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Edited by
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Introduction


There was a participation of delegates from 16 countries. A total of 43 papers were presented to cover various topics of post harvest pest biology, biological and integrated pest management. This is the first meeting on the protection of stored products in Turkey at both national and international level. Although agriculture plays an important role for Turkish economy, preserving agricultural foods and feeds against harmful organisms has only in recent years started to gain attention with specific postharvest activities supported with research resources. Furthermore, the decisions of the Montreal protocol for the phase out of methyl bromide have caused serious concerns of the dried fruit processing industry in Turkey. Therefore, the results of this IOBC meeting are expected to have an important contribution to the postharvest pest control sector in Turkey.

A field trip to GABAY fig processing factory demonstrated the importance of the pest problems in the dry fruit industry. I would like to thank Mr. Menash and Haim Gabay brothers and Eng. Okşan Gülseri for their detailed explanations on the dry fig process in the Aydın region of Turkey and in their facilities. An excursion made to Ephesus permitted the participants to foster collaboration and get better acquainted of each other work.

The present IOBC Working Group on Integrated Protection of Stored Products gained its current structure due to the committed work of Dr. Cornel Adler who convened the IOBC Working Group on Integrated Protection of Stored Products since the meeting in Zurich in 1997, then in Berlin in 1999 and then in Lisbon in 2001.

I thank the local Organizing committee consisted of Prof. Dr. Neşet Kılınçer, TUBITAK, Dr. Mevlüt Emeçk and Dr. A. Gürüar Ferizli of Plant Protection Department of the Faculty of Agriculture of Ankara University and Mr. Sezmen Alper, the Secretary General of Aegean Exporters’ Union. The support provided by Prof. Dr. Nusret Aras, Rector of Ankara University, Prof. Dr. Yetkin Güngör, Dean of Faculty of Agriculture and Prof. Dr. Ali Tokgöz, vice Dean Faculty of Agriculture of the University of Ankara in the realization of this meeting is much appreciated. I would like to mention with thanks the preparations made by Mrs. Sinem Erduran of SETUR, for her help in organizing the Pine Bay, Kuşadası facilities for the IOBC 2003 meeting.

The editing of this book of proceedings was made during my Sabbatical from the Agricultural Research Organization (ARO), ISRAEL, at the Department of Plant Protection, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Centre de Cabrils, Barcelona, SPAIN. I thank Prof. Dr. Ramon Albajes of the University of Lleida, Dr. Rosa Gabarra and Dr. Jordi Riudavets of the Department of Plant Protection of IRTA, Centre de Cabrils, Barcelona, Spain, for inviting me for a sabbatical year and the Ministry of Education, Culture and Sport of Spain for providing the support (reference number SAB2002-0016) that enabled the preparation of this book of proceedings.
I would like to thank my colleagues Dr. Cornel Adler, Dr. Matthias Schöller, Dr. Mevlüt Emekçi, Dr. Ahmet Güray Ferizli, and Dr. Lise Stengård Hansen for their contribution in the editing process, and Dr. Horst Bاثon for his relentless support.

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INTEGRATED PROTECTION OF STORED PRODUCTS
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General topics
What are the implications of climate change for integrated pest management of stored grain in the UK?

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Abstract: CSL continues to develop integrated pest management (IPM) strategies to protect stored durable food crops from biodeterioration. This work draws on a wide matrix of specialist skills, recently re-organised into a large single team that encompasses research into: biology, behaviour and population dynamics of pest and beneficial invertebrates; monitoring tools for pests, other contaminants and physical parameters; the development of optimal drying and cooling strategies; the development of novel, including biological, control agents; and assessment of risk from storage pests and mycotoxins. It is vital that recommendations from this research are sustainable, in order to continue delivering long-term value to the UK industry and the consumer.

A major challenge to sustainability comes from climate change. The existence of climate change is now widely accepted throughout the international community and is seen to pose a great threat to the continued supply of food to the growing world population. A recent study calculates that in parts of the UK, temperatures may rise 5°C on average by the 2080s with major changes to precipitation, depending upon future patterns of global emissions.

This paper discusses the impact of these changes on IPM of stored grain in the UK, with a case study of the effects on cooling strategies, based on CSL aeration and UK meteorological records. It is suggested that the current strategy is robust enough to cope with current warming but that new optimum aeration strategies may be needed in the longer term. One way of extending the time available for cooling is to reduce the moisture content (MC) of stored grain and thus lengthen insect development time. Since extreme weather events will become more common, it will be important to maintain sufficient drying capacity to deal with exceptionally damp years, even if the pattern of early dry harvests were to become established.

Key words: grain storage, Integrated Pest Management, climate change, aeration, grain cooling, grain drying, mycotoxins

Introduction

Cereals and their products supply the majority of the world’s dietary needs. The UK produces in the region of 24 million tonnes per annum, with exports accounting for about 30% of this (Anon., 2003 a). Recently there have been many challenges and changes to UK agriculture, and ongoing international market reform will have implications for many years. On May 30th 2003, the UK Government’s Department for Environment, Food and Rural Affairs (Defra), made public its Science and Innovation Strategy for the next three years (Anon., 2003 b). A key area of this strategy is “Sustainable Farming and Food” that aims for Defra’s scientific activities to ensure that UK agriculture can be economically, environmentally and socially sustainable, to protect weakening farm incomes, protect biodiversity and ensure the highest levels of food safety.
UK integrated pest management of stored grain

Researchers at the Central Science Laboratory (CSL) have been focusing on developing a cost-effective, integrated pest management (IPM) strategy for the storage of durable food crops for the last 15 years. This robust strategy has been developed to reduce chemical inputs, while ensuring that UK grain is free from harmful bio-contaminants. So successful has this approach been that not only does this inform the current Defra storage research programme but it has also been adopted as best practice by the UK grain industry (Armitage and Wildey, 2003). Originally published as the result of a 3-year industry-sponsored study (Wilkin et al., 1990), this strategy has seen a number of refinements, most recently the replacement of organophosphate (OP) surface treatments with diatomaceous earths (DE) (Cook and Armitage, 2002). This evolving strategy can be summarised as –

1. Clean the empty store and monitor with traps before harvest to help minimise potential sources of infestation.
2. Treat the empty store if pests are present with an approved product to control residual pests.
3. Dry cereals to 14.5% moisture content (MC) or less at harvest, to eliminate mites and moulds, and prevent mycotoxin production.
4. Cool the grain at an airflow of 10 m³/tonne/hr to below 15°C within 2 weeks, to prevent Oryzaephilus surinamensis breeding, to below 10°C within a further 2 months, to prevent Sitophilus granarius breeding and to below 5°C by winter to prevent mites breeding and to kill insects.
5. Use automatic fan control with a differential thermostat set at 4-6°C to guarantee achievement of cooling targets and preferably a time clock to select night tariff electricity to cut costs to one-third.
6. Monitoring insect numbers using traps, to check the success of the strategy.
7. Optionally, top dress with an approved product, to kill upward-moving insects and mites that may survive at the surface. This will also prevent any mite infestations that might otherwise occur as the surface absorbs moisture in the winter.

Recent re-organisation at CSL has resulted in the creation of one large multi-disciplined storage team that reflects the integrated programme of research and will bring greater synergy to future work. This may include further optimisation of cooling, drying and trapping techniques, and other elements under development such as biological control (Cox et al., 2003), heat disinfection and controlled atmospheres (Conyers et al., 1996).

Climate change

Another key area identified by Defra is climate change. As part of the UK “Climate Impacts Programme”, a number of climate change scenarios have been modelled depending on the level of global emissions, and are referred to as “Low”, “Medium low”, “Medium high” and “High emissions” scenarios. These mathematical models are available to researchers and are intended to act as a key resource for risk assessment and adaptation strategies (Hulme et al., 2002). It is without doubt that temperature and precipitation patterns in the UK will change over the next 80 years, since much of this has already been determined by historic emissions and inertia in the climate system.

In summary, this report indicates that already there has been a global rise in temperature of 0.6°C over the last 100 years and the temperature has risen by as much as 1°C in central England. The warmest year since global instrumental records began was 1998, and the 1990s was the warmest decade on record in central England, a major cereal production area in the UK. The central England temperature data set is one of the key references used by the UK Meteorological (Met.) office, with records going back to 1659 (Manley, 1974; Parker et al.,
By the 2080s, it is anticipated that global CO₂ concentrations will have increased by 57-143% compared to 1961-90 levels. It is therefore predicted that in the UK –

- Temperatures will increase by 2-3.5°C, with a summer increase as great as 5°C in the South-East of England
- By the 2050s, seasonal patterns are likely to change; spring temperatures arriving 1-3 weeks earlier and winter occurring 1-3 weeks later
- A longer thermal growing season will be accompanied by a decline in the number of air frosts
- Changes to precipitation patterns will result in wetter winters (up to 30% increase) and drier summers (up to 50% decrease).

In addition, seasonal weather extremes are likely to become more frequent, relative humidity (RH) will decrease, reduced cloud cover will result in increased solar radiation and sea-levels will rise.

The aim of this paper is to consider what impact climate change may have on UK storage, with particular focus on the effects on drying and on cooling using ambient air, since this is the backbone on which the IPM strategy is based. References to climate change will be to the Defra study, unless otherwise stated.

Implications for the UK storage strategy of warmer autumns / milder winters

Cooling rates and targets

The model on which current cooling recommendations for low-volume aeration is based, was constructed using 3 key variables (Armitage et al., 1991): -

- Aeration rates; i.e. airflow required to cool 1 tonne of grain, measured in m³/tonne/hr
- Meteorological considerations; temperature data sets for two widely separated regions in England based on a 20 year average and in addition a hot autumn and a mild winter at each location
- Biological considerations; cooling needs to be fast enough to prevent the quickest developing insect completing its life-cycle and to a temperature low enough to prevent the most cold hardy

This model was successfully validated in a number of large-scale field trials. Therefore, depending upon when aeration starts, cooling targets can reliably be set (Table 1).

Table 1. Cooling targets for UK stores using low volume aeration at 10 (m³/tonne)/hr.

<table>
<thead>
<tr>
<th>Latest date to cool to</th>
<th>Cooling starts on 15°C</th>
<th>1 Jul</th>
<th>1 Aug</th>
<th>1 Sep</th>
<th>1 Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
<td>29 Sep</td>
<td>9 Oct</td>
<td>14 Oct</td>
<td>9 Nov</td>
<td></td>
</tr>
<tr>
<td>5°C</td>
<td>8 Dec</td>
<td>8 Dec</td>
<td>9 Dec</td>
<td>1 Jan</td>
<td></td>
</tr>
</tbody>
</table>

(After – Armitage et al., 1991)

An important factor of this research that is often overlooked by the UK industry is achievement of these targets can only be guaranteed using automatic fan control based on the
grain/ambient temperature differential; i.e. the fans are only switched on when the ambient temperature is cooler than the grain. For the UK, the ideal differential in terms of cost and efficiency is 4-6°C. Grain temperatures will therefore follow the daily minimum ambient temperature, and this can be monitored as a series of cooling ‘fronts’ passing through the grain.

It is therefore reasonable to expect that if the current climate change predictions hold true, the following could happen –

- Warmer grain on harvest may cause problems with condensation and accelerate the breeding of initial pests prior to cooling
- Delayed autumn/winter temperatures would lengthen the time required to pass each cooling front giving pests a wider window to complete life-cycles and build populations
- An increase in winter temperatures may limit the lowest temperature achievable with implications to pest survival

With the 1990s being the warmest decade on record, it may already be possible to see whether the cooling strategy has been affected. Of particular interest are the data for harvest through to early winter, which is when the majority of cooling takes place. Using the central England Met. Office data as a reference point, comparison can be made between the warmest years post-1990 and the 30 years previous (1961-90) for this period. By selecting the years that contained the warmest months from July-December, the most problematic years for cooling would have been 1994, 1995, 1999 and 2001 (Figure 1). It is significant that for August – November, the warmest months were 2-3.6°C above the 1961-90 mean, falling outside of this data range.

![Temperature Graph](source: Manley, 1974; Parker et al., 1992 – updated by the Hadley Centre for Climate Prediction and Research, Met. Office, Berkshire, UK)

To test whether our cooling strategy has already been compromised, we have looked at grain cooling records for 1999-2002 at CSL’s experimental grain store, which is located just
to the north of central England. This data has been compiled from three non-related storage projects (diatomaceous earth treatments; storage of malting barley; effect of MC on storage), which all used the same facilities. Data collection and fan control were achieved using either data loggers in conjunction with a differential thermostat or by computer monitoring system using system feedback for differential control. Ambient store records show a similar warming trend as before, although these values are within the upper limits for the central England 1961-90 average (Figure 2). The months January – March have also been included, since these mid-winter temperatures are an important factor in aiding the survival of surface insects.

![Figure 2](image-url)

**Fig. 2.** Comparison of ambient mean monthly temperatures for 1999, 2000, 2001 and 2002 at CSL grain store compared to mean 1961-90 central England temperatures (with ranges) for “cooling” period.

The experiments for which the cooling profiles have been compiled, were all undertaken in 3 m deep open topped steel bins containing 20-25 t of grain. Figure 3 compares the experimental cooling profiles with cooling targets derived from Table 1. With the UK harvest normally completed by October, the original cooling model only provided targets for aeration starting up to and including 1 October. This latter set of targets has therefore been used, since they were the closest available taking into account the later aeration start dates of the CSL experiments. The start of aeration was deliberately delayed to allow introduced populations of insects and mites to become established. For this reason, cooling started after the 15°C target date in each case. Therefore it is only the 10°C and 5°C targets that will be discussed. It is also important to note that the moisture of some of the grain was deliberately high to demonstrate the effects of MC for those experiments.

In the 1999 trial, six bins of wheat at 15.5% MC were cooled uniformly with differential fan control set at 6 °C (Cook and Armitage, 2002). Aeration started on 12 October 1999 (11 days later than target start), the 10°C front was achieved by 21 November 1999 (12 days later than target) and the final front of 5°C (± 1°C) was achieved by 29 December 1999 (on target). Therefore, taking into account the late start, cooling targets were met, despite warmer than average ambient conditions.
In the 2000 trial, six bins of malting barley of differing MC (2 x 13.5%; 3 x 15.5%; 1 x 16.5%) were again cooled using a 6°C differential (Armitage et al., 2002). Fans were automatically switched off once the grain reached 10°C to prevent over-cooling, as required for malting. Aeration started on 9 October 2000 (8 days later than target start) and the higher MC grain cooled faster than the lower MC grain, presumably due to evaporative cooling. The 10°C front passed through the bulks by 17 November 2000, 21 November 2000 and 11 December 2000 for the high, medium and low MC grain respectively. Only the high MC barley cooled on target after taking into account the delayed start to cooling, with the others taking a few weeks more. However, the cooling was still effective in terms of pest prevention as indicated by the significant decline of deliberately introduced populations of *S. granarius* and *O. surinamensis* after 5 and 9 weeks respectively in all the bins (not shown).

The 2001 trial again compared the cooling of 20 t bins of different MC grain, with wheat at either 13.5% (n=2) or 17% (n=2) (Armitage and Cook, unpublished). Aeration started on 17 October 2001 (16 days later than target start), the mean 10°C front was achieved by 16 November 2001 (7 days later than target) and the final front of 5°C (±1°C) for the slowest cooling bins was achieved by 5 January 2002 (4 days later than target). Again, the lower MC bins cooled the slowest, but taking into account the late start of aeration, efficient cooling was still achieved. As seen the previous year, there was a rapid decline in insect species (not shown).

![Fig. 3. Mean cooling profiles for 20 tonne bins during 1999, 2000 and 2001 (n=6, n=6, n=4 respectively) controlled by 6°C differential compared to UK cooling targets if aeration begins 1st October.](image-url)

This small data set suggests that despite warmer than average temperatures in recent years, differential fan control can still achieve efficient cooling. Targets were met despite the reduced number of available fan hours caused by the delayed aeration starts. However, although the strategy has proven robust so far, as the climate warms further there will come a time at which cooling by this method can no longer reach target temperatures and therefore protect the grain. Just how long it will be before this happens must be established urgently.
There is therefore a need for full and detailed impact assessments to be made using the UK climate models. It would also be of benefit to include alternative fan control methods (Noyes and Navarro, 2002) for comparison. For example the latest Australian strategy uses a predictive model that adapts to changing ambient temperatures (Darby, 2003). It may be sufficient to change one of the model’s variables, e.g. airflow rates, but in the worst case, ambient air could be supplemented with refrigeration as is sometimes used in warmer climes (Maier et al., 1989), although the latter would incur greater cost. UK specific validation of these alternatives will be essential, since it can not be assumed these approaches will be as successful under our mild and damp, maritime winter conditions.

Potential effects on invertebrate development and ecology

The pest development basis of the UK cooling model is taken from a summary of life-studies that date back to the 1940s. Most of this research consisted of experiments on ideal artificial diets and under constant laboratory conditions, since some of the commonest UK storage insects such as *O. surinamensis* (Prickett and Muggleton, 1991) do not develop on whole grains. Cosmopolitan in distribution, the UK occurrence of some of these pests can be directly correlated to a higher proportion of broken grains resulting from the introduction of modern combines in the 1960s.

Recent research with a recently collected strain has shown that when bred on the actual commodity (95% whole grain; 5% damaged) a reduction in intergranular humidity gives a much more pronounced reduction in productivity compared to artificial diets (Table 2). Since the original cooling model ignored MC, this means that current assumptions on speed of development gives a built in safety factor to the strategy and that extra time for cooling can be gained by lowering the MC.

<table>
<thead>
<tr>
<th>°C</th>
<th>Minimum development time (days)</th>
<th>% completing development (egg-adult)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Diet 70%</td>
<td>Wheat 70%</td>
</tr>
<tr>
<td>35</td>
<td>17 19</td>
<td>16 19</td>
</tr>
<tr>
<td>30</td>
<td>15 20</td>
<td>16 20</td>
</tr>
<tr>
<td>25</td>
<td>28 34</td>
<td>28 38</td>
</tr>
<tr>
<td>20</td>
<td>59 61</td>
<td>63 67</td>
</tr>
</tbody>
</table>

(Source: Fleming and Armitage, 2003a)

Review of the biology of stored product pests also suggests that an increase in UK temperatures by as much as 5°C could change the status of some species (Table 3.) For example *Rhizopertha dominica*, which causes such devastation to stored grain in the tropics and is very tolerant of a range of insecticides, has the potential to become a major pest in the UK. Species with slow rates of increase such as *Ptinus fur*, could have the opportunity to build into significant populations, that would then be capable of causing major physical damage. The metabolic rate of current major pests may also change as they adapt to climate change, potentially enhancing the threat from these too.
Table 3. Development conditions and rates of increase (per lunar month) for some UK storage insect species

<table>
<thead>
<tr>
<th>Species</th>
<th>*Minimum temperature °C</th>
<th>*Optimum temperature °C</th>
<th>*Optimum rate of increase</th>
<th>Present Status</th>
<th>Future status if 5°C rise?</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. ferrugineus</td>
<td>23</td>
<td>32-35</td>
<td>60</td>
<td>Low impact</td>
<td>High impact</td>
</tr>
<tr>
<td>R. dominica</td>
<td>23</td>
<td>32-35</td>
<td>20</td>
<td>(UK too cool)</td>
<td></td>
</tr>
<tr>
<td>O. surinamensis</td>
<td>21</td>
<td>31-34</td>
<td>50</td>
<td>High impact</td>
<td>High impact</td>
</tr>
<tr>
<td>S. granarius</td>
<td>15</td>
<td>26-30</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. tectus</td>
<td>10</td>
<td>23-25</td>
<td>4</td>
<td>Low impact</td>
<td>Medium</td>
</tr>
<tr>
<td>P. fur</td>
<td>10</td>
<td>21-25</td>
<td>2</td>
<td>(slow increase)</td>
<td>impact</td>
</tr>
</tbody>
</table>

(*Source: Howe, 1965)

Recent research has also shown some UK strains of *S. granarius* to be far more cold-hardy than previously thought (Fleming and Armitage, 2003b). This could cause additional problems for long-term storage. It is therefore apparent that UK researchers cannot rely on historic life studies and in the context of climate change, assessments need to be made in conjunction with up-to-date biological studies. Climate change could also present new opportunities. Milder and wetter winters would give biological control a greater chance of success, with warmer temperatures increasing the activity of parasitic wasps and higher humidity improving the efficacy of entomopathic fungi (Cox, pers. comm.).

**Implications for drying of warmer summers / wetter winters**

Earlier and warmer summers imply earlier harvests, dryer grain and a lesser need for grain drying equipment. This might suggest that the threat from mould contamination, particularly mycotoxin production, would be reduced. However, experience in the drier countries in mainland Europe suggests that decreased reliance on this sort of equipment actually increases the threat from Ochratoxin A (OA) production in damper years. Countries that habitually experience damp harvests, such as Scotland, UK, are usually equipped to deal with this scenario. Since climate change will increase the frequency of extreme weather events, it would be strategically important to maintain sufficient drying capacity in order to deal with the exceptionally damp years, even if the pattern of early dry harvests were to become established.

Warmer summers may also speed-up the high volume ambient-air drying process since the warmer air can be expected to remove more moisture. The target moisture could also be lowered, since the ambient humidity of the drying air might be lower than at present. As we have seen in an earlier section (see above), the drier the grain, the longer the cooling can take. However this can be countered by increased insect mortality and increased development time at lower humidities as already discussed.

Warmer summers and less cloud cover might also initiate reconsideration of the merits of solar-aided drying. At about the time of the exceptional warm and dry summer of 1976, there was considerable activity along these lines (Burrell, 1982). Approaches included using the supplemental heat from roof panels to add the required heat for ambient-air drying, rather than relying on electrical or gas power. Another approach was to mix over-dried with damp grain, to achieve the required MC for ‘safe’ storage. This latter approach could be particularly important following recent advice (Armitage and Wildey, 2003) to avoid using an ambient-air
dryer for grain much above 18%, because the process is likely to be slow enough to permit OA production. Mixing dry grain with freshly-harvested damp grain could be one way to reduce the average MC rapidly to below the threshold for OA production.

Damper weather in late autumn and early winter will seriously affect the ability of ambient-air drying systems to pass a drying front through the grain before fungal deterioration, particularly OA production, exceeds regulatory levels. Solutions for this include mixing damp and dry grain using drying aids such as grain stirrers (augers that mix dry and undried layers during the process) or re-visiting the design parameters of such systems, perhaps requiring higher airflow rates or the addition of more heat, to adequately reduce the RH of the drying air. The use of dehumidifiers or even desiccants may be justified to ensure that EU regulatory levels for OA are not exceeded. Although desiccant beds may not be economically viable at present, they may be justified if they could also be used for cooling stored grain as in sub-tropical Australia (Thorpe and Chen, 2003).

Damper winters might be expected to reduce the opportunities for cooling because of the threat of dampening. However, it is difficult to dampen grain at cooling rates and usually grain reduces in average MC by 0.5% during cooling. Nevertheless this ‘myth’ of dampening grain during cooling has been so much of a deterrent to successful cooling that it has required targeted technology transfer to address the points (Armitage and Wildey, 2003).

More seriously, damper winters might also be expected to increase the degree of moisture uptake by surface grain (Armitage and Cook, 2003). This leads to the flourishing of mite populations at the grain surface and enhances the survival there of storage insects. We have already seen that this threat has merited the addition of a pesticide top-dressing to complement the cooling of grain to ensure pest-free grain. Unfortunately the withdrawal in the UK of OP dust formulations has limited the options for such a treatment. Currently only DEs are available for such an application but resistance to the use of these in certain market sectors has limited their uptake.

**Conclusions – new challenges and new opportunities**

In addition to affecting current UK practice climate change will present new opportunities for growers. For example, longer growing seasons with reduced frosts could lead to an increase in crops such as sunflower, mustard and lupin for oilseeds and bulk production of maize and soya beans. Just as when oilseed rape entered mainstream production in the 1970s, new crop-specific storage strategies will be required. Extreme weather events, will have serious implications for global harvests, and with a growing world population, this can only lead to the stockpiling of food. Earlier this month, the main front page item in a UK broadsheet newspaper drew attention to devastating impact of this year’s (2003) heatwave on grain harvests across Europe. The need to protect the harvested commodity will be greater than ever and if droughts continue to affect harvests in Australia, America and Central Europe, the UK harvest may be increasingly important. With current social concerns it is unlikely the UK will be able to revert to reliance on chemical protectants. There is therefore an immediate need to assess the impact of climate change on our ability to protect stored grains from biodeterioration in a sustainable way. Immediate research needs can therefore be summarised –

- Assess full impact of climate change on current cooling and drying strategies, utilising Defra climate models
- UK specific adaptation studies to include alternative aeration models and alternative cooling/drying technologies
- Underpinning biological studies to predict pest and mould response to climate change
- Continued research into “non-chemical” surface treatments and bio-control
Acknowledgements

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References

Fleming, D.A. and Armitage, D.M. 2003a. Lowering the moisture content of stored grain can gain extra time for cooling to prevent infestation: studies on the development, productivity and survival at two relative humidities of two insect species on whole wheat and an


Significance of hermetic seals, controlled ventilation, and wire-mesh screens to prevent the immigration of stored product pests

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Abstract. Stored product insect immigration triggered by positive chemo-taxis towards an attractive volatile is a major cause of infestation. This paper discusses a hermetic seal and controlled aeration as methods to prevent the formation of a gradient of attractive volatiles around storage sites or food- and feed-processing plants. Furthermore, consumer packages could be protected from attack by a gas-tight seal. The minimum protection needed to avoid infestation is an insect-proof enclosure that mechanically prevents immigration. A study using wire-mesh sieves with pore-sizes between 0.05 mm and 4 mm has been carried out to determine the pore-size needed to prevent the permeation of stored product species of the genera Cryptolestes, Ephesia, Lasioderma, Oryzaephilus, Plodia, Sitophilus, Stegobium, Tribolium, Trogoderma and the common clothes moth Tineola bisselliella. Generally, beetles were found to abstain from oviposition when food substrate was not accessible, while all tested moth species laid large numbers of eggs under similar conditions. A pore size small enough to retain even the neonate larvae of stored product moths was 0.1 mm.

Key words: attractant, gradient, orientation, insect-proof, hermetic seal, aeration, insect screen.

Pest attraction by chemotaxis

Pests enter into storages and premises of food industry either with infested raw products or by immigration. Immigration is always triggered by positive chemo-taxis, i.e. the orientation towards a gradient of attractive volatiles. In stores of harvested plant products in the temperate climate of Central Europe stored product insects rarely occur in the field, and immigration from other stores is probably the most important way of infestation. Even in tropic or sub-tropic climates where some products may be infested by stored product pests in the field, immigration may be an additional source of infestation, because storage pests may be removed or killed during the processes of harvesting, threshing, drying or transportation. However, most on-farm storages today are not built to avoid attracting stored product pests. On the contrary, many silo bins made of sheets of corrugated metal are not sealed at the edge between roof and wall. Old concrete or wooden storage structures may have large openings or cracks in the barn doors that do not close properly, and spilled goods often facilitate orientation and infestation (Fig. 1).

The need for including pest prevention aspects becomes more evident considering that the European policy defines the products as food from the time of their harvest (Council directive 1993/43 EEC and EC regulation 178/2002). This implies that a Hazard Analysis of Critical Control Points (HACCP) is carried out from the first storage site onward to avoid any loss in product quality. Given the competitiveness of the world market today, product quality already plays an important role and this importance will increase in the years to come.

Insect immigration is important in the food industry, particularly when heating, milling or a mixing process kills all live individuals (e.g. after mechanical drying at high temperatures) or when the finished product becomes attractive to pest species other than those occurring in the raw ingredients (e.g. fresh dry pasta in a pasta factory becomes attractive to
Stegobium and Sitophilus spp. after extrusion and drying, while the raw product semolina was attractive to Tribolium spp.

Fig. 1. Unsealed structures and product spills facilitate the immigration of stored product pests

Physical barriers and other methods to prevent infestation

In both storage and product processing facilities a physical barrier could prevent the immigration of pests. The use of physical barriers against urban pests, agricultural pests and forest pests is discussed in Boiteau and Vernon (2001). Physical barriers were defined by Banks (1976) as a structure made up of wood, metal, plastic or any other material (including living barriers) used to obstruct or close a passage or to fence a space. The present paper intends to give a preliminary description of the significance of physical barriers in stored product protection.

Hermetic seal: a hermetic or gas-tight seal could prevent the immigration of pests because it would keep the attractive volatiles within the enclosure. However, in silo bins and warehouses, this would require controlled aeration and cooling of most stored plant products and a proper insulation to avoid condensation problems. When a finished product leaves the food industry on its way to the consumer, the quality of the package determines the level of the risk of insect immigration. A hermetically sealed package or packing under controlled atmospheres would reduce this risk.

Controlled aeration: In food processing facilities, controlled aeration close to doors and openings could prevent the build-up of a gradient of attractive volatiles. Controlled aeration may not be regarded as a physical barrier according to the definition by Banks (1976), but could nevertheless be an effective tool to avoid attracting pests from the environment.

Insect-proof barrier: If a hermetic seal or controlled aeration is not feasible, an insect-proof enclosure could be an alternative. The main difference is that the insect-proof barrier is not gastight but physically excludes insects by having sufficiently small openings and consisting of materials that prevent insect penetration. In warehouses and food processing plants, wire-mesh screens mounted on openings are often the only barrier to insect immigration. Packages need to be sealed to a degree that is insect-proof for invading pests. True
penetrators like Anobiid or Dermestid beetles, however, cannot be prevented by materials like paper, cardboard or polyethylene foils.

Test of the permeability of wire-mesh screens to stored product insects

In order to systematically study the efficacy of various screens, adult stored product insects of various species were placed on the largest sieve of a set of stainless steel wire mesh sieves with pore sizes between 4.5 and 0.05 mm (Fig. 2). Food substrate used for cultures was supplied in the dish below the sieve with the smallest pores. The results of the first set of experiments are given in Fig. 3. Because tests are still being conducted, these results constitute the preliminary data.

Fig. 2. Assembled sieves to test the pore size sufficient to exclude a stored product pest. A rubber gasket between each sieve and adhesive tape on each rim was used to prevent escape of small size insects.

Generally, beetles were found not to lay eggs when the food source was not directly accessible while the moth species tested all laid large numbers of eggs even when at a distance from the food source. This is not surprising because female moths are known to lay eggs even without food, e.g. when they are caught in a sticky trap. The pore size that is small enough to retain even the neonate larvae of stored product moths was 0.1mm. These results confirm the findings of Khan (1982) who determined the minimum pore size allowing Pyralid moth invasion by measuring the diameter of the head capsules of neonate larvae.

In conclusion, insect orientation deserves to be more seriously considered in order to prevent infestation in food and feed items and in order to maintain food quality at its optimum on the way from farm to fork.
Fig. 3. Results on the permeability of wire-mesh screens to various adult stored product insects.
In case eggs were laid, distances covered by neonate larvae are mentioned. *(T.conf.: Tribolium confusum, S.pan: Stegobium panicum, L.serr.: Lasioderma serricorne, O.sur: Oryzaephilus surinamensis, C.ferr.: Cryptolestes ferrugineus, C.pus.: Cryptolestes pusillus, T.gran.: Trogoderma granarium, P.int.: Plodia interpunctella, E.elut.: Ephestia elutella, T.biss.: Tineola bisselliella)*.

**Acknowledgements**

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**References**

Evaluation of insect contamination in food products by immunological detection of chitin

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Abstract: Immunoassay methods for detecting insects, mites, fungi and their fragments as food contaminants were tested using a polyclonal rabbit antiserum against macromolecular chitin. The specificity of the antiserum with chitin was tested using the immunofluorescence technique. Application of the ELISA technique using the homologous antigen (chitin) did not produce positive results. However, when we used heterologous antigens we were able to detect eggs, larvae and adult insects and mites by the indirect method (ELISA-I). The smallest amount of heterologous antigen that gave a positive reaction was equivalent to $3.1 \mu g$ of *Plodia interpunctella* wings in $0.01$ g of rice flour. For wheat flour, the detection sensitivity of this type of antigen decreased to $40 \mu g$ in $0.01$ g of flour, probably due to the presence of gluten that competed with the antigen during fixation to the polystyrene plates and blocking the specific union antibodies. Further research is needed in order to develop the ELISA technique for detecting pest contamination in food products by using the antiserum against chitin.

Key words: immunoassay, ELISA, detecting insects, chitin, food contamination, flour

Introduction

Detecting potential food contaminants helps to ensure the quality of both raw materials and manufactured food products and to eliminate a major problem for food producers, sanitary institutions and the food industry in general. Detecting the presence of insects, mites, fungi and their remains is an important aspect of this quality control. A number of methods have been developed for the detection and quantitative estimation of insects and mites. The usual method employed involves conducting a visual examination of the food product to determine the presence of insects or insect damage. Although this is a relatively simple method for detecting adults and certain developmental stages of insects, it does not provide accurate data on the presence of mites and insect fragments and it is unreliable for the detection of eggs, first instar larvae and hidden insect infestation. Other methods that have been used for many years include the insect fragment test, cracking flotation method, X-ray analysis, and carbon dioxide detection method. Recently an immunological method has been developed that is based on the detection of myosin, an insect muscle protein (Schatzki et al., 1993; Brader et al., 1992). This method provides precise and consistent measurements of insect contamination, it is easy and less time consuming to apply, and it can be used to assay a variety of foods products. However, since myosin degrades quickly, the test cannot be used to detect dead insects several days after death, or insect fragments in milled products (Atui et al., 2003). More recently a rapid immunoassay for the detection of storage mite pests has been developed using specific monoclonal antibodies (Dunn et al., 2003). Therefore, to expand the situations in which immunological methods are effective, it is necessary to find a molecule that is more resistant to degradation and that is present in large quantities in the bodies of insects, mites and fungi.
The cuticle – the outer covering of insects and other arthropods – includes an outer cellular matrix of lipo-protein and chitin, which is secreted and maintained by an inner cellular layer called the epidermis. Together these elements represent the largest body organ of arthropods. The cuticle is composed of a mixture of protein (50%) and chitin (20-50%), with a certain amount of lipid. Chitin is a polymer that mainly consists of the aminosugar N-acetylglucosamine, which is partially deacetylated. It is a naturally occurring polysaccharide, which usually forms a complex with other polysaccharides and with proteins. Chitin is not only the main component in the exoskeleton of insects and other arthropods, but also in the cell walls of fungi. It can also be found in crustaceans, worms, the cyst forms of several protozoan and in some algae. Chitin is not, however, expressed in plant tissues.

