates the handling, storage, and disposal problems of conventionally used post-harvest pesticides. An attractive feature of ozone is that it decomposes rapidly (half-life of 20-50 min) to molecular oxygen without leaving a residue. These attributes make ozone an attractive candidate for controlling insects and fungi in stored products. At low concentrations ozone protected clean surfaces from subsequent fungal contamination and growth, although higher doses are required to kill fungi on contaminated surfaces (Rice et al., 1982). Five ppm ozone inhibited surface growth, sporulation, and mycotoxin production by cultures of Aspergillus flavus link: Fr and Fusarium moniliforme Sheldon (Mason et al., 1997).

Ozone in its gaseous form has been also considered to have a potential to kill insect pests in commodities and was subjected to several research studies (Erdman, 1980; Mason et al., 1997; Kells et al., 2001). High mortality was achieved for adults of the maize weevil, Sitophilus zeamais (Mostsch.), and the red flour beetle Tribolium confusum (Jacqueline du Val), and the larval stage of the Indian meal moth, Plodia interpunctella (Hübner) exposed to ozone concentrations ranging from 5 to 45 ppm (Erdman, 1980; Kells et al., 2001). Erdman (1980) also observed mortality of larvae of the confused flour beetle, T. confusum and the red flour beetle, T. castaneum (Herbst) when exposed to a 45 ppm ozone environment. Other than the aforementioned research, little has been done to determine the susceptibility of life stages of stored product insects against ozone treatments. Our study was therefore designed to test susceptibility of various life stages, T. confusum to gaseous ozone.

Materials and methods

Test insects
Tests were carried out on all life stages (adult, larva, pupa and egg) of T. confusum. All stages were obtained from cultures reared at 26 ± 1°C and 65 ± 5% relative humidity (r.h.) on a diet of wheat flour and brewer yeast using standard culture techniques (Donahaye, 1990). Eggs aged 1-2 days in 9 cm Petri dishes were placed in 3 L jars and then were exposed to the treatments. Larvae were removed from culture jars and exposed to the treatments 21 days after oviposition. Two day old pupae were obtained by daily separation from culture jars and were exposed to the treatments. Newly emerged aged 0-1 day were placed in empty exposure jars and then were exposed to the treatments.

Fumigation chambers
Test chambers consisted of 3 L glass jar, each capped with a metal stopper equipped with entry and exit tubing. A magnetic stirrer placed in the bottom well beneath a wire-mesh disc served to mix the air with the ozone. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps. The desiccators were sealed with silicone vacuum grease.

Ozone fumigation procedures
Ozone generator in laboratory scale was provided from Ozomax Inc., Canada (http://www.ozomax.com). Ozone gas was generated using a laboratory corona discharge ozone generator (Model OZO-1VTT) from purified extra dry oxygen feed gas. Gaseous zone was introduced into the exposure jars using an ozone generator. Pressure in each jar was measured using a 0 to 800 mm Hg vacuum digital gauge (Celesco-model SE-2000, U.S.A.). The 100 mm Hg measure referred to herein is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Prior to each test, twenty larvae, pupae or adults were confined, separately, inside 3 cm diameter by 8 cm long wire-mesh cages. Fifty eggs placed in opened Petri dishes, used per fumigation, to test the effect of the treatment on the eggs,
For a single application of high ozone concentration, the eggs, pupae, larvae and adults of *T. confusum* were exposed to initial ozone concentration of 19.4 mg/l for 2 hours. The insects were first placed in exposure jars and then, the desiccators were briefly evacuated to 760 mm Hg. Afterwards gaseous ozone was flushed into the exposure jar until reaching atmospheric pressure and the insects were exposed to for 2 hours. For intermittently repeated ozone treatments in the presence of commodity, each desiccator was loaded separately with two kg of wheat, and then 50 eggs, 25 pupae, adults and larvae were confined inside wire-mesh cages and inserted into top and bottom position of the commodity. The desiccators were briefly evacuated to 760 mm Hg. Afterwards gaseous ozone was flushed into the exposure jar until reaching atmospheric pressure. Repetitions were made every half hour for 5 hours. Untreated control insects were exposed to atmospheric conditions.

Each test was replicated three times. The gas mixtures in the desiccators were stirred for at least 20 min. For all fumigations, r.h. and temperature were maintained at 65±5% at atmospheric pressure and 30±2°C, respectively. Pressure inside the desiccators was checked at the end of each test. Relative humidity within the desiccators during fumigations was also measured by placing small mechanical hygrometers.

**Data processing and analysis**

After each treatment, larvae, pupae, and adults were transferred to 250-mL jars containing standard diets and were held at 26 ± 1°C and 70 ± 5% r.h. until examined for mortality. The eggs in their Perspex slides were held under the same conditions until the oviposition sites were examined for egg hatch. Mortality counts for adults were made 4-5 days after exposure. For larvae they were based on those insects that had failed to pupate 9 days after exposure. Pupal mortality was based on those pupae that failed to produce adults 9 days after exposure, and egg hatch was counted 7 days after treatment. Mortality data was subjected to Arcsin transformation and then, were analyzed using one-way analysis of variance (ANOVA). The means were separated using the LSD method at 1% level (SAS Institute, 1985).

**Results and discussion**

Comparison of susceptibility of the life stages of *T. confusum* against high concentration of gaseous ozone treatment for 2 hours is given in Fig. 1. Toxicity data for empty space ozone treatments indicated a remarkable difference in susceptibility between the various life stages of *T. confusum*. Ozone treatment resulted in very low mortalities of the adults, pupae and eggs of *T. confusum*, ranging from 4.2 to 14.1 % while only the larvae stage had a high mortality (74%). Adults, eggs and pupae of *T. confusum* were the most tolerant to ozone treatment, while the larvae were easy to kill.

These findings may be compared with several studies on the efficacy of ozone to control insect pests of stored grain. The results obtained by Kells et al. (2001) indicated that high mortality was achieved for adults of the maize weevil, *S. zeamais*, and the red flour beetle, and the larval stage of the Indian meal moth, *P. interpunctella* exposed to 50 ppm ozone for 3 days. In a laboratory study, Mason et al., 1997 reported 5 ppm of ozone resulted in %100 mortality of adult saw-toothed grain beetle, *O. surinamensis* and confused flour beetle after exposure times, of 3 and 5 days, respectively. However, in these studies the time of treatment was too long (3 days), which is much higher than that in our studies. Leesch (2002) reported that different stages (apart from eggs) of *P. interpunctella* were more or less susceptible to laboratory treatment of ozone alone at high concentration (300 ppm) at short exposure time (4 hours). In the same study, adults were most easily killed, followed by larvae, pupae and finally eggs, which were unaffected. Susceptibility of life stages of *P. interpunctella* to ozone
treatment reported by Leesch (2002) is different from those of T. confusum obtained from our study. The different susceptibility of life stages to ozone treatment would appear to be attributed to the difference of insect species tested, ozone application concentration and method of application.

Fig. 1. Comparison of susceptibility of life stages of T. confusum against high concentration of gaseous ozone treatment.

Susceptibility of life stages of T. confusum placed on the top position and submitted to 5-h exposure intermittently gaseous ozone treatment in the presence of 2 kg of wheat is given in Fig. 2. Toxicity data for intermittent half-hour flushed ozone fumigations in the presence of commodity also indicated a remarkable difference in susceptibility of the various life stages of T. confusum. For every half-hour flushed ozone fumigation for 5-h in the presence of commodity there was a significant difference in the mortalities of adults, larvae, pupae and eggs of T. confusum placed in top of two kg of wheat. Ozone treatment resulted in very low mortalities of the adults, pupae and eggs of T. confusum, ranging from 4.2 to 7.3 % while the only larvae stage had a high mortality (44.1%) while the only larvae stage had a high mortality (86.2%).

Susceptibility of larvae of T. confusum placed in top and bottom positions against different application method of ozone treatments in presence of 2 kg of wheat is presented in Fig. 4. Intermittent half hour flushed ozone treatments resulted in almost complete mortality of larvae of T. confusum placed in both top and bottom positions of 2 kg wheat, while the larvae placed in both top and bottom position of 2 kg wheat had a very mortality for only one flushed ozone treatment. These results indicated that gaseous ozone needs to be re-flushed intermittently to keep relevant concentration and thus obtained a higher mortality of the insects. Strait (1998) established that ozone, following fumigation of small-scale grain storage bins (18 kg) containing yellow maize, dispersed throughout the grain mass and was toxic to insects within that mass. A concentration of 50 ppm for 3-days resulted in 100% mortality of adult confused flour beetles and maize weevils, S. zeamais, and greatly reduced emergence of P. interpunctella. Initial movement of ozone through the grain was impeded by a phenomenon described as the ozone demand of the medium (Kim et al., 1999).
Fig. 2. Susceptibility of life stages of *T. confusum* placed at the top position against 5-h exposure intermittently gaseous ozone treatment in presence of 2 kg of wheat.

Susceptibility of life stages of *T. confusum* placed at the bottom position against 5-h exposure intermittently gaseous ozone treatment in presence of 2 kg of wheat is presented in Fig. 3. The larvae placed in the bottom position of two kg of wheat were easily killed, whereas eggs, pupae and adults of *T. confusum* were still tolerant.

Fig. 3. Susceptibility of life stages of *T. confusum* placed at bottom position against 5-h exposure intermittently gaseous ozone treatment in presence of 2 kg of wheat.
All these findings indicate that ozone treatment resulted in a remarkable difference in susceptibility for the various life stages of *T. confusum*. It appears that gaseous zone could also have a problem of penetration through commodity. Many factors influence fumigation, including equipment performance and environmental conditions. The abilities and limitations of ozone penetration through the commodity and insect efficacy are important considerations for scale-up fumigation of commercial facilities.

**Acknowledgements**

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Application of ozone as fumigant to prevent unwanted biological activity in stored grain

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**Abstract:** Many scientific studies have shown that Ozone (O$_3$) has the potential to eliminate biological activity (insects, mould and mites) in stored crops. Studies done in Purdue University indicate that Ozone does not harm enzymes and oils inside the kernels of the grain, and that other features of grains. The purpose was to produce a machine for full scale treatment of stored grain in common silo complexes, and to demonstrate that the treatment – shown effective in the laboratory – can be up scaled to full size silo complexes. CROP-PROTECTOR KS has produced two basic models with different capacities. Field research data were obtained from full scale silos showing that it is possible to obtain a near to 100% mortality of the grain weevil and other species using the CROP-PROTECTOR® machines. However in one silo complex we found it might be difficult to obtain 100% mortality, most likely due to high concentrations of “dust” in the stored grain. Experience indicates that the amount of Ozone necessary for successful treatment may vary from site to site, and that the energy level of the Ozone molecules may play an important role when determining how much Ozone is needed to treat one ton of grain. The potential of Ozone to replace traditional fumigants are considered, and it is concluded that ozonation (using Ozone as a fumigant) is a very effective alternative, which is also considers safer and cheaper.

**Key words:** Ozone, stored grain, insect control, silo treatment
Combination of biological control and CO\textsubscript{2} treatments against \textit{Plodia interpunctella} (Hübner)

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Abstract: The Indian meal moth (\textit{Plodia interpunctella} (Hübner)), is one of the most important pests of stored products and structures in Spain. \textit{Blattisocius tarsalis} (Berlese) (Acari: Ascidae) is a native predatory mite of Lepidoptera eggs. Biological control with \textit{B. tarsalis}, as well as \textit{CO\textsubscript{2}}-comprising modified atmospheres at atmospheric or high pressure, was evaluated in the laboratory. \textit{B. tarsalis} was able to prey on \textit{P. interpunctella} and to reduce its population. Dosages with modified atmospheres, needed for the control of \textit{P. interpunctella}, were also effective for the control of the natural enemy.

Key words: \textit{Plodia interpunctella}, \textit{Blattisocius tarsalis}, biological control, modified atmospheres

Introduction

Biological control is recently receiving attention as an alternative method in pest control because chemical control, which is the most important way of controlling pests in stored products, may cause residue problems when insecticide treatments are intensively applied. Nevertheless, there is not enough information on natural enemies of stored product pests to successfully apply IPM programs (Schöller and Prozell, 2003). Biological control is the use of parasitoids, predators and/or pathogen populations to suppress a pest population, making it less abundant and thus less damaging than it would otherwise be (van Driesche and Bellows, 1996). Some advantages of biological control are that it is innocuous to users and consumers, and harmless to the environment; natural enemies are very effective in locating the pest population and, due to the long history of co-evolution with their host, there is no risk that the pest becomes resistant to its natural enemy as occurs with pesticides. Some disadvantages of biological control are that an intensive surveillance of pest populations is necessary in order to release the natural enemy in the appropriate time, and the need to have a deep knowledge of pest biology for managing both populations; usually, these organisms have a limited shelf life and cannot be stored for medium or long periods of time and also require specific environmental conditions of temperature and humidity in their transportation and storage. In addition, European regulations are not ready yet for insect releases in stored products since to introduce organisms that may be present in the final product may represent a risk of contamination.

The use of carbon dioxide (\textit{CO\textsubscript{2}}) is an alternative to application of methyl bromide (Navarro, 2006). \textit{CO\textsubscript{2}} causes desiccation to insects due to permanent opening of spiracles, and produces pH changes affecting many important metabolic processes (Nicolas and Sillans, 1989). At high pressure, cell walls are broken. The use of \textit{CO\textsubscript{2}} has several advantages: as there is no accumulation of toxic residues after the treatment has been performed, there is no need of a time period between the application of treatment and the consumption of the food product; \textit{CO\textsubscript{2}} is accepted as food additive (E-290), it is organoleptically neutral on the majority of food products, and it is recyclable when used at high pressures (Riudavets \textit{et al.}, 2006).
The objective of our study was to assess the possibility to combine biological control based on the release of *B. tarsalis* for the control of *P. interpunctella* in the milling facilities, and the subsequent application of a CO₂ treatment at the end of the manufacturing process to eliminate both the remaining pests and predatory mites. For this, the specific objectives were to define the rates of *B. tarsalis* to control the population of *P. interpunctella*, and to prove that the CO₂ dose necessary to eliminate the pest is also efficient to control *B. tarsalis*.

Material and methods

**Insects**

Stock colonies of the predatory mite *B. tarsalis* were started with individuals collected on wheat semolina infested with *Tyrophagus putrescentiae* (Schrank) and *Liposcelis bostrychophila* (Badonnel) in Barcelona, North Eastern Spain, and reared on Vermiculite® and *Ephestia kuehniella* (Zeller) eggs as prey (Nielsen, 1999a). The prey species tested in this study were obtained from stock colonies maintained at the IRTA (Cabrils, Barcelona). All laboratory studies were conducted in a climatic chamber at 25±1ºC, 75 % RH and 16:8 h L:D. Transparent and ventilated plastic cages (7.3 cm diameter and 3.9 cm height) were used as experimental arenas for experiments regarding biological control of *P. interpunctella*, and 0.67 ml hard gelatine capsules (Acofarma) for carbon dioxide treatments.

**Biological control of Plodia interpunctella**

To determine the predatory capacity of *B. tarsalis* on *P. interpunctella* eggs, we tested 6 ratios of *B. tarsalis* females/*P. interpunctella* eggs: 0, 0.05, 0.1, 0.2, 0.5 and 1. In each cage we placed a fine layer of organic whole-wheat flour and 0, 1, 2, 4, 10 or 20 females of *B. tarsalis* together with 20 eggs (0-3 days old) of *P. interpunctella*. After 4 days, time necessary for one *B. tarsalis* to destroy approximately 16 eggs of *Ephestia kuehniella* (Nielsen, 1998), 20 g of whole-wheat flour were added in each cage in order to allow the development of the surviving larvae of the moth. The number of *P. interpunctella* pupae was recorded after 6 weeks and the production of webs by the moth (weight in g) was evaluated as well. The presence of these webs could be a big problem in the factory’s machinery. There were 10 replicates per ratio.

**Carbon dioxide treatments**

CO₂ was tested at high pressure (20 atm.; 1–10 min.) and in modified atmospheres at ambient pressure (50% CO₂, 3% residual oxygen and 47% balances of nitrogen; 1–3 days). These CO₂ rates tested are effective to control *P. interpunctella* eggs (Riudavets et al., 2006). For both adult and nymph treatments, we caged each individual mite in a 0.67 ml hard gelatine capsules with approximately 15 frozen eggs of *E. kuehniella*. For egg treatments we caged an individual mite egg alone in a 0.67 ml hard gelatine capsules. There were 20 replicates per treatment, including 20 replicates not treated with CO₂ as the control treatment.

To apply CO₂ at high pressure, 20 hard gelatine capsules were placed in a high pressure chamber with a volume of 2.2 m³ connected to a CO₂ tank. Gas was introduced into the chamber until the desired pressure was achieved. Once the target pressure was reached, it was maintained for the time necessary for each experiment.

To apply modified atmospheres packaging, 5 hard gelatine capsules were placed inside a plastic bag (Cryovac BB4L, of 300 x 210 mm) which was then filled with the desired atmosphere using a vacuum packaging machine (Multivac A 300/16). Modified atmospheres were previously prepared using a gas mixer (Witt KM 100-3M/MEM). A gas analyzer (Abiss model TOM 12) was used to verify the contents of carbon dioxide and oxygen inside the plastic bags, which were kept in a climatic chamber for 1 to 3 days, depending on the experiment. There were 4 bags per experiment.
Egg eclosion was recorded 24 hours and 48 hours after exposure and the number of adults and nymphs alive just 24 hours after the treatment.

One-way analysis of variance (ANOVA) and the Tukey test (SAS, 1999-2001) were performed on the data for predatory capacity of *B. tarsalis* on *P. interpunctella* eggs to test for significant differences between 5 different ratios of *B. tarsalis* females/*P. interpunctella* eggs.

**Results and discussion**

The results obtained in these laboratory experiments have shown the importance of combining biological control and CO₂ treatments, because the synergism that exists between them increases the efficacy of the pest control. Biological control and CO₂ treatments can be complementary techniques used in different points of the mill industry. On the other hand, both are harmless to the environment.

In biological control treatments the mean number of *P. interpunctella* adults decreased by around 31% at a 0.2 predator to host ratio in comparison with the control (Fig. 1). There were no significant differences between 0.2, 0.5 and 1 ratios but there were differences between these ratios and the control treatment. Therefore, a minimum of a 0.2 predator to host ratio was necessary to achieve a visible reduction in moth population by biological control. Although, there were significant differences between 0.05 and 0.1, there were not between these ratios and the control. The number of mites of these ratios was insufficient to produce a significant reduction of the moth population.

In our laboratory conditions, one adult of *B. tarsalis* destroyed from 0.36 *P. interpunctella* eggs (ratio 1) to 2.3 (ratio 0.05) in 4 days. This could be explained by the competition that may exist when the mite population is high, and one egg could be eaten or destroyed by more than one mite individual. According to Nielsen (1999), at 25°C and 12:12 h L:D photoperiod the mean number of destroyed eggs of *E. kuehniella* by a *B. tarsalis* female per day is 4.3 ± 1.45. In another experiment, a maximum consumption of 2.5 eggs/day was reported for females of *B. tarsalis* (Riudavets el al., 2002). In these studies, the eggs were offered to the predator without any other material to interfere. However, the mean number of destroyed *P. interpunctella* eggs obtained in our study was lower than the results obtained by Nielsen and by Riudavets, probably explained by the difficulty of mite adults to find moth eggs on the flour.

The webs produced when the ratio was 1 decreased around 47% comparing to the control treatment (Fig. 2). Between 0.05, 0.1, 0.2, and 0.5 ratios, there were no significant differences but there were differences between these ratios and the control treatment. Although there were no significant differences between ratios 0.5 and 1 in the number of *P. interpunctella* adults, these ratios had significant differences in moth web production (Fig. 2). The reduction of webs is very important because an accumulation may not only contaminate but also clog the machinery of industry.

In Table 1 we can see the exposure time necessary to eliminate *B. tarsalis* with CO₂ at high pressure and with modified atmospheres. The presence of mites could be considered as a contaminant for the final product. However, our results have shown that the exposure time required to eliminate the predator, is shorter than the time required to eliminate all stages of a moth population. As reported by Riudavets *et al.* (2006), an exposure time of approximately 12 days for modified atmospheres and 15 min. for CO₂ at high pressure is necessary.

In conclusion, biological control could be a good alternative to reduce the pest population when the product is stored. Moreover, to keep the pest population low could help to avoid the accumulation of webs that contaminate the machinery and facilities.
Fig. 1. Number of *P. interpunctella* pupae (mean ± standard deviation) obtained from flour at different ratios of *B. tarsalis* females/*P. interpunctella* eggs after 6 weeks in contact. Control treatment without predators. Means with the same letter are not significantly different (P>0.05, Tukey test).

Fig. 2. Web production (g) by *P. interpunctella* on flour (mean ± standard deviation), depending on the ratio of *B. tarsalis* females/*P. interpunctella* eggs tested after 6 weeks in contact. Control treatment without predators. Means with the same letter are not significantly different (P>0.05, Tukey test).

Table 1. Time necessary to achieve 100% of mortality of different stages of *B. tarsalis* with CO2 at high pressure and with CO2-rich modified atmospheres at ambient pressure.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conditions</th>
<th>Stage</th>
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<tr>
<td></td>
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<td>Eggs</td>
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<tr>
<td>CO2 at high pressure</td>
<td>20 bar (100% CO2)</td>
<td>1 min</td>
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<tr>
<td>Modified atmospheres</td>
<td>50% CO2+3% O2 +47% N2</td>
<td>3 days</td>
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References


Effectiveness of DEBBM-P, a new enhanced diatomaceous earth formulation for the control of *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) on stored wheat

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Abstract: To control one of the main insect pests of stored wheat, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae), a new enhanced formulation of a diatomaceous earth (DEBBM-P) was assessed in the laboratory for its efficacy against this insect pest. The new enhanced formulation DEBBM-P combining diatomaceous earth (DE) and the plant extract bitterbarkomycin (BBM), developed by Diatom Research and Consulting Inc., Canada, was applied at 75, 100 and 125 ppm on wheat and the bioassay was conducted in the laboratory at 30°C and 65 % r.h. The adults of the insects were exposed to treated wheat for 14 d and 21 d and the mortality was assessed by counting dead and live adults. After the assessment of the mortality, the replications were again retained for the next 63 d for the emergence of progeny. After 21 d of exposure, the mortality of *Tribolium castaneum* was 100% at all dose rates; also there was no emergence of the progeny. It was concluded that the enhanced DE formulation DEBBM-P is more effective than other DE formulations available in the market against stored grain insect pests.

