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September 20-23, 2005

Edited by
Shlomo Navarro, Cornel Adler, Jordi Riudavets, Vaclav Stejskal
The content of the contributions is in the responsibility of the authors
Participants of the Prague conference, September 2005
Introduction

The IOBC/WPRS (OILB/SROP) Working Group on Integrated Protection of Stored Products met under the auspices of the Research Institute of Crop Production in Prague, the Czech Republic, in the conference facilities of the Institute, September 20-23, 2005.

There was a participation of 46 delegates from 18 countries. A total of 54 contributions (37 oral, 17 posters) were presented to cover various topics of post harvest pest biology, biological and integrated pest management. This is the first meeting with four speakers from the U.S.A. who contributed significantly to the success of the meeting. The decisions of the Montreal protocol for the phase out of methyl bromide have caused serious concerns of the European food processing industries. The changing realities against the use of residue leaving toxic pesticides and the increasing demand for environmentally user friendly technologies resulted in interest in biological control, on the use of semiochemicals and pheromone traps.

A field trip to Plzensky Prazdroj, the leading brewery in central and Eastern Europe was combined to a visit to the Government own winery near by Karlštejn. The brewing technology and the importance of barley quality in the beer making was appreciated by the participants. Such a field trip was an excellent opportunity for fostering cooperation, exchange of useful information, and to get better acquainted of each other's work.

The present IOBC Working Group on Integrated Protection of Stored Products gained its current structure after Dr. Cornel Adler convened the IOBC Working Group on Integrated Protection of Stored Products in Zurich in 1997, then in Berlin in 1999, then in Lisbon in 2001, and then in Kusadasi, Turkey in 2003.

I thank the local Organizing committee consisted of Dr. Vaclav Stejskal, Dr. Zuzana Kucerova, and Dr. Zuzana Pzourkova of Research Institute of Crop Production in Prague. The conference and book of abstract were supported by "Vědecký výbor fytosanitární a životního prostředí (Scientific Committee on Phytosanitary and Environment)," projekt Výzkumný záměr No. MZE-000-2700603 (Project No. MZE-000-2700603). The conference was partially supported by Olympus, Biocent laboratory and DDD service, Prague.

The editing of this book of proceedings was made with the editorial assistance of Dr. Samuel Angel, former researcher at the Israel Agricultural Research Organization who was extremely helpful in guiding me in the editing process of the papers.

I would also like to thank my colleagues Dr. Cornel Adler, Dr. Jordi Riudavets, and Dr. Vaclav Stejskal, for their contribution in the editing process, and Dr. Horst Bathon for his relentless support.

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Implications for integrated storage strategies of food contaminants legislation

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Abstract: Contaminants of grain in storage have been the subject of recent EU legislation and others are likely to come under scrutiny. Pesticide residues and mycotoxins are examples of the former and insects and mites are examples of the latter. Maximum residue levels may limit the efficacy of some pesticides and determine the mode of application. For instance, top-dressing may be questionable, which puts greater reliance on physical control. Regulatory levels for mycotoxins in food have focused attention on ochratoxin A (OTA) as the most important quality determinant for safe storage times for damp grain. This dictates how long it may be held in harvest backlogs before it is passed through a hot-air dryer or before a drying front is completed using an ambient air drying system. A UK project seeks to integrate an EU-funded model of OTA production with an established British drying model. This will determine under which conditions the new quality criteria can be achieved using current design parameters and how these may be improved. EU regulations for food hygiene require that farmers adopt procedures to minimise the risk of biological, chemical and physical contamination. Feed businesses must comply with specific microbiological criteria and adopt procedures to meet specific targets including a permanent written procedure based on HACCP procedures. This could be applied through national guides to good practice, such as HGCA’s ‘Grain storage guide’. However, determination of contaminants depends on accurate sampling and knowledge of distribution of the contaminants in bulks that requires further research.

Keywords: food contaminants, sampling, pesticide residues, mycotoxins, OTA, HACCP, HGCA

Introduction

In the past, biodeterioration of cereals during storage in the developed world has been mainly of interest because of the direct damage caused and losses to the crop. Insects were important because they caused weight loss and heated the grain allowing sprouting and fungal growth at the surface. Fungi caused concern because they also caused weight loss and tainted the grain, as did mites.

Of late however, it is less direct effects that are of greater concern – mites and insects are implicated in health issues, associated generally with allergies. The pesticides associated with them are also held in suspicion; by the consumer because of health fears (organophosphates); operator exposure fears (phosphine); or claims that they affect the climate (methyl bromide). Pesticides are regarded not only as solutions to pest problems but also as chemical contaminants which need to be carefully and accurately administered and rigorously documented. To some extent, this has limited their use as prophylaxis and thrown reliance on physical control options such as drying and cooling. Fungi are now mostly important because of the toxins they produce – aflatoxins and ochratoxins being the most commonly associated with storage, although only the latter can occur in temperate climates such as the UK.

EU regulations have acted in response to concerns about these contaminations; there are regulatory maximum levels for mycotoxins and pesticides and statutory sampling schemes for mycotoxins which impinge directly on storage practice. As yet, there no statutory maximum
levels for insects or mites. There is no tolerance in international trading for insects (although 1 insect per 2 kg is tolerated in the USA) but there are no recommended levels at all for mites. It cannot be long however before statutory limits for arthropod contamination are also laid down since new EU regulations on food (Anon. 2002a) and feed (Anon. 2005) demand risk assessments and the latter depends on the application of HACCP principles, in particular the establishing of critical limits.

**Pesticides**

In the UK, pirimiphos-methyl is one of the two commonest encountered chemical residues which is applied mainly as a fabric treatment in grain stores against residual infestations before grain is harvested but which also are applied directly to grain to protect it or cure infestations in store. Maximum residue limits (MRLs) are laid down in UK statutory instruments (Anon. 2002d; 2002e) as well as by EU legislation. The MRL for pirimiphos-methyl, 5 ppm, is very close to the application rate on grain of 4 ppm, so there is little margin for error.

A UK strategy was developed largely to replace the use of admixture chemicals by a preventative approach to infestation. This ensures the grain is dried to moisture contents (m.c.s) that would not support mites and moulds, and cooled quickly enough to prevent insects completing their life-cycle, to temperatures below their development thresholds. However, this still left the grain surface vulnerable as insects could overwinter there, due to temperature fluctuations in the mild UK maritime climate, while mites could still flourish there as the m.c. gained equilibrium with the atmosphere and increased by 2-3% above the bulk m.c.

The physical strategy based on cooling and drying was therefore supplemented by the addition of a top-dressing of a pesticide dust which was convenient to apply to static bulks as prophylaxis or as a curative measure. However, the dust formulations of the pesticides were withdrawn as the pesticide companies did not feel the cost of providing the registration authorities with new data was justified by the size of the market. This void was filled with the advent of diatomaceous earths (DEs) which were also dusts and could be applied in a similar way although to date, they have not found the same size of market, partly due to reluctance from some end-users and partly because of the slow action on pests and the unfamiliarity of growers.

Liquid formulations are still widely available and used in the UK, both for fabric treatments and also for application to the grain but they are not recommended for top-dressing, mainly because of fears about over-dosing locally and exceeding the MRLs. Until this limitation can be overcome, given the slow uptake of the only alternative, DEs, it is likely that a great deal of UK grain will still be treated using residual pesticides as prophylactic treatment.

**Fungi**

Mycotoxins are fungal metabolites produced to give certain species of fungi competitive advantage over others. Unfortunately, some have been proven to have ill effects on human health and may even be genotoxic carcinogens. Their production is usually related to certain moisture and temperature conditions, related to the growth requirements of different species. Mycotoxins in cereals have two origins, being formed either in the field during the growth or in store. The former include fumonosins, trichotheecenes (e.g. deoxynivalenol) and zeralenone that are derived from *Fusarium* spp. Although there is conflicting opinion, it is generally accepted that these are not usually produced in storage, except perhaps during advanced
decay. However, propionic acid treatment of damp grain may select for *Fusarium* spp. (Burrell et al., 1973)

During storage, different fungi predominate and the main concerns then centre on toxins produced by the relatively xerophilic fungal genera (that prefer dry conditions); *Penicillium* and *Aspergillus* (*Eurotium*) which may produce ochratoxins and aflatoxins. It is the latter group, that is currently of greatest concern in the UK since temperatures and moisture contents here do not favour the former. It is generally considered (Cahagnier et al., 2005) that the mycotoxins are formed during mycelial growth and may be related to ergosterol production. The organism responsible for ochratoxin A (OTA) does not grow below 80% r.h. (18% m.c. in cereals) and does not produce the toxin below 85% r.h. (19% MC in cereals) (Northolt and Bullerman, 1982) and is therefore most likely to grow immediately after harvest in backlogs before the grain is hot-air dried or during ambient-air drying when a drying front passes slowly through a bulk over a period of 7 days to a month. The process of moisture migration in unventilated heaps and of uptake by the surface of atmospheric moisture during damp winters should also not be ignored (Armitage and Cook, 2003).

**EU regulatory levels for the storage mycotoxins in food are:**
- 4 ppb total aflatoxins of which aflatoxin B1 must not exceed 2ppb (Anon. 2001)
- 5 ppb (OA) (Anon. 2002b)
- Currently regulatory levels for animal feed are being considered and these are likely to be a limit of 0.05 – 0.1 ppm for OTA and 5 – 20 ppb for aflatoxin B1 (Dr Luis Conchello personal communication)

**Sampling protocols**

For OA, EU Commission directive 2002/26/EC (Anon. 2002c) recommends 100 samples of 100 g be taken from bulks between 50 and 1500 tonnes resulting in aggregate samples of 10 kg. Similar guidelines are laid down for detection of aflatoxins. The EU requirement is that the entire aggregate sample should be ground using a process demonstrated to achieve complete homogenisation and the replicate laboratory/working sample be taken from the homogenised material. Three replicate samples are taken for enforcement purposes by the trade (defence) referee and compliance judged on analysis of one enforcement sample. The origin of mycotoxin formation will naturally have an effect on the sampling process. It may be assumed that field mycotoxins may be mixed in transport and during conveying as the grain goes into store and therefore may be relatively homogeneous. On the other hand, storage mycotoxins are likely to be locally distributed and will only be formed when the grain is stored for some time above the threshold for growth of the mycotoxin-producing fungi. This is only likely to occur on undried moist grain during harvest backlogs and during the ambient-air drying process when grain at the top of the bin remains close to its original moisture content until the drying front passes through. In this case, the mycotoxins will be primarily at the top of the bin (or floor store) if the grain is not ‘turned’ after drying.

The sampling and analysis directives which accompany the regulations are addressed to enforcement agencies (i.e. local and port health authorities) to enable them to enforce the regulations adequately. These agencies are therefore required to use these sampling regimes but there is no direct requirement for others to do so. How others ensure that the commodities they produce and sell comply with the regulations is a matter for them to determine, and to provide evidence in their due diligence defence if an official control sample finds them wanting. It is recognised that the official sampling regime is onerous and the directives state that it is acceptable as a defence if an alternative sampling regime is used that can be shown to be "substantially equivalent" to the official one.
A critical study (Wilson et al., 1999) compared the suitability of four aflatoxin sampling plans to detect OTA by sampling coffee and wheat. These were the USDA plan, the Dutch code of practice and the UK plan for groundnuts and the EC plan for aflatoxin. Only the EC sampling plan for aflatoxins, based on taking 20-100 incremental samples of 30 g, depending on lot size, produced representative samples for OTA in wheat. However, there are no studies to describe the distribution of mycotoxins in grain bulks, and few to validate alternative less labour-intensive sampling strategies, which would also be adequate for detecting other contaminants and this must remain a priority for future storage research.

**Prevention**

We have identified grain above 19% m.c. as being vulnerable to OTA formation and the two main critical control points being pre-drying harvest backlogs (hot air drying) and the time spent before a drying ‘front’ passes through a bed of grain (ambient air drying). Both of these require knowledge of the time available at a range of moistures and temperatures before the critical limit laid down by EU regulations is exceeded. Ideally, a direct model of OTA formation would enable us to determine these critical times at different m.c.s and temperatures but to date, no such model exists.

However, an indirect model has recently been established. Jonsson et al. (2000) observed, using respirometry, that fungal growth has a sigmoid development, with an initial lag phase, followed by an exponential phase when toxin formation commenced. They were therefore able to define maximum safe storage times with respect to mould growth at a range of temperatures and m.c.s which could be used to determine safe drying times and check whether current recommendations for ambient-air drying are adequate.

In the past, the critical limits for fungal growth were ‘visible fungi’ and the critical quality thresholds applied to grain drying engineering design were either visible mould or a critical loss of germination. The Jonsson et al. (2000) model suggested that neither of these were likely to be adequate for achieving compliance with the regulatory thresholds for mycotoxins in food for grain above 18% m.c., where typical ambient air drying rates are designed to pass the drying front through the bulk in 10 days.

Therefore, a current Home-Grown Cereals Authority research project is incorporating these new safe storage times with regard to mould growth and mycotoxin production with existing models of ambient-air drying in the UK in order to identify under which conditions current engineering design is likely to be able to prevent mycotoxin formation and those where it will be unsuccessful in doing so and where other strategies to increase drying speed will be necessary. These other strategies may include part-drying to below the critical m.c. for OTA formation before the process is completed by ambient-air drying, grain stirring to mix dried and moist layers, thus reducing the time spent above the critical limit for OTA production, and increasing the specification for drying fans or reducing bed depth to speed the process of moisture removal.

Initial simulations were run for 1951-1971 (Years 1-20 in Fig 1.), drying from 20% to a maximum m.c of 16.5 and an average m.c. of 14.5% in a 3 m bed. In these computed simulations, the fan was run continuously at 0.058 (m$^3$/s)/tonne and the heater was turned on if the r.h. after the fan went above 70%. Using the new model of OTA production, in 5 out of 20 years it was predicted that a spoilage index of 1 will be exceeded while no visible mould was predicted, and the germination loss was less than 2% in all years.
Fig. 1. Effect of spoilage criterion on success of drying wheat over 20 years in a typical dryer (Spoilage index less than 1 means grain is not spoiled).

**Insects and mites**

There is no tolerance of insects in trade, although the difficulty of detecting low levels of infestation makes this difficult to impose, and in the USA, infestations of up to 1 per 2 kg are permitted. These thresholds are based on the assumption that detection of small numbers of insects in samples indicates relatively large infestations which are likely to have developed to damaging levels before transport by sea can be completed. No thresholds have been formally set for mites although informal levels of 100 per kg have operated in the pet food trade and 500-1000 per kg in intervention storage. However these were arbitrary limits which were not based on particular science and after transport and conveying, the likelihood of detecting many live mites would be much reduced, as the soft-bodied animals do not survive such activities.

The USFDA issued new regulatory guidelines in 2001 which categorises stored product mites as Class 1 chemical contaminants (the highest level) having concluded that mites can present a serious risk to health (Olsen, 1998). They also cause symptoms such as scouring and reduced weight gain in animals to which they are fed so there are welfare issues as well. In humans, they can cause dermatitis, and urticaria by contact, rhinitis and asthma by inhalation and anaphylaxis by ingestion. Mite numbers in cases where food anaphylaxis was noted ranged from 4,000 – 7,000 per kg which compares with maximum levels found in UK products of 11-21 per kg and limits in the USA and Italy of 1.5 per kg (Wildey, CSL: Pers. Com).

As we have seen, there are legislative limits for ochratoxin-A in foodstuffs, and MRLs for pesticides but none yet for arthropods. In the light of EC regulations below, which demand the application of HACCP principles including critical limits, it seems inevitable that legislative limits for arthropods will shortly be set.
EU Regulations

Regulations (EC) No. 1642/2003 (Anon. 2003) lay down principles of food law which apply also to feed for food-producing animals. Regulation (EEC) No. 689/1992 (Anon. 1992) requires intervention cereals to be free from live pests (including mites) at every stage of their development, even though there are no methods by which this can be demonstrated or achieved.

Specific attention must be drawn to Regulations (EC) No. 183/2005 (Anon. 2005). These lay down the requirements for feed hygiene (most UK cereals are used for feed). These stipulate that procedures should keep contaminants as low as reasonably achievable and apply at the level of primary production (farmers) as well as other feed business operators.

Most importantly, this demands a permanent written procedure based on HACCP procedures as follows:

a) identify any hazards
b) identify critical control points
c) establish critical limits separating acceptability from unacceptability
d) establish monitoring procedures
e) establish corrective action
f) establish procedures to verify a-e
g) documentation and records

Tables 1-3 illustrate some knowledge gaps that become apparent when such an HACCP approach is applied. The regulation also suggests that ‘Member states should encourage the development of national guides’ whose ‘dissemination and use should be encouraged by the competent authorities’. These should be…’developed and disseminated by feed business sectors….having regards to relevant codes of practice of the Codex Alimentarius’.

Table 1. HACCP analysis of some storage issues. I. Store Preparation.

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Critical Control Point</th>
<th>Actions</th>
<th>Gap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungal spores Carry over of <em>P. verrucosum</em> may encourage OTA</td>
<td>Pre-harvest: Residues on floor, in conveyer, cleaner, dryer etc</td>
<td>-Clean load points into conveyer -eg pits -Run system until empty and then for some time after -Thorough cleaning -Disinfection</td>
<td>-Innoculation sources of OTA unknown -Transmission by arthropods unknown -Effectiveness of disinfection not established -Critical limits unknown</td>
</tr>
<tr>
<td>Residual arthropod infestation</td>
<td>Period between sale and taking in new harvest</td>
<td>-Clean (sweep, vacuum, hose, steam) -Monitor -Fabric treat (IF insects still exist) -Monitor again -Admix grain on intake (IF insects still exist)</td>
<td>-Results of cleaning etc. unproven) -Critical limits (mites not established)</td>
</tr>
</tbody>
</table>
In the UK, Home Grown Cereals Authority (HGCA) has funded Central Science Laboratory to develop a code of practice in ‘The Grain Storage Guide’ (Armitage and Wildey, 2003) in consultation with industry and has a record-keeping aid ‘Grainplan’ nearing completion. The former has consequently been adopted as required reading by various assurance schemes, such as the Assured Combinable Crops Scheme. In the light of regulation 183/2005, the UK national guide will need to be revised to conform to an HACCP format and research based on a GAP analysis will be the basis of a future UK storage research programme.

Table 2. HACCP analysis of some storage issues. II. Drying.

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Critical Control Point</th>
<th>Actions</th>
<th>Gap</th>
</tr>
</thead>
</table>
| Heating, fungal growth and OTA formation during delayed drying | Backlogs due to hot-air dryer throughput | -Part dry in 1st pass to below -18%  
- Cool undried grain to slow deterioration  
- Continue drying ‘on-floor’ if dryer capacity can’t cope | -Insect development during cooling of damp grain unknown  
- Critical limits of fungi in the absence of toxins not established |
| Formation of OTA | Slow drying in grain above 18% | -Drying front must progress faster than safe storage time (shorter at higher mcs)  
- Late autumn drying will need added heat or stirring | - OTA limit and safe drying time varies for feed and food.  
- Late harvest grain is often damper but also available ambient air is unsuitable for drying.  
- Mixing dried and undried grain may be a solution but untried |

Table 3. HACCP analysis of some storage issues. III. Storage

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Critical Control Point</th>
<th>Actions</th>
<th>Gap</th>
</tr>
</thead>
</table>
| Mites and fungi increase, insects survive in damp surface layers | 1-3 months after harvest | - M.c. increase is inevitable  
- Dryer bulk results in a dryer surface  
- Mites can be controlled and insects prevented by DE  
- Monitor surface for pests and m.c.s | - Whether OTA is formed at the surface of cooled bulks as a result of mc uptake or mc translocation is unknown  
- More treatment options required  
- Thresholds for arthropods not established |
| ‘Hot spots’ in dry grain started by weevils | 3 or more months after harvest | - Increased frequency of cooling removes metabolic heat and prevents weevil development  
- Increase insects traps and temperature probes to locate activity centre  
- Pesticide treatment may be required if infestation spreads | - Integration of measurement/ monitoring and decision support required  
- Localised nature of hidden infestation makes detection difficult  
- Treatment options limited |
Implications to Integrated Pest Management

Integrated Pest Management (IPM) is more than simply monitoring and trapping pests (and other contaminants). IPM is a comprehensive system designed to implement pest control in an informed and sustainable way through ongoing risk assessment utilising the widest armoury of control options. Wherever possible these options should be energy efficient, with least impact on the consumer and the environment.

The UK has gone a long way towards achieving this through industry implementation of the Grain Storage guide, which is underpinned by robust research and development. However, introduction of more stringent regulatory requirements and the move within Europe to adopt HACCP based procedures is highlighting some important knowledge gaps and challenges to current control measures, both on a national and European level. It is therefore vital that the research community responds to these new drivers in order to continue supporting this important industry sector.

Acknowledgements

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and The Pesticides (Maximum Residue Levels in Crops, Food and Feeding Stuffs) (Scotland) amendment (No. 2) Regulations 2002 (Statutory Instrument No 489).


Food safety and on-farm grain storage

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Abstract: An occasional survey in the German federal states of Brandenburg, Sachsen-Anhalt and Mecklenburg-Vorpommern, showed that 12 of 20 farms sold their grain directly after harvest. In farms storing grain, multi-purpose buildings often served for storage for periods of 3-12 months. These buildings posed no barrier to the immigration of insects, mites, and vertebrates. Grain was mostly not cleaned from dust, husks and other contaminants due to the lack of equipment. Cracks and crevices in walls and floors, wooden beams and high roofs with a multitude of dust collecting surfaces complicated cleaning prior to storing the new harvest. These conditions allow many pest species to establish permanently. Grain drying units, blowers and air ducts used in grain aeration facilities for cooling using ambient air, were found to be scarce. All grain samples taken were found infested at least with one insect species. Among the pests found in grain samples from on-farm storages were Cryptolestes ferrugineus, Oryzaephilus surinamensis, Pyralis farinalis, Sitophilus granarius, Typhaea stercorea, Tenebrio molitor, mites and booklice. Mouldy grain could be found close to walls, doors, openings, aeration ducts, in cracks or metal profiles. Poorest storage conditions were found in farms specialised on market crops other than grain and farms storing grain for feeding life-stock. It was concluded that on-farm storage may pose a risk to grain quality down the grain processing chain, that it may be advisable to define minimum storage quality standards, and to provide a better incentive for high-quality storage. A significant improvement could be the definition of a minimum standard of gastightness for European grain silo bins.

Key words: stored products, infestation, contamination, insects, hygiene

Introduction

It is generally accepted that a great risk for durable plant products, stored under appropriate conditions is the attack by stored product insects. In the case of stored grain, species like Oryzaephilus surinamensis, Sitophilus granarius, S. oryzae, Cryptolestes ferrugineus, or Ephhestia elutella may infest the harvested products. If temperatures allow development and a sufficient number of individuals is present, the metabolism of these insects will lead to an increase in moisture and temperature and the development of a hot spot with increased metabolic activity and more rapid insect development. The increased moisture content in this area will allow the development of booklice and mites, as well as of fungi like Aspergillus spp. or Penicillium spp. which cause a further increase in temperature and moisture and can lead to the production of highly toxic and dangerous mycotoxins. Thus, insects can give rise to a dangerous contamination of food. However, according to the professional background required by the risk assessment panels, stored product insects are at present not dealt with by the expert groups of the European Food Safety Authority.

The regulation (EC) 178/2002 (Anon. 2002) on food safety, describing the tasks of the European Food Safety Authority, defines all products intended for human consumption in unprocessed or processed condition as food from the time of harvest on (art. 2). The regulation (EC) 852/2004 on food hygiene requires the application of the principles of Hazard
Analysis of Critical Control Points (HACCP) in all stages of food production except primary production. In the Corrigendum of June 2004 (Anon. 2004), on-farm storages are exempted from this requirement with the comment that HACCP "... at the level of primary production is not yet generally feasible. However, guidelines to good practice should encourage the use of appropriate hygiene practices at farm level" (Art. 11).

On-farm grain storages are probably one of the points in grain handling, most critical from the perspective of food safety, because infestations overlooked here can cause severe problems further down the grain processing chain. The purpose of the research presented in this paper was to study on-farm storages of grain and to describe basic needs and possible improvements to the storages found.

**Materials and methods**

In an occasional study, 20 farms were contacted in the German Federal States of Brandenburg, Mecklenburg-Vorpommern and Sachsen-Anhalt in summer 2005. Farms that stored grain were visited, storages were inspected and grain samples were taken. In the laboratory, these samples were kept at 25°C and 65-75 % r.h. Insects found were identified using the taxonomic guide by Weidner (1982).

**Results and discussion**

From the 20 farms contacted, 8 stored their grain for periods longer than 3 months. None of the farms visited had cleaned their grain from dust and spelt. Grain dryers were not available and grain of the 2004 harvest had not been dried. All storage facilities were horizontal storages. One of the storages was equipped with an aeration unit that can help to reduce grain temperature and moisture in early fall and winter. Temperature was not recorded by the storage keepers, monitoring traps for stored product insects were not found in any of the storages visited.

While in general, the roofs of the structures visited were water proof, the connection between wall and roof or the walls themselves had openings. Barn doors were open or left a gap big enough for rodents to enter. Two farms stored parts of their grain in open sheds with at least one wall missing. Among the pests found in grain samples taken from these storages were mites, booklice, Cryptolestes ferrugineus, Oryzaephilus surinamensis, Pyralis farinalis, Sitophilus granarius, Tenebrio molitor, and Typhaea stercorea.

One reason for these rather poor storage conditions found could be that adverse changes in grain quality are not immediately visible due to a decrease in temperature soon after harvest. An insect infestation may remain undetected without severe consequences until spring of the following year. A lack in attention may thus not lead to immediate financial losses due to lower product quality, because low temperatures during the winter season may hide a low or moderate infestation until most of the harvest is sold.

In their study of organic farms Prozell et al. (2004) visited 15 different storage sites of 5 organic farms in Germany and found at least one insect pest species in each of the storages. These results are confirmed by the findings of this study, where the majority of farms were conventional ones. One can conclude that insect infestation seems to be a frequent problem in on-farm grain storage at present and that better storage structures, hygiene measures and IPM could considerably reduce the risk of infestation. It may be useful to determine the quality of primary storage structures and the level of infestation in on-farm grain storages on a broader level in a joint European research project. This could be important because an infestation during on-farm storage may cause a considerable reduction in product quality and higher costs for pest control further down in the grain processing chain.
**Why do farmers store their grain?**

Either the farmer speculates on better grain prices later in the year or just before the next harvest or the stored grain can be used for feeding livestock which again may help the farmer to achieve a better income. In both cases it would of course be important to keep product quality high and infestation levels low. As could be seen in the market value of wheat grain late in 2004 and early in 2005, there were hardly any gains to be made by storing grain because prices did not increase, probably due to the good harvest in 2004 keeping the grain market sedated.

It may thus be recommended to farmers that cannot improve their storage facilities to sell their grain directly after harvest in order to avoid infestation and the loss of product quality. Those farmers that intend to store animal feed or food grain will need to improve storage conditions.

**What could be done to improve on-farm storage conditions?**

A basic requirement for storage structures is smooth surfaces that are easy to clean and dry. The immigration of rodents and insects could be prevented by well-sealed metal doors and by avoiding shrubs, weeds, machinery or any other cover outside of storages in a distance of at least 5 m. Also inside the building unnecessary items should be removed to prevent serving refuge for both vertebrate and invertebrate pests. Grain cleaning is most desirable, when coupled with a suitable aeration system within the grain bulk, it can reduce the risk of local grain heating. Drying and subsequent aeration cooling would help to reduce the potential of development of insect pests in the grain bulk. Traps and regular temperature control would be important tools to monitor grain quality. Occasional customer audits could be an incentive to farmers to improve storage conditions. A minimum level of gas-tightness, verified by pressure-testing should become a prerequisite for grain storages. An overview on storage requirements is given by Proctor (1994) who also states under the aspect of sealing:

> “When a new grain store is being planned, there should be no question as to whether or not it should be possible to seal it effectively to make it air-tight for fumigation. The benefits of sealed stores are such that the small costs involved during initial construction (negligible in many cases) should not warrant consideration. …”

It appears remarkable that these recommendations were made for developing countries, but are not yet a commonly accepted standard in industrial countries. Australia, as a major grain exporting country, developed a standard of gas-tightness for silo bins as early as 1975 (Proctor, 1994). A later survey showed that many silo bins became leaky over time due to leaky seals and could not keep pressure for a half-life of minimum 5 min (from an initial pressure of 25 mm water column) (Newman, 1994). Nevertheless, structures built under the aspect of gastightness can be a barrier against the immigration of pests and thus an important step in terms of pest prevention. It thus appears as an important task to define minimum requirements for good grain storage in Europe and to adapt the standard of gas-tightness for European silo bins.

**References**


Insects in food in the Jewish religion as a motive for pest control

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Abstract: Judaism forbids the consumption of most types of animals with the exception of a restricted number of species which are specifically mentioned in the Bible. There is a strict injunction against eating insect-infested food, and this is a powerful motive for pest control operations in the whole system of food production acceptable by Judaism, particularly in Israel. The purpose is to supply food free from insects and insect fragments. Most of the public in Israel consumes kosher food, which has a rabbinic authorization according to Jewish dietary laws. Consequently these religious authorities are very powerful in controlling the food industry and the marketing system. In recent years, a monitoring system has been implemented using hundreds of religious inspectors who have been specially trained in entomology. This control system is mandate to permit food plants to obtain a "kashrut" certificate. Pest control is carried out mainly by monitoring: from the crops in the field, through post-harvesting procedures to the acceptance of the raw materials in the food factories, the objective being to prevent inclusion of insects into the food process. In the food industry, the insect monitoring is carried out throughout the production, packaging and storage procedures. The combination of monitoring by inspectors equipped with the essential professional knowledge and the motivation, from deep religious convictions, with the increasingly sophisticated monitoring systems, brings excellent results in achieving clean food with negligible infestation, acceptable by the "kashrut" authorities.

Keywords: Jewish religion, pest control, infested food, kosher food, kashrut certificate, food insects

Introduction

Judaism forbids the consumption of most types of animals except those specifically mentioned in the Bible. There is a strict injunction against eating insect-infested food.

“And every creeping thing that creepeth upon the earth shall be an abomination; it shall not be eaten” (Leviticus XII, 41) (Fig. 1). From the Hebrew version it is known that “creeping things” means insects or other crawling animal.

In the Christian religion there are no “forbidden foods”. Islam forbids the consumption of pork, but there are no references in the Koran concerning insect-infested food. However, we can assume that both Islam and Christianity would clearly regard infested food as inedible. In other religions it is not forbidden to eat infested food. In fact, in some cultures, insects are commonly eaten as an important source of protein.

The strict Jewish prohibition against eating infested food is a powerful motive for pest control operations. The Biblical commandment is the basis of the oral commandment, known as “Halacha”, which is the interpretation of this religious commandment and the practical way to carry it out. The Halacha was written by the Jewish religion leaders, the rabbis, over a period of almost 2000 years. According to the Halacha, a religious control system is in operation today by the religious authorities, in order to monitor and prevent the the inclusion of insects and insect fragments in food.

Amongst the Jewish people there are several main streams of religion: secular, traditional, orthodox and ultra-orthodox. However concerning infested food there is a
consensus of all streams with the ultra-orthodox, who desire to obtain food absolutely free of insects and insect fragments.

The Israeli standards permit a tolerance of a reasonable minimum of insects in food but they do not deal with insect fragments and this does not satisfy the religious authorities.

Fig. 1. Hebrew and English version of Leviticus section describing creeping things.

Fig. 2. "kashrut" authorization seal from rabbinate in Israel.

Most of the public in Israel consume kosher food, which has a rabbinic authorization according to Jewish dietary laws. Almost all the food plants in Israel require a "kashrut" authorization in order to function economically, and they are under the control of the religious authorities, who are very powerful in controlling the food industry and the marketing system (Fig. 2).
The human religious control system

The control system includes hundreds of inspectors who are employed by the food plants. These inspectors are trained in the identification of insects in food, biology of insects and in monitoring methods (Fig. 3). In most of the large factories the inspectors are permanent employees, while for each of several smaller factories there are inspectors who visit them regularly. The range of known control methods is not sufficient and some are unsuitable for achieving the minimum expected level of infestation. Judaism, which relies on a Biblical verse: “Take ye therefore good heed unto yourselves” (Deuteronomy IV, 15), is very aware of human health and the risk of using chemical insecticides.

Fig. 3. Guide book for the religious inspectors about insect infested food.

Therefore the way to achieve food, including fresh produce, free of insects and insect fragments as far as possible is by monitoring and prevention measures. Monitoring is carried out throughout all stages of food production:

1 – During the growing period in the field.
2 – Post harvesting of the crops in the storage facilities.
3 – Acceptance of the raw products in the food factories, in order to prevent insect fragments in the final product.
4 – Monitoring within the plants, along the production lines, as well as packaging and storage.

The inspectors in the food plants are well acquainted with the factories and all the processes there. The inspections are carried out at all critical points in the food plants which are potential foci for infestation (Fig. 4).
Fig. 4. Religious inspector checking potential foci of infestation.

Fig. 5. Religious inspectors checking traps for insect monitoring.

Fig. 6. Number of Lasioderma serricorne trapped in 12 checkpoints per month in a factory using both inspectors and trap monitoring systems.

In many factories, which are working under strict quality assurance conditions, there are sophisticated trap monitoring systems (Fig. 5). There are several well-known disadvantages to these monitoring systems. Traps are only for adults and flying stages, range of operation and active period is very short, there are no pheromone traps for all species etc. The monitoring by the inspectors compliments the trap monitoring systems particularly in the regions where
these systems cannot cover efficiently. The operations by both inspectors and the trap monitoring systems enable a rapid and early detection of infestation foci and immediate control, producing excellent results (Fig. 6). The monitoring traps are an important accessory measure to the work of the inspectors. However, in some factories there is an illusion that these traps alone are sufficient and the human factor is neglected.

The inspectors are also responsible for; (i) Sealing the factories to prevent insects entering from outside. (ii) Cleanliness throughout all the factory operations, both inside and outside, to prevent infestation foci.

A large proportion of both the raw materials and processed products are imported into Israel. If food industries throughout the world want to receive a "kashrut" certificate for export to Israel, the same monitoring processes need to be carried out abroad. This monitoring is carried out either by the local Jewish religious authorities, or by visiting Israeli inspectors.

The religious "kashrut" authorities are also aware of the need to provide solutions and improvements in the food industry concerning insects. They are also involved in improvements in monitoring systems and prevention methods.

**Conclusion**

The combination of monitoring by inspectors with the essential professional knowledge and the motivation, from deep religious convictions, with the increasingly sophisticated monitoring systems, brings good results in achieving clean food with negligible infestation, acceptable by "kashrut" authorities.

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European network on biological control of pests in stored products – COST Action 842, WG 4

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Abstract: In June 2005 the final meeting of the European COST Action 842 was held. COST (an intergovernmental framework for European CO-operation in the field of Scientific and Technical research) has made it possible for a group of researchers to meet on a regular basis during the 5½ years this action has been functional. Originally derived from two applications, COST 842 was entitled “Biological control of pest insects and mites with special reference to Entomophthorales”. Working Group 4 dealt with “Biological control of arthropod pests in stored products”. WG 4 had six meetings during its lifetime, most of the meetings with 20-25 delegates. The activities of WG 4 have been reported in written proceedings; they will become available on a website.

Key words: COST Action, European network, biological control, stored product pests

Introduction

In June 2005 the final meeting of the European COST Action 842 was held. COST (an intergovernmental framework for European CO-operation in the field of Scientific and Technical research) has made it possible for a group of researchers to meet on a regular basis during the 5½ years this action has been functional. The title of COST Action 842 was “Biological control of pest insects and mites with special reference to Entomophthorales”. It was originally based on two applications, one on the subject mentioned in the title, and one on biocontrol of storage pests, initiated by Eva Zdarkova, CZ. This last subject was covered by Working Group 4 “Biological control of arthropod pests in stored products”.

Meetings

Working Group 4 has held six meetings during its lifetime, most of the meetings with 20-25 delegates. Participants from a total of 13 countries participated in the meetings during the course of the COST Action. Each of the meetings focused on a specific sector related to storage of durables from storage facilities through to processing. The possibilities of developing biological control against the main pests in each sector were discussed and evaluated. Where possible, each meeting included a field trip to a facility of relevance to the specific topic of the meeting, e.g. a grain store, flour mill, bakery or pasta factory. The subjects dealt with at the meetings are described below.

Lisbon 2001

The first meeting took place in September 2001 and was organised by CEFA/IICT in Lisbon, Portugal. This meeting was attended by 10 delegates and served to define the scope of the
group’s work. It was decided that each of the following meetings would focus on a specific sector in the chain from storage of raw materials, through processing to storage of the processed durable products. The important pests in each sector were identified and the potential of biocontrol was evaluated.

**Prague 2002**
This meeting took place at RICP in Prague, Czech Republic in May 2002. Twenty-two delegates attended the meeting. The specific sector was grain storage: grain production in different countries, type of storage facilities, important pests and pest control methods. Natural enemies of the main pests in grain stores were described and the potential of applying biocontrol in grain stores was evaluated.

**Berlin 2003**
In December 2003 18 delegates met in Berlin, Germany, for a meeting organised by BBA. The specific topic was flour mills and bakeries; the production capacity in each country was described, important pests identified and the potential of biocontrol evaluated. In addition, each delegate, submitted information that was collated in a general presentation on the legal aspects involved with applying biological control agents in food storage and processing facilities.

**Athens 2004**
The 4th meeting of WG 4 took place in May 2004 in Athens, Greece, with 24 delegates, and was organised by the Agricultural University of Athens. The pests related with storage of nuts, cocoa, spices and dried fruits were described and the potential of biocontrol for this specific sector was evaluated. This meeting included a workshop on storage mite identification.

**Barcelona 2004**
Twenty-three delegates met in Barcelona, Spain, in October 2004 for a meeting arranged by IRTA. The specific topic was the pest situation in pasta processing and the possibilities of biocontrol in this sector. Furthermore, the group discussed the potential of combining biocontrol as an element of IPM with the principles of HACCP (Hazard Analysis – Critical Control Points) in food processing facilities.

**Locorotondo 2005**
The final meeting of the whole COST Action was held in Locorotondo, Italy in June 2005 and attracted 26 delegates from WG 4. The programme of this meeting contained an important joint session with the three other working groups, during which the potential of using entomopathogens for stored product pest control was presented and discussed.

**Short Term Scientific Missions (STSM)**
The COST system contains an opportunity to obtain funding for exchange of expertise through short visits to other research institutes. Seven members of WG 4 used this possibility during the course of the action.

**Proceedings and web site**
Proceedings from each of the meetings are available (Zdarkova et al. 2001; 2002; Hansen et al. 2004a; 2004b: 2005). These proceedings contain valuable reviews of biocontrol against
pests in, for example grain stores, dried fruit and spice storage and in food processing facilities. A website is being constructed to facilitate access to this information (http://cost842.csl.gov.uk/).

A final resolution was prepared that specifies WG 4’s opinion of research priorities in this field and identifies situations where biological control could be a valuable component of an integrated pest management strategy. Three situations were considered to hold most promise: control of beetle, moths and mites in empty stores and in bulk stored grain, and protection of packaged goods against moths. The final resolution will be distributed to policy makers and the research community. A new COST action is being prepared because of the continued interest in this field.

Conclusion

The COST system has meant that the scientific community in Europe involved in this subject has been able to meet on a regular basis and thoroughly evaluate the potential of using biocontrol against stored product pests. The proceedings from WG 4’s meeting contain valuable reviews of research conducted in this field, and a network that has been established that provides a valuable starting point for future collaboration in this field.

Acknowledgements

We wish to acknowledge the efforts that Eva Zdarkova, RICP, Czech Republic, has put into initiating this COST Action and chairing WG 4 during its first two years.

References


Insecticidal effect of diatomaceous earth applied alone or in combination with Beauveria bassiana and beta cyfluthrin against Sitophilus granarius on stored wheat

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Abstract: Three diatomaceous earth (DE) formulations, SilicoSec, PyriSec and Insecto were tested for their effectiveness against adults of the granary weevil, Sitophilus granarius (L.) (Coleoptera: Curculionidae) on stored wheat under laboratory conditions, at 25°C and 65% r.h. Three series of tests (bioassays) were carried out. In Test 1, 500 ppm of each DE was tested alone or in combination with 1000 ppm of dry conidia of the entomopathogenic fungi Beauveria bassiana (Balsamo) Vuillemin (Hyphomycetes: Moniliiales). Mortality was assessed after 24 h, 48 h, 7 d and 14 d of exposure, while the treated substrate was also examined for progeny production, 45 d after the 14 d count. In Test 2, beta cyfluthrin, at 0.4 ppm was tested alone or in combination with each of the three DEs, at 500 ppm. In this test, weevil mortality was assessed after 7 d of exposure. Finally, in Test 3, beta cyfluthrin at the rates 0.2 and 0.8 ppm was tested alone or in combination with 500 ppm of SilicoSec, while mortality and progeny production counts were performed as in Test 1. In Test 1, the presence of DEs increased the effectiveness of B. bassiana, especially at exposures ≥7 d. Hence, after 7 d of exposure, S. granarius mortality was >93% on wheat treated with the fungal/DE combinations, but did not exceed 51% on wheat treated with B. bassiana alone. Furthermore, the presence of DEs decreased progeny production on the fungus-treated wheat. The additive effect on B. bassiana was the same for the different DEs. The same trend was observed in Test 2, where after 7 d of exposure DE increased the effectiveness of beta cyfluthrin. In this test, weevil mortality was 51% on wheat treated with beta cyfluthrin alone, but exceeded 75% when DEs were present. In Test 3, the presence of SilicoSec increased the insecticidal effect of beta cyfluthrin and decreased progeny production only in the case of the lowest dose rate. Hence, after 7 d of exposure, weevil mortality on wheat treated with 0.2 ppm of beta cyfluthrin with or without SilicoSec was 36 and 78%, respectively, while the respective figures for 0.8 ppm were 95 and 94%.

Key words: diatomaceous earth, Beauveria bassiana, beta cyfluthrin, Sitophilus granarius, stored wheat

Introduction

The granary weevil, Sitophilus granarius (L.) (Coleoptera: Curculionidae) is among the most important insect pests of stored grains. It is a primary pest, which means that this species is capable of infesting with great ease sound and undamaged grain kernels (Aitken 1975). It is an internal feeder, since the females lay their eggs inside the kernels where total immature development occurs causing serious infestations (Aitken 1975). For its control, as in the case
of the other stored-grain insect species, two main categories of pesticides are applied: fumigants and residual grain protectants. However, both substances can harm human health and the environment, and the consumer’s demand for residue-free food, has prompted researchers to evaluate alternative, ecologically-compatible, control methods.

Diatomaceous earths (DEs) are considered as one of the most promising alternatives to traditional pesticides in stored-grain (Korunic 1998, Subramanyam and Roesli 2000, Arthur 2003). DEs are composed of the fossils of diatoms, which occurred during the Eocene and Miocene periods (Korunic 1998). They have low mammalian toxicity, and can be applied with approx. the same technology as grain protectants. Several DE formulations are now commercially available (Subramanyam and Roesli 2000) and several studies document that many of these formulations are very effective against a wide range of stored-grain insect species (Aldryhim 1990, 1993, Korunic 1998, Subramanyam and Roesli 2000, Arthur 2000, Fields and Korunic 2000, Mewis and Ulrichs 2001, Fields et al. 2003, Athanassiou et al. 2003, 2004a, 2005a, b, Athanassiou and Kavaliaratos 2005, Kavaliaratos et al. 2005). However, the use of DEs has one serious disadvantage: DEs reduce the bulk density of the grain (Korunic 1998, Korunic et al. 1998). Generally, for a satisfactory level of grain protection, DEs should be applied at very high dose rates, usually >500 ppm, while grain protectants can be effective at even below 10 ppm. There are two possible solutions to this implication; first, newer DEs which can be applied at lower rates (Subramanyam and Roesli 2000) and second, the combination of DEs with other, IPM-compatible control methods (Lord 2001, 2005, Stathers 2003).

In the present work, we evaluated the potential of using three commercially available DE formulations in combination with the entomopathogenic fungus Beauveria bassiana (Balsamo) Vuillemin (Hyphomycetes: Moniliales) or the pyrethroid beta cyfluthrin, against S. granarius adults on stored wheat. Apart from direct mortality, progeny production in the treated substrate was assessed.

Materials and methods

Insects. Granary weevils were obtained from a culture held at the Danish Pest Infestation Laboratory (DPIL), and cultured for five generations at the Benaki Phytopathological Institute, on whole soft wheat (var. Dion) at 25°C and 65% r.h. All adults used in the tests were <2 wk old.

Formulations. The three DE formulations used in the tests were Insecto™, SilicoSec® and PyriSec®. Insecto (Insecto Natural Products Inc., Costa Mesa, CA, USA) is a DE formulation of marine origin containing 86.7% SiO₂, 10% food-grade additives and ≤3% crystalline silica (Subramanyam et al. 1994). SilicoSec (Biofa GmbH, Münsingen, Germany) is a DE formulation of freshwater origin containing 92% SiO₂, 3% Al₂O₃, 1% Fe₂O₃, and 1% Na₂O. Finally, PyriSec (Agrinova GmbH, Obregheim/Mühlheim Germany) contains 1.2% natural pyrethrum (25%), 3.1% piperonyl butoxide and 95.7% SilicoSec.

Commodity. Untreated, clean and infestation-free soft wheat (var. Dion) with little dockage (0.8%) was used in the tests. This commodity was obtained from the 2004 Greek harvest. Its moisture content, as determined by a Dickey – John moisture meter (D.-J. Multigrain CAC II, Dickey-John Co, USA), was 12.6%.

Fungus. B. bassiana isolate used was 678, originally isolated from Musca domestica (house fly), obtained from the culture collection at DPIL in Denmark. The fungus was subcultured on plates with oat meal agar (O3506, Sigma, Germany) for mass production of the fungal conidia. The fungal conidia were collected by scraping the conidial layers formed on the plate surface using a sterilized scalpel.
Insecticide. The beta cyfluthrin formulation used was Buldock 025 SC (Alpha Georgika Efodia AE BE, Greece) which is an emulsion.

Test 1
The fungus was tested at 1000 ppm of dry conidia, while the three DEs at 500 ppm. Thus, seven treatments were evaluated, fungus alone, fungus with each DE and each DE alone. For each case, three 20-g wheat samples were treated. An additional series of samples with untreated wheat served as control. Each sample was placed in a glass Petri dish, and 20 S. granarius adults were introduced in each sample. Then, all samples were placed in incubators set at 25°C and 65% r.h. The desired relative humidity level was maintained by using saturated salt solution of sodium chloride, as recommended by Greenspan (1977). Dead adults were counted after 24 h, 48 h, 7 d and 14 d of exposure. After each interval, all adults, dead and alive, were removed from the treated substrate and the dishes were left at the same conditions for a further period of 45 d after which the number of offspring (F1 weevils) was recorded. Each experiment was repeated three times, by preparing new samples each time. Temperature and humidity throughout the tests were monitored by using HOBO data loggers (HOBO H8, Onset Computers, Pocasset, MA, USA).

Test 2
The experimental conditions were as above. A 0.6 kg quantity of wheat was sprayed by using a CLK-608 sprayer of 450 mL capacity (China Kunli Plastic Sprayer & Bottle Manufacturer) in order to apply 0.4 ppm of beta cyfluthrin. From this quantity, twelve 20-g samples, were taken and placed in Petri dishes. In the first nine, in groups of three, SilicoSec, PyriSec and Insecto were added at 500 ppm, while in the remaining three no DE was applied. An additional 0.6-kg lot was sprayed with distilled water and served as control. The number of tested insects/sample and the conditions were as above, but in this case, mortality was assessed only after 7 d of exposure, while no progeny production counts were performed. The test was repeated three times by preparing new lots each time.

Test 3
In this test, two 0.6 kg lots were treated as above, with 0.2 and 0.8 ppm of beta cyfluthrin. Six 20 g-samples were taken from each lot. To the first three samples, 500 ppm of SilicoSec was added, while the rest were not treated with DE. An additional lot of untreated wheat was sprayed with distilled water. From this lot, six samples were taken; 500 ppm of SilicoSec were added in each of the first three, while the rest served as control. Mortality and progeny production counts were performed as above. This test was also repeated three times with new lots each time.

Data analysis
Generally, control mortality was very low but when necessary, mortality counts were corrected by using Abbott’s (1925) formula. For each test, the arcsine-transformed mortality data were analyzed separately for each exposure by using an one-way Anova, to indicate significances among treatments. The same analysis was performed for progeny production counts. Progeny production counts were not introduced in the analysis, since a preliminary Anova indicated that significantly more progeny were found in the untreated dishes than the treated ones (P<0.0001). Generally mean progeny production in the untreated dishes was (average from Tests 1 and 3) 81.3 ± 14.8 weevils/dish. Means were separated by using the Tukey-Kramer (HSD) test, at a probability level of 5% (Sokal and Rohlf 1995).
Results

Test 1
After 24 h of exposure, despite the fact that significant differences were found between treatments (df=6, 56; F=3.75, P=0.0033), mortality was low and did not exceed 7% (Fig. 1A). At the 48 h exposure interval, significant differences were found among treatments (df=6, 56; F=11.08; P<0.0001), but mortality was still low (<4%) on wheat treated with the fungus alone (Fig. 1B), while in combinations of *B. bassiana* with PyriSec, mortality reached 15%. After 7 d of exposure, significant differences were also noted (df=6, 56; F=95.47; P<0.0001), while, with the exception of PyriSec alone, all treatments were significantly more effective than *B. bassiana* alone (Fig. 1C). In addition, all combination treatments were significantly more effective than DEs alone. Hence, on wheat treated with the fungus/DE combinations mortality was 93-96%, while on wheat treated with Des alone it was only 62-81%. Furthermore, it should be noted that, weevil mortality was significantly higher on wheat treated with Insecto alone, than with SilicoSec or PyriSec. Finally, also after 14 d of exposure, significant differences were found between treatments (df=6, 56; F=40.63; P<0.0001). Significantly less weevils were dead on wheat treated with the fungus alone than in the other treatments (Fig. 1D). All adults were dead in the case of the combination treatments, while on wheat treated with DEs alone mortality ranged between 89 and 92%. Significant differences were also noted regarding progeny production counts (df=6, 56; F=42.72; P<0.0001). More than 35 adults/sample were recorded on wheat treated with the fungus alone, while in the other treatments progeny did not exceed 3 adults/vial (Fig. 2).

Test 2
Significant differences were noted among treatments (df=3, 32; F=10.18; P<0.0001). The presence of DEs significantly increased mortality in comparison with the application of beta cyfluthrin alone (Fig. 3). Almost half of the exposed weevils were still alive after 7 d of exposure on wheat treated with the fungus alone, while on wheat with DEs mortality was 75-81%. No significant differences were noted between the three combination treatments.

Test 3
After 24 h of exposure, significantly more *S. granarius* adults were dead on wheat treated with beta cyfluthrin, with or without SilicoSec, than on wheat treated with SilicoSec alone (df=4, 40; F=12.16; P<0.0001), but mortality in this exposure did not exceed 13% (Fig. 4A). Similar trends were also noted after 48 h of exposure (df=4, 40; F=18.10; P<0.0001), but mortality increased notably, with the exception of SilicoSec alone (Fig. 4B). The presence of DE significantly increased the insecticidal effect of the lowest dose of beta cyfluthrin, while no additive effect was noted for the highest beta cyfluthrin dosage. Hence, mortality on wheat treated with 0.2 ppm of beta cyfluthrin did not exceed 24%, while the simultaneous presence of SilicoSec gave 40% mortality. At the 7 d exposure interval, as above, significantly more adults were dead on wheat treated with 0.8 ppm of beta cyfluthrin with or without SilicoSec, where mortality was >93% (df=4, 40; F=50.68; P<0.0001) (Fig. 4C). In contrast, the mortality caused by the application of 0.2 ppm of beta cyfluthrin alone was 35%, while the combination of this dosage with DE gave 77% mortality. Finally, at the 14 d exposure, significant differences were noted among treatments (df=4, 40; F=51.44; P<0.0001), and mortality was 100% in the case of wheat treated with the highest beta cyfluthrin dosage (Fig. 4D). Also as noted previously, SilicoSec increased the mortality caused by 0.2 ppm of beta cyfluthrin. As far as the progeny production is concerned, significant differences were noted (df=4, 40; F=3.73; P=0.0113). Significantly more weevils were found in the case of 0.2 ppm of beta cyfluthrin alone in comparison with the other treatments, with the exception of SilicoSec alone (Fig. 5).
Fig. 1. Mean mortality (± SE) of *S. granarius* adults exposed for 24 d (A), 48 h (B), 7 d (C) and 14 d (D) on wheat treated with *B. bassiana* (BB) alone or in combination with SilicoSec (Sil), PyriSec (Pyr) and Insecto (Ins) (means followed by the same letter are not significantly different; HSD test at 5%).
Fig 2. Mean progeny production (weevils/sample ± SE) of *S. granarius* adults on wheat treated with *B. bassiana* (BB) alone or in combination with SilicoSec (Sil), PyriSec (Pyr) and Insecto (Ins) 45 d after the removal of the parental adults (means followed by the same letter are not significantly different; HSD test at 5%).

Fig. 3. Mean mortality (± SE) of *S. granarius* adults exposed for 7 d on wheat treated with 0.4 ppm of beta cyfluthrin alone or in combination with SilicoSec (Sil), PyriSec (Pyr) and Insecto (Ins) (means followed by the same letter are not significantly different; HSD test at 5%).

**Discussion**

Our results indicate that SilicoSec, PyriSec and Insecto can be used with success against *S. granarius* on stored wheat, which is in accordance with previous studies regarding the same species (Subramanyam and Roesli 2000, Mewis and Ulrichs 2001). These DEs have proven effective against adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Athanassiou et al. 2004b, 2005a, b), the confused flour beetle, *Tribolium confusum* Jacquelin...
Fig. 4. Mean mortality (± SE) of *S. granarius* adults exposed for 24 d (A), 48 h (B), 7 d (C) and 14 d (D) on wheat treated with beta cyfluthrin at two dose rates, 0.2 and 0.8 ppm, alone or in combination with SilicoSec (DE) (means followed by the same letter are not significantly different; HSD test at 5%).
Fig 5. Mean progeny production (weevils/sample ± SE) of *S. granarius* adults on wheat treated with beta cyfluthrin at two dose rates, 0.2 and 0.8 ppm, alone or in combination with SilicoSec (DE) 45 d after the removal of the parental adults (means followed by the same letter are not significantly different; HSD test at 5%).

du Val (Coleoptera: Tenebrionidae) (Vayias and Athanassiou 2004, Athanassiou et al. 2005b, 2005a) and the lesser grain borer, *Rhizopertha dominica* (F.) (Coleoptera: Bostrychidae) (Athanassiou and Kavallieratos 2005, Kavallieratos et al. 2005). Moreover, based on the present findings, DEs provide an additive effect in the insecticidal effect of *B. bassiana*. Lord (2001) first reported that the presence of the DE formulation Protect-It synergized with *B. bassiana* against larvae of *R. dominica*. The author suggested that DE particles may inactivate certain epicuticular lipids which play an inhibitory role in the fungal attachment, germination and penetration. Also Akbar et al. (2004) found that the same DE synergized with *B. bassiana* against larvae of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and that the presence of DE increased the attachment of the fungal conidia on the insects’ cuticle. Athanassiou (2004) reported that a *B. bassiana*-based formulation, Naturalis® [(*B. bassiana* 7.16% w/v, i.e. 2.3x10^7 conidia/mL)], gave better results against adults of *S. oryzae* and *T. confusum* when admixed with Insecto. The results of the present work support the above observations, and clarify that this additive effect seems to be irrelevant of the DE formulation, since all DEs had the same effect in combination with *B. bassiana*. Apart from parental mortality, the progeny production decreased noticeable. Despite the fact that the physiological basis of this synergism has not been investigated in detail, we assume that a DE/fungal formulation could be effective for a wider range of species.

Inert materials have been used as carriers in dry conidial formulations of entomopathogenic fungi (Moore and Higgins 1997, Moore et al. 2000, Batta 2004), but their effect on fungal efficacy is highly influenced by the fungal species. For instance, Batta (2004) found that the addition of inert materials, such as charcoal or ash, increased the effectiveness of *Metarhizium anisopliae* (Metschikoff) Sorokin (Deuteromycotina: Hyphomycetes) against *S. oryzae* adults. On the other hand, Michalaki et al. (2005) reported that the effectiveness of a *M. anisopliae*/SilicoSec combination against *T. confusum* larvae was negatively affected at some temperature and r.h. levels, and positively affected at others. Since the effectiveness of *B. bassiana* is also affected by temperature and r.h. (Akbar et al. 2004, Lord 2005), and given
that many isolates are available (Wakefield et al. 2002) our data correspond only to the specific experimental conditions.

The second finding of the present series of bioassays is the fact that, in some cases, the presence of DEs increased the effectiveness of beta cyfluthrin. In Test 2 it was evident that, as in the case of *B. bassiana*, this additive effect was provided by all of the three DEs tested. However, in Test 3 this trend was observed only at the lowest dosage of the pyrethroid. From the combined data of Tests 2 and 3, we conclude that DE increased the effect of beta cyfluthrin at dosages ≤0.4 ppm. In contrast, nothing is gained by the addition of SilicoSec on wheat treated with 0.8 ppm of beta cyfluthrin. Previous studies on the same commodity have shown that beta cyfluthrin was effective against adults of *S. oryzae* (Athanassiou et al. 2004b) and *T. confusum* (Athanassiou et al. 2004c). A synergism of deltamethrin with the silica gel “Gasil 23D” and the DE “Protect-It” was reported for the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) by Barbosa et al. (1994) and Stathers (2003). However, in our results this was not evident in the case of PyriSec, which combines silicon dioxide, natural pyrethrum and piperonyl butoxide, given that mortality was similar for all DEs tested. In a recent study, Athanassiou et al. (2004a) compared PyriSec with other DE formulations against *S. oryzae* and found that PyriSec provided faster mortality. Generally, DEs are more slow-acting in comparison with traditional insecticides, and according to the present results, *S. oryzae* mortality was faster in the beta cyfluthrin-treated wheat.

Le-Patourel and Singh (1984) when studying the joint action of the amorphous silicas “Cab-O-Sil M5” or “Aerosil R972” and the pyrethroids permethrin, cypermethrin and deltamethrin against *T. castaneum* adults found that low concentrations of the insecticides caused an increase of the insecticidal activity of silicas. On the other hand, the authors stated that an increase in the pyrethroid dosage increased the rapid knock-down of the exposed beetles, and this immobilization resulted in the decrease of dust particles picked up by the insect bodies. Our results correspond to this observation, and probably this is why DE did not cause any additive effect on the insecticidal effect of the highest beta cyfluthrin dosage.

Progeny production decreased in the case of the lowest beta cyfluthrin dosage. This may suggest that 60 d after treatment, this dose may partially lose its efficacy. In contrast, as inert materials DEs can retain their insecticidal effect for a long period. For instance, Athanassiou et al. (2005b) found that the three DEs tested here could be used with success against *S. oryzae* on wheat and barley for a period >300 d after their application. Thus, this may be one of the reasons, why SilicoSec reduced *S. granarius* emergence on wheat treated with the lowest beta cyfluthrin dosage.

The combination of DEs with other substances may be a solution to the drawback of high application rates of DEs (Arthur 2003, Stathers 2003). Entomopathogenic fungi could be one possible compound with great potential for combination with DEs. In addition, the admixture of DEs with low doses of low mammalian toxicity insecticides, such as the pyrethroids, is one of the alternatives proposed for further investigation. For both combinations, additional tests are needed to examine their effect in a wider range of cases (species, commodities, temperature, r.h., etc.).

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The importance of food supplements for parasitoids of stored product pests: the case of *Venturia canescens* (Hymenoptera: Ichneumonidae).

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Abstract: Laboratory studies were conducted on the effect of honey-feeding on progeny production and longevity of adults of *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) parasitizing larvae of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Experimental adults lived under various temperature regimes with access to hosts. Provision of adult parasitoids with honey resulted in a significant increase in production of total offspring at all temperatures. Highest mean fertility of honey-fed wasps was recorded at 20°C, followed in decreasing order by 25, 30 and 15°C. At all temperatures honey-fed wasps lived significantly longer (almost three times) than their starved conspecifics. The experimental findings are analyzed with the objective of improving the effectiveness of *V. canescens* as a biological control agent of stored product pests.

Key-words: *Venturia canescens*, *Ephestia kuehniella*, adult feeding, longevity, progeny production, life tables, stored products, biological control, parasitoid

Introduction

Parasitoids have often been used against agricultural and horticultural pests during the last decades. However, their application for the control of stored product pests remains very limited. One of the main reasons for this phenomenon is not only that the level and reliability of control may be insufficient but also the very low economic injury level of stored products (Schöller et al., 1997). Therefore, optimization of biological control methods is essential for their practical implementation. One of the most common methods to improve parasitoid effectiveness in storage facilities is to provide them with suitable food supplements. Importance of adult feeding for parasitoids of stored products pests has been demonstrated in many recent studies (Wäckers, 1996; Wäckers et al., 1998; Schmale et al., 2001), where it was clearly shown that adult feeding causes significant increase of egg production, number of offspring and longevity. This is even more apparent in species which do not feed on the haemolymph of their hosts (host-feeding), like the moth parasitoid *Venturia canescens*.

*Venturia canescens* is a thelytokous, koinobiont, solitary endoparasitoid of lepidopterous larvae. Its host range includes many moth species, mainly pyralids, whose larvae are pests of stored products (Salt, 1976). Several laboratory and field studies have examined its potential against Pyralids (Ahmad, 1936; Corbet and Rotheram, 1965; Harvey and Thompson, 1995; Harvey et al., 1996; Harvey and Vet, 1997; Schöller, 2000a; 2000b; Heinlein et al., 2002). However, it should be mentioned that there is no commercial application of *V. canescens* as biocontrol agent to date.