Chitin is strongly cross-linked and is highly resistant to chemical attack. It is insoluble in water and also in most organic solvents. It is stable in response to radiation and very resistant to denaturation and bacterial degradation. However, some fungi and bacteria produce chitinase and are able to use it to satisfy both their carbon and nitrogen requirements (Aumen, 1980; Cody et al., 1990; Godoy et al., 1983). Degradation involves the breaking of chemical bonds between the sugar residues that comprise the chitin molecule. Due to the high resistance of chitin, this molecule will remain present in insect-infested food products even after industrial processes like milling.

The present study is one of the first attempts to investigate the potential use of immunoassay methods based on the detection of chitin for measuring low levels of insect, mite and fungus contamination in food products and, particularly in processed products like flour.

Material and methods

Antigen preparation, production of antiserum, purification, and conjugation of immunoglobulin (IgG)

Chitin from crab shells was triturated using a politron homogenizer (Kinematic) and suspended in phosphate-buffered saline (PBS) at a concentration of 1mg/mL. Polyclonal antiserum was raised in California x New Zealand rabbits following an injection schedule similar to that used by Walker et al. (1990). Rabbits were intramuscularly injected with the antigen at 2-week intervals over a period of 78 days and at a concentration of 1mg/mL, emulsified with an equal volume of incomplete Freund adjuvant. Bleeding started one week after the final injection. IgG were purified of antiserum by ammonium sulphate precipitation, dialysis and fractionation in a DEAE Sephacel column (Cambra et al., 1983). An aliquot of the purified immunoglobulin was conjugated with alkaline phosphatase using glutaraldehyde (Clark and Adams, 1977). Dilutions of IgG and conjugates were kept at -20ºC with equal volumes of glycerol. Pre-immunized serum was used as a negative control in the different serological assays.

Indirect Immunofluorescence (IFI) Microscopy

The specificity of the antiserum was tested using the immunofluorescence technique (Coleno, 1968). Samples previously dried and fixed with methanol on slides were incubated for 30 minutes at room temperature, first using specific antiserum and then – after washing – with goat-anti-rabbit IgG conjugated with fluorescein isothiocyanate. Slides were observed microscopically (× 400 magnification) using UV light.

ELISA

Tests were basically performed by applying the methodology described by Clark and Adams (1977). The different ELISA procedures were carried out on polystyrene plates using 100µl per well for each of the solutions: antigen, antiserum, purified IgG or conjugated with alkaline
phosphatase, with incubation periods of 3 h at 37º C and 16 h at 4º C. Plates were washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween-20 between each step. An enzymatic reaction was developed by adding 150µl per well of the substrate solution (1 mg/mL of p-nitrophenyl phosphate in diethanolamine buffer, pH=9.8) and incubating at room temperature. Colour was measured at 405 nm in a Multiskan ELISA reader and four wells were used for each condition assayed. Samples were deemed positive for chitin detection when absorbance values were twice as high as the average absorbance values of the controls and higher than 0.2. Bovine serum albumin (BSA) was used as a negative control.

**Indirect ELISA.** Different samples were tested by ELISA: chitin from crab shells served as homologous antigen and *Tribolium confusum* (Coleoptera: Tenebrionidae) eggs, the eggs, wings and legs of *Plodia interpunctella* (Lepidoptera: Pyralidae), mites (*Tyrophagus putrescentiae*) and conidia from the fungus *Penicillium notatum* were used as heterologous antigen. Detected chitin triturated in different buffers with a wide PH range (2-10) was compared. We also compared fixation to the plates of chitin triturated in PBS pH=7.2 and sonicated for 5 min. The supernatant of aliquots previously centrifuged at 2000g for 10 min. or samples dried at 60º C were also compared. The use of non-fat milk powder at 2% in PBS after antigen fixation as blocking agent was tested. After antigen fixation, the specific chitin antiserum diluted in PBS was incubated as an intermediate antibody before the addition of goat anti-rabbit conjugated.

Polystyrene plates MaxiSorp, Covalink-NH and PoliSorp (NUNC) alone and MaxiSorp plates precoated with synthetic polynucleotides with different binding capacities such as poly-L-lysine, poly-L-proline, poly-L-glicine and Poly-glutamic acid were compared for fixing the chitin.

Three different molecules (saccharose, cellulose and polyvinyl) with similar chemical structures to chitin were tested in order to discard cross-reactions.

The fixation of antigens on nitrocellulose, charge-modified nylon and polyvinylidene difluoride membranes by a dot spot procedure based on the method described by Hawkes et al. (1982) were all tested as previously described employing indirect ELISA but also using nitro blue tetrazolium as a reaction substrate.

The antiserum titre was determined in twofold dilutions of up to 1:204.800 using both the homologous and the heterologous antigen.

**Direct ELISA.** Plates were coated according to the optimal conditions established for indirect ELISA and the specific antibodies conjugated were tested at different dilutions in PBS.

**Double antibodies sandwich ELISA.** Plates coated with 1µg/mL of purified IgG in carbonate buffer (CB) were incubated in PBS with several dilutions of antigen. Plates were then incubated using the antibody conjugated diluted in PBS.

**Sensitivity detection.** Sensitivity detection of chitin was determined by testing heterologous antigens mixed with rice or wheat flour. Half dilutions of *P. interpunctella* wings in either rice or wheat flour were analyzed by ELISA-I using the antiserum diluted at 1:1000 in PBS. Samples were prepared by homogenizing 0.01 g of rice flour in 1 mL of PBS.

**Results and discussion**

**Indirect Immunofluorescence (IFI) Microscopy**

The antiserum showed a high specificity to chitin. At a dilution of the antiserum of 1/100, the conjugated antibody emitted fluorescence with the homologous antigen, chitin, at a concentration of 10 pg/mL but not with the negative controls. Probing for chitin components in the exoskeleton of insects using the antiserum resulted in staining of the outlines of wing
veins. Walker et al. (1991) also found good results using the same serologic technique when applying polyclonal antiserum against chitin to detect *Pneumocystis carinii*, an opportunistic pathogen present in the human lung tissue of more than 60% of patients with acquired immunodeficiency syndrome.

**ELISA**

Acceptable results were only obtained with the ELISA-I method by introducing suitable modifications and using heterologous antigens. However, slight or null fixation to the polystyrene plates was obtained in the detection of homologous antigen chitin. These results were probably due to two main reasons: either the molecules of chitin did not become fixed to the plates or they were lost during the washing processes because of their high molecular weight (105 - 106 Daltons).

The usual method for fixation to polystyrene plates using direct incubation in Carbonate Buffer (CB) (PH = 9.6) gave null OD values when both the homologous antigen and the heterologous antigen were tested (Table 1 and 2). Negative results were also obtained for the rest of the PH assessed with the homologous antigen (Tables 1 and 3) and for HCL and NaOH with the heterologous antigen (Tables 2 and 3). The only significant differences were obtained for PBS (PH = 7.2) between the heterologous antigen and the negative controls (BSA) (Tables 2 and 3).

<table>
<thead>
<tr>
<th>Table 1. Effect of coating conditions at different pHs on ELISA-I values (Absorbance at 405 nm) (Means ± SEM, n = 4) obtained using chitin (homologous antigen) in comparison with BSA.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin</td>
</tr>
<tr>
<td>Carbonate Buffer (pH = 9.6)</td>
</tr>
<tr>
<td>Phosphate-buffered Saline (pH = 7.2)</td>
</tr>
<tr>
<td>Acetate Buffer (pH = 4.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Effect of coating conditions at different pHs on ELISA-I values (Absorbance at 405 nm) (Means ± SEM, n = 4) obtained using parts of insects (heterologous antigen) in comparison with BSA.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wings and legs of <em>P. interpunctella</em></td>
</tr>
<tr>
<td>Carbonate Buffer (pH = 9.6)</td>
</tr>
<tr>
<td>Phosphate-buffered Saline (pH = 7.2)</td>
</tr>
<tr>
<td>Acetate Buffer (pH = 4.5)</td>
</tr>
</tbody>
</table>

Preparation of the samples prior to coating, involving the sonication, centrifugation or drying described in the material and methods section, did not improve test results. Even after preparation of the sample, chitin molecules probably remained too large to fix on the polystyrene plates.

Neither polystyrene plates with an affinity for hydrophobic molecules (PoliSorp, NUNC) nor polystyrene plates incorporating free radicals in order to fix molecules through covalent unions (Covalink-NH, NUNC) improved previous results (Table 4). None of the tests
performed with pre-coated plates resulted in the fixation of homologous or heterologous antigens. Synthetic polymers are normally effective with IgG and proteins, but in our case, as chitin is a molecule with a low polarity and high molecular weight, fixation was not improved.

Table 3. Effect of coating conditions at different pHs on ELISA-I values (Absorbance at 405 nm) (Means ± SEM, n = 4) obtained using several heterologous antigens in comparison with BSA.

<table>
<thead>
<tr>
<th></th>
<th>Phosphate-buffered Saline (pH = 7.2)</th>
<th>HCl (pH = 2)</th>
<th>NaOH (pH = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. confusum eggs</td>
<td>0.932 ± 0.005</td>
<td>0.287 ± 0.017</td>
<td>0.112 ± 0.036</td>
</tr>
<tr>
<td>P. interpunctella wings</td>
<td>0.831 ± 0.009</td>
<td>0.746 ± 0.008</td>
<td>0.146 ± 0.012</td>
</tr>
<tr>
<td>Penicillium Conidia</td>
<td>0.361 ± 0.013</td>
<td>0.306 ± 0.010</td>
<td>0.100 ± 0.003</td>
</tr>
<tr>
<td>Chitin</td>
<td>0.192 ± 0.016</td>
<td>0.104 ± 0.004</td>
<td>0.103 ± 0.013</td>
</tr>
<tr>
<td>BSA</td>
<td>0.155 ± 0.004</td>
<td>0.039 ± 0.004</td>
<td>0.117 ± 0.047</td>
</tr>
</tbody>
</table>

Table 4. Effect of coating conditions at different pHs and different polystyrene plates on ELISA-I values (Absorbance at 405 nm) (Means ± SEM, n = 4) obtained using chitin (homologous antigen) and mites (heterologous antigen).

<table>
<thead>
<tr>
<th></th>
<th>Carbonate Buffer</th>
<th>Phosphate-buffered Saline</th>
<th>Phosphate-buffered Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxi Sorp</td>
<td>0.042 ± 0.005</td>
<td>0.032 ± 0.003</td>
<td>0.605 ± 0.032</td>
</tr>
<tr>
<td>Poly Link</td>
<td>0.026 ± 0.008</td>
<td>0.009 ± 0.004</td>
<td>0.434 ± 0.091</td>
</tr>
<tr>
<td>Poly Sorp</td>
<td>0.010 ± 0.001</td>
<td>0.003 ± 0.002</td>
<td>0.245 ± 0.011</td>
</tr>
</tbody>
</table>

As shown in Fig. 1, there was no cross-reaction between the antiserum and other linear polymers with structures similar to chitin. Unspecific reactions aside, absorbance values were low for all the molecules tested except heterologous antigen.

In the case of the dot spot procedure, none of the procedures involving membranes were adequate. Fixation of homologous and heterologous antigens to membranes was low due to losses during the washing process. Moreover, unspecific reactions made it effectively impossible to distinguish between the antigens and the controls.

The chitin antiserum did not provide satisfactory results for the detection of chitin by either the EILSA-D or ELISA-DAS techniques using conjugates prepared with alkaline phosphatase. Absorbance values obtained with both homologous and heterologous antigens were low irrespective of the coating concentrations and conjugation dilutions used. Several experiments proved that IgG and enzyme conjugation occurred and also that immunoglobulin was successfully purified. The lack of reaction with ELISA-D and ELISA-DAS must therefore be imputed to either a loss of capacity to recognize the antigen by the conjugated IgG - as evidenced in other studies (Clark, 1981) - or to spatial structure impediments in the antigen–antibody union. Similar detection difficulties were recorded with an antiserum obtained for detecting dsRNA using as antigen a synthetic polymeric molecule of polyinosinic – polycitidilic acid (Poly I-Poly C) (Aramburu et al. 1991).
Fig. 1. Absorbance at 405 nm (Means ± SEM, n = 4) on ELISA-I obtained using *P. interpunctella* wings in comparison with different linear polymers and BSA.

From our results, it can be concluded that the best antigen fixation was obtained using polystyrene plates (MaxiSorp) to which heterologous antigen in PBS had been added and which had been incubated for 4h at 37ºC. However, in some experiments absorbance values were high in controls with BSA due to unspecific fixations. When we added non-fat milk powder at 2%, OD values in the controls were low. With this blocking agent, background was avoided without interfering with the antigen–antibody reaction.

Since one of the main inconveniences in detecting chitin by ELISA was the difficulty in fixing this kind of antigen to the polystyrene plates, we had to use one heterologous antigen to determine the antiserum titre (Fig. 2). The method used was indirect ELISA on polystyrene plates coated with *P. interpunctella* wings at a concentration of 0.4 mg (2 wings)/mL and with 2% powdered skimmed milk as a blocking agent. Antiserum was used at successive half dilutions in PBS between 1/100 and 1/204800. The antiserum obtained made it possible to discriminate between heterologous antigen and BSA at a dilution of 1/204800 (Fig 2).

**Sensitivity of detection by ELISA-I of heterologous antigen in flour.** The minimum concentration of the antigen detected in rice flour was a dilution of 1/64 of a *P. interpunctella* wing in 0.01 g of flour (Fig. 3): this corresponds to a weight of 3.1 µg of antigen. In wheat flour sensitivity was lower. The minimum concentration of the antigen detected was 0.2 *P. interpunctella* wings in 0.01 g of flour (Fig. 4): this corresponds to a weight of 40 µg of antigen. If we consider a wing as an insect fragment and we express data in terms of 50 g of flour, the antigen detected would respectively represent approximately 78 and 1000 fragments of insects for rice and wheat flour. The detection value in the case of rice wheat is similar to the level considered the maximum defect action level (DAL) by the U.S. Food and Drug Administration (1998) and established as a standard quality control for flour destined for human consumption (75 or more insect fragments per 50 g). The sensitivity in the case of wheat flour was much lower than the DAL. Differences between rice and wheat flour could be due to the presence of gluten, a protein found in the endosperm of the starch grain, which developed in the wheat flour when mixed in watery solutions. Gluten behaves as a viscous-elastic solid. During the ELISA analysis, the starch dissolves and remains as a mixture that coagulates in presence of heat and remains fixed in the wells, thereby making it difficult for the union of the antigen and the plaques. Gluten is not present in rice flour.
Fig. 2. Chitin antiserum titre determined by ELISA-I using *P. interpunctella* wings as heterologous antigen in comparison with BSA used as a control.

Fig. 3. Antigen detection threshold with chitin antiserum by ELISA-I. The plates were coated with half-dilutions of *P. interpunctella* wings in Phosphate-buffered Saline (PBS), and 0.01 g of rice flour and the antiserum were added at a 1:1000 dilution. The ELISA values were measured at Absorbance 405nm (Means ± SEM, n = 4).
Fig. 4. Antigen detection threshold with chitin antiserum by ELISA-I. The plates were coated with half-dilutions of *P. interpunctella* wings in PBS, and 0.01 g of wheat flour and the antiserum were added at a 1:1000 dilution. The ELISA values were measured at Absorbance 405 nm (Means ± SEM, n = 4).

In conclusion, the detection of chitin to determine the contamination of food products by insects and other organisms could prove a useful method on account of its simplicity and speed with respect to other traditional methods of insect detection. With only one antiserum, it may be possible to detect all of the developmental stages of insects, whether dead or alive, and even insect fragments. Despite these advantages, using chitin as an antigen is not easy on account of its chemical characteristics. Chitin is a low reactive molecule, which makes it difficult to use in a high number of serologic technical modalities. Consequently, the method used in this work needs to be optimized in order to reduce the unspecific and crossed reactions and to improve the fixation of the antigen to the polystyrene plates.

**Acknowledgements**

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**References**


Coleno, A. 1968: Utilisation de la technique d’immunofluorescence pour le dépistage de 


The use of IPM for cockroach (Dictyoptera: Blattaria) control

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Abstract: Cockroaches (Dictyoptera: Blattaria) are generally not considered storage pests, but they may live in stores, or visit them, particularly in tropical regions, where storage conditions frequently correspond to cockroach preferences for warm and moist conditions. Cockroaches can transmit diseases, cause allergies and produce persistent offensive odours that may affect food’s flavour, and so their contact with food must be prevented. Until recently, cockroach control relied almost exclusively on repeated applications of synthetic insecticides. However, owing to the development of resistance to insecticides; the repellent effect that most have towards cockroaches; and the increasing public concern towards environment, control with insecticides has been improved, and other control methods have been developed and implemented. IPM is considered the solution for cockroach control. Available data in literature about cockroach control management concern different urban structures, but not specifically the storage environment. For this review, monitoring and control methods that can be used in stores were selected, as well as data that should be further explored for cockroach control in such environment.

Keywords: cockroach, Blattaria, monitoring, IPM

Introduction

Cockroaches are one of the most important groups of urban and industrial pests. Most cockroaches are tropical and subtropical in origin (Truman, 1961), where they are predominant, although they are found all over the world (Claude & Frazier, 1969; Davies & Richards, 1977). In general, cockroaches prefer a moist environment with a relatively high degree of warmth (Truman, 1961), but there are species with a considerable resistance to cold (Beatson & Dripps, 1972; Short & Edwards, 1991).

Of the 3500 described cockroach species, approximately 50 did establish a close relationship with man (Cornwell, 1968; Koehler et al., 1987; Brenner, 1991; Pomes et al., 1998). These species are classified as peridomestic and domestic. The survival of peridomestic species (e.g: Periplaneta sp. and Blatta sp.) is not obligatorily linked to humans (Brenner, 1991): most live outside, feeding on decaying vegetation, and when food or weather conditions become unfavourable they move indoors (Piper & Frankie, 1978; Swadener, 1995a). On the other hand, domestic species (e.g: Blatella germanica and Supella longipalpa) are strictly domestic, intimately associated with humans and their structures (Brenner, 1991).

Cockroaches can be a medical problem, owing to the numerous pathogenic organisms they carry (Jung & Schaefer, 1952; Roth & Willis, 1960; Rueger & Olsen, 1969; Frishman & Alcamo, 1977; Cornwell & Mendes, 1981, Rivault et al., 1993, 1994; Vythilingam et al., 1997). They are intermediate hosts of pathogens that cause meningitis, hepatitis, typhoid fever, scarlet fever, yellow fever, Asiatic cholera, pneumonia, tetanus, tuberculosis, poliomyelitis, leprosy, Coxsackie, mouse encephalitis, cerebrospinal fever, diphtheria, undulant fever and anthrax (Graffar & Mertens, 1950; Claude & Frazier, 1969; Frishman & Alcamo, 1977; Vythilingam et al., 1997).
These insects are also responsible for allergic reactions (Richman et al., 1984). The allergens, present in their bodies, act in humans when ingested in contaminated food, inhaled (insect materials incorporated into house dust), or by dermal contact with contaminated surfaces (Bennett, 1977; Brenner, 1991). According to Squillace et al. (1997), Leung (1998) and Pomes et al. (1998), cockroach sensitisation constitutes a significant risk factor for asthma development or aggravation.

Cockroaches are also responsible for the production of persistent offensive odours, which may affect food’s flavour and may cause nausea or vertigo (Hafez & Afifi, 1956; Piper & Frankie, 1978; Piper et al., 1983; Brenner, 1991).

**Monitoring and control methods**

*Monitoring*

Outdoors, cockroaches hide and breed on the ground, under dead leaves, debris and rocks; in piles of logs and firewood; under the bark of dead or alive trees; in abandoned nests of ants, termites, birds, rats, and burrows of other rodents; and in trash accumulation of any kind (Roth & Willis, 1960; Fleet et al., 1978; Brenner, 1991). Inside structures, they are mainly found in dark places, in almost any crack or crevice, showing a thigmotactic behaviour (Truman, 1961; Bennett, 1977; Metcalf & Metcalf, 1993; Ebeling, 1991).

Cockroaches can be monitored by visual counting (with or without a flushing agent) or by trapping (jar traps and sticky traps). Monitoring with sticky traps is the most used procedure (Moore & Granovsky, 1983; Ballard & Gold, 1984; Ebeling, 1991; Swadener, 1995a; Kaakeh & Bennett, 1997; Stejskal, 1998). Traps can be baited with food attractants or pheromones. Many lures are already commercially available, provided by trap manufacturers.

Aggregation pheromones (APs) are more useful than sex pheromones, because they are not strictly species specific: individuals of one species are more responsive to their own pheromones, but some degree of interspecific aggregation was demonstrated (Piper, 1977). Additionally, APs attract all developmental stages and both sexes. However, pheromones are not good lures for *B. germanica*, because in this species they are not sensed as volatiles; they are detected by touch (Ritter & Persoons, 1975; Ebeling, 1991; Tsai & Lee, 1997). On the other hand, Liang et al. (1998) demonstrated that the synthetic sex pheromone of *Supella longipalpa* female was highly attractive to males, and could provide a powerful lure.

Traps must be placed where roaches are likely to be found, i.e. near principal outdoor and indoor harboursages, and where cockroaches normally travel, i.e. along the intersection of two surfaces (e.g. the juncture between the floor or ceiling and the wall, or the back edge of a shelf) and along the perimeter of objects rather than out in the open (Ebeling, 1991; Swadener, 1995a). Traps placed just a few inches away from an intersection of surfaces catch significantly fewer roaches than traps placed in corners or along walls (Reierson & Rust, 1977).

Traps location must be changed periodically, in order to renew cockroach exploratory activity (Reierson & Rust, 1984). Hunger greatly increases their exploratory activity (Ballard et al., 1984), and so alternative food sources must be removed from the area being monitored, or the access to them must be made difficult. In areas where water is scarce, cockroaches are more attracted to traps containing water, in sponge-stoppered vials (Ross, 1981).

The greatest number of cockroaches is caught during the first 24 h of trap exposure, and trap catches decrease in frequency, as time progresses (Moore & Granovsky, 1983). If no individuals are caught in a trap after two nights, its position should be changed (Piper et al., 1983). Traps are usually checked, at least, weekly (Rust & Reierson, 1977a, 1978; Ballard & Gold, 1984; Coler et al., 1984; Benson & Zungoli, 1989; Ebeling, 1991), however, if many
individuals are caught, attention must be made to the decreasing availability of sticky surface inside traps.

**Control with insecticides**

Target treatments use less amount of insecticide than general ones, being also safer to humans and beneficials. They also allow the reduction of treated areas, what is specially important where food is stored. In the “Crack-and-Crevice Technique”, small amounts of insecticide (liquid, dust or gel) are introduced into cracks and crevices. Several toxic baits are already commercially available: they combine a selective pesticide with one or more attractants and, eventually, feeding stimulants, and are formulated as gels, pellets, pastes, dusts, aerosolised or in containerised form (Barson & Lole, 1981; Ebeling, 1991; Huang, 1995; Smith et al., 1996, 1998; Robinson, 1996; Fischer et al., 1997; Krzeminska et al., 1997; Suiter, 1997). Cockroaches are more likely to feed on the bait if there is little alternative food available, which means that sanitation is essential before baiting commences, and that stored food products must not be accessible.

The target places are aggregation sites, travel routes, potential entry areas inside buildings and around them. Perimeter insecticide treatments constitute a barrier between the structure and peridomestic cockroach populations, but their killing effect often diminishes very quickly owing to the insecticide degradation (Piper & Frankie, 1978).

An efficient horizontal insecticide transmission was observed on *B. germanica* and *Blatta orientalis*, owing to the consumption of the faeces and bodies of adults primary killed by a toxicant; the insecticidal activity of the cadavers was maintained during several weeks (He et al., 1998; Patourel, 2000). Small nymphs, especially 1st instars, which forage infrequently, and therefore have less direct contact with insecticides, are particularly affected (Kopanic & Schal, 1997). Nymphs eat significantly more adult faeces when food is far from the shelter (Kopanic & Schal, 1999), and so, once again, sanitation is essential.

The repellency that many organophosphates, carbamates and pyrethroids cause in cockroaches is well known (Goodhue & Linnard, 1952; Ebeling et al., 1966, 1967; Bennett & Wright, 1971; Moore, 1972; Reieerson & Rust, 1977; Rust & Reieerson, 1977a, b; Kaakeh & Bennett, 1997). However, cockroaches can be encouraged to remain on treated surfaces for longer periods by the addition of, for example, aggregation pheromones to conventional insecticides (Rust & Reierson, 1977a,b; Glaser, 1980; Miller et al., 1997).

Owing to cockroach resistance to pesticides, especially in *B. germanica*, these products should be used as an alternative to, or integrated with, other control agents. Kaakeh et al. (1997) observed that *B. germanica* individuals were killed significantly faster by imidaclorpid, after a topical application of spore suspensions of *Metarhizium anisopliae* (Deuteromycetes). Some plant extracts must be carefully used: a formulation from Chinese medicine did not reveal toxic effects against pyrethroid-resistant strains, because a pyrethroid was detected in its composition (Komagata & Motoyama, 1998).

Boric acid, diatomaceous earth and silica gel are considered efficient in cockroach control, and are commercially available. Boric acid, a stomach poison, has low toxicity to cockroaches, but also has little or no repellency to them (Ebeling et al., 1966, 1967; Barson, 1982; Barson & Lole, 1981). Cockroaches avoid thick deposits and so light dustings, barely visible, are better than heavy dustings (Swadener, 1995b).

**Other chemicals for cockroach control**

Repellents can be used to protect some areas from cockroaches or to direct their movements into chosen places. Repellent materials may be applied as package treatments: Mallis et al. (1961) treated beer cartons with commercial repellents and found more than 90% repellency
for 5 weeks and 64% repellency after 5 months. These products can also be applied on the floor and walls around the areas where products are stored.

Many of the most effective repellents to *B. germanica* were found among the simple alkylcarboxamides that can induce 100% repellency, during 10 or more days (McGovern & Burden, 1985). Methyl neodecanamide, already incorporated in commercial cleaning products, is a highly effective repellent to *B. germanica* (Kinscherf *et al*., 1996) and *P. americana* (Ho & Goh, 1996). Some major constituents of essential oils, safrole and isosafrole, are repellents to *P. americana* nymphs (Ngoh *et al*., 1998) and several plant extracts also showed repellency to cockroaches, as for example *Curcuma longa*, *Artemisia tridentata*, *Juniperus virginiana* and *Citrus* sp. (Zingiberaceae, Asteraceae, Capparaceae and Rutaceae, respectively) (Appel & Mack, 1989; Yadav *et al*., 1994; Ahmad *et al*., 1995; Martin & Guldan, 1998; Liao, 1999).

It was also observed that the regurgitate produced by *Litoprosopus futilis* (Lepidoptera: Noctuidae) larvae, when disturbed (Smedley *et al*., 1993), and the leaf extracts of *Dicerandra frutescens* (Labiatae) (Eisner *et al*., 1990) have an irritating effect on *P. americana*.

Commercial neem-based products have a fago-inhibitor effect in cockroaches, causing nymphal growth retardation and death, and reduction of adult reproduction, with the possibility of population long-term control by permanent baiting with baits containing these products (Adler & Uebel, 1987; Richter *et al*., 1997). Extracts of *Ajuga reptans* (Labiatae) have also a phagodeterrent effect (Richter & Birkenbeil, 1987).

Lufenuron, an Insect Growth Regulator, prevents the hatch of oothecae in *B. germanica* and acts as a disrupter of moultng in nymphs. Populations of this species declined 3 to 4 months after the start of bait or spray treatment, and were completely eradicated after 12 months (Mosson *et al*., 1995). Jensen & Schenker (1999) observed that populations of *B. germanica* were reduced by more than 95%, within 4 to 6 weeks after treatment. However, for *B. orientalis*, treatments were much less effective, requiring higher doses. Recently, a chitinase inhibitor (argifin) was isolated from the fungi *Gliocladium* sp. (Deuteromycetes) which arrests the moult of cockroach larvae (Omura *et al*., 2000).

Artificial diet containing the plant hormone 22S, 23S-homobrassinolide, or extracts of *Brassica napus* L. (Cruciferae) show a moult retarding effect in *P. americana* (Richter *et al*., 1987). It was also observed that steroid alkaloid conessine, brassinosteroids and extracts of different species of Rutaceae interfere with cockroach moult regulation (Richter *et al*., 1997). Treatments with hydroprene, a Juvenile Hormone Analogue (JHA) resulted in abnormal non-reproductive cockroach adults (Ebeling, 1991). The combination of conventional insecticides and pyriproxyfen (another JHA) resulted in a decreased number of individuals and in an increased percentage of sterile adults in a *B. germanica* population, (Scharf *et al*., 1997; Yu *et al*., 1999). Additionally, treatments with hydroprene against *P. australasiae* not only were efficient, but also had no negative impact on biological control programs that were being conducted against other pests (Bell *et al*., 1999).

Some of the active ingredients mentioned here are already available on the market. Apart from the sexual and aggregation pheromones already mentioned, others may be explored in the future for cockroach control. Krivosheina & Shatov (1995) observed the existence of trail pheromones in *B. orientalis* and *P. americana*, and Miller *et al.* (1996) observed a trail following behaviour in *B. germanica*. Farine *et al.* (1997) demonstrated the emission of an alarm pheromone in the cockroach *Eurycotis floridana* (Walker).

**Habitat modification**

Cockroach reservoir populations in the vicinity of storage structures should be greatly suppressed or eliminated, and so all materials that constitute hiding and breeding places
around buildings should be kept at distances up to 30 m from them, or stored in durable, securely covered containers (Fleet et al., 1978; Piper & Frankie, 1978; Piper et al., 1983). Additionally, outdoor populations must be discouraged from entering urban structures by sealing cracks in foundations and exterior walls; sealing or caulking around windows, doors, ventilation units, pipes and other openings. This can be supplemented by placing screens behind any heating or cooling vents and caulking around the edges of the screen; and by inspecting incoming merchandise (Piper & Frankie, 1978; Piper et al., 1983; Swadener, 1995a).

Within structures, conditions must be made unsuitable for cockroach survival and so, all hiding areas, food and water sources must be eliminated. Harbourages where cockroaches hide and breed (mainly cracks and crevices) must be eliminated (with caulk and paint) or possibly modified, so that they do not constitute protective places or are more accessible for pest control (Piper & Frankie, 1978; Piper et al., 1983; Swadener, 1995a). Poor hygiene encourages cockroach presence (Rivault & Cloarec, 1996), and makes control difficult, because food resources compete with trap lures and insecticidal baits. Additionally, hunger increases exploratory activity (Ballard et al., 1984), increasing the probability of trap catch or contact with insecticides. Water leaks should be fixed, and adequate drainage and ventilation provided (Swadener, 1995a). According to the World Food Program (1983), cockroaches will attack almost any kind of food stored in damp and unhygienic conditions. The implementation of grid like shelving may be advantageous (Metcalf & Metcalf, 1993) because it hinders cockroach movement. Clutter accumulations should be eliminated because they provide undisturbed shelters free of insecticide residues (Rivault & Cloarec, 1996). Any extra materials that must remain inside storage structures should be confined to containers with tight lids.

**Physical control strategies**

An air flow directed into cockroach harbourages significantly repelled all stages of *B. germanica* (air velocities equal or superior to 2.0 m/s) and of *P. americana* and *P. fuliginosa* (equal or superior to 4.0 m/s) from those areas (Appel et al., 1997; Oswalt et al., 1997a; Appel & Smith, 1999). This method also desiccates cockroaches (Oswalt et al., 1997b), which become more sensitive to toxicants and are more likely to consume water based insecticidal baits (Appel, 1992; Appel & Benson, 1995).

Vacuuming can be useful for removing cockroaches, oothecae and debris out of harbourages (Kaakeh & Bennett, 1997; Valles, 1997). Zeichner et al. (1998) used heat (46°C during 45 minutes) to control *B. germanica*, and cockroaches were vacuumed up when they emerged from harbourages. Steam cleaning or carbon dioxide fumigation are also useful in killing cockroaches (Swadener, 1995b). Ballard et al. (1984) observed that ultrasounds increased significantly *B. germanica* population activity, and electronic devices that emit ultrasounds, acting as cockroach repellents, are already available on the market.

**Mass capture**

Traps alone, do not reduce cockroach populations to acceptable levels (Ballard & Gold, 1984; Benson & Zungoli, 1989); they must be integrated with other control methods (Barak et al., 1977, Piper et al., 1983; Lukawa & Manokore, 1997). According to Moore & Granovsky (1983), *B. orientalis* appears to be controlled more easily by sticky traps than other species, because of its high likelihood of being caught (it has a sluggish behaviour), long life cycle and relatively low reproductive potential. Traps have the advantage of catching oothecae together with adults, while residual insecticides frequently leave them untouched inside the harbourages (Barak et al., 1977).
**Biological control**

A wide diversity of cockroach natural enemies exists (Roth & Willis, 1960; Suto et al., 1979; Archbold et al., 1986; Lipa et al., 1991; Koehler et al., 1992; Elzinga, 1994; Palacios & Jimenez, 1997, Zukowski et al., 1998; Libersat & Moore, 2000). Conservation of natural enemies can be achieved by switching from residual sprays to baits whenever possible.