Key words: DEBBM-P, enhanced diatomaceous earth, efficacy, *Tribolium castaneum*, stored wheat

Introduction

The red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) is one of the major secondary grain insect pests, cosmopolitan in nature especially in Pakistan. It has great impact on the economy of Pakistan and should not be underestimated (Hamed and Khattak, 1985). Grain is a living entity which is heavily affected by adverse biotic and abiotic factors resulting in qualitative and quantitative damage to grains (Ahmed, 1995). In Pakistan, estimated weight loss during storage is approximately 4 % and account for about 1000 million rupees loss contributing more than 24 % to Gross Domestic Product (GDP) each year (Hashmi, 2001). The diatomaceous earths have a very low mammalian toxicity (Athanassiou *et al*., 2003, 2004) consist of skeletal fragments of marine or fresh water diatoms and act generally as desiccants when applied on the cuticle of insects. Ultimately, the insect dies due to the excessive loss of water from the body (Korunic, 1998; Subramanyam and Roesli, 2000; Mewis and Ulrichs, 2001). A given diatomaceous earth should have a large oil absorption capacity and the chemical composition of DE is ideal when it has a high purity of amorphous silica of a uniformly small (less then 10/μ) particle size that contains very little clay, and less than 1 % crystalline silica (Allen, 1972; Calvert, 1930; Katz, 1991). Natural DEs are classified as Generally Recognized As Safe (GRAS), are also feed additives or even food additives (Anonymous, 1991). In stored product protection, diatomaceous earth is most useful in treating cracks, wall crevices, wall voids and attics to repel insects and deny harborage in these areas. It is effective against pests that live in close association with humans such as cockroaches, silverfish, mites, ants, houseflies, spiders, bedbugs, fleas and crickets (St. Aubin, 1991). There are many DE formulations available in the market but many of them are
effective only at high dose rates like 400 to 1000 ppm and higher against stored grain insect pests (Subramanyam and Roesli, 2000). It is already a public concern that the high dose rates of the DE’s may cause respiratory problems among workers (Athanassiou and Korunic, 2007) but also may reduce the flowability of the grains and reduce bulk density (Fields, 1999). Recently Diatom Research and Consulting Inc., Guelph, Canada developed a new synergized and enhanced DE formulation (DEBBM-P), which is a combination of diatomaceous earth (DE) and a Chinese plant extract (bitterbarkomycin, BBM) (Korunic, 2007). Bitterbarkomycin (BBM) is extracted from the root bark of the plant Celastrus angulata Maxim. (Celastraceae). This is a perennial shrub or woody climber in tropical and temperate zone. DEBBM-P has a low mammalian toxicity with an oral LD50 for rat higher than 4500 mg/kg (Athanassiou et al. 2006, Korunic, 2007). In the present study we planned to evaluate this new enhanced DE formulation against T. castaneum in terms of mortality of adults and reduction in progeny production.

Materials and methods

A trial was designed in the laboratory of Grain Research Training and Storage Management Cell (GRTSMC), University of Agriculture, Faisalabad, Pakistan. 

Test insect

Tribolium castaneum (Herbst) adults used were taken from a culture that was kept in the laboratory on wheat at 28°C, 60% r.h. and continuous darkness. A mixed culture of unsexed adults was used in our laboratory trial, assuming equal distribution of both sexes.

Enhanced DEBBM-P formulation

DEBBM-P was obtained from Diatom Research and Consulting Inc., Guelph, Canada. It is a powder formulation which is a combination of 90% diatomaceous earth and 0.05% active ingredient of the Chinese plant extract (bitterbarkomycin).

Bioassay

Of the DEBBM-P formulation, three concentrations were tested: 75, 100 and 125 ppm, at 30°C and 65 % r.h. DEBBM-P in the respective concentration was added to a jar containing 500g of wheat grains and then mixed by shaking well in a jar for two minutes. The 500g of treated wheat grains were equally distributed among five glass jars each then containing 100 g of grain). Fifty adults were introduced into each jar and the opening of the jar was covered with muslin cloth. For comparison, control jars were maintained with untreated wheat. After the exposure of 14 and 21 d the dead and the live adults were counted and after each data recording the grains were sieved to separate the dead and the live ones. For the progeny assessment the jars after taking the data of 21 d exposure were retained again in the incubator until they were re-opened after 63 d and the adults emerged were counted.

Statistical analysis

Mortality was calculated by pooling the numbers of dead and alive insects across every replicate and correcting them with the mortality in untreated control (Abbott, 1925). The data were submitted to a one-way analysis of variance (ANOVA) with SPSS statistical package and the means were separated by using the Sidek test, at P = 0.05 (Sokal and Rohlf, 1995).

Results and discussion

After 14 d exposure interval, the results indicated that the DEBBM-P was effective against Tribolium castaneum where the mortality was 97.6% at 75 ppm, which increases with the
increase in dose and at 100 ppm it reaches 99.2% (Fig. 1). It was also shown that at the highest dose the mortality reaches up to 100% at the 14 d exposure interval.

![Fig. 1. Mean mortality (% ±SE) of T. castaneum adults on treated wheat with DEBBM-P after 14 days exposure](image)

After 21 d of exposure, all the dose rates gave 100% mortality and so they all were statistically at par with each other (Fig. 2). Exposure time has great impact on efficacy of DE and efficacy is also influenced by several abiotic factors including temperature, humidity, etc. (Le Patourel, 1986; Cook and Armitage, 2000; Fields and Korunic, 2000). Our present study on the evaluation of insecticidal effects of enhanced diatomaceous earth against T. castaneum showed a similar interdependence of factors and is in accordance with previous laboratory studies conducted in different parts of the world (Rigaux et al. 2001; Kavallieratos et al. 2005; Collins and Cook, 2006; Athanassiou et al. 2007). Several formulations of diatomaceous earths are available today to be used against pests in different countries of the world like Africa, Canada, USA etc. Other researchers tested different DE’s such as Insecto (DE plus food grade bait), PyriSec (DE plus pyrethrum) and SilicoSec (DE) at dosages of up to 1000 ppm to control the stored grain insect species (Fields and Korunic, 2000, Athanassiou et al., 2004, 2005) in contrast, in the present study the dose rates applied on the substrate against T. castaneum were exceptionally low. In this case where we used new enhanced DEBBM-P formulation, the dose rate was very low and even after 14 d exposure interval the 125 ppm gave the 100% mortality of the insect pest.

Other studies indicated that Tribolium spp. are the most tolerant species regarding DE treatments (Arthur, 2000; Fields and Korunic, 2000; Athanassiou et al., 2004, 2005; Wakil et al., 2005) which enhances the value of the presented results, but it seems obvious that the tested admixture of a normal DE with an insecticidal plant extract was considerably more effective in controlling T. castaneum.

In reducing the progeny production, the DEBBM-P formulation was also very effective and at 125 ppm there was no emergence of F1 adults (Fig. 3). At lower dose rates, there was marginal production of progeny as at 75 and 100 ppm with 2.8 and 1.2 adults on average, respectively, in comparison to controls where it was 24.8.

In Pakistan there is a wide range of plant flora and already a lot of work has been done on the efficacy of plants and plant extracts against stored grain insect pests (Zaidi, et al.,
2003; Anwar et al., 2005; Hasan et al., 2006 a,b). In future, these types of studies could provide more knowledge about the synergism of DE’s with plant for the safer management of stored product insects. On the basis of the present study it was concluded that the new enhanced diatomaceous earth formulation is more effective at very low dose rate as compared to the traditional DE’s available in the market and in this way these new formulations may be supportive in reducing the health and environmental hazards caused by conventional DE products and other insecticides.

Fig. 2. Mean mortality (% ±SE) of *T. castaneum* adults on treated wheat with DEBBM-P after 21 days exposure

Fig. 3. Progeny production (no of adults emerged ±SE) of *T. castaneum* in wheat treated with DEBBM-P (means followed by the same letter are not significantly different; Sidek test at p = 0.05)

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Distribution and efficacy of aerosol insecticides in commercial facilities

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Abstract: Aerosol insecticides are being viewed as a potential alternative to fumigations in commercial milling, processing, and storage facilities. Although there are a number of insecticides and delivery systems available for use, there are little published data regarding efficacy and performance in actual commercial sites. Factors such as the specific insecticide and distribution system, susceptibility of the target pest, availability of a food source, the configuration of equipment, machinery, and processed food products inside a facility, and seasonal history of pest populations can all affect insect control when using aerosols. Data from current field studies will be used to illustrate and describe the impact of these factors and how they can affect efficacy of aerosol insecticides.

Key words: insects, stored-products, aerosols, insecticides, control

Introduction

Aerosol insecticides, also known as fogging and ultra-low-volume applications (Peckman and Arthur, 2006) have historically been used as part of management programs for stored-product insects in milling, processing, and storage facilities (Childs, 1967; Gillenwater et al., 1971; Cogburn and Simonaitis, 1975). During the past several decades, fumigation treatments became more common, and aerosols were not used as frequently. Now that methyl bromide is being phased out world-wide as a result of international agreement (Fields and White, 2002), aerosols are being advocated as a viable alternative to methyl bromide and other fumigants. Aerosols are usually safer than fumigants and do not require the extensive downtime associated with fumigations. The equipment and insecticides used for aerosol treatment can vary, but common to all is a process of atomization of liquid material into small particles ranging from 5 to 50 microns in size. The application equipment must effectively distribute the insecticide throughout the area being treated. Differences in the insecticides and the delivery systems used for those insecticides can also affect resultant control. Data from field trials are presented to illustrate how methods of exposure and the dispersion of aerosols inside a facility can affect survival of stored-product insect species exposed to aerosols. Selected studies will be reviewed and discussed in relation to: 1) assessing efficacy and dispersion of aerosols; 2) effects of life stage and food material on susceptibility of Tribolium spp.; and 3) aerosol penetration into obstructed and hidden areas within facilities.

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1 This paper reports the results of research only. Any mention of a proprietary product, chemical trade name, or company does not constitute a recommendation or endorsement by the U. S. Department of Agriculture.
Materials and methods

The first field trial was conducted inside an abandoned warehouse that was once part of an active milling facility. The warehouse had a total volume of about 12,500 m³ and was empty except for some debris. There was a fan on the wall at the north end which could not be sealed, which resulted in some air movement from the outside. An aerosol application system was installed with two nozzles that were about 12 and 27 m from the south wall, below the roofline. Individual exposure arenas consisted of the bottom portion of standard plastic Petri dishes (62 cm²) lined with filter paper. Ten 1-2-week-old adult Tribolium confusum, all from pesticide-susceptible colonies maintained at the USDA-ARS Grain Marketing and Production Research Center (GMPRC) in Manhattan, Kansas, were placed in each of 36 dishes. Two dishes were put at each position as shown in Fig. 1. Companion dishes were also placed in an adjoining room next to the warehouse to serve as untreated controls.

The aerosol applied was a mixture of 0.7% pyrethrins, 5.0% piperonyl butoxide synergist, and 94.3% other ingredients, with CO₂ propellant to facilitate dispersion. All doors from the empty warehouse to the adjacent areas were sealed with polyethylene plastic. The insects were exposed to the aerosol for two hours, at which time the room was aerated and the exposure dishes were collected. Beetles in each of the two dishes at the 18 positions were visually classified as either knocked down (on their backs and incapable of extended movement) or running (upright and mobile). Beetles from one set of the exposed dishes were then transferred to new Petri dishes to eliminate effects from continual exposure, and the original exposure dishes were discarded. The beetles in the other set were left in the original dishes in which they were exposed. All dishes were put in cardboard boxes, covered, and returned to the GMPRC, where they were held on a laboratory countertop. The percentage of active beetles was assessed again at 1, 2, 3, 7, and 14 d after the beetles had been removed.

Figure 1. Layout and dimensions of empty warehouse where field trials were conducted for Experiment 1. Dimensions are in meters, numbers represent sites on the floor where adult T. confusum were exposed to the pyrethrin aerosol, and asterisks are the approximate positions of the spray nozzles.
from the warehouse. For all of these assessments, those adults that were mobile and running were considered to have “survived” exposure. Four separate trials were conducted and all procedures for each replicate were as described above. Data were analyzed using the Statistical Analysis System (SAS Institute, 2001).

The second field trial was conducted in a room measuring about 17,000 m$^3$ which was inside a large commercial food warehouse. The test insects were *Tribolium castaneum* and *T. confusum*, which are often more tolerant to contact insecticides than other stored-product beetles, however, the order of susceptibility between these two species is often dependent upon the specific insecticide and application method (Arthur, 1998a). Both of these species can be found world-wide in mills and processed food warehouses, and therefore it is important to know the relative susceptibility of these *Tribolium* species to aerosol insecticides.

The room contained pallet stacks of different food products, including canned and packaged food, along with boxed non-food items. An aerosol application system, which dispensed insecticides at an approximate particle size of 15 microns, was installed inside the facility. There were two dispensing nozzles hung from the ceiling at approximately 17 m from each end along the long axis of the room (Fig. 2). The experimental unit was the bottom portion of a standard plastic Petri dish, which measured approximately 89 by 15 mm (area of 62 cm$^2$), which was painted white and lined with filter paper to minimize any potential effect of repellency by the plastic in the dish. The test insects were 4-week-old larvae, pupae, and adults of *T. castaneum* or *T. confusum*. All immature and adult insects were from the pesticide-susceptible cultures at the GMPRC.

![Figure 2. Layout and dimensions of empty warehouse where field trials were conducted for Experiment 2. Dimensions are in meters, numbers represent sites on the floor where adult *T. confusum* and *T. castaneum* adults, larvae, and pupae were exposed to the pyrethrin aerosol, and asterisks are the approximate positions of the spray nozzles.](image)

For each of five replicate trials, two sets of dishes containing either 10 adults of each beetle species, in separate dishes either with 250g of whole wheat flour or without flour (4 dishes total) were placed at 15 locations within the room (Fig. 2). In addition, separate dishes
containing either ten 4-week-old larvae of each species or ten pupae, with 250 mg of flour (4 dishes total for immature stages), were placed in the same positions. Positions 1-5 were along the north wall, positions 6-10 were in the center of the room, and positions 11-15 were along the south wall. The dishes that were along the side walls were usually placed between the wall and stacked pallets. The insecticide used in the trials was a 1% active ingredient [AI] pyrethrin formulation (2% composition of piperonyl butoxide synergist), with a labelled maximum application rate of 23.4 g of formulation/28 m³ of headspace. At each trial, 80% of the maximum use rate of the insecticide was applied in the room. Separate sets of adults and larvae of each species (10 individual dishes for each species and life stage with the same numbers of individuals as described for the treatments) were placed in the warehouse office as untreated controls.

The time required for the aerosol system to dispense the insecticide was 20 minutes, and the dishes were left in the test room and the office until they were picked up the next morning and returned to the GMPRC in Manhattan. Dishes containing the adults that were exposed to the aerosol were held on a laboratory countertop, in the same dishes in which they were exposed, and at 1 and 2 weeks post-treatment were classified as running, knocked down, or dead (failure to move when touched with a probe). Dishes containing the immature life stages were held until 7-10 days after all immatures in the untreated control dishes had emerged as adults. The General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS Institute, 2001) was used to analyze the data.

The third field trial was conducted in the room described in experiment 2, but in this test the aerosol that was sprayed was the insect growth regulator (IGR) methoprene (Diacon II®) at the label rate of 3 ml of formulation per 280 m³ of headspace area. The test insects used were 4-week-old larvae of *T. castaneum*, primarily because preliminary tests with methoprene applied as a contact insecticide indicates that *T. castaneum* is more susceptible than *T. confusum*. Plastic Petri dishes were lined with filter paper and prepared as previously described and contained approximately 250 mg of flour, along with 10 larvae. Open site treatments (dishes in unobstructed locations) were put in ten positions in the approximate center of the room (Fig. 2), ca. 9 m from either of the side walls. The sites that were chosen to represent obstructed areas were underneath pallets of goods, and the dishes were set 0.3 to 0.6 m from the edge of the pallet. As before, ten dishes containing larvae and food were placed in the warehouse office.

A fourth and final field trial was conducted in a flour mill where the insecticide was a mixture of the pyrethrin formulation described in experiment 2 and the label rate of methoprene. For this field trial, concrete treatment arenas were constructed in the bottom portions of standard plastic Petri dishes, with a measured area of 62 cm². Procedures for constructing these concrete arenas have been previously described (Arthur and Hoernemann, 2004). Because of concerns about insects escaping exposure dishes, *T. castaneum* pupae were exposed in this trial. Dishes containing 10 pupae, along with 250 mg of flour, were shipped to the mill manager, who placed 5 dishes in open areas, 5 dishes underneath pieces of equipment, and 5 dishes in hidden areas on the same floor of the mill prior to activation of the aerosol spray. As usual, a set of 10 untreated dishes were held in the mill office as untreated controls. The insecticide was dispensed in accordance with the label rate. Two hours after the fog was dispensed, the dishes were picked up and shipped back to the GMPRC, where they were held for adult emergence. Four replications were done in this field trial.

**Results and discussion**

In the first field trial, knockdown of *T. confusum* was 99 to 100% in all dishes, but there was recovery from knockdown during the two-week holding period. In addition, survival was
greatly increased in those beetles exposed to the aerosol and transferred to new dishes compared to beetles held in the same dishes in which they were exposed (Table 1). The beetles that were held in the same dishes apparently continued to be affected by the aerosol that was absorbed by the filter paper, compared to the other set where the beetles were removed from the exposure dishes and placed on clean unexposed filter paper.

The effect of dish position (Fig. 1), indicating uneven distribution of aerosol particles in the warehouse, is illustrated in Table 2 using data on survival of *T. confusum* held in the same dishes in which they were exposed, 14 days post-treatment. Since the beetles were exposed in three separate rows along the east and west walls and in the center, data were analyzed for differences in position within a row. Survival was greater at the northern end of the warehouse than at the southern end. Differential deposition of the aerosol particles could have resulted from movement of outside air through the vents of the exhaust fan on the north wall, thereby causing the particles to drift toward the south end of the warehouse.

Table 1. Percentage (mean ± SE) survival of adult *T. confusum* exposed to pyrethrin aerosol and transferred to unexposed dishes versus survival of beetles held in the same dishes in which they were exposed. Values are an average of 18 exposure positions within the warehouse. Means within the same row (days post-exposure) followed by a different lower-case letter are significantly different (*P* < 0.05, PROC t-test, SAS Institute).

<table>
<thead>
<tr>
<th>Days Post-Exposure</th>
<th>Survival in dishes exposed to pyrethrin aerosol</th>
<th>Survival when transferred to unexposed dishes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.1 ± 0.1a</td>
<td>0.4 ± 0.2a</td>
</tr>
<tr>
<td>1</td>
<td>1.1 ± 0.8a</td>
<td>1.5 ± 0.9a</td>
</tr>
<tr>
<td>2</td>
<td>1.8 ± 1.1a</td>
<td>3.8 ± 0.6a</td>
</tr>
<tr>
<td>3</td>
<td>5.2 ± 2.2b</td>
<td>10.8 ± 3.2a</td>
</tr>
<tr>
<td>7</td>
<td>21.7 ± 4.3b</td>
<td>83.2 ± 3.3a</td>
</tr>
<tr>
<td>14</td>
<td>25.3 ± 4.4b</td>
<td>79.6 ± 3.1a</td>
</tr>
</tbody>
</table>

Table 2. Percentage (mean ± SE) survival of adult *T. confusum* exposed at 18 positions in the warehouse and held in the same dishes at which they were exposed. Means within columns followed by different lower-case letters are significantly different (*P* < 0.05, Waller-Duncan k-ratio t-test, SAS Institute).

<table>
<thead>
<tr>
<th>West Wall Dish Positions</th>
<th>Percent Survival (mean ± SE)</th>
<th>Center Dish Positions</th>
<th>Percent Survival (mean ± SE)</th>
<th>East Wall Dish Positions</th>
<th>Percent Survival (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0 ± 0.0b</td>
<td>7</td>
<td>37.5 ± 20.6ab</td>
<td>13</td>
<td>0.0 ± 0.0b</td>
</tr>
<tr>
<td>2</td>
<td>0.0 ± 0.0b</td>
<td>8</td>
<td>20.0 ± 16.8ab</td>
<td>14</td>
<td>5.3 ± 3.2b</td>
</tr>
<tr>
<td>3</td>
<td>5.3 ± 3.2b</td>
<td>9</td>
<td>0.0 ± 0.0b</td>
<td>15</td>
<td>12.5 ± 12.5b</td>
</tr>
<tr>
<td>4</td>
<td>15.0 ± 11.9b</td>
<td>10</td>
<td>5.0 ± 5.0ab</td>
<td>16</td>
<td>5.0 ± 5.0b</td>
</tr>
<tr>
<td>5</td>
<td>65.0 ± 23.6a</td>
<td>11</td>
<td>42.5 ± 20.1ab</td>
<td>17</td>
<td>17.5 ± 17.5b</td>
</tr>
<tr>
<td>6</td>
<td>77.5 ± 22.5a</td>
<td>12</td>
<td>55.0 ± 26.2a</td>
<td>18</td>
<td>67.5 ± 22.8a</td>
</tr>
</tbody>
</table>

Mortality of untreated control adults in the second field trial was virtually 0, and nearly all immatures in controls emerged as normal adults. No corrections for control mortality were necessary to analyze the treatments. There was no effect of exposure position on survival, knockdown, and mortality of adult *T. confusum* or *T. castaneum* at 1- and 2-weeks post-treatment, either when dishes from all 15 positions were grouped for analysis or when each
set of dishes (north and south walls and the center) were analyzed separately \((P > 0.05)\). This lack of position effect indicates an even dispersion of aerosol particles throughout the room where the tests were conducted. Data for position were combined and analyzed again for differences between species and the presence of food during exposure (Table 3). At 1 and 2-weeks post-exposure, survival (i.e., running) was greater in \textit{T. confusum} than \textit{T. castaneum} exposed with food, but not for the exposures without food. Mortality was always greater for \textit{T. castaneum} compared to \textit{T. confusum}, while knockdown was always greater in \textit{T. confusum} than in \textit{T. castaneum}. \textit{T. castaneum} was the more susceptible species, and there were no differences in knockdown or mortality, and no differences in survival, knockdown, or mortality between \textit{T. castaneum} exposed with and without food. Larvae of both species were much more susceptible to the pyrethrin aerosol compared to the adults. None of the exposed larvae of either species emerged as normal adults. Emergence of exposed \textit{T. confusum} pupae was sporadic and averaged \(0.06 \pm 0.06\) and \(0.17 \pm 0.12\%\) at 1 and 2 weeks post-treatment, respectively, while no exposed pupae of \textit{T. castaneum} emerged as adults.

Table 3. Percentage survival, knockdown, and mortality at 1 and 2 weeks post-treatment for adult \textit{T. confusum} and \textit{T. castaneum} exposed with and without food. For each of the measured variables, differences between species \((P < 0.05)\) exposed with and without food are shown by different upper case letters (rows), while differences between weeks post-exposures within each food/species treatment combination \((P < 0.05)\) are shown by different lower-case letters (columns) (PROC \textit{t}-test, SAS institute).