This study deals with the effect of adult feeding on progeny production and longevity, at four different temperatures. The findings are analyzed on the basis of improving the efficiency of *V. canescens* as biocontrol agent against stored products pests.
Materials and methods

Host and Parasitoid cultures

Larvae of the Mediterranean flour moth *E. kuehniella* were used as hosts. The host species was reared in incubators at 25°C with a L:D 16:8h photecycle and 65±5% R.H. Culturing was undertaken using clear plastic boxes (17 x 11 x 5cm) containing 200-250g of semolina with 250-300 host eggs. This allowed host larvae to develop with excess food throughout larval life. The original population of the parasitoid *V. canescens* had been collected in flourmills near Athens, Attiki Co.

*Venturia canescens* was also reared in plastic boxes (as for *E. kuehniella*). Approximately, two hundred 4th-5th instar larvae from host culture were placed in each box together with 10 adult wasps. This procedure was repeated every 4 days. Boxes were left until adult wasps hatched. To segregate parasitoids for experiments, parasitized hosts were removed from the culture and placed individually in Petri dishes at 20°C.

Effect on number of progeny

Full grown moth larvae (5th instar) were placed in groups of 100 into a large modified Petri dish (diameter 12cm). Air circulation was achieved through a hole (diameter 4cm) in the lid, covered with nylon mesh. The dishes were left undisturbed for 24h before being presented to parasitoids, in order to permit release of mandibular secretions (e.g. silk) which contain kairomones that elicit probing behavior by *V. canescens* (Corbet, 1971).

The following day, newly emerged adults were collected and placed individually in a dish and either were given no access to food or were provided with honey *ad libitum* smeared on the inside of the dish. Each parental wasp was transferred daily to another Petri dish identical with the previous one. Larvae in the previous dish were transferred at 25°C to large glass jars containing an excess of food medium to complete development and emerging adult parasitoids or moths were counted. The procedure was carried on until the parental wasp died.

To study the effect of honey-feeding on the number of progeny produced, the offspring of 10 honey-fed and 10 starved adults, were counted at four constant temperatures (15°, 20°, 25° and 30°C).

Effect on longevity

Adult longevity of cohorts of 35 honey-fed or starved individuals exposed to a range of constant temperatures (15°, 20°, 25° and 30°C) was measured. A constant supply of honey and hosts (50 mature L5 larvae of *E. kuehniella*) was achieved by transferring daily each experimental adult to a Petri dish identical with the initial one. Parasitoid longevity was determined by checking daily (or every 8 hours in the case of cohorts kept at 30°C).

Statistical analysis

Data were subjected to analysis of variance at α = 0.05. Means were separated using the Tukey – Kramer HSD Test (Sokal and Rohlf, 1995) and all statistical analyses were performed using the statistical package JMP v.4.0.2 (SAS, 1989).

Results

Feeding on honey resulted in a remarkable increase in offspring at 15°C (75.8%), 20°C (352.9%), 25°C (258.8%) and 30°C (112.3%). The differences in number of progeny among fed and starved wasps were significant at all temperatures (Table 1) (15°C: *F*=18.71; df =1, 18; *P*=0.0004, 20°C: *F*=149.02; df =1, 18; *P*<0.0001, 25°C: *F*=167.64; df =1, 18; *P*<0.0001, 30°C: *F*=55.02; df =1, 18; *P*<0.0001).
Table 1. Mean number of progeny produced by female *V. canescens* at various constant temperatures, supplied daily with 100 full grown larvae of *E. kuehniella* (65 ± 5% R.H., 16:8 L:D)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>n</th>
<th>Honey-fed mean ± S.E.</th>
<th>range value</th>
<th>Starved mean ± S.E.</th>
<th>range value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10</td>
<td>35.7 ±3.62 aA</td>
<td>22-58</td>
<td>20.3 ±0.82 bA  F</td>
<td>16-25</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>108.6±6.89 aB</td>
<td>78-136</td>
<td>24.0±0.73 bB F</td>
<td>19-26</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>82.9±4.58 aC</td>
<td>67-108</td>
<td>23.1±0.54 bB F</td>
<td>19-25</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>51.8±3.51 aD</td>
<td>36-71</td>
<td>24.4±1.14 bB F</td>
<td>20-30</td>
</tr>
</tbody>
</table>

n: number of parental females.

α: means in a column followed by the same capital letter are not significantly different; means in a row followed by the same small letter are not significantly different (Tukey-Kramer HSD Test, α: 0.05)

F: differences proved to be significant but were minor and biologically meaningless

Honey-fed adults lived significantly longer than their starved counterparts at all experimental conditions (15°C: $F=293.62; df =1, 68, 18; P<0.0001$, 20°C: $F=78.14; df =1, 68; P<0.0001$, 25°C: $F=134.61; df =1, 68; P<0.0001$, 30°C: $F=76.93; df =1, 68; P<0.0001$). Food supply resulted in almost three times increase of longevity at all temperatures (Table 2).

Table 2. Longevity of honey-fed and starved adults of *V. canescens* in days (mean ± S.E.) lived at various constant temperatures (65 ± 5% R.H., photoperiod: 16L:8D).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>n</th>
<th>Honey-fed mean ± S.E.</th>
<th>range value</th>
<th>Starved mean ± S.E.</th>
<th>range value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>35</td>
<td>42.80 ± 1.54 aF A</td>
<td>21-55</td>
<td>14.00 ± 0.67 bA</td>
<td>9-21</td>
</tr>
<tr>
<td>20</td>
<td>35</td>
<td>15.60 ± 1.10 aB</td>
<td>5-30</td>
<td>5.26 ± 0.39 bB</td>
<td>2-9</td>
</tr>
<tr>
<td>25</td>
<td>35</td>
<td>7.37 ± 0.38 aC</td>
<td>5-13</td>
<td>2.19 ± 0.23 bC</td>
<td>1-6</td>
</tr>
<tr>
<td>30</td>
<td>35</td>
<td>4.19 ± 0.31 aC</td>
<td>0.5-8</td>
<td>1.29 ± 0.11 bC</td>
<td>0.3-2.3</td>
</tr>
</tbody>
</table>

n: number of experimental adults

α: means in a column followed by the same capital letter are not significantly different; means in a row followed by the same small letter are not significantly different (Tukey-Kramer HSD Test, α: 0.05)

F: differences proved to be significant but were minor and biologically meaningless

Discussion

Wasps with access to honey produced significantly more progeny than starved ones. Similar conclusions have been made not only for *V. canescens* (Harvey et al. 2001) but for many other synovigenic parasitoids (Wäckers, 1996; Wäckers et al., 1998; Schmale et al., 2001). Beling (1932) was the first to suspect the importance of adult feeding on *V. canescens* when she observed newly-emerged wasps leaving and then returning later on to the host habitat with nectar droplets in their mouthparts.

During the present study honey supply resulted in significantly increased longevity of *V. canescens* adults in all cases. Similar observations have been made in earlier studies (Beling, 1932; Ahmad, 1936; Matsumoto, 1974). Starved adults lived 3.5 days at 23°C while fed adults
lived as long as 40 days (Ahmad, 1936). Furthermore, Beling (1932), recorded significant effects of food type on longevity, with adults fed on sugar solution living up to 57 days and honey-fed wasps living up to 72 days.

Adult feeding (on sugar or hosts) can have strong effects on parasitoid fitness parameters such as longevity, lifetime fecundity, survival, searching efficiency, overall activity and other related parameters (Godfray, 1994; Jervis et al., 1996; Jervis and Kidd, 1999). This has been verified not only for *V. canescens* (Ahmad 1936; Harvey et al., 2001; Eliopoulos, 2003), but also for many other parasitoids of stored product pests, such as *Uscana lariophaga* Steffan (Trichogrammatidae)(van Huis et al., 1990), *Anisopteromalus calandrae* Howard (Wäckers, 1996; Wäckers et al., 1998; Schmale et al., 2001), *Dinarmus basalis* Rondani (Schmale et al., 2001) (Pteromalidae), *Heterospilus prosopidis* Viereck (Wäckers et al., 1998; Schmale et al., 2001) and *Habrobracon hebetor* Say (Braconidae) (Nickle and Hagstrum, 1981).

These conclusions, in combination with the fact that a lack of suitable food has been regarded as an important factor for poor performance of many biocontrol agents (Wolcott, 1942) may justify the use of food supplements in suitable release sites to augment the efficacy of released parasitoids and other natural enemies (Schöller et al., 1997; Schöller, 1998; Wäckers et al., 1998). However, not only laboratory but mainly field studies are needed to investigate methods for food supply. Such food supplements promise substantial improvement in biological control efficacy in storage ecosystems.

References


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Amylase inhibitor is without any adverse effect on parasitoid *Venturia canescens*

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**Abstract:** The amylase inhibitors (AI) are suitable candidates for transgenic plants to increase their resistance to insect pests. Detail study of suppressive effects of AI on target and non-target insects is necessary before their incorporation into GMO plants. Therefore, we tested the effect of selected AI inhibitor (acarbose) on pest flour moth (*Ephestia kuehniella*) and its parasitoid wasp (*Venturia canescens*) in laboratory experiments. Various concentrations of AI were incorporated into diet for larvae of *E. kuehniella* and their mortality was observed to find sublethal doses of AI. The larvae influenced by sublethal concentrations of AI (0.001 and 0.0001%) were parasitized by the wasp. The morphological parameters of the wasps treated by AI and control were compared. The AI in the range from 0.1 to 0.01% caused 100% mortality of *Ephestia kuehniella* larvae, and the concentration AI 0.001% suppressed larval weight increase and prolonged developmental period. We did not observe any suppressive effect of AI on *Venturia canescens*; but we found differences in morphological parameters between wasps treated on (i) control larvae and on larvae fed on 0.001% AI and (ii) 0.0001% AI. Measured parameters of the wasp influenced by AI 0.0001% were in most cases significantly higher (i.e. weight, hind tibia length and wing size). These results demonstrate the possibility of the combination of biocontrol and AI that would provide higher pest control efficiency than these methods used individually.

This work was supported by the grants GACR 522/04/1286, COST-1P04OC842.20 and MZE-000-2700063.

**Keywords:** amylase inhibitors, resistance to insect, acarbose, *Ephestia kuehniella, Venturia canescens*
Age specific fecundity and survivorship of *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) at different temperatures

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**Abstract:** The effect of temperature on fecundity and survivorship of *Cephalonomia tarsalis* (Fabr.) was studied at 5 constant temperatures (21°C, 24°C, 27°C, 30°C and 33°C) in temperature-controlled chambers. The adult longevity was temperature and sex dependent. Adults lived longer at lower temperatures and females lived longer than males. Mean longevity of males was 4-8 days over temperature range of 21°C-33°C, females mean longevity increased from 43 days at 30°C to 82 days at 21°C. Egg production was non-linear, temperature dependent. Highest mean fecundity of 110 eggs per female was recorded at 27°C, lowest mean fecundity of 32 eggs per female was found at 21°C. The rate of egg laying was highest at 30°C and lowest at 21°C.

**Key words:** *Cephalonomia tarsalis*, temperature, fecundity, survivorship

**Introduction**

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae), is a cosmopolitan stored-product secondary pest whose adults and larvae cause damage. This pest is considered as a key pest of stored and processed grain in the Czech Republic. Fumigants and contact biocides are recommended and broadly used in control programs against this pest. Because of a ban of methyl bromide, cases of pesticide resistance, the risk of toxicity against humans and the risk of insecticide residues, there is a run for alternative control means. One of the possible alternatives is the use of natural enemies. *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) is both a predator and an ectoparasitoid of larvae and pupae of sawtoothed grain beetle. This parasitoid is naturally present in stored products in the Czech Republic (Lukáš, 2002). The basic biology of the parasitoid was described by Powell (1938). Some aspects of behavioral traits of this parasitoid were studied by Howard at al. (1998) and Cheng at al. (2004a, 2004b). Lord (2001) conducted experiments on the compatibility of this parasitoid with an entomopathogenic fungus (*Beauveria bassiana*) and Lukáš & Šambergerová (2005) evaluated the sensitivity of this parasitoid to deltamethrin. *C. tarsalis* is studied in our laboratory with the aim to assess its suitability for biological control program with regard to temperature requirements. Recently, Lukáš & Stejskal (2005) determined lower developmental thresholds (LDT) and the sum of effective temperatures (SETk) for eggs, larvae, pupae and overall development of this parasitoid. Lukáš (2005) described its temperature dependent functional response. Nevertheless, little is known about the effect of temperature on other life history traits of *C. tarsalis*. The aim of this study was to describe an influence of temperature and age of *Cephalonomia tarsalis* on its fecundity and survivorship.

**Material and methods**

Cultures of *Cephalonomia tarsalis* and *Oryzaephilus surinamensis* originated from stored wheat samples obtained from a warehouse close to Prague in 2002. *O. surinamensis* was
reared on rolled oats and *C. tarsalis* was reared on fourth instar larvae of *O. surinamensis* in wheat. Both cultures were maintained in climatic chambers at a constant temperature of 30°C, a relative humidity of 75-80% and a photoperiod of 16:8 (L:D).

Age-specific fecundity of mated females of *C. tarsalis* and survivorship of both males and mated females of *C. tarsalis* was studied at different constant temperatures of 21°C, 24°C, 27°C, 30°C and 33°C in temperature controlled chambers. Newly emerged males and females of *C. tarsalis* were placed in a plastic jar containing 10 g of wheat and provided with 10 fourth-instar larvae of *O. surinamensis*. At each temperature, twelve replicates were made with one male and one female of parasitoid adult per replicate. The pre-oviposition period, number of laid eggs and survivorship of males and females of *C. tarsalis* was recorded daily until the parasitoid had died. Hosts were renewed (replacement of parasitized hosts) daily until the female died.

The analysis of the influence of temperature and age on the pre-oviposition period, number of laid eggs and oviposition period of *C. tarsalis* was done by means of GLM logit analysis using the R freeware statistical package (http://cran.at.r-project.org) assuming quasipoisson distributed residuals to adjust over dispersion and asymmetries in the distributions. Survival analysis was used to analyze the mortality in dependence of age and temperature. The data were fitted to a maximal model and then less significant variables were progressively withdrawn from the model until obtaining a minimal appropriate model (i.e. a simplified model in which all terms are significant) (Crawley, 2002). Examination of the residuals confirmed the fit of the models to the data.

**Results**

The number of eggs laid on individual host varied between 1 and 4, but 1-2 laid eggs prevailed. The pre-oviposition period was nonlinearly temperature dependent: days of pre-oviposition $\sim$ temperature + temperature$^2$, intercept = 17.877±2.644 (1s.e., p<0.05), temperature = -1.241±0.203 (1s.e., p<0.05), temperature$^2$ = 0.022±0.004 (1s.e., p<0.05)) (Fig. 1). Females that oviposited first did so at 30°C between 1-2 days. The latest start of oviposition was witnessed in females kept at 21°C at an age of 2-8 days. An increase in the pre-oviposition period of 1-6 days was recorded at 33°C. The risk of death increased with age. The adult longevity was temperature and sex dependent. Adults lived longer at lower temperature and females lived longer than males. The overall mean longevity of males was 4-8 days at a temperature range between 21°C and 33°C (Fig. 2). At 21°C males lived up to 17 days. Mean longevity of females varied between 43 days at 30°C and 82 days at 21°C (Fig. 3). An individual female lived more than 90 days at 21°C. The oviposition period was negatively linearly temperature dependent: days of oviposition $\sim$ temperature, intercept = 5.098±0.403 (1s.e., p<0.05), temperature = -0.051±0.016 (1s.e., p<0.05)) (Fig. 4). Females oviposited 54 days on average at 21°C but 33 days at 30°C. Egg production was nonlinearly temperature dependent (glm(quasipoisson): days of oviposition $\sim$ temperature + temperature$^2$, intercept = -16.945±4.557 (1s.e., p<0.05), temperature = 1.567±0.353 (1s.e., p<0.05), temperature$^2$ = -0.028±0.007 (1s.e., p<0.05)) (Fig. 5). Up to 198 eggs were laid by an individual female during its lifetime at 27°C. A maximal mean fecundity of 110 eggs per female was recorded also at 27°C but only 32 eggs per female (mean) was laid at 21°C. Fig. 6 shows the mean age specific fecundity at 21°C, 24°C, 27°C and 30°C. Table 1 summarizes parameter estimates of this model. The rate of egg laying was highest at 30°C and lowest at 21°C. The model predicts no egg laying under a temperature of 19°C, continuing egg laying in the range of 21°C-27°C by *C. tarsalis* females older then 45 days and the end of egg laying at an age of 35days at 33°C.
Fig. 1. Observed number of days prior to start of oviposition of *Cephalonomia tarsalis* at different constant temperatures (21°C, 24°C, 27°C, 30°C and 33°C; n=12 for each treatment).

Fig. 2. Product-limit estimates of survival function for males of *Cephalonomia tarsalis* at different constant temperatures (21°C, 24°C, 27°C and 30°C; n=12 for each treatment).
Fig. 3. Product-limit estimates of survival function for females of *Cephalonomia tarsalis* at different constant temperatures (21°C, 24°C, 27°C and 30°C; n=12 for each treatment). Data of 21°C, 24°C and 27°C are right censored.

Fig. 4. Observed duration of oviposition (days) of *Cephalonomia tarsalis* at different constant temperatures (21°C, 24°C, 27°C, 30°C; n=12 for each treatment).
Fig. 5. Observed total number of laid eggs of *Cephalonomia tarsalis* at different constant temperatures (21°C, 24°C, 27°C, 30°C; n=12 for each treatment).

Fig. 6. Mean age (A) specific fecundity (E) of *Cephalonomia tarsalis* at different constant temperatures (T=21°C, 24°C, 27°C, 30°C; n=12 for each treatment); glm (E~A*T+A^2+T^2), r^2=0.68).
Table 1. Parameter estimates for generalized linear model of mean age specific fecundity of *Cephalonomia tarsalis* at different constant temperatures.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-3.014e+01</td>
<td>3.622e+00</td>
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</tr>
<tr>
<td>age</td>
<td>3.541e-01</td>
<td>3.214e-02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>temperature</td>
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<td>2.842e-01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>age^2</td>
<td>-2.622e-03</td>
<td>3.167e-04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>temperature^2</td>
<td>-3.299e-02</td>
<td>5.543e-03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>age:temperature</td>
<td>-1.052e-02</td>
<td>1.120e-03</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Discussion**

Temperature affects population trends of poikilotherms through its effect on developmental times, fecundity and survival (Hagstrum & Milliken, 1988). These parameters are essential parts of models simulating host-parasitoid dynamics (Flinn & Hagstrum, 1995; Throne at al., 1998). The use of such models is of great importance when introducing biological control programs in practice. It allows right timing of parasitoids with respect to status of host population and environmental conditions. The results show that temperature strongly influenced survivorship and fecundity of *C. tarsalis*. The optimal temperature range for reproduction is between 24 and 30°C. Preliminary results (not shown) from further experiments at more extreme temperatures suggest no reproduction at 18°C and very limited reproduction at 36°C. These findings are in accordance with lower and higher developmental thresholds (LDT and HDT) for development of *C. tarsalis*. LDT for eggs of *C. tarsalis* is 18.6°C (Lukáš & Stejskal, 2005) and HDT for overall development of *C. tarsalis* is slightly above 36°C (not published data). Nevertheless, even outside of these temperature boundaries *C. tarsalis* paralyze and host-feed on larvae of *O. surinamensis* (not published data). Flinn (1991) found highest age specific fecundity of *Cephalonomia waterstoni* at 35°C, no fecundity at 40°C and very low fecundity at 21°C. *C. tarsalis* seems to be about 5°C more cold tolerant and less adapted to high temperatures than *C. waterstoni*. Moreover, females of *C. tarsalis* survived longer than females of *C. waterstoni* at similar constant temperatures. Results of this study suggest that this parasitoid could be effective against populations of *O. surinamensis*.

**Acknowledgements**

This work was supported by grant GACR No. 522/04/P169.

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Control of *Sitophilus granarius* in grain using a combination of parasitoids and entomopathogenic fungi – preliminary results

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**Abstract:** Several studies have investigated the potential of larval parasitoids for control of *Sitophilus granarius* (L.) (Coleoptera: Cucurionidae) in stored grain. However, as the longevity of the adult weevils, and thus the oviposition period, is very long, efficient control of *S. granarius* necessitates a strategy that will target both the adult weevils and the larvae. The presentation will report on preliminary results of a “semi-field” study involving a combination of larval parasitoids and entomopathogenic fungi against *S. granarius*.

A wide range of isolates of entomopathogenic fungi have been screened for their impact on *S. granarius* imagines. An isolate of *Beauveria bassiana* has been selected for further studies. Two species of larval parasitoids have been included in the study: *Lariophagus distinguendus* Förster and *Anisopteromalus calandrae* (Howard) (both Hymenoptera: Pteromalidae). A trial was set up to simulate an infestation in a grain store using plastic containers each containing 9 kg of wheat infested with *S. granarius*. The trial involved the following: i) addition of *L. distinguendus*, ii) addition of *A. calandrae*, iii) addition of *B. bassiana*, and combinations of the natural enemies. The trial ran for a total of 24 weeks.

**Key words:** Grain stores, *Sitophilus granarius*, biological control, *Lariophagus distinguendus*, *Anisopteromalus calandrae*, *Beauveria bassiana*

**Introduction**

The granary weevil *Sitophilus granarius* is a widespread pest in grain stores in temperate regions. Biological control of this pest is estimated to have good chances of success in grain stores due to the protected environment and the long storage period. Cox and Wilkin (1998) found that given a choice, the consumer would prefer food to be free from pesticide residues providing that this did not lead to lower standards of food hygiene or the presence of insects in food. A biological control programme for grain would need to live up to these requirements.

Several biological control agents are relevant for biological control of *S. granarius*. Larval parasitoids have been investigated for this purpose, e.g. *Lariophagus distinguendus* (Reppchen et al. 2003; Steidle & Schöller 2002; Schöller 2002) and *Anisopteromalus calandrae*, this species most often on other *Sitophilus* species (Press 1992; Lucas & Riudavets 2002). As the oviposition period of *S. granarius* extends over many months, new pest individuals will emerge over a long period even if the larvae are controlled. A combination of the parasitoids with entomopathogenic fungi to control the adult weevils was suggested as a strategy to solve this. Entomopathogenic fungi, notably *Beauveria bassiana*, have recently been studied for use against a range of stored product pests (Moore et al., 2000; Lord, 2001) and may be applicable against granary weevils.
The present paper reports on a study in which combinations of these three biocontrol agents were tested in a set-up that simulated a grain store, i.e. plastic containers with 9 kg of grain. The investigation ran for 24 weeks; the results obtained after the first 16 weeks are presented below.

**Material and methods**

The investigations were carried out in plastic containers (11.5 l) each with 9 kg of wheat grain. Adults of *S. granarius* (2 specimens per kg) were added to each container. The following combinations of natural enemies were used: *L. distinguendus* alone, *A. calandrae* alone (both: 1.5 parasitoids per kg), *B. bassiana* alone (2 x 10^6 spores per g grain), and each of the parasitoids in combination with the fungus. The fungal spores were distributed as a surface treatment. The containers were placed at 20°C, 70% r.h., with a slight airflow through each container. The densities of live and dead insects were investigated after 7, 16, 20 and 24 weeks, 3 replicates each time.

**Results and discussion**

*Effect of Beauveria bassiana on initial weevils.*

After 7 weeks the mortality was recorded for weevils in untreated containers and fungus-treated containers to check whether the fungus had any effect at the chosen inoculum concentration and at the abiotic conditions presented above. The control mortality was 6% while 64% of weevils in fungus-treated containers had died.

*Population development after 16 weeks*

The population development at the first examination is shown in Table 1. The pest density in the untreated controls had increased more than 50-fold. The lowest density was found in the containers with *L. distinguendus* alone, whereas the densities in the other containers were intermediate.

The examinations that are planned at 20 and 24 weeks will elucidate the ability of the different natural enemies to control *S. granarius* over a longer period of time. In addition, the investigation will elucidate the persistence of *B. bassiana* and the effect of the fungus on the natural enemies.

Table 1. Mean density of *Sitophilus granarius* per kg grain in combination with different natural enemies after 16 weeks (20°C, 70% r.h.). Initial density: 2 *S. granarius* per kg. *L. dist.*: *Lariophagus distinguendus*. *A. cal*: *Anisopteromalus calandrae*. *B. bass.*: *Beauveria bassiana*. Mean values followed by different letters are significantly different at p ≤ 0.05.

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Acknowledgements

The technical assistance of Lars Damberg, Minna Wernegreen and Bodil M. Pedersen is gratefully acknowledged. This study was supported by the Danish Ministry of Food, Agriculture and Fisheries, Directorate for Food, Fisheries, and Agri Business.

References


Interpretation of pheromone monitoring programs for stored-product insects

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Abstract: Effective integrated pest management programs are needed for food processing and storage facilities and this requires improvements in our ability to monitor pest populations and use this information to target interventions in both time and space. The use of pheromone traps to monitor pests is increasing in the food industry, but adoption has been hampered by lack of information on how to effectively implement and interpret pheromone-monitoring programs. In this presentation, how factors such as landscape structure, and pest behavior and ecology, impact pheromone trapping and the targeting of pest management will be discussed. Critical factors include determining temporal dynamics of pest populations, spatial distribution of subpopulations in the landscape, and pest movement patterns. How these factors can be assessed in the field will be discussed and their impact illustrated using data collected in and around food processing facilities.

Key words: pheromones, monitoring, behavior, ecology, integrated pest management

Introduction

The foundation of a successful integrated pest management (IPM) program is an effective monitoring system that supplies information on not only the number and type of pests present, but also detects changes in pest populations over time and locates foci of infestation and routes of entry (Burkholder, 1990). With this information, pest management decisions can be made based on monitoring data; rather than calendar based applications that may or may not be optimally temporally and spatially targeted. Many stored-product insect pheromones have been identified, a range of traps and lures are commercially available for stored-product insects, and use of pheromone monitoring is increasing in commercial facilities (Phillips et al., 2000). However, many questions remain about how to best implement and interpret these monitoring tools; from issues such as how many traps are needed and which types work best, to fundamental issues concerning the relationship between pheromone trap captures and actual pest population density, distribution, and level of product infestation (Arbogast and Mankin, 1999). To accurately interpret pheromone monitoring programs, we need to better understand stored-product insect population structure and dynamics in the spatially and temporally patchy landscapes where food is processed and stored.

Landscape and stored-product insect population structure

The importance of landscape structure and how organisms interact with spatial and temporal landscape heterogeneity has come to the forefront of ecology. This perspective is making inroads into pest management as well. If, as it has been argued, the foundation of an effective IPM program is an understanding of pest ecology, then this understanding needs to be
developed within the appropriate landscape structure and at the appropriate scale for that pest. This perspective may be especially important for post harvest IPM, where the focus on sanitation, structural modification, and targeted pest control tactics can be considered in ecological terms as manipulating the landscape structure to make it less favorable for pest population establishment, growth, and persistence.

Environments created or modified by humans tend to be highly fragmented landscapes consisting of a mosaic of resource patches that are separated from each other by barriers to movement or by a matrix of less hospitable habitat (Wiens, 1976). Landscape structure and dynamics in turn influence processes such as population dynamics, spatial distribution, and movement patterns of the organisms living in the landscape (Turner, 1989; Wiens, 1976; Wiens et al., 1993). Populations can be made up of interconnected subpopulations (e.g., metapopulations) occupying different resource patches, and the degree of movement among patches is what defines the type of population structure and the scale at which individuals can be considered to be from the same population (Harrison and Taylor, 1997). When there is considerable movement among patches and population trends within patches are correlated with each other, this is considered just a single patchily distributed population; but, as the level of recruitment from within a patch increases relative to immigration from other patches, the population becomes more of a true metapopulation. A range of intermediate scenarios may be especially relevant to stored-product insects, particularly source-sink metapopulations where populations in higher quality (source) patches persist and produce individuals that immigrate into lower quality (sink) patches and enable subpopulations to persist in these less favorable locations.

It is important to consider the spatial scale over which pest subpopulations are distributed and the degree of movement among patches, because this determines the proportion of individuals exposed to treatment, the potential for recolonization, and the probability of being captured in a pheromone trap. It can be argued that stored-product pests of the grain and food industries are pests in large part due to their effectiveness at finding and exploiting the temporally and spatially fragmented landscapes within food facilities and within which food facilities are located. Targeting pest management to patches that are occupied by insects can increase the probability of suppressing the pest population, while reducing the cost of management and risk of negative non-target consequences (Brenner et al., 1998). Spatial scale of subpopulation distribution also has implications for using pheromone-monitoring programs to evaluate treatment efficacy. For example, when pest subpopulations are interacting over spatial scales larger than an individual structure, rapid pest resurgence after treatments are applied to that structure could occur due to recolonization from other source patches. Depending on the spatial scale over which pest subpopulations are distributed, the sources of recolonization may be patches within the structure that were not controlled; patches onsite, either inside or outside the structure, that were not treated; or patches from offsite, perhaps due to a broader area-wide population structure (Campbell and Arthur, in press).

**Use of pheromone monitoring to evaluate population structure**

Multiple studies have used pheromone monitoring to show that stored-product insects have spatially and temporally patchy distributions inside structures (Arbogast et al., 2000; Arbogast et al., 1998; Campbell et al., 2002; Hansen et al., 2004), and even around the outside of structures (Campbell and Muller, 2004). Although studies measuring stored-product insect dispersal ability are limited, they indicate that many stored-product insects are highly mobile and capable of moving among food patches (Campbell and Arbogast, 2004; Campbell et al., 2002; Chesnut, 1972; Fadamiro, 1997; Hagstrum and Davis, 1980). Stored-product pests are often trapped outside grain storage and processing structures and sometimes far away from
these structures (see references in Campbell et al. (2004) and Campbell (2006)). These data suggests the capability for long distance flight, but may also indicate localized movement of feral populations. Studies have also suggested that insect immigration into facilities can be important (Campbell and Arbogast, 2004; Campbell and Mullen, 2004; Hagstrum, 2001; Toews et al., 2006).

In some cases, broad spatial scale or area-wide processes may be important influences on pest population dynamics within food storage and processing structures, but our understanding of the ecology and behavior of stored-product insects at these spatial scales is still limited. Exploitation of resource patches outside of grain storage systems (e.g., woody plant parts) and capture in forested areas suggests that area-wide processes may have an important influence on the spatial distribution and population dynamics of the larger grain borer, *Prostephanus truncatus* (Horn), in stored grain (Hill et al., 2002). In a recent study in Kansas, USA, the spatial and temporal patterns of pheromone trap capture in an agricultural landscape suggested that *Rhyzopertha dominica* F. may over winter in wooded areas and serve as an important source of infestation of new harvested and stored wheat in the spring (Ching’oma, 2006). Outdoor pheromone monitoring in different habitats around the city of Manhattan, Kansas USA, indicated that different species had different temporal patterns of capture in different habitats (Campbell et al., unpublished data). For example, *Plodia interpunctella* (Hübner) was captured outside in higher numbers and earlier in the year in residential areas than near grain storage and processing locations or in agricultural landscapes surrounding the city. These findings suggest that broader landscape processes may be more important then generally recognized in pest management, but certainly this is an area that needs additional research.

**Using population structure to help interpret pheromone monitoring programs**

In evaluating treatment efficacy in food facilities, the spatial scale over which pest subpopulations interact determines the proportion of individuals exposed to treatment and the potential for recolonization. When pest subpopulations are interacting over spatial scales larger than the area being treated, rapid pest resurgence can occur and pheromone trapping may not accurately indicate impact of treatment. Data from a wheat flour mill can be used to illustrate these points (Campbell and Arbogast, 2004). Even within a facility, using pheromone traps to evaluate the impact of a treatment on the pest population density can be challenging because pheromone traps are capturing individuals dispersing between resource patches and therefore may not accurately reflect the true impact pest population. Data from studies conducted in experimental sheds can be used to illustrate the potential interpretation problems (Toews et al., 2005).

Seasonal trends in stored-product insect trap capture, relationships between trap captures inside and outside and between pheromone trap capture and product infestation, and impact of fumigation on pest populations at a flour mill were assessed (Campbell and Arbogast, 2004). The findings from this study suggest that pest populations can fall into one of two general patterns in terms of their spatial distribution and movement patterns. In the first pattern, source patches for the insects lay over a spatial scale greater than the mill itself and there was movement of insects across this larger spatial scale linking activity patterns inside and outside the mill. At this location, this pattern is applied to *P. interpunctella*, among other species. A similar pattern of activity has been observed at other locations in the same region (Doud and Phillips, 2000; Campbell and Mullen, 2004), but, in other locations, this pattern may apply to other species and may or may not apply to *P. interpunctella*. As a consequence of this pattern, both indoor and outdoor trap captures tended to cycle according to seasonal environmental changes; declining in the fall even in the absence of treatment.
Fumigation treatments did not appear to impact trap captures of these species, probably because of high rates of immigration. Pheromone trap captures within the mill for these species indicated the potential for infestation and the capacity for insects to immigrate, but did not accurately reflect the current level of infestation within the mill.

The second observed pattern suggests source patches for the insects lay over a spatial scale contained primarily within the mill itself with pheromone traps capturing primarily insects moving among these internal patches. This pattern was seen with the major pest of this mill, the red flour beetle (*Tribolium castaneum* (Herbst)). Trap captures tended to be lower outside compared to inside and followed a pattern of sharp decline after fumigation treatment and then steady increase in numbers until the next fumigation. This rebound, other than potentially the rate of increase, was not impacted by season and outside trap capture levels.

Red flour beetle was the primary species recovered in product samples and pheromone trap captures were correlated with numbers in the product samples. Rebound after fumigation may result from persistence of individuals within some of the patches within the mill and, probably to a lesser extent, movement of new individuals into the mill either actively or in infested products. This pattern is likely to be important for other species in other locations; especially for species with limited mobility such as sawtoothed grain beetle. It is also more likely to be important in buildings that are tightly sealed with limited immigration from outside sources.

Even at the finer spatial scales that occur within storage structures, pheromone traps may not accurately reflect the true population size or the efficacy of different management tactics because infestations occur in hidden areas and traps are capturing individuals moving among these hidden patches. Because it is typically not possible to assess the true population size in commercial facilities, studies were conducted in pilot-scale warehouses where the locations and numbers of resource patches could be controlled, true population levels could be directly measured, immigration and emigration could be managed, and environments replicated (Toews et al., 2005). Food patches under shelves were infested with known quantities of mixed life stages of *T. castaneum*. Insects in food patches, number of dead adults on the floor, and insect captures in traps were recorded weekly. There were always more dead insects and fewer beetles captured in pheromone traps in warehouses treated with pyrethroids than in untreated warehouses or warehouses treated with insect growth regulators (Toews et al., 2005). However, direct sampling of pest populations in food patches generally showed no overall differences in the number of larvae, pupae, or adults regardless of treatment. Thus, at least under this set of conditions, pheromone traps were not accurately reflecting the true pest population in the pilot-scale warehouse and thus did not show the true impact of the insecticide treatment.

**Conclusions**

Pheromone monitoring is a powerful tool for pest management programs in food processing facilities. However, interpretation of pheromone monitoring programs can be challenging due to the spatial structure of food facilities and the insects within these facilities. Understanding pest population structure and spatial scale over which pest populations interact can help with both the selection and targeting of pest management strategies and with the implementation and interpretation of pheromone monitoring programs. There is a clear need for more research addressing these issues because of the increased reliance of IPM programs in the food and grain industries.
References


Mating disruption of stored product moths: toward commercial development

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Abstract: Mating disruption (MD) of the Indianmeal moth was suggested and proven on a small scale in the laboratory more than 30 years ago, but no commercial scale treatments have been demonstrated. We studied mating disruption of *Plodia interpunctella* in a controlled field situation in which ZETA was loaded into high release-rate bags that were distributed throughout the test site to disrupt male orientation and mating. A „switching” experiment was done using two similar chicken houses in which adult moths were released weekly and monitored by trapping with virgin females. Reproduction was monitored with oviposition dishes. MD was implemented in one house while the other remained as an untreated check during the 4-week treatment period. Male responses to females and reproduction were significantly suppressed by MD. Mean male trap catch during MD was 1.6 (±7.2 SE) vs. 44.6 (±5.3 SE) in non MD checks. The mean larval count in oviposition dishes during the four week period before the MD was 181.8 (±26.3 SE), whereas the mean larval count during MD was 82.5 (±35.4 SE). Subsequent experiments to test efficacy in commercial food facilities were conducted in warehouses located around the U.S. Each location had pairs of non-mating disruption (control) and mating disruption (treatment) buildings. Infestations of almond moth, *Cadra cautella*, and Mediterranean flour moth, *Ephestia kuehniella*, were also studied. Most treated locations displayed significant decreases in moth activity after on set of MD, while untreated buildings showed typical seasonal increases. Migration of moths into treatment facilities from other areas may account for lower treatment effects. Testing continues on deployment density of MD lures while commercial partners pursue government registration.

Keywords: mating disruption, *Plodia interpunctella*, trapping, *Cadra cautella*, *Ephestia kuehniella*, commercial scale treatments
The influence of environmental structure on trap efficacy in food industry pests: preliminary study

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Abstract: The effect of structural complexity on the dispersion behaviour of food industry pests was estimated by means of sticky traps. The German cockroach, *Blattella germanica* L., was used as a model of a highly mobile pest species frequently associated with food facilities. The experiments were performed in an isolated chamber (3x2m) with controlled temperature and dark-light regime that contained sticky traps arranged in a grid pattern. Cockroach shelter was located at the edge of the chamber. The increasing complexity of environment was simulated by installation of wooden boards-barriers between trap area and cockroach shelter. The catch of cockroaches in traps was recorded for 24 hrs. It was found that increasing complexity decreased the cockroach catch dramatically. In structurally complex environment, all traps falsely indicated zero presence of cockroach pest thereby generating an alarming message: the results of pest monitoring by traps may be completely misleading under certain conditions. We discuss the possibility of using fractal geometry for the description of an irregular surface and for modelling insect dispersal in a complex, food industry environment.

Key words: insect monitoring, trap efficacy, dispersion behaviour, sticky traps, *Blattella germanica*, food industry

Introduction

Monitoring is claimed to be one of the main pillars of various pest risk management strategies (e.g. IPM and HACCP) (Schal and Hamilton, 1990, Stejskal, 2002a, Stejskal, et al. 2004). Although we have a large variety of monitoring tools with a capacity to generate huge amounts of data, we are still facing problems of interpretation of these monitoring records. It should be stressed that the interpretation of trapping data may differ according to the specific aim of pest monitoring. What are the main purposes of monitoring in food industry plants and commodity stores? Within the framework of IPM, monitoring is mainly used for three purposes: (i) detection of pests (presence/absence), (ii) estimation of pest population density, and (iii) location of pest by spatial analysis; the latter may include “Pierce’s triangular method” (Pierce, 1994, Mueller, 1998) and contour mapping (Brenner et. al., 1998, Arbogast, et al. 2004, Trematerra, et al. 2004). Traditionally, pest control scientists and technicians claim that in most cases, simple analysis of numbers captured provides adequate information to estimate pest presence/absence, population density and to precisely target the infestation foci. Thus the above mentioned “traditional monitoring idea” may be formally expressed as an unsubstantiated belief that the pest dispersal is uniform in all directions from the infestation focus and the catch gradually decreases with a dispersal distance. As a result, the traps with the highest load of pests indicate proximity of the foci of infestation while empty traps cannot be expected nearby the focus of infestation. However, these assumptions may be frequently violated in food industry facilities where the irregularity of the environment is enormous and reveals high spatio-temporal variation (Stejskal, 2002b, Campbell et al., 2002, Campbell and Arbogast, 2004, Trematerra et al. 2004).
Therefore, the aim of this preliminary study was to present an example of influence of increasing irregularity/complexity of structure of environment on the trap catch of the German cockroach as a model species.

**Material and methods**

Experiments were conducted in a climatized chamber of 2 x 3 m (D:L 12:12, 26°C) in 3 replicates per each experimental design. The walls of the chamber were treated with grease to prevent insect escape. Fifty male individuals of the German cockroach (*Blattella germanica* L.) were inserted into a closed plastic box-shelter, containing a surplus of water + food for 24 hours. After 24 hours the entrance to the shelter box was opened. The effect of structural complexity on the dispersion behavior of food industry pests was estimated by means of the Lo-Line *Cockroach* trap (DDD-Servis, Prague CZ) (Stejskal, 1998). Traps were exposed for 24 hours. We used two experimental designs: **Design “A”** – sticky traps were arranged in a regular grid pattern with cockroach shelter located at the edge of the chamber (Fig. 1); **Design “B”** – sticky traps were arranged in a regular grid pattern while cockroach shelter was located at the edge arena and separated by 2 erected wooden board- barriers (Fig. 2).

**Results and discussion**

**Fitting design “A” with the „traditional monitoring idea”.** Fig. 1. shows that in an environment without irregularities (simulated by the design B ) trapping fulfilled all expectations of the “traditional monitoring idea”: traps positively indicated presence of pests; the catch declined with a distance from shelter thereby (via gradient) indicating position of „pest focus“; traps indicated population density since the pooled catch from all traps almost reached 100% of the insects released.

**Fitting design “B” with the „traditional monitoring idea“.** Fig 2 shows that in an environment with irregularities (simulated by design B) trapping results contradicted all expectations of the “traditional monitoring idea”: traps falsely indicated pest absence; the catch spatial arrangement did not indicate the proper position of „pest focus“; traps falsely indicated „zero“ population density.

The results of this preliminary work showed the influence of increasing complexity on cockroach catch. The traps provided sufficient and reliable information on the level and location of pest population in a structurally simple and homogenous environment. However, an absolutely opposite statement is true for structurally complex environment. When we added structural complexity (i.e. erected wooden boards) between pest shelter and traps, the traps falsely indicated zero pest presence although the German cockroach was traditionally believed a highly mobile pest. It was notable that we found “0” presence even in those traps located in the shortest (i.e. less than 50cm) distance from the pest shelter taken here as a focus of infestation. Therefore, this preliminary study brings an alarming message: the results of monitoring by traps may be completely misleading under certain conditions. Feeding such data into geographical contour analysis would be a typical example of “garbage in – garbage out” approach of using statistical/ mathematical modeling. The problem of poor sensitivity of sticky traps to detect low populations of the German cockroach was recently reported by Willimas et al (2005) as a result of large scale pest control study in US urban environment. The authors stated that “...few cockroaches were trapped in the schools throughout this study (only 23 of 354 traps that were deployed for 12-mo captured cockroaches), and the trapping data suggest only spotty and unpredictable infestations.”
Theoretically, in a non-homogenous environment we can expect lower catch in the proximate than the distant traps, provided that the distant traps are more easily accessible for walking insects than the proximate traps. Thus, interpretation of pest trapping in food industry facilities probably cannot be solely based on the critical assumptions coined by the „traditional monitoring idea“ due to a strong effect of environmental irregularities on insect dispersal. Consequently, the question is emerging how to measure the irregularities of the environment and include them into pest dispersal models. One way to describe the irregular shapes is fractal geometry. Fractals are defined as irregular objects revealing self similarity - most objects are only statistically self similar. The crucial idea on fractal geometry was developed by famous French mathematician Benoit Mandelbrot in the late 1960s and early 1970s; he proved the fact that nested shapes can be identified in a great many natural systems and in several branches of mathematics. In fractal environment absolute distance depends on the scale that is used for measurement. In the case of pest dispersal, perception of distance depends on the size („scale“) of the animal (Weis and Murphy 1988). Fig. 3 shows how increase of structural complexity (measured as fractal dimension of the environment) nonlinearly increases the travel distance for various developmental stages of the German cockroach. Fig. 3 clearly indicates that size-related perception of distance should be implemented into pest dispersal models for various food industry environments.

Fig. 1. Spatial distribution of catch of German cockroach in a simple environment without any barriers. Number in the circle means average No. (3 replicates) of cockroaches (N per replicate = 50) trapped per Lo line trap after 24 h. exposure.

It also should be stressed that not only the size of animal and physical structure of environment but also other factors may influence pest dispersal, thereby affecting trap efficacy (e.g. presence of highly attractive shelter lured with arrestants or attractants from conspecifics, repellent surface, etc.). Previously we demonstrated profound effects of presence of
food on dispersal behavior and trap catch of *Tribolium* sp. (Stejskal, 1995). The presence of shelter or food decreased the trap efficacy by 80% even in a small arena.

Fig. 2. Spatial distribution of catch of German cockroach in structurally complex environment with two board-barriers. Number in the circle means average No. (3 replicates) of cockroaches (N per replicate = 50) trapped per Lo line trap after 24 h. exposure. Traps indicate zero catch.

Fig. 3. Relationship between structural complexity of environment (measured as fractal dimension of the environment ranging from 1 /=flat surface/ and 1,7 /=very rough surface/) and the distance to be traveled by various developmental stages of the German cockroach (1-nymph stage 1, 2- nymph stage 3, 3- nymph stage 5 4 - adult).
The obtained results indicate the enormous complexity of pest monitoring in food industry and that there is a lot of work in front of us to be able to correctly interpret trapping data in a structurally complex environment.

Acknowledgment

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References


First evaluation of the efficiency of a pheromone trap as a monitoring tool

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Abstract: The foundation of a successful integrated pest management (IPM) program is an effective monitoring system that supplies accurate information on the size and changes in pest populations over time. For many important stored-product insects, pheromones have been isolated and traps are now commercially available. However, many questions remain about the use of these traps as a monitoring tool, from the very practical issues such as how many traps are needed and which types work best, to the fundamental issues concerning the relationship between pheromone trap captures and actual pest population density, distribution, and level of product infestation.

In this communication we present for the first time preliminary data evaluation of pheromone trap efficiency under controlled laboratory settings. In order to achieve controlled conditions a 170 x 65 x 45cm olfactometer was designed and built with a vent producing a 0.1-0.3 m/second wind velocity. The traps were attached to the wall below the vent.

Two insect species were tested: the cigarette beetle (Lasioderma serricorne F.) and the tropical warehouse moth (Cadra (Ephestia) cautella (Walker 1863)) using three commercial pheromone traps. For the moth we used "Gachon®" from "Fuji" and "Biostop®" traps and for the cigarette beetle we used "New Serrico®" from "Fuji" traps. The preliminary results indicate that the capturing efficiency of the traps in a mixed population of 100 females and male tropical warehouse moths after 24 h. averaged about 10% and after 72 h. it increased to 30%. In all tests, male and also females were captured in a ratio of 2:1 up to 5:1 males to females. For the cigarette beetle the capturing efficiency was about 25% on average after 72 h. Different levels of capturing efficiency were obtained when the position of the cigarette beetle traps was changed. The capturing efficiency increased to about 40% when the traps were located on the floor 30 cm from the vent wall and decreased to about 10% when the traps were hanging in the air 30 cm from vent.

These preliminary results indicate that additional studies are needed to evaluate the efficiency of available commercially traps in order to use them effectively as a monitoring tool in for the reliable indication of size and changes of a pest population over time.

Key words: monitoring, pheromone trap, IPM, storage pest control
Pheromones and kairomones for detection and control of indoor pyralid moths

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Abstract: Three pyralid moths, the Mediterranean flour moth (Ephestia kuehniella), the almond moth (Ephestia cautella) and the Indian meal moth (Plodia interpunctella), infest food products all over the world and cause severe problems in factories, shops and households. For health and environmental reasons chemical control becomes more and more restricted. We here present some promising results offering efficient detection and control of these species based on semiochemicals. The pheromone mating disruption technique (MD) was employed in three mills, a chocolate factory, a pet shop and in a pet food warehouse during 7-21 months, including localities with infestations of all three species. Catches of monitoring traps decreased instantaneously and measured air concentrations of the main pheromone component, released from the MD-dispensers, increased immediately. Fewer moths were observed in the localities and the number of complaints from customers of mill products decreased. In the chocolate factory, it was possible to use traps baited with water to obtain an additional and independent measurement of the E. cautella population level. Catches in these traps showed a decrease in population density. From the MD-experiments we can conclude that this technique has a large potential for controlling all three moth species. For more efficient monitoring and evaluation of control measures, we have done a series of studies to improve pheromone traps and to make trapping of E. kuehniella and P. interpunctella females possible (E. cautella females are readily caught by water traps). Improved pheromone baits for E. cautella and P. interpunctella and blends potentially attracting females will be presented.

Key words: Ephestia kuehniella, Ephestia cautella, Plodia interpunctella, monitoring, mating disruption, semiochemicals

Introduction

About 150 insect species have adapted to feed on stored food products or other dried organic material and many of them cause problems worldwide (Plarre & Vanderwel, 1999; Rees, 2004). Most species belong to the order Coleoptera, but also a number of lepidopteran species can complete their whole life cycle in stored products (Rees, 2004). Three pyralid moths, the Mediterranean flour moth (Ephestia kuehniella), the almond moth (Ephestia cautella) and the Indian meal moth (Plodia interpunctella), infest food products all over the world and cause severe problems in factories, shops and households (Rees, 2004).

All three species have similar pheromones and the main component, (Z,E)-9,12-tetradecadienyl acetate, is used widely in monitoring traps (Table 1). Until recently population suppression has mainly been done by treatment with chemical insecticides, e.g. methyl bromide. For health and environmental reasons such chemical control becomes more and more restricted and the need for alternative methods is urgent. We here present promising
results from published and ongoing studies offering efficient detection and control of these species based on semiochemicals.

Table 1. Published composition of sex pheromone for three pyralids (relative amounts compared to main component). Proportion in parenthesis indicates that no published results confirming the behavioural activity have been found.
Data from http://www-pherolist.slu.se/pherolist.php.

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</table>

**Monitoring**

Pheromone traps are sensitive tools for answering *When? Where?* or *How many?* insects that occur. For practical or economical reasons the same one-component bait is usually used for all three of these pyralid species. This implicates that for at least two of them the lure is suboptimal, and catch efficiency is unsatisfactory for many users. For this reason we have investigated more complete pheromone blends and shown that for at least *P. interpunctella* and *E. cautella* the catches increase when additional compounds are added. By using the four-component blend for *P. interpunctella*, shown in Table 1, the catch in a pet food storage was increased more than four times (Zhu et al., 1999). Similarly, when (Z)-9-tetradecenyl acetate was added to the bait, the catch of *E. cautella* in a chocolate factory was six-fold (unpublished data).

In some situations there are drawbacks with using pheromone traps that only attract males. For instance, when pheromone-based mating disruption (MD) is used, the pheromone monitoring may indicate the degree of male “confusion” by a drop in catch (trap shut-down), but not necessarily an actual population density decrease. When MD is used to control pest insects of growing crops, the measurement of success rate is the reduction in damage. However, for stored product insect pests such measurements are not easily obtained, as there usually is a steady flow of products in factories and storage facilities. Thus, it would be valuable to develop methods catching the target species by other means than sex pheromones. In our studies we exploited the observation that almond moths were attracted to various chocolate products in a factory. Chocolate derived odours could potentially generate stimuli of high signal to noise ratio, if used in mills or pet food storages.

Both the almond moth and the Indian meal moth, males as well as mated females, were readily attracted to three different types of chocolate, and to extracts of them, when tested in a flight tunnel (Olsson et al., 2005). Several compounds elicited electrophysiological response and upwind flight when tested alone. Three substances, phenylacetaldehyde, ethyl vanillin and nonanal, were most active and when combined resulted in 30-60% landings in flight tunnel tests (Fig. 1) (Olsson, 2005; Olsson et al., 2005). In a similar study of the Mediterranean flour moth, phenylacetaldehyde, benzyl alcohol and nonanal stimulated oviposition (Olsson et al., 2006).

Several years ago Chow et al. (1977) showed that buckets containing tap water with detergent caught large numbers of moths, most likely both *E. cautella* and *Plodia inter-
punctella. More recently this was reproduced for E. cautella in a chocolate factory in Sweden with approximately the same number of males and females being caught (Ryne et al., 2002). Whether the males were caught because they were attracted to the water, or because trapped females might have released pheromone, can not be positively concluded. However, in this species, use of water traps may be a powerful tool to obtain pheromone-independent population density estimates.

### Population suppression

The pheromone mating disruption technique (MD) was tested previously for control of the target moth species, but it has not yet become a commercial alternative. We employed MD by releasing (Z,E)-9,12-tetradecadienyl acetate at a rate of about 2 mg/100m³ per day in three mills, a chocolate factory, a pet shop and a pet food warehouse during 7-21 months. AgriSense delta or funnel traps were used to measure degree of male confusion. Traps were baited with either the commercial one-component lure (EP 100, AgriSense, UK) or a two-component lure ((Z,E)-9,12-tetradecadienyl acetate and (Z,E)-9,12-tetradecadienol (1:0.4)) prepared in our lab. In the chocolate factory, we used traps baited with water to obtain an additional and independent measurement of the E. cautella population levels.

Catches in monitoring traps decreased instantaneously (Fig. 2) and measured air concentrations of the main pheromone component, released from the MD-dispersers, increased immediately. Fewer moths were observed in the localities and the number of complaints from customers products decreased in the most intensively investigated mill. Catches in the water traps showed a decrease in population density, although not as dramatic as the pheromone trap catch indicated (Ryne et al., 2006).

### Conclusions

Based on the studies referred to above and on results from ongoing experiments, we conclude that pheromone monitoring baits can become more efficient in detecting infestations and that
the MD technique has a large potential for controlling all three moth species. The main challenge for future research is to develop reliable methods to estimate the success rate. This is especially true for *P. interpunctella* and *E. kuehniella*, for which an independent measurement of population density is still lacking. Potentially the chocolate derived volatiles may serve this function, if the bait composition is optimised and put in a trap suitable for catching females. For *E. cautella*, water traps seem to be reliable for population monitoring and potentially also useful for mass trapping (Ryne et al., 2002).

![Graph showing catch of male *E. cautella*](image)

**Fig. 2.** Catch of male *E. cautella* in pheromone monitoring traps before and during mating disruption treatment in a chocolate factory.

**Acknowledgements**

We thank Cam Oehlschlager, ChemTica Ltd., Costa Rica, for providing the mating disruption baits. This study was part of the research programme Biosignal (Pheromones and kairomones for control of pest insects), supported by the Foundation for Strategic Environmental Research (Mistra) and Cerealia R&D.

**References**


Contribution of spatial analysis for precision Integrated Pest Management of beetle pests in a semolina-mill

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Abstract. By utilizing food-bait traps we evaluated the presence and the spatio-temporal distribution of insect-pests in an industrial semolina-mill, with particular attention to the adults of Tribolium confusum J. du Val, Typhaea stercorea (L.) and Tribolium castaneum (Herbst). The confused flour beetle was collected on all the sampling dates, with two main peaks in the second half of June-beginning of July and in the first half of August. The hairy fungus beetle was most abundant from the second half of May to the beginning of July and in the first half of September. The presence of the rust-red flour beetle had a main peak in June. After the annual fumigation of the mill, carried out in the second half of August utilizing methyl bromide, only a small number of captures of the two Tribolium species occurred until the end of the sampling period. In contrast T. stercorea remained to colonize the mill until the end of November. The contour maps drawn to represent the spatial distribution of T. confusum before the fumigation treatment showed that the highest abundance foci of adult population were confined to the corners of the milling area and in the base of the adjacent walls of every floor, where a large amount of dust and debris usually accumulate. Adults of T. stercorea were confined in the I and the II floors, the main moisture areas of the mill contaminated with mould. Almost the same distribution as T. confusum was found for T. castaneum, but this species was observed only in the upper floors of the mill, especially in the VI floor. The indications obtained by spatial analysis and representation of spatio-temporal distribution were used in pest management procedures (precision IPM) after the fumigation, based essentially on the improvement of good sanitation practices. Furthermore, the initial monitoring plan was adapted, decreasing the number of food-traps (from 85 to 66) and optimizing the trap positions in the structure.

Key words: Spatial analysis, precision IPM, beetle pests, semolina-mill.

Introduction

Precision Integrated Pest Management (IPM) programs require accurate and comprehensive monitoring of pest populations, monitoring methods that not only detect infestation, but also estimate its degree and location. Thus monitoring is able to guide the timing and targeting of control applications, eliminates the need for routine preventive treatment, reduces the area treated with insecticides, and aids in the application of non-chemical methods (Arbogast & Mankin, 1999).

During recent years, the introduction of spatial analysis in applied entomology opened new possibilities to study and manage the spatial distributions of stored product pests (Arbogast et al., 1998; Brenner et al., 1998). This analysis can provide crucial information to improve the monitoring and precision targeting control methods. It has been recently used in various stored products, types of food industries, and storage facilities, such as flour-mills, feed-mills, warehouses and commodity facilities, against several moth and beetle pests (Arbogast & Mankin, 1999; Arbogast et al., 2000a,b, 2002a,b, 2004; Campbell et al., 2002; Trematerra & Sciarretta, 2002, 2004; Trematerra et al., 2004, 2005; Athanassiou et al., 2005).
By incorporating spatial analysis and comparative risk assessment into a standardized pest management operation, it is possible to: quantify the spatial aspects of pest populations and their inherent risk; improve a precise targeting of intervention technologies; evaluate and compare the intervention technologies; reduce pesticide use; simplify reporting and archiving, and; shift the operations to proactive monitoring and preventing pest problems.

In the present paper, we evaluated, by utilizing food-bait traps, the spatio-temporal distribution of insect pests in an industrial semolina-mill. With particular attention to confused flour beetle *Tribolium confusum* J. du Val, hairy fungus beetle *Typhaea stercorea* (L.), and rust-red flour beetle *Tribolium castaneum* (Herbst), the most abundant species, the present paper focused on the effectiveness of the spatial analysis in improving pest management procedures, such as good sanitation practices, and in optimizing the pests monitoring plan.

**Material and methods**

**Study area**
The observations were conducted between the second half of May 2003 and the first half of April 2004 (Sampling Period), in an industrial semolina-mill located in Apulia Region, Southern Italy. The mill was a large building of 18,000 m³, with seven floors (I, II, III, IV, V, VI and VII), processing 500 tons of *Triticum durum* Desf. (hard wheat) per day.

**Traps and data collection**
Food-bait traps were used to collect insect pests during the Sampling Period; the traps were 10x20 cm and made of welded plastic mesh of 3 mm aperture, folded and sealed to form a pouch for food materials. Each bag contained 30 g of mixed spelt, maize, rolled oats, broken hazelnuts, broken carobs, raisins and dried bananas (Trematerra, 1985).

To monitor insect populations, from 21 May to 12 August 2003 (1st Sampling Interval) and from 28 August to 18 December 2003 (2nd Sampling Interval) 85 traps were placed in the semolina-mill, while from 18 December 2003 to 14 April 2004 (3rd Sampling Interval) the number of traps used was 66 (Fig. 1).

The position of the traps were first defined by the distance measured from corners or external walls of the mill, and then the coordinates \(x, y\) were assigned to the trap position for use in the subsequent spatial analyses.

Traps were placed on 21 May 2003 and checks were conducted until 14 April 2004. A fumigation treatment of the mill, with methyl bromide, was realized on 13 August 2003. For this reason the traps were removed on 12 August and re-installed on 28 August.

Single traps were left at sampling sites for two weeks in the 1st and 2nd sampling Intervals and for one month in the 3rd Sampling Interval, and then were replaced by new ones. Before the traps were re-used, they were held at -20°C for at least a week to kill any arthropods that remained in the food-bait. Identification of insects present in the food traps was carried out in laboratory.

During the surveys, in every sampling date visual inspections were carried out to monitor the presence of insect pests and to verify the general cleanliness and orderliness.

**Spatial analysis**
As samples were taken from seven floors of the same semolina-mill, to determine the spatial dependence stratified mode variograms were computed, with \(x, y\) representing the coordinate axes of the trap position in the horizontal directions (expressed in meters), and \(z\) values representing the number of the stratum, as in Pebesma & Wesseling (1998).

Spatial interpolations of trap catch counts of pest adults were carried out by means of Surfer version 8.02 (Golden software, Golden, Colorado, USA), using a linear model with.
zero nugget, as suggested by Brenner et al. (1998) who explain this type of analysis in more detail.

By interpolating the data with linear kriging, the software produces a dense grid of values. The interpolation grid obtained is used to produce a contour map, which shows the configuration of the surface by means of isolines representing equal density values. A base map showing the plan of each floor, with the same coordinate system, was placed on top of the contour map. In the case of sampling intervals with low catch numbers, the spatial analyses were carried out using the default of the software, in order to obtain an indication of the spatial distribution of the insect pests, to be compared with the results of the other Sampling Intervals.

![Fig. 1. Schematic representation of the semolina-mill, with trap location, floor surface area and number of traps in each floor.](image)

Furthermore a normalized variable was obtained by converting cumulative catches, observed in all traps during the whole Sampling Period, in catch probability by means of a derived indicator, following Brenner et al. (1998). This procedure, called indicator kriging, enabled us to focus on the areas with important insect densities by minimizing the effect of an unusual trap count and by leaving out low-density zones. The trap counts were sorted in descending order and expressed as proportions of the pooled counts. An indicator score of “1” was given to all traps with catches that exceeded a critical proportion (cumulative frequency distribution), that we set at 0.90. A score of “0” was given to the remaining traps. Interpolation of scores yields a contour map with isolines ranging from 0 to 1.

The geostatistical techniques were used to represent the spatial distribution of the most abundant insect pests collected in every sampling date. The contour maps obtained were sent to the management personnel of the semolina-mill in reports with comments regarding also the results of the visual inspections.

In this paper the spatial analyses by means of linear kriging were applied to the total catch data of *T. confusum*, *T. stercorea* and *T. castaneum*, obtained in the 1st Sampling
Interval, 2nd Sampling Interval and 3rd Sampling Interval, respectively. The indicator kriging was analyzed for the accumulated trap counts of the three pests referred to the 1st and the 2nd Sampling Intervals.

**Results**

A total of 1476 insect specimens were captured in the food-bait traps, belonging to 14 taxa, mainly Coleoptera. Most abundant species was *T. confusum* (with 1021 adults), followed by *T. stercorea* (with 167 adults) and *T. castaneum* (with 125 adults). The majority of insects were found in the traps before the fumigation treatment, in the 1st Sampling Interval (1283 adults); after the treatment, in the 2nd and the 3rd Sampling Intervals, only few specimens were trapped (193 adults) (Fig. 2).