Oothecal parasitoids (Hymenoptera) that have wide distribution, and the most potential for controlling cockroach populations, include *Tetrastichus hagenowii* (Eulophidae), *Comperia merceti* (Encyrtidae), *Evania appendigaster* (Evaniidae) and *Prosevania punctata* (Evaniidae) (Fleet et al., 1975; Piper et al., 1978; Coler et al., 1984; Hagenbuch et al., 1988). Good results were obtained with *C. merceti* (Coler et al., 1984) and *T. hagenowii* (Piper & Frankie, 1978). Some parasitoids are already available commercially (Swadener, personal communication).

The nematodes *Steinernema feltiae*, *S. carpocapsae* and *S. scapterisci* demonstrated potential to control cockroaches (Locatelli & Parleaz, 1987; Koehler et al., 1992; Grewal et al., 1993; Mathur et al., 1996). Nematodes can be delivered to cockroaches in bait stations. In the group of species most closely associated with man, *B. germanica* and *S. longipalpa* are the most susceptible to infection (Koehler et al., 1992).

Fungi can be ideal for cockroach biocontrol because the habitat of these insects promotes initial fungal infection and its subsequent spread (Mohan et al., 1999). *Metarhizium anisopliae* is already used as a biological control agent of cockroaches, in a bait station, available in the market. Cockroaches expose themselves to the pathogens as they crawl through the station and afterwards they transmit the disease to others. This fungus appears not to infect warm-blooded animals, fish, or bees (Swadener, 1995b). Good results were also obtained with *Beauveria bassiana* (Ascomycota) (Nong et al., 1996; Mohan et al., 1999). These last authors suggest the use of spore formulations in food baits. Pachamuthu et al. (1999) observed that insecticides affected negatively the growth and sporulation of a *M. anisopliae* strain. Attention must be also made to fungal strains since there are differences in their pathogenicity (Nong et al., 1996; Zukowski & Bajan, 1996; Zukowski et al., 1998).

In the opinion of Suiter (1997), due to the harshness, ecological instability and physical impediments associated with the indoor and outdoor environments where cockroaches are found, releases of biological control agents will necessarily be periodic and inundative.

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**References**


Ebeling, W., Reierson, D.A. & Wagner, R.E., 1967. Influence of repellency on the efficacy of blatticides. II. Laboratory experiments with German cockroaches. – J. Econ. Entomol. 60(5), 1375-1390.


Kaakeh, W., Reid, B.L., Bohnert, T.J. & Bennett, G.W., 1997. Toxicity of imidacloprid in German cockroach (Dictyoptera: Blattellidae) and the synergisme between imidacloprid and *Metarhizium anisopliae* (Imperfect fungi: Hyphomycetes). – J. Econ. Entomol. 90 (2): 473-482.


Action thresholds and statistical quality control to manage food industry pests

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Abstract: It is proposed to implement the framework of Statistical Quality Control (SQC) to manage pest-problems in the food industry and stored-product environment by using control charts: (i) non-dynamic control charts and (ii) dynamic control charts. The main goal of quality control is to find an “average” pest infestation level - so called “Central Line“ (CL) - that is related to the instant quality of pest control. Then the variability of pest infestation above upper control limits (UCL) and CL is gradually decreased via improvement of the quality and targeting of pest control.

Keywords: quality control, stored product pests, action threshold

Introduction

Agricultural pest control IPM theory claims that the “action threshold” (AT) or economic threshold (ET) is lower than the “economic injury level“ (EIL) (Fig. 1A) (Stejskal 2001, Stejskal and Lukáš 2003) Stored-product, urban and food-industry pests are tolerated at the zero or near-zero population level. When responsive pest control strategies are used instead of preventive ones, inherently AT or ET should be non-zero and higher than EIL (Fig. 1B) (Stejskal, 2003). This indicates that the traditional agricultural concept is not always an optimal decision-making tool for urban and food industry environment. Therefore, in this work, it is proposed to implement the framework of Statistical Quality Control (SQC) to manage pest-problems in the food industry and stored-product environment.

Materials and methods

The data were taken from monitoring *Ephestia kuehniella* in a small flour mill in the Czech Republic (South Bohemia ) (Stejskal and Lukáš, 2002). We used pheromone traps (Ekovet) located at 10 places inside the mill (Lukáš and Stejskal 2003). For quality control analysis specialized software (QC-Expert 2.5) was used.

Results

The evaluation of variability of pest control process at various sites of the flour mill was done by X-bar control chart. Fig. 2 shows the level of a pest-control process (estimated as No. of pests per pheromone trap) at various sites in the flour mill. A center line (CL) of the control chart is drawn to represent the average value of the quality characteristic when the process; i.e. „pest control process“ in this case is stable. Two lines are drawn on the control chart to represent the upper (UCL) and lower control limits (LCL). Points not lying on the CL are called defects or non-conforming cases.
Fig. 1. Economic Injury Level (EIL) and Action Threshold (AT) for urban and stored-product pests

A - Traditional agricultural model (Stern et al., 1959) of Economic Injury Level (EIL) and Action Threshold (AT) or Economic Threshold (ET).

B - Responsive strategy of the control of urban pests implies that for EIL = 0 the AT or ET is inherently higher than EIL or Aesthetic Injury Level (AIL). Arrows indicate pesticide treatment: S-1 eradication possible; S-2 total eradication impossible due to technical or resistance constraints.

Fig. 2. Statistical quality control: The evaluation of variability of pest control process at various sites of the flour mill by X-bar control chart

```
Number of Groups = 10
Target = 55.2
Lower Limit = 42.0926777
Upper Limit = 68.3073223
Number beyond limits = 1
Number violating runs = 0
```

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**Diagram Notes:**
- **Group Summary Statistics**
- **Point „out of control“**
- **Site (=Group)**
- **Group Summary Statistics**
- **Number of Groups = 10**
- **Target = 55.2**
- **Number beyond limits = 1**
- **Number violating runs = 0**

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*Fig. 2.* Statistical quality control: The evaluation of variability of pest control process at various sites of the flour mill by X-bar control chart.
Discussion

It is proposed to implement the framework of Statistical Quality Control (SQC) to manage pest-problems in the food industry and stored-product environment by using control charts: (i) non-dynamic control charts and (ii) dynamic control charts. The main goal of quality control is to find “average” pest infestation level – so called “Central Line” (CL) – that is related to the instant quality of pest control. Then the variability of pest infestation above UCL and CL is gradually decreased via improvement of the quality and targeting of pest control.

Conclusions

SQC control charts can help to identify the sites where the pest control process is “out of control”.

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References

Lukáš, J. and Stejskal, V. 2003: Computer-based image analysis to estimate the area of sticky trap occupied or contaminated by pests. – Plant Protection Science, 39, 2: 52-60.


Pest biology, faunistics, storage technology and losses
Bruchids (Coleoptera: Bruchidae) on peas (Pisum sativum L.): species, geographical distribution and effect on host varieties

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Abstract: A total of 32 samples of stored threshed dried peas (Pisum sativum L.) infested by insects were collected in different areas of Portugal. Bruchids (Coleoptera: Bruchidae) were the most encountered species with a ratio of 96%. Bruchus pisorum (L.) was the dominant species found in all the 32 samples with a ratio of 93%. Other species also detected in 6 samples from distinct geographic origins were Bruchus rufimanus Boh., Bruchus tristiculus Fahr., Acanthoscelides obtectus (Say), Spermophagus sericeus (Geofigr.) and Zabrotes subfasciatus (Boh.). While only B. pisorum was found to emerge from the pea samples (incubated at 27°C and 70% RH), the other species were detected, loose, on peas. The mean number of attacked seeds in 6 varieties of peas was compared and significant differences were found.

Key-words: bruchids, peas, Bruchus pisorum, Pisum sativum

Introduction

Grain legume (Leguminosae) seeds are very rich in proteins, and constitute one of the major protein sources for man and other animals. Peas (Pisum sativum L.), in addition to human consumption, have also been used in the production of food for aquaculture (Cruz et al., 2001), aviculture (Wiryawan & Dingle, 1999) and for pig breeding (Brand et al., 2000). Recently, it was discovered that pea seeds have a repellent action against pests of stored cereals (Fields et al., 2001) and an anti-fungicidal effect on Aspergillus niger van Tiegh. (Almeida et al., 2000).

One of the main threats in relation to the production and storage of peas is the presence of bruchids (Coleoptera: Bruchidae), which have grain legumes as preferred hosts (Regato, 1961; Southgate, 1979). According to literature, Bruchus pisorum (L.) is the most frequent and abundant bruchid species in peas (Parisi & Govoni, 1998; Girsch et al., 1999; Quisenberry et al., 2000; Hardie & Clement, 2001), as indicated by the common names: “pea weevil” or “pea beetle”.

A survey of bruchids on peas was conducted in Portugal in order to define which species are present in the country. The number of attacked seeds in different pea varieties was also evaluated, in order to detect resistance/susceptibility to bruchid attack.

Materials and methods

Survey on Bruchids

Samples of stored threshed dried peas with symptoms of insect infestation were collected in different regions of Portugal, in October and November of 2001 and 2002, a total of 32 samples in seven districts (Fig. 1).
The weight of the samples was relatively low (a mean of 100 g), because they were collected mostly at small producers’ farms, where small amounts of seeds are stored for the next season. In addition to the samples of peas, insects found loose on the seeds were also collected.

In the laboratory, peas were cleaned from insects and incubated at approximately 27° C and 70% RH, during 6 weeks. Emerged insects were collected. Dead bruchids were dislodged from the seeds, by putting them inside water during 24 hours, followed by mechanical destruction of the seeds. Bruchids were identified (Ramos, 1977; Weidner & Rack, 1984).

Fig. 1. Map of Portugal showing the seven sampling locations

Bruchid attack on pea varieties
Six varieties of dried peas were used in the test, namely: Ucero, GP 3916, Ucieza, Frilene, Solara and Luna.

The different varieties had been cultivated in a randomized complete block design (3 blocks) and, at harvest, in each block, the dried peas of each variety were stored in separate bags. In the laboratory, from each bag, a 250 g sample was collected in a total of 18 samples.

In each sample, seeds presenting symptoms of bruchid attack (windows or emergence holes) were counted. A mean of 1380 seeds per sample was analysed.

The number of attacked seeds was compared in two groups of varieties: (1) Ucero, GP 3916 and Ucieza; (2) Frilene, Solara and Luna. The reason for this procedure was the temporal distance in the analysis of group 1 and 2 (4 months). Data were transformed by the square root and were analysed for a significance level (α) of 0.05. ANOVA was followed by the Tukey test (adapted to block design) for the multiple comparisons of means.

Insects found loose inside the bags were also collected and identified.

Results

Survey on bruchids
In the 32 samples collected, 826 insects were identified, and 96% of them were bruchids. Other insect species not belonging to Bruchidae (4% of the identified insects) were found only in 3 samples.
Regarding bruchids, 93% of the individuals were *Bruchus pisorum*, found in all 32 samples. The other species detected were: *B. rufimanus* Boh., *B. tristiculus* Fahr., *Acanthoscelides obtectus* (Say), *Spermophagus sericeus* (Geoffr.) and *Zabrotes subfasciatus* (Boh.), found in 6 samples, from different geographic origins.

Only *B. pisorum* was found in incubated samples. The other species were found loose on stored peas.

**Bruchid attack on pea varieties**

All the insects collected were bruchids, and 94% of them were *B. pisorum*. The percentage of attacked seeds varied between 7 and 12%, depending on the variety with a mean of 9.2%.

In the comparison of the mean number of attacked seeds, in group 1, there were no significant differences between Ucero and Ucieza and between Ucieza and GP 3916, and, in group 2, significant differences were observed between the varieties tested (Table 1).

Table 1. Mean number of attacked seeds in each variety.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Variety</th>
<th>Ucero</th>
<th>Ucieza</th>
<th>GP3916</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>86.0 a</td>
<td>155.7 ab</td>
<td>182.3 b</td>
</tr>
<tr>
<td>Group 2</td>
<td>Solara</td>
<td>66.7</td>
<td>Frilene</td>
<td>Luna</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>117.3</td>
<td>163.7</td>
</tr>
</tbody>
</table>

Error mean squares, for the transformed data: 1.80 (group 1), 0.41 (group 2). Means followed by the same letter are not significantly different (α=0.05).

**Discussion and conclusions**

It is concluded that bruchids constitute the main insect pest threat to stored peas, with *B. pisorum* as the most frequent and abundant species. This situation is the same throughout Portugal. Thus, any pest management strategy to protect stored peas must have this species as the main target.

Infestation levels found on the pea samples of different varieties were relatively low, however the seed market demands a zero infestation level. To accomplish this level, the most used control method is pesticide application. In Portugal, the registered pesticides for stored peas are malathion and methyl bromide. For a reduction of pesticide application, others must be considered, at least, to be used as a complement.

Currently, in relation to pea protection, the most used alternative to pesticides is the utilization of resistant varieties. It is known that the colour, shape and size of pods and seeds; the thickness and texture of the tegument; and the chemical composition of those elements affect the oviposition of adults and penetration of young larvae (Parr *et al.*, 1998; Bhattacharya & Banerjee, 2001). Alfa-amylase inhibitors in seeds are also capable of retarding larval development and leading to their death, by causing difficulties in the use of starch in the seeds as an energetic source by the larvae (Morton *et al.*, 2000; Franco *et al.*, 2002).

First attempts in order to detect the sources of resistance to bruchids in pea varieties were conducted, using the mean number of attacked seeds as an evaluation parameter. Significant differences were detected. Research will be continued, for an in-depth evaluation. Other parameters are being evaluated, namely the weight and the germination capacity of attacked and non-attacked seeds.
Other control methods are also under consideration to be used in storage conditions, in Portugal: apart from the survey of bruchid parasitoids that is being conducted, an evaluation of plant extracts, oils and powders to control *B. pisorum* is being planned.

**References**


Survey of storage moths in “Moscatel Málaga” variety raisins

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Abstract: This study consists of a survey of insect species that infest and cause damage in raisins of the variety “Moscatel Málaga”. Five pyralid species, Ephestia calidella, E. cautella, E. elutella, E. figulilella and Plodia interpunctella and a gallerid Corcyra cephalonica were detected. The most abundant species were found to be P. interpunctella and C. cephalonica followed by E. elutella.

Key words: raisins, Pyralid moths, Gallerid moth, Corcyra cephalonica

Introduction

The production of raisins of the variety “Moscatel Málaga” is limited to the Axarquía region in the Málaga province of southern Spain. This production is a handmade process that begins in the field, as early as the end of August, in proximity to the vineyards, where the grapes are naturally dried, and then the raisins are cut. By the end of September, the farmers begin to send the raisins to the warehouses for the quality selection and conditioning before dispatching to the market.

During this process, the raisins are exposed to infestation by insects. This work is a co-operative study between Empresa Pública para el Desarrollo Agrario y Pesquero de Andalucía, Citagro and Universidad de Córdoba to determine the insect species that can infest and cause damage to the raisins in Southern Spain.

Material and methods

Traps baited with Plodia interpunctella and Ephestia cautella pheromones were placed in the field and the warehouses. Raisin samples were taken to determine larval infestation. Moth adults were identified by morphological characteristics and when necessary the male genitalia were analysed. For the identification of larvae, we used morphological characteristics (Carter, 1984; Tremblay, 1986) and then the species were confirmed after adult emergence.

Results and discussion

Individuals of five pyralid species, Ephestia calidella, E. cautella, E. elutella, E. figulilella and Plodia interpunctella, known as pests of dry fruits (Balachowsky, 1972; Tremblay, 1986), were caught in traps in the study areas. The most abundant species was E. elutella in all areas, together with E. figulilella in the field and P. interpunctella at the warehouse during conditioning and before shipment to the market. No E. cautella larvae were found in neither raisin samples from the field nor from warehouses, but the larvae of the other species as well as those of Corcyra cephalonica appeared, although differences in quantity were observed.
Nevertheless, since the infestation begins in the field, during the natural drying process, it would be interesting to study the seasonal abundance of each of the species (Athanassiou and Eliopoulos, 2003; Buchelos, et al, 2003; Trematerra, 1983; 2002). From our data the most important Lepidoptera species infesting raisins of the variety “Moscatel Málaga” are *P. interpunctella* and *C. cephalonica* followed by *E. elutella*. To the best of our knowledge, this is the first record of *Corcyra cephalonica* in Spain.

**References**


The response of different wheat varieties to angoumois grain moth, *Sitotroga cerealella* (Oliv.)

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**Abstract:** The relative susceptibility of six commercial varieties of wheat viz. Auqab-2000, Kohinoor-83, Iqbal-2000, MH-97, Chenab-2000 and Inqlab-91 to infestation by *Sitotroga cerealella* (Oliv.) was studied under laboratory conditions following completely randomized design. The data were recorded on the basis of moth emergence, moisture content, weight loss, developmental period, percent damage, and chemical characters of the grain. The variety MH-97 was found to be relatively least susceptible to the attack of *Sitotroga cerealella* (Oliv.) followed by Kohinoor-83. The variety Iqbal-2000 was relatively highly susceptible to the insect followed by Inqlab-91, Chenab-2000 and Auqab-2000. The results further revealed that none of the varieties was completely immune to the attack of *Sitotroga cerealella* (Oliv.). Positive and significant co-relation was found between total number of moths emerged and percent damage, weight loss, moisture contents, protein and carbohydrates. Positive and highly significant co-relation was found between percent damage and weight loss. Negative and non-significant co-relation was found between number of moths emerged and fat percentage. The loss of weight is also negatively co-related with the carbohydrate content of grain. To sum up, the comparative susceptibility of these varieties was found to be in the following order: MH-97 < Kohinoor-83 < Chenab-2000 < Auqab-2000 < Inqlab-91 < Iqbal-2000.

**Key words:** Wheat, varietal response, Angoumois grain moth, *Sitotroga cerealella*

**Introduction**

Besides coleopterous insect pests, the Angoumois grain moth, *Sitotroga cerealella* (Oliv.) is a serious pest of cereal grains (Ayerney, 1982; Khattak et al., 1996). It is cosmopolitan in distribution. The economic losses caused by this insect have been reported to range from 13.1 to 24.0 % (Gerbierge and Goldhein, 1957 and Moore et al., 1966).

Depending upon the physico-chemical characters, cereal varieties vary in susceptibility to *Sitotroga cerealella* (Oliv.) as reported by workers like Pandey et al. (1980), Khattak and Shafique (1981, 1986), Rodriguez and Pla (1983), Hameed et al. (1984), Shazali (1987), Ratnasudhakar (1989), Tirmzy et al. (1989), Gillani and Irshad (1990), Iqbal and Irshad (1993) and Hameed and Khattak (1997). The resistant varieties have been observed to affect the biology of insects. The shape and size of grain also protein, carbohydrate and fat content of the grain, presence of enzyme inhibitors and antioxidant have been reported to be the most important factors in terms of susceptibility to stored grain insects (Pandey et al., 1980, Khattak and Shafique, 1981, Rubbi and Begum, 1986, Dhaliwal et al., 1989, Riaz et al., 1992, Charjan et al., 1994, Almedia and Murta, 1995, Khattak et al., 1996 and Ram and Singh, 1996). In Pakistan, serious attention has not yet been given to the biochemical aspect of cereal resistance to *S. cerealella*. 

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**Materials and methods**

Studies on relative resistance of six wheat varieties viz. Kohinoor-83, Inqlab-91, MH-97, Iqbal-2000, Auqab-2000 and Chenab-2000 were carried out in the Grain Storage Laboratory of University of Agriculture, Faisalabad (Pakistan). Grains of the test varieties were conditioned for two weeks before experimentation. The moisture content and grain size were determined before treatment. Freshly laid moth eggs were counted with the help of a binocular microscope and pieces of paper carrying 100 eggs each were placed in each of the 200 g samples of all the test varieties placed in glass jars and covered with a muslin cloth. Treatments were replicated four times. The jars were kept at 28±2 °C and 65±5% R.H. Moth emergence started 22 days after the introduction of eggs and continued up to 35 days. The development period was recorded on the basis of 1st adult emergence. The experiment was continued up to 70 days when the F2 (second generation) moth emergence was completed. Moths of first (F1) and F2 generations were counted. Population built up, percent damage and weight loss were calculated according to the following formula given by Khattak et al. (1996). Protein, fat, crude fiber and moisture contents were determined according to A.O.A.C. (1984).

\[
\text{% damage} = \frac{\text{wt. of control sample} - \text{wt. of sound grain in the sample}}{\text{wt. of control sample}} \times 100
\]

\[
\text{% loss} = \frac{\text{wt. of control sample} - \text{wt. of (sound + damaged) grain in the test sample}}{\text{wt. of control sample}} \times 100
\]

**Results and discussion**

None of the tested varieties was found to be completely immune to the infestation of *S. cerealella*. Similar results were reported by Pandey *et al.* (1980), Khattak and Shafique (1981), Ratnasudhakar (1989), Tirmzy *et al.* (1989) and Khattak *et al.* (1996). Iqbal-2000 was found to be highly susceptible (24.85% grain damage and 19.75% weight loss) and was significantly different from all the other test varieties. Kohinoor-83 and Chenab-2000 did not differ significantly from each other. Variety MH-97 showed minimum percent grain damage (7.28%) and weight loss (5.81%). This was followed by Kohinoor-83 (10.80% and 8.25%) which was significantly less susceptible than the remaining varieties Iqbal-2000, Inqlab-91, Chenab-2000 and Auqab-2000 with 24.85, 21.42, 18.06 and 13.90 percent damage respectively were significantly different from one another. With respect to percentage weight loss Iqbal-2000 (19.75) and Inqlab-91 (15.81) showed higher weight loss whereas, MH-97 showed the minimum percent weight loss (5.81%). Auqab-2000 (9.85%) and Chenab-2000 (10.09%) did not differ significantly from each other.

On the basis of grain size, the data showed (Table-1) that the varieties Auqab-2000 and Inqlab-91 with large grain size (1243 and 1211 number of grains/50 grams respectively) were found to be significantly more susceptible to *Sitotroga cerealella* than the varieties MH-97 and Kohinoor-83 with smaller grain size (1649 and 1313 number of grains/50 grams respectively). It appears that variation in the susceptibility is most probably due to the physical nature of the grains. These results are in conformity with those of Khattak and Shafique (1981).

Data given in Table-2 revealed that the moisture content of the grain is positively and significantly correlated with the percent damage, weight loss and total number of moths emerged. These findings are comparable with those of Prakash *et al.* (1979), and Hameed *et
Table 1. Data regarding varietal resistance / susceptibility of wheat varieties against *Sitotroga Cerealella* (Oliv.).

<table>
<thead>
<tr>
<th>Varieties</th>
<th>% Moisture content</th>
<th>Population build up</th>
<th>Grain size</th>
<th>% Grain damage</th>
<th>% Weight loss</th>
<th>% Protein content</th>
<th>% Fat content</th>
<th>% Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auqab-2000</td>
<td>10.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>266.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1243&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.90&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.55&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kohinoor –83</td>
<td>9.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>214.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1313&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.84&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>10.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>469.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1715&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.92&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MH-97</td>
<td>9.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141.8&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1649&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.287&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.84&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chenab-2000</td>
<td>10.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>350.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1268&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>10.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>420.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1211&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 2. Correlation matrix between different parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture contents</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of moth emerged (35 D)</td>
<td>0.2550</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of moth emerged (70 D)</td>
<td>0.4018</td>
<td>0.7009**</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. of moth emerged</td>
<td>0.4003</td>
<td>0.7174**</td>
<td>0.9997**</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain size</td>
<td>-0.1724</td>
<td>-0.4627*</td>
<td>-0.7798**</td>
<td>-0.7768**</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental period</td>
<td>0.2607</td>
<td>-0.0226</td>
<td>0.0965</td>
<td>0.0924</td>
<td>-0.1455</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent damage</td>
<td>0.4183*</td>
<td>0.6968**</td>
<td>0.9961**</td>
<td>0.9957**</td>
<td>-0.7668**</td>
<td>0.0931</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent weight loss</td>
<td>0.3721</td>
<td>0.5064*</td>
<td>0.5546**</td>
<td>0.5574**</td>
<td>-0.5964**</td>
<td>-0.0235</td>
<td>0.5861**</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>0.6926**</td>
<td>0.3513</td>
<td>0.4834*</td>
<td>0.4836*</td>
<td>-0.0382</td>
<td>-0.0916</td>
<td>-0.5101**</td>
<td>0.4268*</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>0.1929</td>
<td>-0.3518</td>
<td>-0.2949</td>
<td>-0.2989</td>
<td>0.3441</td>
<td>0.0262</td>
<td>-0.3080</td>
<td>-0.3434</td>
<td>0.1746</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>0.5523**</td>
<td>0.2908</td>
<td>0.5299**</td>
<td>0.5275</td>
<td>-0.0478</td>
<td>0.0208</td>
<td>0.5229**</td>
<td>0.0823</td>
<td>0.7452**</td>
<td>0.2472</td>
<td>1.0000</td>
</tr>
</tbody>
</table>
al. (1984) who reported that the moisture contents had significant effect on the development of stored grain insects. Our findings are, however, not in line with those of Rodriguez and Pla (1983) and Almeida and Murta (1995) who found a negative correlation in between the R. H. and the daily number of eggs laid by a female and at the same time the grains was also significantly decreased by the infestation of S. cerealella. There is positive correlation between the total number of moths emerged, weight loss and percentage damage and these results are similar to the findings of Gillani and Irshad (1990).

Loss of protein, fat and carbohydrate contents is also an indication of susceptibility in addition to the main factors like weight loss, percent damage etc.

In the present study Iqbal-2000 was found to be highly susceptible having protein contents higher than that of the least susceptible variety MH-97. A positive correlation between the weight loss and protein contents of the grain (value 0.4268) has been observed. The results of present study are thus not in the line of Ragumoorthy and Gunathilagaraj (1988) who reported that resistant varieties had higher protein contents.

An inverse correlation was observed between numbers of moths emerged or weight loss and the fat content of grains. Our results support results of Hameed et al. (1984) who reported a negative correlation between weight loss and the carbohydrate content of grains. In summary the comparative susceptibility of the tested varieties was found to be in the following order:


References


Monitoring and spatial distribution of insect pests infesting a paddy rice storage facility

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Abstract: This paper describes the phenology and spatial distribution of the insect fauna collected in a paddy rice storage facility over two years, with emphasis on the most abundant pests. The experiment, using 19 food-bait traps, was realized in the county of Massaranduba, State of Santa Catarina, Brazil. During the whole survey, Sitophilus oryzae (L.) was by far the most abundant species in the storage facility; other primary pests, such as Rhyzopertha dominica (F.), or secondary pests, such as Cryptolestes ferrugineus (Steph.) and Oryzaephilus surinamesis (L.), also were collected in remarkable numbers. In general, the species showed a variable distribution and, depending on pest and year, all parts of the facility appeared infested. In fact, pest populations were present both in the processing and silos area, at least in one of the two years survey. Variations between year I and II were analyzed, showing a strong decrease of total population numbers in year II, depending on species considered.

Key words: Spatio-temporal analysis, insect pests, rice storage

Introduction

Existing or potential new technologies for detecting the presence of insects and estimating insect population levels include pheromone traps, sampling devices, acoustic sampling methods and chemical tests which detect live or dead insects through the presence of enzymes. Computer-assisted decision support systems have also been developed which estimate insect population growth and spatial distribution of insects as a function of environmental factors (Hagstrum & Flinn, 1996; Trematerra, 2002).

The introduction of spatial analysis in applied entomology opened new possibilities to study and manage the spatial distribution of stored-product pests (Arbogast et al., 1998; Brenner et al., 1998). In the analysis of data, algorithms as variograms are used to estimate a population density at locations not sampled and the so-obtained spatial distributions are represented graphically by means of interpolated maps. This analysis can provide crucial information for improving monitoring and precision targeting control methods and has recently been used in flour-mills, feed-mills, warehouses and commodity facilities against several moth and beetle pests (Arbogast & Mankin, 1999; Arbogast et al., 2000a-b, 2002a-b; Campbell et al., 2002; Trematerra & Sciarretta, 2002 and 2004; Trematerra et al., 2004).

Our research had the objective to describe the phenology and spatial distribution of main pests trapped in a paddy-rice storage facility over two years, with major emphasis on spatio-temporal dynamics of the most abundant species, focusing on the effectiveness of the cleaning and other IPM measures adopted during the monitoring period.
Materials and methods

Study area
The experiment was carried out in a paddy rice storage facility in the County of Massaranduba, State of Santa Catarina, Brazil, from November 1997 to October 1999. The structure consists of 10 metal silos and a processing area, formed by several sheds, connected to one another (Fig. 1). Silos are grouped in two areas and each group is connected to a single bucket elevator: silos A-D are located in the north-western corner and silos E-J are positioned in the southern side of the facility; both silos receive husked rice from different farms. The processing area is located in the eastern side of the facility close to the silos and consists of several rooms.

Traps and data collection
Nineteen food-baited cage traps were used during the monitoring period. The bait was a 30 gr mixture of 1 part of wheat germ, 2 parts of broken corn kernels, 2 parts of whole corn kernels and 2 parts of rice kernels, previously sifted to remove foreign matters and frozen to eliminate possible insect infestation (Paula et al., 2002). The food mixture was placed at the bottom of the cage (10x20x30 cm) and removed every fifteen days to count the insect capture. Traps were placed in the beginning of November 1997 and checks were conducted every fortnight until the end of October 1998 (year I survey); after a one-month stop, monitoring began again until the end of October 1999 (year II survey).

Spatial analysis
Spatial analysis was carried out using Surfer version 8.02 (Golden software, Golden, Colorado, USA) with \( x \) and \( y \) representing the coordinates of the trap position in the building (expressed in meters), and \( z \) the trap catch counts. By interpolating \( z \) values, surfer produces a dense grid of values. The interpolation algorithm used was linear kriging with zero nugget. The interpolation grid obtained is used to produce a contour map, which shows the configuration of the surface by means of isolines representing equal \( z \)-values. A base map showing the plan of the paddy rice storage facility, with the same coordinate system, was placed on top of the contour map. A normalized \( z \) variable is obtained by converting annual sum of trap catches in catch probability by means of a derived indicator, following Brenner et al. (1998). This procedure enables us to focus 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 annual counts. An indicator score of “1” was given to all traps with catches that exceeded a critical proportion, that we set at around 85% (cumulative frequency distribution); a score of “0” was given to the remaining traps. The interpolation of scores yields a contour map with isolines ranging from 0 to 1.

Results
A total of 45,612 insects were captured in the traps, belonging to the orders Dermaptera, Hemiptera, Lepidoptera, Coleoptera and Hymenoptera. The Coleoptera species prevailed, with: *Sitophilus oryzae* L. (72.33%), *Cryptolestes ferrugineus* (Steph.) (8.93%), *Rhyzopertha dominica* (F.) (8.62%), *Oryzaephilus surinamensis* (L.) (2.34%), *Tribolium castaneum* (Herbst) (1.30%), and *Gnatocerus cornutus* (F.) (0.93%); species in other coleopteran families and of other orders represented 5.55%.

During the first year 28,542 specimens were captured. The by far most abundant species was *S. oryzae* (20,124 specimens), followed by *R. dominica* (3,403), *C. ferrugineus* (1,813), *Carpophilus* spp. (1,034), *O. surinamensis* (1,007), *Ephestia* spp. (388), *T. castaneum* (275),
and *G. cornutus* (86). During the second year, 17,070 insects were collected. The most abundant species was again *S. oryzae* (12,866 specimens), followed by *C. ferrugineus* (2,262), *R. dominica* (528), *Carpophilus* spp. (416), *G. cornutus* (338), *T. castaneum* (317), *Ephestia* spp. (77), and *O. surinamensis* (62).

**TRAP SITE**

1 - Entrance  
2 - Grain pit-conveyor belt  
3 - Large water tank 1  
4 - Large water tank 2  
5 - De-hulling place 1  
6 - De-hulling place 2  
7 - Rice grading room  
8 - Firewood deposit  
9 - Oven 1  
10 - Oven 2  
11 - Rice husks storage barn  
12 - Roof of the office  
13 - Pre-cleaning machine  
14 - Bucket elevator-load/unload  
15 - Dryers  
16 - Bucket elevator-load/unload  
17 - Oven 3  
18 - Silo J  
19 - Inside the office

Fig. 1. Plan of the paddy rice storage facility. Capital letters indicate silos site; *x*,*y* axis are expressed in meters.

![Plan of the paddy rice storage facility](image)

Fig. 2. Temporal dynamic pattern of captures of *Carpophilus* spp., *C. ferrugineus*, *O. surinamensis*, *R. dominica* and *S. oryzae*.

**Spatial distribution of main pests**

For both years, indicator variables were calculated to the catch data of the species mentioned above.

Spatial distribution of these pests for year I is depicted in Fig. 3. *Carpophilus* specimens were found next to the silos A-D, around the pre-cleaning machine, in the rice husks storage
room and the oven 1. *C. ferrugineus* infestations were located mainly around silos I-J and the oven 3. Other hot spots were the grain pit with the conveyor belt and the dryers. *Ephestia* adults were caught near the silos A-D and in an area comprising entrance, grain pit with the conveyor belt, office, water tanks 1 and 2 and de-hulling place 1. *G. cornutus* populations were distributed in the zone from silos E-J to the entrance, including the grain pit with the conveyor belt and the rice husks storage room. Other hot spots were around the de-hulling place 1, the pre-cleaning machine, the dryers and in the rice grading room. *O. surinamensis* presence was restricted to the silo J and to a secondary focus on the roof of the office. *R. dominica* had three main infestation foci: the first one from the silos I-J to the entrance in the processing area, the second one from the grain pit with the conveyor belt to the de-hulling places 1 and 2, the third one in the zone of silos A-D. *S. oryzae* occurred widespread from the silo zone to the pre-cleaning and dryers area. Other more limited infestation foci were localized in the grain pit with the conveyor belt area, around the water tanks and in the de-hulling place 2. *T. castaneum* had its maximum of infestation near silo J, near the grain pit with the conveyor belt, around the oven 1, in the de-huling place and in the zone of pre-cleaning machine, dryers and firewood deposit.