<table>
<thead>
<tr>
<th>Time</th>
<th>Variable</th>
<th>\textit{T. confusum}</th>
<th>\textit{T. castaneum}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week post-exposure</td>
<td>Survival</td>
<td>No Food 4.5 ± 1.7bA</td>
<td>3.5 ± 1.3aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food 13.1 ± 3.3aA</td>
<td>2.3 ± 1.0aB</td>
</tr>
<tr>
<td></td>
<td>Knockdown</td>
<td>No Food 56.5 ± 4.2aA</td>
<td>5.4 ± 1.2aB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food 46.5 ± 4.3aA</td>
<td>1.9 ± 0.6aB</td>
</tr>
<tr>
<td></td>
<td>Mortality</td>
<td>No Food 38.9 ± 4.3aB</td>
<td>91.0 ± 2.2aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food 40.4 ± 4.4aB</td>
<td>96.5 ± 1.2aA</td>
</tr>
<tr>
<td>2 weeks post-exposure</td>
<td>Survival</td>
<td>No Food 3.7 ± 1.7bA</td>
<td>1.3 ± 0.7aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food 9.5 ± 2.7aA</td>
<td>0.1 ± 0.1aB</td>
</tr>
<tr>
<td></td>
<td>Knockdown</td>
<td>No Food 11.4 ± 2.6aA</td>
<td>0.0 ± 0.0aB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food 11.2 ± 2.4aA</td>
<td>0.0 ± 0.0aB</td>
</tr>
<tr>
<td></td>
<td>Mortality</td>
<td>No Food 84.9 ± 3.2aB</td>
<td>98.7 ± 0.7aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food 79.3 ± 3.8aB</td>
<td>99.9 ± 0.1aA</td>
</tr>
</tbody>
</table>

In the third field trial, emergence of adult \textit{T. castaneum} in the untreated controls placed in the office of the food warehouse was \(81.3 \pm 2.3\%\). In contrast, there was only 1 emerged adult in all dishes exposed in open areas and 1 emerged adult from the dishes set underneath the pallets. We only tested a single “obstructed” position, so perhaps different results would have been obtained if dishes were set further back into the area underneath a pallet. In the fourth and final field trial conducted in the mill, emergence of adults in untreated controls was \(92.4 \pm 1.8\%\). Adult emergence of pupae exposed in open, obstructed, and hidden areas was \(2.0 \pm 1.1, 1.2 \pm 0.6\), and \(36.8 \pm 6.9\%\), respectively. There was excellent penetration of the aerosol into the obstructed areas, and even some penetration into the hidden areas.
Conclusions

The results of these field trials show that pyrethrin and methoprene aerosols can be used effectively to control *Tribolium* spp. in milling and storage facilities. However, it is difficult to compare performance of aerosol systems and insecticides when they are used in different facilities because of the uniqueness of both the application systems and the internal configurations within a particular site. However, common results with these systems indicate that *T. confusum* is more tolerant to aerosol insecticides compared to *T. castaneum*, and the presence of food material will have more of an effect on control of adult *T. confusum* than adult *T. castaneum* due to the individual susceptibilities of the two species. The impact of food material on increasing survival of adults of these species exposed to contact insecticides has also been documented (Arthur, 1998b, 2000), which emphasizes the importance of sanitation in conjunction with insecticide application.

References


Evaluation of the knockdown activity of some pyrethroids on different types of surfaces against larvae of *Plodia interpunctella* (Hbn.) (Lepidoptera: Pyralidae)

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**Abstract:** The knockdown activity after 10, 15, 20, 30, 45 min and 1, 2, 24, 48 h and the mortality after 24 and 48 h against mixed populations of the 2nd instar and the 4th instar larvae (♀♀; ♂♂) of *P. interpunctella*, were evaluated. Larvae were placed in contact for 30 min on glass (g), vitrified tiles (vt), and porous tiles (pt), treated with two concentrated emulsifiable formulations (100 g solution/m²) based respectively on deltamethrin (15 mg a.i./m²) and microcapsulated permethrin (25:75) (300 mg a.i./m²) and an aerosol formulation (100 g/m²) of synergized tetramethrin (180 mg a.i./m²) with piperonyl butoxide (950 mg a.i./m²). Tests were carried out 24 h after the treatment.

It was found that the 2nd instar larvae were more susceptible to the above active ingredients than the 4th instar ones; the lowest mean percentages of knockdown activity and mortality were recorded on pt. The mean percentages of knockdown activity of the 2nd instar larvae on g and vt ranged from 93 to 100% for tetramethrin and from 56 to 100% for deltamethrin; they were always lower than 25% for permethrin. Means of knockdown activity, higher than 90% were recorded on pt from 1 to 48 h with deltamethrin while they were lower than 37% with the other active ingredients. The mean percentages of knockdown activity of the 4th instar larvae on g and vt with deltamethrin were respectively 89-93% for ♀♀ and 98-88% for ♂♂ after 48 h. With tetramethrin a lower knockdown activity for both sexes was recorded on vt (♀♀: 33%; ♂♂: 50%) compared to g (♀♀: 90%; ♂♂: 93%). Permethrin caused a mean percentage of knockdown activity after 1 h of 9-10% for ♀♀ and of 6-38% for ♂♂, while after 48 h they were 49-46% for ♀♀ and 46-51% for ♂♂. The highest knockdown activity on pt was observed for deltamethrin after 2 h (♀♀: 85%; ♂♂: 49%). The mean mortality of the 2nd instar larvae, higher than 80% after 48 h, was recorded on g and vt for tetramethrin and deltamethrin. The mean mortality was lower than 5% for permethrin. A mean mortality of 65% was observed on pt treated with deltamethrin, while it was less than 20% with tetramethrin and permethrin. The mean mortality of the 4th instar larvae was less than 35% for all active ingredients and tested surfaces.

**Key words:** Indian meal moth, knockdown, pyrethroids, treated surfaces.

**Introduction**

Larvae of *Plodia interpunctella* (Hbn.), when mature, tend to leave food and they are often found in machinery, storehouse walls and industrial environments, in search of places for pupation. These surfaces are preferably treated with photo stable pyrethroids, with long persistence. In most of tests reported in the literature, the contact time during which the insect is obliged to stay on the surfaces treated with pyrethroids, is for some hours and, in a few cases, even some days. These data can be useful when all the surfaces are treated uniformly and the insects have no chance to avoid exposure to the active ingredient (Arthur & Peckman, 2006); but in industrial sites the exposure time is usually shorter. In fact many of these active ingredients show a flushing and repellent effect and the insects tend to leave the treated...
surfaces. The knockdown activity of these pyrethroids is fast and it causes reduced adhesion of the larvae to the surface so that in consequence, they fall down from walls, ceilings and vertical surfaces.

Arthur & Peckman (2006) state that “As insecticide applications are reduced and targeted to specific areas where there is a problem, it becomes necessary to establish efficacy data for short exposure intervals”. In this work the knockdown activity of formulations based on some pyrethroids, namely deltamethrin, microcapsulated permethrin and synergized tetramethrin with piperonyl butoxide, were tested on *P. interpunctella* larvae of different ages. The pyrethroids were distributed on surfaces characterized by different absorbent characteristics for a contact time of 30 min.

Deltamethrin and permethrin (described respectively in 1974 and in 1973), are two photostable pyrethroids that are non-systemic insecticides with contact and stomach action. Permethrin has also a slight repellent effect. Tetramethrin (1981), is a light-sensitive pyrethroid, with contact and knockdown activity, often used in mixture with other insecticides and synergists such as PBO. These active ingredients are used for general, spot and crack-crevice residual treatments on surfaces.

**Materials and methods**

The efficacy of two concentrated emulsifiable formulations (100 g solution/m²) based respectively on deltamethrin (15 mg a.i./m²) and microcapsulated permethrin (25:75) (300 mg a.i./m²) and of an aerosol formulation (100 g/m²) based on synergized tetramethrin (180 mg a.i./m²) with piperonyl butoxide (950 mg a.i./m²) was evaluated. Tests were carried out on different types of surface: glass (Ø: 10 cm), vitrified tile (sides: 20 cm), porous tile (sides: 20 cm). Products were distributed over the surfaces from a distance of 30 cm. Tests were carried out 24 h after the treatment at 23±2°C and 30±5% R.H. under a fume hood.

*P. interpunctella* was reared on an artificial diet (Locatelli & Limonta, 2004) in a thermostatic room (26±1°C; 70±5% R.H.; L:B 16:8.) at Istituto di Entomologia agraria - Università degli Studi di Milano. Tests were carried out on larvae of two different instars, namely, the 2nd instar mixed population (♂♂: 2,5±0,6 mg; length 3,01±0,54 mm) and the 4th instar (♀: 14,8±1,2 mg; length: 9,08±0,46 mm – ♂: 19,7±1,8 mg; length: 9,90±0,58 mm). Larvae were tested, respectively, 7-8 and 14-15 days after egg deposition.

Groups of 20 larvae, after being anesthetized with CO₂ for 30 seconds, were placed on different surfaces for 30 min in an artificially enlightened environment (25±2°C and 60±10% R.H.). The area occupied by larvae was delimited by a polypropylene cylinder with an open base (Ø: 6 cm; h: 8 cm) and with the top covered with fine mesh (120 mesh).

At the end of the tests individuals were placed in a polypropylene cylinder (Ø: 6 cm; h: 8 cm), with 1 g of artificial diet, and were transferred to a thermostatic room (25±1°C; 70±5% R.H.; L:B 16:8). After 24 and 48 h the knocked down individuals were counted with a stereoscopic microscope. The individuals that showed evident signs of intoxication, such as uncoordinated movements or difficulty in moving, were considered as knocked down. Those individuals that, when touched with a paint-brush failed to show contraction of abdominal legs, abdomen or head, were considered dead (Lloyd & Hewlett, 1959).

Five replications were carried out for each test as well as for the control. The mean percentages were subjected to angular transformation arc-sine, and then subjected to ANOVA and to Duncan’s multiple range test (P<0.05) (SPSS 13.0 for Windows).
Results

The mean percentage of larval survival of both instars, under conditions similar to the test, but placed on an untreated surface was higher than 95% even after 48 h.

The mean percentages of knockdown activity of the 2nd instar larvae are shown in Fig. 1, and for the 4th instar larvae (♂♂ and ♀♀) respectively, in Figs. 2 and 3.

Fig. 1. Mean* percentages of knockdown activity (±S.E.), observed after 10, 15, 20, 30, 45 min, 1, 2, 24 and 48 h, on larvae of the 2nd instar of *Plodia interpunctella* (Hbn.) placed, for 30 min of contact, on treated glass, vitrified and porous tiles at 25°C and 70% R.H.

* The same letters show homogeneous subsets for a confidence interval of 95% (Duncan's multiple range test; P<0.05).
Fig. 2. Mean* percentages of knockdown activity (±S.E.), observed after 10, 15, 20, 30, 45 min, 1, 2, 24 and 48 h, on larvae of the 4th instar (♀♀) of *Plodia interpunctella* (Hbn.) placed, for 30 min of contact, on treated glass, vitrified and porous tiles at 25°C and 70% R.H.

* The same letters show homogeneous subsets for a confidence interval of 95% (Duncan's multiple range test; P<0,05).
Fig. 3. Mean* percentages of knockdown activity (±S.E.), observed after 10, 15, 20, 30, 45 min, 1, 2, 24 and 48 h, on larvae of the 4th instar (♂♂) of *Plodia interpunctella* (Hbn.) placed, for 30 min of contact, on treated glass, vitrified and porous tiles at 25°C and 70% R.H.

* The same letters show homogeneous subsets for a confidence interval of 95% (Duncan's multiple range test; P<0.05).
The mean percentage of knockdown activity of the 2nd instar larvae of *Plodia interpunctella* for all surfaces treated with microcapsulated permethrin was lower than 25% except after 48 h on the porous tile (30). It was found that after 10, 15 and 20 min contact with the treated surface, there were significant differences between the mean percentage of knockdown activity of deltamethrin and tetramethrin+PBO on glass; the mean percentages were higher than 93% for tetramethrin+PBO, while for deltamethrin a progressive increase in the mean percentage from 56 to 74% was recorded. No significant differences were observed between the mean percentage of knockdown activity from 1 to 24 h on vitrified tile and on glass from 30 min to 48 h, with the mean values being higher than 84%. For deltamethrin a steady increase in the knockdown activity from 23% (10 min) to 100% after 2 h from the end of the treatment was noticed on the porous tiles. After 24 and 48 hrs the mean percentages were respectively 91 and 94%. The mean percentage of knockdown activity of tetramethrin+PBO, always on the porous tiles, was lower than 40%.

The mean percentages of knockdown activity of males and females of the 4th instar larvae were similar: they were respectively 35-81% on glass and vitrified tiles treated with deltamethrin for ♀♀♀ and 64-81% for ♂♂ after 20 min and after 48 h 89-93% for ♀♀♀ and 98-88 for ♂♂. They were 93-73% for tetramethrin after 30 min for ♀♀♀ and 98-79% for ♂♂. After 48 h a lower knockdown activity of both sexes was observed on vitrified tiles (♀♀♀:
33%; ♂♂: 50%) compared to glass (♀♀: 90%; ♂♂: 93%). Permethrin caused a mean percentage of knockdown activity of 9-10% for ♀♀ and of 6-38% for ♂♂ after 1 h and of 49-46% for ♀♀ and of 46-51% for ♂♂ after 48 h. The highest knockdown activity was observed for deltamethrin after 2 h on porous tiles (♀♀: 85%; ♂♂: 49%), while after 48 h it was 25% for ♀♀ and of 18% for ♂♂. It was lower than 20% for other active ingredients. The mean percentages of mortality of the 2nd and the 4th instar larvae are given in Fig. 4.

After 24 h the highest mean percentage of mortality of the 2nd instar larvae was observed on vitrified tiles treated with deltamethrin (56), while no significant differences were recorded between deltamethrin (41) and tetramethrin+PBO on glass (31). After 48 h the mean percentages of mortality on glass and vitrified tile, treated respectively with tetramethrin+PBO (84-78%) and deltamethrin (85-93%), were not significantly different. The mean percentage mortalities on all surfaces treated with microcapsulated permethrin were lower than the ones observed on deltamethrin and tetramethrin+PBO. On porous tiles, mean percentages mortalities of 64% after 48 h from the end of the treatment were observed for individuals placed in contact with deltamethrin, while the mean percentages for the other ingredients were lower than 20%.

The mean percentages mortality of the 4th instar larvae on non absorbent surfaces, treated with tetramethrin+PBO and deltamethrin were not higher than 25% after 24 and 48 h. On porous tiles the mean percentage survival of individuals treated with different active ingredients was very similar to the control. For all the active ingredients, no significant differences between the 2 sexes were recorded except after 48 h when a greater mortality was observed, for males, on glass treated with deltamethrin (♀♀: 4%; ♂♂: 25%) and permethrin (♀♀: 20%; ♂♂: 34%) and for females, on vitrified tiles treated with permethrin (♀♀: 33%; ♂♂: 21%).

Conclusions

The mean percentages of knockdown activity of the 2nd and the 4th instars of Plodia interpunctella (Hbn.) 2 h after contact for 30 min on the different surfaces treated with deltamethrin were, on the whole, higher than the ones that were recorded after 48 h, mostly on porous surfaces. When the contact time failed to allow the insect to absorb a quantity of insecticide sufficient to kill it, a high percentage of knockdown activity was observed after a few hours from the end of the treatment. However, afterwards the insect was able to recover and survive thanks to the presence of oxidase and esterase enzymes that deactivated the active ingredient.

A high knockdown activity was recorded on non absorbent surfaces, 10 min after treatment, with tetramethrin+PBO, according with product properties, which remained almost steady during all the observations (48 h) for the 2nd instar larvae on glass and vitrified tiles and, for the 4th instar larvae, only on glass.

The knockdown activity of microcapsulated permethrin was very slow and increased progressively up to 48 h even though it remained insufficient.

None of the 3 tested formulations gave a good performance with regard to knockdown activity on the absorbent surface.

A contact time of 30 min on vitrified tiles, porous tiles and glass treated with deltamethrin, permethrin microcapsulated and tetramethrin+PBO was not sufficient to cause a mortality of 95%. After 24 and 48 h, for the 4th instar larvae, mean percentage mortalities were lower than 35% on glass and vitrified tile. Less than 10% mortality on porous tiles, was observed for all pyrethroids. Similar results were observed on 3rd instar larvae of P.
*interpunctella* placed on different surfaces treated with deltamethrin for 5 min (Locatelli et al., 2006).

The active ingredients used in this work on all the tested surfaces, caused higher mortality of the 2nd instar larvae compared to the ones of the 4th instar, even though their efficacy was limited.

Arthur (1997b, 1999) observed that, at a contact time (insect-surface) greater than 6 h, wandering fifth instar larvae of *P. interpunctella* showed a reduced insecticidal susceptibility to deltamethrin dust and cyfluthrin wettable powder. Consequently, the insecticidal concentrations sufficient to control adult Coleoptera such as *Tribolium confusum* J. du Val and *T. castaneum* (Herbst) were not sufficient to obtain complete mortality of *P. interpunctella* larvae.

Many studies were carried out in which eggs were placed in contact with treated foodstuffs (Perez-Mendoza & Aguilera-Peña, 2004, Bengston et al., 1987). However the results failed to provide information about the doses required to control mature larvae, pupae and adults. The residual insecticides in fact were essentially used to control crawling insects. As *P. interpunctella* larvae grow in age and weight, the concentrations can become insufficient (Arthur 1997b, 1999). Moreover in many tests the treatment was carried out by topical application (Subramanyam & Cutkomp, 1987), namely, direct contact with the active ingredient, whereas larvae of Lepidoptera are hidden inside foodstuffs, cracks, crevices and in protected locations so that contact with the insecticide occurs indirectly while the insects wander over the treated surfaces.

The percentage survival of larvae of both ages on porous tiles was greater than that on glass and vitrified tiles.

The effect of treated surfaces (such as concrete, wood, plywood, ceramic tile, glass, galvanized metal, plastic films, jute bags, etc.) on residual persistence and efficacy has been tested in many works (Williams et al., 1983; Chadwick, 1985; Yadav & Jha, 1985; Yadav, 1986; Giga & Canhao, 1991; Arthur, 1997a, 1999; Arthur & Peckman, 2006). All the authors agreed in pointing out, that on porous surfaces such as concrete a loss of activity of the active ingredient is more rapid compared to not absorbent surfaces such as ceramic. Moreover, the porous surfaces are sometimes characterized by an alkaline pH which favours a rapid degradation of the insecticidal molecule. Similar observations were made also on active ingredients belonging to the group of organophosphate insecticides (Burkholder & Dicke, 1966; Williams et al., 1982) and of carbamates (Williams et al., 1982).

Undoubtedly it would be preferable to emphasize on the formulation label the necessity of increasing the concentration of the active ingredients when the treatment is carried out on absorbent surfaces. This is not always mentioned by producers of pesticides in their instructions for use.

The percentage mortality in the above experiments was negligible because larvae, - due to the knockdown effect of the active ingredient and to the repellent activity of some pyrethroids, - remained on the treated surface, particularly if this was vertical, for less than 30 min. The considerable percentage of surviving larvae and the possibility that the following generation would enter into contact with the treated surfaces without being controlled increases the risk of the development of resistance to pyrethroids.

**References**


Preliminary investigations about tolerance to phosphine in *Tribolium* strains (Coleoptera; Tenebrionidae) in Italy

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**Abstract:** The Detia® Degesch Resistance Test Kit has been used to investigate the behaviour of *Tribolium* spp. adults in a phosphine atmosphere. This kit is a rapid laboratory bioassay which determines the phosphine tolerance of adult insects. Tests have been made on 4 strains of *Tribolium confusum* and 4 strains of *T. castaneum*. Three strains of each species were collected in different food industries while the fourth was a laboratory strain, reared for several years free of any chemical treatment. The concentration of 3,000 ppm of phosphine used in the tests has a narcotic effect on non-resistant insects within a few minutes, while insects with low phosphine susceptibility are still active after this time period. The results show significant differences related to the phosphine tolerance, particularly with reference to *T. confusum* strains. This laboratory kit is useful to recognize the resistance before a treatment with phosphine.

**Key words:** *Tribolium* sp., phosphine, tolerance, resistance, time to narcosis

**Introduction**

The growing use of phosphine fumigants in stored product protection all over the world has been followed by reports from various countries on resistant insect strains (Champ & Dyte, 1976; Zettler & Cuperus, 1990; Tyler *et al*., 1983; Irshad & Iqbal, 1994; Zettler & Arthur, 1997; Anisur Rahman & Shahjahan, 2000; Mordkovich, 2004). The phenomenon was first observed as cross-resistance in *Sitophilus granarius* (L.) following selection with methyl bromide (Monro *et al*., 1961) and in *Tribolium castaneum* (Herbst) selected with phosphine (Winks, 1969). The toxicity of phosphine to insects and its practical application are well documented (Bond *et al*., 1969; Wainman *et al*., 1975).

As for Italy, the status of insecticide resistance of *Tribolium* strains is unknown: the only data deal with two cases of resistance to lindane and malathion in *Tribolium confusum* Jacquelin du Val and *T. castaneum* strains coming from abroad (Contessi, 1989).

The aim of this study is to investigate the presence of *T. castaneum* and *T. confusum* strains tolerant to phosphine, using a laboratory test developed by Detia® Degesch GmbH. This test is quicker than other tests (for example Bell *et al*., 1994) and is useful to get a quick feedback on the susceptibility of insects to phosphine (Steuerwald *et al*., 2006). Therefore, it was decided to monitor the behaviour and the activity of insect pests, particularly those present in a defined phosphine containing atmosphere. A concentration of 3,000 ppm of phosphine has a narcotic effect on non-resistant insects within a few minutes, while insects with low phosphine susceptibility are still active after this time period. Three strains of *Tribolium confusum* and 3 strains of *T. castaneum* collected in different food industries have been tested with this method and then each species has been compared to one laboratory strain.
Materials and methods

The Detia® Degesch Resistance Test Kit consists of a 100 mL syringe, a cannula with a rubber hose, a 5 L flexible plastic canister and the test kit pellets of magnesium phosphide. In order to generate a phosphine containing atmosphere in a plastic bag, 50 mL of water were put into the canister, then two pellets were added, the canister was closed immediately with the lid and was shaken carefully (Steuerwald et al., 2006). Two pellets of magnesium phosphide produce a concentration between 4,000 ppm and 6,000 ppm. The real concentration ($C_1$) was measured with a Dräger Tube®; to determine the level of dilution needed for a concentration of 3000 ppm ($C_0$) in the syringe, the atmosphere generated in the canister was diluted with air. The amount ($V_{bag}$) which had to be taken from the plastic canister was calculated with the formula $\left( \frac{C_0 \text{ (ppm)} \times 100 \text{ (mL)}}{C_1 \text{(ppm)}} \right) = V_{bag} \text{ (mL)}$. The difference to 100 was the amount of air ($V_{air}$) which was needed to adjust 3,000 ppm in the syringe: $100 - V_{bag} = V_{air}$.