![Graph showing the number of adults trapped over time for different sampling dates and species](image)

**Fig. 2.** *Tribolium confusum*, *Typhaea stercorea* and *Tribolium castaneum* adults collected in the food-bait traps placed in the semolina-mill (white arrows: general cleaning; grey arrow: general cleaning and annual fumigation).

The confused flour beetle was collected on all the sampling dates. In the 1st Sampling Interval the species population had two main peaks, one in the second half of June-beginning of July (248 adults trapped) and a second in the first half of August (273 adults trapped). After the fumigation of the mill only a small number of captures occurred until mid April 2004.: A total number of 29, in the 2nd Sampling Interval and 15 adults in the 3rd Sampling Interval. Hairy fungus beetle catches were obtained especially from the second half of May until the beginning of July (29, 28 and 27 adults were trapped on the three sampling dates of this period), in the 1st Sampling Interval. After the fumigation treatment the adults of *T. stercorea* remained to colonize the mill until the beginning of December, during the entire 2nd Sampling Interval, in which it was most abundant in the first half of September (21 adults trapped); However, in the 3rd Sampling Interval no specimen was collected. The presence of the *T.*
*castaneum* was continuous during the 1st Sampling Interval, with a main peak in the first half of June (37 adults trapped). In the 2nd Sampling Interval only 2 trap catches were obtained. In the 3rd Sampling Interval the species was present with 14 adults trapped.

Figures 3, 4 and 5 show the contour maps obtained by geostatistical analysis using linear kriging applied to the total trap catches of *T. confusum*, *T. stercorea* and *T. castaneum*, obtained respectively in each Sampling Interval.

In Fig. 6 are reported the maps obtained by the analysis of indicator kriging of cumulated data of the three species observed in the first two Sampling Intervals.

In the 1st Sampling Interval the greatest portion of *T. confusum* adults was found on the VII, VI and IV floors. In every floor, the highest abundance foci of population were confined to the corners of the milling area and in the base of the adjacent walls, where a large amount of dust and debris usually cumulate. This is true especially for the same corners on the VII, VI, V, IV, III and II floors. On the other hand, the area encompassed by the contours appeared to involve the machinery, located in the central zones of the floors, which were not relevant to the sampling process. In the 2nd and the 3rd Sampling Intervals it was possible to draw only isolines ranging from 1 to 5. By means of indicator kriging, it can be observed that *T. confusum* population appeared to have a more extended distribution on the VII, VI, V and IV floors, compared to the other floors, where the presence of the pest was limited to the same corner as the main foci in the upper floors.

In the 1st and the 2nd Sampling Intervals, adults of *T. stercorea* was confined to the II floor and in the I floor, the main moisture areas of the mill contaminated with mould. The maps obtained using the indicator kriging illustrate this distribution of the population in a clearer manner.

Almost the same distribution of *T. confusum* was found for *T. castaneum* during the 1st Sampling Interval, but this species was observed only on the upper floors of the mill, with a most abundant presence on the VI floor, compared to the other areas of the mill.

In this sampling interval mixed populations of *T. confusum* and *T. castaneum* were found in the traps distributed on the VII, VI, V and IV floors. In the 3rd Sampling Interval the few captures of the species were confined to a corner on the VII and the IV floors. The representation of the indicator kriging shows that on the upper floors *T. castaneum* had a smaller distribution compared to *T. confusum* (Fig. 6).

**Comments and conclusions**

The results and the indications obtained by means of geostatistical techniques were used to improve the pest management procedures applied in the semolina-mill, first of all using good sanitation practices, in an IPM approach. This was possible combining the results of the spatial analysis with the risk assessment obtained based on the information received from the management personnel of the mill and by what was observed during visual inspections carried out on every sampling date.

As reported by the laboratory personnel of the mill, *T. confusum* and *T. castaneum* were the insects that caused most problems of contamination in the semolina production. Thus the attention of the pest management procedures focused on these two species. The visual observations on the distribution of insect pests reflected the same indications compared with what found out by the traps.
Fig. 3. Contour maps of *Tribolium confusum* distribution by means of kriging applied to total trap counts obtained in each Sampling Interval.

Fig. 4. Contour maps of *Typhaea stercorea* distribution by means of kriging applied to total trap counts obtained in each Sampling Interval.
Fig. 5 – Contour maps of *Tribolium castaneum* distribution by means of kriging applied to total trap counts obtained in each Sampling Interval.

Fig. 6 – Contour maps of *Tribolium confusum*, *Typhaea stercorea* and *Tribolium castaneum* distribution by means of indicator kriging applied to cumulated trap counts obtained in the 1<sup>st</sup> and the 2<sup>nd</sup> Sampling Interval (the cumulative frequency distribution expressed as percentage is 0.9).
In the case of the two *Tribolium*, both the results of the visual inspections and the geostatistical analyses of the trap catches showed that the highest abundance foci were found in particular zones of the floors, almost in every sampling date; This was true especially for the data of the 1st Sampling Period in which these beetles were present to a greater extent. The pest populations were mostly confined to the corners and in the bases of the walls of the milling areas. In these points debris, dusts and residual food material were generally deposited (sometimes in amounts up to 5 cm high), particularly on 17.VI, 1.VII, 28.VII, 12.VIII, and 11.IX sampling dates. For these sampling dates, especially for the first four of them, before the fumigation treatment, a higher number of specimens was found compared with the other sampling dates, when the mill was cleaned. As reported in Fig. 2, cleaning operations were realized before 15.VII, 28.VII and 26.IX sampling dates by sweeping and vacuuming.

During the 1st and the 2nd Sampling Intervals, the general housekeeping in the mill wasn’t conducted regularly but only on the occasion of maintenance of the equipments or before the annual fumigation with methyl bromide.

Using the results of the pests monitoring and the maps of spatial distribution of the most abundant insect species obtained during the 1st and the 2nd Sampling Intervals, in December 2003 cleaning schedules were established by the management personnel of the semolina-mill, planning a general housekeeping of the milling areas per month, as reported in Fig. 2.

By means of the maps with the spatial distribution of the two *Tribolium* species, it was found that in the VII, VI, V, IV, III and II floors the flour beetles were most abundant in the same corner of the milling areas and in the bases of the next walls. This corner is directly exposed to south, so it is the warmer zone of the mill. In this corner, from VII to IV floors there are machines which produce dusts that are hard to remove, while on III and II floor in the same corner there are electric boards that produce heat. It is also possible that infestation was transmitted vertically between floors, because there are pipe-ducts near the walls. Consequently, the sanitation practices, both the routine and the general cleaning of the mill, were focused especially to the points of the milling area of each floor with the highest infestation foci, the corner exposed to south and the bases of the adjacent walls.

Regarding the insect pests monitoring, the results of the geostatistical analyses of trap catches were useful to optimize the initial sampling plan during the 3rd Sampling Period, decreasing the number of food-bait traps from 85 to 66, and changing the trap positions in the structure. These actions were finalized to simplify the insect pests monitoring procedures, without decreasing the effectiveness of this tool as a base component of the IPM program established in the semolina-mill (see Fig. 1).

With these measures, insect pest presence was maintained at a lower level during the following months, compared to the previous years.

The next step to a complete precision IPM approach in the pest management of the mill will be a more significant reduction of the pesticide use, with a further implementation of precision targeting in the chemical control methods based on the indications obtained utilizing the geostatistical tools.

References


Insect populations in a feed mill for horses in Portugal

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Abstract: Trials were carried out in a feed mill for food horses from 7 January 2004 to 20 January 2005 in order to identify the insect species and to study pest populations. Several type of unbaited traps and traps baited with pheromones and food lures were used: Dome traps for crawling insects, Thinline, funnel traps and Lasiotrap for flying insects, probe and pitfall traps for insects developing within the stored products. Following surveys, 47 insect species were identified. Some of them were caught throughout the year, others were seasonal. Considering that the products after the micronizer, to transform cereals and beans to flakes, were free of insects, probes were used for sampling horse-bean stored as micronized product, stored in bulk, and the non micronized black oat which was stored in big bags.

The results demonstrated that the feed mill suffered important infestations of several insect species. High populations of *Ephestia* spp. were present throughout the year. It appeared the major risk should be their development within the machinery than attacking the stored products. Although the micronizer can provide a product free of insects, the horse-bean stored in bulk, after a while presented populations of several stored insect species. The most important insect pests caught in the stored products were *Oryzaephilus surinamensis* and *Sitophilus zeamais*. Eight species of parasitoids were also identified and the existence of these natural enemies may enable the development of a biological control program.

Key words: stored product, feed mill, sampling, pests, beneficials

Introduction

A feed mill is a human-made, unstable ecosystem with a continually replenished energy supply. Most of the food energy is transferred to farm animals through product sales. Some of the energy, in the form of dust and other residues, remains in the mill and provides sustenance for feed-mill pests (Mills, 1992). Stored-product insects are a serious problem in animal feed, damaging and contaminating it. Without an IPM strategy, these insects may be resident in the feed mill and related stores and infest the incoming product. Without any effective counter measures, the pest abundance may increase significantly, and some of their population will disperse to invade other stored products or clean areas. Effective IPM strategies require good sanitation, inspection of incoming goods, monitoring stored-product pests, removing infested stock and judicious application of control methods (Arbogast et al. 2000).

The present study was carried out in a small feed mill, in Portugal that produces only horse feed and uses the micronizer to transform cereals and beans to flakes. Several stored-products were used, including: black oats, carob, alfalfa and sugar beet are directly mixed (with or without been milled) while maize, barley, wheat, soya bean, pea and horse bean are passed through the micronizer, which reaches a temperature of about 110ºC for 90 seconds,
which are then transformed into cereal and bean flakes to be mixed into a final product. The end-use is to national and international horse consumption. In 2003, the manager was faced with high insect infestations and in 2004, a sampling program was developed in order to identify the insect species, to study pest populations in the facility and in the products with and without micronizer action and to help the manager in decision-making.

**Material and methods**

*Experimental sites*

Trials were carried out from 7 January 2004 to 20 January 2005 in a feed mill for horses (Fig.1), located near Lisbon.

![Plant diagram](image)

**Silos floors**

- 1st floor
- 2nd floor
- 3rd floor

Fig. 1. Plant of the feed mill including the three floors of the silos

Two products, black oat and sugar beet, stored in big bags and one product micronized, horse bean, stored in bulk after micronization, were sampled over a range of weeks (Table 1).

<table>
<thead>
<tr>
<th>Product sampled</th>
<th>Type of storage</th>
<th>Number of weeks</th>
<th>Not micronized</th>
<th>Micronized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black oat</td>
<td>7-5 big bags (1.1 ton)</td>
<td>46</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sugar beet</td>
<td>One big bag (1.1 ton)</td>
<td>8</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Horse</td>
<td>In bulk on the floor</td>
<td>28</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>
**Sampling insect populations**

For black oat and sugar beet, one probe (Stogard Probe II, Trécé, Salinas, CA, USA) and two pitfall traps (AgriSense, UK) per big bag (at a surface and 15 cm depth) were used. Both types of traps were applied without lure. For horse bean, six probes without lure were placed in the bulk.

For the facility, several types of traps were employed: for crawling insects, Dome traps with food oil (Trécé, Salinas, CA, USA) to attract several species of crawling and flying insects, Thinline traps (Trécé, Salinas, CA, USA) with pheromone combo for *Plodia, Ephestia, Lasioderma serricorne* (F.), *Trogoderma granarium* (Everts) and *T. variable* (Ballion); for cigarette beetle, Lasiotraps with sex pheromone lure (AgriSense, UK) and funnel traps with sex pheromone lure for *Ephestia* spp. and *Plodia*. The sticky traps and pheromone lures were replaced every sixth week and the insects were collected and identified fortnightly. The number of traps used, in the facility and the stored-products, and the sampling periods are shown in Table 2. One Thinline, one Lasiotrap and one funnel trap were placed in each room of the feed mill, in the three floors of the silos and in two stores; two Dome traps were used in the mill section and in the three floors of the silos.

Table 2. Type of traps used, with and without lure, in the facility and in each product during trials.

<table>
<thead>
<tr>
<th>Local</th>
<th>Type of trap with/without lure</th>
<th>Sampling period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dome</td>
<td>Thinline</td>
</tr>
<tr>
<td>Facility</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Black oat</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Horse bean</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

**Environmental conditions**

One thermohygrograph was used from 7 January to 15 May 2004 and from 10 September to 28 November 2004. One multi-channel data logger with five temperature sensors was used from 2 July to 10 September 2004.

The temperature ranged from 10.0°C (first week of May) to 27.3°C (mid September) and its mean was about 17.5±5.9°C. The mean relative humidity was 79.5±6.3% and its minimum and maximum was 54.5% (last week of September) and 83.8% (mid January 2004), respectively. The minimum might be lower as during summer season it was not possible to register all the data.

**Spatial pattern of moths**

The spatial pattern of moths in the facility was mapped in three and two dimensions for visual assessment and interpretation (Kaluzny, *et al.* 1998).
Results

The total number of each insects caught in the traps used in the feed mill and inside of each product samples are presented in Table 3. From the total, 29 species of beetles were caught and identified. All species were seasonal excepting *L. serricorne*, *Sitophilus* spp. and *O. surinamensis*, collected throughout the year. Five species of moths were registered but only *E. kuehniella* was caught throughout the year. The eight species of wasps and the two species of Hemiptera were seasonal. The majority of beetles and moths were granivores. Five identified species were exclusively mycetophagous (from Cryptophagidae, Lathridiidae and Mycetophagidae families), the wasps were larvae parasitoids of beetles and moths and the carabid beetles, and bugs collected were general predators.

Although three species of psocids were identified, very few individuals were trapped during the trials.

Table 3. Insect taxa and total numbers of each insect species caught in the feed mill and in each stored-product sampled during trials.

<table>
<thead>
<tr>
<th>Order/ Family</th>
<th>Species</th>
<th>Feed mill</th>
<th>Black oat</th>
<th>Sugar beet</th>
<th>Horse bean</th>
</tr>
</thead>
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<td></td>
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<td><em>Cryptophagus perrisi</em> Brisson</td>
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<td>20</td>
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<td>2</td>
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<tr>
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<td>0.03</td>
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<td></td>
<td><em>Typhaea stercorea</em> (L.)</td>
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<tr>
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<td>0.01</td>
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<td>–</td>
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<td>Order/ Family</td>
<td>Species</td>
<td>Feed mill Total</td>
<td>Black oat Total</td>
<td>Sugar beet Total</td>
<td>Horse bean Total</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-----------------</td>
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<td>Tenebrionidae</td>
<td><em>Tribolium castaneum</em> (Herbst)</td>
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<td>1.08</td>
<td>616</td>
<td>5.85</td>
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<td></td>
<td><em>T. confusum</em> J. du Val</td>
<td>11</td>
<td>0.06</td>
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<td><em>Gnatocerus cornutus</em> (F.)</td>
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<tr>
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<td>–</td>
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<td>0.01</td>
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<tr>
<td>Lepidoptera</td>
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<td>77.67</td>
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<td><em>E. kuehniella</em> Zeller</td>
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<td>–</td>
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<td>0.14</td>
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<td>–</td>
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</tr>
<tr>
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<td><em>C. tarsalis</em> (Ashmead)</td>
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<td>0.04</td>
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<td></td>
<td><em>Bracon hebetor</em> Say</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>0.01</td>
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<td>0.01</td>
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<td>–</td>
<td>1</td>
<td>0.01</td>
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<tr>
<td></td>
<td><em>Lariophagus distinguendus</em> (Förster)</td>
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<td>–</td>
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<td>0.10</td>
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<td>3</td>
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<td>0.01</td>
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<td><em>Liposcelis decolor</em> (Pearman)</td>
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<tr>
<td></td>
<td><em>L. bostrychophila</em> Badonnel</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lachesilla pedicularia</em> (L.)</td>
<td>+</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Total**

|       | 17269 | 1.00 | 10525 | 1.00 | 237 | 1.00 | 2119 | 1.00 |

**Facility**

At the facility 17,268 insects were caught. The moth population was the most abundant, followed by the cigarette beetles population. A total of 14,025 moths were caught. *E. kuehniella* was significantly the most abundant species and occurred throughout the year, followed by *P. interpunctella*. The remaining moth species were occasional. Several peaks occurred during the year and the most significant, of 68 moths/trap/week occurred in August (Fig. 2). Adult males of Pyralidae followed the aggregated pattern (Fig. 3 and 4): The major moth trap catches were registered in the micronizer room (more than 40 moths/trap/week); followed by the package room and the first floor of the silos (21-30 moths/trap/week), both without marked differences. The stores for final product were the less infested areas (fewer than 9 moths/trap/week).

A total of 2,612 *L. serricorne* adults were caught. The two major peaks, of 22 and 26 cigarette beetles/trap/week, occurred in mid August and at the beginning of September (Fig. 5).
Fig. 2. Moth population: total of insects caught and relative density in the feed mill during trials.

Fig. 3. Spatial pattern of moths in the feed mill during trials.
Fig. 4. Spatial pattern of moths at 3D in the feed mill during trials; x axis corresponds the width of the feed mill plant; y axis corresponds the length of the feed mill plant; and z axis corresponds the mean number of moths caught by traps.

**Stored-Product**

**Black oat and sugar beet:** In stored black oats, a total of 10,525 insects were caught and the most abundant insect species were *O. surinamensis*, and *S. zeamais*. *O. surinamensis* was dominant until November when it was surpassed by *Sitophilus* spp. populations which can be related to different response to changes in abiotic conditions (Fig. 6).

In stored sugar beet, a total of 237 insects were caught and identified. The main species, *Cryptophagus cellaris*, belonged to the Cryptophagidae family and like mycetophagous they are indicator of fungus presence (Fig. 7).

**Horse bean flakes:** In stored horse bean a total of 2,119 insects were caught. *O. surinamensis*, was the dominant species followed by *T. castaneum* and *T. confusum* (Fig. 8). The peaks presented in the picture were not related to population fluctuations in population development but to product reception and shipment: receipts after micronization converting to flakes were without insects, but those products that remained on the floor, started to be infested until the delivered product became infested. After trials, the horse bean was stored in big bags of 1.1 tonnes.

**Discussion**

With the mean temperature of 17.5°C, the feed mill ecosystem is conducive for survival, growth and reproduction of stored product insects, because of year-round warm conditions of some production areas, open stores and utilization of various feed ingredients of cereal and non cereal origin (Mills, 1992). The great number of stored-product in feed mills and related storage has been reported by several authors (Platt et al, 1998; Arbogast et al., 2000; Trematerra & Fiorilli, 2000; Roesli et al, 2003). More than 44 insect species were identified and the majority of insect taxa were granivorous as the most abundant insect populations belonged also to important stored-product pests.
Fig. 5. Cigarette beetle population: total of insects caught and relative density in the feed mill during trials.

High populations of Pyralidae moths were present throughout the year and it seemed the major risk should be their development within the machinery, then attacking the stored products. There were especially higher trap catches in the micronizer room, where the environmental conditions were warmer, because of the source of heat from the micronizer.

_Ephestia kuehniella_ was dominant and present in relatively high densities throughout the year of trials. Studies conducted in a feed mill in Italy also reported large populations of _E._
*kuehniella* (Trematerra & Fiorilli, 2000). The different records of trap catches in each place of the feed mill suggested that moth populations followed an aggregated spatial pattern. *Lasioderma serricorne* were present in abundant populations but the source of infestation was uncertain.

![Graph showing total trap catches of *Gnathocerus cornutus*, *Cryptophagus perrisi* and *C. cellaris* in stored sugar beet.](image)

Fig. 7. Total trap catches of *Gnathocerus cornutus*, *Cryptophagus perrisi* and *C. cellaris* in stored sugar beet.

![Graph showing the relative density of *Oryzaephilus surinamensis* and Tenebrionidae spp. in micronized horse bean stored in bulk on the first floor of the silos.](image)

Fig. 8. The relative density of *Oryzaephilus surinamensis* and Tenebrionidae spp. in micronized horse bean stored in bulk on the first floor of the silos.

In stored products samples, the most important insect pests caught were *Oryzaephilus surinamensis* and *Sitophilus zeamais*. Following Nansen et al. (2004) *Sitophilus* can coexist with other insect species also granivorous. *O. surinamensis* is a serious pest of stored-products, although it is unable to attack whole grain, it can attack grain previously damaged
by *Sitophilus*. Low populations were present at the beginning of trials and it can be assumed that black oat was not infested before was brought into the store but became infested from infested products previously stored or from residual populations within the store. Also, during the micronizer action, the product reached 110°C during 90 seconds and should kill all insect stages (Adler, 1998). The horse-bean stored in bulk on the floor after been micronized presented free of insects but the number of sampling units clean of insects declined over time as the number of insect taxa and insect density increased over time as the horse bean remained stored on the floor. It appears that, *L. serricorne*, *Oryzaephilus* spp and *Sitophilus* spp are very common pests in feed (Platt et al., 1998; Arbogast et al., 2000; Roesli et al., 2003). Studies conducted at pet stores found a large number *Oryzaephilus* sp. and the highest trap catch was near the horse feed, which may have been the source of infestation (Arbogast et al., 2000). The larval parasitoids associated to the main insects pests registered were identified and encountered in fairly low numbers. It is well documented that *A. calandrae, L. distinguendus* and *B. hebetor* can act with a high rate on host population suppression (Lukas & Riudavets, 2002; Nansen et al. 2004). The existence of these natural enemies may encourage the development of a biological control program.

**Acknowledgements**

The authors express their gratitude: to INTACOL (Sobralinho, Portugal) for allowing the trials in the feed mill; to Bill Lingren from Trécé (Salinas, EUA), for supplying the Storgard Dome, Probe II and Thinline traps used in these studies; to Zuzana Kučerová from Research Institute of Crop Production (Prague, Czech Republic) from Psocoptera identification; to Alberto Vargues, from National Agronomic Station (Oeiras, Portugal), to lend the multi-channel data logger.

**References**


Insect fragments in flour: Relationship to Lesser Grain Borer (Coleoptera: Bostrichidae) infestation level in wheat and rapid detection using near-infrared spectroscopy

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Abstract: The grain milling industry routinely checks wheat flour for insect fragments to determine whether the number found is below the U.S. Food and Drug Administration (FDA) defect action level of 75 fragments/50 g flour. However, the standard chemical extraction method used to detect insect fragments in flour is costly and time-consuming; thus, a rapid detection method is desirable. In addition, little is known about differences in the number of fragments produced from different stages of different insect species. In this study, we determined that wheat infested with a single pre-emergent adult lesser grain borer, Rhyzopertha dominica (F.), contributed 28 times and 10 times as many fragments as wheat infested with a single larva or pupa, respectively. Using regression models that we developed from these data, we predicted that 1-kg samples of wheat with more than 20 kernels infested with pre-emergent adult borers would be above the FDA defect action level for insect fragments. Similarly, it would take an infestation level of 380-640 kernels (in a 1-kg sample) containing larvae or pupae to exceed the defect action level. We also determined the accuracy and sensitivity of near-infrared spectroscopy (NIRS) for detecting insect fragments in flour using three different NIR-spectrometers. The number of insect fragments predicted by NIRS was correlated with the actual number of fragments in test samples. NIRS was less precise than the standard flotation method, but it has the advantages that it is rapid, non-destructive, does not require extensive sample preparation, and can be automated for a more sophisticated sampling protocol for flour.

Keywords: Insect fragments, flour, detection, near-infrared spectroscopy

Introduction

The U.S. Food and Drug Administration (FDA) has established a Defect Action Level (DAL) of 75 insect fragments per 50 g of flour as the regulatory standard for quality control (FDA 1997). Immature stages and pre-emergent adults of internal grain feeding insects may not be removed from grain by cleaning before milling; as a result, these stages are the main source of insect fragments in wheat flour (Brader et al. 2002). However, the relationship between internal infestation of wheat kernels and the number of insect fragments produced in flour has not been described well. Sachdeva (1978) reported that wheat infested with granary weevil adults produced 3× more fragments than wheat infested with weevil larvae. The standard method used by the FDA for detection of insect fragments (AOAC 1996) is labor intensive and expensive because it involves milling, extracting, and microscopically examining the number of insect fragments produced (Glaze and Bryce 1994). Thus, development of a fast and reliable alternative method is needed by the milling industry.
In a previous study, we found that a near-infrared (NIR) spectrometer with a spectral range of 400-1700 nm was able to predict accurately whether flour samples contained less than or more than 130 insect fragments (Perez-Mendoza et al. 2003). Although that study had limited success detecting insect fragments at the FDA defect action level, NIR spectrometers with extended wavelength ranges are now available and may improve detection accuracies. In this study, we re-examined this method with instruments that extend the near infrared region tested to 2500 nm.

The objectives of this study were: (1) to characterize the relationship between different levels of internal wheat infestation with larvae, pupae, or pre-emergent adults of the lesser grain borer, *Rhyzopertha dominica* (F.), and the number of insect fragments produced in flour milled from that wheat; and (2) to compare the accuracy of three NIR spectrometers for determining number of insect fragments in the flour produced from the infested wheat. *R. dominica* is a common and destructive pest of stored wheat in the U.S. (Hagstrom et al. 1994) that develops and feeds inside grain kernels and is the main source of insect fragments in wheat flour. Eggs are laid on the surface of grain kernels and, after hatching, the first instars bore into the grain (Elek 1994). Larvae pupate inside the kernels, and adults remain inside the kernels for several days after eclosion (Hagstrom and Flinn 1994). We refer to these newly eclosed adults still in the kernel as pre-emergent adults.

**Materials and methods**

**Insects**
Kernels infested with lesser grain borers were obtained from a laboratory strain reared on whole kernel, hard red winter wheat, *Triticum aestivum* L. Insect cultures were started by placing 200 unsexed adults into 200 g of wheat, adjusted to 13.5% moisture content by adding distilled water as needed, in 800 mL glass jars capped with screen/filter paper lids. Jars were held in a rearing chamber at 30±1°C and 70±5% r.h. with a 12:12 L:D photoperiod. All founding adults were removed by sieving after 7 d. After 21 days, kernels containing larvae, pupae, or pre-emergent adults were detected by x-ray analysis (Throne 1994). Infested wheat kernels were placed in aluminum dishes which were placed in a mechanical convection oven (Precision Scientific Inc., Chicago, IL) maintained at 130°C for 30 min to kill the insects. After cooling at room temperature, the desired number of infested kernels with each life stage was added to batches of uninfested wheat to complete 100 g samples. Samples were conditioned to 15% moisture content for 1 wk before milling.

**Levels of Insect Infestation**
Ten levels of infestation were tested to determine the fragment contribution of each stage of insect development (Table 1). The level of infestation was adjusted according to the insect stage and the expected fragment contribution based on a preliminary study (data not shown). The infestation levels used produced flour samples with fragment counts below and above the FDA defect action level.

**Milling**
Individual wheat samples were milled on a Brabender Quadrumat Sr. mill (type 12-10-N87, C.W. Brabender Instruments, Hackensack, NJ). Temperature of the rolls was maintained at 31.1-32.2°C during milling. The milling efficiency (% flour yield) of this mill was around 60%; therefore, milling produced flour samples of about 60 grams each.

**Determination of Insect Fragment Counts Using the Standard Flotation Method**
The standard flotation method used by the FDA (AOAC 1996) was used to determine the number of fragments produced in the flour samples. This method was scaled up to collect
insect fragments in 60 ± 5 g of flour samples (Perez-Mendoza et al. 2003). Fragments in five replicates of each infestation level for each insect stage were determined.

**Detection of Insect Fragments Using Near-Infrared Spectroscopy (NIRS)**

Three near-infrared spectrometers were used to collect spectral data from the wheat flour samples containing varying levels of insect infestation.

**Perten Diode Array 7000**

The DA 7000 (Perten Instruments Inc., Springfield, IL) collects absorbance spectra over a range of 400 to 1690 nm. Each flour sample was poured into a 12.5-cm-diameter sample ring above the 12.5-cm-diameter fixed sample viewing area. The thickness of the flour sample was about 1.2 cm. The light beam comes from below the sample viewing area and penetrates the flour sample. Each spectrum saved was an average of 15 spectra collected in about three seconds.

**Cognis-QTA™ FT-NIR**

The QTA™ system (Cognis, Cincinnati, OH) collects spectra in the 830 to 2500 nm wavelength range. Flour samples were placed directly in a rotating cup. Two replicates were collected for each flour sample. The instrument was set to automatically average the spectra of 100 scans into one spectrum for each sample being scanned. Prior to development of the calibration model, the resulting spectra from the two replicates of each sample were averaged yielding one spectrum that represented that specific sample.

**Foss NIR Systems 6500**

The Foss NIR Systems 6500 (Foss NIRSystems, Silver Spring, MD) scanning monochromator collects spectra from 400 to 2500 nm. The instrument has a sample transport device that allows the sample in a quarter cup to be moved vertically past the light source. Flour samples were poured and leveled in the quarter cup and secured flush using a white board material before placing the sample in the transport device. Each sample was scanned 64 times, and the data were saved as a single spectrum.

**Statistical Analysis**

Equations describing the relationship between infestation level and the number of insect fragments produced in flour recovered with the standard flotation method were fit to the data using TableCurve 2D (SYSTAT Software Inc. 2002). We used 95% prediction limits of the fitted equations to determine the maximum number of infested kernels that could be milled and still be below the FDA defect action level, which was calculated as the lowest number of infested kernels whose upper 95% prediction limit was below 90 fragments in 60 g of flour (= 75 fragments in 50 g of flour).

**NIR spectra were analyzed by partial least squares (PLS) regression (Martens and Naes 1989) using PLSPlus/IQ software (Galactic Industries 2003). Calibration models for each insect stage infesting the grain (larvae, pupae, or pre-emergent adults) and for each NIR spectrometer were developed using PLS (9 individual models). Five test samples of each insect-stage infestation level were scanned (n = 150). Finally, a calibration model that included combined data from the three insect stages (larvae + pupae + adults) was developed. The relationship between NIRS-predicted number of insect fragments and actual number of fragments in the flour samples (as estimated by the flotation method) was determined by using TableCurve 2D (SYSTAT Software Inc. 2002). We used inverse prediction to estimate the actual number of fragments in a sample based on NIRS estimates, including 95% confidence limits on the estimates. We used these confidence limits from the inverse predictions to determine the maximum number of fragments that could be present in flour based on NIRS predictions and still be below the FDA defect action level, which was calculated as the lowest number of actual fragments whose upper 95% confidence limit was below 90 fragments in 60 g of flour (= 75 fragments in 50 g of flour).
Results

Effects of Insect Infestation Level on Fragment Counts

Larvae: Larvae produced the fewest fragments (Table 1). The predicted number of insect fragments produced by a single wheat kernel infested with one larva was 0.6 [95% confidence limits (CL) = 0.23 – 0.93; 95% prediction limits (PL) = 0 – 27.2]. The number of insect fragments increased with increasing infestation levels. This relationship was described by the equation (SE’s in parentheses):

\[ y = 0.5775 (\pm 0.175) \times x^{1.126 (\pm 0.0627)} \]  

Equation 1

where \( y \) = number of fragments and \( x \) = number of infested kernels (\( r^2 = 0.95 \)) (Fig. 1A).

Based on the 95% prediction limits for this equation, the maximum number of infested wheat kernels with larvae that millers can accept to produce flour that meets the FDA Defect Action Level is 64 infested kernels in 100 g of wheat (95% PL = 35.3 – 89.8 fragments in 60 g of flour).

Fragments contributed by pupae: Fragment counts in flour prepared from wheat samples infested with different numbers of kernels infested with pupae were intermediate between those produced by wheat samples infested with larvae or adults (Table 1). The predicted number of insect fragments produced from a single wheat kernel infested with one pupa was 1.6 (95% CL = 0.90 – 2.4; 95% PL = 0 – 22.1). The relationship between the number of kernels infested with pupae and the number of insect fragments produced in flour was described by the equation:

\[ y = 1.646 (\pm 0.372) \times x^{1.025 (\pm 0.0568)} \]  

Equation 2

where \( y \) = number of fragments and \( x \) = number of infested kernels (\( r^2 = 0.95 \)) (Fig. 1B).

Based on the 95% prediction limits for this equation, the maximum number of infested wheat kernels with pupae that millers can accept to produce flour that meets the FDA Defect Action Level is 38 infested kernels in 100 g of wheat (95% PL = 47.7 – 89.2 fragments in 60 g of flour).

Table 1. Mean (± SEM, \( n = 5 \)) number of insect fragments recovered in flour from wheat samples (100 g) infested with three stages and different infestation levels of the lesser grain borer, *Rhyzopertha dominica*.

<table>
<thead>
<tr>
<th>No. of infested kernels</th>
<th>Larvae: No. of fragments (( \pm ))</th>
<th>Pupae: No. of fragments (( \pm ))</th>
<th>Pre-emergent adults: No. of fragments (( \pm ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 ( \pm 0 )</td>
<td>0 ( \pm 0 )</td>
<td>0 ( \pm 0 )</td>
</tr>
<tr>
<td>10</td>
<td>8.6 ( \pm 1.1 )</td>
<td>5 ( \pm 10.6 )</td>
<td>1 ( \pm 14.0 )</td>
</tr>
<tr>
<td>20</td>
<td>12.0 ( \pm 1.8 )</td>
<td>10 ( \pm 17.6 )</td>
<td>2 ( \pm 38.2 )</td>
</tr>
<tr>
<td>40</td>
<td>28.8 ( \pm 2.8 )</td>
<td>15 ( \pm 25.4 )</td>
<td>3 ( \pm 65.8 )</td>
</tr>
<tr>
<td>60</td>
<td>66.8 ( \pm 3.6 )</td>
<td>20 ( \pm 37.6 )</td>
<td>4 ( \pm 94.6 )</td>
</tr>
<tr>
<td>80</td>
<td>78.8 ( \pm 7.7 )</td>
<td>30 ( \pm 50.2 )</td>
<td>5 ( \pm 135.4 )</td>
</tr>
<tr>
<td>100</td>
<td>110.0 ( \pm 8.5 )</td>
<td>40 ( \pm 71.8 )</td>
<td>6 ( \pm 167.4 )</td>
</tr>
<tr>
<td>120</td>
<td>126.2 ( \pm 9.7 )</td>
<td>50 ( \pm 97.8 )</td>
<td>7 ( \pm 201.2 )</td>
</tr>
<tr>
<td>140</td>
<td>144.2 ( \pm 6.2 )</td>
<td>60 ( \pm 100.6 )</td>
<td>8 ( \pm 225.4 )</td>
</tr>
<tr>
<td>160</td>
<td>178.0 ( \pm 8.3 )</td>
<td>70 ( \pm 131.6 )</td>
<td>9 ( \pm 276.8 )</td>
</tr>
</tbody>
</table>
Fragments contributed by pre-emergent adults: Fragment counts for flour prepared from wheat samples infested with different numbers of kernels infested with pre-emergent adults were the highest compared with those produced in wheat samples infested with larvae or pupae (Table 1). The mean number of insect fragments produced by an individual infested wheat kernel containing one adult was 14.0 ± 1.6 (predicted number of insect fragments = 16.7; 95% CL = 13.8 – 19.6; 95% PL = 0 – 42.8). The relationship between the levels of internal wheat infestation with adults and the number of insect fragments produced in flour was described by the equation:

\[ y = 16.69 \pm 1.43 \times x^{1.273 \pm 0.0430} \]

Equation 3

where \( y \) = number of fragments and \( x \) = number of infested kernels (\( r^2 = 0.98 \)) (Fig. 1C). Based on the 95% prediction limits for this equation, the maximum number of wheat kernels infested by newly eclosed adults that millers can accept to produce flour that meets the FDA Defect Action Level is 2 infested kernels in 100 g of wheat (95% PL = 14.0 – 66.7 fragments in 60 g of flour).

![Graphs showing the relationship between number of infested kernels/100 g of wheat and number of insect fragments produced](image)

Fig. 1. Relationship between number of infested kernels/100 g of wheat with larvae (A), pupae (B), and pre-emergent adults (C) and number of insect fragments detected in milled flour samples by using the standard flotation method. Solid lines are from equations 1, 2, and 3.
Prediction of Insect Fragments in Flour by NIRS: Cognis-QTA™ FT-NIR: NIR spectra generated with this spectrometer correlated well with the actual number of insect fragments present in flour samples produced from wheat infested with larvae, pupae, or newly eclosed adults of the lesser grain borer (Table 2, Fig. 2). Based on inverse predictions, a 60-g flour sample that had a maximum of 56, 59, 44, or 45 insect fragments, based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be below the FDA defect action level of 75 fragments in 50 g of flour (upper 95% confidence limit would be below 90 fragments in 60 g of flour). Samples with 125, 116, 145, or 136 insect fragments or more based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be above the FDA defect action level of 75 fragments in 50 g of flour (lower 95% confidence limit would be above 90 fragments in 60 g of flour). It follows that one would not be able to determine whether the number of insect fragments in a sample was above or below the action level when NIRS predicts 57 – 124, 60 – 115, 45 – 144, or 46 – 135 insect fragments in 60-g samples containing larvae, pupae, pre-emergent adults, or all stages combined, respectively.

Table 2. Equations describing the relationship between the number of insect fragments present in flour and the number of insect fragments predicted by using NIRS.

<table>
<thead>
<tr>
<th>NIR spectrometer</th>
<th>Stage</th>
<th>Wavelength range used in model (nm)</th>
<th>$n$</th>
<th>Equation Parameters $^a$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$a \pm SE$</td>
<td>$b \pm SE$</td>
</tr>
<tr>
<td>Cognis-QTA</td>
<td>Larvae</td>
<td>1055 - 2500</td>
<td>47</td>
<td>12.54 ± 3.97</td>
<td>0.8684 ± 0.0402</td>
</tr>
<tr>
<td></td>
<td>Pupae</td>
<td></td>
<td>48</td>
<td>5.644 ± 3.26</td>
<td>0.9087 ± 0.0487</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td></td>
<td>50</td>
<td>13.73 ± 5.84</td>
<td>0.9016 ± 0.0384</td>
</tr>
<tr>
<td></td>
<td>All$^b$</td>
<td></td>
<td>146</td>
<td>14.10 ± 2.84</td>
<td>0.8478 ± 0.0261</td>
</tr>
<tr>
<td>Pertem 7000</td>
<td>Larvae</td>
<td>550 - 1700</td>
<td>48</td>
<td>6.152 ± 3.82</td>
<td>0.9224 ± 0.0387</td>
</tr>
<tr>
<td></td>
<td>Pupae</td>
<td></td>
<td>46</td>
<td>7.514 ± 4.15</td>
<td>0.8649 ± 0.0612</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td></td>
<td>49</td>
<td>9.722 ± 6.40</td>
<td>0.9241 ± 0.0417</td>
</tr>
<tr>
<td></td>
<td>All$^b$</td>
<td></td>
<td>141</td>
<td>14.25 ± 3.78</td>
<td>0.8171 ± 0.0348</td>
</tr>
<tr>
<td>Foss 6500</td>
<td>Larvae</td>
<td>650 - 2250</td>
<td>46</td>
<td>11.47 ± 5.01</td>
<td>0.8818 ± 0.0497</td>
</tr>
<tr>
<td></td>
<td>Pupae</td>
<td></td>
<td>48</td>
<td>7.881 ± 3.74</td>
<td>0.8473 ± 0.0571</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td></td>
<td>48</td>
<td>31.29 ± 9.52</td>
<td>0.7742 ± 0.0622</td>
</tr>
<tr>
<td></td>
<td>All$^b$</td>
<td></td>
<td>145</td>
<td>51.22 ± 4.87</td>
<td>0.3933 ± 0.0438</td>
</tr>
</tbody>
</table>

$^a$ Relationship is $y = a + bx$, where $y$ is NIRS-predicted number of fragments and $x$ is actual number of fragments.

$^b$ Larvae + Pupae + Adults.

Perten Diode Array 7000: NIR spectra generated with this spectrometer correlated well with the actual number of insect fragments present in flour samples (Table 2, Fig. 3). Based on inverse predictions, a 60-g flour sample that had a maximum of 56, 51, 39, or 29 insect fragments based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be below the FDA defect action level of 75 fragments in 50 g of flour (upper 95% confidence limit would be below 90 fragments in 60 g of flour). Samples with 122, 121, 147, or 147 insect fragments or more based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be above the FDA defect action level of 75 fragments in 50 g of flour (lower 95% confidence limit would be
above 90 fragments in 60 g of flour). It follows that one would not be able to determine whether the number of insect fragments in a sample was above or below the action level when NIRS predicts 57 – 121, 52 – 120, 40 – 146, or 30 – 146 insect fragments in 60-g samples containing larvae, pupae, pre-emergent adults, or all stages combined, respectively.

_Foss NIR Systems 6500:_ NIR spectra generated with this spectrometer correlated well with the actual number of insect fragments present in the flour samples (Table 2, Fig. 4). Based on inverse predictions, a 60-g flour sample that had a maximum of 49, 51, 19, or 8 insect fragments based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be below the FDA defect action level of 75 fragments in 50 g of flour (upper 95% confidence limit would be below 90 fragments in 60 g of flour). Samples with 132, 119, 181, or 165 insect fragments or more based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be above the FDA defect action level of 75 fragments in 50 g of flour (lower 95% confidence limit would be above 90 fragments in 60 g of flour). It follows that one would not be able to determine whether the number of insect fragments in a sample was above or below the action level when NIRS predicts 50 – 131, 52 – 118, 20 – 180, or 9 – 164 insect fragments in 60-g samples containing larvae, pupae, pre-emergent adults, or all stages combined, respectively.

![Fig. 2. Relationship between NIRS-predicted and observed number of insect fragments in flour samples produced from wheat infested with larvae (A), pupae (B), pre-emergent adults (C), and the three life stages combined (D). Calibration models generated with the Cognis-QTA NIR-instrument. Reference line shows perfect correlation.](image-url)
Discussion

Wheat kernels infested with a single pre-emergent adult contributed about 28× and 10× as many fragments as wheat kernels infested with a single larva or pupa, respectively. This may be due to the fact that the larval and pupal exoskeletons are weakly sclerotized, compared to the adult stage, and only the most heavily sclerotized structures of their bodies are able to resist the milling process or the hydrochloric acid used for the standard flotation method.

The number of insect fragments detected with the standard flotation method was directly proportional to the infestation level, similar to results found by Harris et al. (1952) and Atui et al. (2002). Contrary to our results, Brader et al. (2002) found no strong correlation between the fragment counts and the level of infestation by late instar larvae of granary weevil, *Sitophilus granarius* (L.), perhaps because of their sampling protocol, which included subsampling. They infested batches of 250 g of wheat with 0 to 60 infested kernels and prepared sub-samples of 50 g, which they assumed would have homogeneous distributions of 0 to 12 infested kernels. Russell (1988) showed that the insect distribution in sub-samples taken from the same grain sample is not homogeneous. In the case of insect fragments, Brader et al. (2002) showed that both false positive and negative counts occurred in results from three laboratories with the standard flotation method. The standard flotation method requires highly trained technicians in microanalytical entomology to recognize insect fragments in flour (Kurtz and McCormack 1965).

![Diagram](image-url)

Fig. 3. Relationship between NIRS-predicted and observed number of insect fragments in flour samples produced from wheat infested with larvae (A), pupae (B), pre-emergent adults (C), and the three life stages combined (D). Calibration models generated with the Perten 7000 NIR-instrument. Reference line shows perfect correlation.
We developed equations describing the relationships between the number of insect fragments produced and the level of insect infestation for each lesser grain borer life stage. These equations will be useful in predicting the maximum level of internal infestation that can be accepted by millers to produce flour with insect fragment counts below the FDA defect action level. If the grain is mainly infested with pupae and larvae, the level of allowable maximum infestation fluctuates from 380 to 640 infested kernels/kg, respectively. But when the grain is internally infested primarily with pre-emergent adults, the level of maximum infestation is reduced to less than 20 infested kernels/kg. However, this last scenario may be less likely to occur in the milling industry because kernels infested internally with pupae and pre-emergent adults are weak and are more easily broken open by using impact machines, and then exposed insects may be removed by screens or aspiration (Sachdeva 1978, Mills and Pedersen 1992, Brader 1997). On the other hand, kernels containing insects in the early stages of development may not be broken open by the impact machines because they have not been sufficiently weakened by the insect (Sachdeva 1978, Mills and Pedersen 1992). As a result, several researchers have reported that most of the insect fragments present in flour are produced by the immature stages of the internal feeding insects. Our equations will be useful in sampling programs to determine how many insect fragments would be expected to be produced in flour milled from a sample of wheat, based on the number of internal insects of each stage detected in that sample of wheat.
NIR spectra generated using the three spectrometers were correlated with the actual number of insect fragments present in flour samples prepared from wheat infested with larvae, pupae, or pre-emergent adults. The QTA spectrometer gave the best estimates of insect fragment levels in flour samples in our tests. For the model combining data for all life stages, the QTA spectrometer would not be able to determine whether fragment counts were above or below the defect action level when actual fragments in a 60-g sample were between 46 and 135; similar levels for the Perten and Foss spectrometers were 30 – 146 and 9 – 164. The QTA spectrometer may have yielded the best predictions of the number of fragments in samples compared with the calibration models generated with the other two spectrometers because the QTA spectrometer continuously mixed the flour sample while collecting spectra whereas the other spectrometers sampled a static sample. In addition, the QTA and Perten spectrometers collect spectra for the whole sample, while the Foss spectrometer collects spectra for only a portion of the sample. Although the spectrometers are not highly accurate in determining number of insect fragments in flour samples, they provide results very quickly and could be used to screen a large number of flour samples. When the spectrometers indicate that either small numbers or large numbers of fragments are present, the standard flotation method would not need to be used. If the spectrometers are not able to determine whether the number of insect fragments is above or below the defect action level, then more samples could be processed quickly using NIRS or the standard flotation method could be used as a follow-up test.

Both the standard flotation method and the NIRS methods can detect and quantify the number of insect fragments produced by the lesser grain borer in wheat flour. The flotation method is more precise, but it is destructive, time consuming, expensive, and requires highly trained technicians. In contrast, although NIRS is less precise, it is rapid, non-destructive, does not require extensive sample preparation, and could easily be automated for a more sophisticated sampling protocol for large flour bulks.

Acknowledgments

We thank Laura McLaughlin for milling the wheat samples, and Ann Redmon for excellent technical assistance. We also gratefully acknowledge Perten Instruments, Cognis, and Foss NIRSystems for providing instrumentation for this study. Mention of trade names or commercial products in this article is solely for providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Monitoring of bruchids (Coleoptera: Bruchidae) in stored broad beans (Vicia faba L.)

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Abstract: Samples of threshed dried broad beans (Vicia faba L.) were collected in different places of Portugal, and bruchids (Coleoptera: Bruchidae) found infesting those seeds were identified. Independently of the geographical location, Bruchus rufimanus was the dominant species in Portugal. Parasitoids were also found inside the seeds. The percentage of attacked seeds in eight varieties of broad beans was compared. No differences in infestation levels were detected among the tested seed varieties.

Key words: bruchids, broad beans, Bruchus rufimanus, Vicia faba

Introduction

Legumes (Leguminosae) are a valuable source of proteins for both humans and animals, and constitute an important element in the diet of a large section of the world’s population, particularly of people in developing countries.

The seeds of broad beans (Vicia faba L.) are rich in proteins (30% of their dry weight) and contain most of the amino acids necessary for animal and human nutrition (Smartt, 1976). Legume seeds are particularly attacked by bruchids (Coleoptera: Bruchidae). Larvae bore into, and feed within seeds causing weight loss, decreased germination potential and reduced commercial value.

Several species of Bruchidae develop in V. faba seeds and cause high seeds losses (Desroches et al., 1995). One of them is the broad bean weevil, Bruchus rufimanus (Boh.). It is a univoltine species. Females oviposit on the green pods during spring, and the adults which emerge from the seeds undergo a reproductive diapause in winter, and enter the crop at the beginning of the flowering period (Tran & Huignard, 1992; Tran et al., 1993). This species is not able to reproduce in stores (Desroches et al., 1995).

A survey of bruchids in stored broad beans was conducted in Portugal in order to evaluate the presence of these insects in the country. Additionally, seeds of different varieties of broad beans were analysed in order to detect possible resistant varieties to bruchids attack.

Materials and methods

Survey on bruchids
A total of 70 samples of threshed and dried broad beans with symptoms of insect infestation were collected from stores located in different regions of Portugal, in October and November of 2001, 2002 and 2004 (Fig. 1).
Samples were relatively small, with a mean of 40 seeds/sample, because they were collected mostly at small producers’ farms, where small amounts of seeds are stored for the next season. Insects found loose on the seeds were also collected.

In the laboratory, the broad beans were cleaned from insects on their surface and incubated at approximately 27°C and 70% r.h., and after 6 weeks, emerged insects were collected. Those insects remaining inside closed windows or inside emergence holes, were dislodged from the seeds, by putting them into water for 24 h. Followed the mechanical destruction of the seeds, bruchids were identified.

Fig. 1. Geographical origin of broad bean samples collected in the study from different districts of Portugal. Legend to the number of samples per district: Aveiro (A) 3, Beja (B) 6, Braga (BR) 1, Bragança (BC) 6, Castelo Branco (CB) 12, Coimbra (C) 1, Évora (E) 1, Faro (F) 8, Funchal (FU) 1, Guarda (G) 11, Leiria (L) 4, Portalegre (P) 2, Santarém (SA) 2, Setúbal (SE) 7, Viana do Castelo (VC) 1, Vila Real (VR) 2, Viseu (V) 2.

Parasitoids detection
In the samples collected to survey bruchids, small emergence holes were found on the seeds, much narrower than those recognized as bruchids emergence holes. Those seeds were put into water during 24 hours, and were mechanically destroyed. The individuals found inside, in connection with those holes, were collected.
Bruchids attack on broad bean varieties


The varieties were bred in the National Plant Breeding Station (Elvas-Portugal): they were cultivated in a complete randomized block design (3 blocks) and, at harvest, in each block, the dried broad beans of each variety were stored in separated bags. In laboratory, a 300 g sample was collected from each bag, for a total of 24 samples.

In each sample, seeds presenting symptoms of bruchids attack (windows or emergence holes) were counted. A mean of 363 seeds per sample were analysed.

The percentage of attacked seeds was compared by ANOVA for a significance level (α) equal to 0.05.

Insects inside closed windows were mechanically dislodged as described above and those found loose inside the bags were also collected. The insects were then identified.

Results

Survey on bruchids

In the 70 samples collected all over the country, 100% of emerged bruchids and of those dislodged from closed windows were *Bruchus rufimanus*.

Other bruchids detected were *B. pisorum* and *Callosobruchus sp.*, they were found loose on stored broad beans.

Parasitoids detection

Inside the seeds, in association with the small holes, the only adults found were Hymenoptera (72 individuals). The species are being identified. First results indicate the presence of *Triaspis thoracica* Curtis (Braconidae).

Bruchids attack on broad bean varieties

All the collected infested samples contained *B. rufimanus*.

The percentage of attacked seeds varied between 6% and 19%, with a mean of 12%, depending on the variety (Table 1).

In relation to the percentage of infested seeds, there were no significant differences (α=0.05) between the varieties tested.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.06</td>
<td>0.09</td>
<td>0.10</td>
<td>0.11</td>
<td>0.13</td>
<td>0.13</td>
<td>0.14</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Error mean square: 0.003

Discussion

In this survey, conducted in several regions of Portugal, on stored broad beans, the only species that developed in broad beans was *Bruchus rufimanus*, also known as “broad bean weevil”. Since the literature indicates that this species does not develop a second generation in dry seeds, it was assumed that the insects detected in storage, must have originated from eggs oviposited in the field. Damages occur in the field, mainly owing to the feeding activity of the larvae, and in storage, owing to the emergence holes left by the adults when they emerge from the seeds.
Samples were collected after 2 months of storage, just before planting the next season crop. If samples had been taken at the beginning of storage, the possibility of having caught other bruchid species seems improbable. This is so because earlier oviposition dates in the field and/or a shorter developing period seems not possible. Those species would have been trapped inside the closed bags where farmer stored them, and they would have been collected as “loose insects” during the survey.

Resistance of Leguminosae to bruchids may be originated during host selection for oviposition, or by the inability of larvae to penetrate the seed, owing to the physical or chemical characteristics of the seed coat, or by the inability or delay of larvae development, owing to chemical properties in the cotyledon (Thiery, 1984; Boughdad et al., 1986; Oigiangbe & Onigbinde, 1996). The occurrence of some anti-nutritional factors (i.e., protease inhibitors, lectins, phytates, tannins, saponins) hamper the nutritional potential of legume proteins by interfering with the intake, availability or metabolism of nutrients (Fernández-Quintela et al., 1997). This is valid both for humans and bruchids. Differences have been detected between varieties of *V. faba* in the presence/absence of some anti-nutritional factors, and in their concentration, both in the seed coat and inside the cotyledons (Desroches et al., 1995; Makkar et al., 1997), which can be considered, at least in part, responsible for the different bruchids infestation rates observed in those studies.

In the present work no differences in bruchids infestation were detected between the varieties tested.

There are several methods of storage to control the bruchids or to prevent their development during storage. Among the well known methods to mention, are an initial fumigation, storage in a modified atmosphere, cooling or freezing the seeds after harvest. Any of these methods could provide a solution to avoid the aesthetic damages caused by adults’ emergence holes on the seeds, since larvae development initiated in the field would be stopped. These methods, well known for their effectiveness and lack of adverse effect on the organoleptic or germination qualities of the seeds, would need to be investigated for their adaptation as appropriate technologies in Portugal.

**Acknowledgements**

The authors are grateful to the producers who kindly collaborated in this work, and to Dr. Ahmed Boughdad (Meknès, Marocco) and Dr. Marcelino De los Mozos Pascual (Centro Albaladejito, Spain) for helping in bruchids identification.

**References**


The application of immunochemical methods in detection and traceability of arthropod contaminants in stored food

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Abstract: HACCP (Hazard Analysis and Critical Control Point) needs methods for the detection, identification and quantification of food contaminants, not only of living pests, but also of their products (like allergens, and quinone secretions). The main benefit of molecular methods consists in their ability to detect not only current infestation but also the residues of past infestations. These methods are applicable not only to whole arthropods or their fragments, but also to their metabolites (toxins or allergens). We reviewed the published results of application of antibodies on detection of 4 stored-product mites, 4 stored-product beetles and 1 cockroach species or their allergens. ELISA techniques were demonstrated to have a potential to detect stored product arthropods as well as their hazardous products. The immunological approach in combination with antibodies-based microarrays, based on highly parallel simultaneous analyses, has shown its potential for large-scale analyses. Such a screening technology would allow simultaneous detection of contaminants like allergens of microbial and arthropod origin as well as mycotoxins and pathogens.

Key words: food, allergens, mite, insect, antibodies

Introduction

One of the main political EU priorities is to maintain a high standard of food quality and safety. This priority area is aimed at assuring the health and well-being of the European consumers through higher food quality, improved control of food and related environmental factors (Jardine et al., 2003). Food safety is frequently endangered by the presence of various hazards including food contaminants and additives reaching unsafe levels. Contaminants, i.e. substances unintentionally added to food and present therein in the form of residues from production, processing, transport and storage must be kept at the lowest possible levels (Sanders, 2003). Potential hazards associated with food and regulations to control these hazards are identified. The hazards include biological agents (microorganisms, pests and their metabolites); chemicals (e.g. toxins); or hazards of physical nature, such as ground glass or metal fragments.

During storage, agricultural commodities are often attacked by various pest organisms such as birds, rodents, insects, mites, and micro organisms. More than 600 beetles species and 70 species of moths among insects, 355 mites species, 40 rodents species and 150 micro fungal species have been reported to be associated with various stored products (Rajendran, 2002; 2005). The infestation of food by stored product arthropods (mites, psocids, beetles, cockroaches and moths) strongly decreases the safety of food, especially by the productions of allergens and carcinogen secretions (Ladisch et al., 1967; Ahmed 1998; Alanko et al., 2000, Arlian, 2002; Chambers, 2003). A variety of methods are currently developed and used to detect the stored product pest arthropod hazards. Detection of arthropod infestation is: (i) necessary to ensure a supply of wholesome food to the consumers (EU priorities), (ii) the
foremost step in pest management in stored food, and (iii) a tool to assess effectiveness of the 
application of pesticides and other treatments (Thind & Clarke, 2001, Cambell et al., 2004; 
Rajendran, 2005). The most common detection and monitoring methods for stored product 
arthropods consist in (i) trapping in various kinds of traps and (ii) sampling and extraction (cf. 
Hagstrum et al., 1990; Dowell et al., 1999; Thind, 2000; 2005; Cambell et al., 2004; 
Rajendran, 2005).

HACCP (Hazard Analysis and Critical Control Point) needs methods for the detection, 
identification and quantification of food contaminants, not only of living pests, but also of 
their products (like allergens, quinone secretions) (Raspor, 2005). Antibody-based methods, 
such as ELISA, are widely used to diagnose detectable levels of virus diseases and identify 
bacterial and fungal pathogens. New nucleic acid-based assays, mainly based on the 
polymerase chain reaction (PCR), are now available to identify many of the most important 
viruses and bacteria as well as eukaryotic pests and diseases (Liu-Stratton et al., 2004). The 
availability of this new technology opens up outstanding perspectives for the field of 
molecular diagnostics in its broadest sense. For example, diagnosis of all medically important 
pests on one single micro-array chip with an area of less than 5 cm² may now be possible. 
Micro-array-based molecular diagnostics is therefore an extremely powerful tool that will 
significantly advance modern agricultural diagnosis (Wang et al., 2002; Beuzen et al., 2000). 
The boom of molecular techniques not only provides promising methodical approaches for 
the analysis of hazards, but will contribute to the elimination of hazards in the food chain. 
Such techniques can be used to target control actions to problem areas thereby reducing costs, 
damage and pesticide usage.

The recent studies are focused to develop specific antibodies against stored product 
species and optimized immunochemical detection techniques that allow detect and quantify 
pests including their metabolites. The boom of molecular techniques provides promising 
methodical approaches not only for the analysis of stored product arthropods hazards, but also 
contributes to their elimination. In this review, we included the principles of immunochemical 
techniques as well as their application in identification of arthropod contaminants during food 
storage and processing.

**Molecular based methods for detection of stored-product arthropods**

Diagnostic methods, which have the accuracy limited to taxonomical knowledge, personal 
skills and instrumentation, are called classical or conventional. In contrast, methods based on 
unequivocal parameters, such as species specific molecules or DNA/RNA sequences that 
allow eliminating of subjective assessment are named modern; methods based on the two 
mentioned markers are called molecular methods.

Dead, hidden arthropods and their faeces represent an equally hazardous contamination. 
The allergen of stored product arthropods could be used as an example of the food 
contamination by hazardous products (Table 1). Up to present time 10 species of mites, 2 
species of cockroaches 2 species of beetles are known as allergen producers. Allergens can 
persist and accumulate in stored products independently of pest population density. For 
example, the actual size of mite population does not directly correspond to allergen levels 
(Danielsen et al., 2004). If mites are fumigated, the production of their allergens will 
discontinue, but dead mites, faecal pellets, and cuticle fragments still contain allergens (Tovey 
et al., 1981).

This contamination, however, is hardly detectable by conventional methods usually 
determining the occurrence and/or density of arthropod pests. There is therefore an urgent 
need for an objective, rapid and accurate method, which would detect and trace these
contaminants. An alternative approach is to detect these specific pest products by means of molecular methods. An ideal diagnostic test should possess the following properties: accuracy, sensitivity, simplicity, rapidity, cheapness, and safety. Sensitivity and accuracy are vital, but a test should be also cheap, easy to perform, and give swift results to find wide application. The more sensitive and specific methods for detecting pathogens/pests are currently based either on antibodies (Abs), which recognize particular antigens (i.e. allergens, toxins), or on nucleic acid probes, which target genomic sequences characteristic of the pest arthropod.

Table 1. The allergen contaminants of arthropod origin endangered stored food. (WBH – whole body homogenates; ?? allergens were not characterized by biochemical methods)

<table>
<thead>
<tr>
<th>Species</th>
<th>contaminant</th>
<th>WBH</th>
<th>Faeces</th>
<th>kit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acarus siro</td>
<td>Aca s 13 fatty acid binding prot</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td>Glycyphagus domesticus</td>
<td>Gly d 2</td>
<td>+</td>
<td>+</td>
<td>1D-8 anti Group 2</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
</tr>
<tr>
<td>Lepidogyphus destructor</td>
<td>Lep d 2</td>
<td>+</td>
<td>+</td>
<td>1D-8 anti Group 2</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
</tr>
<tr>
<td></td>
<td>Lep d 5</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lep d 7</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lep d 10 tropomyosin</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lep d 13 fatty-acid binding protein</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td>Tyrophagus putrescentiae</td>
<td>Tyr p 2</td>
<td>+</td>
<td>+</td>
<td>1D-8 anti Group 2</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
</tr>
<tr>
<td></td>
<td>Tyr p 13 fatty-acid binding protein</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td>Thyreophagus entomaphagus</td>
<td>??</td>
<td>?</td>
<td>?</td>
<td>Musken et al. 2003</td>
<td></td>
</tr>
<tr>
<td>Cheyletus eruditus</td>
<td>??</td>
<td>?</td>
<td>?</td>
<td>Neto et al. 2002</td>
<td></td>
</tr>
<tr>
<td>Blatella germanica</td>
<td>Bla g 1</td>
<td>+</td>
<td>+</td>
<td>10A6 anti Bla g 1</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
</tr>
<tr>
<td></td>
<td>Bla g 2 aspartic protease</td>
<td>+</td>
<td>+</td>
<td>7C11 anti Bla g 2</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
</tr>
<tr>
<td></td>
<td>Bla g 4 calycin</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bla g 5 glutathione transferase</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bla g 6 troponin C</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td>Periplaneta americana</td>
<td>Per a 1 Cr-PII</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per a 3 Cr-PI</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per a 7 tropomyosin</td>
<td>+</td>
<td>-</td>
<td><a href="http://www.allergen.org">www.allergen.org</a></td>
<td></td>
</tr>
<tr>
<td>Sitophilus granarius</td>
<td>??</td>
<td>?</td>
<td>?</td>
<td>Herling et al. 1995</td>
<td></td>
</tr>
<tr>
<td>Ephestia kuehniella</td>
<td>??</td>
<td>?</td>
<td>?</td>
<td>Armentia et al. 2004</td>
<td></td>
</tr>
</tbody>
</table>

**Nucleic acid-based methods**

Nucleic acid probes, which hybridize with DNA or RNA sequences characteristic of a particular pathogen, represent a more recent development arising from recombinant DNA technology. The sensitivity of the nucleic acid hybridization assay is comparable to that of immunochemical tests such as ELISA, and, similarly to ELISA, the results can be quantified. The limits for the detection by nucleic acid probes can be greatly enhanced by using the PCR to synthesize multiple copies of a particular nucleotide sequence.