Spatial distribution of the eight pests for the year II is reported in Fig. 3. *Carpophilus* catches were concentrated near the oven 1 and around the pre-cleaning machine and the dryers. In the case of *C. ferrugineus*, infestation was in the whole silos area, reaching the entry zone of the processing area and the de-hulling place 1. Specimens of *Ephestia* were found near the silos E-H, around the bucket elevator, the entrance, the grain pit with the conveyor belt, the water tanks 1 and 2, the rice grading room and the pre-cleaning machine. *G. cornutus* presence was confined to the silo J and to the de-hulling place 1, the pre-cleaning machine and dryers; a weak focus was observable at the roof of the office, as well. *O. surinamensis* was present around silo J and in bounded spots in the following zones: entrance, grain pit with the conveyor belt, oven 1, water tank 2, de-hulling place 2. *R. dominica* distribution presented several defined hot spots: silo J, grain pit with conveyor belt, water tank 1, de-hulling place 2, rice grading room and firewood deposit. As in the previous year, *S. oryzae* infestation was widespread in the zone of silos. Other limited foci were located around the water tanks, in the de-hulling place 2, in an area covering the grain pit with the conveyor belt, the rice husks storage room and the oven 1. *T. castaneum* adults were located near the silos E-J, near the entrance, around the grain pit with the conveyor belt and in the zone of pre-cleaning machine, dryers and firewood deposit.

**Spatio-temporal dynamics of main pests**

When monthly trap catches were high enough, the monthly sums of trap catches were calculated and used as the z variable to compare the spatio-temporal dynamics. Monthly temporal contour maps were produced for *Carpophilus* spp., *C. ferrugineus*, *O. surinamensis*, *R. dominica* and *S. oryzae*; in contrast, for *Ephestia* spp., *G. cornutus* and *T. castaneum* low catches did not allow the construction of monthly maps.

High presence of *Carpophilus* spp. was detected from December 1997 to May 1998 and from March to April 1999 (Fig. 2). Contour maps were produced for the year I survey (21 Nov-22 Jan; 21 Mar-22 Apr; 2 Jul-27 Aug) and the year II survey (5 Feb-2 Jun) (Fig. 4). Location of infestations during the year I changed from a month to another, but were always connected to small parts of the processing area or around silos A-D. In 1999, a single main focus was observed around the pre-cleaning machine in March and April maps.

Highest number of *C. ferrugineus* occurred in November-December 1997 and April-May 1998 during the I year survey, while during the II year numbers were very low, except for the month of April (Fig. 2). Monthly contour maps were depicted for the I year survey (7 Nov-18 Dec; 23 Jan-19 Feb; 23 Apr-25 May) and for the II year survey (4 Mar-30 Jun) (Fig. 5).
Fig. 3. Probability contour lines, obtained by converting the annual trap catches, showing spatial distribution for *Carpophilus* spp., *Cryptolestes ferrugineus*, *Ephestia* spp., *Gnatocerus cornutus*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum*. Cumulative frequency distribution (cfd), expressed as percentage, for each species is given. Probability levels (Prob.) are indicated in the scale. Contours are not shown for low density areas, with cfd < 0.5; x and y axis are expressed in meters.
Spatio-temporal distribution from November 1997 to February 1998 showed a concentration of populations mainly in the processing zone, but with a variable location of foci (oven 3, entrance, grain pit with the conveyor belt). Afterwards, distribution strongly reduced in this area, while increased in the zone of silo J, to become stable in that location. In 1999, a strong infestation around silos E-G was limited to the month of April; other foci were localized in the entrance and in the de-hulling place 1.

*O. surinamensis* was detected mainly in the period of April-August 1998; during the II year, catches were very low (Fig. 2). Spatio-temporal distribution was depicted for the I year survey (21 Mar-2 Jul; 31 Jul-27 Aug) (Fig. 6). A stable locus was detected near the silo J during all the time. Weak loci during August and September were in the grain pit with the conveyor belt, in the de-hulling place 2 and on the roof of the office.

*R. dominica* populations were high during all months of the I year, with the maximum in November-December 1997 and again in October 1998; in the II year, infestation decreased to lower levels (Fig. 2). Spatio-temporal dynamic is shown by monthly contour maps for the I year (21 Nov-22 Jan; 23 Apr-25 May; 25 Sep-22 Oct) (Fig. 7). At the beginning of the survey infestations were located in the area of silos A-D, in the grain pit with the conveyor belt and in the de-hulling place 2. From the end of January, distribution changed and remained localized near the silo J, while in the processing area no catches were detected. In August, spatial location of *R. dominica* changed again, disappearing from silos area and having locus foci again in the processing area (near the entrance, in the grain pit with the conveyor belt and in the de-hulling place 2).

*S. oryzae* had very high presence during all the monitoring period. In the I year, maximum was in November and December 1997; in the II year, catches increase during the last months of the monitoring (Aug-Oct 1999) (Fig. 2). Contour maps of the I year showed different trends in the various areas of the paddy rice storage facility (Fig. 8). At the beginning of the survey, hot spots were around silos E-H, near the dryers and the grain pit with the conveyor belt. In following months, distribution changed radically: new foci appeared in the processing area, near the water tanks and the pre-cleaning machine, instead the infestation near silos E-H disappeared quickly. From the end of March, catches in the processing area decreased, while new infestations grew in the silos area (silos A-D and J). Distribution changed from July onward remaining limited in several zones of the processing area. During the II year (Fig. 8), *S. oryzae* important presence in the silos area was detected only in April and July (silos A-D), while a stable hot spot was located in the grain pit with the conveyor belt.

**Discussion**

The pest species collected showed a rather variable distribution and, depending on pest and year, all parts of the facility appeared infested. In fact, in the various cases observed, pest populations were present both in processing area and in silos area, at least in one of the two Nov-18 Dec; 23 Jan-19 Feb; 23 Apr-25 May) and for the II year survey (4 Mar-30 Jun) (Fig. 5). Spatio-temporal distribution from November 1997 to February 1998 showed a concentration of populations mainly in the processing zone, but with a variable location of foci (oven 3, entrance, grain pit with the conveyor belt). Afterwards, distribution strongly reduced in this area, while increased in the zone of silo J, to become stable in that location. In 1999, a strong infestation around silos E-G was limited to the month of April; other foci were localized in the entrance and in the de-hulling place 1. years survey. Large extension of distribution in silos area has to be interpreted as an interpolation effect since few traps were deployed there.
Fig. 4. Spatial distribution of *Carpophilus* spp. during the year I survey (21 Nov-22 Jan; 21 Mar-22 Apr; 3 Jul-27 Aug) and the year II survey (5 Feb-2 Jun). Shades of grey indicate the number of insects captured. *X* and *y* axis are expressed in meters.

Fig. 5. Spatial distribution of *C. ferrugineus* during the I year survey (7 Nov-18 Dec; 23 Jan-19 Feb; 23 Apr-25 May) and the II year survey (4 Mar-30 Jun). Shades of grey indicate the number of insects captured. *X* and *y* axis are expressed in meters.
Fig. 6. Spatial distribution of *O. surinamensis* during the I year survey (21 Mar-2 Jul; 31 Jul-27 Aug). Shades of grey indicate the number of insects captured. *X* and *y* axis are expressed in meters.

Fig. 7. Spatial distribution of *R. dominica* during the I year survey (21 Nov-22 Jan; 23 Apr-25 May; 25 Sep-22 Oct). Shades of grey indicate the number of insects captured. *X* and *y* axis are expressed in meters.

Fig. 8. Spatial distribution of *S. oryzae* during the I year survey (7-20 Nov; 19 Dec-22 Jan; 23 Apr-25 May; 3-30 Jul) and the II year survey (9 Apr-5 May; 1-21 Jul; 2 Sep-20 Oct). Shades of grey indicate the number of insects captured. *X* and *y* axis are expressed in meters.
Analyzing the distributions, the various zones of the rice facility appeared to have different susceptibility to insect infestations. The zone around silos I-J was attractive only to *Carpophilus* and *Ephestia* species, high infestations were not found. In the case of *G. cornutus*, *O. surinamensis* and *T. confusium* (in year II), and *C. ferrugineus*, *R. dominica* and *S. oryzae* (in both years), populations had high density, in some cases the highest of the facility, frequently connected to locations at the entrance of the processing area. Silos A-D and silo E-H were found infested less frequently than silos I-J, but high density foci in these zones were detected for many species, particularly *S. oryzae*. Grain pit and conveyor belt were almost always found infested, except for *O. surinamensis* (year I), *G. cornutus* (year I) and *Carpophilus* spp. (both years). Similarly, also the de-hulling places 1 and 2 where zones of high infestation, often connected with various nearby sites, particularly place 2 with *G. cornutus* and *T. castaneum* (year I), *C. ferrugineus*, *O. surinamensis*, *R. dominica* and *S. oryzae* (both years). The pre-cleaning machine and the nearby dryers represented a focus of infestation in several cases (*C. ferrugineus* and *S. oryzae* during the year I, *Ephestia* spp. during year II; *Carpophilus* spp., *G. cornutus* and *T. castaneum* in both years), usually connected each other or with neighbouring areas. Among the three oven present in the facility, oven 1 resulted more infested respect others (*Carpophilus* spp. and *T. castaneum* in the year I; *Carpophilus* spp., *O. surinamensis* and *S. oryzae* in year II), maybe due to the vicinity of the grain pit. Near the large water tanks 1 and 2, foci were observed only for *Ephestia* spp., *R. dominica* and *S. oryzae*, usually linked to grain pit or de-hulling place infestations. Other sites, such as the rice husks storage barn, the rice grading room, the firewood deposit and the office, were rarely found infested.

At the beginning of the survey, no control measures were used, residues such as old bags and garbage were spread in the processing area; moreover, the rice was not homogenous because it was received from many different farms, and at different times. Basic cleaning measures were adopted inside and outside the storage facilities as soon as the results of the first survey were obtained. The sanitation measures included the elimination of piles of old sacks, garbage and other materials, mainly in the zones where largest insect infestations were detected, i.e. in the receiving area of paddy rice, around the grain pits and the pre-cleaning machines. Further, cleaning of the silos before filling, and insecticide application on the silo walls, were adopted. The efficacy of these measures can be evaluated with a comparison between the years I and II data, that show a strong decrease of population numbers in the second year. Nevertheless, variations in number and in spatial distribution were not uniform, but changed in some unexpected ways. As for *S. oryzae* numbers decreased of 36% in the second year, but spatial distribution did not shrink, with a reduction in the zone of the pre-cleaning machine and the dryers, probably related to constant curative practices, and an augmentation in the rice husks storage room and oven 1, probably shelter sites. Regarding *C. ferrugineus*, numbers increased in the year II, and spatial distribution partly changed, appearing in the area of silos A-H. Also *G. cornutus* and *T. castaneum* populations grew in the second year, but, in the first case spatial distribution resulted narrower, whereas in the second case a new hot spot appeared around silos E-H. Decreasing of population corresponded, for *Carpophilus* spp. and *R. dominica* to a reduction in infestation extent, while, for *Ephestia* spp. and *O. surinamensis*, to an increased distribution.

The changes of spatial distributions are probably strongly influenced by the pest management practices; in fact, species submitted to a pressure due to the curative intervention adjust to new surroundings, maintaining low level population in less treated zones. To obtain an in-depth effect of measures, also shelter sites, that represent hidden infestation foci ready to quickly re-colonize the remaining sites of the structure, should be identified and monitored. Spatial analysis allows locating these persistent infestation foci that can determine in a new spreading of infestation.
By using the monthly produced spatial map, monitoring practices can be directed with a high degree of precision. This would allow the early detection of pests in shelter sites, before they move to infest the grain mass in other areas of the facility or inside the silos, and help to make the most efficient and cost effective decisions about management and control strategies.

References


Sitotroga cerealella (Lepidoptera: Gelechiidae), development parameters of a strain from maize stores in West Africa

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Abstract: In certain areas of West Africa the production of maize has replaced millet and sorghum as the major crop. Much of this development has occurred in areas where maize stores are exposed to a high risk of attack by insect pests, e.g. the Northern Guinea Savanna. These pests include the larger grain borer Prostephanus truncatus (Horn) (Coleoptera: Bostrichidae), the maize weevil Sitophilus zeamais Motschulsky (Coleoptera: Cucurbitidae) and the Angoumois grain moth Sitotroga cerealella (Olivier). The biology and ecology of P. truncatus and S. zeamais in the humid coastal regions of West Africa has been intensively studied at the International Institute of Tropical Agriculture (IITA) in Benin and weather driven demographic simulation models have been developed in collaboration with the Danish Institute of Agricultural Sciences and Natural Resources Institute to support integrated pest management of these pests. These models will be further developed to include other climate zones and other pests, such as S. cerealella. However, the population dynamics, destructive potential and ecological relations of S. cerealella on maize in this region are not well understood. It appears from the literature that great variation in development parameters exists among strains from different continents. In the present study the life history parameters of a West African strain of S. cerealella have been investigated using a local maize variety. The results of these investigations will be presented and used to examine ecological constraints for the distribution of this pest within the region.

Key words: Sitotroga cerealella, maize stores, Benin, West Africa, life tables, intrinsic rate of natural increase, \( r_m \)

Introduction

The Angoumois grain moth, Sitotroga cerealella (Olivier), is a primary colonizer of stored grain in subtropical and warm temperate climates of the world. Its larvae tunnel inside the kernels, causing substantial damage and making the grain a more suitable place for reproduction of secondary insect pests (Weston and Rattlingourd, 2000). Along with the maize weevil Sitophilus zeamais (Motschulsky), the larger grain borer Prostephanus truncatus (Horn) and the lesser grain borer Rhyzopertha dominica (Fabricius), this species is part of a most damaging pest complex in maize stores of small-scale farmers in West Africa (Markham, 1981; Ayertey and Ibitoye, 1987; Meikle et al., 2002). This complex of damaging insect species has dispersed further within the last 10 to 20 years, partly due to socio-economic changes in the region. Farmers have switched from local millet and sorghum varieties previously planted in the hot dry zones to growing high yielding maize varieties with little natural resistance against pests resulting in an increased use of insecticides against the pests (Markham et al., 1994; Meikle et al., 1998b).
Although *S. cerealella* is a relatively well-studied pest of especially sorghum, its biology, population dynamics and destructive potential as a pest on maize in this region of the world still needs to be better understood.

Previous work in the humid coastal part of Benin on *P. truncatus* and *S. zeamais* has been based mainly on a combination of laboratory and on-farm research, (Holst et al., 2000) surveys and the production of weather-driven demographic simulation models (www.agrsci.dk/plb/nho/vms). This has led to a substantial understanding of the population dynamics of these two species including their ecological constraints along with the development of new options for managing smallholder grain stores. Consequently, this work on simulation modelling has been expanded to cover the central and northern part of Benin as well and to include *S. cerealella* as an additional pest species.

A simulation model is often constructed on the basis of a single set of published life history data or a combination of several studies, but if such data are gathered from other geographic regions they may not be comparable to the pest insect biology in the region for which the model is intended (Weaver and Throne, 1994). For *S. cerealella* life history data on maize are only available from India (Grewal and Atwal, 1967) and USA (Weaver and Throne, 1994). A comparison of these two studies reveals that great variation exists among different strains with respect to the effect of temperature on important life history parameters of the pest.

Therefore, as a first step in the expansion of the simulation model it was decided to collect life history data from a local strain of *S. cerealella* in the laboratory at four relevant temperatures and two levels of humidity. In addition, on-farm research, surveys, etc. were conducted in the central and northern part of Benin. Based on the tentative results presented here the ecological constraints with respect to its competitive power as a pest when compared with *P. truncatus* and *S. zeamais* in Benin will be discussed.

**Material and methods**

Investigations were conducted at the DPIL at combinations of four temperatures (20°, 25°, 30° and 35°C) and two relative humidities (ca 44% and 80% r.h.) maintained by means of saturated salt solutions. This corresponds to 10.2-10.3% m.c. at the low r.h. and 15.9-16.4% m.c. at the high r.h., highest at low temperatures (Meikle et al., 1998a). The investigations were conducted on a colony of *S. cerealella* collected in Nigeria in 2001 with a Benin variety of maize (Gbogbe) as medium.

To determine development time 50 eggs of *S. cerealella* (<24 hours) were transferred individually to pieces of black filter paper (3.5 x 1.5 cm). A single paper with an egg was placed with two acclimatised maize kernels in a small plastic container. The containers were placed in environmental chambers at the experimental temperature and relative humidity. Hatching of eggs was checked daily and presence of dead larvae in the containers was noted. Emergence of adults was checked four times a week until four weeks after the last adult had emerged. Three replicates separated in time were made with each combination of temperature and humidity.

Investigations of the fecundity were conducted by transferring a newly emerged female (<24 hours) and two males to a plastic container as described above, into which a piece of folded black filter paper (3.5 x 1.3 cm, folded 3 times) had been placed for oviposition. The containers were placed in environmental chambers as described above. The females were transferred daily to a new container with an oviposition paper six times a week until the females died. The numbers of eggs on the oviposition papers and on the internal surfaces of the containers were registered. Three replicates with 10 females each separated in time were
made with each combination of temperature and humidity, except for 35°C, as only six females survived under these conditions.

Results

The development time (total development egg to adult) of *S. cerealella* in relation to temperature and relative humidity ranged between 37 days at 30°C, high r.h., to 106 days at 20°C, low r.h. The highest survival during the juvenile stages (61-63%) was found at high relative humidity and temperatures of 25 and 30°C. At 35°C, emergence of adults was negligible, irrespective of r.h. The greatest fecundity (average 123 eggs per female) occurred at 20°C, high r.h.

The data were used to calculate $r_m$, the intrinsic rate for natural increase (Table 1). The highest $r_m$ (0.086 day$^{-1}$) occurred at 30°C, high r.h. At 35°C, low r.h, no specimens survived through the preimaginal stage. At 35°C, high r.h., a value of -0.005 was found for $r_m$ despite the fact that a few moths developed into adults and were able to oviposit. The net reproduction rate, however, was less than one under these conditions and mortality among juveniles was 93%; these factors explain the negative $r_m$ value. Results of this study can be found in Hansen et al. (2004).

Table 1. Intrinsic rate of natural increase ($r_m$) (day$^{-1}$) of a West African strain of *Sitotroga cerealella* in relation to temperature and relative humidity.

<table>
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<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
<th>35°C</th>
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<tr>
<td>Low r.h. (ca 44%)</td>
<td>0.014</td>
<td>0.031</td>
<td>0.051</td>
<td>0.051</td>
</tr>
<tr>
<td>High r.h. (ca 80%)</td>
<td>0.039</td>
<td>0.067</td>
<td>0.086</td>
<td>-0.005</td>
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Discussion

The results support the observation that great variations in life history parameters exist among different strains of *S. cerealella* (Weaver and Throne, 1994). Comparable investigations have been conducted on the pre-imaginal development and survival of a North American strain (Weaver and Throne, 1994) and these factors as well as fecundity in a strain from India (Grewal and Atwal, 1967). The American strain usually had greater survival and quicker development during the larval and pupal stages, but at 30°C, high r.h., the development of this strain took longer time than the African strain. The Indian strain exhibited higher fecundity (in general 30-70% higher) despite the fact that the females had shorter longevity than the African strain under many conditions. At high relative humidity the pre-imaginal survival of the Indian strain was much lower than the African strain; e.g. at 20°, 30° and 35°, high r.h., the survival of the African strain was 52, 61 and 63%, respectively, compared to 20, 36 and 34% in the Indian strain (survival percentage combined from the three categories).

However, the two references mentioned above do not include data on the age-specific survival and fecundity, thus it is not possible to calculate $r_m$. As the African strain is superior to the other strains in some aspects and inferior in others it is not possible to estimate what effect these differences may have on the population development rates of the two strains under different climatic conditions.

The highest population increase rate in the African strain of *S. cerealella* occurs at 30°C and high relative humidity. Grewal and Atwal (1967) conclude that 25°C and 80% relative humidity were most favourable for development, survival and reproduction of the Indian
strain of S. cerealella. In both strains, temperatures higher than 30°C are not favourable for development of this pest.

Within the present project pest surveys were conducted in maize stores in the Southern and Northern Guinea Savannah by the IITA and by Larsen (2003), but surprisingly, S. cerealella was not found in any of these surveys. S. cerealella is known as a major pest across the continent and has been recorded not only in countries in the region, e.g. Togo (R. Markham, pers. comm.), Nigeria (Markham, 1981), Ghana (Ayerty, 1979) and Camaroon (Nonvellier, 1984), but also from Ethiopia (Ferdu et al., 2001) and Zimbabwe (Mvumi et al., 2003). This pest was expected to be present in Benin as well, especially in the more humid areas. In previous studies in maize stores, no specimens of S. cerealella were found (Meikle et al., 2002; Hell et al., 2003). In a study in southern Benin, Borgemeister et al. (1994) mentions that four insect species were common throughout the storage season on maize with husks, none of which was S. cerealella.

The lack of findings of S. cerealella in the surveys conducted in Benin is difficult to understand as it is found in neighbouring countries with similar agro-ecologies. Furthermore, the environmental conditions prevailing in southern Benin are close to the optimal (30°C, high relative humidity) for S. cerealella. Under these conditions the population development potential of S. cerealella \( r_m = 0.086 \text{ day}^{-1} \) is intermediate to those of S. zeamais and P. truncatus \( r_m = \text{values 0.078 and 0.114 day}^{-1} \), respectively) (Holst and Meikle, 2003). However, laboratory studies dealing with the interspecific competition between S. zeamais and S. cerealella on maize revealed that S. cerealella was eliminated by S. zeamais (Chesnut and Douglas, 1971; Ayertey, 1979, Larsen, 2003). Low grain moisture content is expected to be more limiting on the population development of P. truncatus and S. zeamais than of S. cerealella so this species could possibly have an advantage in dryer areas of the region.

Other factors play a role in the distribution of a pest. The role of natural enemies is unknown, and the commodity and mode of storage may also influence the relative importance of different species in different areas. Further studies and surveys, including pheromone trapping, are planned to elucidate the distribution and pest status of S. cerealella within the region.

**Acknowledgements**

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**References**


Holst, N., Meikle, W.G. & Markham, R.H., 2000: Grain injury models for *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Cucurionidae) in rural maize stores in West Africa. – Journal of Economic Entomology 93: 1338-1346.


Detection
Sequential sampling plans for making management decisions about *Lasioderma serricorne* (F.) in stored tobacco using pheromone traps

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**Abstract:** Sequential sampling plans for management decisions about *Lasioderma serricorne* (F.) in stored tobacco using pheromone traps were developed using Iwao's confidence interval methods. The results were obtained from data collected in two green tobacco stores and three processed tobacco stores, from October 1998 to December 2001. The sequential programs developed allow achieving recommendations for intervention reducing up to 67% of the number of pheromone traps observed.

**Key words:** *Lasioderma serricorne*, sequential sampling, Iwao’s confidence interval, spatial pattern

**Introduction**

*Lasioderma serricorne* (F.) is the most serious insect pest to stored tobacco. Mainly, are the larvae that feed on the product and may contaminate it with their excreta, causing damage on tobacco. In addition, when exposed to live insects or to their remains, the smokers and workers may have allergic responses (Almeida, 1956; Ryan, 1996; Bellas, 1999). When cured tobacco is stored, it becomes particularly susceptible to pest populations. Therefore, it is important to develop sampling programs as tools for management of insect populations. Pheromone traps have been used to assess the relative density of *L. serricorne* for classifying it as above or below a specified economic threshold. In such cases, a sequential hypothesis testing provides an alternative to sampling schemes that employ a fixed number of observations per sample.

Sequential sampling plans for classifying the infestation level relative to a threshold has been developed using sequential sampling plans. With this procedure, sample size is dependent on the outcome of each successive observation as it relates to the sum of previous observations in a given set of samples. In general, these plans are as reliable as fixed-sample-size procedures and require fewer observations, which shall be important when cost and time efficiency are important (Nyrop & Simmons, 1985). Sequential sampling can also be used to evaluate the effectiveness of insecticide treatments and to determine whether parasitoids and/or predators are sufficiently effective to avoid intervention (Boivin & Vincent, 1987).

In the present work, based on data collected in two green tobacco and three processed tobacco stores, sequential sampling plans were developed using Iwao's confidence interval methods. In these stores, economic thresholds are being used to control the cigarette beetle, and to reduce the number of samples required in making decisions concerning these control actions.

**Material and methods**

*Collection of L. serricorne sampling data*

Two green tobacco stores and three processed tobacco stores from a processing tobacco factory in Portugal using 14 and 30 pheromones traps, respectively were sampled from 6
October 1998 to 30 December 2000 (New Serrico, from Fuji Flavor Co.). All pheromone traps were observed weekly and the insects counted and identified. Both traps and lures were replaced each sixth week (Carvalho et al., 2001, 2002).

In the green tobacco stores, no insects were caught in 65 sampling data, which corresponds to 910 observations, while in 50 sampling data, that is 700 observations, the traps caught one or more adults. In the processed tobacco stores, at least one insect was caught in 58 sampling data, about 1726 observations, while no insects were registered during 57 sampling data, which corresponds to 1710 observations. From the 50 counts obtained in the green tobacco stores, 29 fitted the negative binomial, 14 fitted the Poisson and eight fitted both distributions. In the processed tobacco stores, from 58 counts, 31 fitted the negative binomial, 12 fitted the Poisson and five fitted both distributions. The $k$ common to the individual distributions fitting the negative binomial model, which method for estimating the parameter $k$ was the maximum likelihood, it was estimated as 0.6412 for the green tobacco stores results and 0.1800 for those obtained in the processed tobacco stores (Southwood, 1978; Ludwig & Reynolds, 1988; Krebs, 1989; Davis, 1994).

Since the data sets of the green tobacco stores contained less than 30 sampling units (14 pheromone traps) (Davis, 1994), the Iwao’s Patchiness regression technique (Lloyd, 1967; Iwao, 1968; Davis, 1994) was carried out for classifying dispersion patterns and to be used in the sampling program (Table 1):

$$x = \alpha + \beta \hat{x}$$

where $\alpha$ = intercept, defined as the index of basic contagious; $\beta$ = slope, defined as the density contagiousness coefficient.

Table 1. Iwao’s Patchiness Regression estimates for adults of *Lasioderma serricorne* caught in tobacco stores using pheromone traps.

<table>
<thead>
<tr>
<th>Stores</th>
<th>$n$</th>
<th>$\alpha \pm SE_\alpha$</th>
<th>$t = \alpha / SE_\alpha$</th>
<th>$\beta \pm SE_\beta$</th>
<th>$t = (\beta - 1) / SE_\beta$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green tobacco</td>
<td>50</td>
<td>-0.318±0.198</td>
<td>-1.610 (P=0.114)</td>
<td>2.834±0.091</td>
<td>20.130 (P=0)</td>
<td>0.95</td>
</tr>
<tr>
<td>Processed tobacco</td>
<td>58</td>
<td>0.840±0.419</td>
<td>2.006 (P=0.050)</td>
<td>3.081±0.171</td>
<td>12.181 (P=0)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*a number of _x_ _x_ _pairs used in the regression

The slope $\beta$ describes the distribution of cigarette beetles and indicates, for both situations, that the individuals were distributed in a strong aggregated pattern as the results obtained from Poisson and binomial distribution.

**Sequential sampling plans for making management decisions about**

*Lasioderma serricorne* in stored tobacco using pheromone traps

Iwao’s confidence interval method was used to develop sequential sampling plans for making decisions concerning *L. serricorne*.

Contrary to sequential probability ratio test (SPRT), Iwao’s procedure uses directly the economic threshold to calculate the decision lines (Boivin & Vincent, 1987; Binns, 1994; Subramanyam & Hagstrum, 1996). The upper and lower decision lines were based on Iwao’s patchiness regression (Table 1) and were calculated as:
Upper decision line:
\[ U_u = n\mu_0 + t\left[\alpha + (1)\mu_0 + (\beta - 1)\mu_0^2\right]^{0.5} \]
Lower decision line:
\[ L_u = n\mu_0 - t\left[\alpha + (1)\mu_0 + (\beta - 1)\mu_0^2\right]^{0.5} \]
where \( n \) = number of samples taken, \( \mu_0 \) = critical pest density (20 for green tobacco, 5 for processed tobacco), \( \alpha \) = y-intercept and \( \beta \) = slope estimates, from Iwao’s patchiness regression, and \( t \) (=1.96) = the distribution statistic at 0.05 probability level.

Iwao’s confidence interval method was developed from information on the parameters: \( \mu_0 \), the economic threshold; \( \mu_1 \), the lower threshold; \( \mu_2 \) the upper threshold; the intercept \( \alpha \) and the slope \( \beta \) from Iwao’s Patchiness Regression estimates. The value of Student’s \( t \), for infinite degrees student, was 1.96, at 0.05 probability level.

When the relative density of cigarette beetles is between the two decision lines a maximum number of samples must be established in order to obtain a limit of sampling units within a predetermined confidence level.

Maximum number of samples:
\[ N_m = \frac{t^2}{d^2} \left[\alpha + (1)\mu_0 + (\beta - 1)\mu_0^2\right] \]
where \( d \) = confidence interval of the estimated mean density.

Results

Green tobacco stores
The mean crowding estimated for cigarette beetles caught in the green tobacco stores was \( \bar{x} = -0.32 + 2.83 \bar{x} \) \( (r^2 = 0.95) \) and shows a very strong linear relationship between the mean crowding and the mean.

Three hypotheses were tested:
\( H_0: \mu_0 = 20 \) insects/trap/week
\( H_1: \mu_1 \leq 20 \) insects/trap/week
\( H_2: \mu_2 \geq 20 \) insects/trap/week.

The hypothesis \( H_0 \) is the economic threshold applied in the green tobacco stores. Below threshold (hypothesis \( H_1 \)) the relative density of cigarette beetles is low and tolerate at this level. Above the threshold (hypothesis \( H_2 \)) the relative density is high and control action is needed.

The upper and lower decision lines for classifying \( L. serricorne \) infestation levels relative to the economic threshold of 20 insects/trap/week are shown in Fig. 1 and Table 2.

The lower decision line intersects the x-axis at eight traps. Therefore, a minimum of eight traps must be observed for classifying the \( L. serricorne \) infestation level. If the total of cigarette beetles caught is below of eight individuals the sampling procedure stops, between eight and 312 individuals more pheromone traps must be observed, and if the trap catches surpassed this number, the sampling procedure stops and control actions are needed.

The space between these curves increases with the amplitude of the degree of precision. If the relative density of the cigarette beetles is equal to the economic threshold of 20 insects/trap/week, a large number of traps must be observed between the calculated limits.

The maximum number of samples, that must be taken in order to determine if the relative density of cigarette beetles is equal to the economic threshold, can be calculated in Iwao’s procedure. The results for different confidence intervals (\( d \)) are presented in Table 3.
Fig. 1. Iwao’s confidence interval method decision lines for classifying level of *Lasioderma serricorne* relative to the threshold of 20 insects/trap/week used in the green tobacco stores.

Table 2. Acceptance limits of hypotheses $H_1$ and $H_2$ for sequential sampling plan according Iwao’s confidence interval method relative to the economic threshold of 20 cigarette beetles/trap/week used in the green tobacco stores.

<table>
<thead>
<tr>
<th>Number of pheromone traps</th>
<th>Lower decision line</th>
<th>Upper decision line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No decision</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>No decision until eight</td>
<td>116</td>
</tr>
<tr>
<td>3</td>
<td>No decision until eight</td>
<td>153</td>
</tr>
<tr>
<td>4</td>
<td>No decision until eight</td>
<td>187</td>
</tr>
<tr>
<td>5</td>
<td>No decision until eight</td>
<td>220</td>
</tr>
<tr>
<td>6</td>
<td>No decision until eight</td>
<td>251</td>
</tr>
<tr>
<td>7</td>
<td>No decision until eight</td>
<td>282</td>
</tr>
<tr>
<td>8</td>
<td>Stop sampling and tolerate this population level</td>
<td>312</td>
</tr>
<tr>
<td>9</td>
<td>Stop sampling and tolerate this population level</td>
<td>341</td>
</tr>
<tr>
<td>10</td>
<td>Stop sampling and tolerate this population level</td>
<td>369</td>
</tr>
<tr>
<td>11</td>
<td>Stop sampling and tolerate this population level</td>
<td>398</td>
</tr>
<tr>
<td>12</td>
<td>Stop sampling and tolerate this population level</td>
<td>426</td>
</tr>
<tr>
<td>13</td>
<td>Stop sampling and tolerate this population level</td>
<td>453</td>
</tr>
<tr>
<td>14</td>
<td>Stop sampling and tolerate this population level</td>
<td>480</td>
</tr>
<tr>
<td>15</td>
<td>Stop sampling and tolerate this population level</td>
<td>508</td>
</tr>
<tr>
<td>20</td>
<td>Stop sampling and tolerate this population level</td>
<td>640</td>
</tr>
<tr>
<td>25</td>
<td>Stop sampling and tolerate this population level</td>
<td>768</td>
</tr>
<tr>
<td>30</td>
<td>Stop sampling and tolerate this population level</td>
<td>893</td>
</tr>
<tr>
<td>35</td>
<td>Stop sampling and tolerate this population level</td>
<td>1017</td>
</tr>
<tr>
<td>40</td>
<td>Stop sampling and tolerate this population level</td>
<td>1139</td>
</tr>
<tr>
<td>45</td>
<td>Stop sampling and tolerate this population level</td>
<td>1259</td>
</tr>
<tr>
<td>50</td>
<td>Stop sampling and tolerate this population level</td>
<td>1379</td>
</tr>
</tbody>
</table>

At different confidence intervals used sampling should not stop and when results are very high a sampling program using pheromone traps could be applied. The most acceptable shall be a maximum of 148 pheromone traps for a confidence interval of $d \pm 4.5$. 
### Table 3. Maximum number of pheromone traps needed at different confidence intervals.