The resistance test was conducted by introducing 20 adults into the syringe; then the syringe was connected with the canister and filled with phosphine atmosphere, adjusting the air volume to reach 3,000 ppm. The activity of the insects was observed after 3, 5, 7, 10, 15 and 20 and the number of knocked down beetles was noted. The total exposure time of insects to phosphine was 30 minutes. The directions for use report that the concentration of 3,000 ppm phosphine has a narcotic effect on non-resistant insects within a few minutes while insects with a low phosphine susceptibility are still active after this time period.

Four replicates of 20 insects were carried out for each Tribolium strain, at 25°C. The reference insects T. castaneum (TCA1) and T. confusum (TCO1) were taken from laboratory stock cultures, where they had been reared for several years free of any chemical treatment. Field strains of T. castaneum were collected in a flour mill in Central Italy (TCA2), in a warehouse of sunflower seeds in Central Italy (TCA3) and in a rice industry in the North of Italy (TCA4), while field strains of T. confusum were collected in a pasta factory (TCO2), in a confectionary industry (TCO3) and in a cocoa industry (TCO4), all located in the North of Italy. Data were analyzed with the probit regression.

Results and discussion

The symptoms of narcosis were described by Winks (1985) during laboratory experiments on T. castaneum adults exposed to phosphine. Immediately after the phosphine was applied, beetles became active. Following a period of hyperactivity, beetles became progressively inactive until they were quite motionless. This phase of progressive inactivity proceeded with beetles rolling over onto their dorsal surface. The same symptoms were noticed in this study and the percentage of narcotized adults of Tribolium strains at fixed observation time is shown in Table 1 and Table 2.

After 3 minutes of exposure to phosphine, among T. castaneum, the laboratory strain (TCA1) shows a significant higher percentage of narcotized adults compared to the other strains (Table 1). By increasing exposure time, the least susceptible strain to phosphine action is TCA2 until 15 minutes of exposure. After 20 minutes no significant differences have been observed (Table 1).

As for as T. confusum, the least tolerant strain to phosphine, after 3, 5 and 7 minutes of treatment, is the laboratory one. After 5 minutes more than 50% of TCO1 adults have been knocked down and the total number of narcotized adults has been observed after 10 minutes. The most tolerant strain is TCO2 which shows the lowest percentage of narcotized adults, even after 20 minutes (Table 2).
Table 1. Percentage of narcotized adults of *Tribolium castaneum* strains after 3, 5, 7, 10, 15, 20 minutes of exposure to 3,000 ppm phosphine at 25°C.

<table>
<thead>
<tr>
<th>strain</th>
<th>% narcotized adults ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 min</td>
</tr>
<tr>
<td>TCA1</td>
<td>7.5 ± 3.3 b*</td>
</tr>
<tr>
<td>TCA2</td>
<td>2.5 ± 1.5 a</td>
</tr>
<tr>
<td>TCA3</td>
<td>2.5 ± 1.5 a</td>
</tr>
<tr>
<td>TCA4</td>
<td>0.0 ± 0.0 a</td>
</tr>
</tbody>
</table>

*Values followed by a different letter, in the same column, are significantly different (P<0.05, ANOVA, Duncan’s test).

Table 2. Percentage of narcotized adults of *Tribolium confusum* strains after 3, 5, 7, 10, 15, 20 minutes of exposure to 3,000 ppm phosphine at 25°C.

<table>
<thead>
<tr>
<th>strain</th>
<th>% narcotized adults ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 min</td>
</tr>
<tr>
<td>TCO1</td>
<td>15.0 ± 2.1 b*</td>
</tr>
<tr>
<td>TCO2</td>
<td>1.2 ± 1.3 a</td>
</tr>
<tr>
<td>TCO3</td>
<td>10.0 ± 4.6 ab</td>
</tr>
<tr>
<td>TCO4</td>
<td>2.5 ± 2.5 a</td>
</tr>
</tbody>
</table>

*Values followed by a different letter, in the same column, are significantly different (P<0.05, ANOVA, Duncan’s test).

The results show that the two strains which are most tolerant to phosphine are TCA2 and TCO2. In the flour mill and in the pasta factory in which they were collected, cereals and silos had been treated with aluminium phosphide several times, but the result was the survival of some insects. The insects that are more difficult to kill may produce offspring that is also hard to kill. These insects are said to be more “tolerant” and if they are repeatedly treated with the same insecticide, a “resistant” strain may be produced. In this study the total exposure time to 3,000 ppm phosphine was 30 minutes: we observed that after this time, narcotized adults were able to recover from narcosis and to lay fecund eggs. Winks (1984) referred that time to narcosis was shorter with higher concentrations and that insects can recover from narcosis if the exposure is not excessive.

Studies on phosphine resistance in *Rhizopertha dominica* (F.) showed that the resistance mechanism is an active exclusion where the insect actively keeps the fumigant away from susceptible sites. Therefore, resistant strains of *R. dominica* take a longer time to succumb to the narcotic effect than susceptible stains do (Price, 1984). In a previous study on *Sitophilus granarius* (L.), the reduction in phosphine absorption concomitant with a lowering of respiration and metabolic rate was suggested as a mechanism of resistance to this gas (Monro *et al.*, 1972).

In another study on the toxicity of phosphine to adults of *T. castaneum*, narcosis was examined for its implications in phosphine resistance because it was thought that narcosis was a form of protective mechanism, in which narcotised insects did not take up as much phosphine (Winks, 1985). Afterwards it was demonstrated that the insects which survived or which remained active were those resistant to narcosis, whereas those that succumbed quickly
were the first to die (Waterford & Winks, 1994). This implies a different mechanism of resistance, as reported by Price (1984). He observed that the major physiological feature of the resistance to phosphine in *R. dominica* is that respiratory metabolism and physical activity appear to be unaffected and oxygen consumption continues unabated (Price, 1980). Normally-respiring, phosphine-resistant *R. dominica* were shown to absorb much less toxicant than their susceptible counterparts, the difference not being attributable to metabolism (Price *et al.*, 1982). Data collected in this study confirm this behaviour because laboratory strains (free from any chemical treatments) show the highest susceptibility to narcosis, while field strains (particularly the two strains collected in the industry where several treatments with phosphine have been made) are more tolerant to phosphine.

The narcotic response to phosphine can be used to indicate the presence of resistant field strains and to decide which treatment should be applied to infested cereals or silos. Pest controllers can vary the phosphine dosage or the exposure time required to kill tolerant and resistant insect strains. This possibility is a big step to prevent the worldwide development and spread of phosphine resistance.

Acknowledgements

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References


Evaluation of a new enhanced diatomaceous earth formulation (DEBBM-P) against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) on stored wheat

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**Abstract:** A new enhanced diatomaceous earth (DE) based formulation (DEBBM-P) developed by Diatom Research and Consulting Inc., Canada, was evaluated for its efficacy against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). The applied concentrations on wheat grains were 75, 100 and 125 ppm at 30°C and 65% r.h. The mortality of adults was assessed after 14 and 21d exposure time to treated and untreated wheat and the number of the progeny was assessed after 60 days. Mortality was higher in the treated substrate as compared to the untreated control. The dose rate of 125 ppm of DEBBM-P gave 100% mortality 14d after treatment and there were no production of the progeny, similarly, the dose rate of 75 ppm gave 100% mortality 21d after treatment and 0.4 adults (F1 generation) emerged per replication in comparison with the untreated controls which had 22.4 emerged adults. The results clearly indicate that the blending of DE’s with other naturally occurring compounds may produce more toxic effect and the synergized DE based formulations may be significantly much effective even applied at low dose rates.

**Key words:** DEBBM-P, enhanced diatomaceous earth, mortality, *Rhyzopertha dominica*, stored wheat

**Introduction**

Due to the hazardous effects of synthetic insecticides on non-target organisms there is a need to search for reduced risk non-chemical alternatives. Diatomaceous Earth (DE) may become an integral part of Integrated Stored Grain Pest Management (ISGPM) program. DE is a dust composed of fossilized bodies of unicellular algae called diatoms. The fine DE dust is mainly made up of amorphous SiO2 which absorbs the epicuticular lipids of the insect cuticle and due to desiccation (Ebeling, 1971; Golob, 1997; Korunic, 1998; Fields and Korunic, 2002) ultimately death occurs. High dose rates of DE affects the bulk density and flowability of the grain (Fields et al., 2002) and long exposure to particles of DE in the air also cause respiratory problems in workers. To mitigate the problems caused with the use of DE, Diatom Research and Consulting Inc., Guelph, Canada developed a new synergized and enhanced DE formulation (DEBBM-P), which is a combination of diatomaceous earth (DE) and a Chinese plant extract (bitterbarkomycin - BBM), (Korunic, 2007). DEBBM-P has a low mammalian toxicity with an oral LD50 for rats higher than 4500 mg/kg (Athanassiou et al. 2005; Korunic, 2007). The specific objective of this study was to evaluate the insecticidal effect of different dose rates of a new enhanced DE formulation called DEBBM-P against adults of *Rhyzopertha dominica* (a most notorious insect pest of stored wheat) and also to assess the progeny production on stored wheat.
Materials and methods

An experiment was designed in the laboratory of Grain Research Training and Storage Management Cell (GRTSM), University of Agriculture, Faisalabad, Pakistan. The Rhyzopertha dominica (F.) adults used were taken from a culture that was kept in the laboratory on stored wheat at 28ºC, 60% r.h. and continuous darkness. A mixed culture of unsexed adults was used in the laboratory trial. A sample of DEBBM-P was obtained from the Diatom Research and Consulting Inc., Guelph, Canada. It is a DE formulation which is a combination of diatomaceous earth and also a Chinese plant extract, bitterbarkomycin (BBM) 0.05% (Athanassiou and Korunic, 2007; Korunic, 2007). There were three concentrations 75, 100 and 125 ppm at 30ºC and 65% r.h. DEBBM-P in the respective concentrations was added to a jar containing 500g of grains of wheat and then mixed by shaking well in a big jar. The 500g of treated grains were then equally distributed among five glass jars containing 100 g of grain in each. Fifty unsexed adults were introduced into each jar and control jars were maintained in untreated wheat. The mouth of the jars was covered with muslin cloth to keep the insects inside and also for sufficient aeration. After 14 and 21d of exposure the grains were sieved and the numbers of live and dead insects were counted. For progeny production the grains were incubated for 60 days and then the emergence was counted. The mortality observed in each treatment was corrected with the mortality in the control (Abbott, 1925). The data were submitted to a one-way analysis of variance (ANOVA) with SPSS software. Means were separated by using the Sidak test, at P = 0.05 (Sokal and Rohlf, 1995).

Results and discussion

The adult mortality increased significantly with exposure interval, although the combined effect of experimental conditions, substrate and enhanced diatomaceous earth is predominant. It was generally shown that after the exposure period of 14 days there was a very high mortality (99.6%) already at dose rate of 75 ppm which increased with an increase in the dose rate i.e. both the high concentrations 100 and 125 ppm gave 100% mortality (Fig. 1). Athanassiou et al. (2005) described that an adequate exposure time is crucially required for the effectiveness of DE, because contact of cuticle with dust increases with the increasing movement of insects.

![Fig. 1. Mean mortality (% ±SE) of R. dominica adults on treated wheat with DEBBM-P after 14 days exposure.](image-url)
After 21 days of exposure interval all the adults were dead (100% mortality) (Fig. 2). It was noted that the enhanced DE formulation (DEBBM-P) gave 100% mortality of the *R. dominica* even at very low dose rates. The high dose rates are not acceptable today because of negative influence on the physical properties of grain, for example bulk density and also for environmental and health reasons (Korunic *et al.*, 1996; Korunic, 1998; Subramanyam and Roesli, 2000). The traditional DE’s are being used at very high rates like Hamel (1997) reported that 100 ppm concentration was not high enough to control *R. dominica*. Similarly, in Italy Contessi and Mucciolini (1997) conducted a large field trial and reported that 600 ppm is required to control the insect pest. Athanassiou and Korunic (2007) also tested this new DE formulation and found the same results that this enhanced formulation of DE is more effective against *R. dominica* as compared to the traditional DE formulations which are currently being used in the world.

![Fig. 2. Mean mortality (% ±SE) of *R. dominica* adults on treated wheat with DEBBM-P after 21 days exposure](image)

The results indicated that there was no emergence of the progeny at the high dose rates (100 and 125 ppm), however, 0.4 offspring were produced where the DEBBM-P was applied at dose rate of 75 ppm (Fig. 3) on stored wheat against *R. dominica* in comparison with the controlled vials where 22.4 number of adults emerged.

Very little data is available on the efficacy of diatomaceous earth against stored grain insect pests under Pakistan conditions till now (Wakil *et al.*, 2006), however, Pakistan is situated in a region with very high temperatures and dry environmental conditions during summer time so the use of DE’s may prove to be an excellent alternative to fumigants (Phosphine) and other traditional insecticides being used in Pakistan in storages. So, there is need of the day to work a lot on the experimentation with both traditional DE’s as well as enhanced DE’s which will surely in future be helpful in exploring the local geological deposits for local DE’s and they will be environmentally safe and economical too.

**Conclusions**

The DE formulation (DEBBM-P) with bitterbarkomycin was highly effective even at low dose rate (75 ppm). This dosage is several times lower than the recommended dosages of currently available commercial DE formulations.
Fig. 3. Progeny production (no of adults emerged ±SE) of *R. dominica* adults on treated wheat with DEBBM-P (means followed by the same letter are not significantly different; Sidak test at \( p = 0.05 \))

**Acknowledgements**

I would like to thank Dr. Zlatko Korunic, Diatom Research and Consulting Inc., 14 Greenwich Dr., Guelph, ON, N1H 8B8, Canada for providing me with the samples of DEBBM-P and protocols for conducting these trials.

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Efficacy of a new grain fumigant: ethyl formate/allyl isothiocyanate for the control of two stored grain beetles, the rice weevil *Sitophilus oryzae* L. and the granary weevil *Sitophilus granarius* L.

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Abstract: The search for new molecules is essential in Europe in response to progressive phasing out of several post-harvest insecticides like dichlorvos, malathion or methyl bromide. Ethyl formate (EF) is a fumigant that is currently used in Australia on dried fruits. The Australian Stored Grain Research Laboratory (SGRL) has recently patented a mixture of EF with allyl isothiocyanate (AITC) 95% / 5% (w/w) to reduce flammability and increase efficacy. It is a volatile liquid at ambient temperature and several trials carried out in Australia have shown very good control against wheat insects. In our laboratory, we began trials on wheat with *Sitophilus oryzae* L. and *Sitophilus granarius* L. because they are the most difficult stored grain insect to control. The mixture was tested with an application rate of 60 g/m³ mixture. Gas concentrations were measured by GC, FID for EF and NPD for AITC. A preliminary test was carried out with small samples of infested grain (200 g) in gastight drums. We obtained successful results with a control of 100% of adults and the most resistant stages (pupae and aged larvae) in less than 24 hours at 20°C. Other experiments were carried out under the same conditions with samples of 12 kg of heavily infested grain in 30-litre drums. Applications were made by introducing the mixture with a syringe on the grain in movement. Under these harsh conditions, high infestation rate and high gastight conditions, a high sorption quickly decreased the concentrations. In addition, anoxia took place very quickly, blocking insect respiration and thus the gas was not allowed to produce its toxic effect on cytochrome c oxidase. The mixture EF + AITC is a promising means to control grain insects and could be registered in integrated protection. However the conditions of its use have to be carefully studied.

Key words: ethyl formate, allyl isothiocyanate, *Sitophilus oryzae*, *Sitophilus granarius*, grain fumigation, gastight conditions, anoxia

Introduction

In Europe, several insecticides were recently phased out (malathion, dichlorvos, methyl bromide). As a result, it is necessary to find new contact insecticides or new fumigants. Currently, even if phosphine (PH₃) is the only registered fumigant available in France, it is not widely used. Ethyl formate (EF) under the trade name VAPORMATE® is currently used in Australia on dried fruits and more recently on grain, mixed with CO₂. Many experiments have been carried out where it was be found that EF is effective against stored grain pests (Muthu *et al.*, 1984). EF and allyl isothiocyanate (AITC) were separately tested with success on grain weevils at the beginning of 20th-century (Neifert *et al.*, 1925). AITC is derived from the plant family, Cruciferae (Tsao *et al.*, 2002). The Australian Stored Grain Research Laboratory (SGRL) conducted several successful trials on wheat in 2005 (Fisherman Islands, QLD) (Ren *et al.*, 2005). In this case, ethyl formate was used with a AITC as synergist: 95% EF and just 5% AITC (w/w). EF presents a very low flash point (-22°C) but it is not a
problem if the lower explosive limit (LEL) of 2.8% is not crossed, which is equivalent to 85 g/m³ (Damcevski & Annis, 2002). The residues decline shortly after fumigation to natural levels without forced aeration (Annis & Graver, 2000). EF shows a very high and safe Threshold Limit Value (TLV): 100 ppm (Fehler! Ungültiger Eigenverweis auf Textmarke.).

Table 1. Comparison of EF characteristics to Phosphine.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Phosphine</th>
<th>Ethyl formate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor</td>
<td>Strong garlic odor</td>
<td>Organic odor</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>34</td>
<td>74.1</td>
</tr>
<tr>
<td>Density (g/L)</td>
<td>1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>-87.7</td>
<td>53</td>
</tr>
<tr>
<td>Flammability</td>
<td>&gt; 2% and 100°C</td>
<td>&gt; 2.8 %</td>
</tr>
<tr>
<td>Mode of action</td>
<td>Superoxyde formation</td>
<td>Inhibition of cytochrome c oxidase</td>
</tr>
<tr>
<td>TLV</td>
<td>0.3 ppm</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Toxicological classification</td>
<td>T +</td>
<td>Xn</td>
</tr>
</tbody>
</table>

According to CSIRO International Patent #WO 2006/066308 A1, EF + AITC has many appealing traits. It shows a very fast action and it is naturally present in soil, oceans, plants, and commodities (Vu & Ren, 2004).

This ethyl formate + AITC mixture was evaluated by CSIRO Entomology in Australia (Ren et al, 2005), in the last experiments on *Sitophilus oryzae* L. in a large scale. Two sealed metal vertical silos (50 t capacity and 60 m³) of wheat were fumigated with an application rate of 80 g/t (95% EF + 5% AITC (w/w)). The application was made by pouring the formulation directly on the top of the wheat. An air recirculation system was used (0.5 kW fan). The temperature of the wheat was 30°C with a moisture content of 12.5%. After five days of fumigation, the hatch was opened, but no forced aeration was used.

Table 2. Results of trial fumigation of wheat using an EF + AITC mixture in Australia.

<table>
<thead>
<tr>
<th>Results after 5 days of fumigation</th>
<th>Silo 1</th>
<th>Silo 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTP (g.h/m³)</td>
<td>790</td>
<td>650</td>
</tr>
<tr>
<td><em>S. oryzae</em> adults killed</td>
<td>100 %</td>
<td>99.5 %</td>
</tr>
<tr>
<td><em>S. oryzae</em> pupae/larvae killed</td>
<td>100 %</td>
<td>99.4 %</td>
</tr>
<tr>
<td><em>S. oryzae</em> eggs killed</td>
<td>100 %</td>
<td>No data</td>
</tr>
<tr>
<td>Color of wheat</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>EF residues (experimental MRL = 0.2 mg EF/kg)</td>
<td>&lt; 0.2 mg EF/kg</td>
<td>&lt; 0.2 mg EF/kg</td>
</tr>
<tr>
<td>AITC residues (experimental MRL = 0.1 mg AITC/kg)</td>
<td>&lt; 0.1 mg AITC/kg</td>
<td>&lt; 0.1 mg AITC/kg</td>
</tr>
</tbody>
</table>
In Silo 1, all stages were controlled, but in Silo 2: 99.5% of adults and 99.4% of pupae and larvae were killed. There were no data for results on eggs. The main difference between the two silos’ conditions was the Concentration Time Product (CTP). The optimal CTP was 650 and 790 g.h/m$^3$, respectively. In neither case did the color of wheat change and EF and AITC residues decreased quickly below the experimental Maximum Residues Levels (Table 2).

After these excellent experimental results in Australia, this mixture was tested in France (LNDS-QUALIS).

**Material and methods**

**Insects**

Sitophilus granarius and Sitophilus oryzae were reared in the laboratory at 25°C (± 1°C) and 60% RH (± 5%). The infestation rate to obtain the emergence of a new generation of insects (F1) was 10 adults/kg of wheat. Each culture started with 320 adults on 32 kg of wheat in 120-litre plastic drums.

**Preliminary laboratory trial (small scale)**

Before carrying out trials on a semi-large scale, the efficacy of the mixture without commodity sorption was verified. The fumigation was conducted in gas-tight plastic drums at 20°C with an application rate of 60 g/m$^3$. Samples of 200 g of infested wheat with mixed aged cultures of Sitophilus oryzae and Sitophilus granarius were put into the drums just before the injection of the liquid mixture. Two exposure times were tested, 24 and 48 hours.

Another experiment was carried out in order to study the possible difference of sorption between the grain infested by S. granarius and that by S. oryzae. Two columns with a capacity of 2.8 L containing 1 kg of infested wheat were treated with the mixture at 20°C with an application rate of 60 g/m$^3$ for an exposure time of 24 hours. The mixture was injected using a syringe through a septum and then the column was shaken for five minutes.

**Trials on a semi-large scale**

Four exposure times were tested: 24, 48, 72 and 96 hours. The wheat was infested by Sitophilus oryzae and Sitophilus granarius. A very high infestation rate was observed in the grain, two months after infestation. There was an average of 600 S. granarius adults per kilogram of wheat and 1100 S. oryzae adults / kg of wheat. One control per species was kept for 96 hours. In 30-litre gas-tight plastic drums, 12 kg of wheat were treated for each replicate, with an application rate of 60 g/m$^3$. This dosage was chosen in order to stay below the low explosive level (LEL) by a large margin. The injection of the mixture was introduced directly into the grain in movement with a syringe through a septum. Each drum was spun for 5 minutes in order to homogenize the mixture into the grain for five minutes. The temperature of the wheat was 20°C and the moisture content was 13%. EF concentrations were measured by a Gas Chromatograph (GC), Flame Ionization Detector (FID) and AITC concentrations by a GC Nitrogen Phosphorus Detector (NPD). After fumigation, the percentage of oxygen in drums was measured by an oxymeter HM16N, and then, the drums were opened. This meter has a 0.1% resolution scale with 1% error. Two-kilogram samples were taken and sifted in order to count the adult mortalities. These samples were then placed in the rear chamber at 25°C (± 1°C) and 60% RH (± 5%), and sifted again to evaluate the mortality rate of hidden stages according to the development period of immature stages (Balachowsky, 1963).
Results

The preliminary trial shows that with the application rate of 60 g/m³ (Table 3), all stages were controlled for both species and both exposure times (24 and 48 hours).