Phillips & Zhao (2003) found that hidden infestation of *Rhyzopertha dominica* and *Sitophilus* spp. in food grains could be detected by DNA markers using PCR at the level of one larva per kg of grain. This approach was not successful for the detection of *Tyrophagus putrescentiae* by PCR with ribosomal DNA primers from the spider mite *Tetranychus urticae* (Navajas et al., 1998; Phillips & Zhao 2003). Zouhar et al. (2005) detected flour mite *Acarus siro* using published rDNA primers. The detection limit was determined as 5 individuals per one gram of flour (Angus et al., 2004).

Up to present time, all these technologies are integrated into micro-arrays. Micro-array technology refers to techniques that allow simultaneous analysis of many thousands of
individual tests. DNA-microarrays were first to boom in medicine, but there are also references related to the diagnostics of pathogens.

**Immunochemical methods**

Antibodies are animal proteins produced in response to the presence of foreign molecules, organisms, or other agents in the body. Antibodies are synthesized predominantly by plasma cells, terminally differentiated cells of the B-lymphocyte lineage, and they circulate throughout the lymph, where they bind to the antigen (Harlow & Lane, 1999).

Antibodies for diagnosis can be obtained from a number of sources. The original antibodies were obtained from the sera of immunized animals. Polyclonal antibodies (Pabs) are raised by injecting antigens into an animal, usually a rabbit, according to an immunization scheme and its collecting blood. Pabs are purified from the raw serum fraction of the blood. They represent a complex mixture of different immunoglobulin types directed towards different antigenic determinants (multiple sites on the antigen) with varying affinities (Werres & Steffens, 1994). In contrast to polyclonal antibodies, monoclonal antibodies (Mabs) are highly selective and unlimited amounts of equal quality can be produced in *in vitro* cultures or in animals. Mabs are made by fusing antibody-producing cells (lymphocytes) from the spleens of an immunized animal (usually mice or rats) with cultured myeloma cells. This generates many hybrid cell lines (hybridomas) each producing a different single (monoclonal) antibody. These individual cell lines are propagated and single monoclonal antibodies are harvested from the supernatants of tissue cultures or ascitic fluids. Mabs have powerful applications in biomedical research, diagnosis, and therapy (Bean, 2001).

As indicated by their name, polyclonal antibodies from antisera bind to a number of epitopes. Pabs are isolated from the serum of an immunized animal. There are typically thousands of different antibodies reacting with numerous epitopes. Polyclonal antisera usually contain most of the immunoglobulin types and a large range of affinities for the different epitopes. For many applications such as electron microscopy, Pabs offer some important advantages over Mabs. For example, when searching for an antigen with multiple epitopes, one polyclonal antibody can reach many binding sites, increasing the detection sensitivity. When the first paper on the process for creating Mabs was published (Köhler & Milstein, 1975), it seemed that Pabs were relict of the past. However, there are limitations to the uses of Mabs, and recent developments in solid phase synthesis of peptides extend the application of Pabs into areas previously thought to be exclusive to Mabs. It is now relatively straightforward to produce monospecific polyclonal antibodies via affinity purification using short peptide antigens that represent a single epitop. In addition, epitopes in nature may change in conformation, resulting in total failure of detection with Mabs (Harlow & Lane, 1999).

Immunochemical tests utilizing the specificity of antigen-antibody reactions are originally base on polyclonal antisera containing a mixture of antibodies, which often reduced the reliability of the test due to non-specific cross-reactions. The development of Mabs, which have narrow specificity against a single type of antigen, has greatly improved the accuracy of such tests. Mabs can, for instance, discriminate between different species, whereas polyclonal sera often react with several species within a genus (Ward *et al*., 2004). Along with improvements in the specificity of immunochemical tests, there have been advances in sensitivity, such that tiny amounts of an antigen can now be detected. Along with being very sensitive, this test is also quantitative, as the intensity of colour is proportional to the amount of antigens (pests, their products) in the tested samples. It can, therefore, be used to estimate the level of infestation as well hazards of infested food.
Application of immunochemical methods for detection of stored-product arthropods

Immunochemical assays involving immuno-diffusion, immuno-osmophoresis and ELISA are currently available techniques for the detection on pest arthropods (Rotundo et al., 2000; Brader et al., 2002; Rajendran 2005) (Table 2). These methods are routinely used in clinical diagnostics tests and are utilized with increasing tendency also in the field of agriculture, e.g. in the detection of pesticide residues, mycotoxins and other hazard factors (Radon et al. 2000; Park et al. 2004; Yu et al. 2005; Zheng et al. 2005). In addition, the epidemic proportion of allergen diseases (Holgate, 1999) induced an increase in the immunodetection of mite and cockroach associated allergens (Table 1) in house dust, and commercial kits have been already developed (Indoor-Biotechnologies, http://www.inbio.com). But in comparison to other pathogenic organisms, immunochemical techniques started to be employed later to detect stored-product arthropods, are less studied and relatively infrequent.

Table 2. The list of species or their products which is possible to detect by ELISA tests.

<table>
<thead>
<tr>
<th>Detection</th>
<th>Antibodies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>individuals, faeces</td>
<td>Pabs</td>
<td>Kudlikova et al. 2004</td>
</tr>
<tr>
<td>Gly d 2 - individuals, faeces</td>
<td>1D-8 anti Group 2</td>
<td>Dunn et al. 2002a</td>
</tr>
<tr>
<td>Lep d 2 - individuals, faeces</td>
<td>1D-8 anti Group 2</td>
<td></td>
</tr>
<tr>
<td>individuals</td>
<td>MAbs + Pabs</td>
<td></td>
</tr>
<tr>
<td>Tyr p 2 - individuals, faeces</td>
<td>1D-8 anti Group 2</td>
<td></td>
</tr>
<tr>
<td>individuals, faeces</td>
<td>Pabs</td>
<td>Kudlikova et al. 2005a</td>
</tr>
<tr>
<td>Bla g 1 - faeces</td>
<td>10A6 anti Bla g 1</td>
<td></td>
</tr>
<tr>
<td>Bla g 2 -faeces</td>
<td>7C11 anti Bla g 2</td>
<td></td>
</tr>
<tr>
<td>individuals</td>
<td>PAbs+MAbs</td>
<td>Stuart et al. 1994</td>
</tr>
<tr>
<td>individuals</td>
<td>PAbs+MAbs</td>
<td>Chen et al. 1993</td>
</tr>
<tr>
<td>individuals</td>
<td>Mabs anti myosin</td>
<td>Quinn et al., 1992</td>
</tr>
<tr>
<td>individuals</td>
<td>Mabs anti myosin (Biotec®, Austin)</td>
<td>Atui et al. 2003a;2003b</td>
</tr>
<tr>
<td>individuals, faeces</td>
<td>Pabs</td>
<td>Kudlikova et al. 2005b</td>
</tr>
</tbody>
</table>

The main benefit of immunochemical methods for the detection of arthropods consists in their multi-level application; (i) assay of food remnants in the gut of predators and hyperparasitism; (ii) assay of different products of mite or insect origin (allergens), (iii) assay of species-specific contamination or (iv) assay of contamination caused by larger taxonomic groups (e.g. mites, beetles, and moths).

For example, Hagler et al. (1997) developed an ELISA technique to detect pink bollworm, Pectinophora gossypiella (Lepidoptera: Gelechiidae) eggs in whole body homogenized adults of the predator Hippodamia convergens (Coleoptera: Coccinellidae). Naranjo & Hagler (2001) successfully utilized ELISA to detect remnants of the prey P. gossypiella in the gut of the predatory bug Orius insidiosus as a tool for the development of a predation model. Pab against the protein fraction of the mite T. putrescenitae enables the detection of remnants of this species in the whole body homogenates of predatory mite Cheyletus malaccensis (Kudliková et al., 2005a).

The mite allergens of the first group Der p 1 and Der f 1 (produced by the house dust mites Dermatophagoides pteronyssinus and D. farinae) can be detected by commercial monoclonal antibodies (Chien et al., 2000). There are commercially available mono/poly abs against the second group of allergens (i.e. rLep d 2, Pab Lep d 2, Pab Tyr p 2) of unknown
biochemical function (Parvaneh et al., 2002, http://www.inbio.com). Danielsen et al. (2004) applied commercial Mab for the detection of production and degradation of Lep d 2 in the grain contaminated by Lepidoglyphus destructor under laboratory conditions. The levels of Lep d 2 correlated with the abundance of the mites.

The detection on species-specific level was carried out mostly by sandwich ELISA formats using a combination of Mabs and Pabs. An exception constitutes the work of Stuart et al. (1994), where specific Mabs against Trogoderma granarium were developed with the exclusive aim to determine individuals caught in a trap to the species level. This assay could rapidly and accurately distinguish T. granarium adults, pupae and larvae from six other Trogoderma species.

The Pabs prepared against antigen of Tribolium castaneum showed no cross reactivities to stored product mites, moths, micro-fungi, but cross reactivity to other Tribolium species (T. destructor, T. confusum) was significant. The Pabs enable to detect Tribolium spp. adults, larvae and eggs as well as the faeces with different sensitivity (Kudlíková et al., 2005b). The Pabs prepared against antigen in the form of protein fraction from whole body homogenates of Acarus siro showed no cross-reactivity with stored beetles, moths, other mite species, micro-fungi, the rearing diet of mites (yeast and wheat diet) and extracts from wheat kernels. Cross-reactivity to closely related species Acarus gracilis was found (Kudlíková et al., 2004).

Mab against a 39kDa allergen (Härfast et al., 1992; 1996) was applied in combination to Pab in the development of a detection technique for the stored product mite Lepidoglyphus destructor (Dunn et al., 2002a). The detection technique rendered satisfactory results in the concentration range from 1 to 100 adult mites per 5 g of grain (Dunn et al., 2002a; b). The same techniques (DAS-ELISA) based on capture Mab and detecting Pab against a specific protein purified from Sitophilus granarius enabled to detect this pest among a mixed population of insects infesting wheat. This species-specific assay allowed detecting the pest quantitatively in laboratory conditions in the range from 5 to 10 adults (Chen et al., 1993).

The Pabs developed against protein fraction from T. putrescentiae showed strong cross-reactivities to mites of Acaridae, Carpoglyphidae and Glyciphagidae family (Klaudyová et al., 2005), but no cross-reaction was found out for stored product insects, micro-fungi. It indicates that these Pabs had a potential to detect stored product mite contamination including the faeces (Kudlíková et al., 2005a).

To detect and measure a wide range of insect contamination, an antibody against the insect muscle protein myosin was prepared (Quinn et al., 1992). The ELISA test sensitivity was about 20ng of purified insect myosin, which means that the test was able to detect 1 adult of S. granarius in 50 g of grain in spiked samples (Quinn et al., 1992). Additional studies showed that this test was not able to detect eggs and stored product mites. A commercial ELISA test (Biotec®, Austin) detecting insect myosin was applied for the detection of contaminated flour prepared from grain infested by R. dominica. The detection limit of the immunoassay was 0.5 insect in a 50g grain sample (Atui et al., 2003a). Atui et al., (2003b) confirmed the adequate stability of myosin during some time in flour for application of the ELISA method in R. dominica detection.

ELISA techniques were demonstrated to have a great potential to detect mites or insects in stored products as well as their hazardous products. In spite of promising results of specificity and sensitivity of newly prepared antibodies, serious difficulties appear with optimizing real grain and flour samples to establish reliability and reproducibility of immunochimical tests. It might be the reason, that up to present time, there is not any commercial kit against stored-product pests, which enables direct detection, and quantification of plant food, and commodities contamination.
The future perspectives of immunochemical detections

Antibodies are highly specific targeting agents and very valuable tools for in vitro diagnostic applications. Miniaturized and parallelized immunoassays are of general interest for all diagnostic applications, where several parameters in an individual sample have to be determined simultaneously from a limited amount of material. New trends in technology, mainly in microtechnology and microfluidics, newly established detection systems and improvements in computer technology and bioinformatics were rapidly integrated into the development of microarray-based assay systems. Biochips have become important tools for life science research. Protein microarrays are still in an early phase of development, but the increase in the number of publications on protein microarrays clearly demonstrates their large potential (Templin et al., 2002).

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Evaluation and characterization of damage produced in packaging films by insect pests

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Abstract: Packaging of food products has an important role as a barrier for insect pest and plastics films are among the most effective materials. In this work, the penetration ability of *Rhyzopertha dominica*, *Sitophilus oryzae* and *Oryzaephilus surinamensis* into different plastic films was assessed. The plastic films studied were polypropylene, polyethylene, and polyester, and a multilayer film (Paper, Polyethylene, Aluminium and Polyethylene). Damages observed in each material were evaluated under a binocular microscope. All three species were able to penetrate the films tested. *Rhyzopertha dominica* was the species with the highest penetration ability. The number of damages produced by all three species were higher in polyethylene than in polypropylene and polyester. In the multilayer film, *R. dominica* showed a similar penetration ability notwithstanding the film side exposed to the insect, since the Aluminium foil was the layer acting as a barrier to avoid the penetration of this species.

Key words: insect pests, food packaging, penetration, damage

Introduction

World expenses on packaging materials and equipment are very high and reach 240,000 million euros per year (Hanlon *et al.* 2000). Cardboard, paper and plastic films are the most important materials used. However, during the last years there has been an increase in the use of plastic and a decrease in the use of paper and cardboard, and nowadays, there is also an increase in the use of new materials such as bio-plastics. World consumption of plastic films for packaging is approximately 100 million tons, with more than 30 different types of materials. The most common plastic materials used are polyethylene, polypropylene and polyester. To combine the characteristics of this plastic films with other materials such as aluminium or cardboard, complex packaging materials and multilayer plastic films have been developed and their use is increasing.

Food products are packaged to protect them against external attacks due to handling, hits or other mechanical actions or against the effects of macro- and micro-organisms. Among macro-organisms, insects are one of the most important affecting the packaged final products. Food packages protect also from contamination of pathogens and prevent changes in the micro flora, or enzymatic reactions, lipid oxidation or hydrolysis, enzymatic or non enzymatic browning, vitamin degradation, changes in colour and physical or sensorial changes because of light, humidity or temperature. Finally, packaging has an important function in defining quantity, communication and presentation of the food product.

The objective of our work was to compare the resistance of several plastic films to insect attack.
Material and methods

Insect pest studied were *Rhyzopertha dominica*, *Sitophilus oryzae* and *Oryzaephilus surinamensis*. Tested films and thickness compared were: polypropylene 25µm and 40 µm (only for *R. dominica*); polyethylene 15µm, 50µm, 75µm and 150µm (only for *R. dominica*); polyester 12µm; multilayer film (paper, polyethylene 15µm, aluminium 7µm, polyethylene 30µm); and cigarette paper as a control treatment. We follow the material and methods described previously by Navarro et al. (1998) consisting in a device of two identical glass cylinders separated by a disc of the tested films and a disc of a wire mesh to provide anchoring points to the insects. Insects were kept inside a climatic chamber at 25ºC for 48 hours and afterwards we evaluated damages produced in each film and in the control. We carried out 5 replicates per tested film and insect specie with 10 insects in each replicate.

Damages found in the packaging materials were evaluated counting the number of completed holes, the number of impacts or intense damages without perforations, and the area covered with any kind of damage, using a logarithmic scale (score 0, no damage; 1, 0-3% of damaged area; 2, 4-9% of damaged area; 3, 10-27% of damaged area; 4, 28-100% of damaged area).

Results and discussion

The number of impacts and damaged area produced by *S. oryzae* with the presence of a wire mesh as an anchoring point in the test cells was much higher compared to the data obtained from cells without wire mesh (impacts, \( F=238; \) d.f.=3,19; \( P<0.001 \); damaged area, \( F=238; \) d.f.=3,19; \( P<0.001 \)) (Table 1). Insects having anchoring points greatly increase their ability to penetrate plastic films compared to the insects which stand free on the surface of the film. Similar results were obtained by Navarro et al. (2005) using the same device and *R. dominica* as the test insect.

Table 1. Number of impacts and damaged area index (mean ± SEM) in polyethylene of 50 µm and 75 µm made by *S. oryzae* with and without a wire mesh before the plastic film.

<table>
<thead>
<tr>
<th>Type of damages</th>
<th>Plastic film</th>
<th>Wire Mesh</th>
<th>Mean±SEM (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impacts</td>
<td>Polyethylene 50 µm</td>
<td>No</td>
<td>0b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>42,0±1,6a</td>
</tr>
<tr>
<td></td>
<td>Polyethylene 75 µm</td>
<td>No</td>
<td>0b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>38,4±2,5a</td>
</tr>
<tr>
<td>Damaged area (^2)</td>
<td>Polyethylene 50 µm</td>
<td>No</td>
<td>2,2±0,2c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>4±0a</td>
</tr>
<tr>
<td></td>
<td>Polyethylene 75 µm</td>
<td>No</td>
<td>2,0±0,3c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>3,0±0b</td>
</tr>
</tbody>
</table>

\(^1\) Means±SEM within a column for each type of plastic film followed by the same letter are not significantly different (\( P>0.05 \), Tukey test)

\(^2\) Damaged area was evaluated using a logarithmic scale (see material and methods)
In cigarette paper, *R. dominica* was the species that made the highest number of perforations ($F=17.09; \text{d.f.}=2.24; P<0.001$) (Table 2) and no differences were found among the number of holes made by *S. oryzae* and *O. surinamensis*. In the tested plastic films the number of holes were lower than in paper (Table 3). Regarding plastic films, *S. oryzae* and *O. surinamensis* only made perforations in the 15 µm polyethylene and in polyester (12 µm) but not in the thinner polypropylene film (25 µm). *Rhyzopertha dominica* was able to produce perforations in all the plastic films tested. In our experiment, significant differences in the number of holes according to the film thickness only were found between the 50 µm polyethylene, the 150 µm polyethylene and the 40 µm polypropylene ($F=2.54; \text{d.f.}=6.32; P<0.05$). Table 4 shows the results corresponding to the number of impacts produced by these 3 species in the different plastic films. Impacts could be considered as potential holes after a longer exposure period. The number of impacts were also higher in polyethylene than in polypropylene and polyester. The number of impacts in polyethylene were higher as thickness increased. Once first holes were made by the individuals kept inside the test cells, the rest of individuals could take advantage and escape through the holes present, which were larger in the thinner films tested. The total area damaged by *S. oryzae* was overall higher than the area damaged by the other 2 species in most of the polypropylene and polyethylene films tested (Table 5). In the polyester film tested, the area damaged by all three species was similar as this plastic is highly resistant to traction, hard, smooth and difficult to damage. *Rhyzopertha dominica* produced a higher level of damaged area in polyethylene and polypropylene than in polyester ($F=12.75; \text{d.f.}=6.32; P<0.001$). *Oryzaephilus surinamensis* produced a higher level of damaged area in polyethylene than in polypropylene and polyester ($F=21.14; \text{d.f.}=4.24; P<0.001$). Probably, the reason for this result was that polyethylene is softer and more flexible than polypropylene and polyester. Several authors have reported clear differences in insect penetration ability depending on film type and film thickness, polyethylene being the least resistant compared to the rest of plastic films (Cline 1978, Highland & Wilson 1981).

### Table 2. Number of holes (mean ± SEM) made by 3 stored product pest species on cigarette paper during 48 hours

<table>
<thead>
<tr>
<th></th>
<th>Number of holes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. dominica</em></td>
<td>20.9±2.1a</td>
</tr>
<tr>
<td><em>S. oryzae</em></td>
<td>3.0±0.6b</td>
</tr>
<tr>
<td><em>O. surinamensis</em></td>
<td>8.7±2b</td>
</tr>
</tbody>
</table>

Means±SEM followed by the same letter are not significantly different ($P>0.05$, Tukey test)

### Table 3. Number of holes (mean ± SEM) made by 3 stored product pest species in 3 different plastic films (n.t. not tested).

<table>
<thead>
<tr>
<th></th>
<th>Polypropylene</th>
<th>Polyethylene</th>
<th>Polyester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25µm 40µm</td>
<td>15µm 50µm 75µm 150µm</td>
<td>12µm</td>
</tr>
<tr>
<td><em>R. dominica</em></td>
<td>1.4±1ab 0.5±0.4b</td>
<td>1±0.4ab 5±1.8a 1±0.8ab 0.6±0.4b</td>
<td>2±0.9ab</td>
</tr>
<tr>
<td><em>S. oryzae</em></td>
<td>0a n.t.</td>
<td>0.8±0.6a 0a n.t. 1±0.7a</td>
<td>1±0.5a</td>
</tr>
<tr>
<td><em>O. surinamensis</em></td>
<td>0a n.t.</td>
<td>1.6±1a 0a n.t. 0.5a</td>
<td></td>
</tr>
</tbody>
</table>

Means±SEM within a line followed by the same letter are not significantly different ($P>0.05$, Tukey test)
Table 4. Number of impacts (mean ± SEM) made by 3 stored product pest species in 3 different plastic films (n.t. not tested).

<table>
<thead>
<tr>
<th></th>
<th>Polypropylene</th>
<th>25µm</th>
<th>40µm</th>
<th>Polyethylene</th>
<th>15µm</th>
<th>50µm</th>
<th>75µm</th>
<th>150µm</th>
<th>Polyester</th>
<th>12µm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R. dominica</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2±0,2c</td>
<td>0,8±0,4c</td>
<td></td>
<td>0,2±0,2c</td>
<td>4±1c</td>
<td>12±1,4b</td>
<td>23,8±2,8a</td>
<td>0,2±0,9c</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S. oryzae</strong></td>
<td>2,8±1,3b</td>
<td>n.t.</td>
<td></td>
<td>0,2±0,2b</td>
<td>42±1,6a</td>
<td>38,4±2,5a</td>
<td>n.t.</td>
<td>2±0,6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>O. surinamensis</strong></td>
<td>0a</td>
<td>n.t.</td>
<td></td>
<td>0a</td>
<td>0a</td>
<td>0a</td>
<td>n.t.</td>
<td>0a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means±SEM within a line followed by the same letter are not significantly different (P>0.05, Tukey test)

Table 5. Average (± SEM) damaged area index evaluated using a logarithmic scale (see material and methods) made by 3 stored product pest species in 3 different plastic films (n.t. not tested).

<table>
<thead>
<tr>
<th></th>
<th>Polypropylene</th>
<th>25µm</th>
<th>40µm</th>
<th>Polyethylene</th>
<th>15µm</th>
<th>50µm</th>
<th>75µm</th>
<th>150µm</th>
<th>Polyester</th>
<th>12µm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R. dominica</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,6±0,2b</td>
<td>2±0a</td>
<td></td>
<td>1,2±0,2b</td>
<td>2,3±0,2a</td>
<td>2,8±0,2a</td>
<td>2,6±0,2a</td>
<td>1±0b</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S. oryzae</strong></td>
<td>2,6±0,2b</td>
<td>n.t.</td>
<td></td>
<td>1,4±0,2c</td>
<td>4±0a</td>
<td>3±0b</td>
<td>n.t.</td>
<td>1,4±0,2c</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>O. surinamensis</strong></td>
<td>1,2±0,2c</td>
<td>n.t.</td>
<td></td>
<td>2±0b</td>
<td>2,8±0,2a</td>
<td>2,4±0,2ab</td>
<td>n.t.</td>
<td>1±0c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means±SEM within a line followed by the same letter are not significantly different (P>0.05, Tukey test)

Fig. 1. Percentage of perforations in each of the layers of a multilayer film (paper, polyethylene 15µm (PE15), aluminium 7µm, polyethylene 30µm (PE30)) produced by adults of *R. dominica* isolated on the paper layer.

In the multilayer film, the ability of *R. dominica* to penetrate from the external side (layer of paper) to the internal side (layer of polyethylene) was similar to the ability of the insect to penetrate from the external to the internal layer. (Figures 1 and 2). From the 100% of perforations found in the first layer only 7% and 4% respectively corresponded to holes crossing all layers. The aluminium foil was the layer acting as a barrier to avoid the penetration of this species, since only 13% and 14% respectively of the holes went in this layer.
Stored product pest species could be divided in two groups according to their capacity to penetrate into the food product and packaging materials. Borers are able to penetrate the packaging materials through the holes produced with their mandibles. Invaders use existing holes, scratches or apertures deriving from faulty sealing to colonize the packages. *Sitophilus* spp., *R. dominica*, *Plodia interpunctella*, *Lasioderma serricorne* and *Stegobium paniceum* are typical borer insects (Highland 1984). In contrast, *Tribolium* spp., *Cryptolestes ferrugineus* and *Oryzaephilus* spp. are considered invaders that are not usually able to penetrate closed packages (Highland 1991). This division is generally useful, although there are some exceptions. Borers could also use existing holes to penetrate into the packages (Cline & Press 1990) and some species classified as invaders might, under certain circumstances, make holes in the packaging films. At different development stages these species could have different ability to penetrate packaging materials (Cline 1978). In our experiment, the adults of *O. surinamensis* were able to penetrate the thinner polyethylene film and a 12 µm polyester film tested.

![Graph](image.png)

**Fig. 2.** Percentage of perforations in each of the layers of a multilayer film (paper, polyethylene 15µm (PE15), aluminium 7µm, polyethylene 30µm (PE30)) produced by adults of *R. dominica* isolated on the polyethylene layer.

**Acknowledgements**

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**References**


The comparison of population growth of stored product mites (Acari: Acaridida) under various temperatures

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Abstract: Stored product mites are of economical and medical importance, their feeding damaged grain and their allergens are dangerous for farmers, food industry workers and also for urban population. The pest potential of stored product mites depends on their population density and their reproduction rate. Temperature is one of the most important abiotic factors influencing reproduction rate. In this study we compare of population growth of three species (Acarus siro, Tyrophagus putrescentiae, Aleuroglyphus ovatus) under temperatures in the range from 5° to 35°C at 85% humidity. The start population was 20 individuals and final population was recorded after 21 days or after 42 days. The experiment was terminated by extraction of mites in modified Tullgren-Berlese funnels and counting the final population density. The population growth was influenced by the temperature, the response was Logan curve with broad optima from 20° to 30°C and the curves were similar for all tested species. The population growth was minimal at temperatures of 32.5° and below 15°C. These results indicate that for multiplication of mite population in stored grain are the most critical temperatures in the range from 20° to 30 °C.

This work was supported by the grant MZE -000-2700063.

Keywords: stored product mites, population growth, Acarus siro, Tyrophagus putrescentiae, Aleuroglyphus ovatus
Kernel-kernel communications and behaviour of *Sitophilus zeamais* Motschulsky

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**Abstract:** To assess the influence of mechanically damaged, insect damaged, and intact maize kernels on the behaviour of adults of *Sitophilus zeamais* Motschulsky in the laboratory, kernel-kernel interaction experiments were carried out. Three series of experiments (with olfactometer assays in arena) were conducted. In the first, mechanically damaged kernels, insect damaged kernels, and intact kernels were placed in traps containing arenas (three-choice tests). In this experiment, the kernels were conditioned in a plastic box with two caps: one cap containing 2 g of a certain “kernel status” (mechanically damaged, insect damaged, or intact kernels), and the other cap with 2 g of whole kernels. Five types of conditioning were assessed: 0, 1, 2, 7 and 14 days. In the second experiment, two trap-devices were placed in the arenas, containing each of the aforementioned conditioned whole kernels in all possible combinations. The conditioning duration tested in this case was 1 and 7 days. In the third test, two choice tests were placed in the arenas, containing fresh or stored conditioned whole kernels, or mechanically damaged kernels, or insect damaged kernels in pairs. Conditioned tests were carried out as above in boxes with plastic capsules containing 2 g of fresh maize (~8 months after harvest) or 2 g of stored maize (~20 months after harvest). Adults of *S. zeamais* are strongly attracted by the insect damaged kernels or by the conditioned kernels damaged by insects, in comparison with the other two-conditioned kernel categories considered. In addition, the conditioned mechanically damaged kernels are more or less attractive than the conditioned intact kernels. Conditioned whole kernels by allelochemicals coming from mechanically damaged kernels revealed less attractiveness if compared to the conditioned insect damaged kernels or to the conditioned intact kernels.

**Keywords:** Kernel-kernel interactions; *Sitophilus zeamais*; behavioural responses; stored maize

**Introduction**

When plants are attacked by insects, volatile chemical signals can be released, not only from the damaged parts, but also systemically from other parts of the plant and this continues after cessation of feeding by the insect. These signals are perceived by olfactory sensory mechanisms in both the herbivorous insects and their parasites. Evidence is mounting that such signals can also affect neighbouring intact plants, which initiate defence by the induction of further signalling systems, such as those that increase parasitoid foraging. Recently, it was found that certain plants can release stress signals even when undamaged, and that these can cause defence responses in intact plants. Virtually nothing is known about the signalling pathways activated in response to herbivores that feed on seeds or roots (Walling, 2000; Pickett *et al*., 2003; Trematerra *et al*., 2005).
Stored grain can vary in quality as a function of biotic and abiotic factors, and volatiles that come from grain can be indicative of the current grain “status” quality (Christensen & Kaufman, 1969; Sinha, 1990). Several studies demonstrate that grain volatiles may have important effects on host selection behaviour in granivores and that these effects may differ substantially among species sharing the same resources (Levinson & Levinson, 1978; Walgenbach et al., 1983, 1987; Walgenbach & Burkholder, 1986; Trematerra & Girgenti, 1989; Phillips et al., 1993).

Olfactory attraction of storage insects can be induced by a blend of volatiles such as the aroma of wheat or other cereals, while aggregation and feeding may be stimulated by less volatile food components including salts, sugars and lipids. Several saturated or unsaturated fatty acids with a chain length of 12 to 18 carbon atoms induce aggregation and/or feeding in adults and/or larvae of some species (Levinson & Levinson, 1978). The granary weevil, *Sitophilus granarius* (L.) was found to assemble in response to the total triglycerides and esters of long-chain fatty acids recovered from wheat, and in response to either oleic, stearic or palmitic acid (Donat, 1970; Nawrot & Czaplicki, 1978). Unprocessed oat stored for one year had increased levels of phenolic acids and aldehydes, and the increase of phenolic acids was most pronounced after storage at high relative humidity (Dimberg et al., 1996). The development of rancidity is generally considered to be a consequence of the deteriorative reactions of lipids, although deterioration of proteins and reactions of phenolic acids should not be excluded. During storage, two distinct reactions of oat lipids take place: 1) the hydrolytic deterioration where triacylglycerols or phospholipids are converted to free fatty acids and 2) the oxidative deterioration where polyunsaturated fatty acids are converted to hydroperoxides and further to secondary oxidation products.

In plants, hydroperoxidation of polyunsaturated fatty acids may be catalyzed by lipoxygenase; the fatty acid hydroperoxides are then metabolized to produce jasmonic acid, a regulatory molecule, the volatile compounds hexanal and hexenal as well as traumatin (wound hormone) and traumatic acid (Hildebrand, 1989; Siedow, 1991). Interestingly, a number of studies have demonstrated that lipoxygenase is activated as a result of wounding, mechanical stress and biotic stress and that lipoxygenase products have an adverse effect on plant pathogenic organisms, thus suggesting that lipoxygenase may have a role in wound healing and pest resistance in plants (Hildebrand, 1989; Mauch et al., 1997).

Older grains, particularly if infested by fungi, are known to have higher fatty acid content than fresh grain. The behavioural trends of stored-product insect species are regulated by the characteristics of the commodity. Hence very little is known for the cue role of the commodity itself (Landolt & Phillips, 1997). For instance, the condition of a given grain kernel may determine the behaviour of a given insect individual. Trematerra et al. (2000) found that wheat kernels damaged by the rice weevil *Sitophilus oryzae* (L.) were more attractive than intact or mechanically damaged kernels, for *Tribolium castaneum* (Herbst), *Tribolium confusum* J. du Val and *Oryzaephilus surinamensis* (L.).

However, intact, mechanically and insect damaged kernels may coexist in a storage facility. One of the main questions in this coexistence can be, if there are specific kernel-kernel interactions that can regulate insect behaviours, as reported in plant-plant interactions by Pickett et al. (2003).

In our experiments, we examined the behavioural responses of adults of the maize weevil, *Sitophilus zeamais* Motschulsky, to several kernel categories of maize, including the interactions between kernels of different status.
Materials and methods

Insects
The adults of *S. zeamais* used in the tests were taken from cultures that were kept in the laboratory in whole maize at 28±1°C and 70±5% relative humidity (r.h.) and continuous darkness.

Experiment 1
Conditioning of kernels have been realized in plastic boxes (9 x 12.5 x 4.5 cm) containing two caps (diam. 3 cm): a cap with 2 g of a certain “kernel status” (mechanically damaged, insect damaged, or intact kernels), and a cap with 2 g of whole kernels to be contaminated by the volatile semiochemicals released from time to time, from the different “kernel status”. According to the test, the kernels conditioning have been realized for different periods of time. All conditioning tests were conducted in controlled rooms set at 27±1°C with 70±5% r.h., and continuous darkness.

For olfactometer assays, the tests were carried out in a cylindrical arena of plexi-glass (45 cm diam. x 30 cm high). In the cylindrical arena three modified Flit-Track M² trap-devices (Trécé Inc, USA) (choice tests) were placed. In each trial 50 adult beetles of mixed sex and age were released at the centre of the arena.

The number of trapped insects was checked 15 h after their introduction in the arena. Nine replicates were performed for each “Case study”, using a total of 450 insects. To measure the different attractiveness of each kernels status, 2 g of conditioned whole kernels were used as bait for each trap. The conditioning period was 0 days, 1 day, 2 days, 7 days, and 14 days. Three traps (three-choice tests) for each Case study were baited with kernels under the following conditions:
- **Case study 1.** Without kernel-kernel conditioning, we compared mechanically damaged kernels; insect damaged kernels; intact kernels.
- **Case studies 2-3-4-5.** With kernel-kernel conditioning (after 1, 2, 7, and 14 days of conditioning, respectively) we compared whole kernels conditioned by semiochemicals coming from mechanically damaged kernels; insect damaged kernels; and intact kernels.

The data were submitted to a two-way ANOVA (for the main effects of kernel status and conditioning duration as well as the interaction). Means were separated by using the Tukey-Kramer HSD test at $\alpha=0.05$.

Experiment 2
The conditions were as above, but two-choice tests were placed in the arena. Six replicates were performed for the Case studies 1 and 2, using 300 insects for each Case.

After 1 day of kernel-kernel conditioning, we compared conditioned whole kernels by semiochemicals coming from:
- **Case study 1A:** mechanically damaged kernels vs. insect damaged kernels.
- **Case study 1B:** intact kernels vs. mechanically damaged.
- **Case study 1C:** intact kernels vs. insect damaged kernels.

After 7 days of kernel-kernel conditioning, we compared conditioned whole kernels by semiochemicals coming from:
- **Case study 2A:** mechanically damaged kernels vs. insect damaged kernels.
- **Case study 2B:** intact kernels vs. mechanically damaged kernels.
- **Case study 2C:** intact kernels vs. insect damaged kernels.

For each Case study data were separately analysed by using the two-tailed t test at $\alpha=0.05$ and n-2 df (Snedecor & Coehran, 1980).
Fig. 1. Plastic box for kernels conditioning (MD = mechanically damaged kernels; ID = insect damaged kernels; I = intact kernels; WK = whole kernels) and circular area used in the experiments (R = insects releasing point; T-D trap-device).

**Experiment 3**

These tests were two-choice tests, set as in the case of Experiment 2. Conditioned tests were carried out in plastic boxes (as above) containing plastic capsules in which 2 g of fresh maize (~ 8 months after harvest) or 2 g of stored maize (~ 20 months after harvest) in “different status” were placed. Two conditioning periods were used: 1 day and 7 days.

After 1 day of kernel-kernel conditioning, we compared conditioned whole kernels by semiochemicals coming from:
- **Case study 1A**: fresh intact kernels vs. stored intact kernels.
- **Case study 1B**: fresh mechanically damaged kernels vs. stored mechanically damaged kernels.
- **Case study 1C**: fresh insect damaged kernels vs. stored insect damaged kernels.

After 7 days of kernel-kernel conditioning, we compared conditioned whole kernels by semiochemicals coming from:
- **Case study 2A**: fresh intact kernels vs. stored intact kernels.
- **Case study 2B**: fresh mechanically damaged kernels vs. stored mechanically damaged kernels.
- **Case study 2C**: fresh insect damaged kernels vs. stored insect damaged kernels.
- For each Case, the data were analysed as in the case of Experiment 2.

For all experiments, all capture counts were transformed prior to analysis as suggested by Trematerra et al. (2000).

**Results**

**Experiment 1**

The overall data showed significant differences between kernel status (df=2.120; F=30.67; P<0.0001) (Table 1). In contrast, no significant differences were noted among conditioning duration (df=4.120; F=0.12; P=0.9743); the interaction was also not significant (df=8.120; F=0.67; P=0.7106). Without kernel conditioning, significantly more adults were found in trap-devices containing the insect damaged kernels, in comparison with those containing the mechanically damaged and the intact kernels. In this case, almost 45% of the total number of *S. zeamais* individuals were found in the traps with the insect damaged kernels. This trend
was also evident in the case of the conditioning kernel categories. Of the weevils released, a similar proportion was found in traps containing the kernels conditioned by semiochemicals coming from the insect damaged kernels. For 0 d: F=8.65, F=0.0015; for 1 d: F=6.26, P=0.0065; for 2 d: F=4.98, P=0.0155; for 7 d: F=4.29, P=0.0254; for 14 d: F=13.91, P<0.0001; and in all cases df=2.24.

Experiment 2
After 1 day of conditioning, significantly more adults were captured in the trap-devices that contained conditioned insect damaged kernels, than in those that contained conditioned mechanically damaged kernels (df=10, t=-2.729, P=0.0212) (Table 2). In contrast, no significant differences were noted between the same traps after 7 days of conditioning (df=10, t=0.452, P=0.6614). Similarly, no significant differences were noted between traps containing conditioned intact and mechanically damaged kernels, either at the 1 day or at the 7 days conditioning level (for 1 d: t=1.686, P=0.1228, for 7 d: t=1.344, P=0.2086, in both cases, df=10). Finally, traps baited with conditioned insect damaged kernels were significantly more attractive than those baited with conditioned intact kernels, for both semiochemicals conditioning periods examined (for 1 d: t=-4.406, P=0.0013, for 7 d: t=-2.457, P=0.0339, in both Cases, df=10).

Experiment 3
After 1 day of conditioning, no significant differences were noted in capture rates among trap-devices, for all cases examined (for Case study 1A: t=0.518, P=0.6157; for Case study 1B: t=-0.254, P=0.8049; for Case study 1C: t=0.668, P=0.5192; in all Cases df=10) (Table 3). After 7 days of semiochemicals conditioning no significant differences were noted in Cases 2A (t=-0.414, P=0.6875) and 2C (t=-0.535, P=0.6042), in both Cases df=10. In contrast, in Case 2B, significantly more weevils were found in traps containing kernels conditioned by allelochemicals coming from stored mechanically damaged kernels, in comparison with traps containing contaminated fresh mechanically damaged kernels (df=10, t=3.194, P=0.0096).

Discussion
Phagostimulatory responses of stored-product beetle species are equally, or even more important behavioural cues than pheromone sources (Landolt & Phillips, 1997; Cox & Collins, 2003; Collins et al. 2004). Several plant-derived volatiles have been proved to be determinative in stored-product beetle behaviour (Phillips et al., 1993; Landolt & Phillips, 1997; Trematerra et al., 1999, 2000; Bashir et al., 2002; Athanassiou et al., 2003).

However, there is still inadequate information on kernel-kernel interactions and behavioural responses in stored-product insects (Trematerra et al., 2005). The present study shows that adult maize weevils S. zeamais are strongly attracted by the insect damaged kernels or by the conditioned insect damaged kernels, than by the other two-conditioned kernel categories considered. In addition, the conditioned mechanically damaged kernels are more or less attractive than the conditioned intact kernels. The same happens, in most combinations tested here, for fresh and stored maize kernels.

There is no clear evidence about the possible indirect defence mechanism adopted by conditioned whole kernels, but in the tests, conditioned whole kernels by allelochemicals coming from mechanically damaged kernels revealed less attractiveness than conditioned insect damaged kernels or to the conditioned intact kernels.

In a previous study, Trematerra et al. (2000) reported that wheat kernels damaged by the rice weevil S. oryzae were more attractive for secondary colonizers than intact or mechanically damaged kernels. This is also true in the case of the primary maize colonizer S. zeamais. Hence, adult maize weevils seek for kernels that were contaminated by semio-
chemicals coming from kernels infested by adults of the same species. As a result, high populations may be built quickly around small sources of infestations. In addition, this behaviour was not influenced by the conditioning duration. This may suggest that the volatile semiochemicals derived from the insect damaged kernels are considerably different than those derived by intact or mechanically damaged kernels. The activity of semiochemicals of contaminated kernels damaged by insects remains the same even if the intact or the mechanically damaged kernels are close to the infested ones.

Table 1. Experiment 1, case study 1-5: behavioural responses of *S. zeamais*.

<table>
<thead>
<tr>
<th>Case study</th>
<th>Kernel status</th>
<th>Intact</th>
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</thead>
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<tr>
<td></td>
<td>Mechanically damaged</td>
<td>Insect damaged</td>
</tr>
<tr>
<td>1A</td>
<td>11.4 ± 1.8b</td>
<td>22.2 ± 1.7a</td>
</tr>
<tr>
<td>1B</td>
<td>13.6 ± 1.6b</td>
<td>20.1 ± 1.6a</td>
</tr>
<tr>
<td>1C</td>
<td>12.3 ± 1.8b</td>
<td>20.9 ± 3.0a</td>
</tr>
<tr>
<td>2A</td>
<td>14.1 ± 1.1b</td>
<td>19.3 ± 2.3a</td>
</tr>
<tr>
<td>2B</td>
<td>12.1 ± 1.3b</td>
<td>21.9 ± 2.0a</td>
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</tbody>
</table>

Table 2. Experiment 2, case study 1A-C and 2A-C: behavioural responses of *S. zeamais*.

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</thead>
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<td>1A</td>
<td>17.3 ± 2.9</td>
<td>29.3 ± 3.2</td>
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<tr>
<td>1B</td>
<td>18.7 ± 3.0</td>
<td>–</td>
</tr>
<tr>
<td>1C</td>
<td>–</td>
<td>30.8 ± 2.1</td>
</tr>
<tr>
<td>2A</td>
<td>20.8 ± 4.0</td>
<td>23.7 ± 4.8</td>
</tr>
<tr>
<td>2B</td>
<td>19.2 ± 1.7</td>
<td>–</td>
</tr>
<tr>
<td>2C</td>
<td>–</td>
<td>27.7 ± 2.8</td>
</tr>
</tbody>
</table>

Table 3. Experiment 3, case study 1A-C and 2A-C: behavioural responses of *S. zeamais*.

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<th>Case study</th>
<th>Kernel status</th>
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</thead>
<tbody>
<tr>
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<td>Mechanically damaged</td>
<td>Insect damaged</td>
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<tr>
<td></td>
<td>Fresh Stored</td>
<td>Fresh Stored</td>
</tr>
<tr>
<td>1A</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1B</td>
<td>24.2 ± 3.5 23 ± 3.2</td>
<td>–</td>
</tr>
<tr>
<td>1C</td>
<td>–</td>
<td>23 ± 2.6 24.5 ± 2.7</td>
</tr>
<tr>
<td>2A</td>
<td>–</td>
<td>23.9 ± 3.0 22.2 ± 2.8</td>
</tr>
<tr>
<td>2B</td>
<td>18.2 ± 2.1 26.4 ± 1.4 *</td>
<td>–</td>
</tr>
<tr>
<td>2C</td>
<td>–</td>
<td>23.9 ± 3.9 22.2 ± 4.1</td>
</tr>
</tbody>
</table>

According to our results, weevils “contaminate and stimulate” the kernels they infest, and this “contamination and stimuli” is permanent and long lasting, since it was not affected by time. From a practical point of view, this means that stock maize that contains some infested
kernels is more likely to be heavily infested by *S. zeamais* adults than maize with intact or broken kernels. In our experiments, the attractiveness of the kernel remains unaffected with time, since the behavioural trends were the same in fresh and stored kernels.

Moreover, conditioning did not change the kernel status. This was not true in the case of mechanically damaged kernels, where stored kernels were more attractive than fresh ones, after 7 days of conditioning. Hence, duration of conditioning may play a key role in the response of weevils to mechanically damaged kernels. However, in storage facilities where newly-harvested and aged maize is present at the same time, all kernel categories examined here are likely to coexist, and, based on our results, fresh and stored maize have the same possibility of being infested by *S. zeamais*.

Based on the results of the present work, the mechanical damage does not add to the “attractiveness” of the kernel, and this kernel is equally “vulnerable” as an undamaged kernel to the infestation by *S. zeamais*. Apparently, this could be attributed to the fact that, in Experiment 1, the presence of semiochemicals coming from infested kernels “dominated” over those from the other two kernel categories. Thus, weevils exhibited a strong preference over the infested kernels, which may not allow the expression of a behavioural preference between the other two kernel categories. However, the results of the two-choice tests in Experiment 2 indicated clearly that these two categories were more or less equally attractive, and that no change occurs with the increase of conditioning duration, at least in the duration examined here.

Nevertheless, insect damaged kernels were more attractive than mechanically damaged ones only at the 1 day conditioning interval; after 7 days of conditioning, mechanically damaged kernels were equally attractive with the infested ones. This may suggest that after 7 days of conditioning, the mechanically damaged kernels are sufficiently influenced by the presence of volatiles derived from the infested kernels, and this makes weevils unable to discriminate between broken and infested kernels. Since this is not in agreement with the results from the three-choice test in Experiment 1, we assume that this behaviour was revealed because in Experiment 2, only two-choices were available to the weevils.

The comparison of conditioned kernels damaged by insect and intact kernels indicated that infested kernels are much more attractive than the latter. In addition, this behaviour is not influenced by the duration of conditioning. This suggests that the simultaneous presence of infested kernels and intact kernels is attractive to adult maize weevils, only due to the presence of semiochemicals coming from the infested kernels, and not due to the interaction between the two kernel status. In other words, there is no behavioural influence between the two kernel types.

Host selection of stored-product beetles is different among primary and secondary colonizers (Phillips et al., 1993; Phillips, 1997; Landolt & Phillips, 1997; Trematerra et al., 1996, 1999, 2000). As for *S. oryzae*, Trematerra et al. (1999) reported that in pheromone traps, damaged kernels might have an additional capacity in attracting rice weevils, if compared to intact kernels. In the maize case of the maize weevil where adults seek for a spot that is suitable for infestation, and then the emission of an aggregation pheromone call additional individuals of the same species (Levinson et al., 1990). Also, the presence of saliva or frass may increase the emission of semiochemicals from specific parts of the seed, such as the germ or the kernel endosperm, which are highly attractive for *S. oryzae* adults. In addition, damaged seeds offer an easier access for weevil feeding (Trematerra et al., 1999).

Insect-kernel interactions have been examined for some species, especially primary grain colonizers. In contrast, very little is known regarding plant-plant or kernel-kernel interactions and our olfactometer assays suggest that these interactions may have a certain impact in
regulating stored-product insect behavioural trends. Insect damaged seeds and mechanically damaged seeds seem to affect other uninfested seeds, a fact which has a direct effect on how an infestation is manifested in the stored-grain ecosystem.

An interesting hypothesis may involve lipoxygenase activity. That lipoxygenase may have a role in pest resistance is particularly suggested by the immediate, massive burst of lipoxygenase products formation upon wounding of plant tissues. For instance, the production of volatile compounds increases dramatically upon the wounding of plant leaves and a major portion of the volatile products are apparently the result of lipoxygenase activity. Moreover, lipoxygenase products such as hexanal have an adverse effect on plant pests (Hildebrand, 1989).

References


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The comparison of allergen classes in stored-product and house-dust mites (Acari: Acaridida)

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*Corresponding author e-mail: hubert@vurv.cz

Abstract. Nowadays, 483 different proteins inducing IgE antibody in human have been investigated from all groups of allergen producing organisms. The allergens derived from the arthropods cover about 25 % of these compounds. Among arthropods, mites are the serious producers of allergens covering about 10 % of total allergens. Up to the present time, 20 classes of allergens and 49 IgE binding compounds have been described in house dust (Dermatophagoides pteronyssinus, D. farinae, D. siboney, Euroglyphus maynei and Blomia tropicalis) and stored product mites (Glycyphagus domesticus, Acarus siro, Lepidoglyphus destructor and Tyrophagus putrescentiae). The dual functionality of allergenic proteases increasing their importance has been discovered recently. Except passive allergen reaction, the cystein (Der p 1, Blo t 1, Der f 1) and serine (Blo t 3, Der p 3, Der f 3, Eur m3, Blo t 6, Der p 6 and Der p 9) proteases are able to provoke allergic reaction due to their enzymatic activity. These enzymes participate in mite digestion of both groups of stored product and house dust mites and accumulate in faeces in a high amount. In the faeces, proteases are still active and stable, because their decomposition rate is very low during time in the house conditions. The spectrum of mite species occurring in stored products is substantially wider than in house dust. Apart from the named species, the protein fractions of many other species are known to bind IgE of sensitive patients. Although biochemical characterization of their allergens is lacking, the unique IgE epitopes for their allergens most likely exist. It increases the medical importance of stored-product mites.

Key words: mite, allergen, storage, anaphylaxis, digestive enzyme

Introduction

The group of Acaridid mites includes extremely diversified species from parazitic to commensally living species in the soil and nests of animals or birds. Through the nests of birds and rhodents, some of these mites penetrated into human homes (Solarz et al. 1997; Solarz, 2003; Solarz & Senczuk, 2003). These „antropogenic“ mites are known as allergen producers. These species are classified into two artificial groups: (i) house dust mites (HDM) including members of Pyroglyphidae and Echimypodidae families living in the house dust and (ii) stored-product mites (SPM / families Acarididae, Glycyphagidae, Chortoglyphidae and Carpoglyphidae) inhabited stored food and feed (Bronswijk & Sinha 1971). The members of both groups are feeding on microscopic fungi, bacteria, and decaying plant cells. Moreover mites are able to feed on dead skin cells or hairs in house dust (Hughes 1976). More than 20 species of mites have been found in households all around the world (Solarz, 2000; 2001). These mite communities are seldom formed by a single species, they more frequently comprise multi-species complexes, usually both Dermatophagoides species (Bronswijk & Sinha 1971) in combination to „SPM“ (i.e. Aleuroglyphus ovatus, Lepidoglyphus destructor, Tyrophagus putrescentiae). In the Mediterranean Euroglyphus maynei and Blomia tropicalis are very common and numerous (Arlian, 2002; Fernandez-Caldas & Lockey 2004). Many SPM species are cosmopolitan in distribution with prevailed occurrence in humid climate.
Under suitable conditions, mites have potential to multiply their density to ten thousands individuals in one gram of food (Willey et al., 1998; Chambers, 2003).

Allergens causing allergic reactions may be inhaled, injected, ingested and absorbed through the skin or mucus membrane. Allergic diseases associated with food contamination, such as asthma, rhinitis, and eczema are reaching epidemic proportions in both the developed and developing world (Holgate, 1999). Mite allergens can cause anaphylaxis and anaphylactoid reactions after the ingestion of mite-infested food (Chambers, 2003; Matsumoto & Satoh, 2004; Sanchez-Borges et al., 2005). Anaphylaxis and asthma can be life threatening and every year deadly cases occur, often in young people, which are avoidable (Holgate, 1999; Edston & van-Hage-Hamstem, 2003). In recent time, many people have become sensitive to strong allergens present in bodies and faeces of house-dust and stored-product mites. The stored-product mites cause hypersensitivity not only in those who work with stored grain such as farm workers, millers and bakers, but they also seriously endanger the health of the city population (Luczynska et al., 1990; Musken et al., 2003).

The increase in the hypersensitivity of consumers to the allergens of stored-product mites requires intensive research focused on the biochemical characterization of these allergens and developing of the detection tools for allergen traceability. Although the majority of allergens have been described in house dust mites, there is enough evidence that identical allergens can present in stored product mites. Among them we focused and reviewed enzymatic allergens, it means digestive enzymes that are due to their dual allergenic function, both passive occurrence and enzymatic activity, responsible for immunological reaction of patients.

The sources of mite allergens

The allergens of mites are present in their bodies and faeces. If the mites are eradicated, the production of their allergens will discontinue, but dead mites, faecal pellets and cuticular fragments still contain allergens (Tovey et al., 1981). Therefore the allergens could persist and accumulate in stored products. The potential risk of allergen contamination is given by the allergen production including concentration of allergens in mites’ bodies and their faeces and by allergen degradation rate. The stability of allergens in the faeces is crucial for their possible accumulation in stored food. The microbial degradation of faeces and biochemical denaturation of the protein components depend on humidity and temperature (Danielsen et al., 2004) but relevant experimental data for allergens are missing so far.

For detection and research of allergens the mite extracts (whole body homogenates (WGH)) are used because the dissection of gut is impossible due to small size of mites, especially in the case of digestive enzymes. The allergens were isolated from extracts of different material including mites, juveniles, eggs, faeces and rearing medium (Thomas et al., 2002). The spent growth medium (SGM) after cultivation of mites substitutes the pure faces for mite allergen studies (Stewart et al., 1991).

The allergen classes and their biochemical characterisation

Allergens that share similar biochemical properties, biological function and have 67% or higher identity of amino acid sequences are assigned to the same group (Thomas et al., 2002). Allergens are designated as major if more than 50% of patients tested have serum IgE that reacts to the allergen.

Nowadays, 483 different human IgE binding compounds have been investigated (www.allergen.org) from all groups of allergen producing organisms (Fig. 1). Among arthropods, mites are the serious producers of allergens covering about 10 % of total allergens. Up
to the present time, 20 classes of allergens and 49 IgE binding compounds have been described in house dust (Dermatophagoides pteronyssinus, D. farinae, D. siboney, Euroglyphus maynei and Blomia tropicalis) and stored product mites (Glycyphagus domesticus, Acarus siro, Lepidoglyphus destructor and Tyrophagus putrescentiae, see Table 1). The recombinant techniques provide a method of examining the allergens without relying on their abundance and stability in the extracts, many allergens were detected directly by the screening of cDNA expression libraries for IgE binding with sera of allergic patients (Thomas et al., 2002). Despite of this, in some cases (groups 5, 7 and 12) the biochemical function of recombinant allergens has not been discovered yet (www.allergen.org).

![Fig. 1. The comparison of the numbers of allergens (based on www.allergen.org).](image)

Except previously named species, the IgE binding proteins have been recognized in Blomia tjibodas, B. kulagi, Acarus farris, Chortoglyphus arcuatus, Glycyphagus domesticus, Thyreophagus entomophagus (Muesken et al. 2003), but their biochemical classification is still missing.

**Group 1: Cysteine proteases**

Cysteine proteases are 25 kDa proteins isolated from Dermatophagoides spp., Euroglyphus maynei and Blomia tropicalis and are presented in both WGH and SGM. The sequences of allergens Der f 1 and Der p 1 showed 80% sequence homology, moreover Blo t 1 showed 35% homology to Der p 1. Mora et al. (2003) proved the existence of unique IgE binding epitope for Blo t 1. Based on immunostaining of D. pteronyssinus sections, secretion and synthesis of Der p 1 in the cells of posterior ventriculus was proved (Thomas et al., 1991; Tovey and Baldo, 1990). Der p 1 and Der f 1 are found in high concentrations in faeces (Tovey et al., 1981) and thus these allergens are frequently detected in high concentrations in house dust containing the faeces (ranging from 100 to 10,000 ng·g⁻¹) (Thomas et al., 2002). The whole body homogenates of stored product mites showed some cystein protease activity (Ortego et al. 2000, Hubert et al. 2006), its function as allergen is speculative.

A recent study has demonstrated that the cysteine protease Der f 1 of D. farinae is a very stable allergen in house dust. Natural decay of Der f 1 was estimated with a half-life of 10 years at housing conditions (Sidenius et al., 2002). However, we expect that protease Der f 1 is not only degraded by microorganisms but also inactivated by denaturation in faeces by their
endogenous activities. The ELISA assay used by Sidenius et al. (2002) can detect the protein allergen without enzymatic activity or even peptide fragments derived from the degraded allergen. As changes in enzymatic activity can be critical for the effective allergen-hazard potential of Der f 1 (see above), quantification by ELISA should be correlated with enzyme activity measurements for the assessment of the allergenic potential.

Table 1. The list of allergens isolated form house-dust and stored-product mites (based on www.allergen.org).

<table>
<thead>
<tr>
<th>Class</th>
<th>molecular weight kDa</th>
<th>biochemical function</th>
<th>species</th>
<th>Acanus siro</th>
<th>Blomia tropicalis</th>
<th>Dermatophagoides farinae</th>
<th>Eriophyes pterygygius</th>
<th>Erythromitus maynei</th>
<th>Glycyphagus domesticus</th>
<th>Lepidoglyphus destructor</th>
<th>Tyrophagus putrescentiae</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>cystein protease</td>
<td>Blo t 1</td>
<td>Der f 1</td>
<td>Der p 1</td>
<td>Der p 1</td>
<td>Gly d 2</td>
<td>Le p d 2</td>
<td>Tyr p 2</td>
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<td>14-18</td>
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<td>Aca s 2</td>
<td>Der f 2</td>
<td>Der p 2</td>
<td>Eur m 2</td>
<td>Gly d 2</td>
<td>Le p d 2</td>
<td>Tyr p 2</td>
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<td>Der p 4</td>
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<td>Eur m 4</td>
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<td>Blo t 7</td>
<td>Der f 7</td>
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<td>Der f 11</td>
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<td>Eur m 14</td>
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<td>Der f 16</td>
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</table>

**Group 2: Lysozyme-like proteins**

This group comprises small neutral to basic non-glycosylated proteins that contain three internal disulphide bridges which are essential for their immunogenicity (Robison et al. 1997). Lysozyme-like proteins represent one of the main groups of allergens of stored product mites, but they are also present in house dust mites. The lysozyme-like proteins were immunochemically localized in the gut and in the faeces of the house dust mites (Jeong et al., 2002).

In the past, these proteins were considered to be lysozymes. However, the purified protein from *D. pteronyssinus* (Der p 2) failed to exhibit any enzymatic activity (Hakkaart et al., 1997; Lee et al., 2004). Recently, Mathaba et al. (2002) studied the structure of these proteins and suggested their bacterial origin.

Cloning of cDNA and protein sequencing strategies has been applied to obtain sequence information for Der p2, Der f2 and Lep d2. Der f 2 and Der p 2 showed 88% sequence homologies, the antibodies against them showed cross reactivity, however the species-specific epitope exists. Eur m 2 has 82% sequence identity to both Der p 2 and Der f 2, revealed also IgE cross-reactivity to these allergens (Smith et al., 2001). The protein sequence of Gly d 2 revealed 79% identity to Lep d 2 and 46% and 41% identity to Tyr p 2 and Der p 2, respectively. IgE cross-reactivity was observed between Gly d 2, Lep d 2, and Tyr p 2, but only limited cross-reactivity was demonstrated between these 3 allergens and Der p 2 (Gafvelin et al., 2001).

The concentration of allergens of group 2 and the number of mites in house dust correlates (Yasueda et al., 1996). The concentration of Der f 2 was 5-fold lower in WGH than in SGM (Thomas et al., 2002). Danielsen et al. (2004) observed the correlation between
The abundance of *Lepidoglyphus destructor* and level of Lep d 2 contamination in stored grains under laboratory conditions. This allergen was probably not cumulated in the faeces.

The polyclonal antibodies for detection of the second group of allergens of stored product mites (Lep d 2, Tyr p 2, and Gly d 2) are produced by Indoor-Biotechnologies (http://www.inbio.com) and are commercially available.

**Group 3: Trypsins**

Trypsins are serine endopeptidases (Mr from 24 to 34 kDa) that preferentially cleave protein chains on the carboxyl side of L-amino acids such as arginine and lysine. Trypsins have been known as allergens in *Dermatophagoides* spp., *Blomia tropicalis* and *Euroglyphus maynei* (Stewart et al., 1992a; b; Ando et al., 1993; Flores et al., 2003). N-terminal sequences of Der f 3 and Der p 3 showed 85% homology and Eur m 3 has 81% identity to Der p 3 and Der f 3 (Thomas et al., 2002).

Based on enzymological studies, the trypsins are highly active enzymes in stored product mites (Bowman, 1981; Ortego et al., 2000; Sanchez-Ramos et al., 2004). It indicates the possible importance of this group of allergen in stored product mites.

The group 3 allergens are known to be in a large amount in SGM and in lower concentration in WGH (Sun et al., 2001). Nothing is known about the stability of trypsins in the faeces in house dust.

**Group 4: α-Amylases**

Amylases (α-1-4-glucan-4-glucanohydrolases) catalyze the initial hydrolyses of α-1-4 linked sugar polymers, such as starch or glycogen, into oligosaccharides, an important step towards transforming sugar polymers into single units that can be assimilated by organisms. IgE epitopes for Blo t 4, Der p 4 and Eur m 4 have been described in *Blomia tropicalis*, *Dermatophagoides pteronyssinus* and *Euroglyphus maynei* (Lake et al., 1991, www.allergen.org). The cDNA sequences of Der p 4 and Eur m 4 were calculated to be 90% identical (Mills et al., 1999). Based on enzyme activity measurement, α-amylases have been detected in all investigated stored-product mites species (Bowman & Child 1982).

**Group 5:**

This group is formed by a low molecular weight allergen (14-15 kDa) with unknown biological function. The sequences of Blo t 5 and Der p 5 showed 43 % homology. However, there are unique IgE epitopes for Blo t 5 (Yi et al., 2004). Der p 5 does not appear to be abundant in house dust (Thomas et al., 2002).