<table>
<thead>
<tr>
<th>Confidence interval (d)</th>
<th>Maximum number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>11956</td>
</tr>
<tr>
<td>1.0</td>
<td>2989</td>
</tr>
<tr>
<td>1.5</td>
<td>1328</td>
</tr>
<tr>
<td>2.0</td>
<td>747</td>
</tr>
<tr>
<td>2.5</td>
<td>478</td>
</tr>
<tr>
<td>3.0</td>
<td>332</td>
</tr>
<tr>
<td>3.5</td>
<td>244</td>
</tr>
<tr>
<td>4.0</td>
<td>187</td>
</tr>
<tr>
<td>4.5</td>
<td>148</td>
</tr>
</tbody>
</table>

**Processed tobacco stores**

The mean crowding estimated for cigarette beetles caught in the three processed tobacco stores was \( \bar{x} = 0.84 + 3.08 \bar{x} \) \( (r^2 = 0.85) \) which shows strong linear relationship between the mean crowding and the mean.

Three hypotheses were tested:

- \( H_0: \mu_0 = 5 \) insects/trap/week
- \( H_1: \mu_1 \leq 5 \) insects/trap/week
- \( H_2: \mu_2 \geq 5 \) insects/trap/week.

The upper and lower decision lines for classifying \( L. serricorne \) infestation levels relative to the economic threshold of five-cigarette beetles/trap/week are shown in Fig. 2 and Table 4.

The lower decision line intersects the x axis at 10 pheromone traps. Therefore, a minimum of 10 traps must be observed for classifying the \( L. serricorne \) infestation level. If the total accumulated of cigarette beetles caught is below two individuals the sampling procedure stops, between two and 98 individuals more pheromone traps must be observed, and if the trap catches surpassed this number, the sampling procedure stops and control actions are needed.

The maximum number of samples was taken in order to determine if the relative density of cigarette beetles is equal to the economic threshold and is presented in Table 5. For a confidence interval of 0.5, the results obtained were too high to be applied in a sampling program using pheromone traps. Using higher \( d \) the maximum number of pheromone traps ranged from 61, for a mean of 5±2.0 cigarette beetles/trap/week, to 27, for a mean of 4±3.0 cigarette beetles/trap/week.

**Discussion**

The most usual methods used to develop sequential sampling plans, for classifying pest status, are the sequential probability ratio test (SPRT) and Iwao’s confidence interval. For the data set, obtained at the two types of stores studied, the spatial pattern of cigarette beetles was evaluated testing two mathematical distributions, Poisson and binomial negative. Although most of the observed counts fitted the negative binomial distribution, this was not the rule, mostly at the processed tobacco stores. Due to this and as the sample size in the green tobacco stores was smaller than 30 sampling units; Iwao’s confidence interval method was used to develop the sequential sampling plans.
Fig. 2. Iwao’s confidence interval method decision lines for classifying level of *Lasioderma serricorne* relative to the threshold of 5 insects/trap/week used in the processed tobacco stores.

Table 4. Acceptance limits of hypotheses H<sub>1</sub> and H<sub>2</sub> for sequential sampling plan according Iwao’s confidence interval method relative to the economic threshold of 5 cigarette beetles/trap/week used in the processed tobacco stores.

<table>
<thead>
<tr>
<th>Number of pheromone traps</th>
<th>Lower decision line</th>
<th>Upper decision line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>No decision</td>
<td>51</td>
</tr>
<tr>
<td>5</td>
<td>until 10 traps were observed</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Stop sampling</td>
<td>98</td>
</tr>
<tr>
<td>11</td>
<td>and tolerate</td>
<td>106</td>
</tr>
<tr>
<td>12</td>
<td>this population level</td>
<td>113</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>120</td>
</tr>
<tr>
<td>14</td>
<td>13</td>
<td>127</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>134</td>
</tr>
<tr>
<td>20</td>
<td>31</td>
<td>169</td>
</tr>
<tr>
<td>25</td>
<td>48</td>
<td>202</td>
</tr>
<tr>
<td>30</td>
<td>66</td>
<td>234</td>
</tr>
<tr>
<td>35</td>
<td>84</td>
<td>266</td>
</tr>
<tr>
<td>40</td>
<td>103</td>
<td>297</td>
</tr>
<tr>
<td>45</td>
<td>122</td>
<td>328</td>
</tr>
<tr>
<td>50</td>
<td>142</td>
<td>358</td>
</tr>
</tbody>
</table>
The Iwao’s procedure has the advantage, compared with SPRT method, of not requiring a characterization of the distribution of the population, which often varies with changes in the density, quality and age of a population, as well as spatial and temporal changes in the environment (Nyrop & Simmons, 1985).

Table 5. Maximum number of pheromone traps needed at different confidence intervals.

<table>
<thead>
<tr>
<th>Confidence interval (d)</th>
<th>Maximum number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,5</td>
<td>980</td>
</tr>
<tr>
<td>1,0</td>
<td>245</td>
</tr>
<tr>
<td>1,5</td>
<td>109</td>
</tr>
<tr>
<td>2,0</td>
<td>61</td>
</tr>
<tr>
<td>2,5</td>
<td>39</td>
</tr>
<tr>
<td>3,0</td>
<td>27</td>
</tr>
</tbody>
</table>

This is the first time that sequential sampling plans are developed for *L. serricorne*, using Iwao’s confidence interval method and pheromone traps. These sampling procedures can reduce the number of traps used from 43%, for green tobacco stores data, to 67%, for processed tobacco stores data, allowing a sampling procedure less expensive in cost and time than the plans based on the fixed number of traps used.

Due to the relative high tolerance to cigarette beetles presence in both type of stores – as shown by the economic threshold values – and the strong aggregation pattern of the population studied, the maximum number of samples required, according to the developed sampling plan, is very high and difficult to apply when pheromone traps are used. Than, if the cumulative number of trap catches falls between the two decisions lines, it is suggested to increase the number of traps to a level at which they do not compete each other, to avoid hiding cigarette beetle from catches.

Acknowledgements

The authors thank the managers of the processing tobacco factory for allowing the trials and providing the pheromone traps. The authors would like to express their gratitude to the late Prof. José Passos de Carvalho, for his valuable help in the field trials.

References


Carvalho, M.O.; Pereira, A.P. & Mexia, A. 2001. Pheromone traps for monitoring *Lasioderma serricorne* and *Ephestia elutella* in stored tobacco in Portugal. – In: Donahaye, E.


Image analysis of occupancy and contamination – Mediterranean flour moth, *Ephestia kuehniella* and parasitoid *Venturia canescens*

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**Abstract:** Traps are tools frequently used for monitoring and control of pests. Therefore it is important to study prerequisites of their use. The instant active area of trap (the instantly available area of a trap that is able to capture a pest) determines trap efficacy. However, the measurement of instant active trap area is difficult. Therefore, we developed a technique of computer image analysis of digital photography for evaluation of instant active trap area, occupancy (area of insect bodies captured on trap) and contamination (area of filth left by pests on traps, such as wing-scales or faeces) of traps. We found that the Mediterranean flour moth, *Ephestia kuehniella* decreased the capacity of the sticky surface more than its parasitoid *Venturia canescens* because of contamination by wing scales. The sticky trap area covered by wing scales was nearly as large as the area occupied by moth bodies. The results indicate that moths are heavy contaminators while parasitoids don’t contaminate sticky traps.

**Keywords:** monitoring, traps, digital image analysis, *Ephestia kuehniella*, *Venturia canescens*

**Introduction**

Monitoring is a keystone of Integrated Pest Management (IMP) in orchards, glasshouses, forestry, field crops or stored products, urban and food industry environment (e.g. Stejskal, 1993; Hagstrum & Subramanyam, 2000; Campbell et al., 2002). Although there are many methods of pest monitoring, currently the trapping method is used most frequently. In addition, some pest control strategies (e.g. “mass-trapping” or “trapping-out” strategies) use traps as direct means of pest population management, in orchards (Sternlicht et al., 1990), forests (Weslien & Lindelow, 1990; Barclay & Van den Driesche, 1984), urban environment (Appel, 1998) and food industry (Trematerra & Battani, 1987).

The crucial condition of successful implementation of mass-trapping or unbiased collection of field trapping-data is a detailed knowledge of the critical conditions of effectiveness in the particular trap (e.g. Campbell & Hagstrum, 2001; Stejskal, 1995). Story (1986) stated that *pest population monitoring is based on carrying out repeated surveys using the same methodology each time so that results can be meaningfully compared*. This means that the good monitoring practice requires not only using the identical type of trap but also using the identical trap with the identical efficacy, which is not always the same. The capacity of a trap belongs among the most important factors influencing the trap-efficacy since it may quickly decline with the increasing occupation by pests. The opposite extreme represents the calendar based regular replacement and destroying of traps, which may be still effective. It is felt that these aspects of trapping are neglected and their omission may lead to misinterpretation of the obtained data, superfluous increase of the cost of pest monitoring (Stejskal, 2002b) or ineffectiveness of mass trapping. The reason is probably the operational one. In the past it has been difficult to measure the pest-occupied or contaminated area of sticky traps by the traditional methods of area measurement (Hargrove, 1988; Benjamin et al., 1968), especially under field conditions. However, the present day technique of digital
photography (Spring, 2000; Weyda, 2002) coupled with the computer image analysis (e.g. Richardson et al., 2001; Daniel et al., 1995) provides a new opportunity for cheap and quick estimation of the instant capacity of traps.

Therefore, the aim of our work is to apply the technique of scientific digital photography and computer-based image analysis for evaluation of the active and inactive (i.e. occupied /contaminated) area of a trap. In this study we measure the contamination and occupancy of pheromone baited sticky traps by Mediterranean meal moths, *Ephestia kuehniella* and its parasitoid *Venturia canescens*. This work is a part of a broader research program that is aimed at the development of the general trapping methodology for agricultural and food industry environment (e.g. Stejskal, 2002b) and financially supported by the by the Czech Ministry of Education (MZE-000 2700 603) and the Czech Ministry of Agriculture (NAZV-project No. QF4071).

**Materials and methods**

Pheromone (ZETA) baited sticky traps, brand name Ekovet® (sticky board area 7.3 x 19.5 cm) were used for trapping of moths and parasitoids.

The traps loaded with pests that were included in this study were obtained from the previous field research: 9 traps with Mediterranean meal moths and *Venturia canescens* were obtained from the study described by Stejskal & Lukáš (2002).

Digital images of traps were obtained by a flatbed scanner (Umax Astra 1200S). The sticky board was fixed on the paper frame to prevent contact of the sticky surface of the trap with the glass surface of the scanner. The acquired digital images were saved in the JPEG format (joint photographic experts group, .jpg), with a colour depth of 16.7 million colours, in a resolution of 300 dpi.. Consequently, the individual digital images were analysed by the SigmaScan Pro 5 software (SPSS Inc., 1999). After processing image to correct defects, enhance important aspects of the image and recognize the objects of interest, the measurement tools in the software package were used to count the total number of selected pixels corresponding with moth and moth scales. The number of counted pixels was then divided by the total pixel count of the image for a determination of moth a moth scales coverage percentage of the trap.

We estimated four parameters of traps: overall active area of trap, instant active trap area, instant contamination of trap and instant occupancy of trap. “Overall Active Area of Trap” (OAAT) was defined as the total sticky area of a fresh and unused trap. “Instant Occupancy of Trap” (IOT) was defined as the area of sticky trap occupied by insect bodies at the moment of taking picture of the trap. “Instant Contamination of Trap” (ICT) was defined as the area of sticky trap contaminated by filth produced by pests such as wing-scales and faeces at the moment of taking picture of the trap. “Instant Active Area of Trap”(IAAT) was defined as free area of sticky trap, which was not occupied or contaminated at the moment of taking picture of the trap. Thus IAAT is simply obtained:

\[
IAAT = OAAT - (ICT + IOT)
\]
Results

New method of measurement of occupancy and contamination of sticky traps

Moths: The image analysis using SigmaScan consisted of enhancing image quality, separating active image colours, filtering, thresholding and measuring operations. First, the image defects were corrected balancing contrast, brightness and eliminating uneven lighting (Fig. 1a). Red-Green-Blue (RGB) colour separation function was used to detach intrusive background grid-like pattern of the moth trap. The dominant colour of the background was found to be a red component ranging from 128 to 225 (Mean=180, SD=22.78). On the opposite site red values of moths ranged from 1 to 99 (Mean=65.6, SD=19.65). Consequently, the red image channel (Fig. 1b) was chosen to process by posterize filter (level 2). The number of colour levels was thus reduced into 2 colours where black area represented overall space covered by moth and their scales. The moth bodies were identified by the same procedure but before posterising the contrast level was increased to maximum. The obtained images were then combined by the logical “average” operation into third image where the moth’s scales were separated. The area corresponding to moths, moth scales and uncovered area was identified by thresholding. The green overlay was assigned to moths (Fig. 1c), the red one to moth scales (Fig. 1d) and blue one to sum of both (Fig. 1e).

Parasitoids: The same algorithm was applied to analyse Venturia canescens occupancy and contamination (Fig. 3).

Occupancy and contamination of traps by moths and parasitoids

Moths: Digital image analysis revealed that the average ratio of area covered by moth bodies and area covered by moth scales was 1.32 (SD=0.38) (Table 3). This ratio was fairly stable over all tested traps (Fig. 3). Roughly, one trapped moth resulted in decrease of available trapping area by 0.8%. The results clearly show that moths are strong trap contaminators since the sticky trap area contaminated by wing scales represents almost 100% of area occupied by moth bodies. Thus in E. kuehniella (EK) IAATEK can be estimated:

\[ IAATEK \approx OAAT - 1.81 \times IOTEK \]  \hspace{1cm} (2)

or more roughly

\[ IAATEK \approx OAAT - 2 \times IOTEK \]  \hspace{1cm} (3)

Table 1 and Fig. 2 show a detailed result of occupancy/contamination analysis of a medium covered trap (39 moths, E. kuehniella), as an example in tables where moths occupied 22% of the active trap area while moth-scales contaminated 16%.

Parasitoids: Digital image analysis revealed that there is no contamination caused by the presence of V. canescens on traps. Thus in V. canescence (VC) IAATVC can be estimated:

\[ IAATVC \approx OAAT - IOTVC \]  \hspace{1cm} (4)

The results indicate that moths are heavy contaminators while parasitoids are weak contaminators of sticky traps.

Discussion

Computer-based image analysis (CBIA) and pest monitoring

The CBIA is used effectively in a broad range of applications, from satellite images to industrial quality control of macroscopic manufactured items, to light and scanning electron microscopy of materials, structures, biological, geological or archaeological specimens, integrated circuits, and so forth (Brosnan & Son, 2002). The processing of the raw image to
Fig. 1. Digital image analysis of occupancy and contamination of sticky trap by *Ephestia kuehniella* – the sequential procedure is composed from 5 steps (a-e). For explanation see Table 1 and text.
enhance interesting details or to extract quantitative information is a vital step in the use of images as scientific data. Generally, these methods are concerned with extracting a few numerical values, such as the number, size, shape or location of objects from the image. In other cases, global structural parameters such as the volume or surface of structures present are of interest.

Table 1. Image analysis of occupancy (area of sticky trap covered by moths bodies) and contamination (area of sticky trap covered by wing-scales) of moth-trap.

<table>
<thead>
<tr>
<th></th>
<th>Number of pixels</th>
<th>Trap area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall active area of trap (sticky trap area)</td>
<td>2 037 075</td>
<td>100</td>
</tr>
<tr>
<td>Occupancy (moth-bodies)</td>
<td>482 599</td>
<td>24</td>
</tr>
<tr>
<td>Contamination (moth-scales)</td>
<td>333 452</td>
<td>16</td>
</tr>
<tr>
<td>Instant active trap area</td>
<td>1 384 649</td>
<td>60</td>
</tr>
</tbody>
</table>

Fig. 2: Digital image analysis of occupancy and contamination of sticky trap by *Venturia canescens*.

**CBIA-based evaluation of moth traps**

We, for the first time, introduce the technique of computer-based image analysis into the measuring of the instant active area of a trap in this work. In this illustrative study we show how to measure the occupancy and contamination of sticky traps in two model species, that
include *V. canescens* (Fig. 2) and the Mediterranean flour moth (Fig. 1). We found that the per individual decrease of the instant active area of the sticky surface was much higher in the pest-moths than in parasitoids because of the extensive contamination of trap sticky surface by wing-scales of moths. The sticky trap area contaminated by moth-scales is almost equal (cca additional 100%) to the area occupied by moths (Tab. 1). This illustrates that the contamination of a trap by the hardly observable (i.e. by a naked eye) wing-scales decreases the instant active area of a trap to a large extent. Clearly, the evaluation of instant active area of a trap purely according the area occupied by moth-bodies may leave an illusory image of the instant active area of a trap. *Venturia canescens* didn’t contaminated the surface of moth sticky traps. This is due to the fact that parasitoids are free of wing-scales and the faeces are composed mainly of sugar and water compounds. Thus the parameter ICT can be neglected in this case.

Table 2. The occupancy (area of sticky trap covered by moths bodies) and contamination (area of sticky trap covered by wing-scales) of moth-trap expressed in pixels (px)

<table>
<thead>
<tr>
<th>trap</th>
<th>moth bodies area [px]</th>
<th>moth scales area [px]</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>482599</td>
<td>333452</td>
<td>1.45</td>
</tr>
<tr>
<td>2</td>
<td>217981</td>
<td>223361</td>
<td>0.98</td>
</tr>
<tr>
<td>3</td>
<td>556867</td>
<td>489630</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>431791</td>
<td>306190</td>
<td>1.41</td>
</tr>
<tr>
<td>5</td>
<td>266424</td>
<td>239422</td>
<td>1.11</td>
</tr>
<tr>
<td>6</td>
<td>335796</td>
<td>278603</td>
<td>1.21</td>
</tr>
<tr>
<td>7</td>
<td>205373</td>
<td>238851</td>
<td>0.86</td>
</tr>
<tr>
<td>8</td>
<td>123524</td>
<td>81623</td>
<td>1.51</td>
</tr>
<tr>
<td>9</td>
<td>201588</td>
<td>91087</td>
<td>2.21</td>
</tr>
</tbody>
</table>

![Graph showing ratios between sticky trap areas covered by moth bodies and wing scales](image)

Fig. 3: The ratios between the sticky trap areas (N= 9) covered by moth bodies and by wing scales of moths.
Conclusions

The new method of measuring trap capacity via computer-based image analysis seems to be promising from the practical point of view since it is easy and quick. This study illustrated the use of CBIA in two types of sticky traps for monitoring the Mediterranean flour moth and *V. canescens*. Once we obtain the data on the instant occupancy, contamination and active area of a trap, it remains to decide whether the trap is efficient enough for a given trapping purposes or whether it is no longer efficient enough and must be replaced. The evaluation of the instant trap efficacy should be based on the pre-estimated relationship between the efficacy of traps and the percent of active area of a trap. We think, however, that these functions are not currently available for most of the traps and pests, which indicates area for additional laboratory and fieldwork.

Acknowledgements

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References


Spss Inc. 1999: SigmaScan Pro 5.0 Users guide. – Chicago: 281 pp.

Spring, K.R. 2000: Scientific imaging with digital camera. – Biotechniques 71-75.


Estimation of population density and spatial pattern of stored paddy rice insect species using un-baited traps

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Abstract: Studies were conducted in five paddy rice stores and one silo used for rice storage to determine the insect species associated with stored rice, their abundance and spatial pattern from November 2002 to April 2003. In each store and silo, un-baited Pitfall and Storgard WB Probe II traps were placed. Additionally, during a period of six weeks, un-baited Pitfall traps were placed in the silo, near each Storgard WB Probe II position to compare quantitative trap catches. In addition, Storgard Dome traps were used, with attractants (food and pheromones) to monitor the occurrence of pest insects on the floor below the aerated silos for grain drying. The traps were observed weekly and the insects counted and identified. The Storgard WB Probe II traps captured significantly more insects and more species than did Pitfall traps. *Ptinus raptor* (Sturm), *Cryptophagus saginatus* (Sturm), *Conionemus constricatus* (Westwood), *Gnathocerus cornutus* (F.) and *Sitophilus zeamais* (Motschulshy) were the most abundant species from about 21 insect species identified. In all five-paddy rice stores, Iwao’s regression analysis pattern suggests a definite tendency of aggregation for the insect species discovered. In addition, insect surveys were conducted in a rice mill. In the stores of the processors, un-baited Storgard WB Probe II and Pitfall traps were placed in the paddy, brown and white rice (bagged storage). To survey insect species in the factory, Stogard Dome traps were placed on the floor, between machinery, and Stogard Thinline traps, on the walls. The most abundant insect species recorded were *Sitophilus zeamais* and *Cryptolestes ferrugineus* (Stevens) and the greatest abundance was discovered in the brown rice.

Key-words: paddy rice, trap, stored product insect, density, spatial pattern, Iwao’s regression

Introduction

Portugal consumes the largest quantity of white rice in Europe, and consequently a large number of farmers and industries are associated with rice production, transportation, and processing. In Portugal, rice is a seasonal crop. Planting of rice takes place in April and harvest is near the end of August. Rice is stored as paddy on farm or in co-operatives in horizontal warehouses or vertical silos, until the end of winter.

Like all stored grains after harvest, rice is permanently at risk of insect infestation. Because of this, those involved in product storage and processing often use direct application of chemicals to the rice for insect control. Due to consumer concerns over the presence of pesticide residues in rice, there is an urgent need to develop integrated pest management strategies to reduce these residual treatments. An important element in developing an integrated pest management strategy to preserve quality of rice, is sampling. By utilizing appropriate sampling procedures, it is possible to detect insect species infesting the commodity, to determine the nature of their populations, such as density and dispersion. This will ultimately help in maximizing the effectiveness of insect control actions. It is also possible to estimate a threshold or a tolerance level for initiating control measures and
consequently this will help in designing cost-effective sampling plans for estimating insect density and categorizing the infestation level.

The aim of this work was to detect insect species from paddy rice stored in bulk on farm warehouses, in silos and in a rice mill. The paddy and brown rice were stored in bulk and the white rice was stored in polyethylene bags. The study included detection of the density, the spatial pattern of the most abundant insect species collected in farm storage and a comparison of the total trap catches from two types of traps without lures.

**Materials and methods**

**Collecting sampling data**

**Farm storage**
To detect and study the relative density and spatial pattern of insects associated with paddy rice; trials were conducted in six storages containing paddy rice, situated in Alcácer do Sal, Portugal. Five of these storages were horizontal and the sixth was a silo. Sampling lasted from 7 November 2003 to 11 April 2003 (Table 1).

**Rice mill**
During the course of the year producers, deliver paddy rice to the rice mill situated in Santiago do Cacém, Portugal. Sampling at this facility, was carried out in the horizontal storage and in the mill from mid April to 18 July 2003 (Table 2).

**Sampling procedure**

**Farm storage**
The number and type of traps used in each place are presented in Table 1. For the paddy rice in bulk, Pitfall and Probe traps (Storgard WB Probe II traps) were used without lures (Table 1). In the silo trial, nine Pitfall traps were placed within approximately 30 cm of the Probe traps to compare trap catch efficacy during nine weeks, from 2 January to 14 March, 2003 (Table 1).

Also at the silo structure site, Storgard Dome traps containing standard attractant oil without pheromone were placed on the floor below the aerated silos for grain drying. These dryers were located adjacent to the main paddy rice silos. Pheromone lures for *Tribolium castaneum* (Herbst) and *T. confusum* du Val was used in these traps only during the first four weeks of trials. The traps were inspected weekly and insects were counted and identified.

**Rice mill**
Bulk storage at the rice mill was sampled using Pitfall and Probe traps without lures. Store 1 contained paddy and brown rice in bulk, Store 2 finished product in polyethylene bags of a capacity of 1.1 tonne: 143 bags of white rice and 90 bags of by-product. Inside the mill, 5 Stogard Dome traps were placed on each of the 3 floors. The traps were placed on the floor among machinery components. Besides the standard attractant oil, pheromone dispensers with new formulation lures to attract *Lasioderma serricorne* (F.), *Tribolium castaneum*, *T. confusum*, and *Trogoderma* species were used and replaced every four weeks. Stogard Thinline traps with new lures for *Lasioderma serricorne*, *Plodia interpunctella* (Hb.) and *Trogoderma species*, were also placed on the factory walls. The traps were inspected weekly from May to July. During each inspection, the insects were counted and identified.

**Environmental conditions**
For determining environmental conditions in the farm stores, a thermo hygrograph was placed in the silo structure and in the rice mill. Temperature probes were used in the paddy rice stored in the farm storages.
Table 1. Farm storage data: locations sampled, quantity of paddy rice in each storage type and number of traps used, and the trial period.

<table>
<thead>
<tr>
<th>Local</th>
<th>Total of paddy rice (tonnes)</th>
<th>Type of trap</th>
<th>Trial period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pitfall</td>
<td>Storgard WB</td>
<td></td>
</tr>
<tr>
<td>Warehouse Store 1</td>
<td>510</td>
<td>10</td>
<td>−</td>
</tr>
<tr>
<td>Warehouse Store 2</td>
<td>590</td>
<td>10</td>
<td>−</td>
</tr>
<tr>
<td>Warehouse Store 3</td>
<td>300</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Warehouse Store 4</td>
<td>240</td>
<td>10</td>
<td>−</td>
</tr>
<tr>
<td>Warehouse Store 5</td>
<td>2017</td>
<td>−</td>
<td>6</td>
</tr>
<tr>
<td>Silos structure</td>
<td>Silo</td>
<td>300</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Floor*</td>
<td>−</td>
<td>12</td>
</tr>
</tbody>
</table>

* Dome traps used on the floor, outside the silos and dryers but inside the building.

Table 2. Rice mill data: locations sampled, quantity of rice in each storage type and number of traps used, and the trial period.

<table>
<thead>
<tr>
<th>Local</th>
<th>Total of rice (tonnes)</th>
<th>Type of trap</th>
<th>Trial period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warehouse</td>
<td>paddy rice</td>
<td>Pitfall</td>
<td>Storgard WB</td>
</tr>
<tr>
<td>Store 1</td>
<td>946</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Factory</td>
<td>brown rice</td>
<td>2000</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>brown rice</td>
<td>−</td>
<td>7</td>
</tr>
<tr>
<td>Warehouse</td>
<td>white rice</td>
<td>257</td>
<td>8</td>
</tr>
<tr>
<td>Store 2</td>
<td>brown rice</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Statistical Analysis

The mean number of adult insects caught each week, was determined and used to calculate the mean crowding \( \hat{x} \) according to the equation created by Lloyd (1967) and expressed as:

\[
\hat{x} = \bar{x} + \left( \frac{s^2}{\bar{x}} \right) - 1,
\]

where, \( \bar{x} \) = mean crowding, \( \bar{x} \) = mean insects \((\bar{x} >0); s^2 = variance of the mean\). The mean and variance necessary to calculate mean crowding can be based on different sample sizes and on several sets of samples obtained from different locations and at various sampling times (Subramanyam and Hagstrum, 1996). The mean crowding is used to describe the mean number of other individuals per individual in an average sample unit and these indices expressed the level of "crowding" in a given unit of habitat. The patchiness linear regression, examines the relationship between the Lloyd's mean crowding \( \hat{x} \) and the mean insects \( \bar{x} \):

\[
\hat{x} = \alpha + \beta \bar{x}.
\]

The y-intercept, \( \alpha \), has been interpreted as the average number of other individuals living in the same sample unit or quadrat per individual as is termed as the index of basic contagion. The measure of clump size is given as \((\alpha +1)\). The slope, \( \beta \), also called the density-contagousness coefficient, is used as a measure of the dispersion of the basic unit and indicates the spatial pattern of the clump (Iwao, 1968; Southwood, 1978; Davis, 1994).
Table 3. Total of adults and the percentage of the total, of each insect species associated to stored product caught in the traps in the stores, silo and on the floor of the silos structure (farmers) and in the store and the rice mill.

<table>
<thead>
<tr>
<th></th>
<th>Farmer’s storage</th>
<th>Rice mill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Warehouse Stores</td>
<td>Warehouse Store</td>
</tr>
<tr>
<td></td>
<td>Paddy rice</td>
<td>Silo</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>% total</td>
</tr>
<tr>
<td><strong>Coleoptera</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthicidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthicus floralis L.</td>
<td>1</td>
<td>2.6E-03</td>
</tr>
<tr>
<td>Bostrychidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ryzopertha dominica (F.)</td>
<td>2</td>
<td>2.7E-3</td>
</tr>
<tr>
<td>Carabidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harpalus rufipes (Deg)</td>
<td>1</td>
<td>2.6E-3</td>
</tr>
<tr>
<td>Cryptophagidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptophagus saginatus</td>
<td>119</td>
<td>0.31</td>
</tr>
<tr>
<td>Curculionidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sitophilus spp</td>
<td>8</td>
<td>0.02</td>
</tr>
<tr>
<td>Laemophloeidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptolestes sp.</td>
<td>1</td>
<td>2.6E-3</td>
</tr>
<tr>
<td>Cryptolestes ferrugineus</td>
<td>132</td>
<td>0.31</td>
</tr>
<tr>
<td>Lathridiidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coninomus constrictus</td>
<td>150</td>
<td>0.39</td>
</tr>
<tr>
<td>Coninomus nodifer (Westwood)</td>
<td>1</td>
<td>2.6E-3</td>
</tr>
<tr>
<td>Coninomus bifasciatus (Reitter)</td>
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<td>0.01</td>
</tr>
<tr>
<td>Mycetophagidae</td>
<td></td>
<td></td>
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<tr>
<td>Litargus balteatus J. Leclerc</td>
<td>7</td>
<td>0.02</td>
</tr>
<tr>
<td>Typheae stercorea (L.)</td>
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<tr>
<td>Nitidulidae</td>
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<td></td>
</tr>
<tr>
<td>Carophophilus dimediatus (F.)</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Ptinidae</td>
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<tr>
<td>Ptinus raptor Sturm</td>
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<td>0.20</td>
</tr>
<tr>
<td>Silvanidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ashaverus advena (Walt)</td>
<td>3</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Farmer’s storage</td>
<td>Rice mill</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>Warehouse Stores</td>
<td>Paddy Rice</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>% total</td>
</tr>
<tr>
<td><strong>Monotoma sp.</strong></td>
<td>1</td>
<td>2.6E-3</td>
</tr>
<tr>
<td><em>Oryzaephilus surinamensis</em> (L.)</td>
<td>11</td>
<td>0.03</td>
</tr>
<tr>
<td>Staphilinidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tenebrionidae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gnathocerus cornutus</em> F.</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Tribolim castaneum</em> (Herbst)</td>
<td>6</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Tribolim cofusum</em> du Val</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hemiptera</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthocoridiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lyctocoris campestris</em> F.</td>
<td>1</td>
<td>2.6E-3</td>
</tr>
<tr>
<td><strong>Hymenoptera</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pteromalidiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anisopteromalus calandrae</em> (Howard)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dinarmus basilis</em> Ashm.</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Lariophagus distinguendus</em> (Förster)</td>
<td>1</td>
<td>2.6E-3</td>
</tr>
<tr>
<td>Chalybidiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cerocephala</em> sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lepidoptera</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelechiidiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sitotroga cerealella</em> (Olivier)</td>
<td>16</td>
<td>0.02</td>
</tr>
<tr>
<td>Pyralidiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Plodia interpunctella</em> (Hb.)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Psocoptera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>389</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Dome traps used on the floor, outside the silos and dryers but inside the building.
Results

Environmental conditions

Farm storage
The mean temperature of paddy rice in the silo was 21.4±2.0°C. In the silo structure, the mean temperature was 14.6±0.4 °C and the relative humidity was 72.5±1.6%. The temperature ranged from 10.5°C during mid January to 17°C in the first week of December. The relative humidity varied from 57% in the first week of April to 80.5%, during the first half of December.

Rice mill
During June and July 2003 the mean temperature in the factory was 25.6±0.4°C and ranged from 24.5°C to 26.5°C. The relative humidity was 60.5±4.1% and varied from 46% to 70%.

Insect detection
The total number of insects and the percentage of the total of each insect species caught in the traps in stores, silo and on the floor of the farm silos structure as well as those insects caught in the stores and the rice mill are presented in Table 3.

Twenty-eight insect species of insects were identified, 21 in the farm storage and nine in the rice mill. Species of Psocoptera were not identified among these species. The percentages shown in the following sections do not result the 100% of the population, there was a residual population that consisted of various storage insects of about 14 species of low percentage each.

In the farm storage the three main species were:
- **Stores** – Coninomus constrictus (39%), Cryptophagus saginatus (31%) followed by Ptinus raptor (20%).
- **Silo**:
  - paddy rice – *P. raptor* (67%), *Gnathocerus cornutus* (20%) and *C. constrictus* (10%).
  - floor – *Sitophilus* spp. (73%), which 92% was *S. zeamais* and 8% *S. oryzae*, followed by *Carphophilus dimidiatus* (10%).

In the rice mill the main species were:
- **Stores**:
  - paddy rice - *Sitophilus* spp., (67%), of which 63% of the total was *S. zeamais* and 27% *S. oryzae*, and *Cryptocestes ferrugineus* (31%)
  - brown rice – *C. ferrugineus* (87%), *Sitophilus* spp. (11%), which 67% was *S. zeamais* and 23% *S. oryzae*, and Psocoptera
  - white rice (big bags) – Psocoptera
- **Factory**:
  - floor – *Sitophilus* spp. (49%), which 61% was *S. zeamais* and 39% *S. oryzae*, *C. ferrugineus* (35%) and *Tribolium castaneum* (13%)
  - walls – the Stogard Thinline caught no insects.