Table 3. Mortality rates of all stages of mixed age cultures of *Sitophilus granarius* and *S. oryzae* in the preliminary trial (small scale) for the application rate of 60 g/m³ of EF.

<table>
<thead>
<tr>
<th>exposure times</th>
<th>species</th>
<th>mortality rate (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. granarius</em></td>
<td>adults: 100%</td>
<td>pupae: 100%</td>
<td>larvae: 100%</td>
<td>eggs: 100%</td>
</tr>
<tr>
<td>24 h</td>
<td><em>S. oryzae</em></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>48 h</td>
<td><em>S. granarius</em></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td><em>S. oryzae</em></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

On a semi-large scale, the concentration of EF decreased very quickly after the injection of the mixture and after 24 hours of exposure time, it remained below 1 g/m³ in all drums (Fig. 1 and

Fig. 3). Generally, in the first hours following the injection, line curves increase slightly. The concentration peak observed never exceeded 12 g/m³ in the drums of *S. granarius* and 7.5 g/m³ in the drums of *S. oryzae*. The AITC concentrations for both species fell very fast in all drums. The maximal values were measured after 1 hour with concentrations between 0.4 and 0.6 g/m³ in the drums of *Sitophilus granarius* and between 0.35 and 0.45 g/m³ for *S. oryzae* drums (Fig. 2 and Fig. 4).

All the data show that concentrations were always higher in the drums of *S. oryzae* than in those of *S. granarius*.

![Fig. 1. Evolution of EF concentrations in the drums of *Sitophilus granarius*, measured by Gas Chromatograph (FID).](image-url)
The ratio between the application rate and the maximum concentration measured was practically the same for EF and AITC. The average maximum EF concentration for all drums was 8.26 g/m³, that is to say, 6.9 times less than the application rate (57 g/m³). The average maximum AITC concentration was 0.47 g/m³, that is to say, 6.4 times less than the application rate (3 g/m³) (Table 4). After 24 h, there remained on the average about 0.75 g EF/m³; that is 11.1 times less than the maximum average concentration. It was in the same range for AITC concentrations, there remained, after 24 h, 0.06 g AITC/m³ on average; that is 7.4 times less than the maximum average concentration (Table 5).
The $O_2$ concentrations in the drum headspaces decreased with the exposure times (Fig. 5). After 48 h, less than 3% $O_2$ was measured in the drums of *Sitophilus granarius* and less than 2% $O_2$ in the *S. oryzae* drums.

The sorption experiment (Fig. 6) revealed that the wheat infested by *S. granarius* sorbed less than that infested by *S. oryzae*. For example, after an 10-hour exposure, EF concentration in the column of *S. granarius* was 5.6 g/m$^3$ and just 2.5 g/m$^3$ for the *S. oryzae* column.

Fig. 4. Evolution of AITC concentrations in the drums of *Sitophilus oryzae*, measured by Gas Chromatograph (NPD).

The adult mortality results for different exposure times showed that practically all adults were controlled (99.8%) after 24 h for *S. oryzae*. A total control of adults was observed in less than 72 h. However, the control presented a mortality rate of 99.9% without treatment. For *S. granarius* adult mortality results, the mortality percentage increased with exposure time, except for the 72 h drum (Table 7). But, a total control was still not reached after 96 h. Less than 50% of adult insects were killed in the control.

Table 4. Ratio between average maximum concentrations and initial application rate for EF and AITC in the first hours after injection

<table>
<thead>
<tr>
<th>application rate</th>
<th>average maximum concentrations</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF 57 g/m$^3$</td>
<td>8.26 g/m$^3$</td>
<td>6.9</td>
</tr>
<tr>
<td>AITC 3 g/m$^3$</td>
<td>0.47 g/m$^3$</td>
<td>6.4</td>
</tr>
</tbody>
</table>
Table 5. Ratio between average maximum concentrations and concentrations after 24 hours for EF and AITC.

<table>
<thead>
<tr>
<th></th>
<th>average maximum concentrations</th>
<th>average concentrations after 24 h</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
<td>8.26 g/m³</td>
<td>0.745 g/m³</td>
<td>11.1</td>
</tr>
<tr>
<td>AITC</td>
<td>0.47 g/m³</td>
<td>0.063 g/m³</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Fig. 5- Oxygen concentrations in the drum headspace before degassing for each replicate.

Fig. 6. Evolution of EF concentrations in the column of S. granarius and S. oryzae.
Table 6. Mortality rate of *Sitophilus oryzae* adults for different exposure times, at 20°C.

<table>
<thead>
<tr>
<th>exposure time</th>
<th>total insects in 2 kg of wheat</th>
<th>mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>1690</td>
<td>99.8</td>
</tr>
<tr>
<td>48 h</td>
<td>1726</td>
<td>99.9</td>
</tr>
<tr>
<td>72 h</td>
<td>3115</td>
<td>100</td>
</tr>
<tr>
<td>96 h</td>
<td>2713</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>1925</td>
<td>99.9</td>
</tr>
</tbody>
</table>

Table 7. Mortality rate of *S. granarius* adults for different exposure times, at 20°C.

<table>
<thead>
<tr>
<th>exposure time</th>
<th>total insects in 2 kg of wheat</th>
<th>mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>1281</td>
<td>64.6</td>
</tr>
<tr>
<td>48 h</td>
<td>1300</td>
<td>96.2</td>
</tr>
<tr>
<td>72 h</td>
<td>1083</td>
<td>90.1</td>
</tr>
<tr>
<td>96 h</td>
<td>1274</td>
<td>98.7</td>
</tr>
<tr>
<td>Control</td>
<td>1034</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

The mortality of pupae and aged larvae (L4/L3) for different exposure times showed that less insects emerged in the 96 h sample than in the control for *S. oryzae* (Table 8), giving an emergence reduction of 39.3%. The emergence reduction was smaller with less than 96 h exposure time,. For *S. granarius*, there were no differences between insects which emerged in the control and in the 96 h drum (Table 9). The emergence reduction for this species was close to zero.

Table 8. Mortality rate for *S. oryzae* pupae and aged larvae for the longest exposure time.

<table>
<thead>
<tr>
<th>exposure times</th>
<th>insects emerged / kg of wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 h</td>
<td>1791</td>
</tr>
<tr>
<td>Control</td>
<td>2950</td>
</tr>
</tbody>
</table>

Table 9. Mortality rate for *S. granarius* pupae and aged larvae for the longest exposure time.

<table>
<thead>
<tr>
<th>exposure times</th>
<th>insects emerged / kg of wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 h</td>
<td>237</td>
</tr>
<tr>
<td>Control</td>
<td>239</td>
</tr>
</tbody>
</table>

**Discussion**

The application rate was successfully tested in the preliminary trial. However, the sorption factor was not taken into account, and the results of the sorption study (Fig. 6) show that the wheat sorbed very quickly after injection, with a difference between the two species. It is difficult to deduce an interpretation from this result, but it is possible that the wheat infested
by *S. granarius* was more spoiled by insects than *S. oryzae* wheat, and therefore, the sorption rate was lower.

EF concentrations (Fig. 1 and Fig. 3), in the first hours following injection, increased slightly in most cases, because during this period, EF was evaporating. This increase corresponds to the EF evaporation period. The application rate was 60 g/m³ which corresponds to 57 g of EF/m³, but the concentrations were never close to this value. They were at a very low level of about 10 g/m³. At the same time as EF was evaporating, there was a huge EF gas sorption by the grain resulting a drastic decrease in concentration.

AITC concentrations also fell very quickly after injection, because this molecule was sorbed by the wheat. Thus similarly to EF, there wasn’t an important AITC peak (Fig. 2 and Fig. 4). EF and AITC maximum concentrations were respectively about 6.9 and 6.4 less than the initial application rates (Table 4).

The AITC and EF concentrations after 24 h showed a similar decrease in a ratio of sorption within approximately the same range (Table 5), respectively of seven to eleven times less than the maximum concentration.

The adult mortality rate seemed to provide very efficient results for *S. oryzae* for all exposure times. The problem was, that, in the control drum, practically all insects were killed (99.9%) after 96 h under the existing gas-tight conditions. In fact, it seems that the anoxia took place very quickly after sealing the drums hermatically. Insect breathing leads to an O₂ decrease and at the same time, a CO₂ increase. Nevertheless, the *S. granarius* control seemed to be less affected by anoxia than the *S. oryzae* control (Table 7). That may have been due to the slower decrease in O₂ concentration in the drum headspace volume than in the *S. oryzae* drums (Fig. 5). The O₂/CO₂ mixture with a low percentage of O₂ (< 4%) and a high percentage of CO₂ has a toxic effect on *S. granarius* and *S. oryzae* (Fleurat-Lessard & Le Torc’h, 1991). It is possible that the *S. oryzae* adult mortality rate is higher than *S. granarius* adult mortality rate, because *S. oryzae* drums reached these harmful conditions faster than the *S. granarius* drums and then remained that way for a longer exposure time. The insect emergence results reveal that, for *S. oryzae*, there was 39.3% emergence reduction after a 96 h exposure time and no emergence reduction for *S. granarius*. So, the efficacy on all stages was bad. Several hypotheses for this can be suggested.

- The application rate was not sufficient, but the dosage applied was higher than the one used in trials carried out in Australia, 60 g/m³ in these experiments represented 150 g/t.
- The fumigation chambers should have been equipped with an air-recirculation system. That was the main difference in the application method between trials carried out in France and in Australia.
- The differences between French and Australian species can have an influence on insect susceptibility.
- The higher temperature of the wheat in Australia (30°C) with high variations in headspace (18-50°C)) than wheat in France (20°C). But the main explanation leading to the lack of efficacy was certainly anoxia, which is due to the high infestation rate. A lot of hypotheses can be given, but this is the most obvious:
- A part of the insects (adults or immature stages) died, because they breathed a lethal dose of insecticide (EF inhibits cytochrome c oxidase (Haristos & Dojchinov, 2003)); or the modification in the drum atmospheric composition (a CO₂ increase and an O₂ decrease) was toxic for them.
- A part of the insects (adults or immature stages) resisted, because the induced conditions caused a slow-down of their respiratory exchanges. Thus they did not breathe a sufficient enough dose to die (Fleurat-Lessard, 1990).
Conclusion

A treatment on a very small scale with a mixture EF + AITC gave successful results with a control of 100% of all stages, in less than 24 hours at 20°C for the two species *S. granarius* and *S. oryzae*. However, sorption was not taken into account. Other experiments were carried out on a semi-large scale with harsh conditions, a high infestation rate and high gastight conditions. A high sorption quickly decreases the EF and AITC concentrations. There is a smaller sorption by the wheat infested by *S. granarius* than that infested by *S. oryzae*. The mortality rates of hidden stages were very low. In addition, anoxia takes place very quickly, and it could explain the lack of efficacy of the fumigation.

More trials have to be carried out to understand the lack of efficacy under the conditions which existed and more particularly, without the problem of anoxia. With this unexpected parameter, it is difficult to distinguish between the insect mortality due to anoxia, and to the fumigant. If a solution concerning the non-controlled insects can be found, the mixture: ethyl formate + allyl isothiocyanate could be a promising insecticide to control stored grain insects. An interesting attribute of the mixture is the AITC fungicidal effect. It will be investigated in order to determine if the mixture can be used in other fields of treatment for stored grain. This insecticide presents many characteristics providing an incentive to pursue further study.

References


Ethyl formate efficacy in combination with low pressure or at atmospheric pressure in mixture with CO₂ against the dried fruit beetle, *Carpophilus hemipterus* (L.) on prunes

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Abstract: The current disinfestation of prunes is carried out with phosphine fumigation, after methyl bromide having been phased out for its action as an ozone-depleting compound. But phosphine fumigation takes time, typically the exposure time above 15°C is between 3 to 10 days according to the temperature. Low molecular weight volatile compounds such as ethyl formate (EF) are produced naturally by many types of fruit and vegetables. They also have been shown to have insecticidal properties. Ethyl formate is currently registered in Australia for dried fruit, but high doses are required to control pests such as the dried fruit beetle, *Carpophilus hemipterus* (L.) (8-h (25°C) at 73g/m³). To increase the EF efficacy, two methods were investigated. At normal atmospheric pressure (NAP), EF is mixed with CO₂ as in VAPORMATE® cylinders, 83.3 wt% CO₂ and 16.7 wt% EF. In a vacuum, pure EF is used at different levels of pressure. One insect species was tested, the dried fruit beetle, *Carpophilus hemipterus* (L.).

At NAP (EF + CO₂), fumigation of eggs showed that 9-h (20°C) exposures at 70g/m³ were more effective than 12-h (20°C) exposures at 50g/m³. Tests with pupae and adult stages, in NAP (EF+CO₂) fumigation showed that 100% mortality was achieved at 6-h (20°C) at 70g/m³; eggs were controlled at 9-h (20°C) at 70g/m³. In vacuum fumigation, adult stages were controlled at 40g/m³ in 2-h (20°C) at 800 mb. For the others stages, the fumigation dosage was doubled, going from 40g/m³ to 80g/m³. Pupae were already controlled, but only 93% of eggs died at 80g/m³ in 2-h (20°C) at 900 mb. Trials are to be done to find out the exposure time and the dosage necessary to kill 100% of the eggs. With both treatments, eggs were the most resistant stage, much more tolerant than pupae, and adults, the most susceptible. In conclusion, the use of EF at NAP either with CO₂ or pure in a vacuum is of great interest to quickly control dried fruit pests.

Key words: ethyl formate, carbon dioxide, vacuum treatment, *Carpophilus hemipterus*, prunes, dried fruit fumigant

Introduction

The prunes came from drying the plum tree variety “prune d’Ente” in large trays at 70°C for 20 to 30 hours, until reaching 20 to 25% moisture content which fits storage conditions (Cangardel, 1976).

During the storage period, a number of insect pests can be found in prunes. The dried fruit beetle, *Carpophilus hemipterus* (L.) Nitidulidae, is an agricultural product pest (Dowd, 1987) that attacks ripe and overripe fruits. Several species of nitidulid beetles are particularly associated with dried fruit, because they are field and storage pests (Navarro et al., 1998).
Prune fumigation was commonly carried out with methyl bromide or now with phosphine, although the use of both chemicals has become increasingly difficult. In 1995, the parties of the Montreal Protocol decided to phase out methyl bromide and this process was completed in the European Union in 2005 (Règlement (CE) No 2037, Anon. 2000). Phosphine fumigation takes time. Typically, the exposure time above 15°C is between 3 to 10 days according to the temperature (Mahon and Ren, 2003). The development of an alternative fumigant is essential.

Ethyl formate (EF) has been considered a candidate for development as a fumigant (Muthu et al., 1984). This liquid organic compound, known since 1925 (Neifert, 1925), has been used for a variety of purposes: a fumigant to disinfect textiles (Busvine & Vasuvat, 1966) and an artificial flavour for drinks (Hilton & Banks, 1997, Desmarchelier, 1999). Ethyl formate is still registered in Australia by Orica Australia® for use on dried raisin disinfection (Ryan and Bishop, 2003). It has certain characteristics that suggest its interest for fumigation. First, it is naturally produced by many types of fruit and vegetables (Nursten, 1970). These low molecular weight volatile compounds can potentially break down into biogenic levels in the tissues of treated commodities (Simpson et al., 2004) and contrary to conventional chemicals, there are only trace amounts of residues found (Muthu et al., 1984). EF has been shown to have insecticidal properties (Aharoni et al., 1987) with a rapid action against insects (2-4 hours) (Ryan et al., 2006). Finally, toxicological studies have shown a relatively low mammalian toxicity (Haritos et al., 2003). However, the applied dosage of EF needed to control fruit pests is, generally, close from the flammable limit (2.8% v/v in air) (Haritos et al., 2006) and dispensed into a large space could result in a destructive explosion (Ryan & Bishop, 2003).

To increase Ethyl Formate efficacy, without increasing the dosage, two methods were investigated. The first one was EF + carbon dioxide (CO₂) in normal atmosphere pressure (NAP). It has been established that CO₂ can enhance fumigant toxicity to insects and increase the flammability limit (Nicolas & Sillans, 1989). The new BOC Ltd. pesticide Vapormate® (16.7% EF w/w and 83.3% CO₂ w/w) registered since March 2005 for stored grain, pulses, fresh products and packaged food, is a new weapon against stored product insects (Ryan et al., 2006). This formulation was designed to be below the flammability limit no matter what concentration may be in the air (Ryan & Bishop, 2003). The second method investigated is EF + low pressure. The fumigation in a vacuum has two advantages: 1. By comparison to methyl bromide, vacuum fumigation is more effective than at NAP and the exposure time may be reduced from 24h to 2h (Bond, 1984). 2. In a vacuum, the level of oxygen (O₂) is very low and reducing the risks of fire caused by EF flammability.

This present work has two main objectives: First to verify at NAP the efficacy of EF mixed with CO₂ against egg, pupal and adult stages of Carpophilus hemipterus (L.), at the same level as the mixture of Vapormate® cylinders (16.7% EF w/w) at 20°C. Second, to determine the efficacy of pure EF used under different levels of pressure against egg, pupal and adult stages of Carpophilus hemipterus (L.) at 20°C.

Materials and methods

Chemicals
The ethyl formate used had a purity of 98% with a specific gravity of 920g/litre, (Sigma-Aldrich Chemical Company). The carbon dioxide was 99.8% food grade, provided by Messer France.
**Target pest**

Dried fruit beetles were reared at 25°C in 70% relative humidity in plastic boxes aerated by a wire mesh on rehydrated prunes. Moist sand was placed in each box to provide pupation sites. To conserve relative humidity in the box, wet sponges were placed over the prunes (Fig. 1).

For the egg stage, adult dried fruit beetles were placed in a plastic box with dried prunes rehydrated under a wet sponge and females were allowed to lay on prunes for 24h. 30 eggs were collected from the prunes with a brush and put down on black paper, then placed into Petri dishes (Ø 55 mm) for each replicate. For the other stages, 30 adults and 30 pupae were collected from insect colonies and placed respectively into glass jars (200ml) with a wet sponge to keep a high level of relative humidity. After treatment, all stages were transferred into the rearing room maintained at 25°C and 70% relative humidity to determine the mortality of the adults, of the eggs on the 3rd day and of the pupae on the 8th day.

![Wet sponge Moist sand](image)

**Fig. 1.** Plastic box for rearing dried fruit beetles, aerated by a wire mesh. To conserve humidity in the box, a wet sponge is placed over the prunes. Moist sand is placed in each box to provide pupation sites

![Vaults](image)

**Fig. 2.** Vaults are equipped with a valve and septum. Pure ethyl formate is applied at the target concentration by injection, with a syringe, via the septum.

**Fumigation**

*EF+CO₂ in NAP*

All experiments were conducted at 20°C for 6h, 9h and 12h. For each exposure time, the jars and Petri dishes were placed in 10.5 litre vaults fitted with a lid equipped with a valve and septum. The carbon dioxide was put into a 5 L Tedlar® bag. A laboratory vacuum pump connected to the vault takes away a volume of air and later, incorporates the same volume of CO₂ dosage. Then, it was introduced into the headspace by the difference in pressure,
measured by a manometer, until reaching the atmospheric pressure. Finally a target concentration of pure EF was applied (e.g. 5, 10... g/m³) by liquid injection with a syringe, via the septum. The air in each vault was mixed using a micro-ventilator put on the vault bottom. Ethyl formate concentrations were monitored by gas chromatography (see Section 2.3.3). At the end of the exposure period, vaults were aerated and insects were placed into a rearing room.

**EF + Low pressure:** All experiments were carried out at 20°C for 2h, 3h and 4h and two dosages 40 and 80 g/m³. For each exposure time, the jars and Petri dishes were placed in 10.5 litre vaults. Each vault was equipped with a valve which was connected to a pump, and measured by manometer in a range of 0 mb (atm. pressure, relative measure) to 1000 mb (full vacuum). A laboratory vacuum pump was used to obtain a value under the target experimental pressure. A target concentration of pure EF was applied by liquid injection, with a syringe, via a septum and bringing the vacuum back to the target pressure. The air in each vault was mixed using a micro-ventilator put on the vault bottom. At the end of exposure period, vaults were aerated and insects were placed into a rearing room.

**Concentration measurements:** Ethyl formate concentrations were measured by using a Varian 3400 gas chromatograph fitted with a flame ionisation detector and run isothermally at 150°C. Injector and detector temperatures were 160°C and 210°C respectively. Samples were injected through a gas valve and compared to standard concentrations.

**Mortality analysis**
Mortality data were analysed by the probit method, using a computer program (SAS Institute, V 8.2) based on the Finney method (Finney, 1971).

The fumigant exposure Concentration Time Product (CTP) was calculated from the area under the concentration-time curve of the measured ethyl formate concentrations throughout the exposure time and expressed as mg h litre⁻¹ using the equation:

$$CTP = \sum_{i=1}^{n} \frac{(C_i + C_{i+1})}{2} \times (t_{i+1} - t_i)$$

Where the concentration observed or calculated is Cᵢ at any time tᵢ and there are n observations.

**Results**

For all the experiments, the sorption is nil, they were no or few leaks in the vaults.

**EF + CO₂ in NAP**
Fumigation of adults and pupae shows that 100% mortality was achieved at 245g/h/m³ (i.e. 30g/m³) and 420g/h/m³ (i.e. 70g/m³) respectively, for an exposure time of 6 Hrs. But only 80.9% of eggs died at the highest CTP tried, 409g/h/m³ (i.e. 70g/m³). The probit’s give the CTP for 50% mortality (CTP₅₀) for each stage. Eggs were the most tolerant life stage to EF + CO₂ fumigation, and adults were the least tolerant stage (Table 2; Fig.3)

A trial on eggs was carried out to determine the total control of this stage at 20°C. The exposure time was increased from 6 hours to 9 hrs and 12 and 90g/m³ was added to the previous dosages (50 and 70g/m³), 100% of mortality occurred at 70g/m³ (CTP: 594gh/m³), for an exposure time of 9 hours (Fig.4).
**EF + low pressure**

Fumigation at low pressure for an exposure time of 2 hours at 20°C shows total control of the adult stage at 40g/m³ and -800 mb, but only 93% of pupae died at the same conditions. The eggs were very tolerant.