**Group 6: Chymotrypsins**

Chymotrypsins are serine endopeptidases that preferentially cleave protein chains on the carboxyl side of aromatic amino acids. Chymotrypsins are allergens about 25 kDa in *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, *D. farinae* in the WGH and SGM of *Dermatophagoides* mites (Thomas et al., 2002).

Based on enzymological studies, the chymotrypsins are presented in stored product mites (Bowman, 1981}; Ortego et al., 2000; Sanchez-Ramos et al., 2004) similarly like trypsins. It also indicates the possible importance of this group of allergen in stored product mites. Nothing is known about the chymotripsins stability in the faeces and in the environment.

**Group 7**

The allergens of Mr from 22 to 34 kDa are described in *Dermatophagoides* species (Der p 7 and Der f 7) and showed mutual sequences homology about 86% (Shen et al., 1997). The biochemical function has not been known yet.
Group 8: Glutathione-S-transferase
About 26 kDa protein found as the allergen produced by *Dermatophagoides pteronyssinus*. Molecular cloning studies of Der p 8 indicate significant sequence identity with glutathione-S-transferase of mouse (O’Neill et al., 1994; 1995).

Group 9: Collagenolytic serine protease
In *Dermatophagoides pteronyssinus*, a serine protease (Der p 9) similar to cathepsin G has been isolated (King et al., 1996). It preferentially cleaves substrates containing either C-terminal phenylalanine or leucine residues (King et al., 1996). The protease is responsible for IgE reactivity. The substrate specificity of Der p 9 indicates the possibility of utilization of collagenous substrates in mammal skin cells (Hamilton et al., 2003).

In addition, the ability to digest collagenous material was suggested by Bowman (1981) for the stored product mites; *Acarus siro*, *Lepidoglyphus destructor*, *Glycyphagus domesticus*, *Rhizoglyphus callae* and *R. roebi*, but the detection on enzyme level is missing.

Group 10: Tropomyosin
Tropomyosins form filaments in the actin groove of muscles and regulate contraction. Tropomyosin is known as allergen of *Dermatophagoides* spp. and *Blomia tropicalis* (Asturias et al., 1998). The sequences of Der p 10 and Der f 10 showed 98% homology and both have 75% homology to other arthropod tropomyosin and 60% to mammalian myosin (Thomas et al., 2002). Tropomyosin is abundant in house dust, but there is a problem to distinguish between mite and other arthropod tropomyosin due to the conservative features of these proteins (Thomas et al., 2002).

Group 11: Paramyosin
Paramyosin is 98 kDa protein of mite muscles (Tsai et al., 1999; 2000) and has been detected as an allergen in *Dermatophagoides farinae* and *Blomia tropicalis*.

Group 12
The proteins about 14 kDa were described based on cDNA analysis from *Blomia tropicalis* (Blo t 12) (Thomas et al., 2002).

Group 13: Fatty acid-binding proteins
These proteins have been known from cDNA sequences from mite *Blomia tropicalis* (Blo t 13) and several stored product species; *Acarus siro* (Aca s 13), *Lepidoglyphus destructor* (Lep d 13) and *Tyrophagus putrescentiae* (Tyr p 13) (www.allergen.org). Molecular weight is about 14 kDa and these proteins are probably fatty acid-binding proteins (Caraballo et al., 1997; Puerta et al., 1999).

Group 14: Vitellogenin/Apolipophorin
The high-molecular-weight group of proteins are high IgE-binding allergens. From partial cDNA clones peptide fragments Mag 1 and Mag 3 were expressed and studied (Thomas et al., 2002). Mag 3 has homology with the lipid transport apolipoporphins and shares structural homology with egg yolk protein vitellogenin (Fujikawa et al., 1996; 1998). Breakdown of the protein yields peptides with stronger IgE binding than does the complete protein (i.e. two peptides fragments of Mr 80-83 kDa and 95-101 kDa) (Arlian, 2002).

Group 16: Gelsolin/villin
About 53 kDa protein isolated from *Dermatophagoides farinae* (Der f 16) belongs to the regulatory proteins from the gelsolin/villin protein family (Kawamoto et al., 2002).

Groups 15 and 18: Chitinases
Chitin is an aminosugar consisting of partially deacetylated 1,4-ß-N-acetyl-glucosamines. Chitinolytic enzymes include chitinase (EC 3.2.1.14), which catalyzes a random hydrolysis of internal bonds in chitin forming smaller oligosaccharides, and ß-acetyl-D-glucosaminidase
(chitobiase, EC 3.2.1.52), which liberates N-acetyl-glucosamine from the non-reducing end of oligosaccharides.

Two chitinases were purified from *Dermatophagoides farinae*. Their molecular weight is 60 and 98 kDa, and these enzymes are proved as allergens (McCall et al., 2001; Weber et al., 2003). In addition, both chitinases were recognized by monoclonal antibodies in the digestive system but not in faecall pellets (McCall et al., 2001; Weber et al., 2003). Aminoacid sequences of these chitinases shared the highest found similarity (30%) to chitinase of *Drosophila melanogaster*. Chitinolytic activity was detected in WGH of a wide spectrum of stored product mites (Bowman & Child, 1982).

**Group 17: EF calcium binding protein**

This 53 kDa protein was isolated from *Dermatophagoides farinae* (Der f 17) (www.allergen.org).

**Group 19: Anti-microbial peptide**

Small 7 kDa peptide isolated from *Blomia tropicalis* (Blo t 19) showed homology to anti-microbial peptides.

**Group 20: Arginin kinase**

This 40 kDa allergen has been described recently in *Dermatophagoides pteronyssinus* (Der p 20) (www.allergen.org).

**Dual activity of enzymatic allergens**

The recent progress on the immunology of mite allergens has clearly demonstrated the importance of their enzymatic activity in the sensitization process. Moreover, it has been hypothesized that proteolytic activity may be a common mechanism for other allergens because more allergenic proteases have been recognized (e.g. Per a 1, Fel d 1) (Thomas et al., 1999). Among the protease allergens, cysteine and serine (trypsin, chymotrypsin and collagenolytic) proteases are known as the allergens of HDM (groups 1, 3, 6 and 9) (Thomas et al., 1991). Their allergenic activity was inactivated by inhibition of protease by appropriate inhibitor (e.g. E64, STI) (Pernas et al., 2000).

The best understood example is the cysteine protease Der p1 from faecal pellets of the house dust mite *D. pteronyssinus*. Proteolytically active Der p 1 has been reported to cleave both CD23 (Hewitt et al, 1995; Schulz et al., 1995; Shakib et al., 1998) and CD25 receptors (Schulz et al., 1998) from the surface of T-immune cells and to change immune response. Proteolytically active Der p 1 is also able to polarize the immune response to another antigen by reducing the Th1 protective response (Comoy et al., 1998). It was shown that an inhibitor of proteolytic activity E64 reduced the ability of Der p 1 to induce IgE production in mice (Gough et al, 1999).

Cysteine and serine proteases of HDM exert profound effects on epithelial cells which will promote allergic sensitization namely by disruption of intercellular adhesion, increased paracellular permeability and the initiation of cell death. The proteases are secreted into environment where they become aerosolized and inhaled. Der p 1 critically contributes to the tissue pathology observed in asthmatic airways as it can increase the passage of proteins across epithelial monolayers (Herbert et al., 1995). This increased epithelial permeability is likely to occur due to cleavage of tight junction proteins (Robinson et al., 1997). In addition, Der p 1 catalytically inactivates a naturally occurring serine protease inhibitor, namely 1-antitrypsin (Kalshheker et al., 1996), which protects the lung epithelial cells from damage mediated by endogenous or exogenous serine proteases (Crystal 1990). Der p 1 is a potent inducer of proinflammatory cytokines from the respiratory epithelium, and its action is
mediated by the protease-activated receptor PAR-1 (Kalsheker et al., 1996; Asokananthan et al., 2002). An adjuvant activity for bystander allergen has also been described for Der p 1 (Gough et al., 2001).

**Concluding remarks**

It is well known that the second group of allergens is the most important among SPM (Hage-Hamsten & Johansson, 1998; Olsson & Hage-Hamsten 2000). These allergens seem to be unstable in the environment and are present in lower amount in the faeces than in mite bodies. It supports the findings that these allergens are not accumulated in stored food.

The proteases as major allergens in HDM are also presented in SPM as was proved by their enzymatic activities. Their allergen reaction is given by their enzymatic activity. Especially cysteine proteases (group 1) are present in high amount in the faeces. These proteins are hardly decomposed in house dust or in the environment. Therefore proteases represent potential enzymatic allergens dangerous for the final consumers of mite contaminated food due to their ability to be accumulated. While serine proteases (groups 3, 6 and 9) are of unknown allergenic potential. The future research should be focused on the screening of allergen potential of cysteine and serine proteases in SPM including development and optimizing of detection methods.

**Acknowledgement**

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The bacteria as food for stored-product mites (Acari: Acaridida)

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Abstract: Stored product mites have been considered as gramni or fungi-vorous, feeding on grain debris, fungi, and yeasts growing in the grain ecosystems. The bacteriophagy (e.g. feeding on bacteria) represents an alternative feeding strategy in the grain ecosystems. However, it has not been reported in stored product mites yet. We analyzed whether bacteria could serve as a food source for stored-product mites. The analyses were based on the (i) activity of lysozyme and (ii) increase of population growth of mites on diets enriched by bacteria. Lysozyme (EC 3.2.1.17) is an enzyme catalyzing the hydrolysis of cell walls of many bacteria, causing cell death lysis, cell wall degradation and enables digestion of bacterial cells. The increase of population growth was observed on the control (rearing) diet and the diet enriched by Microccocus lysodeicticus (5% in the diet). Among tested species the lysozyme activity was highest in middle acid pH (optima from 4 to 5). Based on the observed enzymatic activity we distinguished three levels: (i) high lysozyme activity: Caloglyphus redickorzevi, Tyroborus lini, Tyrophagus brevicrinatus, Acarus gracilis; (ii) intermediate lysozyme activity: Aleuroglyphus ovatus, Glycyphagus domesticus, Tyrophagus putrescentiae; (iii) low lysozyme activity: Carpoglyphus lactis, Acarus siro, Lepidoglyphus destructor. The population growth of Tyroborus lini and Caloglyphus redickorzevi was higher on the diet enriched by Micrococcus lysodeicticus in comparison to control, while we observed no differences in population growth of Acarus siro and Lepidoglyphus destructor. We conclude that species with high lysozyme activity (Tyroborus lini and Caloglyphus redickorzevi ) are able to utilize bacteria as a food source and bacteriophagy seems to be another alternative feeding strategy in stored grain ecosystem.

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Keywords: bacteriophagy, bacteria, food source, stored product mites, grain ecosystems
Nymph’s morphology of *Dorypteryx domestica* (Psocoptera)

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**Abstract:** *Dorypteryx domestica* is an expansive psocid often occurring in synanthropic localities. So far only, adults and eggs have been possible to identify since the description of nymphs has not been available. The morphological study reported here deals with overall appearance and size measurements of particular instars. Five developmental instars are distinguished and described. Morphological characters include mainly number of flagellar segments, number of ommatidia in compound eyes, and level of wing development. Morphological details are illustrated with scanning electron microscope micrographs and macro photos.

**Key words:** *Dorypteryx domestica*, psocid, nymphs, morphology, SEM micrographs

**Introduction**

Psocid *Dorypteryx domestica* commonly occurs in domestic localities in Europe. So far only adults and eggs have been possible to identify, since the description of nymphs has not been available. The brachypterous adults were described by Smithers (1958) and re-described by Lienhard (1998). Kučerová (1997, 1998) described the macropterous forms, and eggs (Kučerová 2002) of this species.

Papers concerning psocid nymph’s morphology are only sporadic. Nymphs of some outdoor psocid species were described e.g. by New (1969). Key to families of last instar larvae of North American psocids was published by Mockford (1987).

The study reported here is focussed on external morphology of nymphs of *D. domestica* and is a part of a project of the comparative morphology of developmental stages of stored-product psocids aimed at facilitating their identification.

**Materials and methods**

*D. domestica* was reared in the laboratory at 25°C and 75 % relative humidity in glass containers (25 mL), with wheat germ as food in continual darkness.

The nymphs used for morphological study were bred separately from the eggs to obtain particular instars. A minimum of 20 individuals of particular instars were measured and examined.

The psocid specimens intended for morphological examination were mounted on slide mounts in Swan medium. Colour observations were made both on live individuals and on microscopic slides. All examined material is from the collection of the Department of Stored Product Pest Control, Research Institute of Crop Production, Prague.

The morphological study deals with overall appearance and size measurements of particular instars. Measurements were made under a light microscope using a micrometer. Morphological details are illustrated with scanning electron microscope (JEOL JSM 6400) micrographs and macro photos (Olympus Camedia C 5050).
Results

Nymph’s description

Basic morphological characteristics typical for brachypterous adults are valid also for all nymphs’ instars of *D. domestica*. Head hypognathous, longer than wide. Lacinia with 4 terminal teeth of various sizes. Prothorax narrower than meso- and metathorax. Abdomen ovoid, paraproct with a strong spine. Legs long and slender. Tibia and tarsus together longer than abdomen, claws with distinct preapical tooth.

Nymphs, contrary to adults have two-segmented tarsi, fore wing-pads (2.-5. instar) without venation and setae on the margin. Genitalia not developed.

Morphological differences between instars

1. instar
   Coloration of body whitish, without reddish bands. Eyes compound from 2 facets. Flagellum with 7 segments. No traces of wing-pads. Body length, about 0.4 mm.

2. instar
   Coloration of abdomen whitish-yellow with only two indistinct slight reddish transverse bands (dorsal surface). Eyes compound from 2 - 3 facets (Fig. 2a). Flagellum with 13 segments (Fig. 2b). Both fore and hind wing-pads (FW, HW) are discernible as lateral micro prominences of meso- and metathoracic segments. FW pad with one seta situated near the tip. Body length about 0.6 mm.

3. instar
   Coloration of abdomen whitish-yellow with two light reddish transverse bands. Compound eyes with 4 - 5 facets (Fig. 2c). Flagellum with 15 - 17 segments. Both fore and hind wing-pads clearly differentiated as small pointed lateral lobs of meso- and metathoracic segments about the same size (Fig. 2d). FW pad with 2 setae situated near the wing angular and the wing tip. Body length, about 1.1 mm.

4. instar
   Coloration of abdomen whitish-yellow with two distinct wide reddish brown transverse bands. Compound eyes with 8 - 11 facets (Fig. 2e). Flagellum with 17 - 19 segments. Pointed fore wings overreach base of abdomen (= extending beyond posterior border of metathorax) and are held laterally (Fig. 2f). FW with 3 setae situated between the wing angular and the wing tip. Wing venation is not developed. HW small and covered by FW. Body length, about 1.4 mm.

5. instar
   Coloration of abdomen whitish-yellow with two deep reddish brown transverse bands. Compound eyes with 17 - 19 facets (Fig. 2g). Flagellum with 25 - 31 segments. Fore wings pointed, reach ca ½ of the abdomen and are situated laterally (Fig. 2h). FW with 6 - 7 setae situated between the wing angular and the wing tip. Wing venation is not developed. HW rudimental covered by FW. Body length, about 1.8 mm.

Nymph’s measurements

The main body measurements of particular instars are summarised in Table 1. The size of all measured characters differs in particular instars.
Table 1. Body size measurements of nymph’s instars (µm).

<table>
<thead>
<tr>
<th>Instars</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IO</td>
<td>146 ± 8.2</td>
<td>170 ± 9.5</td>
<td>210 ± 10.3</td>
<td>230 ± 10.9</td>
<td>289 ± 13.8</td>
</tr>
<tr>
<td>D</td>
<td>23.1 ± 1.6</td>
<td>24.5 ± 2.3</td>
<td>41.2 ± 3.4</td>
<td>60.9 ± 3.8</td>
<td>85.8 ± 3.9</td>
</tr>
<tr>
<td>Ma</td>
<td>92.4 ± 2.1</td>
<td>107 ± 2.9</td>
<td>129 ± 6.4</td>
<td>138 ± 4.5</td>
<td>179 ± 5.1</td>
</tr>
<tr>
<td>f1</td>
<td>64.2 ± 3.0</td>
<td>45.8 ± 4.4</td>
<td>84.9 ± 9.1</td>
<td>75.1 ± 6.3</td>
<td>87.6 ± 4.9</td>
</tr>
<tr>
<td>f2</td>
<td>92.2 ± 4.7</td>
<td>44.0 ± 4.4</td>
<td>88.0 ± 16.5</td>
<td>64.0 ± 9.3</td>
<td>61.8 ± 5.1</td>
</tr>
<tr>
<td>f3</td>
<td>125 ± 5.9</td>
<td>78.3 ± 4.6</td>
<td>91.8 ± 16.3</td>
<td>76.8 ± 10</td>
<td>69.8 ± 10.7</td>
</tr>
<tr>
<td>T</td>
<td>213 ± 3.8</td>
<td>279 ± 17.1</td>
<td>369 ± 35.9</td>
<td>435 ± 23.6</td>
<td>614 ± 26.4</td>
</tr>
<tr>
<td>t1</td>
<td>128 ± 4.4</td>
<td>159 ± 7.8</td>
<td>204 ± 9.4</td>
<td>229 ± 10.8</td>
<td>309 ± 11.3</td>
</tr>
<tr>
<td>t2</td>
<td>86.4 ± 6.5</td>
<td>89.6 ± 8.4</td>
<td>93.7 ± 5.2</td>
<td>101 ± 7.1</td>
<td>120 ± 5.1</td>
</tr>
<tr>
<td>FW</td>
<td>0</td>
<td>45.2 ± 3.2</td>
<td>106 ± 11.7</td>
<td>199 ± 28.6</td>
<td>535 ± 18.8</td>
</tr>
</tbody>
</table>

Legend: IO=minimum distance between eyes, D=anteroposterior diameter of compound eye, f1-3=length of 1.- 3. flagellar segments, Ma= length of mandible, T=length of hind tibia, t1-2=length of 1.-2. hind tarsomeres, FW= forewing length. Measured distances of D, Ma and t1-t2 are marked with arrows in Fig. 1 a - c and FW in Fig. 2f.

Fig. 1. Morphological details of Dorypteryx domestica, measured distances are marked with arrows; a – eye, b – mandible (Ma), c – tarsomeres (t1, t2)

**Discussion**

Described morphological differences of nymphs - mainly number of flagellar segments, number of facets in compound eyes, level of wing-pads’ development, number of FW setae, size of IO, D, Ma, T and FW are suitable for distinguishing of all five developmental instars of brachypterous form of D. domestica. Measurements of flagellar and tarsal segments were done according to accepted practice of adult descriptions. There were distinct size differences in normally developed specimens of particular instars. In the case of specimens somehow damaged during their development (especially antennae) can lead to length variation of segments. According New (1969), lengths and rations of various antennal and leg segments had little value for specific identification of nymphs. Reddish transverse bands of dorsal surface of abdomen of particular instars varied in shape (width) and colour intensity as it was described for adults by Kučerová (1997).
Acknowledgements

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Fig. 2. Morphological details of *Dorypteryx domestica*: a, b – 2. instar (eye, flagellar segment), c, d – 3. instar (eye, wing pads), e, f – 4. instar (eye, wing), g, h – 5. instar (eye, wing).
**Sitophilus granarius** (Curculionidae): outdoor occurrence in vicinity of a grain store

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**Abstract:** The aim of this work was to explore the frequency and abundance of *Sitophilus granarius* occurring outside a grain store. Wheat bait-traps were used for pest monitoring. Bait-traps exchange was carried out monthly throughout the year. Exposed bait traps were analysed in the laboratory and stored for subsequent adult incubation (27°C, 75% r.h.) for 1 month. The long-term outdoor occurrence of *S. granarius* was documented in the Czech Republic for the first time. This highly mobile internal grain feeder was the dominant pest in comparison to 8 other species of Coleoptera found outside the grain store. The weevils were captured in grain baits almost throughout the year. There were differences in weevil’s abundance and frequency depending on bait trap location. The population of granary weevil in baits from hidden infestation (eggs) increased 10 - 50 times after one month of incubation period compared to primary infestation.

**Key words:** *S. granarius*, grain bait traps, outdoor occurrence, hidden infestation, grain residues

**Introduction**

The study reported here is focussed on the internal grain feeder *Sitophilus granarius* and is a part of a project dealing with stored product pest occurrence in grain residues in the Czech Republic (Stejskal & Kučerová, 1996, Kučerová et al., 2003, 2005, Hubert et al., 2006). The granary weevil is one of the most important pests of stored grain capable of causing considerable losses. This species is distributed throughout the temperate regions of the world; being rare in tropical countries. Its distribution is limited more by its commodity associations with cool climate crops than by its direct response to temperature (Haines, 1991).

IPM practices are mostly directed toward detecting and controlling pests within the commodity or inside empty storage facilities. Less attention is addressed to infested indoor and outdoor grain residues (Dowdy & McGaughey 1994, Reed et al., 2003, Arthur et al. 2006). Information on pest population occurrence, ecology, and behaviour in grain residues both inside and outside of stores is important for effective monitoring and control of stored-product pests.

The aim of this work was to study long term frequency and abundance of granary weevil outside a grain store depending on location of grain residues, because such type of information is absent for this economically important pest species.

**Materials and methods**

Wheat bait-traps simulating grain residues (100 g of whole wheat grains) were used for pest monitoring. Traps were placed outside a flat grain store, along the south and the north wall. Traps exchange was done carried out monthly during throughout the year. Altogether 84 traps
were used. Exposed bait traps were analysed in laboratory. Each bait was separately sieved (sieving machine Retsch AS 200 – sieving time 2 min., mesh size - 2 mm). Beetles were then removed and counted. The analysed baits were stored for subsequent adult incubation from hidden infestation for one month (27°C, 75% r. h.).

Results

Grain weevils were found altogether in 40% of grain bait traps outside the grain store. The highest levels of both primary and hidden infestation were during spring and summer (March – August). The populations in traps declined during September to February and rose again in March (Fig. 1). It generally corresponds with the trend of outdoor temperatures (Fig. 2). The population of granary weevil in baits from hidden infestation increased as much as 10 - 50 times after one month of incubation period compared to primary infestation. There were differences in weevil’s abundance and frequency depending on bait trap location (Fig. 3). The primary infestation occurred even during the winter months, but only in south located traps. Primary infestation in winter did not result in development of hidden infestation. Eight more species of stored product beetles were found outside the grain store (Fig. 4), but S. granarius was dominant (56 % of total abundance).

Discussion

The long-term outdoor occurrence of primary pest S. granarius was documented in the Czech Republic for the first time. Weevils were captured during almost the entire year. This species is normally found only in stores, not in open nature (Howe 1965). We suppose that the source of outdoor bait infestation is from the grain stored in the flat grain store. Weevils tend to move towards the top and sides of the bulk (Surtees, 1965). Such movement behaviour probably facilitates their dispersal also to outside the store.
*S. granarius* is a very cold-hardy, and as result, is the dominant species in the temperate regions, but does not breed at temperatures less than 11°C (Rees, 2004). According to Fleming & Armitage (2003) steady temperatures below 5°C were successful in killing all developmental stages within 7 weeks, but fluctuating temperatures between 0 - 10°C prolonged that time to 16 weeks. Mixed-stage infestation of developing grain weevils inside grain could survive fluctuating temperatures that occur at the grain surface during winter in the UK. This is in agreement with our results confirming that weevils can occur in certain suitable outdoor places in grain “residues” also during mild winters (e.g. in south positions with likely higher temperatures on sunny days). Wakefield & Cogan (1999) found, that laboratory specimens of *S. granarius* used in a field trial were not moving much at temperatures below 9°C, but they supposed that weevils were probably able to move less at

Fig. 2. Average outdoor temperatures.

Fig. 3. Primary and hidden occurrence of *S. granarius* in different outdoor bait traps locations (mean number of specimens).
low temperatures than field populations. Our field populations must be mobile at low temperatures because of their detection in outdoor baits in winter, but at such temperatures they were not able to lay eggs (no hidden infestation found). They continued laying again when the temperature reached the minimum limit suitable for egg laying.

Outdoor populations could thus be serious source of re-infestation of stored grain. This hazard is stressed by the fact that only few specimens were able to start further population increase, as was documented in comparison of primary and hidden infestation.

Acknowledgements

Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE 0002700603.

References


**Fig. 4. List of stored product pests (Coleoptera)**

| List of species found outdoor: |
| Cucujidae                |
| Cryptolestes ferrugineus |
| Silvanidae               |
| Ahasverus advena         |
| Oryzaephilus surinamensis|
| Cryptophagidae           |
| Cryptophagus sp.         |
| Mycetophagidae           |
| Typhaea stercorea        |
| Bostrychidae             |
| Rhyzopertha dominica     |
| Tenebrionidae            |
| Tribolium castaneum      |
| Curculionidae            |
| Sitophilus oryzae        |
| Sitophilus granarius     |
Surtees, G. 1965: Ecological significance and practical implications of behaviour patterns determining the spatial structure of insect populations in stored grain. – Bulletin of Entomological Research 56: 201-213.
The secretory cells of digestive tract of *Tribolium castaneum* (Coleoptera, Tenebrionidae) larvae

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**Abstract:** The red flour beetle, *Tribolium castaneum* (Herbst, 1797), is widely distributed and is a secondary pest of grain and cereal products. In the Czech Republic, it belongs to the most abundant and frequent storage pest that is hard to control by pyrethroid insecticides due to tolerance to them. This is why the new insecticides against this pest are developed. They are target against midgut (*Bt* toxins, inhibitors of insects amylases and proteases). To understand the effect of insecticide on the midgut in this study, we explored and described the structure of secretory cells of midgut of *Tribolium castaneum* larvae using optical microscopy.

The larvae, approximately 3 weeks old, were fixed in Bouin-Duboque-Brazil fluid, embedded in paraplast, sectioned (thickness 5-7 µm) and stained in Masson’s triple stain. The histological preparates were observed under Axioskop Zeiss.

The digestive tract of the larvae of *T. castaneum* is composed of 3 parts: stomodaeum (foregut), mesodaeum (midgut), and proctodaeum (hindgut). Stomodaeum forms short and dilated pharynx and narrow oesophagus extends towards the swollen mesodaeum. Mesodaeum of the larval *T. castaneum* is rather simple tube, without any caeca or diverticula. Proctodaeum is divisible into 3 sections. Very small and conical „pyloric chamber“, at the anterior end followed by the ileum which forms an S-shaped bend and spindle-shaped rectum. In the middle part of mesodaeum there are the cylindrical cells. Short middle zone secrets no enzymes. Multi-layer peritrophic membrane is present. The secretory cells were localized into (i) anterior mesodaeum and (ii) posterior mesodaeum. The anterior part of mesodaeum contains tall and columnar cells and the membrane on the luminal side forms microvilli. The apical extrusions at the apical parts of the cells release into gut lumen. The cells of posterior mesodaeum create multicellular crypts and apical extrusions can be also observed here. Both the cells in anterior and posterior mesodaeum contain large nuclei and they are strongly vacuolized indicating strong secretory activity. The described situation corresponds to model species *Tenebrio molitor*. The function of secretory cells as well as the potential for suppressive compounds is discussed.

The study was supported by the grant of MŠMT 1P05ME758.

**Keywords:** digestive tract, secretory cells, midgut, larvae, *Tribolium castaneum*
Development of *Araecerus fasciculatus* De Geer (Coleoptera: Anthribidae) on *Panax ginseng* C. A. Meyer roots

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**Abstract:** *Araecerus fasciculatus* De Geer is a beetle of considerable economic importance. Its occurrence is cosmopolitan, though it is more common in tropical and subtropical countries.  

The aim of this experiment was to study the development of coffee bean weevil on two different kinds of ginseng roots, compared with data obtained on corn grains and coffee beans. Tests were carried out under laboratory conditions at a constant temperature and relative humidity.  

It was observed that the time of development is the shortest on spindle-shaped, bipartite ginseng roots and the number of emerging adults is higher on corn grains and coffee beans. *A. fasciculatus* do not develop on secondary ginseng roots.  

**Key words:** *Araecerus fasciculatus*, *A. coffeae*, *Panax ginseng*, coffee bean weevil, development

**Introduction**

The current name of the coffee bean weevil is *Araecerus coffeae* (Fabricius), but it was known as *A. fasciculatus* De Geer until 1994, when Zimmerman (1994) published notes on the long ignored synonymy of this pest. However, “there is controversy over this synonymy and, as such, the name changes may continue for this important pest species” (Bloem et al., 2002). In the present paper, it is therefore called *A. fasciculatus*.

This widespread stored product pest is polyphagous. It has been reported as an important pest of many plants or plant products, including coffee, cocoa, nutmegs, corn, peanuts, spices, grains, dried fruit, yam, cassava, ginger, turmeric, garlic bulbs, all seeds and grains (Mphuru, 1974; Abraham, 1975; Parker & Booth, 1979; Childers & Woodruff, 1980; Nagano, 1981; Joseph et al., 2001). It can also live to a certain extent on flour, thrives on biscuits and even on ordinary bread, preferring the crumb to the crust (El Sayed, 1940). Its ability to attack a broad spectrum of commodities is enhanced by high moisture content of the food or high relative humidity of the environment, because it is very moisture dependent (Bitran et al., 1978).

A sample of *Panax ginseng* C.A. Meyer roots heavily infested by *A. fasciculatus* was brought to the Istituto di Entomologia agraria, University of Milan (Italy). As no information is at present available on the intrinsic susceptibility of ginseng to coffee bean weevil, the development on two kinds of roots was investigated: spindle-shaped, bipartite roots and secondary roots, and comparison was made with the development on *Zea mais* grains, *Coffea arabica* and *C. robusta* beans. Arbogast et al. (2002) reported their observation on *A. fasciculatus*, pointing out that it is “capable of severely damaging on stored ginseng roots”.

The objective of this paper was to report on the development of *A. fasciculatus* on two different kinds of ginseng roots, compared with data obtained on corn grain and coffee beans.
Materials and methods

*A. fasciculatus* was obtained from a laboratory strain of the Istituto di Entomologia Agraria, at the Università degli Studi di Milano (Italy). The insects were reared on wheat at 26±1°C and 70±5% r.h. and had been maintained for several years free of any chemical treatment.

*Z. mais* grains, *C. arabica* and *C. robusta* beans were used for the experiment, together with two kinds of *P. ginseng* roots: spindle-shaped, bipartite roots and secondary roots. Fifty 10-15-day-old adults of *A. fasciculatus*, both males and females, were released into a 200 mL polystyrene jar. Gonçalves et al. (1976), agreeing with El Sayed (1935), reported that females reach sexual maturity 6 days after emergence and males need less time. According to Autuori (1931) both sexes are sexually mature 2-3 days after emergence. The jars, containing 30 g of corn, coffee or ginseng, were covered with a fine net, to prevent adults from escaping and to allow gas exchange. The jars were put in a climatic room at 26±1°C and 70±5% r.h., which were the same conditions of rearing. The adults were left in the jars for 7 days, to oviposit before being sieved off. Adult progeny was removed and counted every two days. The duration of the test was double the time requested to obtain the first F1 adult. The development period was from egg laying to the time when 50% of the total F1 adults emerged.

The Index of Susceptibility (IS) was calculated using the formula: $\text{IS} = (\log_{e}F/D) \times 100$, where *D* refers to the time of development (= number of days from oviposition to 50% F1 adult progeny) and *F* refers to the total number of F1 adults (Dobie, 1974).

Four replicates were carried out for each kind of sample. Results were submitted to ANOVA and Duncan’s multiple range test (P<0.05). The tests were performed using the SPSS for Windows version 13.0.

Results

No progeny emergence was found on secondary ginseng roots, so the results shown in Table 1 and Figure 1 refer only to the development on the spindle-shaped, bipartite roots.

Results showed significant differences between the number of emerged F1 adults, the period of development (calculated from oviposition to 50% adults’ emergence), and the Index of Susceptibility of *A. fasciculatus* on tested food substrates.

The normalized cumulative emergence curves (Fig. 1) show that *A. fasciculatus* growth was better on *P. ginseng* rather than on coffee and corn. The period of development was, indeed, the shortest on spindle-shaped *P. ginseng* roots but the number of progeny was the lowest (Table 1). The greatest number of progeny was observed on *Z. mais* and *C. arabica*, while the longest period of development was on *C. robusta*.

The highest Index of Susceptibility was on *Z. mais*, the lowest on *C. robusta* and *P. ginseng*.

Discussion

In these experimental conditions, results indicate that *A. fasciculatus* can grow on spindle-shaped, bipartite roots of *P. ginseng*, but not on secondary roots. Newly-hatched larvae bore into the commodity and develop internally (MAF Plants Biosecurity, 2005): The size of secondary roots is too small to allow larval development. However, among the tested food substrates, the number of progeny on ginseng was the lowest and the period of development was the shortest.
C. arabica was more susceptible than C. robusta to infestation by A. fasciculatus, as reported by other authors (Warui, 1977; Rodriguez & Carvalho, 1978; Novo et al., 1999). The highest Index of Susceptibility was observed on Z. mais as compared to other foods. Gonçalves et al. (1976) reported that A. fasciculatus prefers peanuts to coffee beans and corn. Gonçalves et al. (1976) observed that the number of emerged adults on corn and coffee was the same, as pointed out in this trial with corn and C. arabica, but they did not specify the coffee species.

Table 1. Mean of emerged adults, period of development (time required to obtain 50% of emerged adults) and Index of Susceptibility for A. fasciculatus on Z. mais, C. robusta, C. arabica and P. ginseng at 26±1°C and 70±5% r.h..

<table>
<thead>
<tr>
<th></th>
<th>Emerged adults ± SD</th>
<th>Period of development ± SD</th>
<th>Index of Susceptibility ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. mais</td>
<td>58 ± 10 c*</td>
<td>62.50 ± 1.91 b</td>
<td>6.49 ± 0.40 c</td>
</tr>
<tr>
<td>C. robusta</td>
<td>36 ± 9 b</td>
<td>71.50 ± 1.00 d</td>
<td>4.99 ± 0.32 a</td>
</tr>
<tr>
<td>C. arabica</td>
<td>52 ± 6 c</td>
<td>68.00 ± 1.16 c</td>
<td>5.80 ± 0.15 b</td>
</tr>
<tr>
<td>P. ginseng</td>
<td>15 ± 4 a</td>
<td>58.00 ± 1.16 a</td>
<td>4.64 ± 0.44 a</td>
</tr>
</tbody>
</table>

*Values followed by a different letter are significantly different (P<0.05, ANOVA, Duncan’s test).
The development of coffee bean weevil on *P. ginseng* is interesting due to the economic importance of these roots. It will be important to further investigate the quality change in ginseng roots infested by this pest.

**Acknowledgements**

Thanks to Dr Francesco Gattesco, INDENA S.p.A. (Italy) for ginseng roots.

**References**


Susceptibility of *Triticum polonicum* L., *T. durum* Desf., *T. spelta* L. to post-harvest infestations by *Sitophilus zeamais* Motschulsky (Coleoptera: Dryophthoridae, Rhynchophorinae)

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Abstract: *T. polonicum* L. (Polish wheat) is a tetraploid wheat, with a high content of protein, lipids and minerals. Moreover, it is less allergenic than other wheat species and for these reasons it is increasingly cultivated in Italy.

The development of *Sitophilus zeamais* Motschulsky on Polish wheat was examined in laboratory trials at a fixed temperature and relative humidity. These results were compared with the development on *T. durum* Desf. (durum wheat) and *T. spelta* L. (spelt wheat). Tests were carried out with 100 adults of mixed sex in 30 g of kernels, removed from jars after 5 days. New generation adults were checked every day, counted and removed. A greater number of emerged adults was observed on *T. durum*, the lowest on *T. spelta*. The beginning of emergence started earlier on *T. durum* and *T. polonicum* as compared to *T. spelta.*

Key words: *Sitophilus zeamais*, *Triticum* cereals, development rates

Introduction

*Triticum polonicum* L. *sensu* Michalová (2000), belongs to the list of minor cereals of Europe. Originally, it was identified as Kamut but, more recently, taxonomists specialized in wheat have identified Kamut as *T. turgidum*, ssp. *durum* (Stallknecht et al., 1996; Quinn, 1999). It is a tetraploid wheat grown in southern Europe (Mediterranean region) because it requires a warm climate, but the origin of this species is uncertain. It was developed through cultivation and is not found in a truly wild location. Spikes are large, open or dense, and square or rectangular in cross-section. Kernels are very long, narrow, and hard. They thresh free of the glumes (Magness et al., 1971).

The seeds contain significantly higher level of protein and slightly higher levels of lipids and minerals. It is reported as less allergenic - but this has not been substantiated by controlled studies (Facciola, 1990). It is high in gluten (Usher, 1974) and is usually ground into flour (for making bread, pasta, etc.) and also used as a cereal (seed-cooked). The seed is said to have a superior flavour (Facciola, 1990).

The fibre obtained from the stems is used for making paper (Bell, 1988); the starch from the seed is utilized for laundering, and sizing textiles (Uphof, 1959).

The cultivation of Polish wheat is increasing in Italy and it is likely that small farmers will store it using traditional methods. It will then be necessary to consider the susceptibility of *T. polonicum* to insect pests which frequently cause high levels of damage to stored grain. As no information is at present available on the intrinsic susceptibility to storage pests, it was decided to investigate the possibility of infestation of *T. polonicum* to *Sitophilus zeamais* Motschulsky, and to compare it with the susceptibility of *T. durum* and *T. spelta*. 
Materials and methods

*S. zeamais* was obtained from a laboratory strain of the Istituto di Entomologia Agraria, at the Università degli Studi di Milano (Italy), reared on wheat at 26±1°C and 70±5% r.h., which had been maintained for several years free of any chemical treatment.

The cereals used in the experiments were *T. polonicum* and *T. durum* (cv. Navighetor), which are free of the glumes, and *T. spelta* with glumes. Before starting the tests all cereals were placed in a freezer at -20°C for 3 weeks, then at 25°C for one week and finally at -20°C for additional 3 weeks in order to eliminate the possible presence of Arthropods.

To start the experiments, one hundred 10-15-day-old adults of *S. zeamais*, both males and females, were released into a 200 mL polystyrene jar containing 30 g of grains (Table 1). The jars were covered with a fine net, to prevent adults from escaping and to allow gas exchange. The jars were put in a climatic room at 26±1°C and 70±5% r.h., the same conditions of insect rearing. The weevils were left in the cereal for 5 days to oviposit before being sieved off. Adult progeny appearing in the wheat was removed and counted daily. The duration of every trial was double of the time required to obtain the first F1 adult. The development period was calculated from eggs laying to the time when 50% of the total F1 adults emerged.

The weight losses of wheat cultivars caused by larval feeding of the maize weevil were measured. The samples were weighed after oviposition and weighed again at the end of experiments (Kučerová & Stejskal, 1994). An Index of Susceptibility (IS) was calculated using the formula: IS = (log_eF/D)*100, where D means the time of development (= number of days from oviposition to 50% F1 adult progeny) and F means the total number of F1 adults (Dobie, 1974).

Four replicates were carried out for each cereal species. Results were submitted to ANOVA and Duncan’s multiple range test (P<0.05). The tests were performed using the SPSS for Windows version 13.0.

Results

Significant differences between weight losses are shown in Table 1. The highest weight loss was found on *T. durum*. There was also a significant difference in size of F1 adults: The maize weevils grown on *T. spelta* were smaller than those on *T. polonicum* and *T. durum*.

Significant differences were observed in progeny production on the tested cereal as shown in Table 2. The greatest number of progeny was found with *T. durum* and the lowest with *T. spelta*. *T. durum* had the highest Index of Susceptibility, while *T. spelta* had the lowest (Table 2).

Table 1. Mean number of kernels of *Triticum polonicum*, *T. durum*, and *T. spelta* in 30 g of cereal; weight of kernels after experiments (g) and size of F1 adults (mm).

<table>
<thead>
<tr>
<th><em>Triticum</em> species</th>
<th>Number of kernels in 30 g</th>
<th>Weight losses of kernels (g) ± SD</th>
<th>Size of F1 adults (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>polonicum</td>
<td>384 ± 1</td>
<td>1.58 ± 1.19 b*</td>
<td>2.71 ± 0.10 b</td>
</tr>
<tr>
<td>durum</td>
<td>629 ± 3</td>
<td>3.15 ± 0.81 a</td>
<td>2.73 ± 0.12 b</td>
</tr>
<tr>
<td>spelta</td>
<td>596 ± 22</td>
<td>0.78 ± 0.30 b</td>
<td>2.53 ± 0.15 a</td>
</tr>
</tbody>
</table>

Values followed by a different letter are significantly different (P<0.05, ANOVA, Duncan’s test).
Fig. 1. Normalized cumulative mean number of *Sitophilus zeamais* adults emerged from different species of *Triticum* at 26±1°C and 70±5% r.h.

Fig. 2. The daily emergence curves of *Sitophilus zeamais* adults on different species of *Triticum* at 26±1°C and 70±5% r.h.
Table 2. Mean of emerged adults, period of the development (time requested to obtain 50% of emerged adult) and Index of Susceptibility for *S. zeamais* on *T. polonicum*, *T. durum* and *T. spelta* at 26±1°C and 70±5% r.h..

<table>
<thead>
<tr>
<th><em>Triticum</em> species</th>
<th>Emerged adults ± SD</th>
<th>Period of development ± SD</th>
<th>Index of Susceptibility ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>polonicum</em></td>
<td>161 ± 48 b*</td>
<td>38.25 ± 0.50 ab</td>
<td>13.20 ± 0.76 b</td>
</tr>
<tr>
<td><em>durum</em></td>
<td>293 ± 20 c</td>
<td>37.00 ± 0.82 a</td>
<td>15.36 ± 0.47 c</td>
</tr>
<tr>
<td><em>spelta</em></td>
<td>60 ± 30 a</td>
<td>39.75 ± 2.06 b</td>
<td>9.91 ± 1.19 a</td>
</tr>
</tbody>
</table>

*Values followed by a different letter are significantly different (P<0.05, ANOVA, Duncan’s test).*

Furthermore significant differences among the tested cereals were noticed in the duration of the development (from oviposition to 50% adults emergence) of the maize weevil. The shortest development period was on *T. durum* and the longest on *T. spelta* (Table 1).

Since experimental errors are more likely with the daily assessment data, they are presented as cumulative emergence data (Trematerra et al., 1996). The normalized cumulative emergence curves of *S. zeamais* (Fig. 1) show that the insect growth was better on *T. durum* and *T. polonicum* than on *T. spelta*. The curves of daily average emergence differ in shape for the tested *Triticum* species (Fig. 2).

**Discussion**

In these laboratory trials different species of *Triticum* influenced progeny production of the maize weevil. In particular, a low number of emerged adults was observed on the kernels with glumes of *T. spelta*. Süß et al. (1999) observed no emergence or low number of adults of *S. zeamais* on *T. spelta* and *T. dicoccum*. Glumes and inner glumes completely cover their kernels and hinder oviposition and adult feeding. Sinha (1971) pointed out that hulled wheat was highly resistant to *S. zeamais* and *S. oryzae* (L.). Kernels of *T. aestivum* and *Fagopyrum esculentum* without pericarp showed similar susceptibility to the damage of *S. granarius* and *S. oryzae* while the development of *S. zeamais* was better on peeled achenes as compared to soft wheat (Locatelli & Antignati, 1993).

Among kernels free of the glumes, *T. polonicum* was more resistant to infestation by *S. zeamais* than *T. durum*. Kernels of Polish wheat are bigger than those of *T. durum* but the size of F1 adults was the same, although Teotia & Singh (1968) observed that larvae of *S. oryzae* developed better and adults emerging from the largest seeds were larger.

The resistance to insect attacks has been attributed by Russel (1968) and Dobie (1974) to physical and nutritional characteristics of the kernels, such as grain hardness or sugar content. In particular Kućerová & Stejskal (1994) found that the milling cultivars with high quality were 3 times more resistant to infestation by *S. granarius* (L.) than the milling cultivars with lower quality and feed cultivars. Locatelli & Antignati (1993) noticed that the presence of a resistant pericarp on achenes of *F. esculentum* hindered oviposition of *Sitophilus* spp. females.
Acknowledgements

Thanks to Apsov Sementi, Voghera (Pavia, Italy) for providing *T. durum* and *T. spelta* and Istituto Sperimentale per la Cerealicolture, Sant’Angelo Lodigiano (Lodi, Italy) for providing *T. polonicum*.

References

Susceptibility of different kinds of powdered milk to the attack of Pyralid moths

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Abstract: Tests were carried out to determine the susceptibility of four different kinds of powdered milk, used in human nourishment to attack by Cadra cautella (Walk.), Corcyra cephalonica (Staint.), Ephestia kuehniella (Zell.) and Plodia interpunctella (Hbn.). One hundred eggs, 24-48 h old, were placed on substrates (50g); in a thermostatic room (26±1°C; 70±5% r.h.; 16:8 L:D). For each substrate, three replicates were carried out. At the end of each test the mean adult number emerged, the mean developmental period and the mean susceptibility index was calculated. Results were subjected to ANOVA and to Duncas’ multiple range test (SPSS 11.5 for Windows).

Test species were able to complete their development on soya milk. On this substrate, emergence of 50-60 adults were observed except for C. cephalonica where there were 19.0±1.00 emerged adults. C. cautella, E. kuehniella, and P. interpunctella were not able to complete their development on the human nourishment milk (powdered whole milk, powdered reconstituted milk and powdered baby milk). C. cephalonica adults were observed not only on powdered soya milk but also on the whole milk, numbers of adults that emerged were: 17.0±1.00 from powdered baby milk: 40.7±3.84 from reconstituted milk: 32.0±2.00 from other substrates. C. cephalonica showed the ability to use lactose, as an energy source. This sugar, in fact, for the most part is toxic to the moths and beetles of stored products.

Key words: powdered milk, soya milk, Pyralid moths, development, diets

Introduction

Carbohydrates are used by insects mainly as an energy source for glycogen and fat synthesis (Friend, 1958). Sugars may be divided according to their monosaccharide components before being absorbed by midgut. The ability to digest food di- and polysaccharides varies with the species (Waterhouse & Day, 1953; House, 1961).

Pyralid moths Ephestia kuehniella (Zell.) and Plodia interpunctella (Hbn.) require a diet rich of carbohydrates because they are not able to use proteins as their only energy source. (Candura, 1939). P. interpunctella (Hbn.) larvae are attracted by substrates rich in sucrose, fructose, maltose, fucose and melibiose (Backer & Mabie, 1973). Fraenkel and Blewett (1946) observed that the Cadra cautella (Walk.) development is inhibited when glucose is replaced with starch while it is favoured by sucrose. Even Corcyra cephalonica needs glucose as carbohydrate source. Removing glucose from a diet consisting of casein, cholesterol, vitamins, yeast, and mineral salts, reduced the percentage of the surviving larvae by 50% (Uberoi, 1960). C. cephalonica (Staint.) larvae development is favoured if glucose is added to the rearing substrate; this was not observed when casein was added to the diet (Rao et al., 1980).

Some sugars as arabinose, xilose, and galactose may be toxic to the foodstuff pests and they are able to change the action of several proteolytic enzymes (Applebaum & Konijin, 1965; Applebaum, 1966).
As a result of an infestation by *C. cephalonica* on powdered baby milk, the susceptibility of different kinds of powdered animal and vegetable milk to attack by some Pyralid moth species was tested.

**Materials and methods**

Tests were carried out to assay the ability of *Cadra cautella* (Walk.), *Corcyra cephalonica* (Staint.), *Ephestia kuehniella* (Zell.) and *Plodia interpunctella* (Hbn.) to grow on four different kinds of powdered milk: cow’s whole milk, reconstituted milk, baby milk and soya milk. The nutritional substrate composition is reported in Table 1.

Test species were reared at the Agricultural Entomology Institute of Milan on an artificial diet consisting of bran, soft wheat flour, maize meal, wheat germ, yeast, honey, and glycerine, and maintained in a thermostatic room (26±1°C; 70±5% r.h. with photoperiod of 16:8 L:D).

One hundred eggs, 24-48 hours old, were placed on the substrate (50 g) in glass Petri dishes (Ø: 6 cm). The containers, were placed under the same conditions of temperature, relative humidity and photoperiod. Three replicates were set up for each substrate and control. The mean adult number, the mean developmental period and the mean susceptibility index were calculated. ANOVA and Duncan’s Multiple Range (Howell & Games, 1974; Zarr, 1984) Test were used for means and comparison of means.

Table 1. Nutritional composition of test substrates (100g) with values shown as percentage.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Proteins (%)</th>
<th>Lipids (%)</th>
<th>Carbohydrates</th>
<th>Mineral salts (%)</th>
<th>Flour (%)</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>25.0</td>
<td>26.5</td>
<td>40.0</td>
<td>A*</td>
<td>0.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Reconstitute milk (a)</td>
<td>30.0</td>
<td>5.0</td>
<td>48.0</td>
<td>A</td>
<td>A</td>
<td>5.3</td>
</tr>
<tr>
<td>Baby milk (b)</td>
<td>11.5</td>
<td>28.5</td>
<td>46.2</td>
<td>A</td>
<td>A</td>
<td>8.2</td>
</tr>
<tr>
<td>Soya milk (c)</td>
<td>13.7</td>
<td>28.1</td>
<td>A</td>
<td>A</td>
<td>52.5</td>
<td>A</td>
</tr>
</tbody>
</table>

* A: absent, P: present
(a) Screamed milk for only zootechnic use. It includes 90% of dairy products.
(b) Screamed milk. Presence of vitamin A, D₃, E, B₁, B₂, B₆, B₁₂, C, K₁, niacin, biotin, pantothenic acid, folic acid. Linoleic acid (4.60g), linolenic acid (0.47g). Cream. Emulsionants: lecithin, taurine.
(c) Presence of vitamin A, D, E, K₁, B₁, B₂, B₆, B₁₂, C, niacin, pantothenic acid, biotin, folic acid. Linoleic acid (5.10g), linolenic acid (0.51g). Maize syrup, vegetable oils, soya proteins, sucrose, mineral salts

**Results and discussion**

Test species were able to develop on powdered soya milk (Tables 2-5); the highest and the lowest mean adult number observed for *Ephestia kuehniella* and *Corcyra cephalonica* were 64.0±1.53 and 19.0±1.00, respectively.
Table 2. Mean number of *Plodia interpunctella* (Hbn.) adults (±S.E.), mean developmental period (days) (±S.E.) and mean susceptibility index (±S.E.) observed on different kinds of powdered milk.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Mean adult number</th>
<th>Mean developmental period (d)</th>
<th>Mean susceptibility index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Soya milk</td>
<td>53.7 ± 1.33b</td>
<td>29.3 ± 0.33ab</td>
<td>5.9 ± 0.10b</td>
</tr>
<tr>
<td>Reconstituted milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Baby milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control</td>
<td>89.0 ± 0.58a</td>
<td>25.3 ± 0.88a</td>
<td>7.6 ± 0.18a</td>
</tr>
</tbody>
</table>

Table 3. Mean number of *Ephestia kuehniella* (Zell.) adults (±S.E.), mean developmental period (days) (±S.E.) and mean susceptibility index (±S.E.) observed on different kinds of powdered milk.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Mean adult number</th>
<th>Mean developmental period (d)</th>
<th>Mean susceptibility index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Soya milk</td>
<td>64.0 ± 1.53b</td>
<td>48.7 ± 0.33b</td>
<td>3.7 ± 0.20b</td>
</tr>
<tr>
<td>Reconstituted milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Baby milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control</td>
<td>85.3 ± 0.33a</td>
<td>38.0 ± 0.58a</td>
<td>4.9 ± 0.57a</td>
</tr>
</tbody>
</table>

Table 4. Mean number of *Cadra cautella* (Walk.) adults (±S.E.), mean developmental period (days) (±S.E.) and mean susceptibility index (±S.E.) observed on different kinds of powdered milk.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Mean adult number</th>
<th>Mean developmental period (d)</th>
<th>Mean susceptibility index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Soya milk</td>
<td>59.3 ± 2.33b</td>
<td>34.0 ± 0.58a</td>
<td>5.1 ± 0.08a</td>
</tr>
<tr>
<td>Reconstituted milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Baby milk</td>
<td>0.0 ± 0.00c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control</td>
<td>86.7 ± 0.88a</td>
<td>31.0 ± 0.58a</td>
<td>4.8 ± 0.27a</td>
</tr>
</tbody>
</table>

Soya milk is rich in proteins with a high nutritional power while is poor in protein with sulphurate amino-acids (Wolf & Cowan, 1971). Soya products, as flour and seeds, are susceptible to attack by *Cadra cautella*, *C. cephalonica*, *E. kuehniella* and *Plodia interpunctella* (Bhattacharya et al., 1976; Cox & Simms, 1978; Cline, 1982; Locatelli & Biglia, 1995). In these substrates, and in particular in soya flour, polyunsaturated fatty acids and sterols are considered to be present. These are important growth factors for *P.*
_interpunctella_ (Morère, 1971). Foodstuff insects, in fact, require sterols and unsaturated fatty acids in their diet because they are not able to synthesize them (Thompson et al., 1973).

_C. cautella, E. kuehniella_ and _P. interpunctella_ did not develop on powdered cow’s milk. Lactose, in the presence of lactase, is broken down, in the midgut into galactose and glucose. Sharma et al. (1983) found this enzyme in the _C. cautella_ adults midgut. Galactose must be transformed into glucose by the action of several enzymes, before being able to be absorbed. _C. cautella_, however, was not able to grow on powdered milk with lactose, even though provided with specific enzyme. Galactose, probably, was transformed in galactitol, a toxic alcohol, as already observed in _Tribolium confusum_ (du Val).

Table 5. Mean number of _Corcyra cephalonica_ (Staint.) adults (±S.E.), mean developmental period (days) (±S.E.) and mean susceptibility index (±S.E.) observed on different kinds of powdered milk.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Mean adult number</th>
<th>Mean developmental period (d)</th>
<th>Mean susceptibility index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>17.0 ± 1.21d*</td>
<td>77.2 ± 0.97d</td>
<td>2.2 ± 0.09b</td>
</tr>
<tr>
<td>Soya milk</td>
<td>19.0 ± 1.00d</td>
<td>46.7 ± 0.88b</td>
<td>2.7 ± 0.06b</td>
</tr>
<tr>
<td>Reconstituted milk</td>
<td>32.0 ± 2.00c</td>
<td>87.7 ± 0.33d</td>
<td>1.7 ± 0.04c</td>
</tr>
<tr>
<td>Baby milk</td>
<td>40.7 ± 3.84b</td>
<td>58.3 ± 1.20c</td>
<td>2.7 ± 0.09b</td>
</tr>
<tr>
<td>Control</td>
<td>89.3 ± 0.88a</td>
<td>34.7 ± 0.88a</td>
<td>5.2 ± 0.10a</td>
</tr>
</tbody>
</table>

*Values, followed by a different character, are significantly different for an interval of confidence of 95%.

_C. cephalonica_ developed on whole powdered milk, reconstituted powdered milk and powdered baby milk; mean adult numbers were 17.0±1.21, 32.0±2.00 and 40.7±3.84, respectively.

On the other hand, Trematerra (1983) observed, that on powdered milk _C. cephalonica_ development stops already at the egg stage.

Ferreira et al. (1998) observed that some Lepidoptera species are able to hydrolyze complex sugars even though lacking the specific enzyme. Therefore, it is possible that for _C. cephalonica_, an enzyme endowed with a catalytic site, able to recognize and hydrolyze lactose, evolved in _E. kuehniella_ and _P. interpunctella_.

This ability, present mainly in phytophagous Lepidoptera, was acquired during their evolution to defend themselves by the ingestion of toxic substances present in some plants, as for example, alkaloids (Krieger et al., 1971).

References


Injury capability of pests to stored legumes in Namibia

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Abstract: It is recognized that in developing countries the stored product pests endanger both food safety and food security. However, there is a controversy over the extent of weight losses caused by pests to various stored food products. In this work we report unusually high pest related losses (i.e. losses >95%) to stored legumes (Vigna unguicula, Arachis hypogea - Leguminosae) in Namibia. In the inspected samples and stores we found: (i) 100% infestation of legume kernels; (ii) 100% injury of legume germs; (iii) the weight loss of internal content of kernels of Vigna unguicula was >95%. Symptoms of combined injury caused by pyralid moth (Corcyra cephalonica) and bruchid beetles (Callosobruchus subinnotatus) are described and photo-documented.

Key words: legumes, Namibia, weight loss, damage, Vigna unguicula, Arachis hypogea, Corcyra cephalonica

Introduction

Stored-food pests endanger food security and food safety world wide (Haines., 1991, Hill, 2002). In the developed countries, the activity of stored-product pests is mostly associated with presence of allergens (Arlian, 2002) or toxigenic fungi in the infested stored-food commodities (Hubert et. al., 2003, 2004, Stejskal, et al. 2004). In the developing countries, currently the main concern is not the food safety but the food security. In these regions, pests have the potential to cause weight losses that negatively influence the seed availability and food security of the affected human populations. It is widely recognized that in tropical and subtropical countries the high circum-annual temperatures aggravate the problems with stored food pests enabling a long-term exponential increase of pest populations resulting in measurable weight losses. However, there is a controversy over the extent of losses that may cause pest organisms in various stored food products. Some authors (CIAT, 1986, Hill, 2002) claim that pest-related losses of stored food show figures reaching 5-50 %. On the contrary, Golob et. al. (2002) argues that losses usually do not exceed 5% over the storage season. The aforesaid mentioned controversial information generates conclusion that the data enabling the estimation of the capacity of storage pests for injury/loss in various geographical and storage areas is far to be complete.

The objective of the present paper was to: (i) report weight losses exceeding 95% and (ii) describe the symptoms resulting from infestation of stored legumes (Vigna unguicula, Arachis hypogea - Leguminosae) by arthropod food-pests in the northern Namibia.
Materials and methods

The inspection was conducted in two stores in (Ogongo, Omahenene) in northern Namibia in April and May 2004. We inspected highly infested textile bags (designed for 500 kg of agro-food commodities) containing two legumes, cowpea (*Vigna unguicula*) and groundnuts (*Arachis hypogea*). It was not possible to determine the length of storage since both sample were not labelled properly. However, the estimate of storekeepers was 14 months storage period for *Arachis hypogea*, and 22 months for *Vigna unguicula*. We inspected the content of the bags and collected individual insects for their identification. We sampled 100 seeds in each bag (N= 20 bags per each food commodity) and estimated percent on injured (infested) seed/100 g. Injury symptoms of single and multiple species infestation were described and photo-documented by a digital camera (Fig.1).

Results and discussion

In each examined sample of 100 seeds per each of the total of 20 textile bags: (i) 100% of seeds was infested in both *Arachis hypogea* and *Vigna unguicula*; (ii) 100% injury of germs (100 % loss of germination); and (iii) loss of more than 95% of the internal content of all inspected seed of *Vigna unguicula* (Fig.1 A, B). Fig. 1 B, C show extent and symptoms injury caused solely by *Callosobruchus subinnotatus* on *Vigna unguicula* (Fig. 1B) and *Arachis hypogea* (Fig. 1C). The injury marks caused by bruchid beetles represent oval exit holes and white eggs adhered to the surface of the beans by the female beetle. Fig 1A, D show the extent and the symptoms of combined injury caused by the pyralid moth (*Corcyra cephalonica*) and the bruchid beetles (*Callosobruchus subinnotatus*) on *Vigna unguicula* (Fig. 1A), and *Arachis hypogea* (Fig. 1D). Beside oval exit holes caused by the bruchid beetles, the marks include irregular injuries to the surface layer of the legume kernels and contamination by faeces and webbings that are produced by larvae of the pyralid moth *Corcyra cephalonica*.

Such extensive loss (Fig. 1A, B, C, and D) and contamination of legumes by allergenic faeces (Fig. 1 A, D) of pests exactly documents the large pest potential of storage arthropods to stored food and seeds in developing countries. Oerke & Dehne (2004), in their comprehensive work, reviewed estimates of overall losses for various groups of pest organisms and crops: 31, 8 % weeds, 17,6% animal pests, 14,9 % bacteria and fungi, and 3,1% viruses. In stored products, the reported pest-related losses show figures usually ranging from 5-50%. However, Golob et. al. (2002) consider these figures largely overestimated, asserting that the results for the farm level showed losses to be fairly well contained about or bellow 5% over a storage season. They admitted the only exception: were the high losses caused by *Prostephanus truncatus* (Hodges et. al.1983), to which farmers were unaccustomed, and for which locally traditional storage provided ideal conditions. On the contrary, Schamle et al. (2002) gathered documentation that bruchid beetles (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) may cause losses reaching 7.4 % in Colombia, 35% losses in Mexico and Central America, and 13% in Brazil at different storage times (CIAT, 1986). It was found that after 3 months of pests infestation weight losses were 9.7% caused by psocids (*Liposcelis bostrychophila*) (Kučerová, 2002) and 3,2-9,3 % by *Sitophilus granarius* (Kučerová & Stejskal 1994). Our case showed that long-term storage (>1 year) of unprotected legumes may result in weight losses exceeding 95%.

Acknowledgments

This work was supported by the projects MSMT/B - No 40/03-05/ (P. Kosina, P. Holesovska) and MZE -000-2700603 (V. Stejskal).
Fig. 1. Symptoms and extent of infestation of stored legumes (A, B – *Vigna unguicula*; C, D – *Arachis hypogea*) by stored food pests (A, B, C, D – *Callosobruchus subinnotatus*; A, D – *Corycra cephalonica*). For description, see text.

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Use of biogenerated atmospheres of stored commodities for quality preservation and insect control, with particular reference to cocoa beans

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Abstract: Preliminary data for insect control and for quality preservation of stored cocoa beans is presented, as a methyl bromide alternative, by employing a novel approach through the use of biogenerated modified atmospheres. The respiration rates of fermented cocoa beans from Makassar, Sulawesi, Indonesia, were determined under laboratory conditions. Initial insect populations found in these cocoa beans samples consisted of Carpophilus spp., Ahasverus advena, Cryptolestes spp., and Psocids. Respiration rates of cocoa beans at equilibrium relative humidities (r.h.) of 59, 68, and 73% were determined at 26°C in hermetically sealed 1 L capacity jars containing 500 g commodity. At the equilibrium r.h. of 73%, the respiration of the cocoa beans depleted the oxygen concentration to <1% and increased the carbon dioxide concentration to 23% within six days. To obtain a similar oxygen depletion for cocoa beans at 68% equilibrium r.h, a duration of 23 days was required, while for the sealed cocoa beans in equilibrium with 59% r.h. the oxygen concentration after 23 days had only decreased to 10.8%.