Psocoptera were constantly present at low populations and were associated with paddy rice and white rice. High population levels of Psocoptera were also present in brown rice. Additionally, some parasitoids of *Sitophilus* larvae and other coleopterans were caught during trials, primarily *Anisopteromalus calandra* (Howard).
Comparing trap catches of Pitfall and Probe traps

The results of trap catches from Pitfall and Probe traps are presented in Table 4. In the Probe-traps 345 adults of 10 different insect species were caught while during the same period, the Pitfall traps caught 16 adults of five insect species.

Table 4. Insect species associated to stored products caught by nine Pitfall and nine Probe traps at the surface of a paddy rice silo from 2 January to 14 March 2003.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pitfall</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptolestes sp.</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sitophilus zeamais</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Sitophilus oryzae</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Coninomus nodifer</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Coninomus constrictus</td>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>Ptinus raptor</td>
<td>9</td>
<td>228</td>
</tr>
<tr>
<td>Oryzaephilus surinamensis</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ashaverus advena</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gnathocerus cornutus</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Sitotroga cerealella</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Psocoptera</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>16</td>
<td>345</td>
</tr>
</tbody>
</table>

Relative density and spatial pattern of insect species associated with paddy rice in the farmer’s storage

Two population proprieties, namely density and dispersion, were examined for the most abundant insect species present in the farm storage. These species were *Coninomus constrictus*, *Cryptophagus saginatus*, *Gnathocerus cornutus*, *Ptinus raptor* and *Sitophilus* spp..

Density

The relative density of the five insect species caught in silos and stores are presented in Fig. 1. *Ptinus raptor* was the most abundant species collected in the silo and it was detected also in the flat stores and on the floor of silo structure. It was caught until the end of March.

The relative density of *Sitophilus* spp., especially *S. zeamais*, was particularly high in the product remains on the floor under the silo type dryer. It was significantly lower in the product but it was also detected in the traps placed in the paddy rice stored in stores and silo.

*Gnathocerus cornutus* was caught until the end of February, mainly in silo and its population was very low in stores and on the floor of silos structure.

The mycetophagous species, *C. constrictus* and *C. saginatus* were also caught in the traps placed in the paddy rice, in stores and silo. *C. constrictus* was caught during all trials while *C. saginatus* was caught mainly in the stores and until the end of February.
Fig. 1. Relative density of *Coninomus constrictus*, *Cryptophagus saginatus*, *Gnathocerus cornutus*, *Ptinus raptor* and *Sitophilus* spp, caught by traps placed in the stores, silos and in the floor of silos structure.

**Spacial pattern**

The Iwao’s patchiness regression estimates for adults of the five insect species are shown in Table 5. Except for the $\hat{x} - \bar{x}$ pairs of adults of *C. constrictus*, all pairs of the other four insect species followed well the regression.

The slope-$\beta$ classified as aggregated, the spatial pattern of habitat utilization by the adults of the five species: very strong tendency of aggregation for *C. saginatus,*
Sitophilus spp. and C. constrictus; weak but with a definitive tendency of aggregation for adults of P. raptor and G. cornutus.

The y-intercept $\alpha$ negative, obtained for adults of C. saginatus, Sitophilus and C. constrictus, can likely be interpreted as the effects of intraspecific competition among adults in the same colony. For C. constrictus, the results demonstrated a weak probability ($p = 0.13$) for the presence for one adult no influence the presence of another where the clump size ($\alpha+1$) is a single individual. For G. cornutus, this probability is higher ($p = 0.51$) and the results show a very weak tendency for the presence of a colony rather than a single adult. For P. raptor, the values of $\alpha$ ($\alpha+1 = 1+0.78\pm0.31$) suggesting that more than one individual exist together in the same quadrat.

Table 5. Iwao’s patchiness regression for adults of five insect species associated with paddy rice.

<table>
<thead>
<tr>
<th>Insect species</th>
<th>$n^a$</th>
<th>$\alpha \pm SE_\alpha$</th>
<th>$t = \alpha / SE_\alpha$</th>
<th>$\beta \pm SE_\beta$</th>
<th>$t = (\beta - 1)/ SE_\beta$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptophagus saginatus</td>
<td>21</td>
<td>-1.53±0.54</td>
<td>-2.84 (P=0.01)</td>
<td>10.37±1.12</td>
<td>8.36 (P=0)</td>
<td>0.81</td>
</tr>
<tr>
<td>Sitophilus spp.</td>
<td>45</td>
<td>-0.76±0.27</td>
<td>-2.86 (P=0.01)</td>
<td>3.45±0.19</td>
<td>13.07(P=0)</td>
<td>0.89</td>
</tr>
<tr>
<td>Coninomus constrictus</td>
<td>33</td>
<td>-1.32±0.86</td>
<td>-1.55 (P=0.13)</td>
<td>5.97±1.01</td>
<td>4.90 (P=0)</td>
<td>0.53</td>
</tr>
<tr>
<td>Ptinus raptor</td>
<td>38</td>
<td>0.78±0.31</td>
<td>2.56 (P=0.01)</td>
<td>1.21±0.08</td>
<td>2.53 (P=0)</td>
<td>0.86</td>
</tr>
<tr>
<td>Gnathocerus cornutus</td>
<td>25</td>
<td>0.11±0.16</td>
<td>0.66 (P=0.51)</td>
<td>1.25±0.17</td>
<td>1.50 (P=0)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

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

Discussion

The most abundant insect species reported in farm storage (C. saginatus and C. constrictus) tend to be associated with moulds and accumulations of residues (P. raptor) and are usually an indication of deteriorating grain due to poor storage conditions, or lack of an effective management program. However, the main insect species discovered in the rice mill are important rice pests (Sitophilus spp. and C. ferrugineus). S. zeamais and S. oryzae were the two species identified, although S. zeamais was the dominant species in all records from the farm storage to the rice mill.

Results tend to indicate that paddy rice stored on the farms surveyed increases in moisture because of increasing humidity over the storage life. This increase in paddy rice moisture favours mould growth and therefore the presence of the mycetophagous species of insects (Adler, 1998). This implies the risk of mycotoxin production if critical moisture contents are exceeded and it would thus be recommended to avoid such conditions by drying procedures.

When conditions tend to be cooler, trap captures may not truly reflect the actual populations as inactivity of the pest species and/or migration may have affected different species to a different extent. Possibly examining grain samples from various positions of the grain stores may give a more accurate picture in future surveys.

The number of insect species associated with the processing facility compared to the trap captures at farmers storage decreases significantly, while the presence of primary insect pests such as Sitophilus spp, in paddy rice, and C. ferrugineus, in brown rice stand out dramatically. The presence of Psocoptera, especially in the rice mill in brown rice was quite consistent.
The most abundant insect species present in the farmers’ storage, were recorded mainly in the vertical silo, as *G. cornutus* and *P. raptor*; in stores and silo, *C. saginatus* and *C. constrictus*; while *Sitophilus* spp. were discovered mainly in the residues of product under dryers. In addition, the adults of these insect species were distributed in an aggregate pattern. In the colonies, between individuals, *C. saginatus, Sitophilus* spp. and *C. constrictus* may show some repulsion interaction and competition; the presence of an adult of *G. cornutus* does not influence the presence of other and the clump size is a single individual and for adults of *P. raptor*, more than an individual tend to live together in the same quadrant.

The Probe trap was significantly the most efficient trap when compared with the Pitfall trap, regarding the number of adults and the insect species recorded in each type. The efficacy of Probe traps have also been recorded from several authors (Lippert & Hagstrum, 1987; Fargo *et al.*, 1994; Hagstrum et al, 1998; Hagstrum, 2000).

Acknowledgements

The authors express their gratitude to Dr. Jordi Riudavets for his contribution in this study. We also thank APARROZ and paddy rice farmers association for allowing the conductance of the trials in their stores and silos structures and especially the manager and friend João Reis Mendes. We thank SEAR, for allowing the trials in the rice mill, especially Dr. Romano Mancini and Altino Teixeira; and Trécé (Salinas, EUA), specially Bill Lingren and Selina AA-Stewart for their supply of the Storgard Dome and Thinline traps.

References

Evaluation of the efficacy of different kinds of pheromone traps for the monitoring of *Plodia interpunctella* Hbn. (Lepidoptera: Pyralidae)

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*Corresponding author e-mail: sara.savoldelli@unimi.it*

**Abstract:** In food industries, different kinds of pheromone traps are used for the monitoring of *Plodia interpunctella*, but in many cases, it is difficult to relate captured adults with the real density of moth’s population. In this work, the results of laboratory tests of four kinds of pheromone traps have been reported. Three kinds of sticky trap (wing trap, delta trap, strip trap) and a funnel trap have been tested in two Peet & Grady chambers of 28 and 30 m$^3$ with a known number of adults. The wing trap has been tested in three different ways: with only adhesive base, with only adhesive top and with both base and top glued. Captured adults have been counted after 24 and 48 hours. The results are discussed.

**Key words:** *Plodia interpunctella*, pheromone trap, efficacy, monitoring.

**Introduction**

The monitoring has assumed in the last years a more and more important role in the integrated pest management strategies, which suggest a limited use of chemicals to the advance of alternative methodologies. In warehouses and food industries, pheromone traps represent an essential instrument to monitor insect populations and to give useful information for struggle.

On the market, it is possible to find different kinds of moth’s traps; the choice to use one or the other depends on the infesting species, on the environmental characteristics (e.g. much or little dust) and on the area to keep under monitoring.

Usually, the most used traps are those to hang up in the room’s centre at 2-2.50 m from the floor, but there are also traps to be put under the shelves or in confined areas that, as underlined by Mullen & Dowdy (2001), remain out of view in order not to give the impression that the surrounding area is dirty or insect-infested. Besides this trap, there are oil traps that, activated with more pheromones, are able to catch different insects simultaneously.

Whatever the trap’s type may be, in presence of a certain number of trapped insects, it is essential to have an idea of the insect population density, as reported by Pereira *et al.* (2002) who have used the pheromone traps to estimate moth population density at different points within a flour mill. It is also important to know the efficacy of each trap’s type: many researchers have evaluated the effectiveness of some traps in warehouse or laboratory. Cogan & Hartley (1984) have experimented the efficacy of funnel trap in laboratory, on *Ephesia kuehniella* Zell., with a comparison of sticky and non-sticky trap models; a multifunnel trap was compared by Trematerra *et al.* (1994) in a warehouse with the effectiveness of other traps baited with pheromones of some moths and beetles infesting stored products and with the same traps without baits. Papadopoulou & Buchelos (2002) have made a comparison of trapping efficacy for *Lasioderma serricorne* (F.) adults with electric, pheromone, food attractant baited and control-adhesive traps; Ryne *et al.* (2002) have demonstrated the efficacy of water trap that caught more *Ephesia cautella* than pheromone trap without water and more than the one with water plus pheromone. Athanassiou *et al.* (2003) have examined the efficiency of the multisurface trap for the capture of *Ephesia kuehniella*.
The aim of this work was to compare four kinds of traps, commonly used for monitoring the Indian Meal Moth, *Plodia interpunctella* Hbn. (Lepidoptera: Pyralidae), and to estimate, in presence of a known number of males, the catch percentage of each trap. Therefore, it was possible a direct comparison with different traps to establish the most efficient one at the same experimental conditions. It is known, in fact, that there are many factors which may affect the captures: trap design and colour, location in the warehouse, ability to retain the insects after the capture, type of storage facility, food inside the facility, environmental temperature, type of lure, etc. (Levinson & Hoppe, 1983; Trematerra et al., 1994; Mullen et al., 1998).

**Materials and methods**

The four traps tested were funnel, wing, delta and strip traps. The first three are usually used for monitoring the moths in warehouses and food industries, the last one is an experimental trap, consisting of a yellow strip of 0.4 mm thick paperboard, 10 cm width and 25 cm height, with one surface glued. An adhesive trap consisting of vertically suspended strip was successfully used for the monitoring of moths populations in a chocolate factory by Hoppe & Levinson (1979) and in a flour mill by Levinson & Buchelos (1981).

The strip trap can be located where the use of other models are uncomfortable, in particular, it can be used in automatic warehouses, developed in vertical direction to exploit all the space, where various products are stored. The traditional traps cannot be employ for monitoring the high flats of these structures because the height of the shelves makes the capture’s control difficult. On the contrary, the sticky strip trap, baited with pheromone (the lure was applied in the middle of the strip), can be placed in the highest flats; the caught insects are easily evident from below thanks to the vertical position of the trap and its clear colour.

The wing trap has been tested in three different ways: with only adhesive base, with only adhesive top and with both base and top glued. In the second and third case the lure was applied on the top. The wing traps with adhesive top have been tested for applications in dusty environments.

All traps have been activated with a caoutchouc lure impregnated with 0.2 mg of (Z,E)-9,12-tetra-decadenylacetate (TDA) (average daily release 30 µg/day).

The male larva III-IV can be separated because it shows a dark patch of the sketches of male gonads on the abdominal segments.

The tests have been made in two Peet & Grady chambers with a known number of unmated males of *Plodia interpunctella*. Each trap was tested alone, in presence of 10 or 100 unmated 2-3-day-old males (Levinson & Hoppe, 1983), with four replications for each kind of trap. The lure was changed after the fourth repetition.

The tests in presence of 10 males have been made in the Peet & Grady chamber of 28 m³, the tests with 100 males in that of 30 m³, at a temperature of 25°C, relative humidity of 50% and natural light. The caught insects have been counted 24 and 48 hours after the release.

The data have been elaborated with ANOVA and Duncan’s *post hoc* test; the number of captures was expressed in terms of percentage on the total of released males. In the tables 1 and 2, the different letters represent statistically differences with Duncan’s test (P≤0.05)

**Results and discussion**

Duncan’s *post hoc* test showed that there are significant differences in trap’s captures (Table 1). The funnel trap captured the lowest percentage of males. Among the adhesive traps, the highest percentage of captures was with wing and delta traps, the lowest with the strip trap; in
this one, a higher number of adults were captured at the lowest part of the sticky surface, as observed by Athanassiou et al. (2003).

The wing traps with adhesive base and top and with only adhesive top have captured a similar percentage of males as the wing with adhesive base. The adhesive base captured less males than the top (Table 2).

Table 1. Cumulative percentage of captured males of *Plodia interpunctella* in 24 and 48 hours after the release of 10 moths.

<table>
<thead>
<tr>
<th>Trap</th>
<th>% male captured after 24 hours ± SD</th>
<th>% male captured after 48 hours ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Funnel</td>
<td>30±14 a</td>
<td>40±14 a</td>
</tr>
<tr>
<td>Delta</td>
<td>55±13 b</td>
<td>80±8 bc</td>
</tr>
<tr>
<td>Strip</td>
<td>47±10 ab</td>
<td>67±10 b</td>
</tr>
<tr>
<td>Wing (adhesive base)</td>
<td>77±17 c</td>
<td>90±8 c</td>
</tr>
<tr>
<td>Wing (adhesive top)</td>
<td>85±17 c</td>
<td>87±15 c</td>
</tr>
<tr>
<td>Wing (adhesive base and top)</td>
<td>65±13 bc</td>
<td>80±8 bc</td>
</tr>
</tbody>
</table>

Table 2. Cumulative percentage of captured males of *Plodia interpunctella* in 24 and 48 hours after the release of 10 moths with the wing trap (adhesive base and top).

<table>
<thead>
<tr>
<th>Wing trap with adhesive base and top</th>
<th>% male captured after 24 hours ± SD</th>
<th>% male captured after 48 hours ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>17.5±9.6</td>
<td>22.5±9.6</td>
</tr>
<tr>
<td>Top</td>
<td>47.5±9.6</td>
<td>57.5±5.0</td>
</tr>
</tbody>
</table>

It was always noticed that the highest number of captured males was in the first 24 hours. In the second 24 hours, trap’s captures increased on average only of 14% on the total. However, in presence of 10 males, the average percentage of each trap increased if compared to the test with 100 males. Muirhead-Thomson (1991), in earlier experiences with sex traps for *Choristoneura fumiferana* (Lepidoptera: Tortricidae), reported that the trapping effectiveness of a virgin female is affected by population density: with 70 males/acre, the female attraction was ten times higher than when the density was 4000 males/acre.

Levinson and Buchelos (1981) monitored for two years *Ephestia cautella* in a flour mill with TDA-baited traps. During the second year, they observed an increase of trap efficiency that can be related to a relatively low population density, in addition to the increased flight frequency. Sower et al. (1975) suggested that the control of the moths in enclosed environments with aid of the pheromone would have been feasible only when population densities were lower than 0.1 pair/m² wall surface; the effectiveness of a given dose of pheromone markedly increased as population densities of Indian Meal Moth were decreased from 10 to 0.1 pairs/m². Trematerra (1997) said that pheromone traps are generally effective when pests number is very low.

The monitoring with pheromone traps is particularly useful when population density is low because it makes precociously evident an infestation. When population density is high,
the infestation is evident also through a visual inspection, so the number of captures is less important.

Nearly always, in the tests with 100 males, the average percentage of captures was under 50% (Table 3), only the wing trap with adhesive base caught 50.2% in 48 hours. Also in this case the highest number of captured males was in the first 24 hours. It was confirmed that the most efficacy traps were delta and wing traps.

In these tests, the wing trap with only adhesive top was less effective than the wing trap with adhesive base. In the wing trap with adhesive base and top the adhesive base captured more males than the top (table 4).

The experimental strip sticky trap was the less efficient of all the sticky ones: in fact, although *P. interpunctella* has an orientation preference for narrow rectangles (Levinson & Hoppe, 1983), the vertical position is not the best for the moth’s flight.

Table 3. Cumulative percentage of captured males of *Plodia interpunctella* in 24 and 48 hours after the release of 100 moths.

<table>
<thead>
<tr>
<th>Trap</th>
<th>% male captured after 24 hours ± SD</th>
<th>% male captured after 48 hours ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Funnel</td>
<td>20±13 ab</td>
<td>28±12 ab</td>
</tr>
<tr>
<td>Delta</td>
<td>35.5±3.2 cd</td>
<td>43.0±3.2 c</td>
</tr>
<tr>
<td>Strip</td>
<td>14.3±7.3 a</td>
<td>16.5±7.8 a</td>
</tr>
<tr>
<td>Wing (adhesive base)</td>
<td>45.2±5.5 d</td>
<td>50.2±6.7 c</td>
</tr>
<tr>
<td>Wing (adhesive top)</td>
<td>27.2±7.9 bc</td>
<td>30.2±8.7 b</td>
</tr>
<tr>
<td>Wing (adhesive base and top)</td>
<td>34.7±5.8 cd</td>
<td>38.5±7.8 bc</td>
</tr>
</tbody>
</table>

Table 4. Cumulative percentage of captured males of *Plodia interpunctella* in 24 and 48 hours after the release of 100 moths with the wing trap (adhesive base and top).

<table>
<thead>
<tr>
<th>Wing trap with adhesive base and top</th>
<th>% male captured after 24 hours ± SD</th>
<th>% male captured after 48 hours ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>27.0±3.4</td>
<td>30.0±6.5</td>
</tr>
<tr>
<td>Top</td>
<td>7.7±7.3</td>
<td>8.5±7.0</td>
</tr>
</tbody>
</table>

It was confirmed that in presence of a low number of males, the effectiveness of all traps increased. In all tests the highest number of captures was in the first 24 hours.

The funnel trap is not as efficient as sticky traps in capturing Indian Meal Moths, although it is more adaptable in particular situations (e.g. dusty areas) and it has the capacity to contain a lot of insects compared to sticky traps, which become saturated early.

The strip trap has demonstrated a low efficacy compared to other sticky traps. In any case, it can be used in the highest flats of automatic warehouses, where others kinds of traps cannot be useful for the monitoring.

It was confirmed that the wing trap is the most effective for the monitoring of Indian Meal Moth.
The two types of wing traps with adhesive top have demonstrated a similar percentage of captures if compared to the wing trap with adhesive base, although the percentage of the top (in the wing trap with both surfaces glued), with 100 males, was low. They can be applied in dusty environments instead of funnel trap.

These data, associated to those obtained from new monitoring techniques, like spatial and geostatistical analysis, are useful tools to estimate the moths' populations in warehouses and food industries (Arbogast & Manking, 1999; Arbogast et al. 2000; Trematerra & Sciarretta, 2002).

References


Piophila casei L. (Diptera: Piophilidae) monitoring in cheese ripening storehouses

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Abstract: The authors report the results of monitoring tests of the Cheese Fly, Piophila casei L. (Diptera Piophilidae) carried out in Sicilian cheese storehouses by mean different attractants. During summer 2002, the monitoring was carried out with MacPhail traps activated by a water solution (4%) of hydrolyzed proteins, acetic acid, saccharose and, as comparison, only water; during summer 2003, the monitoring was carried out using traps activated with two different concentration of water solution of butyric acid (2 e 4%), acetic acid (4%) compared with the water only. The captures were expressed as the mean numbers of flies caught per trap per week. The data collected were analyzed by ANOVA test.

Key words: cheese fly, monitoring, traps, attractants

Introduction

“Ragusano” is a typical Italian cheese, one of the most ancient of Southern Italy production. The ripening can persist four-six months, in cool and ventilate storehouse; during the ripening phase, the cheese could be infested by Piophila casei L, a cosmopolitan fly, also known as the “cheese fly”.

P. casei is a typical eusynanthropic fly, showing high degree of endophily occurring in various branches of food industry, such as slaughterhouses, other meat factories, poultry farm, storage of fresh hides and fish canneries (Zuska, 1965), but especially it is considered the main pest of cured hams (Fulton, 1953) and dairy products (Zuska, 1965; Hegazi, 1978).

The sanitary importance of P. casei is great, infact it is a typical communicative fly, visiting filth, carrion, stored cow horns hides, bristles (Zuska, 1965); besides it is a vector of several micropathogens, as the human and animals parasite Listeria monocytogenes (Domenichini, 1991). Also P. casei belongs to the cadaveric fauna, as the larvae are often recovered on human bodies after active decay, as the body to dry (Byrd, 2001). The larvae are called cheese skipper for their behaviour, infact they are able to jumping to into the air 3 to 4 inches; infact P. casei larvae grasp small protrusions on the anal segment with the mouth hooks and suddenly release their grip. This mechanism is also utilized during larval migration (Byrd, 2001). The skipping ability is most pronounced in the mature larva and enables it to find a safe pupation place (Simmons, 1927). The cheese skipper could be eaten with infested food and could be responsible for gastric and intestinal myasis (Zumpt, 1965).

The need to elevate sanitary standard in food production and the damage caused to food industries make necessary to improve the monitoring techniques for P. casei.

The specific purpose of this study is to determinate the relative attractiveness of baits components for P. casei.
Material and methods

*P. casei* monitoring was been carried out during the years, 2002-2003, in two traditional ripening storehouses (called farm A and farm B) of South-Eastern Sicily (Italy), using MacPhail traps activated with several attractants. During summer 2002, monitoring was carried out using 12 MacPhail traps for farms activated with water solution (4%) of hydrolyzed proteins, acetic acid, saccharose and water as comparison. Three traps for each component are used. During summer 2003, monitoring is carried out using 12 traps activated with two different concentration of water solution of butyric acid (2 e 4%), acetic acid (4%) and water.

All traps were checked and emptied weekly. Each trap was moved along one position, following a circular exchange plan, to reduce the “site effect” on the captures. The flies collected were counted and identified in the laboratory with a stereomicroscope.

Trap captures were expressed as the mean numbers of flies captured per trap per week. Differences in the capture among the four substances were determined using analysis of variance (ANOVA). Significant ANOVAs were followed by the Duncan mean separation tests (P = 0.05), using Statistica V.5.1. (Statsoft Inc., 1997).

Results and discussion

The total number of *P. casei* flies collected with the MacPhail traps baited with the four substances, during summer 2002, in farm A and B, are reported in Fig. 1. In farm A the population of *P. casei* adult flies was extremely low and weekly trapping data never exceed a total of 31 flies. Unlike farm A, high populations of *P. casei* were present in farm B and weekly trapping data is about of 200 total flies, during September and October.

The largest number of flies collected in farm B is to correlate with the internal farm favourable environmental conditions (both temperature and moisture) for the piophilid, respected with farm A conditions (Figs. 2 and 3).

![Fig. 1. Total number of *P. casei* adult flies caught in farm A and B, during the 2002.](image-url)
The data show that during the summer 2002, significantly more *P. casei* male flies were caught in farm A on traps baited with water solution of saccharose than on traps baited with water solution of proteins hydrolysate and only water (Table 1). On average the traps baited with saccharose captured more than two times (0.55 flies per traps) than the other two traps (respectively 0.17 and 0.21 flies per traps). The response of *P. casei* female flies in farm A shows that no significant differences are in the performance of the four traps.

![Fig. 2. Thermo hygrograph values recorded in farm A during 2002.](image1)

![Fig. 3. Thermo hygrograph values recorded in farm B during 2002.](image2)
The data of experiment in farm B are more interesting, in relation to the high populations of *P. casei*. Significantly more *P. casei* male flies were caught on traps baited with saccharose compared with the traps baited with proteins hydrolysate and only water. Besides significantly more *P. casei* female flies were caught on traps baited with water solution of acetic acid than on traps baited with water only (respectively 10.81 flies per trap and 3.57 flies per trap).

Table 1. Attraction of *P. casei* flies to traps baited with the four substances, in farm A during 2002. Means in the same column followed by the same letter are not significantly different (P=0,05).

<table>
<thead>
<tr>
<th></th>
<th>males</th>
<th>females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyd. Proteins</td>
<td>0,17±0,49 b</td>
<td>0,74±1,50a</td>
</tr>
<tr>
<td>Saccharose</td>
<td>0,55±1,02 a</td>
<td>0,48±0,89a</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>0,31±0,68ab</td>
<td>1,17±2,94a</td>
</tr>
<tr>
<td>Water</td>
<td>0,21±0,52 b</td>
<td>0,52±1,21a</td>
</tr>
</tbody>
</table>

Table 2. Attraction of *P. casei* flies to traps baited with the four substances, in farm B during 2002. Means in the same column followed by the same letter are not significantly different (P=0,05).

<table>
<thead>
<tr>
<th></th>
<th>males</th>
<th>females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyd. Proteins</td>
<td>1,02±1,67 b</td>
<td>6,38±13,93ab</td>
</tr>
<tr>
<td>Saccharose</td>
<td>2,31±4,62 a</td>
<td>5,48± 9,57ab</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>1,95±2,48ab</td>
<td>10,81±19,23 a</td>
</tr>
<tr>
<td>Water</td>
<td>0,95±1,29 b</td>
<td>3,57± 4,64 b</td>
</tr>
</tbody>
</table>

The *P. casei* flies collected with the MacPhail traps baited with the two concentrations of butyric acid, acetic acid and water, during summer 2003, in farm A and B, are reported in figure 4. Also during this summer, in farm A the population of *P. casei* was very low, infect only in two week the traps have caught some flies (2 and 3 flies per trap). This data are in relation with the climatic conditions inside the farm; infect the storehouse is provided with air-conditioning, at constant temperatures of 15± 1 °C. During 2003, in farm B were present a higher populations of *P. casei* than the previous year. The weekly trapping data was of 6000 total flies, during second week of July. This is in relation with the temperature data recorded inside the farm B, infect as figure 5 shows, it is about 23-24°C, during the experiment, besides during the end of May very high temperature are been recorded in Sicily.

The captures of *P. casei* female and male flies in farm A on the four types of traps, during the same period, did not differ significantly (table 3); infect we didn’t catch any *P. casei* females, while very low *P. casei* male are been caught by the traps.

High populations of *P. casei* adult fly are present in Farm B, during 2003. More *P. casei* female than male have been collected, besides significantly more *P. casei* male and female flies have been found on traps baited with butyric acid than water.

The data of experiment shows that traps baited with acetic and butyric acids are effective for the monitoring of the *P. casei* adult flies, these free fatty acids are some of those the cheeses release, during lipolysis that take place in the ripening phase.

A possible future line direction for improving the monitoring technique’s could be to study the attraction of other free fatty acids for *P. casei* adults both in farms and in laboratory.
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A possible future line direction for improving the monitoring technique’s could be to study the attraction of other free fatty acids for *P. casei* adults both in farms and in laboratory.

![Figure 4](image1.png)

**Fig. 4.** Total number of *P. casei* adult flies caught in farm A and B, during 2003.

![Figure 5](image2.png)

**Fig. 5.** Thermo hygrograph values recorded in farm B during 2003.
Table 3. Attraction of *P. casei* flies to traps baited with the four substances, in farm A during 2003. Means in the same column followed by the same letter are not significantly different (P=0.05).

<table>
<thead>
<tr>
<th></th>
<th>males</th>
<th>females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric Acid 4%</td>
<td>0,05±0,22a</td>
<td>0a</td>
</tr>
<tr>
<td>Butyric Acid 2%</td>
<td>0a</td>
<td>0a</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>0,05±0,22a</td>
<td>0a</td>
</tr>
<tr>
<td>Water</td>
<td>0,14±0,65a</td>
<td>0a</td>
</tr>
</tbody>
</table>

Table 4. Attraction of *P. casei* flies to traps baited with the four substances, in farm B during 2003. Means in the same column followed by the same letter are not significantly different (P=0.05).

<table>
<thead>
<tr>
<th></th>
<th>males</th>
<th>females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric Acid 4%</td>
<td>113,48±132,04 a</td>
<td>351,24±465,40 a</td>
</tr>
<tr>
<td>Butyric Acid 2%</td>
<td>79,52± 99,85 a</td>
<td>372,81±598,31 a</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>63,86± 84,56ab</td>
<td>290,86±324,67ab</td>
</tr>
<tr>
<td>Water</td>
<td>16,19± 15,53 b</td>
<td>38,81± 48,66 b</td>
</tr>
</tbody>
</table>

References


Phytochemicals
Comparative potential of powders and essential oils from leaves of *Clausena anisata* and *Eucalyptus saligna* to protect stored grains from attack by *Callosobruchus maculatus* and *C. chinensis* (Coleoptera, Bruchidae)

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Abstract: Powders and essential oils extracted from *Clausena anisata* and *Eucalyptus saligna* leaves along with anethole and cymol respectively identified as their main constituents were tested under laboratory conditions at 25-27°C and 70-75% RH for their ability to protect stored grains from attack by *Callosobruchus maculatus* (F.) and *C. chinensis* (L.). The contact toxicity of the phytochemicals assayed by mixing the dry ground leaves and crude volatile oils with grains or by impregnating the crude oils and pure volatile compounds on filter paper discs showed that the products derived from *C. anisata* leaves were highly toxic to the two insect species. A concentration of 10% of powder from dry ground leaves of *C. anisata* with mung beans(*Vigna mungo*) killed 60% of *C. chinensis* after 48h exposure. A dose of 8µl/40g of *C. anisata* oil on grain was enough to induced 80% and 70% mortality of *C. maculatus* and *C. chinensis*, respectively after 24h exposure. Insect mortality on filter paper discs-impregnated with oils was dose-dependent with the highest dose of 8µl/disc of all oils causing more than 80% mortality of both insects after 24h exposure. All the crude oil extracts and pure volatile compounds were highly repellent to the two insect species tested with overall repellency values in the range of 70-95%.

Résumé: Les poudres et huile essentielles de feuilles de *Clausena anisata* et *Eucalyptus saligna* ainsi que l’anethole et le cymol identifiés respectivement comme étant leurs constituants majoritaires ont été testées au laboratoire pour leur habilité à protéger les grains en stockage contre les attaques de *Callosobruchus maculatus* (F.) et *C. chinensis* (L.). Ces huiles essentielles extraites par hydrodistillation ont été analysées par chromatographie en phase gazeuse (CPG) couplée à la spectrométrie de masse (SM). Les tests de toxicité par contact dans les grains traités par les poudres et huile essentielle ainsi que de contact et de répulsion sur papier filtre imprégné d’huile essentielle à différentes doses ont été réalisés à température et humidité maintenues entre 25-27°C et 70-75% HR. Les résultats montrent que la poudre et l’huile essentielle des feuilles de *C. anisata* sont plus toxique à l’égard de ces deux insectes. La dose de 10% (poids/poids) de poudre de *C. anisata* dans les grains de haricot (var. *Vigna mungo*) a occasionné une mortalité de 60% de *C. chinensis* après 48 heures et la dose d’huile de 8µl/40g de grain de *C. anisata* a occasionné des mortalités respectives de 80 et 70% de *C. maculatus* et *C. chinensis* au bout de 24 heures. La toxicité par contact des huiles imprégnées sur papier filtre varie avec la dose d’huile utilisée. C’est alors que la dose de 0,416µl/cm² pour chacune des huiles a occasionné plus de 80% de mortalité des deux types d’insectes au bout de 24 heures. L’anethole, le cymol et les huiles essentielles des deux plantes ont montré une répulsion assez significative vis à vis de ces deux insectes avec des pourcentages de répulsion moyen entre 70-95%.

Key words: *Clausena anisata*, *Eucalyptus saligna*, essential oil, anethole, cymol, contact toxicity, repellency
**Introduction**

Stored grain protection against insect infestation has always been a great challenge all over the world. The situation is even more complicated in developing countries because of problems such as high cost and erratic supply of conventional insecticides. In addition, their misuse generally induces many environmental hazards such as farmers' health risks and environment pollution. The need to look for alternative methods of insect control have prompted us to initiate a programme of screening African plants extracts for insecticidal activities.

Small scale farmers in the western highlands of Cameroon as well as in many other developing countries usually mix stored foodstuffs with different kinds of plant materials for protection against pest damage (Parh et al., 1998; Hassanali et al., 1990; Poswal and Akpa, 1991). As described in an earlier paper (Tapondjou et al., 2000) the dry ground leaves of some local plants among which *Clausena anisata* and *Eucalyptus saligna* are mainly used in sealed traditional storage structures by communities of the western highlands of Cameroon to protect different variety of grains against insect infestation. The dry leaves of these plants emit strong and persistent aromatic odours for long periods of time thus suggesting their high content of volatile oils. Although a lot of works have been published on the insecticidal activities of the non-volatile extracts and components of *C. anisata* and *E. saligna* (Gebreyesus and Chapya, 1983; Hideo et al., 1990; Noriyuki et al., 1989; Satoshi et al., 1992) very few information is available concerning the insecticidal effects of their essential oils.