Table 1. Mortality of adults, pupae and eggs of *Carpophilus hemipterus* for trials at 6 hour exposure time and at 20°C for several CTPs. Grey boxes indicate that there is no trial for this CTP. For each CTP, for each stage, the total number of insects and the number of dead are indicated.

<table>
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Table 2. Probit transformation of the mortality data (Table 1) for exposures of adults, pupae and eggs of *Carpophilus hemipterus* to ethyl formate and CO₂. The probit regression line is given with intercept and slope. Indicated are also values of slope, CTP₅₀, CTP₉₅, and their superior and inferior limits.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Regression line</th>
<th>Slope</th>
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<th>CTP₉₅</th>
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<tr>
<td></td>
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<td>Value</td>
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<td>D+</td>
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<td>Egg</td>
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Fig. 3. Probit lines showing the mortality (%) of *Carpophilus hemipterus* adult, pupal and egg stages exposed to ethyl formate with CO₂ for an exposure time of 6 hours at 20°C.

Fig. 4. Mortality (%) of eggs of *Carpophilus hemipterus* at two exposure times: 9 hours (dark column) and 12 hours (lighter grey column) at three dosages (50, 70 and 90 g/m³) at 20°C.

Table 3. Mortality of adults, pupae and eggs of *Carpophilus hemipterus* for trials at 2-hour exposure time at 40g/m³ and 20°C for some low pressures. For each pressure and stage, the total number of insects and the number of dead individuals are indicated.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Adult</th>
<th>Pupal</th>
<th>Egg</th>
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</thead>
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<tr>
<td></td>
<td>Total</td>
<td>Dead</td>
<td>% Mortality</td>
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<tr>
<td>Relative pressure (mb)</td>
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<td>-800.0</td>
<td>60</td>
<td>60</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table 4. Probit transformation of the mortality data (Table 3) for exposures of adults, pupae and eggs of *Carpophilus hemipterus* to ethyl formate and low pressure. Probit regression line is given with slope and intercept. Indicated are also the values of slope and their limits, LP50, and LP95.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Regression line</th>
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<th>LP50</th>
<th>LP95</th>
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<td></td>
<td></td>
<td>Value</td>
<td>D-</td>
<td>D+</td>
</tr>
<tr>
<td>Adult</td>
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<td>-0.1</td>
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<tr>
<td>Pupal</td>
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<td>-0.1347</td>
<td>0.9088</td>
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<tr>
<td>Egg</td>
<td>$y = -2.49 + 0.22 \ln(LP)$</td>
<td>0.22</td>
<td>-0.36</td>
<td>0.81</td>
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</table>

Fig. 5. Probit lines showing the mortality (%) of *Carpophilus hemipterus* adult, pupal and egg stages exposed to ethyl formate with low pressure for an exposure time of 2 hours.

The probits give the Low pressure (LP) of 50% mortality (LP50) for each stage. Eggs were the most tolerant life stage to pure EF + low pressure fumigation, and adults were the least tolerant stage (Table 4; Fig.5)

A trial on eggs and pupae, under low pressures, was carried out to determine the total control of these stages at 20°C. The fumigation dosage was doubled, going from 40g/m³ to 80g/m³ and a higher vacuum was added, -900 mb. After an exposure time of 2 hours, pupae were already controlled at -800 mb, but only 93% of eggs died at the highest vacuum at -900 mb (Fig. 6).

**Discussion**

Our results indicate that the dried fruit beetle response to EF depends on experiments as well as life stages. Ethyl formate applied with carbon dioxide provides a rapid control of *Carpophilus hemipterus* (L.) adult and pupae stages in only 6 hours. Pupae died at a CTP of 420gh/m³ and adults died at a CT product of 245gh/m³. The eggs needed a longer exposure time for 100% mortality. The eggs were more tolerant than adults and pupae. The complete mortality of eggs is achieved in a 9-hour exposure time at 70g/m³ with a CT product of 594g*h/m³. The slope and the lethal CTP given by the probit regression lines confirm this result. Nevertheless, the fumigation of eggs showed that a 9-hour exposure time at 70g/m³
(CT product 594 g*h/m³) at 20°C is more effective than a 12-hour exposure time at 50g/m³ at 20°C (CT product of 583 g*h/m³), we can deduce that dosage prevails over exposure time for this fumigant; Hilton et al. (1997) found the same result.

In the first trials, adults, pupae and eggs were tested with an application of pure ethyl formate at low pressures ranging from -200 to -800 mb (relative pressure) for 2-hour exposure times at 40 g/m³ at 20°C. Only adults were controlled at 40g/m³ at -800 mb, the pupae and egg stages were not controlled. The eggs were more tolerant than adults and pupae. The complete mortality of pupae is achieved in the second trial where concentration was doubled from 40 to 80 g/m³, for an exposure time of 2 hours at 20°C for a vacuum of -800 mb, but only 93% of eggs died at a lower level of -900 mb. The slope and lethal CTP given by the probit regression lines confirm this result.

For a same dosage, EF is more efficient with a mixture of CO₂ than in a vacuum. The trials with low pressure do not achieve a total control for all stages.

In all the experiments, the most susceptible stage is that of the adult, and the eggs were more tolerant than pupae contrary to other bibliographic results which find pupae as the most resistant stage (Vincent and Lindgren, 1972 and Hilton and al., 1997).

![Fig. 6. Mortality (%) of eggs (striped bar) and pupae (solid bar) of Carpophilus hemipterus at two low pressures: -800 and -900 mb at 80 g/m³ and 20°C.](image)

**Conclusions**

This study has investigated two solutions to increase EF efficacy without increasing flammability risk. The first one, EF + CO₂ in NAP at the same level as the mixture of Vapormate⁶ (BOC Ltd.) cylinders (16.7% w/w EF in CO₂). The second one, pure EF used at different pressure levels. One insect species was tested, the dried fruit beetle, *Carpophilus hemipterus* (L.) on three stages: adult, pupae and egg.

In NAP fumigation with EF + CO₂ at 20°C, tests with pupae and adult stages for an exposure time of 6 hours, show 100% mortality at 70g/m³; eggs were controlled at 9 hours and 70g/m³. In vacuum fumigation with pure EF at 20°C with a 2-hour exposure time, the adult stage is controlled at 40g/m³ and 800 mb at this vacuum, 80g/m³ is needed for pupae and at this dosage, even with the higher vacuum of 900 mb, only 93% of eggs were killed.
For the same dosage, EF is more efficient with a mixture with CO₂ than in a vacuum. The most susceptible stage is that of the adult, and eggs were more tolerant than pupae.

These preliminary results should be confirmed in further studies. On the basis of rapid kill observed with adults and pupae of *Carpophilus hemipterus* (L.), it is worth extending similar mortality studies, with CO₂ and low pressure to other dried fruit pests like *plodia interpunctella* (Hübner). Studies are to be carried out to understand the behavior of eggs in a vacuum and to take sorption into account in trials with fruits.

To conclude, this study shows that ethyl formate mixed with CO₂ is a good alternative to other fumigants against dried fruit pests, but the results in a vacuum did not give complete control of eggs: more study is needed for this stage.

References


Session 7:
Integrated pest prevention methods during storage, transportation and handling
Contemporary enhancement of post-harvest IPM programs by selected physical methods

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Abstract: During the last decade, dry-food processing industries are facing a major challenge in pest control with the ban of methyl bromide and the changes in food hygiene regulations aimed at consumer health protection, with a drastic reduction of chemical protectants for pest control in post-harvest systems.

In these post-harvest situations, the IPM approach represents the more convenient alternative to chemical control leading to both a reduction of pest infestation losses while complying with ‘good hygienic practices’ for food safety as a part of global HACCP quality assurance system. As an alternative to deregulated chemicals, the implementation of IPM in the dry food processing chains has three major advantages: i/ reduces pesticide residues risk in processed food; ii/ insures the traceability of insect population along the food chain, from raw product storage to ready-to-eat food, through insect population monitoring systems; and iii/ prevents and controls infestation in the post-harvest food processing chain by non-chemical treatments. This last objective mainly relates to the replacement of chemical treatments by physical control methods in IPM programs.

The integration of physical techniques in IPM programs has significantly enhanced the efficiency of IPM programs in the post-harvest chain situations. The most popular physical techniques actually used in IPM are:

- High temperature killing of insect species infesting food processing facilities;
- Packaging design and testing for insect-proof properties;
- Modified atmosphere packaging of high added-value foods;
- Combination of different physical methods for food or feed product ‘soundness assurance’ before packaging (heat, CA, MA, high pressure);
- High-temperature preventative disinfestation of heat-insensitive food and feed products packaged in a plastic enclosure (MW or RF dielectric heating);
- Temporary deep-freezing of leguminous seeds for immediate post-harvest disinfestation.

The more recent advances in the integration of physical techniques into post-harvest IPM programs resulted from the support of specific computer programs. Additionally, the efficiency of physical treatments may be predicted by specific computer programs and more use of physical means in IPM program should help the stored-product quality managers to achieve efficient and customized IPM programs.

Key words: IPM, physical control, heat disinfestation, freezing, physical barrier, insect proof packaging, computer-assisted decision support.

Introduction

The context of IPM in post-harvest situations
Insect pests are a perennial problem in grain storage facilities, food processing plants, feed mills, warehouses, retail stores and households. They infest susceptible commodities, mainly dry vegetable products such as cereal foods. Insect pests can enter the food production chain
at any step of this chain from the producer to the consumer. The presence of stored-product insects in processed food products is unacceptable firstly because a detectable infestation depreciates the hygienic value of the product. It may also reflect on the good manufacturing practices of the manufacturer and have a negative effect on his trade mark image.

Until recently, pest management in post-harvest situations has relied on intensive use of chemical insecticides and fumigants. Strategies for using insecticides with an extended residual action involve risk assessment prior to each application and an evaluation of the results taking into account the economic cost and the potential loss of the markets requiring the ban of post harvest treatments. Consequently, modern pest management in post-harvest situations should be based on a permanent effort to prevent infestations in order to limit the use of residual pesticides to the lowest possible level. Additionally, it is frequently reported in scientific literature that some post-harvest insect pest species are becoming immune to some currently used major chemical insecticides. During the last decade, dry-food processing industries are facing a major challenge in pest control with the ban of methyl bromide (MeBr) a fumigant very largely used for the disinfeation of food processing plants as well as for quarantine purposes. The ban of MeBr or the prohibition of highly volatile insecticides of common use in sanitation of empty warehouses or food storage, processing or marketing facilities (such as dichlorvos) throughout the EU require quality managers and food of feed storekeepers to revise their pest management strategies and to adopt integrated pest management (IPM).

The integrated pest management (IPM) approach constitutes the more convenient alternative leading to both the reduction of the infestation-resulting losses and the compliance with the new regulations for food hygienic quality and safety assurance.

Basically, IPM is an ecologically based system in which all the available control means are evaluated and consolidated into a unified program to manage pest populations so that their economic damage is avoided or at least maintained below an economic injury level (EIL). The EIL is the point where pest suppression becomes economically favourable, i.e. when the cost of treatment is equal to the value of the loss. However, the IPM in post-harvest systems cannot be based on the EIL because the detection of a single insect in a food commodity is considered as a food defect depreciating the food value and leading to the rejection of the infested food from the marketing channel.

**The maximum acceptable defect level concept**

Therefore, every food or feed product destined for trade must be free of insect pests. This requirement is a standard for food sanitary quality and hygiene of in international exchanges as established by the sanitary and phytosanitary agreement of World Trade Organisation (WTO). Consequently, the first specificity of IPM in post-harvest systems is the replacement of the EIL concept by the maximum acceptable defect level (MADL). The MADL is the threshold of density when pest presence in a raw, intermediate, or finished food product can be detected either by visual examination or a standard detection procedure or devices. This may be also the maximum tolerable number of insects that may be found in a grain sample or catch per unit of time (generally each week) in trapping systems installed in warehouses or food processing plants for insect population survey. The determination of the thresholds to start carrying out adequate control action, needs appropriate knowledge by IPM practitioners regarding the biological traits of the most likely insect pest species occurring in different food commodities, as well as knowledge about their respective habits or behaviour and the presumed origin of the initial infestation.

The second specificity of IPM refers to the particular situation of pest control inside a well-defined area delimitated by the structures of industrial buildings (food processing facility, grain storage elevator, food factory, feed mill, and similar facilities) This situation greatly
facilitates the implementation of IPM procedures. Most physical factors can be manipulated in post-harvest situations, especially the environmental conditions (temperature, relative humidity, and if appropriately equipped, atmospheric composition). Moreover, since electric power is generally available at any point in the area where IPM is intended, the implementation of physical control methods requiring an electric power source can easily be carried out.

**IPM as a part of global HACCP quality assurance system**

Today, IPM in the food manufacturing and processing chains may be considered as a part of the global quality assurance system in all the food chains: the hazard analysis and critical control point (HACCP) system. The main focus of an HACCP system is to produce a safe food product. Basically, HACCP systems are a systemic approach to preventing hazards (of biological, physical and chemical origin). Thus, insect pest contamination of food or feed may be considered inside the scope of HACCP systems (table 1). However, pest management was often overlooked in the first application of HACCP procedures application to post-harvest durable food chains (such as cereal products, dried fruits, spices, herbs, vegetal products, pet-food, etc). According to the regulations for food quality and safety action programmes applied for a decade in all developed countries, the issues of pest control and management in post-harvest food chains are more seriously taken into account. For instance, the old ECC Directive “Hygiene” 93-43 (anonymous, 1993) was updated with a new regulation of direct application (anonymous, 2001; 2004a) and with the “Federal Food, Drug, and Cosmetic Act” that was updated recently in the USA (anonymous, 2004b).

Table 1. Including the steps of IPM programs into the 7 steps of the global HACCP quality assurance system (Fleurat-Lessard and Ducom, 2004).

<table>
<thead>
<tr>
<th>Step</th>
<th>HACCP system</th>
<th>IPM step setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Plant design mapping, commodity flow diagramme, localization of all hazard points for food safety</td>
<td>First inspection of the entire plant or warehouse for identification of the nature, location, scale, and intensity of points with potential pest problems</td>
</tr>
<tr>
<td>II</td>
<td>Identification of critical control points (CCPs) with a high risk for food product quality deterioration</td>
<td>Identification of points with potential infestation risks &amp; planning sanitation and cleaning specific procedures at each identified ‘risky’ location</td>
</tr>
<tr>
<td>III</td>
<td>Establishing specific requirements for deteriorative organisms detection and monitoring protocols at identified CCPs</td>
<td>Implementation of a specific monitoring plan. Establishing acceptable pest density and action threshold at identified points with infestation risk</td>
</tr>
<tr>
<td>IV</td>
<td>Establishing critical limits for each CCP (critical economic injury level - or maximum acceptable defect level (MADL))</td>
<td>Analysis of inspection and trapping data for a comparison to maximum acceptable density level (in using population dynamics models)</td>
</tr>
<tr>
<td>V</td>
<td>Establishing corrective actions procedures (when MADL is reached)</td>
<td>Building and application of a pest management plan, site specific, including control strategies, timing, and time frequency</td>
</tr>
<tr>
<td>VI</td>
<td>Laying down records keeping procedures, reporting content and frequency for a good traceability</td>
<td>Recording of control actions and impacts of program implementation (e.g. review of monitoring information)</td>
</tr>
<tr>
<td>VII</td>
<td>Establishing HACCP plan effectiveness verification procedures – Periodical updating (optimizing) HACCP system</td>
<td>IPM program modification for regular improvement based on evaluation of success and failure at control points with infestation risks</td>
</tr>
</tbody>
</table>
The section of global HACCP system dealing with pest management in the post-harvest food and feed chains are now based on the codes for good hygiene and sanitation practices (GHSP) in food or feed plants, already in use for five years in some food chains in France (e.g. in the durum wheat processing industry; anonymous, 2000).

According to the current standards for hygienic and sanitary quality required for any food product, there is a renewed interest in the application of physical and mechanical methods to control insect pests in raw or processed foodstuffs as a substitute for currently used chemical treatments. Thus, the IPM programs applicable to post-harvest pest management and control may be enhanced through an appropriate use of up-to-date physical control methods.

Enhancement of post-harvest IPM programs by selected physical methods

Physical control means (active or passive) are introduced in IPM programs mainly at the step of the corrective action procedures. However, some of the “corrective physical control methods” are also considered as a “hygiene assurance treatment” that may be applied systematically even when infestation risk is at a very low level or not foreseeable (Vincent et al., 2003). Thus physical pest control methods are more likely used for “pest-free assurance program” complementary to the good hygienic “working” practices (in handling, storing, transportation, processing, packaging, distribution, etc.) implemented in the HACCP system.

Insect-proof physical barriers for pest exclusion and infestation risk prevention

Pest-proofing a food-plant is of paramount importance before undertaking a sanitation program. For instance, insect-proof grids should be installed at all the window opening and appropriate insect exclusion systems should be placed at the main plant doors (e.g. pulse-air curtains or automatic closure doors). The interior design of buildings and disposition of equipments in stored product warehouses and food processing plants should facilitate the surveillance of insect presence and limit room-to-room exchanges. A limitation of insect displacement may be obtained by the zoning and the partitioning of buildings and workrooms with different activities or processing different products.

At the end of the food-plant chain, the product is generally packaged for temporary or long-term insect-proof protection. The durable food entering the distribution channels must be protected by insect-proof packaging material up to the official date for consumption-limit-date. For durable food this maximum period spent in the distribution channel may reach 18 months or even longer (e.g. for rice). Since the warehouses of the distribution channels are susceptible to house stored product insects, the packaging material and the package design should protect the food during this period. Consequently, the insect-proof properties of the package of durable food are often submitted to insect resistance bioassays and to aging tests of ageing on the insect-resistant properties of the packages. New multi-layer plastic materials with very good gastightness are available for package design of high added-value foods, enabling the introduction of an inert gas before sealing. Controlled atmospheres or modified atmospheres that can be introduced into the gastight packages are lethal for food insect pests for a limited period of time (Fleurat-Lessard, 1990). Nitrogen-based modified atmospheres are generally preferred to carbon dioxide modified atmospheres for introduction into food packages.

Physical elimination of insect populations already installed

Intense trapping system installed before the period of higher infestation risk are intended to lower population increase rate when population densities are still low (e.g. at the end of the cold season). Either UV light or pheromone traps (Fig. 1) may be used for mass-trapping targeted important pest species. This technique was recommended to delay the use of conventional pest control procedures against phycitid moths in situation of low insect densities (Trematerra, 1994).
Preventative physical control means for pest-free food products

a) Mechanical injury

Insect in stored grain or in cereal flour can be killed by impact during the transportation by a pneumatic conveyor resulting from the violent repeated shocks of kernels against the metal ducts (Paliwal et al., 1999). In flour mills, both grain and flour can be disinfested by passing through “entoleters” that use centrifugal force to throw the grain against a steel surface (Stratil and Wohlgemuth, 1989; Fields et al., 2001). When this equipment is activated on the grain stream prior to milling, the infested kernels break apart and are separated from the intact kernels. This equipment is more often used to kill the eventual insect or eggs infesting fresh flour, before it is packaged or bulk stored.

b) Substitution of cooling aeration for chemical insecticides in stored grain pest control

The population growth of all grain insect species is inhibited when temperature reach below 10°C. Thus, the lowering of grain temperature to this level in a short time after harvest will reduce insect damage to acceptable levels (insect final density kept below the MADL of one insect per kg). Since the cooling process is slow due to the insulating properties of grains, reducing grain temperature to the target level of 10°C requires several aeration periods (Lasseran et al., 1994; Fields et al., 2001).

Fig. 1a. Different design of probe traps (left) and a cup trap (a, b) used for the monitoring of stored grain beetles at the surface of grain bulks.

Fig. 1b. Light trap (c) and pheromone traps: “open box model” with internal sticky surface (d and e); pheromone-baited moth trap “reservoir model” (f).

In temperate climates, each cooling step is generally obtained by 1-2 weeks of aeration, mainly achieved during the night. In Mediterranean or sub-tropical climates, the grain elevators may be equipped with refrigeration units to cool the grain even though ambient air...
temperature is above the grain bulk temperature (Fleurat-Lessard and Vincent, 2005). Automated control systems for automatic running of the aeration process are available to control fan operations, some of them now being aconnected to software packages in decision support systems. These systems can be used to perform the calculations and to produce an optimal design for fans, ducts, etc. Grain cooling aeration has become very popular in Europe mainly because it is a means of preservation of stored grain for a an entire year without the use of persistent insecticide treatment. Cooling aeration is extensively used in European countries for the storage of organic bread-making wheat or rye, for which any post-harvest insecticidal treatment on grain is forbidden. The cost of cooling aeration is about $ 0.20 per tonne, i.e. slightly higher than the cost of an insecticidal treatment ($ 0.15 per T), and cost is much lower than phosphine fumigation ($ 1.2 per T in Europe).

c) Hermetic enclosure and controlled atmospheres
Storing grain in hermetic structures cause a progressive depletion of oxygen by grains' natural respiration and can also kill the insects and prevent eventual re-infestation. Inert gas may be introduced in storage structures to accelerate the oxygen depletion rate and increase the insect mortality process. There are two different ways to control stored-grain insects with inert atmospheres: the injection of controlled atmosphere obtained from an exothermic inert gas generator or the injection of carbon dioxide gas at a concentration higher than 40% (v/v) (Fleurat-Lessard, 1990; 2004).

d) Heat disinfestation
The assurance disinfestation for a food commodity may be obtained by thermal treatment if the commodity is heat-tolerant. Dry foods are dielectric material with low thermal conductivity. Consequently, the rapid heating of this material up to the lethal temperature level for food insect pests needs specific technological solutions. Two techniques are currently used commercially for rapid heat disinfestation of foodstuffs: i) heated air fluidized bed (or heated air pneumatic fluid-lift) and ii) dielectric heating by using microwave or radio-frequency electric fields. For high-temperature fluidized bed treatments, the effectiveness of the disinfestation depends of a number of factors, the most important being the treated product moisture content, the inlet air temperature, the air flow / product flow ratio and the pest to be controlled (Fleurat-Lessard and Le Torc’h, 2001). Pilot and industrial scale heated air fluidized beds were developed in Australia for the disinfestation of export grain (Dermott and Evans, 1978). Similar equipment has been built in France for the disinfestation of durum wheat semolina during fluid-lift transfer to a storage bin. To control insect eggs in semolina, the product is heated at 156 to 200°C for 6 to 7 seconds during fluid-lift conveyance with air (Fleurat-Lessard and Le Torc’h, 2001). The semolina heats to a maximum of 70°C, before being cooled with ambient air before storage. Practical processes have been developed to control food insect pests using microwaves (2.45 GHz) or radio-frequency electric radiations (13.56, 27.12 or 40.68 MHz). Although the application to the disinfestation of food commodities remains a marginal application of this technology of dielectric heating, some pilot scale equipment were developed for the disinfestation of spices, vegetal material, dried fruits, nut fruits, etc. (Nelson, 1996; Fleurat-Lessard, 2001 and Fig. 2; Wang et al., 2003). On an industrial scale, the energy cost of treating more than 4.5 tonnes of in-shell nuts to kill both the codling moth Cydia pomonella and the Indian meal moth Plodia interpunctella larvae is estimated at $0.23 cents/kg (Wang et al., 2006). This technique offers many advantages compared to heated-air treatments in terms of the design of the treatment units and for the possibility to automatize the treatment directly on the on-line flow of the food commodity. However, installation of large scale heat treatment facilities is capital intensive, independent of the energy input cost needed to produce heat. So, it should be reserved for high added-
value foodstuffs (e.g. organic food) that are also heat insensitive durable food products (semolina, rice, dried fruits, nut fruits, spices, herbs, aromatic plant, etc.).

e) Temporary freezing
For the disinfestation of high value food commodities such as “organic” dry beans, temporary freezing can also be used for the disinfestation of food commodities before marketing. The disinfestation of dry beans infested after the harvest by the bean beetle, *Acanthoscelides obtectus*, can be obtain following 50 h exposure at -23°C in a rapid-freezing chamber (Dupuis et al., 2006). Another application of this technique to the disinfestation of beans from bruchid beetles is used commercially in North America (Johnson and Valero, 2003). Even if such freezing temperatures are not reached, the winter temperatures observed in Canada during winter are sufficiently low to kill insects in grain silos equipped with cooling aeration systems. Grain insects cannot survive temperature levels below -15°C more than one month (Fields et al., 1998). Cooling grain to this temperature or below in Canada during winter is easily achievable and affords a cheap physical control method for grain pests.

f) Combination of different physical stresses
The effectiveness of combining different physical treatments to obtain a synergistic effect was demonstrated in two major protocols: the combination of heat and CA or MA and very high pressure and CO2. The first combination increases the mortality rate of insects due to enhanced respiratory demand (Mitcham et al., 2004). For packaged food, the elevation of temperature during exposure to CA or MA can be obtained by heating packaged food by RF or MW dielectric heating. It induces a significant increase in the rate of insect mortality (Fleurat-Lessard, 2001). With the second combination of high pressure (2 MPa) and high concentration of CO2 exposure, the complete kill of stored product insects at all stages can be observed after less than two hours exposure time (Le Torc’h and Fleurat-Lessard, 1991; Reichmuth and Wohlgemuth, 1994; Fleurat-Lessard and Le Torc’h, 2001). Several industrial equipments built on this principle are in use in EU countries for the quick disinfestation of spices or pet-food (Fig. 2).