Under field conditions in a cocoa bean storage facility in Makassar, Indonesia, a hermetically sealed flexible structure containing 6.7 tonnes of cocoa beans at an initial moisture content of 7.3% (70% equilibrium r.h.) was monitored for oxygen concentration and quality parameters of the beans. The measurements showed a decrease in oxygen concentration to 0.3% after 5.5 days. No insects survived the oxygen depleted biogenerated atmosphere. These encouraging results reveal the possibility of utilizing biogenerated atmospheres in integrated pest management (IPM) for the quality preservation (by preventing the development of FFA, molds, and mycotoxins), and insect control of cocoa pests.

Key words: cocoa beans, respiration rate, modified atmospheres, methyl bromide alternatives, IPM, storage pest control

Introduction

Cocoa beans are usually harvested twice yearly in a “main”, and a “secondary” harvest. Timing and duration of the harvests are dependent upon climatic conditions. The beans are treated through a process of fermentation after they have been removed from the fruit. This process is necessary to moderate the initially bitter flavour of the cocoa beans and to develop their typical flavour. By fermentation, the highly bitter tannins present in the beans are oxidized, resulting in the formation of aromatic substances and the development of the typical brown to deep red-brown colour of the cocoa beans. As a result of the heat generated by fermentation, the cocoa beans lose their ability to germinate. Depending on the region,
fermentation of cocoa beans is termed either “dry” or “wet”. In the dry fermentation process, cocoa beans are sun dried for several weeks until the pulp decays, whereas in the wet process, the pulp is washed away and subsequently the cocoa beans are sun dried till the moisture content reaches 7% (wet basis). Wet fermentation demands a better post harvest infrastructure than dry fermentation and produces better quality beans. Most of the beans in Africa (Ivory Coast, Ghana) are handled by wet fermentation, whereas most of the Indonesian beans are dry fermented. The beans are collected by traders and sold to exporters who decide either to sell right away or keep the commodity in storage for several months, to speculate for better market prices. In West Africa, some companies are trying to introduce bulk handling in containers, but most of the trade is still in jute bags of about 70 kg. Climatic conditions in the tropics: high humidity levels of 70 to 90% r.h. and temperatures around 30°C are ideal for storage insects and molds to develop on agricultural products. The most common storage pest in cocoa beans from Indonesia and South America is the cocoa moth or the tropical warehouse moth (*Ephestia cautella*), whereas the dominant species in cocoa beans from West Africa is the rice moth (*Corcyra cephalonica*). In addition, several other storage species are known to infest cocoa beans; among them are storage beetles such as the flour beetle (*Tribolium castaneum*) which is very common. Infestation is a major problem and therefore the beans are frequently fumigated using phosphine or methyl bromide (MB).

Fumigants are widely used for pest control both in cocoa beans and also in other stored products to prevent economic and quality losses caused by these insect pests. However, increased public concern over the adverse effects of pesticide residues in food and the environment has led to their partial substitution by alternative control methods. Consequently, non-chemical and environmentally user-friendly methods of pest control in the post harvest sector are becoming increasingly important. 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). 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).

Biogenerated atmospheres can be achieved in hermetically sealed storage systems, and are based on the generation of oxygen-depleted and carbon dioxide-enriched interstitial atmospheres as a result of the respiration of living organisms. Intermediate moisture contents (at equilibrium air relative humidities of 65 to 75%) of stored commodities are inevitable in tropical climates due to the difficulties in maintaining safe moisture contents for long-term storage. Under hermetic conditions, stored commodities with intermediate moisture contents generate modified atmospheres due to the respiration of the microflora and the commodity itself. The objective of this work was to provide preliminary data on this novel approach of using biogenerated modified atmospheres as a methyl bromide alternative for insect control, and for the quality preservation of stored cocoa beans.

**Materials and methods**

**Laboratory trials**

The laboratory trials were carried out in the Department of Food Science, Israel. Dry fermented cocoa bean samples weighing about 1.2 kg each originating from Makassar, Indonesia were tested for their equilibrium r.h. and infestation level. Infestation levels were determined by sieving the samples through a 10 mesh size US standard sieve and examining the beans for free insects.
The equilibrium r.h. was determined using a humidity tester (Defensor® Novasina model ms1, Switzerland) with box sensor enMBRK-3. The equilibrium r.h. values expressed in this paper as percentages are equivalent to the decimal values in terms of water activity (a_w) which is the ratio of the water vapour pressure in the agricultural commodity to the water vapour pressure of pure water at the same temperature.

The equilibrium r.h. value of the cocoa beans was determined as 82% at a temperature of 25°C. The initial samples were then dried in a drying oven at 45°C to obtain equilibrium r.h. levels of 73, 68 and 59%.

Respiration rates of the cocoa beans were determined after the insects were removed from the beans. Concentrations of oxygen and carbon dioxide were determined using a gas chromatograph (SRI 8610C, SRI Instruments USA) equipped with a thermal conductivity detector, with Porapak Q and Molecular Sieve 5A columns for detecting the oxygen, and the carbon dioxide concentrations. Weighed amounts of 500 g of cocoa beans were placed in 1 Liter capacity glass jars sealed with gastight metal covers equipped with silicon septa for gas sampling. The gas composition within each jar was periodically sampled using a 1 mL "pressure-lock" syringe through the septum. Gas samples were taken according to the respiration intensity of the cocoa beans inside the jars. The jars were stored throughout the respiration tests at 26°C.

Field trial in Makassar, Indonesia

A field trial was carried out in warehouses located in Makassar (Ujung Pandang), Indonesia, starting on June 6 and ending November 17, 2005. The trial consisted of storing locally available bagged cocoa beans in a gas-tight sealed GrainPro® Cocoon of 15 m³ capacity. The Cocoon accommodated 108 jute bags each of 62.5 kg capacity comprising a total of 6.75 tonnes of cocoa beans. The initial moisture content (m.c.) was determined on ten samples taken from the bottom, middle and top layers of the stack. The average m.c. of each sample was taken at the start of the trial in June and at the end of the trial.

Results and discussion

Laboratory trials

Examination of the two samples of cocoa beans received in the laboratory (weighing together 2.45 kg) revealed that there was a significant insect population in the samples. The insects consisted of: adults of Cryptolestes sp. (30 dead and 6 live), adults of Carpophilus sp. (5 live), Acarina (> 20 live), adults of Ahasverus advena (10 dead); Psocidae (> 20 live), adults of field flies (12 dead), Phycitid moths (1 dead larva, 1 dead adult), and adults of Araecerus fasciculatus (2 dead). Such an insect population would require periodic control measures during the storage time of the cocoa beans.

The equilibrium r.h. values were determined on the samples of cocoa beans received from the warehouse in Makassar. This is a convenient and accurate measure as an alternative to the determination of moisture content in vacuum ovens or other methods. According to the ICCO (Anonymous, 1999) the moisture content of cocoa beans should meet the export standard of the country exporting the beans; in general this is around 7.5% but ranges from 5.5% to 8% depending on the country concerned. According to the Transport Information Service (TIS) (Anonymous, 2006), when transporting cocoa beans in containers, care should be taken to ensure that the moisture content of the cocoa beans on packing is approx. 6 – 8%, which corresponds to an equilibrium relative humidity of 55 – 75% (at 26°C). These are

*Mention of trade names or commercial products in this article is solely for providing specific information and does not imply recommendation or endorsement by the Israel Agricultural Research Organization.
values that arouse considerable problems from the outset, because the higher moisture content cited of 8% corresponds to the mold growth threshold of 75%. Moreover, cocoa beans have an elevated fat content which, in conjunction with moisture, results in hydrolytic/enzymatic fat cleavage and self-heating of the cocoa beans. An examination of the low temperature/dew point difference will also show how rapidly the dew point of the cocoa bean cargo is reached on cooling. It is thus recommended to insist on a water content of 6% or less when transporting cocoa beans in containers.

To clarify the relationship between the moisture content and equilibrium r.h. of cocoa beans, Fig. 1 was compiled using the data of Gough (1975) at 27° – 29°C. Data in Fig. 1 are close to the values given by the ICCO but are quite different from data provided by Hall (1960) and TIS (Anonymous, 2006). Therefore, data in Fig. 1 should be viewed as guiding information until additional data becomes available in the literature. Our approach using the equilibrium r.h. values strengthens the view of using the equilibrium r.h. to determine the micro-floral activity in cocoa beans since this activity is dependent on the water activity, which is a more meaningful criterion, and is the decimal of the equilibrium r.h. values.

Fig. 1. Equilibrium moisture content of cocoa beans at 27° – 29°C (Gough, 1975).

Respiration rates determined on cocoa beans free from insects at equilibrium r.h. values of 59, 68, and 73% are shown in Figures 2, 3, and 4, respectively. From these figures it is obvious that the increase in the equilibrium r.h. values caused a significant increase in respiration rates. The respiration rate values for the 82% equilibrium r.h. was not graphically presented in this paper, since the extremely high gas exchange intensity necessitated gas analysis almost every two hours, that would necessitate measurements over a work day period that was not available to the laboratory. However the respiration rate could be calculated and resulted in complete depletion of oxygen within 36 h. In contrast, Fig. 2 shows that at 59% equilibrium r.h., even after 21 days the oxygen level remained at 11%. The respiration rates shown in Figures 3 and 4 reveal that an equilibrium r.h. value between 68% and 73% would
be sufficient for the generation of an oxygen depleted atmosphere within a one to two week period. Such an atmosphere would be suitable for the control of all storage insects. This deduction would imply that for the cocoa beans industry, the repeated fumigations using phosphine or methyl bromide might be superfluous. All that is necessary would be to seal the commodity and let the organisms including the insects themselves to deplete the oxygen to a level where survival would no longer be possible. In the case of phosphine a ten days exposure, now has to be, and is implemented for a successful fumigation due to insect resistance problems. In the case of cocoa beans, the ICCO (Anonymous, 1999) recommended moisture content of 7.5% for storage, would be sufficient to rely on biogenerated atmospheres, since this moisture content is in equilibrium r.h. with about 70% (Fig. 1).

![Graph showing CO2 and O2 concentration over time](image)

*Fig. 2. Respiration rate of cocoa beans at 59% equilibrium r.h. and 26°C.*

**Field trial in Makassar, Indonesia**

Fig. 5 shows the set up of the GrainPro* Cocoon before and after sealing. The Cocoon was kept for about six months inside the warehouse during which period the ambient temperature ranged closely around 30°C. The cocoa bean samples that were taken before and after the storage period showed a slight but significant increase in moisture content from 7.3 to 7.7%. This range of moisture content is equivalent to a range of about 70 to 73% equilibrium r.h. (Fig. 1). According to the laboratory data for the equilibrium r.h. of 73% (Fig. 4), it would be reasonable to expect an oxygen drop within 6 days. Not surprisingly, Fig. 6 shows an oxygen depleted atmosphere within 5.5 days, this being very close to the laboratory data. Such an atmosphere would be sufficient to control the existing insect population. Indeed at the opening of the Cocoon in November, no live insects were recorded on the ten cocoa bean samples.

On the opening of the Cocoon in November, several mouldy sacks were observed on the top of the stack. Although such appearance caused concern, according to the TIS (Anonymous, 2006), if the moisture content is < 6%, cocoa beans become brittle, while at a moisture content of > 8%, there is a risk of vapor and mold damage which cause depreciation which may go as far as total loss due to rot. A fundamental distinction is drawn between two types of moisture damage: sweat damage and vapor damage:
Fig. 3. Respiration rate of cocoa beans at 68% equilibrium r.h. and 26°C.

Fig. 4. Respiration rate of cocoa beans at 73% equilibrium r.h. and 26°C.

**Sweat damage (mould damage):** Recognizable by spots on the bag fabric caused by drops of drip water. Under these spots, there are clusters of cocoa beans covered with white mold and stuck together. In serious cases, the mold penetrates into the kernel of individual beans. As a result, these then smell and taste musty. Such losses are usually limited to only a few bags in a consignment and are caused by the formation of ship sweat below deck, especially at night when the surrounding atmosphere and thus the outer walls of the hold cool down. If the upper
layer of bags in the hold is inadequately covered, the dripping cargo sweat cannot be absorbed, penetrates into the bags containing the cocoa beans and causes the damage described above.

Fig. 5. The GrainPro cocoon, before sealing (left) and after sealing (right) (courtesy of Tom deBruin).

![Image of the GrainPro cocoon before and after sealing](image)

Fig. 6. Changes in oxygen concentration recorded throughout the storage period in Makassar, Indonesia.

![Graph showing changes in oxygen concentration](image)

**Vapour damage**: this is caused by excessive relative humidity in the hold or container. While the cocoa beans have only a thin covering of mold, from time to time the damage affects the entire contents of the bags stowed in a hold. Vapor damage is thus generally much more extensive than sweat damage. Marked mold growth is not normally observable, but aroma and flavour are still considerably degraded. For this reason, care must be taken not only to prevent formation of sweat, but also to ensure favorable relative humidity values in the hold/container.
From the observations made at the opening of the cocoon in November, most probably the observed white mouldy spots on the bags were caused due to condensation that could be attributed to sweat damage, since except for the several bags on the top of the stack the rest of the bags remained without such visible mould spots. These encouraging results have led to additional trials with slightly lower equilibrium r.h. of cocoa beans.

References

Effectiveness of gaseous ozone alone and in combination with low pressure or carbon dioxide against *Ephestia kuehniella* (Zell.) (Lepidoptera: Pyralidae) at short exposure time

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**Abstract:** Toxicity of initial concentration of 300 ppm gaseous ozone either alone or combined with a low pressure of 100 mm Hg and 92% CO₂ against all life stages of *Ephestia kuehniella* (Zell.) at short exposure time (2 hours) was studied. Results indicated that a remarkable difference in corrected mortalities of the treatments for each life stage. For the larvae, adults and pupae, treatments of ozone alone and all in combination with vacuum and CO₂ resulted in complete mortalities except ozone treatment in combination with CO₂ for the pupae by the mortalities ranging from 79.3 to 91.4%. However, although ozone alone had a higher mortality on the eggs by 85.1% than all other treatments any of the treatments did not give complete mortality. Clearly it indicated that the adults and larvae were the most easily killed, followed by the pupae and finally the eggs, which were the most tolerant to ozone treatments. There were very limited mortalities of all stages except the adults (21.9% to 100%), when exposed to either 100 mm Hg or 92% CO₂ for 1, 2 and 4 hours. Ozone alone was found to be effective against all the life stages of the common stored-product insect, *E. kuehniella*, at high initial concentration (300 ppm) and short exposure time (2 hours). However, the use of a low pressure of 100 mm Hg, or 92% CO₂ did not cause synergistic effect on this species as evidenced by no significant decrements in mortalities for all life stages. These results indicate that ozone alone is sufficient to have potential alternative to methyl bromide fumigation for rapid disinfestation of commodities.

**Keywords:** Ozone, fumigation, quarantine, toxicity, carbon dioxide, vacuum, *Ephestia kuehniella*

**Introduction**

During the last two decades methyl bromide (MB) and phosphine (PH₃) have in many situations replaced other fumigants used for disinfestation of stored products such as food grains, grain products, nuts, and animal feed. Now MB is being phased out as a fumigant under the Montreal Protocol because of its ozone depletion potential (UNEP, 1995). Therefore, for the disinfestation of durable stored products, phosphine is an important alternative, having already replaced MB in many situations. However, phosphine is not always appropriate for some uses due to being a dangerous gas, pest resistance (Champ & Dyte, 1976; Zettler *et al.*, 1989; Zettler & Cuperus, 1990) and its requirement of long exposure period (5 days or longer) (Howe, 1973), which makes the chemical unsuitable for rapid disinfestation or quarantine fumigations. Sulphuryl fluoride is used in some countries to disinfest structures, but has relatively poor ovicidal properties and long exposure period
(1 day or more) for quarantine fumigations (Taylor, 1994). MB is apparently the only fumigant available for quarantine treatment of commodities for which rapid disinfestation techniques and a very high degree of insect mortality are essential. The loss of MB could have a significant negative impact on world agriculture, particularly because no available alternatives to MB currently exist for rapid disinfestation of commodities. Thus, there is a critical need to develop new fumigants for quarantine purposes.

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 lasts about 20 minute, depending on the temperature. Thus, it does not accumulate substantially without continual ozone generator (Peleg, 1976; Miller et al., 1978). At room temperature, ozone is nearly colorless gas. Ozone has a pungent, characteristic odor 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 impure solution (Hill & 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 against micro-organisms 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. Attractive aspect 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 were 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 and Fusarium moniliforme Sheldon (Mason et al., 1997).

Ozone in its gaseous form has been also considered to have potential to kill insect pests in commodities and was subjected of 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 (Motsch.), and the red flour beetle Tribolium confusum Jacquelin du Val, and the larval stage of the Indian meal moth, Plodia interpunctella (Hübner) exposed to lower ozone concentrations ranging from to 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, Tribolium castaneum (Herbst.) when exposed to a 45 ppm ozone environment. However, in these studies the time of treatment was three days, which is allowable for control fumigations but not for quarantine fumigation. Other than that research, little has been published to determine whether ozone has potential as a fumigant for eliminating pests from postharvest commodities in food industry. Our study was therefore designed to determine the potential uses of ozone gaseous in controlling postharvest storage and quarantine insects in durable or perishable commodities by evaluating the toxicity of gaseous ozone alone and in combination of low pressure and CO₂ against all life stages of Ephesia kuehniella (Zell.) at short exposure time.

Materials and methods

Test insects
Tests were carried out on all life stages (adult, larva, pupa and egg) of E. kuehniella. All stages were obtained from cultures reared at 26 ± 1°C and 70 ± 5% relative humidity (r.h.) on
a diet of ground wheat, yeast and glycerol using standard culture techniques (Donahaye, 1990). Cultures were started from eggs collected from 3 L empty oviposition jars containing adults of *E. kuehniella*. 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 days 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 jars were sealed with silicone vacuum grease.

**Ozone fumigation procedures**

Ozone generator in laboratory scale was provided from the company Ozomax Inc., Canada (http://www.ozomax.com). Ozone gas was generated using a laboratory corona discharge ozone generator (Model OZO-IVTT) from purified extra dry oxygen feed gas. The output of generator was 5 g/h. Measurement of carbon dioxide (CO₂) was carried out using a Bedfont MB gas monitor Model 415, equipped with a thermal conductivity detector. CO₂ was supplied from a cylinder and was 99 + % pure.

Ozone was introduced as gaseous 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. For eggs, fifty eggs placed in opened Petri dishes were used per fumigation.

For ozone fumigation at atmospheric pressure, the insects were first placed in exposure jars and then, the jars were briefly evacuated to 100 mm Hg followed by flushing with ozone gaseous until restoration of atmospheric pressure. This process lasted 25 seconds. The initial ozone concentration calculated as 300 ppm alone was exposed to all life stages of *E. kuehniella* for 2 hours. For ozone fumigations in combination with low pressure, the jars were briefly evacuated to 100 mm Hg and then the insects were kept to a low pressure of 100 mm Hg for 1, 2 and 4 hours. Afterwards ozone was flushed into exposure jar until reaching atmospheric pressure and was exposed to the insects for 2 hours. For ozone treatments in combination with CO₂, the jars were briefly evacuated to 60.8 mm Hg followed by flushing with CO₂ until restoration of atmospheric pressure so as to obtain a uniform concentration of 92% CO₂ and then the insects were kept under this CO₂ atmosphere for 1, 2 and 4 hours. Afterwards the jars were briefly evacuated to 100 mm Hg followed by flushing with ozone until restoration of atmospheric pressure and then insects were exposed to ozone for additional 2 hours. For ozone treatments in combination with a low pressure and CO₂, initial ozone concentration was also calculated as 300 ppm. In addition separate exposures to 100 mm Hg and to 92% CO₂ alone for 1, 2 and 4 hours were made, and untreated control insects were exposed to atmospheric conditions.

Each test was replicated three times. The gas mixtures in the jars 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 jars was checked at the end of each test. Relative humidity during fumigations was also measured by placing small mechanical hygrometers within the jars.
**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 obtained from the treatments were obtained and were corrected by using Abbott’s formula (Abbott, 1925). Mortality data was subjected to Arcsin transformation and then, were analyzed using one-way analysis of variance (ANOVA). The means were analysed using the LSD method at 1% level (SAS Institute, 1985).

**Results and discussion**

Corrected mortality (%) of all life stages of *E. kuehniella* following treatments exposed to initial concentration of 300 ppm ozone either alone or combined with CO₂ for 2 hours are presented in Table 1. The table shows a remarkable difference in corrected mortalities of the treatments for each life stage of *E. kuehniella*. While the treatments of ozone alone, 1-h CO₂+O₃, 2-h CO₂+O₃, and 4-h CO₂+O₃ had a complete mortality (100%) on the adults, treatments of 1-h, 2-h and 4-h CO₂ alone gave low mortalities ranging from 21.9% to 63.5%. For the pupae, ozone alone resulted in a complete mortality, while treatments of 1-h CO₂+O₃, 2-h CO₂+O₃, and 4-h CO₂+O₃ had a significantly lower mortality than treatments of ozone alone. Treatments of 2-h and 4-h CO₂+O₃ had a significantly higher mortality for the pupae than treatments of 1-h CO₂+O₃, while treatments of all CO₂ alone gave much lower mortality than all other treatments. For the larvae, the treatments of ozone alone and all CO₂+O₃ resulted in a significantly higher mortality than treatments of all CO₂ alone. However, there was not significant difference in mortalities of the treatments of ozone alone and all CO₂+O₃. There was no treatment that gave the complete mortality on the eggs, although treatments of ozone alone resulted in the highest mortality (85.1%) followed by 4-h, 1-h and 2-h CO₂+O₃ with the mortalities of 77.3%, 68.8% and 64.5% respectively. As similar to treatments of CO₂ in combination with O₃, there was no treatment that gave the complete mortality on the eggs, although treatments of ozone alone and 4-h vacuum+O₃ resulted in the highest mortality by the mortalities of 85.1% and 81.4% respectively. It was followed by 1-h and 2-h vacuum+O₃ with the mortalities of 74.9% and 75.8% respectively. Treatments of vacuum alone produced much lower mortality on the eggs than ozone alone and vacuum+O₃.
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 laboratory study, Mason et al., (1997) reported that 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. In these studies the time of treatment was too long (3 days), which is not suitable for quarantine fumigation. However, in our study treatment of ozone alone resulted in complete mortality of all life stages of *E. kuehniella* (except egg stage) for short exposure period (2 hours). In similar to our results obtained from ozone alone treatments, Leesch (2002) reported that laboratory treatment of ozone alone resulted in high mortalities on the pupae of *P. interpunctella* at high concentration (300 ppm) at short exposure time (4 hours).

Table 1. Corrected mortality (%) of all life stages of *E. kuehniella* following treatments exposed to initial concentration of 300 ppm ozone either alone or combined with CO₂ for one to four hours.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Adult (Number of individuals tested)</th>
<th>Pupa (Number of individuals tested)</th>
<th>Larva (Number of individuals tested)</th>
<th>Egg (Number of individuals tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-h CO₂+O₃</td>
<td>100±0 (60) A</td>
<td>79.3±2.9 C (60)</td>
<td>98.3±1.7 A (60)</td>
<td>68.8±1.9 C (150)</td>
</tr>
<tr>
<td>2-h CO₂+O₃</td>
<td>100±0 (60) A</td>
<td>87.9±3.5 B (60)</td>
<td>98.1±1.9 A (60)</td>
<td>64.5±1.4 C (150)</td>
</tr>
<tr>
<td>4-h CO₂+O₃</td>
<td>100±0 (60) A</td>
<td>91.4±1.7 B (60)</td>
<td>98.3±1.7 A (60)</td>
<td>77.3±0.7 B (150)</td>
</tr>
<tr>
<td>Ozone alone</td>
<td>100±0 (60) A</td>
<td>100±0 A (60)</td>
<td>100±0 A (60)</td>
<td>85.1±2.1 A (150)</td>
</tr>
<tr>
<td>1-h CO₂</td>
<td>21.9±2.1 D (60)</td>
<td>10.2±3.4 E (60)</td>
<td>36.7±3.3 C (60)</td>
<td>19.1±1.2 E (150)</td>
</tr>
<tr>
<td>2-h CO₂</td>
<td>27.9±4.0 C (60)</td>
<td>24.0±1.7 D (60)</td>
<td>43.9±2.1 BC (60)</td>
<td>21.3±1.2 E (150)</td>
</tr>
<tr>
<td>3-h CO₂</td>
<td>63.5±2.3 B (60)</td>
<td>29.2±1.7 D (60)</td>
<td>50.2±3.1 B (60)</td>
<td>20.9±1.1 D (150)</td>
</tr>
</tbody>
</table>

Means within a column with the same upper-case letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied for data.

Comparative corrected mortality (%) of all life stages of *E. kuehniella* following treatments exposed to initial concentration of 300 ppm ozone either alone or combined with vacuum and CO₂ for 2 hours is given in Figure 1. For the larvae and adults, treatments of ozone alone and all in combination with vacuum and CO₂ resulted in complete mortalities. For the pupae ozone alone and ozone in combination with vacuum gave a complete mortality, while ozone in combination with CO₂ had lower mortalities than ozone alone and ozone in combination with vacuum. Although ozone alone had a higher mortality on the eggs than all other treatments, any of the treatments did not give complete mortality. Clearly it indicated that the adults and larvae were the most easily killed, followed by the pupae and finally the
eggs, which were the most tolerant to ozone treatments. As similar to our study, Leesch (2002) reported that the adults of Indian meal moth (*P. interpunctella*) were the most susceptible to the ozone treatments, followed by the larvae, pupae and finally eggs, which were unaffected. This finding was also supported by the work of Erdman (1980) who looked at two species of flour beetles.

Most of the insects might be protecting themselves by closing their spiracles when exposed to the fumigants and therefore not be taking in the toxicant. Additions of CO₂ or vacuum application have been used to study the physiology of insects and to find ways to increase their respiration so that the insects are more susceptible to a toxic gas. Many studies have shown that vacuum fumigation or the admixture of CO₂ could increase the toxicity of fumigants, mainly MB and phosphine (Monro *et al*., 1966; Dumas *et al*., 1969; Calderon & Leesch, 1983; Williams, 1985; Donahaye & Navarro, 1989). From our study it can be seen that the use of vacuum or CO₂ with ozone clearly did not result in significant increase of mortalities for all life stages (Fig. 1). It might be argued that low O₂ concentrations did not increase the effect of CO₂ and reduced pressure. This can be attributed to high ozone concentration in ozone alone treatments that caused high mortalities for the adults, pupae and larvae. In the case of egg stage, the results suggest that low pressure and CO₂ did not have a synergistic effect on the eggs when exposed together with ozone. Similarly, Leesch (2002) reported that CO₂ increase the toxicity of ozone (300 ppm) for the egg stage of *P. interpunctella*, which is unaffected by ozone with CO₂.

### Table 2. Corrected mortality (%) of all life stages of *E. kuehniella* following treatments exposing to initial concentration of 300 ppm ozone either alone or combined with vacuum for one to four hours.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Corrected mortality (%)±SE (Number of individuals tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
</tr>
<tr>
<td>1-h vacuum+O₃</td>
<td>100±0 A</td>
</tr>
<tr>
<td>(60)</td>
<td>(60)</td>
</tr>
<tr>
<td>2-h vacuum+O₃</td>
<td>100±0 A</td>
</tr>
<tr>
<td>(60)</td>
<td>(60)</td>
</tr>
<tr>
<td>4-h vacuum+O₃</td>
<td>100±0 A</td>
</tr>
<tr>
<td>(60)</td>
<td>(60)</td>
</tr>
<tr>
<td>Ozone alone</td>
<td>100±0 A</td>
</tr>
<tr>
<td>(60)</td>
<td>(60)</td>
</tr>
<tr>
<td>1-h vacuum</td>
<td>30.8±4.9 B</td>
</tr>
<tr>
<td>(60)</td>
<td>(60)</td>
</tr>
<tr>
<td>2-h vacuum</td>
<td>100±0 A</td>
</tr>
<tr>
<td>(60)</td>
<td>(60)</td>
</tr>
<tr>
<td>3-h vacuum</td>
<td>100±0 A</td>
</tr>
<tr>
<td>(60)</td>
<td>(60)</td>
</tr>
</tbody>
</table>

Means within a column with the same upper-case letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied for data.

In conclusion, ozone alone was found to be effective against all the life stages of the common stored-product insect, *E. kuehniella*, at high concentration (300 ppm) and short
exposure time (2 hours). However, the use of a low pressure of 100 mm Hg, or 92% CO₂ did not exhibit a synergistic effect on this species as evidenced by no significant differences in mortalities for all life stages. These results indicate that ozone alone is sufficient to have potential as an alternative to methyl bromide fumigation of commodities. Clearly, further research is needed to obtain toxicity data on other stored-product insects, on its absorption by different commodities, and on its power of penetration into bulk commodities.

![Graph showing comparative corrected mortality (%)](image)

**Fig. 1.** Comparative corrected mortality (%) of all life stages of *E. kuehniella* following treatments exposed to initial concentration of 300 ppm ozone either alone or combined with vacuum and CO₂ for 2 hours. Means on a bar with the same upper-case letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied for data.

**Acknowledgements**

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**References**


Control of *Sitophilus oryzae* (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) by modified atmosphere created by paddy husk combustion

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Abstract: The smoke generated from paddy husk combustion, was evaluated for its toxicity against adults of Rice Weevil *Sitophilus oryzae* L., Lesser Grain Borer *Rhyzopertha dominica* F. and Red Flour Beetle *Tribolium castaneum* (Herbst.) under laboratory conditions. Adults of these species were put into cloth sacks hung in sealed bottles which were later filled with smoke generated from paddy husk combustion. Insects prepared in the same manner were kept as the control without being exposed to smoke. After different periods of exposure to smoke, response of insects was evaluated in terms of mortality.

A significant lethal effect of the paddy husk combusted gas was observed on the adults of all three insect species tested compared to the untreated control. Adult mortality of 100% was achieved for *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* at 14, 13 and 18 hours of exposure respectively, compared to 5-6.6% mortality in the relevant controls.

The experiments were conducted by changing the carbon dioxide and carbon monoxide concentrations separately. It was revealed that carbon monoxide was directly related to the mortality of the target insects. The recorded mortality was brought about by 5000-6000 ppm of carbon monoxide. This study shows the application of smoke enriched with carbon monoxide generated from the partial combustion of paddy husk as an insect pest management strategy for the insect pests of stored paddy instead of complete combustion and thus saving energy.

Key Words: *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, smoke, paddy husk, carbon monoxide

Introduction

Insect pest management during the storage of paddy claims an important position in pest management as that of pre harvest stage. Experiments conducted by the Institute of Post Harvest Technology (IPHT), Sri Lanka have shown that nearly 80% of the total loss in grains during storage occurs due to insect attacks (Palipane, 2001).

Fumigants are ideal insect pest control measures due to their many advantages such as penetrability into the grain where the alleged insects reside and evenly spreading throughout the grain mass. However, there are several limitations in the application of currently available synthetic chemical fumigants, such as, high toxicity to humans, environmental hazards and high cost. More importantly, there is a risk of resistance development by common insect pests to the currently available fumigants signaling the risk of them being removed from the list of registered fumigants. Hence, it is necessary to explore the alternatives. Paddy husk is ideal in this regard since it is an agricultural by product and is readily available to the farmer in the rice growing areas of the world.
An attempt was made to investigate and ascertain the toxicity of the smoke generated from paddy husk combustion against adults of common insects of stored paddy in Sri Lanka.

Materials and methods

This research was conducted at the Institute of Post Harvest Technology (IPHT), Anuradhapura, Sri Lanka. The research was designed in Complete Randomized Design (CRD) with 3 replicates. Adults of Rice Weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae), Lesser Grain Borer *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) and Red Flour Beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) were used in the experiment.

Unsexed insect adults of one week old and approximately the same size were counted into cloth sacks of thin material (8x10 cm in size) to which a small sample of broken rice was also added as a nutritional medium. Twenty adults were used in one replicate. Cloth sacks containing the insects prepared as above were hung in empty glass bottles (3.5 litres in volume). The paddy husk was combusted in a stove made by metal (Fig. 1) and the gas emanated upon combustion was conveyed through plastic tubes to the bottle in which the test insects were present (Fig. 2).

![Fig. 1. Stove fabricated to generate smoke from partial combustion of paddy husk](image)

The insects were thus exposed to the flue gas generated. When the bottle was completely filled with the smoke, in 15-20 minutes, the gas concentration was detected using Gas Analyzer (Ecoline 6000) and conditions were set to generate more carbon monoxide by pressing paddy husk well inside the stove. When the carbon monoxide concentration was increased and attained more or less constant (5000-6000 ppm) the bottle containing the insects was disconnected and was sealed tightly at the both ends of the plastic tubes by heating and pressing it so that there would be no gas leakage. The exposure periods of insects to the smoke ("sealed periods") were increased until 100% mortality was achieved in the treatment sample (Reichmuth, 1986). Each exposure time was replicated three times. Control samples
were kept sealed for similar time periods, as those of relevant treatment samples, without being exposed to smoke. Mortality was counted 24 hours after each exposure period (Wijayaratne et al., 2004). Treatment mortality data were compared with those of the relevant controls (Abbott, 1925).

Fig. 2. Experimental set-up

**Results and discussion**

Smoke generated from partial combustion of paddy husk contained a gaseous mixture consists of carbon monoxide, carbon dioxide, oxides of nitrogen, hydrocarbons and excess oxygen not used for the combustion. Variation of the gaseous composition as observed during the combustion process is given in table1.

As revealed by Figures 3, 4, and 5, the adult mortality increased gradually with the increase in the exposure period of the insects to the smoke generated from partial combustion of paddy husk irrespective of the species. In the treatment samples, adult mortality of 100% was achieved at 13 hrs, 14 hrs and 18 hrs for *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* respectively whereas in the control samples, mortality of 5% in the first two species and 6.66% in the third species were recorded.

Table1. Composition of smoke generated from partial combustion of paddy husk

<table>
<thead>
<tr>
<th>Gas</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon monoxide</td>
<td>5328 – 6014 ppm</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>3.7 – 1.9%</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>70 – 58 ppm</td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>0.26 – 0%</td>
</tr>
<tr>
<td>Oxygen</td>
<td>17.2 – 19%</td>
</tr>
</tbody>
</table>
When the experiment was conducted by increasing the CO₂ concentration up to 16%, allowing the complete combustion of paddy husk, no remarkable mortality was observed. Strikingly, when the carbon monoxide (CO) concentration was increased while carbon dioxide was at low level, allowing the partial combustion of paddy husk, the insect mortality was also observed to follow the same trend.

Results of this experiment show that there is a definite effect of smoke generated from partial combustion of paddy husk on the mortality of adults of common insect pests of stored paddy in Sri Lanka; *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum*. Furthermore, it is evident that the lethal effect of the smoke was brought about by carbon monoxide.

![Fig. 3. Effect of smoke on *Sitophilus oryzae*](image)

Experiments conducted in Australia and Brazil highlight the use of CO₂, applied as dry ice at a concentration of 60% for the control of stored product insect pests (Banks & Sharp, 1979; Goncalves *et al.*, 2000). This study shows the possibility of using smoke generated from partial combustion of paddy husk for the control of stored product insect pests as an alternative to the chemical insecticides. Paddy husk which is an agricultural by product confers remarkable advantages such as free availability, extremely low cost, environmentally sound nature and low toxicity compared to the commercially available fumigants.

Insect pest control of this nature, using the smoke generated from partial combustion of paddy husk which is an agricultural by product, has never been scientifically addressed in Sri Lanka. The promising results obtained during the laboratory scale study, indicates the possibility of using it as an insect pest management strategy during paddy storage. Also, it confers remarkable advantages to the farmers in the paddy growing areas as indicated above. In this study, the target insects placed inside sacs prepared from thin cloth material were directly exposed to the smoke. They, however, are in between the grains or sometimes inside the grains stored in poly sacks under natural storage conditions. Therefore, the application of this fumigation method has to be attempted under such natural conditions before recommending it as a low cost alternative insect pest management strategy.
Fig. 4 - Effect of smoke on *Rhyzopertha dominica*.

Fig. 5. Effect of smoke on *Tribolium castaneum*.

**Acknowledgement**

The financial assistance by the Sri Lanka Council for Agricultural Research Policy (CARP-12/540/411) is acknowledged.

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Improving the reliability of heat treatments in food industry

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Abstract: A German flour mill was heat treated in August 2005. Heated air was blown into different parts of a 40 m high flour mill building through air hoses. The building consisted of a flour mill built in 1989 and a new mill built in 2002 adjacent to it. Connected to both mills was a packaging area, with just a first floor included as a loft into the 7 m high hall where finished products such as flour, food pellets, and bran were stored.

Bioassay samples containing caged adults and all developmental stages of *Rhizopertha dominica* were distributed in 50 locations of the building. The cages were placed into sealed cotton bags and every other bag was supplied with a data logger recording temperature and moisture contents in 15 min intervals. Infrared (iR)-thermographs were used during the treatment from outside and inside the building to detect areas of heat loss or cold bridges, respectively.

Temperatures achieved in most of the open space and in all of the machinery tested were sufficient to control insects. However, not all test insects were killed during the treatment, the topmost floor in the older mill, as well as several upper floors in the new mill and areas in the cellar did not reach temperatures above 50°C. Another obstacle to the treatment was that the mill had not been emptied and cleaned prior to the treatment, but contained bag stacks with flour, wooden beams, garbage bags with dust and husks and a bucket with water. Some of these materials allowed the survival of insect samples as well as a portion of the natural infestation with *Tribolium confusum* and *Cryptolestes ferrugineus* beetles. In conclusion, it is crucial for the success of a disinfestation to make food industry management aware of the prerequisites of a heat treatment. iR-thermographs, data loggers, manual temperature measurements and a bioassay with insect samples were found to be good and complementary tools to determine the efficacy of a treatment.

Key words: flour mill, infestation, insects, control, hygiene, infrared thermography

Introduction

After the phase out of methyl bromide in industrial countries by the end of 2004, many mills in industrialised countries have to rely on other methods of pest control. A possible option for pest control is the application of high temperatures alone (Fields, 1992, Burks et al., 2000), or in combination with fumigants (Adler, 1997; Bell et al., 2004; Mueller, 1994) or diatomaceous earths (Dowdy, 1999, Dowdy & Fields, 2002). In Germany, two different systems of disinfection using heat alone compete on the market: the use of ex-proof electrical heaters inside a building that circulate and re-heat air (www.thermonox.de) and the use of external burners that blow hot air into the building through a number of large ducts (www.biotech.at). Both systems have their advantages and limitations. While the use of electrical heaters inside a building is more discrete and may allow a more precise temperature control around sensitive machinery and materials, the hot air produced by burners is more cost efficient and it may be possible to treat larger buildings with this system. The volume of a premise that can be treated at once is limited in the former case by the availability of electric...
energy and the running costs of electric heaters and fans suitable to heat spaces as large as 40,000 m³, and in the latter case by the number of hot air-ducts that can be fed into a building through doors and windows. Because inspection is carried out frequently in all parts of the building to check for the even distribution of heat, not all doors and hallways can be blocked by air ducts.

Laboratory studies proved that high temperatures result in reliable control of stored product pests (Beckett et al., 1998, Adler, 2003, Adler, 2004, Adler & Grosse, 2004). Exposure times at 45°C, 50°C and 55°C needed to control various species of stored product beetles and their developmental stages are given in Table 1. With the exposure times needed for a complete disinfestation, heat competes well with the exposure time necessary for fumigation. Even cold buildings, where fumigation would take much longer time can be heat treated, heat loss could be minimised by using electrical heaters inside the premise.

The most difficult task, however, is to secure an even distribution of high temperatures in a large building, to avoid cooler zones or insulating materials that could allow the survival of insects. Dowdy (1999) described the uneven distribution of heat by temperature contour maps. The study presented here was carried out in August 2005 to determine the efficacy of a practical heat treatment with external burners in a German flour mill, to describe critical points and to test tools that may allow a more precise surveillance of the temperatures in different parts of a building.

Table 1. Exposure times (min) needed for complete control of stored product beetles at various temperatures in laboratory tests, and developmental stage found most tolerant to the respective temperature

<table>
<thead>
<tr>
<th>Insect species</th>
<th>45°C</th>
<th>50°C</th>
<th>55°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sitophilus granarius</em></td>
<td>540 (9 h) L</td>
<td>40 L</td>
<td>30 L</td>
</tr>
<tr>
<td><em>Sitophilus zeamais</em></td>
<td>660 (11 h) L</td>
<td>45 A</td>
<td>30 A</td>
</tr>
<tr>
<td><em>Cryptolestes pusillus</em></td>
<td>1200 (20 h) L</td>
<td>65 L</td>
<td>20 L</td>
</tr>
<tr>
<td><em>Tribolium castaneum</em></td>
<td>1800 (30 h) L</td>
<td>35 L</td>
<td>20 L</td>
</tr>
<tr>
<td><em>Lasioderma serricorne</em></td>
<td>2400 (40 h) L</td>
<td>370 E</td>
<td>45 E</td>
</tr>
<tr>
<td><em>Rhyzopertha dominica</em></td>
<td>6000 (100 h) L</td>
<td>370 L</td>
<td>45 L</td>
</tr>
</tbody>
</table>

E = egg stage, L = larval stage, A = adult stage

**Material and methods**

**Structure of the flour mill**

A flour mill in the Southwest of Germany close to the city of Heilbronn was commercially heat treated by Biotech Co. The building consisted of a flour mill built in 1989 and a new mill built in 2002 adjacent to it sharing the same staircase and lift. The older mill consisted of a cellar, a ground floor and ten floors with additional mezzanine floors between the 1st and the 4th floor, resulting in a total of 15 floors. The new mill had a total of 14 floors including mezzanine floors between the 1st and 3rd floor. Connected to both mills there was a packaging hall at the ground floor, with a loft (mezzanine) included into the 7 m high hall where packaging material and finished products such as sacks and packages of flour, food pellets,
and bran were stored as bag stacks on pallets. Heat treated mills plus packaging hall had a volume of approx. 42,000 m³. The 55 grain silos and the flour silo section of the mill (with 52 silos) were not part of the heat treatment. Loading silos for trucks were also heat treated at the end of the procedure but not monitored.

Bioassay
Because literature data (Kirkpatrick and Tilton, 1972, Becket et al., 1998) and laboratory studies (Table 1) had shown that the Lesser grain borer *Ryzopertha dominica* is one of the most heat tolerant stored product pests, adults and all developmental stages of this species were used to test the efficacy of this practical treatment. Total 52 bioassay insect samples were prepared, each bioassay consisting of 50 young adult beetles of mixed sexes and 10 g of wheat grain consisting of 2 g of each developmental stage stemming from weekly cultures kept at at 25±1°C and 65±5% r.h. Test insects were placed into photo film tubes with a fine wire mesh welded into the bottom and the lid of each tube. The plastic container was then placed into a linen bag of 20 X 30 cm. 50 bags were numbered and 2 bags were used as untreated control of which one (U1) was taken along to the treatment, the other (U2) remained under laboratory conditions at 25±1°C and 65±5% r.h. 25 samples (no. 26-50) were distributed in the older mill because it contained floors with wooden planks, older parts of machinery, more potential hiding places and a visible infestation with the Confused flour beetle *Tribolium confusum* and the Rusty grain beetle *Cryptolestes ferrugineus*. 13 samples (no. 13-25) were distributed in the new mill and 12 samples (no. 1-12) in the packing station and bag stack storage site. At the end of the treatment, all samples were collected, taken back to the laboratory, and checked for beetle mortality and adults hatching from treated grain with immature stages for the following 12 weeks at 25°C and 65 % r.h.

Temperature measurement
The building was heated with eight external oil burners and fans forcing the heated air through hoses into the building. Additional fans in the various floors helped to increase the horizontal distribution of heat. Heat was applied from Thursday afternoon to Sunday evening, i.e. for some 78 h, and in this time the old mill, the new mill and the packaging area were heated consecutively. To determine the changes of temperature over time, 25 data loggers were added to each odd number of the 50 bioassay insect samples. These data loggers had been calibrated and programmed to record ambient temperature and moisture contents at 15 min intervals. Subsequently all bags were closed with a Velcro fastener along the seam of each bag, the seam was patent-folded and fixed with a metal wire. The 50 bioassay insect samples and one untreated control sample were placed into a thermo-insulated bag and taken to the flour mill. There, the samples were distributed within the building. In addition to the data loggers used, an electric digital thermometer (GTH 1200 A) was utilised during inspection walks in the building while the heat treatment was carried out in order to determine temperatures in the air, on surfaces, in cracks or crevices and in substrates.

Infrared-thermography
Infrared (iR) thermography is used in a vast variety of fields were it is useful to make temperature changes visible. The advantage of this method is that it depicts temperatures on surfaces rather than only at one limited point. In the morning hours of the third day of the heat treatment, iR thermographs were taken from the outside of the building when the older mill was just cooling down while the new mill was heated. Another set of thermographic pictures was recorded from inside in the seventh flour of both mills.
Results and discussion

Bioassay and temperatures recorded
In the older mill, complete control of test insects was achieved in 20 out of 25 samples. Temperatures were sufficient in all floors and within all parts of machinery. Complete survival of beetles was found in the uppermost floor where a room with the elevator heads was obviously not treated. Manual temperature readings recorded a maximum of 37°C. A room in the cellar with storages of machine parts was also not treated and the sample deposited there gave complete beetle survival. For the application of heat, the survival of insects in a sample hidden among many cables right at the outside wall just below a pipe coming from outside was found more critical. Here a cool draft could be noticed during the heat treatment and this probably allowed beetle survival, although close to the end of the treatment temperatures of above 50°C were recorded by the data logger (sample no. 31). Flour bags and cardboard boxes also allowed the survival of some test insects. Individuals in samples hidden under material directly on the floor were all killed showing that the floors of this mill did not provide any hiding space.

In both untreated control samples all adults survived the time span of treatment. Over a period of 10 weeks adult hatch from the untreated two samples amounted to a total of 493 and 557. Under the given circumstances, it was not possible to distinguish between F1 and F2 generations.

Quite a curious result was found in the room above the lift that was locked throughout the treatment and had thus been heated just indirectly. An insect sample was placed here on top of a fuse box, because beetle tracks had been found in the dust on the floor. Even though data logger temperatures never exceeded 48°C and stayed between 45°C and 48°C for just 41 h, neither beetles nor immature stages survived the treatment. This could be an effect of extremely low relative humidity combined with the effect of temperature. Levels of r.h. were recorded to be as low as 16.1%, possibly because the room was connected to the lift shaft and was thus affected by a constant draft. R.h. in the sample was recorded to be below 30% for a total of 61 h and 45 min. In the new flour mill where even *R. dominica* beetles survived the treatment at similar temperatures a relative humidity of below 30% was achieved for only 26 or 27 h, respectively, at exposure periods to temperatures between 45°C and 47.4°C for 9 h and 6 h, respectively. In laboratory tests, a few beetles had survived 45°C for up to 35 h and larvae had survived for up to 50 h, in one case 80 h. Beckett et al (1998) mention that higher moisture contents are important for insect survival especially at moderately high temperatures. At 50°C, hardly one beetle survived 4 h and hardly one larva survived 6 h of exposure. The film tube with 10 g infested and thus moist substrate may have provided some protection for the beetles saving them from both heat and desiccation for a certain time.

Only 32 of 72 beetles survived exposure to 45°C or higher for more than 840 min (14 h) including 50-54°C for 250 min (sample 31). Only 5 beetles and 116 adults emerging from developmental stages were found to survive temperatures between 45° and 48°C for 29 h (sample no. 45).

In the sample exposed to the highest temperatures (50°C or more for 22 h, 55°C or more for 11 h, max. temperature 59.2°C) one adult *R. dominica* hatched 8 weeks after the end of the experiment. The r.h. recorded was more than 71 h below 30% with a minimum of 12.5%. If this result is not an artefact, this could show that possibly the genetic variability at least of this species may allow survival of temperatures close to 60°C. In future experiments, the fertility of surviving insects, and the resistance F1 generation to high temperatures should be tested.

In the new mill, complete control was achieved in 7 out of 13 samples while complete survival of beetles was noticed in samples deposited from the 5th floor to the topmost floor. This corresponds to the temperature readings of data loggers (no. 21, 23 and 25) that showed
that in these floors temperatures of 48°C or above were not achieved. In a garbage bag filled with husks and dust from grain cleaning in the 8th floor, the temperatures recorded did not even reach 38°C and the bag with test insects and data logger was covered with live *T. confusum* upon retrieval of the sample at the end of the experiment. Manual temperature measurements of air temperature showed a maximum of 43°C.

As mentioned above, heating resulted in a drop of r.h. to 20% and below. It is quite possible that insects are driven away from certain spots in a building not only by high temperatures but by a combination of heat and drought. Thus, moist and cool spots would be preferred to just cooler regions with similar humidity.

Temperatures measured manually inside a pile of rags were found to be up to 17°C below those recorded in the room (44°C vs. 61°C). No survivors, however, were found in the insect sample at this spot. This may be due to the fact that the insect sample was not equally covered by rags from all sides.

In the packaging hall, insects survived in all samples hidden in products, packaging material, an opening in the outer wall or an electric distribution box next to the outside wall. Alive booklice found at the end of the treatment confirm the impression that heat treatments cannot cause complete control in products stored under these conditions. It is thus recommendable to remove all products from the mill or any storage space to be treated with heat. The amount of such products should be reduced as far as possible prior to a treatment. If some products can not be removed from the flourmill, these products should be stored in an isolated storage space and should be directly removed from the flourmill from there. Flour returned from bakeries should not be brought back into the mill because this could be another source of pest introduction. Packages with machinery spare parts, wooden beams, bags or cans with garbage, empty sacks and bags, buckets with water and all other mobile utensils should be removed prior to the heat treatment. Traps could be used to determine the distribution of an infestation in certain floors, but the connection of floors through elevators, openings, and parts of machinery may attract infestation from other sources and not reflect the success of the treatment. Both the miller and the heat operator should carry out joint inspection walks prior to, during, and after the heat treatment in order to share detailed observations and to secure a successful treatment.

**Infrared-thermography**

Pictures taken with an infrared camera from outside revealed zones of heat loss such as faulty insulation in a vertical line along corners of the building and not sufficiently insulated windows (Figure 1). Thermographs taken inside the treated rooms showed cold parts in the outside walls, while all machinery was uniformly heated (Figure 2). This information could be valuable in deciding where to direct the outlets of heated air or where to search for survivors. Good insulators such as rags or thick layers of dust, however, may not be visible in a thermograph and should be removed where possible. A strategy for future heat treatments could be to initially drive insects out of zones where they could hide or find protection into zones where control is easier. Heaters and blowers should be directed towards the potential cold bridges in a building driving insects away from these spots. In consequence, the bio-assay, data loggers, temperature inspection, and iR-thermography proved suitable and complementary tools to evaluate the efficacy of a heat treatment.

**Acknowledgements**

The author thanks Heidi Anders for technical assistance during and after the heat treatment. He is also indebted to Agnes Paul and Sylvia Krause for preparing the insect samples. Jürgen Rexroth and FLIR Systems Co. are thanked for providing the iR-thermographs.
Fig. 1. iR-thermograph of a heat treated flour mill from outside. Bright surfaces are areas where heat is lost to the environment due to faulty or insufficient insulation (vertical corners of the building, windows).

Fig. 2. iR-thermograph from within the flour mill. Machines and elevators are uniformly heated while parts of the outside wall turn out to be cold bridges and potential refuges for insect survival.
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The use of controlled atmospheres for stored product pest control

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Abstract: Insects and other pests in commodities need to be controlled to meet customer requirements. Methyl bromide (MB) and Phosphine fumigants that have been widely used as disinfestation agents have well-documented environmental and other problems. These problems have been overcome in the Netherlands through the commercialisation of controlled atmospheres, heat, and heated controlled atmospheres. These treatments that can be accommodated within the logistical requirements of many import products are safe, affordable, effective, residue-free, and environmentally-friendly alternatives to MB. These treatments have been developed in the Netherlands, and are now being replicated in other locations in Europe and elsewhere, to meet the safety and quality requirements of the regulatory authorities for disinfestation of food and non-food items.

Key words: stored product pest control, controlled atmospheres, heat, QPS, disinfestation, post harvest, environmentally-friendly, methyl bromide, phosphine, sulfuryl fluoride

Introduction

Insects and other pests contaminate and destroy food supplies and annually cause millions of dollars worth of damage to food, food storage and processing facilities. Pests in food in particular are considered a health risk and must be eradicated. Pests can also enter on packaging material such as wooden pallets leading to the accidental introduction of highly damaging quarantine pests which can become costly to eradicate.

There are a wide variety of measures that can be taken to manage the pests. Methyl bromide (MB) is ozone depleting and leaves residues in the treated product. Pests can also be controlled by Phosphine but they are showing resistance to the fumigant. Phosphine requires relatively long treatment periods and, unlike MB and heat treatments, Phosphine is not approved by the International Plant Protection Convention (IPPC) as a treatment for wooden packaging material.

The production of MB dropped by about 70% as from 1 January 2005 due to the implementation of the international agreement under the Montreal Protocol to phase out about 70% of the MB in developed countries. The phase out affected uses that are not categorised as Quarantine and Pre-shipment (QPS) and Critical Users Exemptions such as flour mill fumigation and immediate treatment of foodstuffs when imported to control non-quarantine pests.

Local regulations may also restrict the use of MB for postharvest use, even for QPS that is not restricted under the Montreal Protocol. For example, the European Union limits the amount of MB for QPS to 1,012 tonnes annually for all 25 Member States and, by agreement under the Management Committee operating under Article 18 of Regulation (EC) No 2037/2000, can reduce this amount in the light of the development of alternatives to MB for QPS. Therefore, as more alternatives are developed one could expect MB to become less available for QPS in the EU.

Some disinfestation treatments are carried out in shipping containers before export in order to control pests during transit. The number of containers that is fumigated with toxic
chemicals is increasing (Vroom, 2004). These containers need to be vented on arrival in order to make them safe before unloading. Venting of fumigated containers is likely to be more restrictive in the future in order to minimise harmful emissions to the environment and for workers safety.

Importers and exporters are under intense pressure to find safe and effective alternatives to control insects in commodities that replace the use of MB and other toxic chemicals. This paper describes the commercialisation of controlled atmospheres (CA), heat, and heated-CA for the control of insect pests in a diverse range of food and non-food products.

**Materials and methods; alternatives**

*Controlled Atmospheres (CA)*

CA are based on the establishment of a low-oxygen environment which kills pests. EcO$_2$ BV is using CA to control all stages of insects, rats and mice in food, food associated products, artefacts, silos, food (processing) facilities and barges.

The products are exposed to CA in airtight and climate controlled rooms equipped to handle variable sorts and quantities of products. The temperature, oxygen, carbon dioxide and humidity are controlled in each room within a specified range known to be toxic to the pest. The treatment requires 3 to 10 days, depending on the pest species, commodity, and level of infestation, which is comparable to the time for phosphine fumigations when both treatment and venting time are taken into consideration. The treatment is fully automated and can be initiated, monitored, managed and halted online by computer.

CA is also a highly effective treatment to control insects in artefacts of historical value as this treatment does not affect paper, paint, leather, textile, wood, metal, plastic, ink and varnish. The objects or products can be treated when packed. The level of humidity, in particular, must be closely monitored to preserve the treated objects in agreement with local cultural heritage regulations. The climate chambers are also suitable for the treatment of imported products or objects that might contain pests. Approximately 6,500 m$^3$ of artefacts and furniture were treated in 2003 in the Netherlands by EcO$_2$ BV.

Special service terminals containing the airtight climate rooms can be found throughout the Netherlands, Belgium, United Kingdom, Turkey, Vietnam, India, and in Uganda. In addition, a fleet of mobile installations enables the same treatment with CA to be used in specific locations such as buildings, silos, barges and structures as both preventive and curative measures. The number of treatments in each year has been increasing as shown in Table 1 and Fig. 1. Treated products include: Almond, Anise, Barley, Basmati rice, Brazil nut, Buckwheat, Cabbage seed, Cocoa beans, Cardi seed, Carob, Cashew nuts, Cereals, Chickpeas, Chinese kidney beans, Cinnamon, Clover seed, Coffee, Coriander, Dried apple, Dried apricots, Dried beans, Dried grapes, Dried peach, Dried pepper, Dried red pepper, Dried shrimps, Dried tomato, Dried white beans, Flour, Food additive, Ginger, Grain, Grass seed, Grated coconut, Ground nuts, Ground pepper, Hazelnuts, Honeysuckle, Hibiscus leaves, Indian corn, Juniper berry, Lettuce seed, Mace, Maize, Maize groats,– Marjoram, Millet, Mustard seed, Nutmeg, Nuts, Oats, Onion flakes, Onion powder, Onion seeds, Organic barley, Papua mace morsel, Peas, Pecan nuts, Perilla seed, Pet feed, Pig feed, Pinto beans, Pistachio nuts, Pumpkin kernel, Radish seed, Raisins, Rice, Seeds, Senna, Sesame seed, Sorghum, Soup additive, Soya beans, Soya germule, Spices, Sunflower seed, Tobacco, Thin grapes, Walnuts, White pepper powder, White sorghum and more.

*Case study: Use of CA to control Lasioderma serricorne and Ephesia clutella in tobacco*

In close co-operation with an international tobacco company, a commercial trial was performed using CA in the treatment facility in Ridderkerk (the Netherlands) to control the
Lasioderma serricorne and Ephesia elutella in 7 different types of raw tobacco, different tobacco products and tobacco seed (Rappl & Vroom, 2004). Adult insects were inserted in 7 wooden tubes (300 cm³) (total 87 adults) and wrapped and sealed according current production of the tobacco products (cigarette packs and cigar tubes). Adults, pupae, larvae, and eggs were inserted in 7 containers inside the tobacco bales.

Table 1. Number of silos and vessels treated with heated controlled atmospheres in the Netherlands

<table>
<thead>
<tr>
<th></th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silos</td>
<td>2</td>
<td>10</td>
<td>11</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Vessels</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>18</td>
<td>26</td>
</tr>
</tbody>
</table>

Fig. 1. Commodities treated with Controlled Atmosphere by by EcO2.

Treatment started on 13th of July and ended on 26th of July 2004 using CA based on low-oxygen and raised temperatures. Results of the wooden tubes showed that adults were 100% lethal after treatment with CA. A control tube (not treated with CA), which contained 10 adults, showed 100% vital on the 26th of July. Results of the containers showed that adults, larvae and pupae were 100% lethal on the 26th of July. Second analysis on the 14th of September showed no vitality inside the treated containers. A control container showed adults, larvae and pupae highly vital on the 30th of August when stored in an environment between 20 and 24°C.

Organoleptic analyses showed no negative influences on the different types of cigarettes and cigars treated. Furthermore, tobacco specific N-Nitrosamines were analysed by determination of the following N-Nitrosamines: N-nitrosonornicotine (NNN), N-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB), and 4-methylnitrosamino-1-(3-pyridyl)-1-butanoine (NNK).

After treatment with CA, analyses showed that there were no increase on the determined tobacco specific N-nitrosamines on tobacco types.

The treatment was monitored by using the EcO2 software technology for CA treatment, which monitors the level of oxygen and temperature as showed in figure 2.
Fig. 2. Monitoring temperature (°C) and oxygen concentration (%) in air during CA treatment of tobacco products.

Future and conclusions

EcO2 BV foresees a strong demand for environmentally-friendly pest control treatments as the trend continues toward even stricter regulations on the use of chemicals to control pests and increased consumer awareness aimed at minimising pesticides in the food chain. At the same time, importers are seeking pest control treatments that can be carried out quickly to meet just-in-time supply, and preferably treatments that require no prior authorisation to comply with national legislative requirements.

To meet these requirements, EcO2 BV proposes a new concept to disinfest containers, commodities and even artefacts in a closed circuit within a treatment terminal. A Multi Purpose Terminal (MPT) will be constructed that offers different treatments not only for export containers to meet QPS regulations but also for venting of import containers that have been gassed in transit with toxic chemicals.

The MPT will offer: QPS treatment with low-oxygen and high temperatures, CA for commodities, Heat for wooden packaging materials, CA for containers, MB and phosphine fumigation in conditioned areas with filters, and safe degassing of containers.

CA is an effective alternative that is a safe, affordable, effective, residue-free environmentally-friendly alternative to MB. Infrastructural investment is required in other countries similar to that undertaken by the Netherlands in order to fully substitute MB.

References

Insect pest management in stored products using reduced-risk insecticides

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Abstract: In recent years there has been increased interest throughout most developed countries in replacing older conventional neurotoxic insecticides used in pest management programs, including those used for stored products. Registrations for older compounds are being either withdrawn completely or altered to be applied in reduced application rates in combination with other insecticides. During the last decade in the United States, there have been several new or revised registrations for insecticides used on raw grain and in food storage facilities. Examples of these insecticides include inert dusts, insect growth regulators, bacterial pathogens, and new insecticides that affect metabolic pathways and receptors specific to insects.

Research today includes not only the identification of potential new insecticides that can be used in stored-products, but also a thorough examination of the factors that can affect efficacy of these new insecticides. Physical and environmental factors, differences among target insect species, insecticidal formulations and methods of application, and the economics involved in determining effective application rates are just a few examples of these factors. In addition, some of these new insecticides can be combined for increased effectiveness, and can be specifically targeted for a particular insect species.

Data from personal research studies will be used to illustrate concepts and ideas relevant to the diverse stored-product environments, from raw grain to urban storages. Topics for discussion include physical and environmental factors that affect insecticidal efficacy, methods of targeted applications, research with new insecticides, and new directions for insect pest management programs.