The present work was undertaken to describe the extraction and chemical characterisation of the volatile oils from these plants as well as laboratory bioassays for evaluating their compare toxicity against two stored grain pests: *Callosobruchus maculatus* and *C. chinensis*.

**Material and methods**

**Plant materials and essential oils extraction**

The leaves of *C. anisata* and *E. saligna* were collected in April 1999 during the fruiting season in the Bafou village located in the Menoua Division of the Western highlands of Cameroon. The plants were identified by the botanists of the Department of Plant Biology, University of Yaounde I, Cameroon and the voucher specimens deposited at the Camerounian National Herbarium. After three days drying at room temperature (25-28°C), 1000g of each plant material were ground and subjected to hydro-distillation in an apparatus similar to the Clevenger type extractor for 8h. Oils collected were dried over anhydrous sodium sulphate filtered and weighed yielding 0.9% (w/w) of fair yellow oil for *E. saligna* and 2% (w/w) of a strong sweet smelling and a brownish oil from *C. anisata* leaves. These samples were thereafter kept in the refrigerator until use.

Analysis of the oils collected were carried out by GC-MS on a HP 5890 II gas chromatograph coupled to a HP 5972 mass selective spectrometer using a DB wax fused silica capillary column (60m x 0.25µm film thickness). Cymol (99.5% purity) and anethole (99% purity) used for bioassays were respectively purchased from Fluka Chemika and Aldrich Limited, Germany.

**Insects**

*C. maculatus* and *C. chinensis* were reared in the laboratory at 25-27°C and 70-75% RH in the dark. Parent adults were obtained from laboratory stock cultures maintained at the Federal Biological Research Centre for Agriculture and Forestry, Institute for Stored Product
Protection of Berlin, Germany. The food media used were green peas for *C. chinensis* and mung beans (*Vigna mungo*) for *C. maculatus* obtained in Berlin, Germany.

**Contact toxicity of dry ground leaves, crude essential oils, and pure volatile compounds on grains and on filter paper discs**

The different bioassays were conducted in the laboratory at 25-27°C and 70-75% RH in the dark with unsexed 1-2 days old adults of each insect species.

The fine powder obtained from dry ground leaves of *C. anisata* and *E. saligna* was mixed separately with 50g of grains in 380ml glass jars at four different dosages ranging from 1.3 - 10% (w/w) for the two insect species. The plant product/grain admixtures were thoroughly mixed with a rotary shaker for 20min. The control in each set of treatments consisted of grain containing no plant material and each dosage was replicated four times. The contact effects of each of the crude oil extracts on grains were evaluated by mixing with the rotary shaker 40g of grains contained in a 380ml glass jar with different solutions of each of them obtained by diluting 1, 2, 4, 8, 16 and 32µl of oil in 1ml of acetone. The contact toxicity of the phytochemicals on filter paper was evaluated by treating a 7cm diameter (38.5cm²) Whatman N°1 filter paper disc contained in a 7cm diameter Petri dish with different solutions of the phytochemicals ranging from 0.0032 to 0.200µl/cm².

The control grains and filter paper discs were only treated with pure acetone. Each treatment was replicated four times and acetone was allowed to evaporate for 10min in each case prior to the infestation of treated grains and filter paper discs with twenty insects.

Insect mortality in ground leaves-treated grains were recorded after 48h exposure whereas in the other cases it was recorded after 24 hours exposure. The percentage mortality in each case was calculated by using the Abbott formula (Abbott, 1925). The different lethal doses (LD₅₀) of the phytochemicals were calculated using probit analysis in which probit-transformed proportion mortality was regressed against log10 dose (Finney, 1971).

**Repellency bioassay**

The repellent effect of different crude essential oils and pure compounds against the tested insects was assessed by the area preference test described by McDonald et al. (1970). Test areas consisted of 7 cm Whatman N°1 filter paper disc cut in half. Different solutions were prepared by diluting 1, 2, 4 and 8 µl of each crude oil and 0.5, 1, 2, 4 µl of anethole or cymol in 0.5ml acetone. Each solution was applied to a half filter paper disc (19.5 cm²) as uniformly as possible with a micropipette thus corresponding to the dosages of 0.52, 0.104, 0.208 and 0.416 µl/cm² for crude oils and 0.026, 0.52, 0.104, 0.208 µl/cm² for anethole or cymol. The other filter paper halves used as controls were only treated with acetone and both were air-dried for 10 min to evaporate the solvent completely. Each treated half disc was then attached lengthwise, edge-edge to a control half-disc with cello tape. The remade disc was placed in a Petri dish and 20 unsexed insects of each species were released at its centre. Each treatment was replicated five times. The number of insects present on control (Nc) and treated (Nt) half discs were recorded after 2h exposure.

Percentage repellency (PR) values were calculated as follows:

\[
PR = \left[\frac{(Nc-Nt)}{(Nc+Nt)}\right] \times 100
\]

Mean repellency values were assigned to repellency classes (McDonald et al. 1970; Juliana and Su, 1983) from 0 to V: class 0 (PR< 0.1%), class I (PR = 0.1-20%), class II (PR = 20.1-40%), class III (PR = 40.1-60%), class IV (PR = 60.1-80%) and class V (PR = 80.1-100%).
Results

The volatile constituents of the two crude oil extracts were identified by their retention index and mass spectrum in comparison with those of standard synthetic compounds. The results of the chemical analysis are presented in Table 1. From these results it is clear that the amount of anethole (which is mainly known to occur naturally as trans-anethole) was very high (80.8 %) in Clausena oil. Other constituents detected were trans-isoeugenol methyl ether (11.5 %), δ-terpinene (1.7 %) and germacrene D (1.0 %). Eucalytus oil is mainly consisted of α-pinene (39.4 %), cymol (p-cymen) (31.1 %), 1,8-cineole (9.8 %) and δ-terpinene (9.5 %). The high yield (2 % w/w) of the essential oil obtained from C. anisata and its high concentration of trans-anethole (80.8 %) suggest that this plant could be exploited as potent source of trans-anethole such as anise Pimpinella anisum L. and Illicium verum Hook F. (Saraç and Tunç, 1995; Ho et al., 1997).

Table 1. Main chemical constituents of volatile oils from the leaves of C. anisata and E. saligna.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. anisata</td>
<td>E. saligna</td>
</tr>
<tr>
<td>α-pinene</td>
<td>–</td>
</tr>
<tr>
<td>Sabinen</td>
<td>0.5</td>
</tr>
<tr>
<td>Myrcen</td>
<td>0.7</td>
</tr>
<tr>
<td>1,8-cineol(eucalyptol)</td>
<td>–</td>
</tr>
<tr>
<td>δ-terpinen</td>
<td>1.8</td>
</tr>
<tr>
<td>Cymol(p-cymen)</td>
<td>–</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>–</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>–</td>
</tr>
<tr>
<td>Germacrene D</td>
<td>1.0</td>
</tr>
<tr>
<td>trans-anethole</td>
<td>80.8</td>
</tr>
<tr>
<td>trans isoeugenol methyl ether</td>
<td>11.5</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>–</td>
</tr>
</tbody>
</table>

Insect mortality in grain

Figures 1 and 2 show the variation in percentage mortality of the two insect species on grain treated with different doses of plant materials. E. saligna applied as dry ground leaves was not toxic to C. chinensis after two days exposure whereas its highest dose (10 %) induced more than 35 % mortality of C. maculatus within the same period. The highest dose of C. anisata ground leaves (10 %) killed 60 % of C. maculatus and only 40 % of C. chinensis after 48 h (Fig. 1) thus indicating that C. maculatus is more susceptible than C. chinensis to dry ground leaves of the two plants with C. anisata leaves evoking the higher toxicity. This is further supported by the LD50 values calculated on the second day exposure (Table 2).

As presented on Fig. 2 the highest dose of Clausena oil on grain (8 µl/40g) killed 80 % of C. maculatus and 70% of C. chinensis after 24h exposure whereas the same dose of Eucalyptus oil only killed 55 % and 30 % of these insects, respectively. These results confirmed the high toxicity of C. anisata oil-treated grains against the two insects when compared to Eucalyptus oil.
Fig. 1. Toxicity of dried ground leaves of *C. anisata* and *E. saligna* against *C. maculatus* and *C. chinensis* respectively in mung beans and green peas.

Table 2. LD\textsubscript{50} values calculated for mortality within 2 days of exposure on dry ground leaves-treated grain one day of exposure on filter paper discs and grains-treated with different crude oils and pure compounds.

<table>
<thead>
<tr>
<th></th>
<th><em>C. chinensis</em></th>
<th><em>C. maculatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eucalyptus saligna</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground leaves on grains</td>
<td>-</td>
<td>32.24%</td>
</tr>
<tr>
<td>Essential oil on grains</td>
<td>0.305µl/g</td>
<td>0.193µl/g</td>
</tr>
<tr>
<td>Essential oil on filter paper disc</td>
<td>0.061µl/cm\textsuperscript{2}</td>
<td>0.067µl/cm\textsuperscript{2}</td>
</tr>
<tr>
<td><strong>Clausena anisata</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground leaves on grains</td>
<td>12.56%</td>
<td>6.3%</td>
</tr>
<tr>
<td>Essential oil on grains</td>
<td>0.107µl/g</td>
<td>0.149µl/g</td>
</tr>
<tr>
<td>Essential oil on filter paper disc</td>
<td>0.036µl/cm\textsuperscript{2}</td>
<td>0.0901µl/cm\textsuperscript{2}</td>
</tr>
<tr>
<td><strong>Cymol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxicity on filter paper disc</td>
<td>0.113µl/cm\textsuperscript{2}</td>
<td>0.148µl/cm\textsuperscript{2}</td>
</tr>
<tr>
<td><strong>Anethole</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxicity on filter paper disc</td>
<td>0.004µl/cm\textsuperscript{2}</td>
<td>0.015µl/cm\textsuperscript{2}</td>
</tr>
</tbody>
</table>

**Insect mortality on filter paper**

The variation of percentage mortality of each insect species after 24h exposure to increasing doses of crude oil extracts and pure compounds are shown on Fig. 3 and 4. Insect mortality was dose-dependent. The highest dose used for all the oils (8µl/disc) caused more than 80% mortality of both insects. Fig. 4 revealed that anethole is more effective than cymol (LD\textsubscript{50} in Table 3) as the dose of 1µl/disc induced 70% and 100% mortality of *C. maculatus* and *C. chinensis* with anethole but only 38% and 15% mortality with cymol, respectively.

**Repellency**

All the crude oil extracts and the pure compounds were highly repellent to the two insect species with overall repellency values in the range of 70-100% (Table 3). Repellency was not always dose dependent.
Fig. 2. Toxicity of *Clausena* and *Eucalyptus* oils treated grains against the two insect species after 24h exposure

Fig. 3. Toxicity of *Clausena* and *Eucalyptus* oils-impregnated filter paper discs to the two insect species after 24h exposure

**Discussion**

The toxic effects of the tested plants against the two insect species depended on several factors, among which is the chemical composition of the plant materials and the insects susceptibility. *C. anisata* oil proved to be more toxic than *E. saligna* oil against the two insects. Their chemical analysis (Table 1) revealed that *C. anisata* oil is mainly composed of *trans*-anethole. Whereas *E. saligna* has five main constituents with two of more than 30% (α-pinene and cymol) and three others of less than 15% (1,8-cineole, δ-terpinene and carvacrol).
Table 3. Mean percentage repellency of crude oil extracts and pure compounds on filter paper discs.

<table>
<thead>
<tr>
<th>Dose (µl/cm²)</th>
<th>C. chinensis</th>
<th>C. maculatus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. anisata oil</td>
<td>Eucalyptus oil</td>
</tr>
<tr>
<td>0.052</td>
<td>95 ± 5</td>
<td>67 ± 9</td>
</tr>
<tr>
<td>0.104</td>
<td>90 ± 12</td>
<td>96 ± 9</td>
</tr>
<tr>
<td>0.208</td>
<td>95 ± 8</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>0.416</td>
<td>90 ± 12</td>
<td>90 ± 17</td>
</tr>
<tr>
<td>Over all mean PR</td>
<td>92.2</td>
<td>87.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose (µl/cm²)</th>
<th>Anethole</th>
<th>Cymol</th>
<th>Anethole</th>
<th>Cymol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.026</td>
<td>90±7</td>
<td>100±0</td>
<td>85±9</td>
<td>76±4</td>
</tr>
<tr>
<td>0.052</td>
<td>73±19</td>
<td>95±9</td>
<td>83±13</td>
<td>80±7</td>
</tr>
<tr>
<td>0.104</td>
<td>73±23</td>
<td>90±10</td>
<td>90±7</td>
<td>93±13</td>
</tr>
<tr>
<td>0.208</td>
<td>55±11</td>
<td>95±8</td>
<td>75±5</td>
<td>86±7</td>
</tr>
<tr>
<td>Over all mean PR</td>
<td>72.7</td>
<td>95.0</td>
<td>83.2</td>
<td>83.8</td>
</tr>
</tbody>
</table>

Fig. 4. Toxicity of different dosages of anethole and cymol- impregnated filter paper discs to the two insect species after 24h exposure.

The comparison of the bio-efficacy of *C. anisata* essential oil with anethole against the tested insects shows that the latter is more active than the first (LD₅₀ in Table 2) thus indicating that the activity of this oil may be attributed to anethole whose insecticidal activities have widely been described in the literature (Ho et al., 1997; Bazzoni et al., 1997; Marcus and Lichtenstein, 1979) and to some of its minor constituents such as *trans*-isoeugenol methyl ether whose insecticidal effects are also reported (Obeng-Ofori and Reichmuth, 1997; Laad et al., 1983). The LD₅₀ values in Table 2 show that cymol is less toxic than *Eucalyptus* oil suggesting that the effects of the latter may have been reinforced by those of some other constituents such as α-pinene, 1,8-cineole and terpinol as these compounds have recently been identified as potent insecticides (Ojimelukwe and Adler, 1999).
These findings demonstrate a possible scientific rational for the use of these plants materials for stored product protection against insect infestation by communities of the western highlands of Cameroon.

Acknowledgments

The authors wish to thank the German Academic Exchange Programme (DAAD) and the Third World Academy of Sciences (TWAS) for their financial support of this research project.

References


Fungistatic activity of plant extracts against stored products fungi

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Abstract: There is an increasing public concern about the level of pesticide residues in food, which has encouraged researchers to find alternative solutions to the use of synthetic pesticides. Plant extracts are generally assumed to be more acceptable and less hazardous than synthetic compounds. This means that those extracts could be considered possible alternative antifungal treatments. In this work, we have evaluated the fungistatic activity of six aqueous extracts namely: chamomile (Anthemis nobilis L.), cinnamon (Cinnamomum verum J. Presl.), coriander (Coriandrum sativum L.), malva (Malva sylvestris L.), parsley (Petroselinum crispum Mill.) and peppermint (Mentha piperita L.). They were tested against Aspergillus sp., A. niger, Penicillium sp. and Fusarium culmorum. Results are presented and discussed.

Keywords: plant extracts, fungi, fungicides, fungistatic activity, natural pesticides

Introduction

Nowadays, there is a growing concern and awareness by the public opinion in relation to the use of synthetic pesticides and to the presence of their residues in food. These facts lead to the search for new forms of food protection against contamination by microorganisms.

The active compounds biologically produced by plants are an example of substances considered safe. These plant extracts have a better acceptance by the public opinion and are less harmful to health and environment than the synthetic ones (Jobling, 2000).

In the last years, many studies have been conducted using plants or parts of plants, like thyme, sage, origano, coriander (Pruthi, 1980), clove (Hitokoto et al., 1980; Mabrouk & El-Shayed, 1980), cinnamon, rosemary, lavender (Davidson, 1997), cumin, pepper (Beuchat & Golden, 1989), garlic, onion (Abdou et al., 1972; Ghandi & Ghodekar, 1988), basil, saffron, marjoram and anise (Hitokoto et al., 1980), to study the effect of such plants on fungal growth and mycotoxins production.

In this work, we have evaluated the fungistatic activity of six aqueous extracts analysing their effect on fungal growth. The plants/spices used were: chamomile (Anthemis nobilis L.), cinnamon (Cinnamomum verum J. Presl.), coriander (Coriandrum sativum L.), malva (Malva sylvestris L.), parsley (Petroselinum crispum Mill.) and peppermint (Mentha piperita L.). The extracts were tested against Aspergillus sp., A. niger, Penicillium sp. and Fusarium culmorum.

Materials and methods

Dried leaves of chamomile, coriander, malva, parsley and peppermint were macerated in 100ml of sterile distilled water (sdw). The respective quantities (weight) are indicated in Table 1. The mixtures were strained through cheesecloth so that aqueous extracts were obtained. The cinnamon powder was diluted in 100 ml sdw, and put in an orbital incubator.
(160 rpm), during 48 hours. The extracts were submitted to two centrifugations (8000 rpm), during 20 and 30 minutes, respectively. After this, the extracts were strained through a 0.45 µm sieve under sterile conditions.

Different quantities of these extracts (Table 1) were added to Potato Dextrose Agar media (PDA), and the mixtures were plated in Petri dishes (20 ml each). After solidification, three plates of each extract were inoculated by placing 5 mm diameter discs with fungi in the centre of each plate. They were cut from the margins of actively growing colonies of Aspergillus sp., A. niger, Penicillium sp. and Fusarium 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 28°C, during 8 days, at the end of which, fungal colonies diameters were measured. Results were analysed using ANOVA – 1 factor (p<0.05).

Table 1. Quantities of the plants/spices used, and volume of their respective extracts added to PDA media.

<table>
<thead>
<tr>
<th>Plants/Spices</th>
<th>Weight (g)</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamomile</td>
<td>23</td>
<td>2, 3 and 4</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>20</td>
<td>2 and 3</td>
</tr>
<tr>
<td>Coriander</td>
<td>90</td>
<td>1, 1.9 and 2</td>
</tr>
<tr>
<td>Malva</td>
<td>30</td>
<td>1 and 2</td>
</tr>
<tr>
<td>Parsley</td>
<td>94</td>
<td>2 and 3</td>
</tr>
<tr>
<td>Peppermint</td>
<td>50</td>
<td>2 and 3</td>
</tr>
</tbody>
</table>

Results

In Fig. 1, results of the effect of PDA media supplemented with different aqueous extracts in colonies diameters are represented graphically. The control was PDA media not supplemented with extracts.

The results obtained from the statistical analysis (ANOVA - 1 factor) are summarised in Tables 2, 3, 4, 5, 6 and 7 respectively.

For chamomile and malva, the most effective extracts were the 4 ml and 2ml respectively, by inhibiting totally the growth of all the tested fungi (Table 2 and 5). In relation to cinnamon, the 3 ml extract was the best by inhibiting the growth of A. niger, Penicillium sp. and Fusarium culmorum and diminishing the growth of Aspergillus sp. (Table 3). In the case of coriander, the 1.9 ml extract inhibited totally the growth of Penicillium sp. and F. culmorum. In relation to Aspergillus sp. and A. niger the most effective extract was the 2 ml one (Table 4). In relation to parsley, the 3 ml extract was the most effective one, diminishing the growth of A. niger and inhibiting totally the growth of Aspergillus sp., Penicillium sp. and F. culmorum (Table 6). In the case of peppermint, the 3 ml extract was the most effective, diminishing the growth of Aspergillus sp., A. niger and Penicillium sp. and inhibiting totally the growth of F. culmorum (Table 7). The quantitative results presented in Figure 1 were transformed in qualitative results, which are presented in Table 8.

Discussion and conclusions

The search for new compounds to control fungi in stored products is a promising area of research. 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 is scarce and practically non-existent for the control of fungi in stored products.

Among the six species of plants tested for their potential use for control of stored products fungi, chamomile and malva had the highest in vitro antmycotic activities (Figure 1). The chamomile and malva more concentrated extracts inhibited totally the growth of the four tested fungi, but malva was the most effective, since it has given the same result with a lower concentration (Table 8). Literature references about this issue were not found.
For the other plants, the level of inhibition varied according to the plant species and the fungus tested, as occurred in Azzouz & Bullerman (1982), Aureli et al. (1992), Paster et al. (1995). *Penicillium* sp. and *Fusarium culmorum* showed a high susceptibility in relation to the highest extracts concentrations of the plants, being *Fusarium culmorum* the most susceptible one (Figure 1 and Table 8).

There are very different ways to prevent and suppress the fungi tested using synthetic fungicides (Lisker, 1990), organic acids (Gosh & Haggblom, 1985, Nandi, 1990, Doores,(106,72),(988,772)

Table 2. Statistical analysis for Chamomile data.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Culture Media</th>
<th>Average</th>
<th>Variance</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> sp.</td>
<td>PDA</td>
<td>6.67</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>3.90</td>
<td>0.03</td>
<td>488.42</td>
<td>2.14E-09</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>3.43</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+4ml extract</td>
<td>0.50</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.9</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>PDA+2ml extract</td>
<td>7.86</td>
<td>0.003</td>
<td>9524.53</td>
<td>1.51E-14</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>7.53</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+4ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>3.36</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium</em> sp.</td>
<td>PDA+2ml extract</td>
<td>2.23</td>
<td>0.003</td>
<td>558.63</td>
<td>1.25E-09</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>1.86</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+4ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.63</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fusarium</em> culmorum</td>
<td>PDA+2ml extract</td>
<td>4.1</td>
<td>0.120</td>
<td>977.95</td>
<td>1.34E-10</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+4ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All F values are significant (p<0.05)

Table 3. Statistical analysis for Cinnamon data.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Culture Media</th>
<th>Average</th>
<th>Variance</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> sp.</td>
<td>PDA</td>
<td>6.67</td>
<td>0.083</td>
<td>368.13</td>
<td>5.28E-07</td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>4.43</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>1.27</td>
<td>0.093</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.9</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>PDA+2ml extract</td>
<td>8</td>
<td>0</td>
<td>16653</td>
<td>5.84E-12</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>3.37</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium</em> sp.</td>
<td>PDA+2ml extract</td>
<td>2.33</td>
<td>0.003</td>
<td>601.44</td>
<td>1.22E-07</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>0.57</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.63</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fusarium</em> culmorum</td>
<td>PDA+2ml extract</td>
<td>0.57</td>
<td>0.013</td>
<td>1124.73</td>
<td>3.84E-10</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All F values are significant (p<0.05)
Table 4. Statistical analysis for Coriander data.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Culture Media</th>
<th>Average</th>
<th>Variance</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDA</td>
<td>6.67</td>
<td>0.083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>PDA+1ml extract</td>
<td>4.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+1.9ml extract</td>
<td>3.07</td>
<td>0.0130</td>
<td>307.14</td>
<td>1.35E-08</td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>2.87</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.9</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+1ml extract</td>
<td>7.9</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+1.9ml extract</td>
<td>3.7</td>
<td>0.03</td>
<td>420.02</td>
<td>3.89E-09</td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>2.73</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>3.37</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>PDA+1ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.63</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>PDA+1ml extract</td>
<td>1.03</td>
<td>0.02</td>
<td>612.24</td>
<td>8.69E-10</td>
</tr>
<tr>
<td></td>
<td>PDA+1.9ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>1.03</td>
<td>0.02</td>
<td>11449.75</td>
<td>1.8E-11</td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.63</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium culmorum</td>
<td>PDA+1ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All F values are significant (p<0.05)

Table 5. Statistical analysis for Malva data.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Culture Media</th>
<th>Average</th>
<th>Variance</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDA</td>
<td>6.67</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>PDA+1ml extract</td>
<td>4</td>
<td>0.03</td>
<td>759.56</td>
<td>6.09E-08</td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.9</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>PDA+1ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.63</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>PDA+1ml extract</td>
<td>1.03</td>
<td>0.003</td>
<td>1255.2</td>
<td>1.36E-08</td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.63</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium culmorum</td>
<td>PDA+1ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA+2ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All F values are significant (p<0.05)

Table 6. Statistical analysis for Parsley data.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Culture Media</th>
<th>Average</th>
<th>Variance</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDA</td>
<td>6.67</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>PDA+2ml extract</td>
<td>4.5</td>
<td>0.04</td>
<td>714.19</td>
<td>7.32E-08</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.9</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>PDA+2ml extract</td>
<td>6.63</td>
<td>0.063</td>
<td>509.38</td>
<td>2.01E-07</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>3.37</td>
<td>0.023</td>
<td></td>
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<td>PDA</td>
<td>3.37</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>PDA+2ml extract</td>
<td>1.27</td>
<td>0.013</td>
<td>743.38</td>
<td>6.49E-08</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.63</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium culmorum</td>
<td>PDA+2ml extract</td>
<td>1.97</td>
<td>0.053</td>
<td>956.41</td>
<td>3.06E-08</td>
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<tr>
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<td>PDA+3ml extract</td>
<td>0.63</td>
<td>0.053</td>
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</table>

All F values are significant (p<0.05)
Table 7. Statistical analysis for Peppermint data.

<table>
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<tr>
<th>Fungi</th>
<th>Culture Media</th>
<th>Average</th>
<th>Variance</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDA</td>
<td>6.67</td>
<td>0.083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>PDA+2ml extract</td>
<td>4.47</td>
<td>0.003</td>
<td>273.85</td>
<td>1.27E-06</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>2.3</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.9</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>PDA+2ml extract</td>
<td>7.93</td>
<td>0.013</td>
<td>136.19</td>
<td>1.001E-05</td>
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<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>5.93</td>
<td>0.063</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>3.37</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>PDA+2ml extract</td>
<td>1.7</td>
<td>0.07</td>
<td>157.08</td>
<td>6.58E-06</td>
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<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>0.97</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>7.63</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium culmorum</td>
<td>PDA+2ml extract</td>
<td>1.07</td>
<td>0.023</td>
<td>3031.93</td>
<td>9.66E-10</td>
</tr>
<tr>
<td></td>
<td>PDA+3ml extract</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All F values are significant (p<0.05)

Table 8. Inhibition effect of the extracts tested.

<table>
<thead>
<tr>
<th>Plant/Spice</th>
<th>g/ml</th>
<th>Aspergillus sp.</th>
<th>A. niger</th>
<th>Penicillium sp.</th>
<th>Fusarium culmorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamomile</td>
<td>0.46</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>0.69</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>0.92</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>0.4</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Coriander</td>
<td>1.71</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td></td>
<td>1.8</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Malva</td>
<td>0.3</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td></td>
<td>0.6</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Parsley</td>
<td>1.88</td>
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<td>2.82</td>
<td>+++</td>
<td>++</td>
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<tr>
<td>Peppermint</td>
<td>1.0</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

(++) 100% inhibition of fungal growth; (++) above 50% inhibition of fungal growth; (+) below 50% inhibition of fungal growth; (-) 0% inhibition of fungi growth.

References


Effect of neem oil on predatory ability of *Teretriosoma nigrescens* Lewis on *Prostephanus truncatus* (Horn)

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Abstract: An experiment was conducted to determine whether the use of neem oil in stored maize grain for the control of the larger grain borer, *Prostephanus truncatus* could affect the ability of *Teretriosoma nigrescens* to predate on the pest. Adults of *P. truncatus* were introduced into maize samples kept in quarter litre glass jars and treated with varied amounts of neem oil ranging from 2.5 to 7.5ml/kg maize. The untreated samples served as the control. Adult *T. nigrescens* were introduced into the samples 10 days after the introduction of *P. truncatus* and the samples were kept at 30°C and 70% r.h. After nine weeks, the total number of both *P. truncatus* and *T. nigrescens* was determined. The results showed that neem oil did not negatively affect the predatory effect of *T. nigrescens* and that it slightly enhanced the reduction in *P. truncatus* adult population by *T. nigrescens*. The results also showed that *T. nigrescens* was more tolerant to mortality effect of neem oil than *P. truncatus*. The population increase of *T. nigrescens* was, however, deterred by neem oil. The difference between neem oil treated samples containing *T. nigrescens* and those without decreased with increasing neem oil dosage. It can be concluded that for short-term control of *P. truncatus* within stored produce, *T. nigrescens* can effectively be employed in neem oil treated grain and together give a better control at lower amounts of the oil than either of them alone.

Key words: *Prostephanus truncatus*, Neem, *Teretriosoma nigrescens*, stored product pests, *Azadirachta indica*

Introduction

The larger grain borer, *Prostephanus truncatus*, is a pest of stored products recently introduced to Tropical Africa (Dunstan and Magazini, 1981; Harnish and Krall, 1984) from its natural habitat in Central America and Mexico where it has been recognised for long as a pest. In this natural habitat, *P. truncatus* is normally associated with *Teretriosoma nigrescens* Lewis (Coleoptera: Histeridae) (Rees, 1985), which is believed to check its population. Its introduction to Africa hence provided it with a new habitat with favourable warm and moist climate without the occurrence of the natural enemy making its spread fast and its damage extremely high.

The use of conventional synthetic insecticides in the control of *P. truncatus* is limited by inability of poor local farmers to afford the chemicals, unavailability of the chemicals in rural areas and limited skill in the use of chemicals by the rural farmers. This has led to increased efforts in search of alternative means of control to the use of synthetic chemicals. These alternative means include biological control and the use of natural plant products. In biological control, the utilisation of *T. nigrescens* has been studied and the predator introduced in *P. truncatus*-infested areas in Africa (Richter et al., 1997; Giles et al., 1996). *T. nigrescens* has been reported to have successfully established in West Africa (Borgemeister et
and to have considerably reduced the populations of *P. truncatus*. Richter *et al.* (1997) also reported a reduction in losses due to *T. nigrescens* of up to 81.2% and a reduction in the population of *P. truncatus* of 56.4% in the first year of release. Nevertheless, much as the biological control of *P. truncatus* by the use of *T. nigrescens* may be counted as a success, the problem of *P. truncatus* as a pest of stored produce cannot be said to be conclusively solved. There continues to be a need for complementary strategies to be employed together with the biological control strategies. This was clearly shown by Meikle *et al.* (1999) who reported that despite the decline in infestation by *P. truncatus*, the number of farmers who used field pesticides increased. Even in the original home of *T. nigrescens*, still significant losses were reported (Wright, 1984; Giles and Leone, 1975). Markham *et al.* (1994) also stressed the need for a wide choice of IPM options especially in areas where farmers stored their grain in ventilated granaries. More recently, Meikle *et al.* (2002) have reported that *P. truncatus* infested 54% of stores in Benin although *T. nigrescens* was well established in the region.

One possibility of widening the scope and effectiveness of *P. truncatus* control is by use of neem products, which have shown a wide range of activity against many other insect pests. For such products to be employed in wider IPM strategies, it is important that their effects on beneficial insects such as *T. nigrescens* are assessed. This study was intended to determine the effect of treating maize grains with neem oil, for controlling *P. truncatus*, on the predatory ability of *T. nigrescens*.

### Materials and methods

#### Experimental material

The neem oil used in this study was obtained from the International Centre for Insect Physiology and Ecology, Nairobi, Kenya. It was prepared by pressing the neem seed kernels, collected from different regions of Kenya, using a manual oil expeller that removed oil from the kernels by squeezing, followed by filtering of the resultant oil.

#### Insect culture

The insects used were obtained from cultures maintained at the Institute for Stored Product Protection of the Federal Biological Research Centre for Agriculture and Forestry, Berlin, Germany for many years. *P. truncatus* were reared on shelled yellow maize at 30°C and 70% r.h. in two-liter glass jars, each culture starting with 500 insects on 500g of maize. The one- to seven-day-old adults for use in the experiments were obtained by setting 500 unsexed adults on 500g of maize in two-liter glass jars as above and allowing them to lay eggs for 3-5 days (Detmers, 1993). The adult beetles were then removed by gently sieving the grains through a 3mm mesh sieve to retain the grains and then through a 1mm sieve to separate the beetles from frass. Those that did not come out during sieving were forced out by probing with a plastic fiber. After all the beetles were removed, the grains and the frass were returned into the glass jars and kept at the same conditions until the adults emerged after 25-32 days. These were also obtained by sieving as described above. *T. nigrescens* were reared in similar jars under similar conditions starting with 500 *P. truncatus* and 50 *T. nigrescens* (Pöschko, 1993).

#### Lethal effect of neem oil on *T. nigrescens*

The effect of neem oil on mortality of *T. nigrescens* was assessed on glass beads and on shelled maize grains. Glass beads equivalent to 100g of maize by volume were placed in 250ml glass jars and 0.5, 1, 1.5 and 2ml neem oil were added. The beads and the oil were thoroughly
mixed by shaking, first by hand then by a mechanical roller for ten minutes. Samples without neem oil served as control. Fifty adults of *P. truncatus* and *T. nigrescens* were then introduced into separate samples and kept at 30°C and 70% r.h. Mortality was determined by counting the number of dead beetles after seven days.

**Predatory ability of *T. nigrescens***

50 one-week-old *P. truncatus* adults were introduced into 100g maize samples in 250ml glass jars treated with neem oil as in section 2.1 above. They were allowed to feed and lay eggs for one week before the introduction of ten *T. nigrescens* in each jar. After eight weeks, the total number of both insects, live and dead, was determined. Other parameters recorded were grain weight loss and the total number of grains bored by *P. truncatus*.

**T. nigrescens population increase***

This experiment was intended to establish whether neem oil had a significant effect on the development of *T. nigrescens* as indicated by insect population increase. The experimental samples were set up as in section 2.4, with ten *T. nigrescens* being introduced into *P. truncatus*-infested samples. In each sample, twenty *P. truncatus* larvae were added every day to serve as a source of feed for *T. nigrescens*. After eight weeks, the total number of *T. nigrescens* was determined.

**Statistical analysis***

Values for mortality, number of bored grains, live and dead *P. truncatus*, were transformed according to the function $y = \log (x + 1)$ and analysed by Split-plot ANOVA procedure. Duncan’s Multiple Range Test was utilised for multiple comparisons while Student t-test and Mann-Whitney U-test were used for two-way comparisons.