**Physical treatments for the eradication of insects in food processing plants**
After the ban of methyl bromide that was the fumigant of general use for complete kill of insect infestation in bins, silos, and hard-to-access storage and food processing facilities, the most popular technique replacing MeBr structure fumigation became dry heat treatment. Heat treatment consists in raising the temperature of the food processing facility to 50-55°C and maintaining these elevated temperatures for at least 36 h to kill stored-product insects. Pioneer work to establish the protocol for effective heat treatment of cereal processing plants was done at the Kansas State University in the USA (Mahroof et al., 2002). Two different heating equipments were tried: steam heaters and gas heaters. The heating of cereal food processing facilities at 50-53°C for 24-36 h has been used for a long time to control insects. Since the ban of methyl bromide for the disinfestation of cereal processing plants, an increasing number of major cereal food processors are routinely using “heat fumigation” to eradicate installed insect populations (Heaps and Black, 1994).

**Implementation of physical control measures in post-harvest IPM programs**

*Decision support based on previous knowledge about insect physiological response to physical stress*
Most of the insect species living in post-harvest durable food and feed chains are of tropical or subtropical origin and require fairly high temperatures for optimal development (Fleurat-Lessard, 2004). The rates of development and of population increase are triggered by
temperature. Low temperatures reduce the rate of increase which can go down to zero when the temperature declines below the development threshold. At the other end of the thermobiological scale (Fleurat-Lessard and Le Torc’h, 2001), durable food insect pests cannot survive temperature higher than 60 to 63°C more than a couple of minutes (Dermott and Evans, 1978; Fields, 1992; Banks and Fields, 1995; Fleurat-Lessard and Le Torc’h, 2001; Fleurat-Lessard, 2005). They also have difficulty surviving at a permanent temperature regime higher than 40°C (Banks and Fields, 1995). They can survive only less than 2 h at 50°C and this is this temperature level which is targeted in heat disinfection of cereal primary processing plants (Fields et al., 1997; Fleurat-Lessard and Le Torc’h, 2001; Mahroof et al., 2002). Low temperature below the development threshold (e.g. 15°C) drastically reduces the potential of increase of a majority of post-harvest pest species or may even stop their development. Thus, the air–conditioning in the different workrooms of a food factory at temperature below 18°C will appreciably reduce the risks of insect population burst. This is the normal situation during summer In facilities without cooling aeration of workrooms or other premises where durable food products are stored or processed (temperature levels may increase to a temperature level corresponding to the highest rate of increase for insects, i.e. between 26 and 32°C).

Buildign and implementation of a pest management plan
The elaboration of a comprehensive plan for the prevention for insect intrusion is based on the analysis of infestation risk from the monitoring data and from the simulation of target pest population dynamics in a specified real situation.

The elements that should be included in the pest management plan for effective IPM implementation include:
i/ Information gathering on the facility where the IPM should be applied: insect-proof structure quality, weakness points for insect entry, convenient airtightness for safe fumigation workshop, organisation of the product flow, air-conditioning equipment, etc.);

ii/ Exclusion practices and current good sanitation and cleaning practices (inside and outside the facility); in association with preventive manipulation of physical conditions (e.g. temperature);

iii/ Rhythm of periodical sanitary and hygiene inspections;

iv/ Efficacy level of the monitoring system for insect presence early warning (density and arrangement of traps, periodicity of checking the insect catches, periodicity for the renewal of trapping material, periodicity of visual inspection, etc.);

v/ Validated skills of employees in charge of insect species identification. Appropriate knowledge about insect biology and behaviour and minimal knowledge about the IPM program;

vi/ Detailed information on the MADLs available at each monitoring or control point and in the annex of the IPM preventative plan of actions (related to the intended level of pest control);

vii/ Periodical training of employees so that they have a responsibility in the application of good hygiene practices, and they are aware of the hazards of pesticide treatment application and treatment products (as well as the advantages of using physical control measures);

viii/ Description of the method for the validation of the efficiency of monitoring, verification, or corrective measures (minimal knowledge about indicators used and their calculation method)

ix/ Accompanying the above knowledge, a reporting system is required in order to compile written procedures as well as the calculation method for the efficiency indicators (Fig. 3).
Implementation of IPM procedures in a decision support system

Simulation models of temperature-dependant growth rate have been developed from the knowledge acquired on biological characteristics of the most damaging pest species in a very large range of development conditions (Beckett et al., 1994; Driscoll et al., 2000). These models may be used in computer-assisted decision-support-systems (DSS) in order to determine the safe storage period or to predict the increase of the population density with time (Longstaff and Cornish, 1994; Qin Zonglin et al., 1999; Williams et al., 2006). Some of these DSS's have been built especially for the implementation of IPM program in post harvest systems (Wilkin and Mumford, 1994; Ndiaye and Fleurat-Lessard, 1995; Collins and Bridgeman, 1997). More recent developments in DSS for pest problems management in post-harvest situations deal with stored grain preservation in silos. In Canada, the software “CanStore ®” (Mani et al., 2001); in the USA Stored Grain Adviser Pro® (Flinn et al., 2007); in UK Grainplan® software (Williams, 2006); in Australia PestMan® expert system (Longstaff and Cornish, 1994); and in France QualiGrain© (Ndiaye, 2001; 2005 and Fig. 4) have been developed for grain qualities management (including pest contamination risk).

The advanced DSS aiming at the preventative approach of quality optimal preservation are based on predictive modelling of the delay for quality deterioration. For instance, GrainPlan® storage ‘time calculator for cereals’ (Knight et al., 2005a,b) and the QualiGrain© expert system give advice on the storability of a grain lot and propose different scenarios for optimal preservation of undamaged quality attributes during the longest possible period of storage (Fig. 4 and Ndiaye, 2005). Today, decision support programs and expert systems are important components required for the implementation of IPM in different domains of phytoprotection (Hagstrum and Flinn, 1992; Hagstrum et al., 1999; Flinn et al., 2003; Fleurat-
Lessard and Ducom, 2004; Ndiaye, 2005). When these DSS can be linked with early detection devices such as acoustical or electronic grain probes, the prediction of the storage period during which the density of pest will remain below the critical level for detection, may be also predicted. (Fleurat-Lessard et al., 2006; Flinn et al., 2006; 2007).

**Other conditions for effective and economical IPM procedures application**

The IPM plan aims at a continuous improvement of indoor environmental conditions with an appropriate timing of corrective control measures. In the HACCP approach, the effectiveness of the preventive measures is verified through a periodical review of the different elements and especially the indicators of efficacy of the preventive measures as well as the efficiency of the monitoring tools. A special competent team must be recruited and trained for the implementation of the HACCP or the IPM system in a particular food plant, factory, processing facilities or warehouses.

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The last important element in the IPM procedure effective implementation concerns the training of two different kind of people to take action: i/ the employees in charge of the IPM plan must be trained in practical implementation so that they acquire an awareness of the IPM program for a comprehensive application; ii/ all the personal working in the food processing facility have to be informed about the importance of a right application of all the actions of the IPM program and what role they should play in the infestation risk alert. The training of the IPM practitioner team of a food or feed company can also be carried out by way of computer-assisted learning. Specialized software is especially available for this purpose (Longstaff, 1999; Schöller et al., 2006). Some other DSS software include a consultable knowledge base delivering advice on any scientific or technical topic of concern in an IPM plan implementation (Campbell et al., 2002; Knight et al., 2005a, b).
Discussion

It is generally considered that the most efficient IPM strategy is a combination of control measures resulting in the optimum level of the ratio: efficiency / cost. However, in food and feed chain (from the field to the dish), this ratio fluctuates largely in range because it is directly linked to the commercial value of the commodity needed to be protected. Thus, the market value of intensive farming cereal production is not comparable to a farmer producing of organically grown beans. Each situation is related to different Maximum Acceptable Defect Limit for insect infestation and the optimum thresholds for deciding on the appropriate control measures. The strategy that should be applied may be extremely different in each case. In the case of cereal grain for instance, some insects are tolerable if the population density remains below the detectable threshold. While in the case of high added-value organic beans, only insect-free beans can be marketed for human consumption. Thus, the manager responsible of the HACCP application to his business will firstly consider the value-added that can be afforded by the IPM procedures implementation compared to conventional pest control tactics.

Fig. 4. Example of a computerised approach of stored grain quality integrated management by a logical chain of preventive actions assisted by an expert system (adapted from Ndiaye and Fleurat-Lessard, 1999)

Today, there is a common worldwide will to limit the pest problems in all steps of the post-harvest durable-food chain and in expanding the IPM procedures application. However, the implementation of IPM in the food storage or processing facilities requires prerequisites and investment. The monitoring and the assessment of the indicators of pest presence and population dynamics change with time IPM application in post-harvest systems requires recruitment of personal with high level of knowledge of bionomics and behaviour of the major pest species. More often, starting an IPM program necessitates an improvement of the
design of the structure and modification of material layout inside to facilitate the implementation of the different IPM components. There are generally moderate costs associated with this optimization of the facilities. After this first stage, the practical application of an IPM program should be continuously improved by the use of modern tools facilitating the sanitation procedures as well as the interpretation of monitoring data. At this particular level, this evolution generally means the buying of new equipment and technical updating (e.g. a cooling aeration system for a grain storage facility, or the acquisition of a RF oven “disinfestor” for insect control in in-shell nuts or chestnuts). The improvement of monitoring systems and the introduction of computer software to insure full traceability of IPM program operations are also costly. Although it is easy to demonstrate that the balance investment / efficiency is favourable in most cases, food factory managers are often reluctant to invest a lot of money in these IPM programs development. The other obstacle to overcome is the specific implementation of the recommendations included in the codes for GHSP in any food processing or manufacturing facility. This difficulty may be overcome by increasing availability of computer software dedicated to the training of IPM practitioners or giving valuable advice for correct practical implementation and customization of IPM plans. These decision support systems may also contain expert knowledge in answer to questions and enquiries from the users. However, since the publication of new EU regulations dealing with food quality and safety assurance in all food production chains, IPM programs will become more and more popular. The adoption of IPM programs in post-harvest food chains must be supported by a financially viable pest management industry for IPM product distribution and affordable services.

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Insect-proof packaging to avoid stored product insects

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Abstract: Insects are usually attracted by volatiles emerging through permeable packaging material, improper seals or other leaks in a given package. A number of package types were tested for invasion by neonate larvae of the Indian meal moth Plodia interpunctella and the rice weevil Sitophilus oryzae. Moth eggs could be found close to minute openings in packages, and neonate larvae were able to enter into the product. This was proven by finding intense webbing and grown larvae inside the package after a few weeks at 25°C and 65% r.h. Adult rice weevils were found to invade through existing openings but not to penetrate packaging material. Bags were often found to be leaky around the seams. In some cases product caught in the seam prevented proper sealing. Welding seams parallel to the edge of a bag were found safer than those at a right angle to the edge. Other bags were leaky because they obviously had been punctured by spike wheels during transportation within the facility of the producer. Clips that may be convenient for consumers and attractive in their appearance were found inappropriate as seals against stored product insects if used as the sole means to close a bag. In cardboard boxes leaks were found around the corners when the flaps did not overlap; when folding caused tension in the flaps; when glue had been applied only in spots, and when cartons were perforated to make opening more convenient.

Key words: stored product, invasion, package, seal

Introduction

A limited number of specialised insect species consist of a group best adapted for survival in stored products for two reasons. First, they can actively search for suitable products following sensing of product volatiles. Second, they can live and multiply in rather dry stored goods such as cereal grains, nuts, pulses, herbs or dried fruits without any additional source of water.

With the exception of sub-tropical and tropical climates, stored product insects hardly occur in the field. Moreover, the harvesting process or grain threshing can drive away or kill pests. Thus, in most cases the major pathway of pest infestation is the invasion of insects into the stored product. That is the reason that the steps of harvest, storage, transportation, processing, packaging and distribution can be seen as a continuous process with the chain of pest prevention being just as strong as its weakest link.

Packaging is crucial because an insect-proof package is the only protection the consumer has from pest attack, on the way from the producer. Metal cans and well sealed bags containing a layer of aluminium are generally safe from insect attack because they do not emit volatiles. Also some plastic films can give a good protection provided they are sealed to a high level of gas-tightness and remain undamaged during storage and transport. Nevertheless, insect attack is a major cause of loss in packaged food (Mullen 1994).

Seen from the perspective of a food producer, a food or feed package has to fulfil a number of different and even contradictory tasks:
1. The packaging material should be low in cost to keep the product competitive in the market.

2. The package must fulfil legal requirements such as giving information of the contents, the quantity, shelf life, etc.

3. The package should appear attractive to the consumer.

4. The package should be convenient to open, and depending on the product, to relock.

5. The package needs to be easily assembled, filled and closed in automatized systems.

6. The chosen package should be easy to stack into a larger secondary package and easy to place onto a supermarket shelf.

7. The package should be able to withstand considerable changes in temperature and pressure without changing appearance.

8. The package should have protective properties and help to maintain shelf-life.

The last point refers to a useful characteristic required for stored product protection.

**Invaders and penetrators**

Most stored product insects are invaders. These species invade products through existing openings instead of destroying packaging films (Highland 1991). Typical penetrators are the drugstore beetle *Stegobium paniceum* and the tobacco beetle *Lasioderma serricorne* (Fam. Anobiidae), the larger and lesser grain borer *Prostephanus truncatus* and *Rhyzopertha dominica*, as well as the root borer *Dinoderus bifoveolatus* (Fam. Bostrychidae), beetles and larvae of the dermestid family, and the grown larvae of pyralid moths on their search for a spot for pupation. Other genera such as adults of *Tribolium* or *Sitophilus* may occasionally damage softer materials, especially in the absence of a suitable food substrate (Highland 1984). Data on the typical damages caused by certain stored product insects to various types of packages are given by Schmidt & Özel (1979), Mullen & Mowery (2006), and Riudavets et al. (2007). Non-feeding stages such as adult Anobiid or fully grown pyralid larvae destroy packaging material on their way out of a confinement rather than on the way into it. An experiment was carried out recently to determine if young mated female tobacco beetles penetrate packaging materials in order to oviposit into a suitable substrate. First results indicated that females laid their eggs onto the surface of packaging materials or close to an opening. When an existing opening presented itself, they inserted their ovipositor but did not increase the opening (Adler & Dittrich unpublished data).

The paper presented here reports on invasion tests carried out to test various consumer packages and summarises some of the weak spots found in bags and cardboard boxes.

**Material and methods**

**Insect cultures**

Young adult *P. interpunctella* moths were taken from a culture kept for many years at the institute at 25±1°C and 65±5 % r.h. on a diet of whole wheat bran and broken almonds. Young adult *S. oryzae* weevils came from a culture kept for many years on a substrate of whole wheat kernels at 20±1°C and 65±5 % r.h.

**Invasion test**

Consumer packages were tested for insect invasion by placing at least six packages of a given type into a plastic container (440 mm X 660 mm X 200 mm). The packages were arranged in such a way that each side (front, back, left side, right side, bottom, top) faced down at least once to facilitate insect movement (Fig. 1). To confine the test insects within the container, its top was covered with a plastic film or a plexi glass lid fixed to the upper rim of the box with
double sided sticky adhesive. Preliminary tests had shown that the material of the plastic box and the covering plastic film or lid were antistatic and did not disturb insect movement.

Fig. 1. Arrangement of packages in a plastic container prior to invasion test. The glass jar in the front contains substrate and eggs to determine the suitability of the substrate. In tests with oviposition by moths also the time required for larval development is determined by regular checks of the glass jar.

a) Invasion test with neonate larvae of *Plodia interpunctella*:
100 young adult Indian meal moths (*P. interpunctella*) of mixed sex were introduced into the container and allowed to oviposit at 25°C and 65±5% r.h. until they died. Packages were kept for approx. four weeks until grown larvae could be found in glass jars that had been filled with similar substrate and moth eggs during oviposition time. Then the packages were cut open in the middle and searched for living larvae and webbings.

In case moth larvae were found developing inside the tested packages, the potential entrance points were searched for by testing the empty plastic bags with a liquid stain of Rhodamine red (Fig. 2).

b) Invasion tests with adult weevils of *Sitophilus oryzae*:
In order to test various types of pasta packages, 500 young adult rice weevils (*S. oryzae*) were added into each container containing at least six packages of one type with the various sides facing down. To test the suitability of the substrate, 100 adult young weevils were placed directly onto the pasta of one package in a glass cylinder with two liter volume (Fig. 1). The weevils were left undisturbed for six days at 22°C and ambient relative humidity (40-45 %). At the end of each experiment the plastic box was opened and adult weevils found were counted. Each pasta package was then cut open in the middle, the contents spread on a laboratory tray and searched for weevils.
Results and discussion

In the majority of packages tested, neonate larvae were able to enter through openings in the seams or punctures in polyethylene (PE), polypropylene (PP) or polyvinyl chloride (PVC) bags. Openings in the seams were caused by folds in the welded plastic liners (Fig. 2) or substrate caught in between the two layers during automated filling (Fig. 3). According to practitioners in packaging this risk can be reduced by the utilization of antistatic film. Seams with welding lines parallel to the edge of a bag (Fig. 2) are today often preferred to those with welding lines at a right angle to the edge (Fig. 3). The latter facilitate the formation of canals from outside into the product. Incomplete seals were found in the vertical seams of bags (Fig. 4). Other openings for insect invasion were accidental punctures in a rigid and less flexible polypropylene film probably caused by needle wheels during transportation of the final product. This was deduced from the fact that several punctures in a given bag followed a symmetrical pattern (equal size and distance from one another, e.g. in one line). Here a somewhat more flexible material could help to prevent damages in the future. Some companies even deliberately puncture their bags in order to allow gas exchange at changing temperatures or pressures.

![Fig. 2. Stains in the seam of this PVC bag show openings and canals through which neonate moth larvae may be able to enter into the stored product.](image)

The bags visible in Fig. 4 had been closed at the top with a welded seam, a clip was attached to give the bag an attractive shape and to facilitate closing by the consumer after partial removal of product. In pasta packages from another producer only a clip had been used to close a bag of pasta, this led to an attack of the drugstore beetle *Stegobium paniceum*.

According to Cline and Highland (1981) adult *S. oryzae* could be retained by a sieve with an aperture size of 0.93 mm. While adult *P. interpunctella* were retained by a wire mesh with an aperture of 1.4 mm, adult *S. paniceum* were retained by an aperture of 0.71 mm and neonate larvae of *P. interpunctella* by an aperture of 0.1 mm (Adler 2004).

In cardboard boxes, openings could be found in the corners if flaps which did not overlap properly. Leaks were produced between flaps if in the process of mechanised closing the glue
had been applied only in spots or if fixing the flaps caused some tension in the cardboard. The perforation for convenient opening of a pet food box was found as a spot for oviposition by *P. interpunctella* (Fig. 5). This supports the findings by Barrer and Jay (1980) who stated that grain odors are important triggers for the location for oviposition by *Ephestia cautella*. Mullen (1994) found that female *P. interpunctella* could clearly distinguish between packages containing food vs. non-food items when choosing a site for oviposition.

Fig. 3. Product caught in between films during welding the seam may produce canals that allow the invasion of neonate larvae. In this case an adult moth developed within the almond package.

Fig. 4. Incomplete seals in vertical seams of packages, large enough to insert the point of forceps.
In conclusion, insect-proof packaging remains an important issue in stored product protection. For each product at stake, the producer will need to find a balance between marketing requirements, consumer convenience and stored product protection. Frequent testing of the quality of seals right after packaging can help to minimize the number of consumer complaints.

Acknowledgements

This paper is dedicated to Mrs. Sabine Berger, a friendly and sympathetic colleague, who kept the institute’s insect cultures in perfect condition for more than 30 years and suddenly deceased in March 2007. The author is endebted to Mrs. Agnes Paul for technical support.

References


Aeration, fumigation by Siroflo®/Eco2Fume® and storage in modern bunkers and hermetic platforms under PVC - A review of three ecologically friendly technologies used for grain storage and protection in Cyprus

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Abstract: During the last 25 years three important ecologically friendly technologies were introduced and are successfully used in the grain storage industry by the Cyprus Grain Commission: a) Aeration (co-operation with Israel, from 1980 on), b) Fumigation technologies Siroflo® and Eco2Fume® (co-operation with Australia, from 1996 on) and c) Storage in modern bunker structures (co-operation with Australia, from 2000 on) and in hermetic platforms under PVC (co-operation with Israel, from 1988 on).