Key words: insects, stored-products, insecticides, control

Introduction

The organophosphate insecticides chlorpyrifos-methyl and malathion have historically been used to protect stored raw commodities from insect pests (Arthur 1996). However, problems associated with insecticide resistance, preferences for safer, biologically-based materials, and regulatory issues have led to a decline in conventional insecticides used as grain protectants (Arthur 1996). There has been renewed emphasis on using reduced-risk insecticides to replace these conventional neurotoxins. Examples of these insecticides include natural products, insect growth regulators (IGRs), and insecticides that target specific receptors in insects, pathogens, and viruses. Research today also focuses on how these new products can be used in control programs. In this paper, selected studies from personal research will be reviewed and discussed in relation to several application methods: 1) Combination treatments; 2) Targeted applications for specific species/life stages; and 3) General applications that control all or most of the stored-product pests species commonly encountered in a field situation.

1 This paper reports the results of research only. Mention of a chemical, proprietary product, or trade name does not constitute a recommendation or endorsement by the U. S. Department of Agriculture.
1. Combination treatments: Study 1

Two general classes of insecticides that have received considerable interest in recent years are natural inert dusts composed of diatomaceous earth (DE) (Golob 1997, Korunic 1998, Subramanyam & Roesli 2000), and insect growth regulators (IGRs), which are synthetic compounds that mimic chemicals used in the developmental processes of insects (Oberlander et al. 1997). However, each of these general classes of insecticides has limitations when used in control programs. Methoprene is effective against external feeders of stored grain, but less effective against internal feeders, especially *Sitophilus* spp. (Oberlander et al. 1997). In addition, like any IGR, it does not kill adults. Diatomaceous earth formulations vary widely in their toxicity towards insects, depending on source of material and the specific commercial formulation, grain moisture content, and the target insect species (Subramanyam & Roesli 2000). *Rhyzopertha dominica*, the lesser grain borer, is a major internal feeder of stored wheat that is particularly tolerant to DE compared to other stored-grain beetles (Arthur 2004). Also, efficacy of DE is greatly reduced as grain moisture content and relative humidity increase (Subramanyam and Roesli 2000). In this study, combinations of S-methoprene EC plus DE were evaluated for control of *R. dominica* on hard red winter wheat.

Materials and methods

Five rates of S-methoprene: 0, 0.25, 0.50, 0.75 ppm, and 1.0 ppm, and five rates of the diatomaceous earth (DE) Protect-It®, 0, 75, 150, 225, and 300 ppm (25 treatment combinations), were treated as described in Arthur (2004). Twenty 1-2 week old mixed-sex adult *R. dominica* were exposed in separate vials containing 30 g of wheat, which were in turn placed in plastic boxes containing either saturated NaBr or NaCl to maintain relative humidity at 57 and 75% relative humidity, respectively (Greenspan 1977). There were 5 separate replicates for each treatment combination. The humidity boxes were held in an incubator at 27°C, parent adults were exposed for 3 weeks and then removed from the vials, and mortality assessed. Wheat and contents in the vials were returned to the vials, which were put back into the humidity boxes and the temperature incubator. After 8 weeks, emerged F1 adults were tabulated and the wheat was discarded.

Results and discussion

With no methoprene, survival of *R. dominica* adults decreased with increasing concentration of DE, and was generally greater at 75 than at 57% relative humidity (Table 1). Consequently, there was considerable F1 production in the absence of methoprene, regardless of the concentration of DE (Table 2). However, even a small amount of methoprene combined with DE drastically reduced the number of F1 adults (Table 3). There was no reproduction in wheat treated with 0.5 - 1.0 ppm methoprene. Results indicate the potential of using methoprene in combination with DE to control *R. dominica* on wheat and other small grains.

Combination treatments: Study 2

The use of heat to disinfest structures was first reported in the entomological literature in the early 1900s (Dean 1911, 1913), but the technique was not extensively used for most of the 20th century because conventional fumigant chemicals were the preferred option. However, with the impending loss of the fumigant methyl bromide, there is renewed interest in using heat treatments to control stored-product insects inside milling and processing facilities.
Several recent studies have evaluated heat treatments for control of *Tribolium castaneum*, the red flour beetle, which is a major pest of flour mills (Mahroof et al. 2003, Roesli et al. 2003). Combining or integrating heat with other control strategies would be beneficial for modern pest management approaches for controlling insects inside processing facilities. The pyrethroid insecticide cyfluthrin can be used inside mills and processing facilities, but there are concerns regarding degradation of residues and loss of efficacy when using heat treatments in areas which have been previously sprayed with residual insecticides. In this field trial, concrete treated with cyfluthrin WP was put either inside a flour mill undergoing a heat treatment or in an unheated office to determine effects of extreme temperatures on insecticide efficacy.

### Table 1. Percentage survival of 20 *R. dominica* adults in wheat treated with DE, no methoprene

<table>
<thead>
<tr>
<th>Concentration of DE (ppm)</th>
<th>57% RH</th>
<th>75% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>93.3 ± 4.9</td>
<td>95.8 ± 2.0</td>
</tr>
<tr>
<td>75</td>
<td>88.3 ± 2.1</td>
<td>95.0 ± 2.2</td>
</tr>
<tr>
<td>150</td>
<td>71.7 ± 9.4</td>
<td>82.5 ± 11.2</td>
</tr>
<tr>
<td>225</td>
<td>63.3 ± 8.9</td>
<td>80.0 ± 6.2</td>
</tr>
<tr>
<td>300</td>
<td>50.0 ± 17.1</td>
<td>48.0 ± 12.9</td>
</tr>
</tbody>
</table>

### Table 2. Number of adult *R. dominica* progeny from adults exposed in Table 1.

<table>
<thead>
<tr>
<th>Concentration of DE (ppm)</th>
<th>57% RH</th>
<th>75% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>118.0 ± 21.1</td>
<td>145.0 ± 21.1</td>
</tr>
<tr>
<td>75</td>
<td>89.0 ± 12.4</td>
<td>124.0 ± 12.4</td>
</tr>
<tr>
<td>150</td>
<td>65.2 ± 9.7</td>
<td>85.8 ± 9.7</td>
</tr>
<tr>
<td>225</td>
<td>41.8 ± 5.3</td>
<td>57.8 ± 5.3</td>
</tr>
<tr>
<td>300</td>
<td>36.4 ± 17.1</td>
<td>38.4 ± 9.5</td>
</tr>
</tbody>
</table>

### Table 3. Number of adult *R. dominica* progeny from 20 parent adults exposed on wheat treated with DE, 0.25 ppm methoprene

<table>
<thead>
<tr>
<th>Concentration of DE (ppm)</th>
<th>57% RH</th>
<th>75% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.8 ± 6.1</td>
<td>8.5 ± 7.5</td>
</tr>
<tr>
<td>75</td>
<td>3.0 ± 2.5</td>
<td>2.3 ± 1.2</td>
</tr>
<tr>
<td>150</td>
<td>1.7 ± 1.1</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>225</td>
<td>0.3 ± 0.3</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>300</td>
<td>0.6 ± 0.6</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

### Materials and methods

Seventy concrete disks were prepared as described in Arthur and Dowdy (2003) treated with 2 mg [Al] cyfluthrin WP/m², which was 10% of the low label rate. This rate was used to
simulate natural residual degradation and inactivation on a treated flour mill surface. There were five replicates of 14 dishes, each treated with a separate formulated solution. Half of the dishes in each replicate were placed in an unheated room and the other half at the middle of the third floor of the mill undergoing the heat treatment. Disks from each replicate were removed from the mill and the unheated control room at intervals of 0, 8, 16, 24, 34, 40, and 52 hours after initiation of the heat treatment, and returned to the laboratory. Plexiglass containers were placed on each disk, and bioassays were conducted by exposing 10 mixed-sex 1-2-week adult *T. castaneum* inside each ring. Survival was assessed after exposure intervals of 0 to 8 hours.

**Results and discussion**

There were no differences regarding the time disks were removed from the mill, therefore data were combined. Survival of *T. castaneum* was actually greater on unheated compared to heated concrete treated with 90% of the low label rate of cyfluthrin. Except for *T. castaneum* exposed for 0.5 hours, more beetles survived on the unheated disks in the control room versus the heated disks in the mill, indicating a beneficial effect of heating on cyfluthrin toxicity, instead of a negative effect (Fig. 1). High temperatures could have volatilized residues from the concrete substrate that were more readily absorbed by *T. castaneum*. Insecticides used as crack and crevice or spot treatments in combination with heat may be beneficial when the insecticides flush insects from hidden areas out into the open where they are more accessible to the heat treatment.

![Fig. 1. Percentage (mean ± SEM) survival of *Tribolium castaneum* after 1-8 hours of exposure on concrete disks treated with 2 mg [AI] cyfluthrin WP/m². Disks were either put inside the flour mill that was being heated or held in an unheated room.](image)

**2. Targeted applications for specific species/life stages**

*Plodia interpunctella*, the Indian meal moth, is a major pest of raw foods and packaged food products (Cox and Bell 1991, Campbell et al. 2002). All five larval stages move and feed within the infested commodity, but fifth instars may leave the infested commodity and wander in search of a pupation site. This represents a vulnerable phase in the biology of the pest, however, the wandering-phase larvae are particularly difficult to kill with conventional...
insecticides compared to stored-product beetles (Arthur 1995). Hydroprene and methoprene are juvenile hormone analogs registered for surface treatments to control stored-product pests in the USA. Many of the earlier studies on IGRs and stored-product insect pests were conducted with beetles (Oberlander et al 1997), and there is comparatively less data for susceptibility of *P. interpunctella* larvae. In one laboratory study, wandering-phase larvae were exposed on concrete treated with the label rate of hydroprene, for different times at various temperatures, to quantify mortality and adult emergence. In other tests, moth larvae were exposed on packaging paper treated with the label rate of methoprene. Field trials were also conducted in which larvae were exposed to aerosol applications of methoprene.

Preparation of concrete treatment arenas and application methods of hydroprene are described in Mohandass et al. (2005). Briefly, concrete was treated with the label rate of 0.0013mg[AI]/cm², and larvae were exposed for different time intervals at temperatures of 16, 20, 24, 28, and 32°C. In laboratory tests with methoprene, treatment arenas were constructed in which packaging papers were treated with different concentrations of methoprene (mgAI/cm²). These papers were cut to fit a 100 by 15 mm Petri dish, eggs were put on the treated paper, and the Petri dish placed inside a larger 150 mm Petri dish which contained food media. In field tests of methoprene aerosol, wandering-phase *P. interpunctella* larvae were exposed inside Petri dishes ringed with Vaseline to minimize escape, exposed to the aerosol for 2 hours, then removed and held in untreated containers until adult emergence was complete.

### Results and discussion

In tests with hydroprene, larval mortality generally increased at all of the five tested temperatures as exposure period increased (Fig. 2). Hydroprene sprayed as a surface treatment significantly delayed development time of wandering-phase *P. interpunctella* larvae, caused direct larval mortality, and decreased adult emergence of larvae that were not arrested or killed by hydroprene (Fig. 3). Application of methoprene to packaging surfaces produced similar results when larvae were exposed in laboratory studies (Table 4), and in field trials with methoprene aerosol, no adults emerged from larvae exposed to the label rate. These studies show that the wandering-phase *P. interpunctella* larvae are susceptible to insect growth regulators, and these insecticides could be used to specifically target this vulnerable life stage.

### Table 4. Percentage adult emergence from eggs exposed on Kraft paper treated with different concentrations of methoprene IGR (emergence in controls was 87.6 ± 3.1%).

<table>
<thead>
<tr>
<th>Concentration [AI] in Mg/cm²</th>
<th>Percentage of adult emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 10⁻⁴/cm²</td>
<td>76 ± 4.5</td>
</tr>
<tr>
<td>4 x 10⁻⁴/cm²</td>
<td>65 ± 4.7</td>
</tr>
<tr>
<td>8 x 10⁻⁴/cm²</td>
<td>37 ± 5.4</td>
</tr>
<tr>
<td>2 x 10⁻³/cm²</td>
<td>5 ± 1.4</td>
</tr>
<tr>
<td>6 x 10⁻³/cm²</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
3. Complete treatments to control all species

One promising new grain protectant is the bacterial pesticide Spinosad, which is registered world-wide for control of various field crop pests. Several laboratory tests have shown that it is very effective on stored-grain beetles (Toews and Subramanyam 2003, Toews et al. 2003), and residues seem to be very persistent on stored wheat (Fang et al. 2002). Controlled aeration will limit insect development in stored wheat (Arthur and Flinn 2000, Reed and Arthur 2000), but populations can still rise above the threshold for export grain in the United States, which is 2 insects injurious to grain/kg. In this field test, treatment of wheat with 1 ppm Spinosad was compared to aerated and unaerated wheat.

Materials and methods

Six cylindrical steel bins, with capacity of 30 metric tons, were used in the study. Two bins were untreated and unaerated, two were aerated with controlled aeration at set points of 23.9, 18.3, and 7.2 °C, at an approximate rate of 0.0026m3/s/m3., and two contained wheat treated with 1 ppm Spinosad as described by Flinn et al. (2004). Wheat was loaded into the bins in July, and every month until October, 400 adults each of *T. castaneum*, *R. dominica*, and *Cryptolestes ferrugineus*, the rusty grain beetle, were introduced into each bin.Bins were sampled monthly for insect populations, for complete details on the sampling procedure see Flinn et al. (2004).

![Fig. 2. Mortality of *P. interpunctella* larvae exposed to hydroprene, 1.9 x 10^{-3} mg [Al]/cm^2](image.png)

Results

Populations in aerated bins were generally much lower than in the untreated unaerated control bins, but *R. dominica* populations did exceed the threshold of 2 insects/kg of wheat (Table 5). Density of this species increased in control bins after only a couple of months and peaked earlier than the other two species, which could explain why aeration did not completely suppress *R. dominica*. Insect populations of all three species were essentially 0 in bins treated with Spinosad during the entire study. Spinosad appears to have excellent potential as a grain
protectant and has been labeled for stored wheat in the USA by DowAgroSciences, but will not be marketed until international Codex tolerances are issued for residues on grain and marketing procedures and requirements have been finalized. Spinosad will also be certified for use on organic grain.

Fig. 3. Adult emergence from larvae exposed to hydroprene at $1.9 \times 10^{-3} \text{ mg [AI]/cm}^2$

Table 5. Adult populations (per kg) in untreated unaerated (control) wheat and aerated wheat held from July to January in Kansas. Populations of all insects in bins treated with Spinosad averaged < 0.1/kg.

<table>
<thead>
<tr>
<th>Species</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. ferruginus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.2 ± 0.5</td>
<td>1.8 ± 0.3</td>
<td>4.1 ± 0.7</td>
<td>3.7 ± 1.0</td>
<td>3.3 ± 1.1</td>
<td>5.9 ± .01</td>
</tr>
<tr>
<td>Aerated</td>
<td>0.2 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td><em>R. dominica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.2 ± 0.1</td>
<td>8.9 ± 0.8</td>
<td>57.7 ± 5.6</td>
<td>39.1 ± 4.5</td>
<td>25.5 ± 3.2</td>
<td>28.9 ± 5.3</td>
</tr>
<tr>
<td>Aerated</td>
<td>0</td>
<td>2.2 ± 0.7</td>
<td>11.7 ± 1.7</td>
<td>12.4 ± 4.1</td>
<td>4.0 ± 2.7</td>
<td>3.1 ± 1.8</td>
</tr>
<tr>
<td><em>T. castaneum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.3 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>7.9 ± 1.2</td>
<td>22.3 ± 3.3</td>
<td>28.8 ± 5.5</td>
<td>75.7 ± 3.1</td>
</tr>
<tr>
<td>Aerated</td>
<td>0</td>
<td>0.3 ± 0.1</td>
<td>1.6 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>

**Summary**

Reduced risk-insecticides show potential for incorporation into management programs for stored products, but there are some limitations to consider. The cost for synthesis and manufacturing of these products is much more expensive than production costs associated with most organophosphates and carbamates. When the registration costs are added, the treatment costs will limit application rates compared to older chemicals. Grain protectants will therefore be applied at much lower rates, for example 1 ppm instead of 6 ppm, which means efficacy may be limited. With this reduced application rate, there may be a need for more investigation of combination treatments to control all species that could be encountered during a storage period, particularly in raw stored grains. Species variability may be more of a problem when the insecticide does not control all pest species. Spot or targeted treatments
may be necessary in large storage areas containing processed and packaged foods, rather than broad-scale applications of surface treatments or whole-plant treatments. However, even with these limitations, reduced-risk insecticides will be more important in the future, considering the anticipated decline of conventional chemicals available for use in control programs.

References


Diatomaceous earth surface treatment for stored wheat

Frank H. Arthur, Erika A. Vardeman, James R. Nechols, and James F. Campbell

Abstract: Diatomaceous earth (DE) can be used as a surface treatment in stored wheat to control pest infestations. However, it is not known how the thickness of the DE-treated wheat layer or grain temperature impacts effectiveness. When adult *Rhyzopertha dominica* (F.), lesser grain borers, were released in experimental towers containing untreated wheat or wheat admixed with DE to a surface layer depth of 15.2, 22.9 or 30.5 cm, they were able to penetrate all DE layers and oviposit in the untreated wheat below. However, survival was significantly reduced in adults exposed to DE. Survival decreased both with increasing depth of the DE-treated wheat and with exposure interval. Temperature had no effect on adult survival, but significantly more progeny were produced at 32 than at 27°C. Progeny production was inversely correlated with the depth of the DE-treated layer. Vertical distribution patterns of parental beetles were not significantly different among treatments or exposure intervals; however more insects were found at greater depths at 32 than at 27°C.

Key words: lesser grain borer, *Rhyzopertha dominica*, diatomaceous earth, wheat, control, movement

Introduction

*Rhyzopertha dominica* (F.), the lesser grain borer, is a serious pest of stored raw commodities worldwide, and most commonly infests grain after it is harvested and stored (Potter, 1935). Initial infestation of the grain mass generally occurs at the surface and then spreads downward (Hagstrum et al., 1994; Vela-Coiffier et al., 1997; Hagstrum, 2001). Control of *R. dominica* is difficult because most of the life cycle is spent inside the grain kernel. Historically malathion was used as a grain protectant, but numerous studies have shown resistance to this insecticide (Subramanyam & Hagstrum, 1996) and other organophosphates used for control (Zettler & Cuperus, 1990; Arthur, 1992; Guedes et al., 1996).

New formulations of diatomaceous earths (DE), a reduced risk insecticide, are being developed to suppress insects in stored grain. Diatomaceous earth is a natural product mined from deposits of fossilized diatoms (Quarles, 1992). The dust interferes with water transpiration by absorption of cuticular lipids and causes desiccation (Korunic, 1998; Subramanyam & Roesli, 2000). Diatomaceous earth can affect physical properties of grain (Korunic et al., 1996, 1998), and it is often used as a treatment to the grain surface to alleviate some of these effects. Diatomaceous earth is thought to attach to the cuticle of *R. dominica* as it disperses downward from the grain surface through the treated layer, and into the grain mass. If populations of *R. dominica* do not obtain a lethal dose while passing through the treated layer, then this approach may not be effective. Therefore, the depth of the treated layer is a critical factor. Variation in temperature can also influence the rate at which beetles move

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1 This paper reports the results of research only. Mention of a proprietary product or insecticide does not constitute a recommendation or endorsement by Kansas State University or the U. S. Department of Agriculture.
through grain (Flinn & Hagstrum, 1998); in turn, this could influence the exposure of beetles to the DE-treated layer. The objectives of this test were to: 1) determine to what extent adult *R. dominica* disperse through different depths of grain treated with DE; and 2) determine progeny production and F₁ adult survival when *R. dominica* are exposed to different combinations of DE-treated layer depth and temperature.

**Materials and methods**

The experiment was conducted in two walk-in environmental growth chambers set to 27°C and to 32°C. Relative humidity in each chamber was maintained at about 50% with a range of 45.5 to 64.4%. Experimental units were constructed using 7.6 (8.9) cm inside (outside) diameter PVC pipe. Individual rings measuring 7.6 cm in height were cut from the pipe, and 12 of these were taped together with duct tape to form 24 vertical towers measuring 91.2 cm. Twelve towers were placed in each of the two temperature chambers. Three towers per chamber served as controls (no DE treatment). These towers were filled with approximately 2.6 kg hard red winter wheat, which extended to the top of the 3rd ring, leaving a head space of 15.2 cm. The remaining 9 towers contained wheat in which the top 15.2, 22.9, or 30.5 cm of the wheat was treated with 400 ppm DE.

One hundred one- to two-week old adult *R. dominica* were placed on the top surface of the wheat in each tower, and then the tower was closed with a PVC cap. Insects were exposed for 7, 10, or 14 d in each treatment (untreated wheat and the three surface layer depths of DE) for a total of 12 treatment combinations at each temperature. Each of five replicates consisted of four treatments (control plus three depths of DE), two temperatures, and three exposure intervals, for a total of 24 towers.

After each exposure interval, the individual rings of the towers were taken apart and the wheat in each ring was sifted and mortality assessed. The wheat then was placed back into the containers and held for 8 weeks under the original temperature conditions. Data were analyzed using the Proc Mixed or General Linear Model (GLM) SAS procedure (SAS Institute, 2002). Raw data were transformed by arcsine square root and statistical analyses were performed on the transformed data. Treatment means for the various statistical tests were separated using the Waller-Duncan k-Ratio t-test. All tests of significance were done at the P=0.05 level. For all tables, means within columns followed by different letters are significantly different (P < 0.05, Waller-Duncan k-ratio t-test) (SAS Institute, 2002).

**Results**

Survival in the DE treatments was significantly lower than in the untreated control, declined with increasing depth of the DE layer, and declined as exposure interval increased from 7 to 10 days (Table 1). The vertical distribution of live *R. dominica* was significantly affected by DE treatments but not by temperature or exposure interval. There was no consistent pattern in the dispersion of *R. dominica* among DE treatments or between DE treatments and the control.

Likewise, there was no difference among DE treatments in the vertical displacement (mean distance traveled) of *R. dominica* at either 27° or 32°C, however, when live and dead adults were combined, there were significant differences among, treatments in the vertical displacement of lesser grain borers at each temperature (Table 2). More insects were found at greater depths at 32° than at 27°C, but there was no significant difference in mean vertical displacement of live and dead adults between DE-treated and untreated wheat.
The percentage of live adults within versus below the DE layer was significantly influenced by DE treatment depth and temperature but not exposure. At both temperatures, there was a general trend for an increase in the percentage of live beetles within the DE layer, and a decreasing percentage below the DE layer (Table 3). At 27°C, more live beetles were found at the 22.9 and 30.5 cm depths than at 15.2 cm, whereas at 32°C, treatments were more similar (Table 3). At 32°C, the percentage of live beetles below versus above the DE layer was greater than at 27°C.

Table 1. Mean (± SEM) percentage survival adult *R. dominica* when exposed for different durations to diatomaceous earth (DE)-treated and untreated wheat.

<table>
<thead>
<tr>
<th>Treatment (DE depth)</th>
<th>Exposure Interval (days)</th>
<th>7</th>
<th>10</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Control</td>
<td>99.8 ± 0.2 a</td>
<td>99.6 ± 0.4 a</td>
<td>99.7 ± 0.2 a</td>
<td></td>
</tr>
<tr>
<td>15.2 cm</td>
<td>66.0 ± 5.5 b</td>
<td>56.5 ± 7.0 b</td>
<td>49.1 ± 7.7 b</td>
<td></td>
</tr>
<tr>
<td>22.9 cm</td>
<td>37.2 ± 5.3 c</td>
<td>28.8 ± 6.6 c</td>
<td>26.2 ± 6.0 c</td>
<td></td>
</tr>
<tr>
<td>30.5 cm</td>
<td>26.9 ± 4.3 c</td>
<td>18.2 ± 3.4 c</td>
<td>14.8 ± 3.2 c</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Mean (± SEM) vertical displacement of combined live and dead adult *R. dominica* at different depths of diatomaceous earth-treated wheat and in untreated wheat.

<table>
<thead>
<tr>
<th>Treatment (DE depth)</th>
<th>27°C</th>
<th>32°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Control</td>
<td>4.6 ± 0.4 a</td>
<td>5.9 ± 0.4 a</td>
</tr>
<tr>
<td>15.2 cm</td>
<td>3.6 ± 0.4 ab</td>
<td>4.5 ± 0.4 ab</td>
</tr>
<tr>
<td>22.9 cm</td>
<td>3.1 ± 0.3 b</td>
<td>4.5 ± 0.4 b</td>
</tr>
<tr>
<td>30.5 cm</td>
<td>3.4 ± 0.2 b</td>
<td>4.2 ± 0.3 b</td>
</tr>
</tbody>
</table>

Table 3. The effect of depth of the DE-treated layer and temperature on the mean (± SEM) percentage of live adult *R. dominica* found within and below the DE-treated layer.

<table>
<thead>
<tr>
<th>Treatment (DE depth)</th>
<th>Percentage of live Lesser Grain Borers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27°C</td>
</tr>
<tr>
<td></td>
<td>Within DE</td>
</tr>
<tr>
<td>15.2 cm</td>
<td>44.5 ± 6.5</td>
</tr>
<tr>
<td>22.9 cm</td>
<td>66.7 ± 7.7</td>
</tr>
<tr>
<td>30.5 cm</td>
<td>69.2 ± 6.5</td>
</tr>
</tbody>
</table>
The percentage of dead adults below versus above the DE layer was significantly affected by the DE but not by temperature or exposure interval. The percentages of dead adults were significantly higher within the DE-treated layer at all three depths than the untreated wheat below, and the percentage of dead lesser grain borers within the DE layer was greatest at 30.5 cm. (Table 4).

**Table 4. The effect of depth of the DE-treated layer on the mean (± SEM) percentage of dead *R. dominica* within and below the DE-treated layer.**

<table>
<thead>
<tr>
<th>Treatment (DE depth)</th>
<th>Percentage of dead <em>R. dominica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within DE</td>
</tr>
<tr>
<td></td>
<td>Below DE</td>
</tr>
<tr>
<td>15.2 cm</td>
<td>75.9 ± 2.8 ab</td>
</tr>
<tr>
<td>22.9 cm</td>
<td>75.0 ± 2.7 b</td>
</tr>
<tr>
<td>30.5 cm</td>
<td>83.4 ± 2.6 a</td>
</tr>
</tbody>
</table>

Total production of F1 adults was significantly affected by the DE treatment and exposure interval, but not by temperature. The number of progeny produced increased as exposure interval increased for all DE treatments and for the untreated control. Progeny production decreased with increasing depth of the DE-treated layer, and the most progeny were produced in the untreated control (Table 5).

**Table 5. The effect of depth of the DE-treated layer and exposure interval on mean (± SEM) number of adult *R. dominica* progeny.**

<table>
<thead>
<tr>
<th>Treatment (DE depth)</th>
<th>Exposure Interval (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>3305.7 ± 1146.8 a</td>
</tr>
<tr>
<td></td>
<td>4766.2 ± 1359.4 a</td>
</tr>
<tr>
<td></td>
<td>6259.4 ± 1123.2 a</td>
</tr>
<tr>
<td>15.2 cm</td>
<td>1702.3 ± 903.8 ab</td>
</tr>
<tr>
<td></td>
<td>2769.5 ± 1261.4 ab</td>
</tr>
<tr>
<td></td>
<td>3713.5 ± 1530.9 ab</td>
</tr>
<tr>
<td>22.9 cm</td>
<td>1017.0 ± 553.7 ab</td>
</tr>
<tr>
<td></td>
<td>1706.9 ± 936.6 ab</td>
</tr>
<tr>
<td></td>
<td>2265.6 ± 1123.7 ab</td>
</tr>
<tr>
<td>30.5 cm</td>
<td>502.0 ± 271.4 b</td>
</tr>
<tr>
<td></td>
<td>683.3 ± 453.9 b</td>
</tr>
<tr>
<td></td>
<td>1814.8 ± 1118.3 b</td>
</tr>
</tbody>
</table>

Population growth rate was determined by dividing the number of progeny from each tower by the initial number of 100 *R. dominica* adults. The lowest population growth rates were observed for the deepest DE layer (Table 6). As depth increased, population growth rates decreased. There appeared to be a trend for increasing population growth rate as exposure interval increased (Table 6).
Discussion

Movement of *R. dominica* is usually a slow downward process that occurs mainly in the center of the grain mass. In our study, adults were vertically distributed throughout most of the strata within the 7- to 14-day exposure period. In addition, activity of *R. dominica* can increase at higher temperatures and have a higher rate of dispersal (Surtees, 1965).

Table 6. The mean (± SEM) population growth rate between the P₁ and F₁ generations of adult *R. dominica* after release in stored wheat treated with different depths of diatomaceous earth (DE) or on untreated wheat.

<table>
<thead>
<tr>
<th>Treatment (DE depth)</th>
<th>Exposure Interval (days)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Untreated</td>
<td>32.1 ± 11.0 a</td>
<td>47.9 ± 14.0 a</td>
<td>62.2 ± 14.4 a</td>
<td></td>
</tr>
<tr>
<td>15.2 cm</td>
<td>17.3 ± 9.4 ab</td>
<td>29.2 ± 13.9 ab</td>
<td>37.0 ± 15.3 ab</td>
<td></td>
</tr>
<tr>
<td>22.9 cm</td>
<td>8.3 ± 3.9 ab</td>
<td>17.1 ± 9.4 ab</td>
<td>21.5 ± 10.4 ab</td>
<td></td>
</tr>
<tr>
<td>30.5 cm</td>
<td>5.2 ± 2.7 b</td>
<td>6.2 ± 4.0 b</td>
<td>18.3 ± 11.1 b</td>
<td></td>
</tr>
</tbody>
</table>

The results of this study showed that the DE treatments did not prevent *R. dominica* from penetrating into untreated wheat, which is further compounded by the fact the *R. dominica* is difficult to kill with DE compared to other stored-grain beetles (Fields & Korunic, 2000). Mortality of parental adults was directly related both to the depth of the DE-treated layer and exposure interval. Several other studies have shown a positive relationship between time exposed to DE and mortality for other species of stored-product beetles (Arthur, 2000). Both initial mortality and final mortality were directly related to exposure interval to DE. In our study, the relatively higher percentage of dead borers within the DE layer compared to the live borers suggests that dying beetles may have remained to a greater degree in the upper (treated) strata than those that survived exposure to the DE.

Higher temperatures generally lead to greater mortality of stored-product beetles when exposed on grain or surface substrates (Aldryhim, 1993; Korunic, 1998). In our study, temperature did not significantly affect mortality of the initial adult lesser grain borers, but it did affect the proportion of progeny produced, therefore temperature may have had an effect on oviposition, pre-adult survival, or both. In contrast to other stored grain beetles, lesser grain borers generally reproduce and develop optimally at higher temperatures (Howe, 1950; Hagstrum, 1996).

Our study demonstrates that the lesser grain borer was capable of penetrating a DE-treated surface layer and reproducing in the untreated wheat. Increasing the depth of the treated layer resulted in greater adult mortality, reduced progeny production, and also decreased population growth rates. Using DE in conjunction with other control methods, such as aeration or insect growth regulators, may be necessary to give economic levels of control when using surface treatments to stored grains.
Acknowledgments

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References


Surtees, G. 1965: Ecological significance and practical implications of behavior patterns determining the spatial structure of insect populations in stored grain. – Bulletin of Entomological Research 56: 201-213.


Ovicidal activity of various essential oils against Confused Flour Beetle, *Tribolium confusum* Jacquelin duVal (Coleoptera: Tenebrionidae)

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**Abstract:** In this study, the ovicidal activity of vapour of essential oils from laurel (*Laurus nobilis*), fennel (*Foeniculum vulgare*), oregano (*Origanum dubium*), onion (*Allium cepa*), yarrow (*Achillea millefolium*), peppermint (*Mentha piperita*), juniper berry (*Abies balsamea*), eucalyptus (*Eucalyptus globulus*), fir needle (*Juniperus communis*), garlic (*Allium sativum*), nutmeg (*Myristica fragrans*), citronella (*Cymbopogon winterianus*), pine (*Pinus sylvestris*), anise (*Pimpinella anisum*), rosemary (*Rosmarinus officinalis*), turmeric (*Curcuma longa*) were evaluated against eggs of confused flour beetle (*Tribolium confusum* Jacquelin duVal). Eggs of *T. confusum* were exposed to a dose of 100 µL/L air of all essential oils for periods of 24, 48 and 72-h. Vapours of laurel, yarrow, peppermint, juniper berry, eucalyptus, fir needle, nutmeg, citronella, pine, rosemary and turmeric essential oils were found to have a low ovicidal toxicity to eggs of *T. confusum* at all exposure times by <20% of corrected mortality. Whereas, garlic, onion, fennel, anise and oregano essential oils indicated a strong ovicidal activity by varying from 42.2% to 100% of corrected mortality at 24-h exposure time. Probit analysis data on eggs of *T. confusum* resulted in LT$_{90}$ values of 1.1, 22.1, 22.4, 13.8 and 51.1-h at a dose of 100 µL/L air for garlic, onion, anise, oregano and fennel respectively. On the basis of LT$_{90}$ values, toxicity of vapour of essential oils to eggs of *T. confusum* in descending order was: garlic < oregano < onion < anise < fennel. Essential oil from garlic with 0.12 g h/L of Ct product was found to be the most promising one by a closer Ct product value to the most commonly used commercial fumigant, methyl bromide (0.05 g h/L).

**Key words:** Essential oils, ovicidal activity, egg, fumigant toxicity, *Tribolium confusum*, bio-fumigant

**Introduction**

The application of various synthetic insecticides and fumigants to grain storage over the years has led to a number of problems, including the development of insecticide resistance in stored grain insect pests in various parts of the world (Champ & Dyte, 1976; Georgiou & Lagunes-Tejeda, 1991). Another concern is the accumulation of pesticides (including fumigant) residues in treated grain (Snelson, 1987). Therefore, there has been recently a growing interest in research concerning the possible use of plant extracts as alternatives to synthetic insecticides (Raja *et al*., 2001). Essential oils are among the best-known substances tested against insects. These compounds act as fumigants (Risha *et al*., 1990; Rice & Coats, 1994; Regnault-Roger & Hamraoui, 1995; Shaaya *et al*., 1997), contact insecticides (Saxena *et al*., 1992; Weaver *et al*., 1994; Schmidt & Strelcke, 1994), repellents (Saim & Meloan, 1986; Ndungu *et al*., 1995; Plarre & Reichmuth, 1997), antifeedants (Harwood *et al*., 1990) and may effect some biological parameters such as growth rate, life span and reproduction (Gunderson *et al*., 1985; Stamopoulos, 1991; Saxena *et al*., 1992; Regnault-Roger & Hamraoui, 1995). Most of these substances were tested against insects attacking stored products in order to
establish new control practices with lower mammalian toxicity, high volatility, and low persistence in the environment.

Essential oils are potential sources of alternative compounds to currently used fumigants. Various studies have demonstrated fumigant activity of various essential oils against various stored product insects (Shaaya et al., 1991; Shaaya et al., 1997; Saraç & Tunç 1995; Tunç et al., 2000; Lee et al., 2003; Andronikashvili & Reichmuth 2002; Kalinovic et al., 2002). Toxicity of various essential oils and their volatile constitutes against the adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) indicated that two essential oils, eucalyptus and rosemary had potent fumigant activities (Lee et al., 2001). The investigation of fumigant activity of essential oils from anise *Pimpinella anisum*, cumin *Cuminum cyminum*, eucalyptus *Eucalyptus camaldulensis*, oregano *Origanum syriacum* var. *bevanii* and rosemary *R. officinalis* on eggs of two stored-product insect species, the confused flour beetle and the Mediterranean flour moth showed the highest mortalities caused by essential oils from anise and oregano (Tunç et al., 2000). The essential oils of the laurel and rosemary indicated insecticidal activity against *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *S. oryzae*, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), ranging from LD<sub>50</sub> values of 7 to > 15 µL/L air respectively (Shaaya et al., 1997).

Many plant extracts and essential oils may be an alternative source of stored-product insect-control agents (Hill & Schoonhoven, 1981; Konstantopoulou et al., 1992; Desmarchelier, 1994; Shaaya et al., 1997) because they constitute a rich source of bioactive chemicals. Their major constituents, monoterpenes, are also of interest to industrial markets because of other potent biological activities in addition to their toxicity to insects (Basilico & Basilico, 1999; Crowell et al., 1992; Kubo et al., 1994). As the essential oils are intended to be used like fumigants to disinfect commodities, they should have the ability to kill all stages of insects. In this context, eggs are of particular concern, because they may exhibit a higher tolerance to chemical agents (e.g. Phosphine) (Bell and Wilson, 1995) than active stages. Therefore, in order to better understand the response of egg stage to essential oils and especially their vapours or their constituents, there is a need for systematic investigations of the effect of different essential oil concentrations and exposure periods on stored-product insects.

In this study, we report ovicidal activity of sixteen essential oils against confused flour beetle, *Tribolium confusum* Jacquelin duVal (Coleoptera: Tenebrionidae).

**Materials and methods**

**Insect culture**
Tests were carried out on eggs of *T. confusum*. Eggs were obtained from laboratory cultures reared at 26 ± 1°C and 65 ± 5% relative humidity (r.h.) on a mixture of wheat flour, bran and yeast (Donahaye, 1990). Eggs were separated from oviposition jars by sieving daily. Eggs for exposure to treatments were transferred into the glass tube. The glass tube, each containing 50 eggs aged 1-2 days were exposed to each treatment.

**Essential Oils**
Essential oils from laurel (*Laurus nobilis*), fennel (*Foeniculum vulgare*), oregano (*Origanum dubium*), onion (*Allium cepa*), yarrow (*Achillea millefolium*), peppermint (*Mentha piperita*), juniper berry (*Abies balsamea*), eucalyptus (*Eucalyptus globulus*), fir needle (*Juniperus communis*), garlic (*Allium sativum*), nutmeg (*Myristica fragrans*), citronella (*Cymbopogon winterianus*), pine (*Pinus sylvestris*), anise (*Pimpinella anisum*), rosemary (*Rosmarinus officinalis*), turmeric (*Curcuma longa*), were evaluated against eggs of confused flour beetle
(T. confusum). Essential oils extracted by stem distillation method were provided commercially from ATL Canada Company. After purchase, the essential oils were collected in sealed glass containers and were refrigerated in the dark at 4°C until their use.

Bioassay tests and experimental procedures

Eggs of T. confusum were collected from the cultures by using US standard sieve mesh #70 and placed in 25 ml glass vials containing rearing food. The glass vials were covered with a fine mesh to enable penetration of any volatilise emanating from the essential oils. Fifty eggs were used in each replicate. Bioassays were carried out in 1 L glass jar with a metal cover that served as fumigation chambers kept at 25±1°C and 60±5% r.h. Sodium Nitrite solution was placed in small glass Petri dishes with 7 cm diameter to provide 60±5% constant r.h., in the glass jar.

A constant dose of 100 µL/L air of each essential oil was applied throughout all bioassay tests. Essential oils were introduced as a liquid into the bottom of the fumigation chamber using 100-1000 µL micro-pipette. Eggs of T. confusum kept in the glass vials, were then transferred separately into the fumigation chamber, which were sealed by screwed lids. Eggs were exposed to essential oils for 24, 48 and 72-h, and each treatment was replicated three times. For each treatment, control jars were exposed to only atmospheric conditions. After each exposure period, eggs were transferred into "pits" drilled into Perspex exposure slides, each slide containing 50 pits. When filled, the slides were covered with a cover glass to retain the eggs (Navarro & Gonen, 1970).

Essential oils over 20% of corrected mortalities were subjected to bioassay tests at a dose of 100 µL/L air for four or five exposure times ranging from 0.25-h to 48-h. Each treatment was replicated three times and two control treatments were put for each trial. The procedures before and after exposure were the same as in above mentioned bioassay tests.

Data Processing and Analysis

After each treatment, the eggs in their Perspex slides were held at 26 ± 1°C and 65 ± 5% r.h. until the oviposition sites were examined for egg hatching. Egg hatch was checked 7 days after treatment for mortality counts. Mortality data obtained from bioassay tests were corrected by using Abbott’s formula (Abbott, 1925). Data obtained from each zero dose control and exposure time-mortality responses were subjected to probit analysis by using maximum likelihood program software (POLO-PC) (LeOra Software, 1987) to determine LT50s (Lethal Time50), LT90s (Lethal Time90) and their respective 95% confidence intervals. Differences in toxicity were considered significant when 95% confidence intervals did not overlap. The slopes and intercepts of exposure time-mortality regressions for each tested insect were compared with the POLO-PC maximum-likelihood procedures (LeOra Software, 1987). The concentrations x time (Ct) products (g h/L) to obtain 90% mortality of the eggs of each insect were calculated using the LT90 values derived from probit analyses and the concentration applied.

Results

Bioassay tests indicated that vapours of various essential oils had variable toxicity to eggs of T. confusum when exposed to a dose of 100 µL/L air for 24, 48 and 48-h exposure times. Corrected mortalities (%) of T. confusum eggs exposed to a dose of 100 µL/L air of various essential oils for 24, 48 and 48-h exposure times are presented in Table 1. Vapours of laurel, yarrow, peppermint, juniper berry, eucalyptus, fir needle, nutmeg, citronella, pine, rosemary and turmeric essential oils were found to have no or a low ovicidal toxicity to eggs of T. confusum at all exposure times due to their low corrected mortalities ranging from 0 to 17.2%.
Prolonged exposure time from 24 to 72-h did not also resulted in an increase of mortality of the eggs. However, garlic, onion, fennel, anise and oregano essential oils indicated a strong ovicidal activity by varying from 42.2% to 100% of corrected mortalities at 24, 48 and 72-h of exposure times. For these essential oils, increasing exposure time from 24 to 48-h achieved a higher mortality of the eggs, while increasing exposure time from 48 to 72-h did not resulted in a higher mortality of the eggs (except for essential oil of fennel). Therefore, these findings indicated that prolonged exposure times would be required to achieve the complete mortality in the eggs of *T. confusum*.

Table 1. Corrected mortalities (%) of *T. confusum* eggs exposed to a dose of 100 µL/L air of various essential oils for different exposure times.

<table>
<thead>
<tr>
<th>Essential Oils</th>
<th>Corrected Mortality (%)±SE</th>
<th>Exposure Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Turmeric</td>
<td>4.65±0.71</td>
<td>6.81±0.71</td>
</tr>
<tr>
<td>Pine</td>
<td>12.31±0.72</td>
<td>14.49±1.91</td>
</tr>
<tr>
<td>Fir needle</td>
<td>3.29±1.26</td>
<td>6.22±2.64</td>
</tr>
<tr>
<td>Rosemary</td>
<td>0</td>
<td>3.03±1.51</td>
</tr>
<tr>
<td>Laurel</td>
<td>6.02±1.55</td>
<td>12.40±1.55</td>
</tr>
<tr>
<td>Yarrow</td>
<td>3.62±0.72</td>
<td>8.69±1.25</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>17.21±5.12</td>
<td>15.75±0.73</td>
</tr>
<tr>
<td>Citronella</td>
<td>4.34±1.25</td>
<td>5.07±1.44</td>
</tr>
<tr>
<td>Peppermint</td>
<td>4.34±1.25</td>
<td>5.79±1.91</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>11.82±1.24</td>
<td>10.86±1.25</td>
</tr>
<tr>
<td>Juniper berry</td>
<td>2.89±0.72</td>
<td>0.72±0.72</td>
</tr>
<tr>
<td>Garlic</td>
<td>83.85±3.05</td>
<td>89.47±2.43</td>
</tr>
<tr>
<td>Onion</td>
<td>95.69±2.48</td>
<td>99.28±0.71</td>
</tr>
<tr>
<td>Anise</td>
<td>79.92±1.89</td>
<td>94.20±1.91</td>
</tr>
<tr>
<td>Fennel</td>
<td>42.02±5.93</td>
<td>89.85±5.65</td>
</tr>
<tr>
<td>Oregano</td>
<td>92±3.05</td>
<td>97.82±1.25</td>
</tr>
</tbody>
</table>

Table 2. LT<sub>50</sub> (h) and LT<sub>90</sub> (h) values for eggs of *T. confusum* exposed to a dose of 100 µL/L air of essential oils from garlic, onion, anise, fennel and oregano.

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>n</th>
<th>Slope±SE</th>
<th>LT&lt;sub&gt;50&lt;/sub&gt; (hours) (Confident Limits)</th>
<th>LT&lt;sub&gt;90&lt;/sub&gt; (hours) (Confident Limits)</th>
<th>λ&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>150</td>
<td>0.17±2.03</td>
<td>0.86 (0.78-0.91)</td>
<td>1.06 (0.98-1.24)</td>
<td>21.183</td>
</tr>
<tr>
<td>Onion</td>
<td>150</td>
<td>0.28±0.50</td>
<td>12.64 (11.05-14.29)</td>
<td>22.11 (18.81-29.13)</td>
<td>31.261</td>
</tr>
<tr>
<td>Anise</td>
<td>150</td>
<td>0.92±0.86</td>
<td>15.98 (14.84-17.09)</td>
<td>22.37 (20.77-24.53)</td>
<td>8.5634</td>
</tr>
<tr>
<td>Fennel</td>
<td>150</td>
<td>0.27±0.42</td>
<td>25.42 (22.54-27.92)</td>
<td>51.14 (45.24-61.23)</td>
<td>19.423</td>
</tr>
<tr>
<td>Oregano</td>
<td>150</td>
<td>0.11±0.10</td>
<td>5.69 (4.06-7.18)</td>
<td>13.82 (11.25-18.63)</td>
<td>105.07</td>
</tr>
</tbody>
</table>
Probit mortality regression data (LT\textsubscript{50} and LT\textsubscript{90} values (h)) for eggs of *T. confusum* exposed to a dose of 100 µL/L air of essential oils from garlic, onion, aniseed, fennel and oregano are shown in Table 2. Probit mortality regression data indicated a remarkable difference in toxicity between the essential oils (Table 2) against eggs of *T. confusum*. LT\textsubscript{90} values of 1.1, 13.8, 22.1, 22.4 and 51.1-h at a dose of 100 µL/L air for essential oil of garlic, oregano, onion, anise and fennel respectively (Table 2). LT\textsubscript{90} value for essential oil from garlic was significantly lower than those of rest of the essential oils, since 95% confidence intervals (CLs) of garlic essential oil did not overlapped those for rest of the essential oils. LT\textsubscript{90} value for fennel essential oil was also significantly higher than those for rest of the essential oils, since 95% CLs did not overlapped. Therefore, essential oil from garlic was the most toxic to the eggs with LT\textsubscript{90} value of 1.1-h at a dose of 100 µl/l air while essential oil from fennel was the least toxic to the eggs with LT\textsubscript{90} value of 51.1-h at a dose of 100 µl/l air (Table 2). Toxicity of vapours of essential oils to the eggs of *T. confusum* in descending order was found as garlic < oregano < onion < aniseed < fennel.

**Discussion**

Due to their high volatility, many plant extracts and essential oils have fumigant action (Basilico & Basilico, 1999; Shaaya *et al.*, 1997). The results in the present study indicated that essential oils of laurel, yarrow, peppermint, juniper berry, eucalyptus, fir needle, nutmeg, citronella, pine, rosemary and turmeric had very low fumigant toxicity against the eggs of *T. confusum*. However, vapour of essential oils of garlic, onion, fennel, aniseed and oregano essential oils indicated a strong ovicidal activity on eggs of *T. confusum*. Compared with the investigation of Shaaya *et al.* (1993) our results indicated a similar toxicity of the essential oil of oregano against eggs of *T. confusum*. As similar to our results, Tunç *et al.* (2000) found that essential oil from rosemary and eucalyptus had also a low toxicity to eggs of *T. confusum* by a mortality of only 25% and 10% respectively at a concentration of 98.5 µL/L air at 72-h exposure time, while vapour of aniseed essential oil had a high ovicidal activity by a mortality of 90%. However, Tunç *et al.* (2000) reported a much lower toxicity of essential oil of oregano against eggs of *T. confusum* in comparison of the results presented here. The different toxicity of essential oil from oregano against eggs of *T. confusum* would appear to be attributed to the difference of the species of oregano tested or seasonal changes in the chemical composition of the essential oil, as reported by Müller-Riebau *et al.* (1996, 1997).

Ct products of 4.98, 2.26, 2.34, 1.33 and 0.12 g h/L air for essential oils from fennel, aniseed, onion, oregano and garlic, respectively were required to obtain 90% kill of the eggs. These findings may be compared with several studies on the two most commonly used fumigants, methyl bromide and phoshine for eggs of *Tribolium* spp. Whilst methyl bromide requires Ct products of 0.05 g h/L to obtain 90 % of kill of the eggs of *T. castaneum* at 26 ºC (Mostafa *et al.*, 1972), phoshine requires Ct products of 0.007g h/L to achieve 90% of kill of the eggs of *T. confusum* at 25ºC (Lindgren & Vincent, 1966). It appears that all tested essential oils were less toxic than phosphine and methyl bromide. However, essential oil from garlic was found to be the most promising one by a closer Ct product value to methyl bromide.

Although essential oils from fennel, aniseed and onion had a fumigant toxicity against eggs of *T. confusum* with different efficacies, they need so high dosages to obtain the complete mortality of eggs of *T. confusum* compared to the most commonly used commercial fumigants, methyl bromide and phoshine. Therefore, it may not be desirable to use such high dosages of these essential oils on their own as a commercial bio-fumigant against stored-product insect. However, essential oil from garlic may have good potential as an alternative
bio-fumigant to methyl bromide or phosphine fumigation of commodities. Clearly, the isolation and identification of the bioactive compounds in essential oil from garlic could be important in their potential application in controlling stored-product pests. Therefore, further investigations are needed to identify biological activity components of essential oil from garlic and their possible synergism by testing combinations of potentially active fractions.

Acknowledgements

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Efficacy of *Plectranthus glandulosus* and *Steganotaenia araliacea* leaf powders from Cameroon as post-harvest grain protectants against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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**Abstract:** Powdered leaves from *Plectranthus glandulosus* and *Steganotaenia araliacea*, collected in the Adamawa province of Cameroon, were tested under laboratory conditions for their ability to protect stored maize from attack by *Sitophilus zeamais* Motschulsky. Maize grains were admixed with the powders at four rates (0.5, 1, 2 and 4% (w/w)) for the assessment of mortality over a 32-day period, as well as for \(F_1\) progeny production, population increase and damage evaluation. Weevil mortality increased over time for the two plant powders at all content levels. A maximum 100% mortality was recorded only for *P. glandulosus* at contents of 4 and 2% at 16 and 32 day post exposure, respectively. 16-day LD\(_{50}\)s were 0.86% for *P. glandulosus* and 1.72% for *S. araliacea*. The two powders considerably reduced \(F_1\) progeny production, population increase and grain damage, with complete protection of the grains provided by *P. glandulosus* at contents of 1% or higher. These results suggest that powdered leaves of *P. glandulosus* and *S. araliacea* may be of high value in grain storage against *S. zeamais*, especially in subsistence agriculture where the plants are locally available to farmers with little resources to meet the high cost of pesticides.

**Key words:** *Plectranthus glandulosus*, *Steganotaenia araliacea*, *Sitophilus zeamais*, powder, toxicity, progeny.

**Introduction**

Maize is an important source of carbohydrate in the tropics (Niber, 1994). It is the most important food crop in Cameroon where it is grown in all the ten provinces that make up the country. To some farmers in the country, maize provides their only possibility to market economy. The climatic conditions in the tropics favour the development and proliferation of storage pests which cause considerable damage in storage and constitute an obstacle to processing (Mutlu & Hountonji, 1990). Prominent among storage insects, is the Maize Weevil, *Sitophilus zeamais* Motschulsky, which causes considerable damage to stored maize worldwide. Heavy infestations of this pest may cause weight losses of as much as 30-40% in stored maize (CAB International, 1999). Nukenine *et al.* (2002) recorded weevil damage on stored maize ranging from 1.63-89.2% (mean = 33%) at the village level, in the Adamawa province of Cameroon.

The use of synthetic insecticides to prevent or control insect infestation has been very successful (Bengston *et al.*, 1980; Thaung & Collins, 1986; White *et al.*, 1997; Arthur *et al.*, 2004). Global concern with the health and environmental impacts of synthetic pesticides, from both consumers and government agencies, is being translated into political action in the form of heightened restrictions and limitations on the use of these products, especially in the production of food crops (Isman, 2001). Further, the majority of farmers in Africa are
resource-poor and have neither the means nor the skills to obtain and handle pesticides appropriately (Saxena et al., 1990). These demerits of synthetic insecticides have created a window of opportunity for the introduction of alternative pesticides, provided their relative safety to humans can be established. Among potential reduced-risk pesticides are botanical insecticides and antifeedants, that is, products based on powders, extracts or purified substances of plant origin. Given that the majority of plants with demonstrated insecticidal activity are tropical and subtropical, and the cost of synthetic insecticides is a significant impediment to their use, it is in developing countries where botanical insecticides are most likely to be adopted on a large scale (Isman, 2001).

Most of the grain produced in sub-Saharan Africa comes from small scale farmers, many of whom use different kinds of plant products for insect control (Poswal & Akpa, 1991; Bekele et al., 1996; Tapondjou et al., 2000; Nukenine et al., 2003). In recent years, research has focussed on bioactivity, application methods, cost-effectiveness and sustainable use of botanical pesticides against pests (Regnault-Roger & Hamrroui, 1993; Talukder & Howse, 1995; Fatope et al., 1995; Ngamo et al., 2001; Tapondjou et al., 2002, 2005; Koona & Njoya, 2004). Since the major focus in developing nations is on cost, crop protection products with even modest efficacy will be embraced if they are readily available and less expensive than conventional pesticides. Plant powders would thus be an attractive option to these farmers since they are locally available, could be produced with limited skills and knowledge, and their use entails little or no financial expenditure in most cases. However, information on the insecticidal efficacy and dosages of the plant powders would significantly benefit the farmers.

*Plectranthus glandulosus* Hook f. (syn. *Coleus laxiflorus* (Benth.) Roberty) (Lamiaceae) and *Steganotenia araliacea* Hochst (Araliaceae), are plants whose leaves are commonly used to protect stored grains against insect attacks, as mosquito repellents and anthelmintics in Cameroon (Nukenine et al., 2003; Musongong et al., 2004).

Essential oil from the dried leaves of *P. glandulosus* from Adamawa province, Cameroon were much richer in oxygenated monoterpenes (84.6%) as compared with monoterpene hydrocarbons (13.0%) (Ngassoum et al., 2001). *Cis*-piperitone oxide (35.1%), fenchone (21.6%) and *trans*-piperitone oxide (12.6%) were predominant among the oxygenated monoterpenes. The most represented monoterpene hydrocarbon was terpinolene. In the Adamawa province, the plant is used in folk medicine for the treatment of colds and sore throat (Ngassoum et al., 2001). Although products derived from many species of the Lamiaceae family have shown various biological activities against stored product insects including *Sitophilus* spp. (Jembere et al., 1995; Sarac & Tunc, 1995; Dales, 1996; Modgil & Samuels, 1998; Mazzonetto & Vendramim, 2003, Khajuria & Malik, 2003; Andronikashvili & Reichmuth, 2003), no work concerning powders from *P. glandulosus* and stored insect pests are documented. Further, studies on the extracts against arthropods are either uncommon or inexistent. Limonene + β-phellandrene (11.78-35.90%), α-pinene (4.89-11.40%), sabine (9.40-25.98%), β-caryophyllene (2.0-14.91%) and cryptone (3.06-16.60%) were identified as the major constituents of the leaf essential oil of *S. araliacea* harvested from different areas of Benin and Togo in west Africa (Moudachirou et al., 1995). Petroleum and ethanol extracts, as well as powders from the leaves of this plant showed significant toxic and repellent effect against *Tribolium castaneum* (Herbst) (Abubakar et al., 2001). Musongong et al. (2004) demonstrated that crude extracts from the leaves of *S. araliacea* could be of value as an anthelmintic in cattle. There is a dearth of information about the insect control properties of *S. araliacea* and no study has reported on *S. zeamais*.

In this paper we report the results of an investigation of the effects of leaf powders from *P. glandulosus* and *S. araliacea* as toxicants and repellents against adult *S. zeamais*. The ability of these plant materials to inhibit progeny production, reduce damage, and suppress population increase of the weevil was also examined.
Materials and methods

Insects
Sitophilus zeamais were reared on maize in a controlled temperature and humidity chamber (25±1°C and 60% r.h.) in darkness. Parent adults were obtained from laboratory stock cultures (about 30 years old) at the Institute for Stored Product Protection, Berlin, Germany.

Plant Materials
The leaves of P. glandulosus and S. araliacea were collected in July 2004 from Ngaoundere located in the Vina Division of the Adamawa province (plateau) of Cameroon. The identity of the plants was confirmed at the Cameroon National Herbarium in Yaounde, where voucher samples were deposited. Leaf samples from each plant species 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 the crushed leaves were ground until they passed through a 0.2 mm sieve and then stored in a freezer at -18°C until needed.

Toxicity and progeny production
For each of the two plants, 0.25, 0.5, 1 and 2 g of powders were separately introduced into 50 g of maize in 250 ml glass jars to give the dosages (contents) 0.5, 1, 2, and 4% (w/w), respectively. The maize grains were disinfested in a freezer at -18°C for two weeks and conditioned in the experimental room for at least 7 days before use. Each jar was then closed with a perforated metal lid. The plant product/grain admixtures were thoroughly mixed with a rotatory shaker (Multifix GmBH, Germany) for 10 min. Control for each set of treatments consisted of grain containing no plant material. Each treatment was repeated four times. A lot of 20 insects of mixed sexes and 1 to 14-day-old were added into the jars containing the treated or untreated grains. Adult mortalities were recorded 1, 2, 4, 8, 16 and 32 days post-inestation. After each recording session, but for the 8th day mortality determination, the contents of the glass jars were immediately discarded. After counting dead insects on the 8th day, the live and dead insects were dissected to determine the sex, while the powders were discarded. The grains were left in the jars and the counting of F1 adults was done once a week for five weeks commencing 6 weeks post-infestation. Preliminary experiments showed that adult emergence usually started after five weeks.

Population increase and damage
Similar contents (0.5, 1, 2 and 4% (w/w)) of the leaf powder/100 g of grain were admixed as described above. A lot of 50 insects 1 to 14-day-old of mixed sexes were introduced into each jar containing treated or untreated grain. Control for each set of treatments consisted of grain containing no plant material. Each treatment was repeated three times. After three months, the number of live and dead insects was determined for each jar. Damage assessment was done by measuring the weight of the sieved powder and that of the grains without powder (final weight). The amount of grain powder (frass plus faeces) formed was expressed as the total powder minus the weight of plant powder used. Percent weight loss was determined as follows: [(initial weight-final weight)/(initial weight)] x 100. The number of damaged (grains with characteristic hole) and undamaged grains in a randomly selected 100 grain sample from each jar were counted and percent grain damage was estimated.

Data analysis
Data on % mortality, number of F1 progeny, % reduction in adult emergence, number of live and dead insects, % damaged grains, grain powder weight and weight loss were subjected to the analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS institute, 2003). Arcsine[(square root(x/100)], square root (x + 0.5) and log10(x + 1) values
were used for ANOVA with data on mortality and % reduction in $F_1$ progeny, grain powder, and live insects, respectively. Fisher’s Protected Least Significant Difference (LSD) test was applied for mean separation. Linear regression was conducted to define dose-response relationships when correlations between dose and test parameters (mortality and $F_1$ progeny) were found to be significant (SAS, 2003). Probit analysis (Finney, 1971; SAS, 2003) was applied to determine lethal contents causing 50% ($LD_{50}$) and 95% ($LD_{95}$) mortality of $S. zeamais$ at 8, 16 and 32 days exposure period. Abbott’s formula (Abbott, 1925) was used to correct for control mortality prior to Probit analysis.

**Results**

**Toxicity**
The two plant powders were generally toxic to adult $S. zeamais$, although the toxic action was slow. There were significant differences ($P<0.001$) in mortality among plants, contents and exposure time (Fig. 1). Control mortality was generally significantly lower than those from each treatment for $P. glandulosus$ (day 2-32) and $S. araliacea$ (day 4-32). Mortality increased with ascending contents and exposure periods. However, these trends were not evident before 16 and 8 days post-exposure for $S. araliacea$ and $P. glandulosus$, respectively. Complete control (100% mortality) was obtained only with $P. glandulosus$ 16 (4%) and 32 (2 and 4%) days post-exposure. This plant also achieved over 50% mortality with contents greater than 1% as from the 8th day after exposure. At a content of 4%, almost 50% mortality was attained in four days. $S. araliacea$ nevertheless provided good control of $S. zeamais$, recording high mortalities of 90 and 93%, 32 days after exposure for the contents 2 and 4%, respectively. By this time, more than 60% mortality was recorded for all the tested contents, unlike $P. glandulosus$, for which only 30% mortality was achieved with the content 0.5%.

![Graphs showing mortality of $S. zeamais$](image)

*Fig. 1. Mortality of $S. zeamais$ (mean ± S.E.) over 32 days when exposed to maize admixed with different contents of powders from two plant species ($P. glandulosus$ and $S. araliacea$) at 25°C and 60% r.h.*
Between the 8th and 32nd days after exposure, LD<sub>50</sub> and LD<sub>95</sub> values reduced with increasing exposure periods respectively from 1.58-0.69 and 8.40-1.43 for <i>P. glandulosus</i> and 13.39-0.27 and 671.81-5.16 for <i>S. araliacea</i> (Table 1). Both LD<sub>50</sub> and LD<sub>95</sub> values were higher for <i>S. araliacea</i> than for <i>P. glandulosus</i>, 8 and 16 days post-exposure. Surprisingly, the former recorded a lower LD<sub>50</sub>, but not LD<sub>95</sub> value than the latter at 32 days after exposure. Regression slopes were higher for <i>P. glandulosus</i> as compared with <i>S. araliacea</i>, irrespective of time post-exposure (Fig. 2). Linear regression of mortality on content between day 8 and 32 were all significant for both plants with <i>R</i><sup>2</sup> values ranging from 0.78 (<i>S. araliacea</i>, day 32) to 0.99 (<i>P. glandulosus</i>, day 8). No significant <i>χ</i><sup>2</sup> values were obtained.

Table 1. Toxicity of powders from <i>P. glandulosus</i> and <i>S. araliacea</i> to <i>S. zeamais</i> at 8, 16 and 32 days after treatment, at 25 °C and 60% r.h.

<table>
<thead>
<tr>
<th></th>
<th>&lt;i&gt;P. glandulosus&lt;/i&gt;</th>
<th>&lt;i&gt;S. araliacea&lt;/i&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 8</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt; (95% f.l.)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58 (1.38-1.83)</td>
<td>13.39 (6.53-85.18)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LD&lt;sub&gt;95&lt;/sub&gt; (95% f.l.)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.40 (6.17-13.06)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>671.81 (98.71-120909)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;χ&lt;/i&gt;&lt;sup&gt;2&lt;/sup&gt; (&lt;i&gt;P&lt;/i&gt; &gt; &lt;i&gt;χ&lt;/i&gt;&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>2.17 (0.3376)</td>
<td>1.68 (0.4302)</td>
</tr>
<tr>
<td><strong>Day 16</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt; (95% f.l.)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86 (0.79-0.94)</td>
<td>1.72 (1.25-2.54)</td>
</tr>
<tr>
<td>LD&lt;sub&gt;95&lt;/sub&gt; (95% f.l.)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.77 (1.55-2.12)</td>
<td>102.73 (29.08-1965)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;χ&lt;/i&gt;&lt;sup&gt;2&lt;/sup&gt; (&lt;i&gt;P&lt;/i&gt; &gt; &lt;i&gt;χ&lt;/i&gt;&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>2.24 (0.1984)</td>
<td>0.12 (0.9421)</td>
</tr>
<tr>
<td><strong>Day 32</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt; (95% f.l.)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67 (0.61-0.73)</td>
<td>0.27 (0.12-0.42)</td>
</tr>
<tr>
<td>LD&lt;sub&gt;95&lt;/sub&gt; (95% f.l.)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43 (1.24-1.75)</td>
<td>5.16 (3.26-12.68)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;χ&lt;/i&gt;&lt;sup&gt;2&lt;/sup&gt; (&lt;i&gt;P&lt;/i&gt; &gt; &lt;i&gt;χ&lt;/i&gt;&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>2.40 (0.3006)</td>
<td>1.33 (0.5154)</td>
</tr>
</tbody>
</table>

<sup>a</sup> are considered significantly different when 95% fiducial limits (f.l.) fail to overlap.

<sup>b</sup> Outside range of contents tested.

Fig. 2. Relationship between <i>S. zeamais</i> mortality and content of powders from <i>P. glandulosus</i> and <i>S. araliacea</i> 8, 16 and 32 days after treatment.
Progeny production
Results showed significant differences ($P < 0.001$) among treatments in cumulative emerging adult $F_1$ insects for $P. glandulosus$ and $S. araliacea$ (Table 2). The number of adults emerging reduced with ascending contents. Nevertheless, all the plants caused a significant reduction in progeny production relative to the control, which was dose dependent. Only $P. glandulosus$ at the highest tested content of 4% achieved total suppression of $F_1$ progeny emergence. The level of suppression of $F_1$ progeny emergence increased with ascending contents (0.5 to 4%) from 48.4 to 83.9% and 23.3 to 100% for $S. araliacea$ and $P. glandulosus$, respectively.

Table 2. $F_1$ progeny production of $S. zeamais$ in grains treated with two plant powders, at 25°C and 60% r.h.

<table>
<thead>
<tr>
<th>Content (g/100 g of grain)</th>
<th>Mean number of $F_1$ adult progenya</th>
<th>% reduction in adult emergence relative to controlb</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P. glandulosus$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 45.01 ± 5.12 a</td>
<td>0 e</td>
<td></td>
</tr>
<tr>
<td>0.5 34.4 ± 7.52 a</td>
<td>23.3 ± 14.17d</td>
<td></td>
</tr>
<tr>
<td>1 15.1 ± 1.90 b</td>
<td>66.2 ± 3.64 c</td>
<td></td>
</tr>
<tr>
<td>2 6.3 ±3.11 c</td>
<td>86.2 ± 6.91b</td>
<td></td>
</tr>
<tr>
<td>4 0 d</td>
<td>100 ± 0 a</td>
<td></td>
</tr>
<tr>
<td>$F^b$ 27.01***</td>
<td>34.16***</td>
<td></td>
</tr>
<tr>
<td>$S. araliacea$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 41.6 ± 9.57 a</td>
<td>0 d</td>
<td></td>
</tr>
<tr>
<td>0.5 22.2 ± 5.82 b</td>
<td>48.4 ±7.19 c</td>
<td></td>
</tr>
<tr>
<td>1 11.7 ± 1.56 bc</td>
<td>70.6 ± 3.85 b</td>
<td></td>
</tr>
<tr>
<td>2 8.0 ± 1.56 c</td>
<td>80.85 ± 0.56 ab</td>
<td></td>
</tr>
<tr>
<td>4 5.9 ± 1.60 c</td>
<td>83.9 ± 4.71 a</td>
<td></td>
</tr>
<tr>
<td>$F^b$ 14.25***</td>
<td>67.43***</td>
<td></td>
</tr>
</tbody>
</table>

*Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Fisher’s Protected least significant difference); Each datum represents the mean of four replicates.

* **P** $< 0.001$

Population increase and grain damage
The rate of increase of the population of $S. zeamais$ was significantly reduced by plant powders from $P. glandulosus$ and $S. araliacea$ regardless of content (Fig. 2). In fact, $P. glandulosus$ at contents above 0.5% completely suppressed the populations of the weevil. No live insects were recovered after three months, but the 50 weevils introduced were all dead. At the lowest tested content, 0.5%, $S. araliacea$ reduced the rate of the population increase of the weevil by more than half, while the highest content, 4%, inhibited the population growth by roughly 10 folds.

The two powders significantly reduced grain damage from the weevil attack (Table 3). As expected, at dosages above 0.5%, $P. glandulosus$ averted weevil damage. The samples treated with the plant powders showed no grain damage, did not practically produce grain powders and lost almost no weight. In general, the samples treated with $S. araliacea$ suffered less damage than the control but with no significant differences among dosages, for each damage parameter (damaged grains, powder weight, and weight loss).
Discussion

Our investigations on the toxicities of leaf powders from *P. glandulosus* and *S. araliacea* in the Adamawa plateau, Cameroon to *S. zeamais* showed that the powders possess some toxic components which could cause significant mortalities of the weevil. However, the toxic action of the powders was slow and efficacy varied among species, which was not surprising since the two plants belong to different families. The toxicity of plant materials to insects depends significantly on the chemical composition, which varies across species, an even more so across families (Mwangi *et al.*, 1992; Weaver & Subramanyam, 2000; Tapondjou *et al.*, 2005). It is well documented also that powdered leaves generally act slower than the extracts or the pure compounds (Adebayo & Gbolade, 1994; Tapondjou *et al.*, 2003).

The speed of action of the powder from *P. glandulosus* seems to be greater than that from *S. araliacea*, because it achieved 100% mortality within 16 days while *S. araliacea* caused only 65% within the same period. This contention is further supported by the lower LD$_{50}$ and LD$_{95}$ values of *P. glandulosus* as compared with those of *S. araliacea*, 8- and 16-day post-exposure. Additionally, during the same exposure periods, steeper slopes were recorded for *P. glandulosus* than *S. araliacea*, all $R^2$s were significant while the $\chi^2$ values were not significant. All these facts lend further credence to the faster action of *P. glandulosus* relative to *S. araliacea*. The non significant $\chi^2$s show that the observed mortality did not deviate significantly from the expected and that the regression models were well fitted (Finney, 1971). According to the $R^2$s, between the 8th and 32nd day after exposure, 91-99% and 78 to 93% mortality of *S. zeamais* could be attributed to the action of *P. glandulosus* and *S. araliacea*, respectively. With the significant linear relationships of mortality on content, using lower contents and increasing the exposure time in field applications, could lead to complete control of *S. zeamais* by both plants.

![Graph of P. glandulosus and S. araliacea](image)

**Fig. 3.** Suppression of population increase of *S. zeamais* in maize admixed with different contents of powders from two plants and stored for three months at 25°C and 60% r.h. (bars with similar letters at the top for live or dead insects, are not significantly different at P < 0.05, Fisher’s Protected LSD)
Table 3. Damage parameters of *S. zeamais* in maize admixed with different contents of powders from four plants and stored for three months at 25°C and 60% r.h.

<table>
<thead>
<tr>
<th>Content (g/100 g grain)</th>
<th>Damaged grains (%) a</th>
<th>Powder weight (g) a</th>
<th>Weight loss (%) a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. glandulosus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20 ± 1.5 a</td>
<td>1.55 ± 0.049 a</td>
<td>6.0 ± 0.26a</td>
</tr>
<tr>
<td>0.5</td>
<td>3 ± 1.7 b</td>
<td>0.11 ± 0.111 b</td>
<td>0.7 ± 0.52b</td>
</tr>
<tr>
<td>1</td>
<td>0 b</td>
<td>0 b</td>
<td>0.4 ± 0.06b</td>
</tr>
<tr>
<td>2</td>
<td>0 b</td>
<td>0 b</td>
<td>0b</td>
</tr>
<tr>
<td>4</td>
<td>0 b</td>
<td>0 b</td>
<td>0b</td>
</tr>
<tr>
<td><em>F</em> b</td>
<td>75.2***</td>
<td>41.97***</td>
<td>96.21***</td>
</tr>
<tr>
<td><strong>S. araliacea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>31 ± 5.2 a</td>
<td>2.52 ± 0.632a</td>
<td>11.5 ± 2.46a</td>
</tr>
<tr>
<td>0.5</td>
<td>21 ± 8.2 ab</td>
<td>1.21 ± 0.559b</td>
<td>5.0 ± 1.80b</td>
</tr>
<tr>
<td>1</td>
<td>14 ± 0.6 b</td>
<td>0.92 ± 0.048bc</td>
<td>3.2 ± 1.5b</td>
</tr>
<tr>
<td>2</td>
<td>12 ± 3.0 b</td>
<td>0.76 ± 0.391bc</td>
<td>3.0 ± 0.22b</td>
</tr>
<tr>
<td>4</td>
<td>6 ± 3.2 b</td>
<td>0.14 ± 0.068d</td>
<td>1.1 ± 0.25b</td>
</tr>
<tr>
<td><em>F</em> b</td>
<td>4.01*</td>
<td>5.72*</td>
<td>6.71***</td>
</tr>
</tbody>
</table>

a Means within a column followed by the same letter are not significantly different at P = 0.05 (Fisher’s Protected least significant difference); Each datum represents the mean (± S.E.) of three replicates.

b * P < 0.05, *** P < 0.001

The botanicals showed a significant effect on the reproductive cycle in which *F*₁ progeny were reduced, and this was generally dose depended. Greater inhibition of *F*₁ progeny production was provided by *P. glandulosus* (23.3-100%) than *S. araliacea* (48.4-83.9%). These results compare favourably with the findings of other workers. Crude powders from *Lantana camara* and *Tephrosia vogelii* reduced *F*₁ progeny of *S. zeamais* by over 75% (Ogendo et al., 2003). Leaf powders of *L. multiflora* reduced egg-laying by only 24.9% in *C. serratus*, as compared with 100% by *Chenopodium ambrosioides* (Delobel & Malonga, 1987). Tapondjou et al. (2002) showed that low (0.8%) and high doses (6.4%) of leaf powders from *C. ambrosioides* reduced *F*₁ progeny in *S. zeamais* by 29 and 100%, respectively. The powdered dried leaves of *Ocimum kilimandscharicum* of the family Lamiaceae like *P. glandulosus* reduced *F*₁ progeny in *S. zeamais* by almost 100% and 100% for the dosages 2 and 10%, respectively (Jembere et al., 1995).

Because the condition of the substrate and the experimental chamber were uniform for all treatments and control, and emergence was recorded in all control samples, differences in emergence between the control and treated samples could rightly be attributed to the deleterious effects of the plant powders on *S. zeamais*. Further, *F*₁ progeny was expressed as number produced by 10 females, so differences in sex ratios among the samples could not explain the observed differences in the number of adults emerging, between the control and treated samples on the one hand, and among the treated samples on the other hand. However, there is a slight possibility that the broad range of insect ages (1-14 days) might have influenced the number of *F*₁ progeny produced, especially as *S. zeamais* adults produce eggs from 3-4 days after emergence, even though mating occurs on the first day (Fleurat-Lessard, 1988). But this factor may not be significant, because the same age range was used for all samples, which should lead to a cancellation or significant attenuation of the effect. Future studies may consider weevils that are older than three days with a narrower age range. Plant
powders can affect all stages of developing beetles (Boeke et al., 2001). Although, the samples treated with *P. glandulosus* and *S. araliacea* powders recorded significant mortalities within the 8-day-period provided for oviposition, this parameter could not adequately explain the differences in *F*<sub>1</sub> progeny, as no offspring were produced with 4% *P. glandulosus*, but the mortality was less than 50% by the 4<sup>th</sup> day after exposure. Higher contents (2 and 4%) of *S. araliacea* inhibited *F*<sub>1</sub> progeny production by over 80%, but achieved less than 10% mortality during the same period. Mortality did account for differences in progeny production in other studies involving *S. zeamais* and *S. oryzae* with botanicals (Xie et al., 1995; Tapondjou et al., 2002). Plant powders do not penetrate grains and *S. zeamais* eggs and larvae are only found inside the seeds, hence are not significantly exposed to plant powders. This contention precludes egg and larval mortality as a result of treatment with the powders. Also, many plant powders are known to be effective against oviposition in stored-product beetles (Boeke et al., 2001), so the observed effects on *F*<sub>1</sub> progeny could be caused by inhibition of egg-laying. Higher contents of powders could also act as a mechanical disturbance to oviposition, whereas all active contents by virtue of their chemical composition could have an adverse effect on ovarian development, fecundity, and fertility (Xie et al., 1995; Boeke et al., 2001). Further tests are needed to differentiate these possible modes of action.

Weaver et al. (1992) reported that dried leaves of *Tetradenia riparia*, a perennial minth from the family Lamiaceae, suppresses population size in bruchid species. Our results show that leaf powders from *P. glandulosus* curbed the rate of population increase of *S. zeamais* more than did *S. araliacea* during 3 months of storage, with total suppression achieved by the former at contents between 1 and 4%. The suppression of weevil populations is likely to be due to the adverse effects of the plant powders on oviposition, fertility and fecundity. The efficacy of dried leaves of *T. riparia* against bruchids is governed primarily by decrease in fecundity and to a lesser extent by the fertility of the parental females (Weaver et al., 1992). Adult mortality might have partly influenced the reduction in the rate of population increase, especially for *P. glandulosus*, which recorded 50 dead adults (the 50 introduced) for the higher contents (1-4%), but no live weevil. Surprisingly, control mortalities of 48 (*P. glandulosus*) and 49 weevils (*S. araliacea*) were recorded. Intra-specific competition is most likely to be responsible for the observed control mortalities. The protection of the grains against *S. zeamais* damage by the plant powders indicates that these materials, especially *P. glandulosus* could be of value in storage protection against the weevil. Because at higher contents (2 and 4%), no grain damage nor weight loss was recorded with *P. glandulosus*, this plant may possess antifeedant properties. This argument is further substantiated since less than 50% mortality was achieved with this plant for the highest contents 4 days after exposure. Even at 8 days after exposure, less than 60% mortality was recorded for the content 2%. The results of this study is consistent with the findings of Jembere et al. (1995), where whole and powdered dried leaves of *O. kilimandscharicum* significantly protected grains against *S. zeamais* damage.

*Plectranthus glandulosus* and *S. araliacea* leaf powders have shown good prospect for the protection of stored grains against *S. zeamais*, at least in subsistence agriculture. The application rates of 1:100 for *P. glandulosus* (complete protection) is low and 1:50 for *S. araliacea* (80% progeny reduction and over 50% mortality in 16 days) is within range compared to 1:40 reported by Delobel & Malonga (1987) using *C. ambrosioides* powdered leaves against *C. serratus* on groundnut. The range of 1:10 to 1:50 was reported by Jembere et al., (1995) using *O. kilimandscharicum* powdered leaves against *S. zeamais* and *Rhyzopertha dominica* (F.) on maize and sorghum, respectively. A range of 1:16 to 1:63 was reported by Tapondjou et al., (2002) using *C. ambrosioides* powdered leaves against *S. granaries*, *S. zeamais* and *Prostephanus truncatus* on wheat and maize. Koona & Njoya, (2004) used 1:50
Lantana camara powdered leaves against S. zeamais on maize. In countries like Cameroon and other developing nations, where the tested two plants are abundant and money is scarce, the use of the powdered leaves for storage protection against insects should be promoted. However, more research involving field trials, mode of action, activity spectrum, persistence, and mammalian toxicity may be needed before the recommendation of these plants to farmers.

Acknowledgement

E.N. Nukenine was supported by the Alexander-von-Humboldt Foundation, which is gratefully acknowledged. Sincere gratitude to The Federal Biological Research Centre for Agriculture and Forestry, Institute for Stored Product Protection, Berlin for providing laboratory facilities and materials for this work. The authors are also thankful to the Staff of the Institute, especially Mrs Anders, Krause, Paul and Reichmann, and Mr Rassmann whose assistance made this work possible.

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Niber, B.T. 1994. The ability of powders and slurries from ten plant species to protect stored grain from attack by Prostephanus truncatus Horn (Coleoptera: Bostrichidae) and Sitophilus oryzae L (Coleoptera: Curculionidae). – Journal of Stored Products Research 30: 279-301.


Comparative efficacy of different grain protectants against Tribolium castaneum under two sets of temperature and humidity

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Abstract: Experiment was conducted to compare the efficacy of different grain protectants viz. cypermethrin, deltamethrin, neem leaf extract and Acorus calamus oil at the concentrations of 100, 100, 1000 ppm and 30 µL/L, respectively against Tribolium castaneum under two sets of temperature and humidity viz. 28°C and 35°C at 55% and 68%. Mortality of T. castaneum was observed for exposure periods of 24, 72 and 168 h. Results showed that maximum mortality was observed against deltamethrin at 28°C and 68% r.h. at exposure period of 168 h followed by cypermethrin. Acorus calamus oil was proved to be least effective at 35°C and 55% r.h. at an exposure period of 24 h. Results further showed that overall effect of humidities were non significant on the mortality of T. castaneum.

Key words: Tribolium castaneum, cypermethrin, deltamethrin, neem leaf extract, Acorus calamus oil, Humidity, Temperature

Introduction

Tribolium castaneum (Herbst) causes heavy damage to stored products in hot and humid conditions. Although this insect is incapable of feeding on sound grains, it does considerable damage to flour and flour by-products and also to grains damaged by other pests. Larvae are always found hidden in food. The adults however, are active but are mostly concealed in flour. In case of severe infestation, the flour turns greyish and mouldy, and develops pungent, undesirable odour. It becomes unfit for human consumptions (Atwal, 1976).

For the control of stored grain insect pests, grain protectants and fumigants have been in use during the last many decades. These chemicals have gone ineffective due to the development of resistance particularly in T. castaneum (Saxena & Singh, 1995). Toxic residues of these chemicals pose risks to human health and to the environment. Therefore, plant extracts, which have been traditionally used as grain protectants merit re-evaluation. These plant extracts are not only effective against stored grain insect pests but also safe to human and to the environment.

Rhizomes of Acorus calamus L. commonly known as sweet flag have many insecticidal properties against a wide variety of insect-pests. The powder and extracted oil of rhizomes act as contact or stomach poison, antifeedants and repellents. The toxic and sterilizing effects of vapours of rhizomes oil against certain insects have been reported (Saxena & Mathur, 1976). Its most effective components are β-asaron (Streloke et al. 1989).

Over 120 plants and plant products can be used for the control of stored product insect pest (Dales, 1996), out of these neem leaves have been successfully tested in the laboratory which gives an indication of their potential usefulness as stored product protectants.

Pyrethroids possess many desirable properties including high toxicity to insects (target animals), low toxicity to mammals (non-target animals), photo stability, high degradability...
and application at minimum dose. Hence they are to supplement and possibly to replace some of the conventional organochlorine, organophosphate and carbamate insecticides.

In the present study insecticidal activity of different doses of Acorus calamus L. oil, neem extract, cypermethrin and deltamethrin are reported against T. castaneum.

Materials and methods

The insects were collected from the godowns of Punjab Food Department located in Faisalabad. T. castaneum was reared on wheat in one L capacity glass jars in an incubator maintained at 30±2°C and 60±5% relative humidity (r.h.) To maintain homogeneous population, 100 adult insects were released in glass jars containing sterilized wheat. All adults were separated by sieving the wheat after three days, and wheat containing the eggs was placed again in glass jars and covered with muslin cloth.

The rhizomes of sweet flag, Acorus calamus, were collected from northern hilly areas of Pakistan where it grows naturally. Rhizomes were cleaned, dried and ground to fine powder (30 mesh) and then extracted with n-hexane in the soxhlet extraction apparatus. Extracts were concentrated in a rotary evaporator and finally made solvent free in vacuum desiccators to obtain pure oil. The oil was stored in a refrigerator at 4°C.

Acetone was used as a solvent for Acorus calamus oil. Different dilutions were prepared as follows: 999 mL of acetone + 1 mL of oil = 1 L of solution; 1 mL of solution = 1 µL of oil

For 100% solution, one kg fresh neem leaves were ground in 1 L water (1/1 : w/w) to make a paste. The paste was stained using porous cloth and filled in a glass bottle: 999 mL of acetone + 1 mL fresh neem leaf extract = 1 L of solution; 1 mL of solution = 1 µL of fresh neem leaf extract

Single dose/concentration of the plant extracts and pyrethroids were used. The dose used for fresh neem leaf extract and Acorus calamus oil was 50 µL whereas, 100 ppm concentration of cypermethrin and deltamethrin was used.

Effect of the plant extracts and pyrethroid insecticides on mortality of T. castaneum was evaluated at two sets of temperatures (28°C and 35°C) and humidities (55% and 68%).

Petri dishes were used as exposure chambers. A filter paper was used in each Petri dish as experimental material. One Petri dish was used as control/check along with each treatment. The plant extracts and pyrethroid insecticides used in the experiment were sprayed thoroughly on the filter papers placed in the Petri dishes by using micropipettes. After applying each treatment on the filter papers, the Petri dishes were left exposed to open air for a few minutes so that acetone used to make different concentrations may evaporate. Twenty adults of T. castaneum were put in each Petri dish on the filter paper and the Petri dishes were covered tightly. In this experiment four incubators were used. Out of four incubators, two were set at 28°C. In one incubator r.h. was 55% and in the other incubator r.h. was 65%. Remaining two incubators were set at 35°C, one with 55% r.h. and other with 68% r.h. Humidity was maintained in incubators by placing saturated solution of calcium nitrate and ammonium nitrate. In each incubator 20 Petri dishes each Petri dish containing 20 adults of T. castaneum were placed.

At the end of each exposure period (24 h, 72 h and 168 h after the spray), Petri dishes were opened. The number of adults that had been knocked down were counted and separated from live adults. Statistical analysis of mortality data was carried out. The effect of treatments was computed following CRD analysis of variance.
Results and discussion

Overall ANOVA indicated that temperature \((F = 178.45, d.f. = 1)\), different chemicals used \((F = 450.10, d.f. = 3)\) and different exposure times \((F = 960.53, d.f. = 2)\) had the significant effect, however, the humidity has the non significant effect \((F = 0.07, d.f. = 1)\) on the mortality of \(T. castaneum\). The interactions of temperature and humidity \((F = 4.83, d.f. = 1)\) and temperature and chemicals \((F = 12.54, d.f. = 3)\) had the significant effect on the mortality of \(T. castaneum\) but the interaction of temperature, humidity and exposure times had the non significant effect on the mortality of \(T. castaneum\) \((F = 0.55, d.f. = 2)\).

Percent mortality of \(T. castaneum\) against Biosal \((Azadirachtin indica)\) concentration was statistically significant at different exposure periods. It is evident from the Table 1 that maximum mortality of \(T. castaneum\) was recorded after 168 h of spray (6.51%), which was statistically followed by the mortality percentage of the insect under study after 72 h (2.81%). Minimum mortality of \(T. castaneum\) was recorded at 24 h of spray (0%), which was statistically different from all other exposure times under study. These results are in agreement with those of Seck et al. (1991), Khaire et al. (1992), Susa & Karnavar (1993), Xie et al. (1995), Suss et al. (1997), Imtiaz et al. (1999), Srivedi & Dhingra (1999), Sharma et al. (1999), and El-Lakwah et al. (1999), who concluded that Biosal increased the percent mortality of stored grains insect pests with the increase of exposure time.

Table 2 shows that temperature has significant effect on the mortality of \(T. castaneum\). Maximum mortality of \(T. castaneum\) was recorded after 28°C of spray (6.94%), which was statistically different and higher than at 35°C (4.60%). Interaction of temperature and chemicals was significant and Deltamethrin performed better at 28°C and gave the mortality (11.8%), however, least mortality was given by \(A. calamus\) oil at 35°C, which is (1.60%). These results conform those of Srivedi & Dhingra (1997), Shawir & Mansee (1997) and Paneru et al. (1997) who concluded that deltamethrin is more effective below 28°C and less effective above 30°C.

A perusal of data indicated that at 28°C deltamethrin caused maximum mortality (11.8%). Followed deltamethrin, was cypermethrin that caused higher mortality (9.36%). The lowest mortality (2.79%) was caused by \(A. calamus\) oil at 28°C. Similarly at 35°C deltamethrin caused maximum mortality (8.07%) and lowest mortality (1.60%) was caused by \(A. calamus\) oil at 35°C. Overall means indicated that deltamethrin was more effective at 28°C and \(A. calamus\) oil was least effective against \(T. castaneum\) at 35°C.

Different exposure periods showed statistically significant results on the mortality percentage of \(T. castaneum\) (Herbst). Maximum mortality percentage of \(T. castaneum\) (Herbst) was recorded with cypermethrin after 168 h of spray (13.07%), which was statistically followed by the mortality percentage of the insect under study after 72 h (9.31%) respectively. Minimum mortality percentage of \(T. castaneum\) (Herbst) was recorded at 24 h of spray (1.13%). These results confirm the findings of Imtiaz et al. (1999), Chander et al. (2000), Tabassum et al. (1996) and Karnataka (2000) who concluded that cypermethrin decreased the population of stored grains insect pests at different exposure periods.

Table 1 shows that mortality caused by different chemicals at different exposure times has significant effect. A perusal of data indicated that deltamethrin caused maximum mortality percentage of \(T. castaneum\) was recorded after 168 h of spray (15.68%), which was significantly different and higher than all other exposure periods under study. It was followed by the mortality percentage after 72 h (12.5%). Minimum mortality of \(T. castaneum\) was recoiled at 24 h of spray (1.63%). These results are in line with those of Lorini & Galley
Ahmad et al. (2001), Sinha & Saxena (2000) who concluded that deltamethrin helped in reducing the population of stored grains insect pests in wheat.

Table 1. Comparison of mean values of percent mortality of *Tribolium castaneum* (Herbst) caused by different chemicals at different exposure times, temperatures and relative humidities.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Exposure (h)</th>
<th>Temperature °C</th>
<th>Means (% mortality at two temperatures and two humidities)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>28 °C 35 °C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Humidity (55%)</td>
<td>Humidity (68%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28 °C 35 °C</td>
<td>Humidity (55%)</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>24</td>
<td>1.25 1.50</td>
<td>1.50 1.00</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>11.61 12.12</td>
<td>7.00 6.50</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>14.57 15.08</td>
<td>11.56 11.07</td>
</tr>
<tr>
<td>Means</td>
<td>(Temperature, r.h., and exposure time)</td>
<td>9.36 b 6.32 d</td>
<td>7.84 B**</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>24</td>
<td>1.50 2.00</td>
<td>1.50 1.50</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>15.75 16.50</td>
<td>9.25 8.50</td>
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<td></td>
<td>168</td>
<td>17.52 17.52</td>
<td>14.53 13.12</td>
</tr>
<tr>
<td>Means</td>
<td>(Temperature, r.h., and exposure time)</td>
<td>11.8 a 8.07 c</td>
<td>9.93 A</td>
</tr>
<tr>
<td>Biosal</td>
<td>24</td>
<td>0.00 0.00</td>
<td>0.00 0.00</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>3.00 3.50</td>
<td>2.50 2.25</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>7.90 8.44</td>
<td>5.10 4.59</td>
</tr>
<tr>
<td>Means</td>
<td>(Temperature, r.h., and exposure time)</td>
<td>3.81 e 2.41 f</td>
<td>3.11 C</td>
</tr>
<tr>
<td><em>Acorus calamus</em></td>
<td>oil</td>
<td>24 72 168</td>
<td>0.00 2.25 5.86</td>
</tr>
<tr>
<td>Means</td>
<td>(Temperature, r.h., and exposure time)</td>
<td>2.79 f 1.60 g</td>
<td>2.19 D</td>
</tr>
</tbody>
</table>

* Means sharing similar characters are statistically non-significant (P>0.05).
** Lower cases represent differences among means of interacting two variables and upper cases show the differences among overall mean values.
A. calamus oil was the least effective, which gave least mortality, however, statistically significant results were observed regarding mortality percentage of *T. castaneum* at different exposure periods. Maximum mortality percentage of *T. castaneum* was recorded after 168 h of spray (4.64%), which was statistically different and higher than all other exposure times under study. It was followed by the mortality percentage of 72 h (1.94%) and minimum mortality percentage of *T. castaneum* was recorded at 24 h of spray (0%). These results are in conform with those of Risha (1993), Chander *et al.* (2000), Rasool *et al.* (2002), Reddy and Reddy (2000), Rahman & Schmidt (1999), Kumari *et al.* (1999) and Paneru *et al.* (1997) who concluded that population of stored grain insect pests reduces with the increase in exposure time rather than increase in dosage.

Table 2 shows that mortality at different exposure times and temperatures had significant effect. A perusal of data indicated that after 168 h at 28°C gave the maximum mortality 11.63%. After 72 h at 28°C the mortality was 8.40% which is higher than mortality after 24 h and lesser than after 168 h. In conclusion, overall means indicated that with the increase of exposure time mortality has increased and in case of temperatures, 28°C has more effect on mortality of *T. castaneum*.

### Table 2. Comparison of combined mean values of the two humidities caused by the tested four chemicals on percent mortality of *Tribolium castaneum* (Herbst) at two temperatures and exposure times.

<table>
<thead>
<tr>
<th>Exposure Times</th>
<th>Temperature</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28°C</td>
<td>35°C</td>
</tr>
<tr>
<td>24 h</td>
<td>0.78 d*</td>
<td>0.59 d</td>
</tr>
<tr>
<td>72 h</td>
<td>8.40 b</td>
<td>4.88 c</td>
</tr>
<tr>
<td>168 h</td>
<td>11.63 a</td>
<td>8.32 b</td>
</tr>
<tr>
<td>Mean</td>
<td>6.94 A</td>
<td>4.60 B</td>
</tr>
</tbody>
</table>

* Means sharing similar characters are statistically non-significant (P>0.05).

** Lower cases represent differences among means of interacting two variables and upper cases show the differences among overall mean values.

### References


Fumigant activity of monoterpenoids against the rice weevil, *Sitophilus oryzae* (L.)

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**Abstract:** Results of the assays on fumigant activity of 6 major monoterpenoid constituents of essential oils from aromatic plants grown in Turkey namely, carvacrol, 1,8-cineole, menthol, \(\gamma\)-terpinen, terpinen-4-ol and thymol against adults of the rice weevil, *Sitophilus oryzae* (L.) are presented. Doses between 5.8 and 184.8 mg/L air and exposure periods of 24-96 h were used. All 6 constituents showed fumigant activity in varying degrees against adults of *S. oryzae*. The most active constituent was \(\gamma\)-terpinen which achieved 100% mortality at 46.2 mg/L air and an exposure of 96 h. This was followed by thymol and carvacrol which achieved 99.2 and 96.4% mortalities, respectively, at the same dose and exposure period. While terpinen-4-ol caused mortalities of 99-100% at 184.8 mg/L air and exposures of 24-96 h, the constituents 1,8-cineole and menthol achieved mortalities of up to 95 and 61%, respectively, at any dose and exposure time.

**Key words:** monoterpenoids; stored-product insect; fumigant activity; *Sitophilus oryzae*

**Introduction**

Plant extracts have been an important weapon of man in his struggle against pests attacking himself and his crops since earlier ages. Though there has been a decline in efforts to develop plant derived natural products into pesticides, by Second World War, this trend has been reversed in recent years due to public concern over health and environmental safety of many pesticides in use. Furthermore it became evident that plants are a vast storehouse of chemical substances that can be used as agrochemicals. It is estimated that there are at least 250 000 different species of plants in the world today and that only about 10% of plant species have been examined chemically, so the possibility for further exploration is enormous (Benner, 1993). On the other hand, stored product insects are adapted to live in confined environments. Therefore, they may have had limited evolutionary association with plants' producing compounds that possess insecticidal activity. Thus their susceptibility to such compounds which were evolved against herbivory is of high probability (Weaver et al., 1994).

Essential oils and their monoterpenoid constituents, deserve special attention among substances of plant origin that were shown to have insecticidal activity. A review of the relevant literature indicates that essential oils and monoterpenoids exert multi-faced control activity on insects. They were reported to possess toxic and repellent activity against insect pests (Saraç and Tunç, 1995a,b). However they may, essentially, be useful as insecticides in fumigation against pests in confined environments such as storehouses, beehives, even greenhouses (Erler and Tunç, 2005). Thus it is no surprise that the wealth of literature on the pest control potential of essential oils and their constituents accumulated in recent years dealt mainly with their use as fumigants against stored product insects (Tunç, 1996).
Essential oils from aromatic plants grown or naturally occurring in Turkey have been screened for their fumigant activity against different stages of various stored product insects (Saraç and Tunç, 1995b; Tunç et al., 2000). The aim of this study was to assay fumigant activity of essential oil constituents individually for reliable and reproducible data.

Materials and methods

The rice weevil, *Sitophilus oryzae* (L.) was reared on wheat grains. Adults of ≤2 weeks old *S. oryzae* were used in the tests. Insect rearing and all experimental procedures were carried out at 26±1°C, 65±5% r.h. and in continuous darkness.

The essential oil constituents were purchased from various manufacturers. Their source and purity was as follows: carvacrol, Fluka, 97%; 1,8-cineole, Sigma, 99%; menthol, Riedel-de Haen, 99.5%; γ-terpinen, Sigma, 97%; terpinen-4-ol, Aldrich, 99% and thymol, Merck, 99%.

Glass jars of 650 ml capacity with screw top lids were used as test chambers. The constituents diluted in 200 µl acetone were applied on a blotting paper strip measuring 3x8 cm using an automatic pipette. Only acetone was applied to control papers. The strip was attached to the under side of the jar’s lid and acetone was evaporated for 14-22 seconds before the lid was tightly fitted. Preliminary dose range finding tests were done for each constituent to include doses that gave high mortalities against the test insect stage. Therefore 4 doses at varying ranges, between 5.8-184.8 mg/L air, were used depending on the constituent. Twenty adults of *S. oryzae* confined in nylon gauze bags containing rearing food were exposed to the essential oil constituents. In each experiment, each bag was counted as a replicate. Three replicates were used for each concentration and exposure time combination in all experiments and experiments were repeated twice, thus the total number of tests for each concentration and exposure time was 6.

After exposure for 24 or 48 or 96 h, bags containing insects were taken out of the jars and mortality counts were made 3 days later. Mortality data were corrected for natural mortality in controls and were subjected to probit analysis to estimate LT<sub>50</sub> and LT<sub>99</sub> values (Sokal and Rohlf, 1973).

Results

All monoterpenoid constituents tested, carvacrol, 1,8-cineole, menthol, γ-terpinen, terpinen-4-ol and thymol, showed fumigant activity in varying degrees against adults of *S. oryzae*. Mortality of the test species exposed to 6 constituents was dose and exposure period dependent.

Only two constituents, γ-terpinen and terpinen-4-ol, achieved 100% mortality against *S. oryzae* adults at doses of 46.2, 92.4 (only the first) and 184.8 mg/L air (the latter) and varying exposure periods (Table 1). The exposure periods needed to achieve 99% mortality or LT<sub>99</sub> values (h) of various constituents at indicated doses, as mg/L air, were as follows: 1,8-cineole at 184.8 mg/L, 288.4 h; carvacrol, γ-terpinen and thymol at 46.2 mg/L, 281.8 h, 63.1 h and 97.7 h, respectively, and terpinen-4-ol at 92.4 mg/L, 114.8 h.

Discussion

There are numerous reports on the insecticidal activity of essential oils and their constituents. However, many of them are based on the fumigant activity of essential oils rather than their constituents. In this study, the fumigant toxicity of 6 monoterpenoid constituents was tested
against the rice weevil adults. The choice of constituents for inclusion in the study was based on their being major constituents of essential oils used in previous studies (Saraç and Tunç, 1995b; Tunç et al., 2000).

Table 1. Mortality and LT values of *Sitophilus oryzae* adults exposed to vapours of monoterpenoids.

<table>
<thead>
<tr>
<th>dose (mg/L air)</th>
<th>% mortality (mean±S.E.)</th>
<th>exposure period (h)</th>
<th>LT_{50} (h)</th>
<th>LT_{99} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>carvacrol</td>
<td>24</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>5.8</td>
<td>10.1±1.6</td>
<td>23.9±3.6</td>
<td>25.2±3.0</td>
<td>354.8</td>
</tr>
<tr>
<td>11.6</td>
<td>21.9±2.0</td>
<td>31.6±2.8</td>
<td>35.9±2.7</td>
<td>302.0</td>
</tr>
<tr>
<td>23.1</td>
<td>49.2±2.5</td>
<td>51.7±3.5</td>
<td>65.2±2.7</td>
<td>30.2</td>
</tr>
<tr>
<td>46.2</td>
<td>84.9±2.3</td>
<td>90.6±2.5</td>
<td>96.4±2.7</td>
<td>4.2</td>
</tr>
<tr>
<td>control</td>
<td>0.8±0.8</td>
<td>1.7±1.1</td>
<td>4.2±1.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1,8-cineole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.1</td>
<td>14.5±2.5</td>
<td>18.0±3.5</td>
<td>18.8±2.0</td>
<td>*</td>
</tr>
<tr>
<td>46.2</td>
<td>18.0±3.1</td>
<td>23.2±3.1</td>
<td>31.9±2.5</td>
<td>416.9</td>
</tr>
<tr>
<td>92.4</td>
<td>45.3±3.7</td>
<td>56.5±3.5</td>
<td>68.7±3.0</td>
<td>30.9</td>
</tr>
<tr>
<td>184.8</td>
<td>77.8±4.1</td>
<td>90.5±3.9</td>
<td>94.7±1.9</td>
<td>6.6</td>
</tr>
<tr>
<td>control</td>
<td>2.5±1.1</td>
<td>3.3±1.7</td>
<td>5.8±2.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>menthol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.1</td>
<td>4.0±2.1</td>
<td>9.5±2.9</td>
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<td>18.9±2.9</td>
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<td>223.9</td>
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<tr>
<td>92.4</td>
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<td>24.9±3.3</td>
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<tr>
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<td>60.7±3.2</td>
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</tr>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>γ-terpinen</td>
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</tr>
<tr>
<td>11.6</td>
<td>19.6±3.3</td>
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</tr>
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<td>92.4</td>
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<td>100.0±0.0</td>
<td>100.0±0.0</td>
<td>**</td>
</tr>
<tr>
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<td>1.7±1.7</td>
<td>2.5±1.1</td>
<td>5.8±1.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>terpinen-4-ol</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>23.1</td>
<td>10.3±2.4</td>
<td>11.5±2.9</td>
<td>20.4±2.5</td>
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<td>53.5±3.2</td>
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<td>*</td>
</tr>
<tr>
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<td>5.0±2.6</td>
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</tr>
<tr>
<td></td>
<td>thymol</td>
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<td></td>
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</tr>
<tr>
<td>5.8</td>
<td>8.7±2.4</td>
<td>23.9±4.3</td>
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<td>99.2±0.9</td>
<td>16.2</td>
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<tr>
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<td>2.5±1.1</td>
<td>0.8±0.8</td>
<td>-</td>
</tr>
</tbody>
</table>

*estimated LT values were too high.

**LT values could not be estimated due to 100% mortality at all exposure periods used.
Results of this study show that the activity of monoterpenoid constituents against the adults of *S. oryzae* varied according to the source of the constituent. The monoterpenoids tested are classified into four groups depending on their chemical nature: Group 1 alcohols; menthol and terpinen-4-ol, Group 2 ethers; 1,8-cineole, Group 3 hydrocarbons; γ-terpinen, and Group 4 phenols; carvacrol and thymol. When comparing their fumigant action against the test species, it can be concluded that hydrocarbons and phenols are most active against *S. oryzae* adults. These differences in activity of the various constituents can be used in the search for more potent compounds for the control of this species.

Some of the constituents investigated in the present study, e.g., carvacrol and thymol, were found promising repellents and were suggested to be used both as fumigants and repellents against stored product insects. In closed chambers these constituents provided promising repellencies, 49-80%, for one month or longer against adults of *T. confusum* at concentrations achieving 99% mortality against adults and/or eggs of the same insect (Tunç and Erler, 2003).

The essential oils and their constituents are intended to be used like fumigants to disinfect commodities. Therefore, they should have the ability to kill all stages of insects. Certain pest species or their certain stages may exhibit a higher tolerance to these compounds than others. Although it was demonstrated that such tolerance could be overcome by increasing the dose, it may, however, not be a good option in cases where dose increases of up to 4 times are needed. For instance, in a previous study, a dose of 23 mg/L anethole was sufficient to obtain 99% mortality at 35 h, or a shorter period against adults and eggs of *T. confusum*, adults of *S. oryzae*, and eggs of *E. kuehniella*. However, a dose of 4 times higher, 92 mg/L, and yet a 2-3 times longer exposure, 89 h, would be necessary to achieve the same in larvae of *E. kuehniella* (Tunç & Erler, 2000).

The penetration and distribution, and absorption and desorption properties of the essential oils or their constituents in the bulk of commodities has not been sufficiently investigated. Such characteristics may effect both the success of control and the quality of the commodity treated. Shaaya et al. (1997) showed that greater amounts of essential oils or constituents would be needed when fumigating in the presence of the commodity (wheat) than in space fumigation without the commodity to achieve the same level of control. Therefore, it may be that fumigation treatments using essential oils are more suitable for empty structures. As for treatment with commodities more studies would be needed to identify niche application of essential oils and their constituents. Another concern is the extent to which it would be possible to remove the odours of essential oils or monoterpenoids from the commodity after treatment and their possible effects on the organoleptic, nutritional and technological qualities of the treated food. However, it may be possible to use these compounds successfully as repellents for preventing infestation of food packages in storage. An additional possibility could be to apply essential oils for disinestation of structures used in storing or processing or transporting of commodities, particularly in situations where high standards of worker and environmental safety are sought.

Aromatic plants contain, in general, essential oils at low quantities. Therefore, considerably large quantity of plant material would have to be processed in order to obtain essential oils in quantities sufficient for commercial scale applications. Screening of essential oil constituents for their fumigant activity may encourage the breeding of plant varieties that produce the active constituents in elevated amounts (Tunç et al., 2000). Although it may be practical to grow sufficient plant material for local use in less developed countries, it is likely that it would be preferable to synthesize the required compound rather than to launch a product which had to be extracted from whole plant material in the developed world. Another option is to use a plant product as a basis for synthesis, since such products, in general,
intrinsically lack all necessary characteristics to compete with the best synthetic agrochemicals (Benner, 1993). There are several advantages of using monoterpenoids as lead compounds for new insecticides. The first is their availability. They exist abundantly in some plants, marine algae, and insects. They can be easily modified to various derivatives in the laboratory to improve their bioactivity. The second is their wide spectrum of bioactivity. These compounds have been shown to have fumigant and insect growth regulatory insecticide properties and to possess attractant, repellent and reproduction inhibiting potential on insects. Thirdly, monoterpenoids are relatively a safe group of natural products. Generally, they are safe for humans and other mammals, and are considered environmentally safe (Tsao et al., 1995).

Acknowledgements

The author is thankful to Prof. Dr. İrfan Tunç (Dept. of Plant Protec., Faculty of Agriculture, Akdeniz University, Antalya, Turkey) for comments on the manuscript. This study was financially supported by the Scientific Research Projects Administration Unit of Akdeniz University.

References

Influence of deltamethrin on *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae)

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**Abstract**: The contact insecticide deltamethrin is broadly used in stored product pest management against saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) in Czech Republic. *Cephalonomia tarsalis* is an ectoparasitoid of *O. surinamensis*. In a dose response experiment with *C. tarsalis* we have studied its sensitivity to deltamethrin. We found that males and females of *C. tarsalis* were not equally sensitive to deltamethrin. Males were significantly more sensitive then females. The LC50 values at 24 h after exposure to deltamethrin were 0.758% for males and 1.201% for females.

**Keywords**: *Cephalonomia tarsalis*, deltamethrin, sex, susceptibility

**Introduction**

Contact insecticides are broadly used in stored product pest management. Due to resistance, toxicity and residues risk of these types of insecticides, there is a search for alternative control means. Biological control would be a possible complementary solution.

Contact insecticides and fumigants are primarily used to control the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), which is considered a secondary pest, is widespread. Both larvae and adults of this pest cause important damage to stored products and particularly to cereal and processed grain, dried fruits, and nuts. Its occurrence and damage increased significantly over the past decade. The insect is now considered a key pest of stored grains. In the Czech Republic, a commonly used contact biocide in stored products and in food and feed processing facilities against this pest is deltamethrin.

*Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) is both a predator and an ectoparasitoid of larvae and pupae of saw-toothed grain beetle Powell (1938). Although *C. tarsalis* reportedly uses several different stored product beetle hosts, it appears to be primarily associated only with the saw-toothed grain beetle (Howard et al., 1998). This parasitoid is naturally present in stored products in Czech Republic (Lukáš, 2002) and is considered as promising natural control agent of *O. surinamensis*. Although interactions between beneficial insects and pesticides are intensively studied in field crops, orchards, vineyards and glasshouses (Croft, 1990), little is still known about beneficial species in a stored product environment.

Therefore, the aim of our study was to assess the influence of deltamethrin (K-Othrin 25W) on *C. tarsalis* with the purpose of determining its susceptibility by determining the lethal concentrations in the range of LC10 and LC99.
Material and methods

Cultures of *C. tarsalis* and *O. surinamensis* originated from stored wheat samples obtained from warehouse near Prague in 2002. *O. surinamensis* was reared on rolled oats and *C. tarsalis* was reared on fourth instar larvae of *O. surinamensis* in wheat. Both cultures were maintained in climate controlled chambers at a constant temperature of 30°C and relative humidity of 75-80% at a photoperiod of 16:8 (L:D). Freshly emerged adults (18±6 h after emergence) were used for the experiments.

The dipping technique was adopted for the bioassay with adult males and females of *C. tarsalis*. The test solutions of the insecticide were prepared by dilution of appropriate amount of K-Othrine 25WP with distilled water containing 5% Tween 80 to make concentrations of 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5% and 2%. Prior to dipping, tested insects were anesthetized for 10 seconds with CO2. The insects were then dipped for 2-3 s in an insecticide solution and left to air dry at 25°C. The treated insects were individually introduced into a test tube (90x15 mm) and closed by cotton wool. For control tests, insects were dipped in distilled water containing only Tween 80. At each insecticide concentration, three replications were made with 5 individuals per replicate for males and five replications were made with 5 individuals per replicate for females. Experiments were carried out with 120 males and 200 females for each LC50. Individuals which did not respond to pencil tip prodding were judged to be dead. Mortality was determined 24 h after treatment. No mortality was observed in the control insects during experiments. All bioassay tests were conducted at 25±1°C, at a photoperiod of 16:8 (L:D).

The analysis of the influence of deltamethrin on mortality of *C. tarsalis* was carried out by means of GLM logit analysis using the R freeware statistical package (http://cran.at.r-project.org) assuming quasibinomial distributed residuals to adjust overdispersion and asymmetries in the distributions. First, the data were fitted to a maximal model and then, less significant variables were progressively withdrawn from the model until a minimal appropriate model was obtained (i.e. a simplified model in which all terms are significant) (Crawley, 2002). Examination of the residuals confirmed the fit of the models to the data.

Results

Males and females of *C. tarsalis* were unequally sensitive to deltamethrin (F=6.1, df=1,13, p<0.05) - males were more sensitive than females (Fig. 1). Logit results with log(dose+1) transformation for males were n=120, slope = 4.1143 ± 0.4833, intercept = -2.3505 ± 0.3681, LC50 = 0.758 ± 0.069 % (±SE). Logit results for females with log(dose+1) transformation were n=200, slope = 4.1143 ± 0.4833, intercept = -3.2457 ± 0.3924 , LC50 = 1.201 ± 0.059 % (±SE). The lethal concentrations for males and females of *C. tarsalis* are summarized in table 1.

Discussion

The sex of the animals was found to be an important factor explaining the effect of deltamethrin on mortality of *C. tarsalis*. A possible explanation would be the difference in size between males (1.0805 ± 0.009 mm) and females (1.268 ± 0.005 mm) (Cheng et al., 2004). Nevertheless Baker & Weaver (1993) did not find any differences between males and females of *Anisopteromalus calandrae* exposed to malathion, despite the fact that males were significantly smaller than females. The fact that females of *C. tarsalis* are less sensitive to
deltamethrin than males, would also indicate a potential for rearing a resistant strain of this parasitoid under laboratory conditions.

Table 1. Estimates of lethal concentrations (LC) of deltamethrin for *Cephalonomia tarsalis* males and females. The LC<sub>50</sub> values were calculated based on the mortality at 24h.

<table>
<thead>
<tr>
<th>Deltamethrin</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. %</td>
<td>±SE</td>
<td>conc. %</td>
<td>±SE</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;10&lt;/sub&gt;</strong></td>
<td>0.074</td>
<td>0.103</td>
<td>0.290</td>
<td>0.076</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;25&lt;/sub&gt;</strong></td>
<td>0.374</td>
<td>0.074</td>
<td>0.685</td>
<td>0.059</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>0.758</td>
<td>0.069</td>
<td>1.201</td>
<td>0.059</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;75&lt;/sub&gt;</strong></td>
<td>1.249</td>
<td>0.091</td>
<td>1.875</td>
<td>0.075</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td>1.878</td>
<td>0.129</td>
<td>2.754</td>
<td>0.099</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;99&lt;/sub&gt;</strong></td>
<td>3.930</td>
<td>0.233</td>
<td>5.724</td>
<td>0.169</td>
</tr>
</tbody>
</table>

Fig. 1. Observed proportion of mortality for males (full circles) and females (open circles) of *Cephalonomia tarsalis* after 24 hours by exposure to different concentration of deltamethrin (expressed as log(dose+1)) using logit transformation.

Although beneficial organisms are generally considered to be more susceptible to insecticides than their hosts (Croft, 1990), they undergo the same process of developing resistance to insecticides. For example Baker & Weaver (1993) found a strain of *A. calandrae* resistant to malathion, and Baker & Throne (1995) studied resistant strains of *Habrobracon hebetor*. Žďárková (1997) described differences in resistance to phosphine between different strains of the predatory mite *Cheyletus eruditus*. It was also shown that some strains of this predator are more tolerant to certain acaricides than its prey and the predator can be released
one week after the acaricide treatment. Use of pesticide resistant parasites and predators would be a promising strategy in pest management systems integrating chemical and biological control technologies (Schoeller, 1998). This approach would have some advantages over traditional chemical control or biological control applied alone. Releasing parasites and predators after application of low dose of a pesticide at an appropriate time would increase the time between two successive applications, decrease the contamination of environment by pesticide residues, decrease the probability of resistance development, and considerably prolong efficacy of both treatments.

Acknowledgements
The study was supported by the grant GACR No. 522/04/P169 and VYZKUMNY ZAMER MZe 0002700603

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Antifungal activity of plant extracts

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Abstract: The use of natural products with therapeutic properties is as ancient as human civilisation. Nowadays, there is a growing concern and awareness by public opinion regarding the use of synthetic pesticides and to the presence of their residues in food. These facts have lead to a search for new forms of food protection against contamination by microorganisms. In this work we evaluate fungistatic activity of six aqueous extracts namely: French lavender (Lavandula stoechas L.), ginger (Zingiber officinale Roscoe), malva (Malva sylvestris L.), oregano (Origanum vulgare L.), pau D’arco (Tabebuia impetiginosa (Mart.) Standley) and rosemary (Rosmarinus officinalis L.). They were tested against Aspergillus candidus, A. niger, Penicillium sp. and Fusarium culmorum. Results are presented and discussed.

Key words: natural products, fungi, natural pesticides, fungicides.

Introduction

The excessive and indiscriminate use of organic pesticides has resulted in effects on soil health, humans’ health hazards, toxicity to useful non-targeted animals and environmental pollution (Roy & Dureja, 1998). Further, the use of synthetic pesticides to control post-harvest deterioration of food commodities is restricted, due to their possible carcinogenicity, teratogenicity, high and acute toxicity, and long degradation periods (Lingk, 1991).

Therefore, alternatives to synthetic pesticides are needed from microbial and plant sources. Effective phyto compounds are expected to be far more advantageous than synthetic pesticides, as they decompose easily, are not environmental pollutants, and possess no residual or phytotoxic properties (Rao, 1990; Tewari, 1990; Badei et al., 1996; Bishop & Thornton, 1997).

Some assays have been made, to study their effects on fungi growth, and mycotoxins production, using plants or parts of plants. These studies were carried out using lavender, rosemary, cinnamon (Davidson, 1997; Magro et al., 2004), anise, marjoram, saffron, basil (Hitokoto et al., 1980), clove (Mabrouk & El-Shayeb, 1980; Beg & Ahmad, 2002; Ponce et al., 2003), coriander, sage, oregano, thyme (Pruthi, 1980), cumin, pepper (Beuchat & Golden, 1989), garlic and onion (Ghandi & Ghodekar, 1988).

In the present work, the fungistatic activity of six aqueous extracts were evaluated by analysing their effect on fungi growth. The plants used were: French lavender (Lavandula stoechas L.), ginger (Zingiber officinale Roscoe), malva (Malva sylvestris L.), oregano (Origanum vulgare L.), Pau D’arco (Tabebuia impetiginosa (Mart.) Standley) and rosemary (Rosmarinus officinalis L.). The extracts were tested against Aspergillus candidus (Link) A. niger (van Tieghem), Penicillium sp. (Link) and Fusarium culmorum (W. G. Smith) Sacc.
Material and methods

Dried leaves of French lavender, malva, oregano, rosemary and ginger tubers and pau D’arco bark were macerated in 100 mL of sterile distilled water (sdw). The pau D’arco (bark) was put in 100 mL sdw, for 48 h. The respective quantities of plants used are indicated in Table 1. The mixtures were strained through cheesecloth so that aqueous extracts were obtained.

The extracts were submitted to two centrifugations (8600 G), for 20 and 30 minutes, respectively. After this, the extracts were strained through a 0.45 µm sieve under sterile conditions.

Different volumes of these extracts (Table 1) were added to Potato Dextrose Agar media (PDA), and the mixtures were plated on Petri dishes (20 mL each).

After solidification, three plates of each extract were inoculated by placing 0.5 cm diameter discs with fungi in the centre of each plate. They were cut from the margins of actively growing colonies of *A. candidus, A. niger, Penicillium* sp. and *F. culmorum* isolated from cereals collected in warehouses, in S. Tomé e Príncipe islands (Africa). Plates of PDA without aqueous extracts were the controls. All the plates were put inside incubators, at 28ºC, for 8 days, at the end of which, fungal colonies diameters were measured.

Results were analysed using one way ANOVA (p<0.05) (Table 2).

<table>
<thead>
<tr>
<th>Plant</th>
<th>Weight (g)</th>
<th>[ ] (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>French lavender</td>
<td>44</td>
<td>0.79 and 0.88</td>
</tr>
<tr>
<td>Ginger</td>
<td>100</td>
<td>1.0, 2.0 and 3.0</td>
</tr>
<tr>
<td>Malva</td>
<td>30</td>
<td>0.3 and 0.6</td>
</tr>
<tr>
<td>Oregano</td>
<td>25</td>
<td>0.25</td>
</tr>
<tr>
<td>Pau D’arco</td>
<td>60</td>
<td>0.6 and 1.14</td>
</tr>
<tr>
<td>Rosemary</td>
<td>50</td>
<td>0.5 and 1.0</td>
</tr>
</tbody>
</table>

Results

Results of the effect of PDA media supplemented with different aqueous extracts in colonies diameters as well as the results obtained from the statistical analysis are presented in Table 2. The value 0.50 cm is the inoculum diameter.

French lavender extract at a concentration 0.88 g/mL was the most effective, reducing the growth of *A. candidus, A. niger, Penicillium* sp. and *F. culmorum*.

For ginger and malva extracts, the most effective concentrations were the 3.0 g/mL and 0.6 g/mL respectively, totally inhibiting the growth of all the tested fungi.

In the case of oregano, the only tested concentration was 0.25 g/mL. This concentration was capable of reducing the growth of the four fungi tested but it would be necessary to complement this study with a larger number of concentrations.

The pau D’arco extract with 1.14 g/mL was the best, it caused total inhibition of *F. culmorum* and *Penicillium* sp. growth, and reduced growth of *A. candidus* and *A. niger*.

Rosemary extract was the most effective at 1.0 g/mL, which reduced *F. culmorum, Penicillium* sp. and *A. candidus* growth. However, this extract did not show any efficacy in the growth of *A. niger*.

The quantitative results presented in Table 2 were transformed into qualitative results, which are presented in Table 3.
Table 2. Results of the effect of PDA media supplemented with different aqueous extracts on colonies diameters (cm), including statistical analysis (one way ANOVA).

<table>
<thead>
<tr>
<th>Plant</th>
<th>[ ] (g/mL)</th>
<th><em>Fusarium culmorum</em> (cm)</th>
<th>F</th>
<th><em>Penicillium sp.</em> (cm)</th>
<th>F</th>
<th><em>Aspergillus candidus</em> (cm)</th>
<th>F</th>
<th><em>Aspergillus niger</em> (cm)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>French lavender</td>
<td>0.79</td>
<td>1.13</td>
<td>1.26</td>
<td>567.1**</td>
<td>2.66</td>
<td>488.8**</td>
<td>5.06</td>
<td>211.2**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.88</td>
<td>0.63</td>
<td>1.00</td>
<td></td>
<td>2.23</td>
<td>6.67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>7.63</td>
<td>3.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ginger</td>
<td>1.00</td>
<td>2.10</td>
<td>1.33</td>
<td>943.3**</td>
<td>5.40</td>
<td>730.4**</td>
<td>7.56</td>
<td>9431.1**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.43</td>
<td>1.03</td>
<td></td>
<td>4.53</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>0.50</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>7.63</td>
<td>3.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malva</td>
<td>0.30</td>
<td>2.26</td>
<td>1.03</td>
<td></td>
<td>4.0</td>
<td>759.6**</td>
<td>0.50</td>
<td>11449.8**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>0.50</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>7.63</td>
<td>3.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oregano</td>
<td>0.25</td>
<td>5.50</td>
<td>2.23</td>
<td></td>
<td>6.16</td>
<td>4.5**</td>
<td>4.06</td>
<td>1889.3**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>7.63</td>
<td>3.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pau D’arco</td>
<td>0.60</td>
<td>2.83</td>
<td>1.97</td>
<td></td>
<td>4.93</td>
<td>261.6**</td>
<td>5.17</td>
<td>697.9**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.14</td>
<td>0.50</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>7.63</td>
<td>3.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosemary</td>
<td>0.50</td>
<td>6.70</td>
<td>2.57</td>
<td></td>
<td>6.03</td>
<td>64.9**</td>
<td>8.00</td>
<td>3*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>2.83</td>
<td>1.53</td>
<td></td>
<td>5.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>7.63</td>
<td>3.36</td>
<td></td>
<td>6.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** F values are significant (p<0.05); * F values are not significant (p<0.05)
Table 3. Inhibition effect of the extracts tested.

<table>
<thead>
<tr>
<th>Plant</th>
<th>[g/mL]</th>
<th>Aspergillus candidus</th>
<th>Aspergillus niger</th>
<th>Penicillium sp.</th>
<th>Fusarium culmorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>French lavender</td>
<td>0.79</td>
<td>++*</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>0.88</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ginger</td>
<td>1.00</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Malva</td>
<td>0.30</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Oregano</td>
<td>0.25</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pau D’arco</td>
<td>0.60</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1.14</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Rosemary</td>
<td>0.50</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>+</td>
<td>–</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

*Inhibition of fungi growth (+++) = 100%; (++) = above 50%; (+) = below 50%; (-) = 0%.

Discussion and conclusions

Worldwide, post-harvest losses have been estimated up to 50%, much of this due to fungal and bacterial activity. The problem is more complicated in developing countries, where post-harvest losses are often severe due to the lack of adequate drying facilities and lack of proper handling that may include lack of advanced technologies such as refrigerated and/or controlled atmosphere storage facilities (El-Ghaouth, 1997).

The development of fungicide resistant strains of pathogens and the revocation of registration of some of the more effective fungicides have generated an interest in the development of safer alternatives to synthetic fungicides that are both effective and economically feasible (El-Ghaouth, 1997).

Natural compounds produced by the secondary metabolism of plants are potentially an important source of new types of fungicides. The knowledge about natural antifungal compounds from plants for the control of fungi in stored products, is scarce and practically non-existent.

Among the six species of plants tested for their potential use for control of stored products fungi, ginger and malva had the highest in vitro antifungicidal activities (Table 3). The high concentrations of ginger and malva extracts caused total inhibition of the four tested fungi. Malva extract was the most effective, since the same result was obtained at a lower concentration. Literature references regarding this effect of malva were not found.

For the other plants, the level of inhibition varied according to the plant species and the fungi tested, as observed also by Azzouz & Bullerman (1982), Aureli et al. (1992), and Paster et al. (1995). Penicillium sp. and F. culmorum showed a high susceptibility to the majority of high concentrations of the extracts.

References


Pruthi, J.S. 1980. Spices and condiments: chemistry, microbiology, technology. – Advances in Food Research, Suppl. 4: 463 pp.