Aeration is extensively used in Cyprus to prevent negative changes in grain quality during storage. The energy needed under Cyprus weather conditions to reach a reduction of grain temperature by 10°C is about 1.0 kW/tonne (0.15 €/tonne), which negligibly increases the grain price.

Fumigation of grain in all metal and concrete silos is carried out by using the flow-through fumigation technology Siroflo®/Eco2Fume® (gas mixture of 2% PH3 in 98% CO2 in cylinders) maintaining a constant phosphine concentration of 45 ppm for 14 days. Fresh air is blown into the structures after fumigation to remove any PH3 residues. Fumigation by using Siroflo®/Eco2Fume® has many advantages in comparison to traditional grain protection methods by using phosphine tablets or liquid insecticides: this method provides a constant and fully controlled PH3 gas concentration in non airtight storage structures, full automation of fumigation, improved safety in working environment, grain free from pesticide residues, more effective and cheaper grain protection, no need in transferring grain into an emptied bin, no need in handling dangerous residues of phosphine tablets. A weak spot is the low PH3 concentration at the top 5-15 cm of treated grain and the long duration of fumigation. The cost for fumigation of 2200 tonnes grain in a vertical bin of 10.5 m diameter by using Siroflo®/Eco2Fume® is 0.15 €/tonne in comparison to 0.15-0.16 €/tonne which is the cost for grain treatment with liquid insecticides or with phosphine tablets, plus 0.1-0.3 €/tonne energy expenses for grain transportation into an emptied bin needed when the last two methods are used. Nowadays in Cyprus phosphine tablets or liquid insecticides are not used any more for treating grain. Although there is a great deal of demand for Eco2Fume® in North America, Australia and China, it seems that for some reasons Cytec, the only manufacturer of Eco2Fume®, will not proceed with its re-registration in EU. This obligates the CGC to look for other grain protection alternatives with at least the same advantages.

In Cyprus, beside vertical silos, modern bunker structures under UV PVC are also used to face abnormal situations, when additional storage space is required (e.g. in order to take advantages of low prices in the international market since Cyprus imports more than 90% of its needs in grain, or when there is a large local production). The bunker solution for grain storage has disadvantages and advantages. Negative aspects: high need in manpower for bunker operation, restriction in filling and opening during rainy days, danger of moisture condensation at the bunker apex. Advantages: successful protection of grain against damages caused by rain, birds and rats, ability to protect huge quantities of grain in a low-cost way, ability to take advantages of low prices in the international grain market, successful fumigation without pesticide residues. Bunkers are low cost structures (≈7.5 €/tonne capacity).
The collaboration among countries, organizations, companies and scientists is a powerful tool for the development, introduction and implementation of advanced technologies in the grain industry.

Key words: Environment, grain, fumigation, Siroflo, Eco2Fume, phosphine, aeration, storage, hermetic, bunkers.

Introduction

The collaboration among research centres, companies, organizations and scientists for the developments of advanced technologies in the field of grain storage and grain protection and for transferring and implementation of these technologies by trade companies and other countries is a powerful tool for more effective grain handling and trade, for more healthy products and for more sensitive and careful attitude to the environment (Champ, 1998). Countries and companies with limited funds for research through cooperation may have access to advanced technologies. The Cyprus Grain Commission, following this concept, since 1980 has introduced and successfully used three advanced technologies which are also ecologically friendly to environment:

1. the Aeration of stored grain in cooperation with the Volcani Center (Israel, 1982)
2. the Fumigation of stored grain by using the Fumigation technology Siroflo® in cooperation with the CSIRO and the Eco2Fume® technology (gas mixture of 2% PH₃ in 98% CO₂ in cylinders) in cooperation with BOC (Australia, 1996)
3. the Storage of grain in modern bunkers in cooperation with the CSIRO and SACBH (Australia, 2000) and the grain storage in hermetic Platforms in cooperation with the Volcani Center (Israel, 1987).

The aim was to solve serious problems that the grain storage industry of Cyprus faced by using advanced technologies which are effective, non expensive, friendly to the environment and costumers and improve the safety in the working place.

The introduced technologies in Cyprus

Aeration of grain

In Cyprus the climatic conditions are favorable for a rapid development of insects during storage of grain. The mean maximum and mean minimum temperatures during summer are 37.6°C and 22.4°C and during winter are 13.5°C and 4.0°C respectively. Warm conditions last for few months. Local grain is harvested with temperature usually above 30°C. The temperature of grain imported from April until October usually is above 24°C. Grain is kept in silos from one up to 7 months. Under these conditions the control of grain temperature is a critical issue.

The principles of grain aeration in Cyprus were laid down by Prof. Shlomo Navarro in 1982 during an FAO mission to assist the Cyprus Grain Commission. The objective of this mission was to disseminate the grain storage management technology to strengthen the existing infrastructure in Cyprus in the use of aeration and chilling of grain by refrigerated air (Navarro and Noyes, 2002). The “Aeration of Grain in Subtropical Climates” prepared for FAO was the leading guidance for the aeration of grain in Cyprus (Navarro and Calderon, 1982).

Aeration systems are installed and successfully operated in all metal and concrete silos, in a total 120 thousand tonnes capacity. The ambient air is used for grain aeration, particularly during night. Thermostats, hygrostats, time switches are used for aeration control, an automatic aeration controller system based on air and grain wet bulb temperatures is under consideration to be used as well.
Under the Cyprus weather conditions the mean energy used for grain aeration to reach a reduction of grain temperature by 10°C is about 1.0 kW/tonne (about 0.15 €/tonne), which negligibly increases the grain price by about 0.1-0.3%.

Every year 10-15% of stored grain is aerated in order to prevent damages caused by insects, fungi and moisture condensation. Proper and timely aeration resulted in a substantial reduction in the use of liquid insecticides for grain protection. The combination of two technologies, the aeration of grain and the fumigation using the Siroflo®/Eco2Fume®, is a powerful tool for good grain quality management under the dry and warm Mediterranean conditions of Cyprus.

The fumigation technology Siroflo®/Eco2Fume®

In Cyprus all metal and concrete grain silos are not airtight. In these non-sealed storage structures it was impossible to maintain adequate phosphine concentrations for a successful fumigation. This caused many problems: ineffective fumigations with solid phosphine tablets, conditions for development of insect resistant to phosphine, increased quantities of liquid insecticides used for grain treatment, the necessity to keep additional storage space for transferring grain for treatment, workers’ safety and residue problems. There was a vital necessity to find a solution.

Siroflo®, a CSIRO (Commonwealth Scientific Industrial Research Organization, Australia) developed and patented technique, is a low positive pressure distribution system for fumigation of grain in situ with Eco2Fume®, a 2wt% phosphine in 98wt% liquid Carbon dioxide mixture in cylinders which is patented by British Oxygen Corporation Gases, (BOC Australia), transferred later to CYTEC, Canada.

The Siroflo® fumigation technique was firstly implemented on a commercial basis in Australia in 1988 after a series of trials in vertical silos that began in 1985 (Winks, 1992; Winks and Russell, 1996).

In 1995, after an agreement between CSIRO/BOC Australia and the Cyprus Grain Commission, the Siroflo® system using the Eco2Fume® mixture was introduced in Cyprus and was installed in a complex of eight vertical, non-airtight, corrugated hopper bottom metal silos of 1000 cubic meters capacity each. Nowadays the Siroflo®/Eco2Fume® technology is installed and successfully used in all metal and concrete non-airtight grain silos of Cyprus.

Eco2Fume® mixed with air is introduced into the silo through the existing aeration ducts. By using the gas pressure regulator and the flow meter control it is possible to adjust the concentration of phosphine gas in the grain mass. Several bins are connected to the Siroflo® system and it is possible to fumigate several bins simultaneously.

In Cyprus a fumigation with Siroflo®/Eco2Fume® lasts 14 days and a constant and fully controlled 45 ppm PH₃ concentration is maintained in the grain mass inside the bin during fumigation.

The quantity of Eco2Fume® mixture needed for fumigation does not depend on the grain quantity, but on the bin diameter. The smaller the bin diameter, the less the amount of phosphine needed for fumigation of the same grain weight (table 1). The weight of a.i. of phosphine used for fumigation by using the Siroflo®/Eco2Fume® technology is few times less (e.g. 4-8 times smaller) in comparison to the weight of phosphine needed for fumigation of the same grain quantity by using the traditional method of phosphine tablets (table 1 and 2).

The fumigation of grain by using the Siroflo®/Eco2Fume® technology has obvious advantages in comparison to the other two main methods of grain protection, the grain treatment with liquid insecticides and the grain fumigation with solid phosphine (tablets etc). In trials it was proven that insect mortality using the Siroflo®/Eco2Fume® fumigation is very high. This is difficult to reach by treating grain with liquid insecticides or by fumigating it with phosphine tablets in non airtight structures (Varnava et al., 1998).
The Siroflo®/Eco2Fume® technology, in comparison to the grain treatment with liquid insecticides or the grain fumigation using solid phosphine, is not only more effective, but it is also more economically feasible. In Cyprus the cost per tonne for chemicals to be used for grain protection by using the above three methods is approximately the same (0.15 €/tonne), but a fumigation with the Siroflo®/Eco2Fume® technology is carried out in situ, while grain must be transferred into an emptied bin in order to be treated with liquid insecticides or solid phosphine, that additionally increases the energy costs by 0.1-0.3 €/tonne (Table 2).

Table 1. Comparison of fumigation parameters in bins of various diameters by using the Siroflo®/Eco2Fume® technology.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Limassol-Bin type A</th>
<th>Limassol-Bin type B</th>
<th>Larnaca-Bin type A</th>
<th>Larnaca-Bin type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bin capacity, tonnes</td>
<td>2200</td>
<td>550</td>
<td>2200</td>
<td>800</td>
</tr>
<tr>
<td>Bin diameter, m</td>
<td>10.5</td>
<td>4.5</td>
<td>15</td>
<td>8.2</td>
</tr>
<tr>
<td>Bin height, m</td>
<td>41</td>
<td>41</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>PH3 concentration, ppm</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of fumigation, days</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressure in Siroflo® system, Pa</td>
<td>500</td>
<td>500</td>
<td>560</td>
<td>460</td>
</tr>
<tr>
<td>Flow of Eco2Fume® mixture, lt/min</td>
<td>1.2</td>
<td>0.3</td>
<td>2.5</td>
<td>0.84</td>
</tr>
<tr>
<td>Eco2Fume® mixture in cylinders, kg</td>
<td>45.2</td>
<td>12.1</td>
<td>94.0</td>
<td>31.0</td>
</tr>
<tr>
<td>Weight of used Eco2Fume® mixture, kg</td>
<td></td>
<td>904</td>
<td>242</td>
<td>1880</td>
</tr>
<tr>
<td>Weight of used phosphine, a.i., g</td>
<td></td>
<td>904</td>
<td>242</td>
<td>1880</td>
</tr>
</tbody>
</table>

Table 2. Cost comparison of grain fumigation with Siroflo®/Eco2Fume® technology, grain treatment with liquid insecticides and fumigation with solid phosphine.

<table>
<thead>
<tr>
<th>Grain fumigation with Eco2fume (mixture 2% PH3 + 98% CO2) using Siroflo System</th>
<th>Grain treatment with liquid insecticides</th>
<th>Grain treatment with solid phosphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity, tonnes</td>
<td>2200</td>
<td>2200</td>
</tr>
<tr>
<td>Silo diameter, m</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>PH3 stable concentration, ppm</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>Duration of fumigation, days</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Pressure in Siroflo® system, Pa</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Flow of mixture, lt/min</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Weight of used mixture according to flow, kg</td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td>Cost of Eco2Fume® mixture, liquid and solid insecticides</td>
<td>€ 7.3/kg</td>
<td>€ 12.8/lt</td>
</tr>
<tr>
<td>Cost of fumigation and grain treatment, €/tonne</td>
<td>0.11</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The total expenses for the installation of the Siroflo® system in 24 concrete bins with a total of 40 thousand tonnes capacity was approximately € 65,000.-. This increases the fumigation cost by 0.08 €/tonne in case of one fumigation of 40000 tonnes every year for twenty years use of system.
Fumigation using Siroflo®/Eco2Fume® has many advantages in comparison to traditional grain protection methods by using phosphine tablets or liquid insecticides. Based on 10 years experience in using Siroflo®/Eco2Fume® technology in Cyprus, this method provides:

- constant and fully controlled PH₃ gas concentration during grain fumigation in non-airtight structures
- a successful grain fumigation in a non-sealed silo
- an easy and automatic phosphine application process
- treatment of grain in-situ (no need to move grain and therefore no machine-equipment wear, no electric-power consumption, no need in extra emptied silo space, no insect distribution etc.)
- improved safety in working environment
- grain free from pesticide residues
- no necessity in liquid insecticides for grain treatment
- no need in handling dangerous to environment disposed residues of phosphine tablets and liquid insecticides
- more effective and cheaper grain protection in comparison to grain treatment with liquid insecticides and solid phosphine.

The following may be considered as weak points of the Siroflo®/Eco2Fume® technology:

- low PH₃ concentration at the top 5-15 cm of grain in the bin (note: risk of selecting for resistance)
- long duration of fumigation process (14 days)
- dependence of this fumigation technology on the use of phosphine, a chemical which is facing the increasing threat of the development of resistance in many regions.

Nowadays in Cyprus phosphine tablets or liquid insecticides are not used any more for treating grain against insects.

Recently the EU started a new procedure for re-registration of all chemicals used in member-countries, including Cyprus (Directive 91-414 EC). All phosphine formulations and phosphine mixtures are considered as separated items and must pass again this procedure as well. Although there is a great deal of demand for Eco2Fume® in North America, Australia and China, it seems that for some reasons Cytec, the only manufacturer of Eco2Fume®, will not proceed with its registration in EU. This obligates the CGC to look for new grain protection alternatives with at least the same advantages of Siroflo®/Eco2Fume® technology.

**Storage in hermetic Platforms and in modern bunkers under UV PVC**

The building of bunkers and platforms for grain storage under PVC in Cyprus aims to:

- increase the grain storage capacity of the country in a fast and inexpensive way
- take advantage of low prices in the international grain market and balance the fluctuations of international prices and currency, since Cyprus imports more than 90% of its needs in grain
- receive the local grain production at a faster pace and protect it against damages caused by adverse weather conditions

In 1987 the Cyprus Grain Commission, in collaboration with the Volcani Center, Israel built two Platforms with concrete pavement and a 1m high peripheral retaining wall. Their dimensions are 75 m long, 25 m wide with a prospected peak at a height of 7 m and with a capacity of about 4,000 tonnes. This Cyprus version of bunker is based on the reinforced concrete pavement and on the use of heavy-duty UV-proof PVC liners of 700 micron thickness, welded together to provide a continuous cover of the grain. The edges of this cover
overlap with the polyethylene under liner that is laid over the floor to provide a gas tight structure for hermetic storage. This design in Cyprus differs from the Israeli version primarily in the concrete construction of platforms; it also differs from the Australian version primarily in the lighter reinforced PVC over liners which are sewn together to provide a continuous covering that is not sufficient gas tight for hermetic storage. The Cyprus Platform version is designed for prolonged grain storage under hermetic conditions (Navarro et al., 1992).

Barley of maximum 11% moisture content was stored up to 34 months in these hermetic bunkers erected on platforms in Cyprus. The CO$_2$ and O$_2$ concentrations of the intergranular air within the bunkers were changed during hermetic storage. There was a rapid decrease in O$_2$ from 21% up to 5% and an increase in CO$_2$ concentration from 0.03% up to 10% over the first two months followed by fairly stable levels for the rest of the storage period. Of 450 samples examined for infestation, live insects were recorded from only 34 samples during the storage period. All infested samples were recorded during the first year. No samples containing live insects were recorded at the end of storage except few samples from the grain bulk surface. No insecticides were used during 34-months hermetic storage in platforms. After one year of hermetic storage the total grain losses were below 0.3 %, after 34 months of hermetic storage the grain losses were less than 1%, mainly due to grain damage caused by moisture condensation at the apex of the grain bulk. The PVC over liner remained with low gas-permeability retained its mechanical characteristics and was suitable for reuse. The bunker storage successfully protected grain against insects, bird and rodent attack and provided safe storage during the rainy season (Varnava et al., 1994, Varnava and Mouskos, 1996).

In 2000 the Cyprus Grain Commission, in cooperation with CSIRO (Scientific Industrial Research Organization, Australia) and SACBH (South Australian Cooperative Bulk Handling Ltd-AusBulk Ltd), built six Australian-type bunkers with a total capacity of 70 thousand tonnes based on 0.60 t/m$^3$. The bunkers are 30 m wide, 130 m long and about 10 m high each. Their pavement is compacted crushed rock of 15 cm thickness which provides a suitable surface with only minor annual grading and re-rolling of the surface, since these structures are seen as filled and emptied only once each year. The Bunker have corrugated steel walls of 1.5m high with fabricated “A” frame supports secured to the ground by iron pegs. Sheets of 100% polyester base cloth UV PVC coated on both sides are used for cover. Basic sheet characteristics are: 520 micron thickness, 600 g/m$^2$ weight, anti-glare ice-blue colour, 3 years guarantee period, 37m long and 12m wide, non permeable to water and low gas permeability. Sheets are sewn together in order to create a sealable strong seam by two passes of the sewing machine and by sealing the seam with an appropriate sealant (silicone etc.). Polyethylene sheet of 200 micron covers the bunker floor, overlaps the side walls and is generally discarded after each use. The side walls are covered with a polyethylene coated reusable fabric which extends from the ground and interlocks with the top tarp to create a gastight and water-proof seal. The top and bottom covers are easily secured to the walls by overlapping the covers and holding them in “Z” purling with timber rail and clamp plate. For loading grain into bunker and for unloading into trucks a drive over hopper and a stacker of 300 tonnes/hour loading capacity with a min. 24 m length boom at 26º, operating from outside of the bunker, are used. A front end loader is used for feeding the stacker during unloading grain into trucks.

The total expenditures for the design, machinery, walls, sheets, apparatus etc. for the construction of six bunkers are € 525 thousand or € 7.5 /tonne capacity (Table 3).

For the operation of bunkers, particularly during covering and uncovering works with sheets, a considerable number of employees is needed (max. 8-10 employees). The total labour for both filling and emptying a bunker of 12 thousand tonnes capacity, including
covering and uncovering works, is about 14 days (8-10 hours working day) or about 1000-1400 total man-hours.

Table 3. Expenditures for design, machinery, walls, apparatus etc. for the construction of six Australian type Bunkers of total storage capacity 70 thousand tonnes, x1000 €.

<table>
<thead>
<tr>
<th>Type of Expenditures</th>
<th>Cost</th>
<th>average lifespan years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunker walls, supporting “A” metal frames etc.</td>
<td>75</td>
<td>20</td>
</tr>
<tr>
<td>Drive over hopper, stacker of 300 tonne/hour capacity</td>
<td>135</td>
<td>20</td>
</tr>
<tr>
<td>8 Aeration fans, aeration ducts, aeration controller etc.</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>Data loggers, software, cables</td>
<td>8</td>
<td>5-10</td>
</tr>
<tr>
<td>Top UV PVC grain cover sheets (double)</td>
<td>150</td>
<td>4x2</td>
</tr>
<tr>
<td>Bottom polyethylene floor sheets</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Design, packing, insurance, erecting, testing, training, commissioning etc.</td>
<td>115</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>525</strong></td>
<td>-</td>
</tr>
</tbody>
</table>

7.5 €/tonne capacity

Barley of moisture content between 12% and 13.5% was stored in these bunkers several times. In case the storage started in summer and lasted until next spring, the grain losses, particularly due to moisture migration and condensation at the bunker apex, were usually between 0.3% and 1.5%. When grain storage starts in April-May, and lasts for 6-8 months until December, i.e. before the low ambient air temperatures cause moisture condensation at Bunker the apex leading to grain damage, the grain losses are below 0.2%.

A well sealed bunker is a good structure for fumigation with solid phosphine. In Australia two ways of fumigation with solid phosphine are used in bunkers. Through fumigation ports, opened on the top sheet surface, phosphine strips or plastic trays with phosphine tablets are inserted under the sheet into grain and ports are sealed again (Banks and Sticka, 1981; Sacbh/AusBulk, 1999). A fumigation of bunkers in Cyprus is carried out in a different way. Special openings consist of plastic pipes of 10 cm diameter with closing cups are fixed on the Bunker walls. Phosphine tablets mixed with grain are placed in 1.5 m long plastic perforated pipes of 8 cm diameter which have the one edge pointed and the other edge can be closed with a cup. The pipes with the mixture of phosphine and grain are inserted through the openings on the walls into the grain in bunker. After completing fumigation, pipes with decomposed tablets are easily removed from the Bunker without leaving disposed residues of phosphine tablets. In a well sealed bunker the PH₃ concentration remains usually higher than 50 ppm for at least 15 days by using doses of 0.5 g a.i. PH₃/tonne.

Based on our experience the bunker solution for grain storage has disadvantages and advantages. Negative aspects may be considered:

- the low automation in filling-emptying bunkers, particularly for covering and uncovering them, which leads to the need of considerable manpower
- the restriction in filling and opening during rainy days
- the danger of condensation at the apex of bunker if there is considerable difference between the grain temperature inside bunker and platform and ambient air.

Advantages of bunker technology may be:

- the successful protection of grain against rain, birds and rats damages and reinfestation
• the ability for safe storage from April until December with negligible losses (less than 0.2%) under Cyprus weather conditions
• the ability to protect huge quantities of grain in a low-cost way
• the ability to take serious advantage of low prices in the international grain market, since Cyprus imports more than 90% of its needs in grain
• the successful fumigation in bunkers by using phosphine tablets without leaving pesticide residues in grain
• the ability of successful long-term storage of grain in hermetic platforms without the use of chemical insecticides
• the low capital expenses for building bunkers and platforms which are 10-20 times lower in comparison to capital expenses for building flat stores, metal or concrete silos.

Conclusions

1. The collaboration among countries, organizations, companies and scientists is a powerful tool for the development, introduction and implementation of advanced technologies in the grain industry.
2. Fumigation technologies Siroflo®/Eco2Fume®, Aeration technology and the storage of grain in modern Bunkers and hermetic Platforms are:
   • ecologically friendly methods which may effectively be used in Integrated Grain and Pest Management for grain storage and protection, for grain without chemical residues and for careful attitude to environment.
   • low-cost solutions for grain storage and protection under certain conditions.
   • good solutions which may have improved safety in working environment.
   • successful alternatives to methyl bromide and they may reduce or even exclude in some cases the use of contact insecticides for grain protection.

References