**Results***

**Lethal effect of neem oil on *T. nigrescens***

The effect of neem oil on mortality of *T. nigrescens* in comparison to *P. truncatus* is given in Figure 1 on glass beads and in Figure 2 on maize grains. In both cases, the mortality of *T. nigrescens* was lower than that of *P. truncatus*, although the difference was much more on maize grains than on glass beads. The mortality of both *P. truncatus* and *T. nigrescens* was higher on glass beads than on maize grains. On glass beads, the mortality of *P. truncatus* reached 100% at 10ml/kg maize volume equivalent of beads while that of *T. nigrescens* was at 20ml/kg. The least amounts that caused mortality were 1 and 2.5ml/kg for *P. truncatus* and *T. nigrescens* respectively. On maize grain, the mortality of *P. truncatus* reached 100% at 20ml/kg while that of *T. nigrescens* reached a maximum of 57.6% at this dosage. The least amounts that caused mortality on maize grains were 1 and 10ml/kg for *P. truncatus* and *T. nigrescens*, respectively.

**Grain weight loss and damage***

Data for grain weight loss and number of grains bored by *P. truncatus* both in the presence and absence of *T. nigrescens* at different dosages of neem oil are given in Figure 3. Both weight loss and number of bored grains were significantly reduced by treatment with neem oil (Split-plot ANOVA, $p < 0.001$). Values for the number of bored grains were transformed using the function $y = \log (x + 1)$. For both weight loss and the number of grains bored, all the treated samples resulted in significantly lower values than the control (DMRT, $p = 0.05$). Both values showed a very similar pattern, decreasing with increase in dosage and the effects
of all dosages were significantly different from one another. The interaction between neem oil and \textit{T. nigrescens} was also significant \((p < 0.001)\) for both weight loss and grain damage.

![Graph](image1.png)

**Fig. 1.** Mortality of \textit{P. truncatus} and \textit{T. nigrescens} exposed to neem oil treated glass beads for a period of seven days at 30°C and 70\%r.h. Data are means of five replicates. Bars within the same treatment dosage denoted with the same letter are not significantly different (Student t-test, \(p > 0.05\)).

![Graph](image2.png)

**Fig. 2.** Mortality of \textit{P. truncatus} and \textit{T. nigrescens} exposed to neem oil-treated maize grains for a period of seven days at 30°C and 70\%r.h. Data are means of five replicates. Bars within the same treatment dosage denoted with the same letter are not significantly different (Student t-test, \(p > 0.05\)).

\textbf{\textit{P. truncatus} population}

The mean number of live and dead insects in samples treated with various dosages of neem oil and either containing or not containing \textit{T. nigrescens} is given in Figure 4. The effects of both neem oil and presence of \textit{T. nigrescens} were significant (Split-plot ANOVA, \(p < 0.001\)) for both live and dead insects. Treated samples resulted in significantly lower values than the control (DMRT, \(p = 0.05\)). For live insects, only the difference between the number from 7.5ml/kg and the other dosages was significant for samples without \textit{T. nigrescens}. For dead insects, the highest number for samples containing \textit{T. nigrescens} was recorded at 7.5g/kg dosage level followed by the control while for samples without \textit{T. nigrescens} the values were in the order \(2.5 < 0 < 5 < 7.5\)ml/kg, the difference between them being significant.
Fig. 3. Effect of neem oil on maize grain (a) weight loss and (b) boring by *P. truncatus* on 100g maize kept at 30°C and 70% r.h. with and without *T. nigrescens*. Data are means of five replicates. Bars denoted by the same letter are not significantly different, capital letters for comparison within (DMRT, *p* = 0.05), and small letters between (Student t-test and Mann-Whitney U test, *p* < 0.05), treatments, respectively.

Fig. 4. Numbers of (a) live and (b) dead *P. truncatus* in 100g of maize treated with different dosages of neem oil and kept at 30°C and 70% r.h. with and without *T. nigrescens*. Data are means of five replicates. Bars denoted by the same letter are not significantly different; capital letter for comparison between dosages (DMRT, *p* = 0.05) and small letters for comparison within dosages (Student t-test and Mann-Whitney U test, *p* > 0.05).

*T. nigrescens* population

The mean number of *T. nigrescens* was compared between samples in which larvae were added and those in which they were not. The effect of neem oil on the resulting number of *T. nigrescens* was significant (ANOVA, *p* < 0.001) for samples without additional larvae but not for those with additional larvae. For samples without additional larvae, there was no significant difference between dosage levels, which were all significantly lower than the control. Apart from the control, the difference between the number of *T. nigrescens* obtained from all dosages with and without additional larvae was significant (Mann-Whitney U-test, *p* < 0.05) with the samples that received additional larvae resulting in higher numbers than those that did not (Figure 5).
Fig. 5. Numbers of *T. nigrescens* in samples treated with different dosages of neem oil with and without additional larvae after eight weeks. Data are means of five replicates. Bars denoted by the same letter are not significantly different; capital letters for comparison between dosages (DMRT p = 0.05) and small letters for comparison within dosages (Student t-test and Mann-Whitney U test, p > 0.05).

**Discussion**

The results of this study show that *T. nigrescens* adults were significantly less susceptible to neem oil than adult *P. truncatus* both on glass beads and on maize grains. The real reason for this difference is not known, but could be due to the visibly difference in body surfaces between the two insects. *P. truncatus* has a rough rugged body surface while *T. nigrescens* is extremely smooth. It is possible that more oil could be held on the rough body of *P. truncatus* and hence easily blocks the trachea as suggested by Helwitt (1975) than on that of *T. nigrescens*. This is also supported by the fact that the difference between the two decreased as the dosage of the oil increased. At high dosages such as 20ml/kg, there was sufficient oil to adversely affect both insects equally.

At dosages that allowed the survival of the two insects, *T. nigrescens* managed to significantly control the population of *P. truncatus* both in the presence and absence of neem oil. The total number of *P. truncatus* resulting from *T. nigrescens* predation in the absence of neem oil was 29 from the initial number of 50 after eight weeks and compared favourably with those reported by Rees (1985, 1990). The difference in the number of *P. truncatus*, weight loss of the infested maize grains and number of damaged grains between the presence and absence of *T. nigrescens* was generally not significant apart from at 2.5ml/kg dosage level. From these results, it may seem unnecessary to use both neem oil and *T. nigrescens* simultaneously because of the non-significant difference between the presence and absence of *T. nigrescens*. However, it is important to note that *T. nigrescens* is normally released into the surroundings where it is intended to control *P. truncatus* both in the surroundings as well as inside the stores just in case *P. truncatus* finds its way there. So, it is more likely that one may supplement the effect of *T. nigrescens* with the effect of the oil, but not vice-versa. It should also be noted that generally, the oil caused a higher control on its own than *T. nigrescens*. Under such circumstances, the lack of significance becomes important since in a situation where a farmer does not expect satisfactory results from the effect of *T. nigrescens*, he may supplement its effect with that of neem oil with the confidence that he will not adversely affect the predator.

Initially, neem oil seemed to affect the development of predator larvae just as it affected prey larvae. However, when additional prey larvae were supplied to the predator as additional
nutrition, predator populations increased normally. This may hence imply that in treated samples, the population of *T. nigrescens* was limited by the absence, or shortage, of *P. truncatus* larvae. This conforms with the work of Detmers (1993) who reported that *T. nigrescens* could not lay eggs in the absence of, or in the presence of very few, *P. truncatus* larvae. Provision of additional larvae to *T. nigrescens* confirmed that their larvae are not adversely affected by neem oil at the tested dosages. Previous reports showed that *T. nigrescens* is susceptible to the insecticides used to control *P. truncatus*, both pirimiphos-methyl and permethrin (Golob *et al*., 1990). This may limit the application of *T. nigrescens* in areas that utilise these chemicals on a large scale. Neem oil could hence be considered for use where *T. nigrescens* can be established or is already well established.

The difference in the susceptibility of the two larvae may lie in the fact that their sources of nutrition are different. The results from this study suggest that *P. truncatus* larvae are disrupted from feeding by the antifeedant properties of neem oil and hence starve to death. This may explain the ability of the predator to survive despite its feeding on the affected larvae, which did not incorporate significant amounts of neem oil. These results also confirm that there is no contact toxicity of neem oil on the larvae of *T. nigrescens* under these conditions.

The effects of a number of neem products on various beneficial and non-target species have already been reported severally, although mostly involving pests other than those of stored products. Among Coleoptera, *Coccinella septempunctata*, an important predator of aphids has been reported not to be adversely affected by neem seed cake powder and neem oil (Kaethner, 1991). Spraying of the sorghum aphid, *Melanaphis sacchari* (Srivastava and Parmar, 1985) as well as the green peach aphid, *Myzus persicae* (Eisenlohr *et al*., 1992) did not affect the coccinellids and syrphids. Among egg-parasitoids, *Telenomus remus*, a parasitoid of *Spodoptera litura*, was also not affected by neem products (Fernandez *et al*., 1992). However, negative effects on parasitization were observed when *Trichogramma pretiosum* was treated with neem seed cake powder in the laboratory, but not in the field. Mansour *et al.*(1997) showed that Neemguard, a commercial neem product, was highly toxic to *Tetranychus cinnabarinus*, a phytophagous mite, but not harmful to two of its predators. Against *Trichogramma japonicum*, two neem products were reported to exhibit less detrimental effects than all the chemical pesticides tested (Borah *et al*., 2001). The effect of neem products on beneficial and non-target species therefore seems to be largely by coincidence or natural adaptation. It can hence be concluded that *T. nigrescens* can be effectively utilised in the control of *P. truncatus* together with neem oil at dosages up to 7.5ml/kg. Under such conditions, neem oil does not affect the larvae or the adults of *T. nigrescens*, but it could enhance its effect in the control of *P. truncatus*.

**Acknowledgement**

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**References**


Rees, D.P. 1990. Further studies on the ability of *Teretriosoma nigrescens* to prey on insect pests of stored products and to feed on stored food and beverage crops. – In: Boeye, J., Wright, M., Laborius, G.A., (Eds.), Implementation of and further research on biological control of the larger grain borer. Proceedings of an FAO/GTZ Co-ordination Meeting, Eschborn, Germany, pp. 96-103.


The assessment of toxicity of the *Melia azedarach* seed oil against stored product insects

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**Abstract:** Vapour of chinaberry tree, *Melia azedarach* L. seed oil caused 53 and 94% mortality in the adults of the rice weevil, *Sitophilus oryzae* (L.) and the last instars of the Mediterranean flour moth, *Ephestia kuehniella* Zeller larvae, respectively, at 135 µl/l air, and 168 and 144 h exposure, respectively, in the tests done using doses of 27-135 µl oil/l air and exposures of 72-168 h. Almost no toxicity was, however, detected against the confused flour beetle, *Tribolium confusum* du Val adults. Using the doses of 24.6-196.8 µl oil/l air and exposure periods of 24-96 h the oil achieved 85 and 74% mortality in *T. confusum* and *E. kuehniella* eggs, respectively, at 196.8 µl/l air and 96 h. The residual toxicity of the oil was very low against *T. confusum* adults and achieved only 13% mortality, at the highest dose, 0.32 µl/cm², in the tests conducted by exposing the insects for 5 days on filter paper impregnated with the oil. Fumigant and residual activities of *M. azedarach* seed oil against stored product insects was compared with that of essential oils from several aromatic plants.

**Key words:** *Melia azedarach,* toxicity, *Sitophilus oryzae,* *Tribolium confusum,* *Ephestia kuehniella*

**Introduction**

The chinaberry tree, *Melia azedarach* L. (Meliaceae) is a deciduous tree native of India and China, but widely cultivated as a street tree in warmer countries including South Europe. The oil from seeds is found to be suitable for oil lamps, and seeds themselves are used to make rosaries and beads. Medicinal and insect deterring properties of its root bark, leaves and flowers were reported. (Davis, 1967; Kunkel, 1975). Although compounds from its close relative the neem tree, *Azadirachta indica* A. Juss, have been studied extensively in the last decade *M. azedarach* seems not to attract the attention it deserves as a promising source of natural pesticides (Valladares et al., 1997).

Growth retardant, repellent and toxic effects of extracts from fruits and seeds of *M. azedarach* against insects only recently were subject of scientific studies. Ethanolic extracts of *M. azedarach* fruits deterred feeding by larvae and adults of the elm beetle, *Xanthogalleruca luteola* (Müller) (Coleoptera: Chrysomelidae); larvae died without molting forced to feed on treated elm leaves; spraying with the extract led to negative effects on survival and development of larvae but no effects were seen on adults (Valladares et al., 1997). Ethanol extracts of *M. azedarach* fruits showed repellent and growth retarding effects against nymphs of the vector of chagas disease, *Triatoma infestans* Klug. (Hemiptera: Reduviidae) (Valladares et al., 1999). Aqueous extract of *M. azedarach* fruits lowered significantly larval populations of the pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) (Abou-Fakhr Hammad, 2000). Aqueous extract of *M. azedarach* seeds was reported to be effective, causing 78% mortality, against the larvae of chickpea, *Cicer arietinum* L., leafminer *Liriomyza cicerina* (Rond.) for 15 days (Hincal et al., 2000).

The active compounds of *M. azedarach* extracts are limonoids and best known limonoid is azadirachtin which was extracted from neem. Limonoids are most often found in the family Meliaceae and are known to exhibit insecticidal, insect growth retardant and antifeedant...
effects. Bohnenstengel et al. (1999) isolated three meliacarpins (limonoids) from leaves of *M. azedarach* which exhibited insecticidal activity comparable to that of azadirachtin toward larvae of Egyptian cotton worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae).

The present investigation was undertaken to assess vapour and residual toxicity of *M. azedarach* seed oil against stored product insects. No studies are known so far investigating the toxicity, in particular vapour toxicity, of extracts of *M. azedarach* against these insects.

**Material and methods**

**Vapour toxicity**

Two to three week old adults of the confused flour beetle, *Tribolium confusum* du Val and the rice weevil, *Sitophilus oryzae* (L.) and last instars of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, and 0-24 h old eggs of *T. confusum* and *E. kuehniella* were used in the tests. *T. confusum* was reared on a mixture of wheat flour, bran and yeast; *E. kuehniella* on wheat flour, bran, yeast and glycerol and *S. oryzae* on wheat. Insect rearing and all experimental procedures were carried out at 26±1 °C and 65±5 r.h.

*M. azedarach* seeds were obtained from fruits of trees grown in Antalya and the oil was extracted from ground seeds by water steam distillation. Glass jars of 370 and 650 ml with screwed lids were used as test chambers for the adults and larvae, and for the eggs, respectively. The oil was applied on a blotting paper stripe which was attached to the lower side of jar’s lid. Five oil doses between 27-135 for the adults and larvae, and four doses between 24.6-196.9 µl/l air for the eggs, and four exposure periods between 72-144 or 96-168 for the former, and 24-96 h for the latter were used. Adults and larvae were exposed in small nylon gauze bags supplied with rearing food. Twenty individuals introduced into each bag were counted as one replicate. Three replicates were used for each dose and exposure time combination. Modified cloning plates (Nunc, Denmark) were used for exposure of eggs (Tunç et al., 1997). A set of the exposure device consists of a bottom plate which has 60 microwells, each for accommodation of one egg, a cover plate which has a hole over each microwell and a piece of serigraphic cloth between two plates to avoid escape of hatched larvae while allowing air circulation through the holes on cover plate. Sixty eggs on each plate represented three replicates each consisting of 20 eggs for each dose and exposure time combination. All experiments were repeated twice so that each dose X exposure time was replicated 6 times.

After exposure, bags or plates were taken out of the jars and final mortality counts were made 7 days later for the adults and larvae, and 11 and 9 days later for the eggs of *T. confusum* and *E. kuehniella*, respectively. Mortality data were corrected for the mortalities in controls.

**Residual toxicity**

For residual toxicity, test insects were exposed on circular filter papers (dia. 85 mm) impregnated with five doses of oil between 0.02-0.32 µl/cm² dissolved in 1 ml acetone. Each dose was replicated 3 times and experiments were repeated 2 times. For controls only acetone was applied. After evaporation of acetone, filter papers were placed into plastic petri dishes (dia.85 mm) and 20 *T. confusum* adults were exposed for 5 days. The covers of Petri dishes had wide gauze-covered openings allowing air circulation in order to avoid vapour activity of *M. azedarach* oil. Mortality counts were made after fifth day.
**Results and discussion**

**Vapour toxicity**

Vapour of *M. azedarach* seed oil caused 53 and 94% mortality in adults of *S. oryzae* and larvae of *E. kuehniella*, respectively, at a dose of 135 µl/l air and exposure periods of 168 and 144 h, respectively, but almost no mortality in *T. confusum* (Fig.1). Vapour activity of the oil was lower against adults of *S. oryzae* but comparable against larvae of *E. kuehniella* when compared with that of the essential oils of anise, *Pimpinella anisum* L.; eucalyptus, *Eucalyptus camaldulensis* Dehn.; *Thymbra spicata* L. and *Satureja thymbra* L. On the other hand only essential oils of anis and eucalyptus showed high activity against adults of *T. confusum* (Saraç and Tunç, 1995a).

The mortalities obtained against eggs of *T. confusum* and *Sitophilus oryzae* and larvae of *Ephestia kuehniella* exposed to vapour of *Melia azedarach* seed oil.

The mortalities obtained against eggs of *T. confusum* and *E. kuehniella* were 85 and 74%, respectively at 196.8 µl/l air and 96 h (Fig. 2). Among the essential oils of anise; cumin, *Cuminum cyminum* L.; eucalyptus; oregano, *Origanum syriacum* L. and rosemary, *Rosmarinus officinalis* L. those that showed higher vapour activity compared to *M. azedarach* seed oil were only anise against eggs of *T. confusum* and only anise, cumin and oregano against eggs of *E. kuehniella* (Tunç et al., 2000).
Residual toxicity
A very low residual activity, only 13% mortality, was detected against adults of *T. confusum* exposed to filter paper impregnated with a dose of 0.32 µl *M. azedarach* seed oil/cm² for 5 days (Table 1). Among the essential oils of anise, eucalyptus, *T. spicata* and *S. thymbra* only anise exhibited high residual activity, 96% mortality, against adults of *T. confusum* at above dose. The mortality caused by others was in the range of 21-31% (Saraç and Tunç, 1995b).

![Figure 2](image)

**Fig. 2.** Mortality of eggs of *Tribolium confusum* and *Ephestia kuehniella* exposed to vapour of *Melia azedarach* seed oil.

<table>
<thead>
<tr>
<th>dose (µl/cm²)</th>
<th>0</th>
<th>0.02</th>
<th>0.04</th>
<th>0.08</th>
<th>0.16</th>
<th>0.32</th>
</tr>
</thead>
<tbody>
<tr>
<td>mortality (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Table 1. Mortality of *Tribolium confusum* adults exposed to filter paper impregnated with *Melia azedarach* seed oil

Conclusion
In conclusion *M. azedarach* seed oil seems to possess inadequate vapour or residual toxicity against adults and eggs of stored product insects used in the present study at the given test doses and exposure periods. However, mortality data on the larvae of *E. kuehniella* may indicate that larval stages of these insects may be sensitive sufficient to provide control. The reports mentioned in the introduction also indicate that extracts of *M. azedarach* have an insecticidal potential against the larvae of insects feeding on plants.

References


Valladares, G., Defago, M.T., Palacios, S. & Carpinella, M.C., 1997: Laboratory evaluation of Melia azedarach (Meliaceae) extracts against the elm leaf beetle (Coleoptera: Chrysomelidae). – J. Econ.Entomol. 90: 747-750.

Biological control
The discrimination ability of parasitised hosts by *Venturia canescens* (Gravenhost) (Hymenoptera: Ichneumonidae)*

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Abstract: How long the first parasitisation deters further oviposition by *Venturia canescens* (Gravenhost) (Hymenoptera: Ichneumonidae) was investigated with the last instar larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as host. The parasitised host larvae of *E. kuehniella* were individually exposed to adult female *V. canescens* for a five minute period. The parasitoid’s ability to discriminate parasitised hosts was defined at twelve different time intervals (5, 10 and 30 min., 5, 10, 15, 20, 24, 28, 32, 36 and 40 h) after the first parasitisation. When parasitised hosts were presented after 5 and 10 min. from the first parasitisation, the parasitoids spent most of the time in ‘escape from host’ and ‘avoidance from host’ behaviours, and rejected parasitisation second time. However, at eight time intervals (from 30 min. to 32 h.) from the first parasitisation, the ratios of the host rejection decreased gradually and the ratios of superparasitism increased gradually. After 36 and 40 h from the first parasitisation, no discrimination was observed. As a result, an egg laid before 36 and 40 h acted no longer as an effective ovipositional deterrent, and for this reason all the hosts were superparasitised at these time intervals.

Key words: host discrimination, superparasitism, behaviour, *Venturia canescens*, *Ephestia kuehniella*

Introduction

The parthenogenetic ichneumon wasp *Venturia canescens* (Gravenhost) (Hymenoptera: Ichneumonidae) is a solitary internal parasite of the caterpillars of various species of Phycitid moths (Salt, 1961). The parasitoid is able to recognise the parasitised hosts, and consequently rejects them as unsuitable sites for oviposition (Fisher, 1961). Salt (1961), showed that the ability of parasitoids to distinguish hosts containing eggs of conspecifics from those which are unparasitised occurs in the major families of parasitic Hymenoptera. This ability is known in the literature as host discrimination. Many hymenopteran parasitoids are able to distinguish between parasitised and unparasitised hosts, through the application of external or internal markers at oviposition (Salt, 1937; Guillot & Vinson, 1972; van Lenteren, 1976; Hubbard et al., 1987; Wolk & Mackauer, 1990; van Alphen & Visser, 1990). Solitary wasps usually reject hosts that have been previously marked by them or by conspecifics (Harvey et al., 1993). Many parasitoids are able to discriminate between parasitised and unparasitised hosts, but superparasitism is a common feature in nature (van Alphen & Visser, 1990). Superparasitism occurs when a host contains more parasitoid eggs or larvae than are able to develop successfully to adults (Wylie, 1965; van Lenteren, 1976; van Alphen & Nell, 1982: Waage, 1986; van Alphen, 1988; Bai & Mackauer, 1990; van Alphen & Visser, 1990; Harvey et al., 1993).

The aim of the work presented in this paper was to determine how long the first parasitisation deterred further oviposition by *V. canescens*. 
Materials and methods

*Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Venturia canescens* (Grav.) (Hymenoptera: Ichneumonidae) were reared at constant 25 ± 1°C in a temperature room with a 16:8h light and dark photoperiod and 60-70 % relative humidity.

There are two approaches to determine whether parasitoids are able to discriminate between parasitised and unparasitised hosts. One is to dissect hosts (Salt,1961), the other is using behavioural criteria (van Alphen & Visser, 1990). In this study, behavioural criteria of *V. canescens* were used. All the adult wasps used in the experiment were 3 days old. This was due to prevent different searching activity depending on age and parasitisation capacity of the parasitoids. These adult wasps were fed with honey and water each day regularly before the experiment. All the experiments took place at 25 ± 1°C with an overhead illumination. Throughout the trial healthy adult wasps and healthy last instars of the host of equal size were used. An area was constructed by inverting a sterile Petri-dish (9 cm diameter) over a circular Whatman filter paper (12.5 cm diameter). One wasp was placed in the area with one 29 day-old host larva, and the larva was parasitised. After 5, 10 and 30 min, 5, 10, 15, 20, 24, 28, 32, 36 and 40 h, the parasitised larvae were individually presented to another parasitoid with the same method, and the discrimination ability of the parasitoid after these twelve different time intervals was determined. Experiments were repeated 10 times for each time interval. Fresh filter paper and sterile Petri-dishes were used for each trail, and forceps were cleaned with 70 % alcohol before picking up the caterpillar. The behavioural activities were enumerated according to Harrison et al. (1985) as follows:

**Stabbing:** Before stabbing, the ovipositor is flexed downwards and forwards. The ovipositor is then quickly inserted into the caterpillar like a hypodermic needle.

**Contact with host:** With either legs or antennae.

**Probing:** The ovipositor is unsheathed and flexed forwards beneath the abdomen. The tip of the ovipositor is repeatedly brought into contact with the host or substrate. No insertion of the ovipositor occurs.

**Searching:** Searching behaviour involves directed locomotion. The wasp turns quickly and repeatedly, presumably in response to the odour emitted by the host, until it comes in contact with the host. The antennae are continually vibrating in the vertical plane and their terminal segments are contacting the substrate or host.

**Avoidance:** This normally occurs when a wasp meets a previously parasitised host.

**Escape:** The wasp becomes positively phototactic and ceases directed searching behaviour on the floor of the Petri dishes. A wasp walking on the sides and top of the inverted Petri dish is counted as attempting to escape from the arena.

**Cleaning:** Cleaning behaviour is commonly observed directly after egg-laying or after contacting already parasitised hosts. The antennae, legs and ovipositor are groomed. This process may involve receptor cleaning, especially on the antennae and ovipositor.

**Cocking:** Cocking can be observed prior to egg-laying or after an egg has just been laid. The ovipositor is swung above the abdomen in a characteristic movement and then returned to its normal position. This action positions a single egg in a groove at the tip of the ovipositor in preparation for egg laying (Rogers, 1972).

**Not searching:** This is a broad category including two main types of behavioural pattern. The first is resting when the wasp remains still. This is more often observed after egg-laying and cleaning. The second is when the wasp walks around the area in a
slow, undirected manner in contrast to searching behaviour which is a more active and directed movement.

Results and discussion

How long the first parasitisation deterred the further oviposition by *V. canescens* was defined using last instars of *E. kuehniella*. When unparasitised hosts were presented, the parasitoids spent most of the time in ‘searching for host’ behaviour. As a result, the wasps accepted all the host larvae as suitable hosts, and parasitised them for a five minute period. However, when parasitised larvae were presented to the parasitoid in 12 different time intervals after the first parasitisation, depending on time the ratios of host discrimination decreased, but the ratios of superparasitism increased gradually. These values are shown in Fig. 1 and Table 1.

![Graph](image)

Fig. 1. The ratios of host discrimination and superparasitism by *Venturia canescens*.

Five and 10 min. after the first parasitisation, the wasps recognized the hosts which had been parasitised. The wasps spent most of the time in ‘escape from host’ and ‘avoidance from host’ behaviours, and discriminated all of the 10 hosts parasitising them secondly. As a result, superparasitism was not observed at these two time intervals. This suggested that the effect of the egg laid by the first parasitisation acted as an effective ovipositional deterrent.

The wasps discriminated 9 of the 10 hosts after 30 min. and 5 h, 8 of the 10 hosts after 10 h, 7 of the 10 hosts after 15 h, and 6 of the 10 the hosts after 20 and 24 h. from the first parasitisation. These results showed that the ratios of host discrimination decreased gradually depending on time, however, the majority of the hosts was still not superparasitised after 24 h from the first parasitisation. The wasps discriminated only 2 of the 10 hosts after 28 and 32 h from the first parasitisation. In other words, 8 of the 10 hosts were superparasitised. This indicated that the effect of the egg laid by the first parasitisation extremely decreased in its ability to deter egg-laying at these time intervals, and eighty percent of the hosts were superparasitised. The wasps discriminated none of the 10 hosts after 36 and 40 h from the first parasitisation.
parasitisation, and superparasitised all of them. This result suggested that the effect of the egg laid by the first parasitisation became completely inactive after 36 h and later after the first parasitisation.

Table 1. The ratios of host discrimination and superparasitism *Venturia canescens*.

<table>
<thead>
<tr>
<th>Time intervals after first parasitisation (n)</th>
<th>Host discrimination (%)</th>
<th>Superparasitism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minutes</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>10 minutes</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>30 minutes</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>5 hours</td>
<td>10</td>
<td>90</td>
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<tr>
<td>10 hours</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>15 hours</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>20 hours</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>24 hours</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>28 hours</td>
<td>10</td>
<td>20</td>
</tr>
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<td>32 hours</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>36 hours</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>40 hours</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

*V. canescens* is able to recognize parasitised *E. kuehniella* larvae, and consequently rejects them as unsuitable hosts for oviposition (Fisher, 1961). In this study, after 5 and 10 min. from the first parasitisation, the wasps recognized all of the hosts which had been parasitised and discriminated them. However, after 30 min. through 32 h from the first parasitisation, the recognizing ability of the parasitoid decreased gradually and got lost after 36 h from the first parasitisation. Because of this, the wasps accepted parasitised *E. kuehniella* as suitable hosts, and the ratios of superparasitism increased depending on time.

In order to be able to reject a parasitised host, the parasitoid must be able to recognize a change taking place in the host after oviposition (Harrison et al., 1985). Recognizing a change in the host may be accomplished in several ways by the wasp. The wasp may deposit an external pheromone on the host or in its vicinity, during or after oviposition, or the wasp may inject an internal marker pheromone into the host during oviposition. Alternatively, the developing egg may emit a substance, or there may be a change in concentration of body fluids, or the formation of a ‘new’ substance by the host as a reaction to parasitisation (van Lenteren, 1976). Mudd et al. (1982) extracted Dufour’s gland secretions from *V. canescens*, applied to the cuticle of *E. kuehniella* larvae. They suggested that the heneicosane, a major component of Dufour’s gland, acted as an external marker pheromone causing the wasps to leave the area or patch. Harrison et al. (1985) applied Dufour’s gland secretions of *V. canescens* to the cuticle of *E. kuehniella* larvae as an external marker pheromone. They suggested that this gland secretion acted as an oviposition deterrent.

Our results confirmed that the effect of the egg laid by the first parasitisation acted as an external pheromone. Depending on time, however, effect of Dufour’s gland pheromone decreased, and so the ratio of superparasitism increased. In addition to this result, the parasitoids showed different behaviours in response to parasitised hosts depending on time. These results may be used for determining the effect of superparasitism and future studies on the adaptiveness of superparasitism for *V. canescens*. 
References


Seasonal occurrence of dried fig pests and their parasitoids in a fig warehouse in Greece

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Abstract: Dried fig pests and their parasitoids were surveyed at a fig warehouse in Kalamata (Peloponnese, Southern Greece), from September 2000 until September 2001. Coloured cardboard sticky traps were suspended at three heights (10, 30 and 100 cm above the fig mass). These traps were baited with TDA, the male attractant of several stored-product Pyralidae, and inspected for captured insects at 15-day intervals. The colours of the adhesive surfaces were white, yellow, green and brown. Twenty-seven insect taxa were recorded during the entire monitoring period, belonging to the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera. The most abundant species were Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), Carpophilus hemipterus (L.) (Coleoptera: Nitidulidae) and the Hymenopteran parasitoids Habrobracon hebetor (Say) (Braconidae) and Cephalonomia tarsalis (Ashmead) (Bethylidae). Plodia interpunctella was not found in traps during winter months, while the other three species were active during the entire monitoring period. For these four species, traps just above the fig mass (at 10 and 30 cm) captured significantly more individuals in comparison with traps that were higher (100 cm), with the exception of C. tarsalis, for which no significant differences were noted between traps suspended at 30 and 100 cm. Considerable variation on colour preference was noted among species. Brown traps were the less attractive for the four aforementioned species. More H. hebetor and C. tarsalis individuals were recorded in traps which were placed at more illuminated areas of the warehouse, in comparison with traps in less illuminated areas. Finally, a noticeable proportion (approx. 40 %) of C. hemipterus individuals was captured in the figural outlines of the adhesive surface.

Key words: stored figs, Plodia interpunctella, Carpophilus hemipterus, Habrobracon hebetor, Cephalonomia tarsalis, TDA, parasitoids

Introduction

The annual dried fig production in Greece approaches 8,000 tons. This quantity is stored and processed in Greek storage facilities. During storage, figs are attacked by numerous pests, which, in cases of heavy infestations, can cause serious quantitative losses and qualitative degradations, making the product commercially unacceptable. Although several studies have been published so far concerning the presence of insect species in Greek stores, most data are about grain and grain products, while very few data is available for stored figs. Buchelos (1985) lists a number of species, belonging to Lepidoptera and Coleoptera, that are related with stored dried fruit. Apart from pest species, several parasitoid species have been recently reported from Greek storage facilities. Eliopoulos et al. (2002a) published the first study concerning the presence of hymenopterous parasitoids in Greek storage facilities. In that study, ten parasitoid species are reported in stored dried fruit (figs, currants, sultanas). In a recent study, Athanassiou and Eliopoulos (2003), using traps to monitor the seasonal abundance of insect pests in stored currant in southern Greece, noted that a considerable proportion of the total number of individuals found were parasitic wasps. It is now well established that some of these species are potential biological control agents, and could be
incorporated in IPM-based control strategies in stored dried fruit. However, there is still inadequate information about the seasonal abundance of insect species (pests or parasitoids) that occur in these products. As a result, «blind» chemical treatments are often applied, without estimating insect presence or infestation level. Thus, additional experimental work is needed for the improvement of insect detection and estimation of population densities in these commodities.

In this study, we used pheromone-baited traps for long-term monitoring of insect populations in a warehouse which is used for fig storage in southern Greece. In addition, we evaluated several factors that affect captures, such as trapping location or trap characteristics.

**Materials and methods**

The study was conducted in Kalamata (Peloponnes, Southern Greece), which is one of the main fig productive areas of Greece. A horizontal (flat) warehouse was used for experimentation, which covered an area of approx. 460 m² (29.4 X 15.6 m). This warehouse, used each year for fig storage, was made of bricks and had a solid roof. At the southern walls, there were two glass windows and a door, usually kept closed. Approx. 310 tons of figs were stored, most of them from the previous years’ harvest, but there was also newly harvested product (approx. 90 tons) placed into the warehouse in early fall 2000. Most of the figs were in net-like sacks (50 kg each), but there were also small bulks of old product. The product covered equally the southern half of the storage area, at a height of 1.5 m, while the other half was empty.

In 17 September 2000, 36 traps were hung above the fig mass, by using wires across the store room’s roof. The traps were rectangular adhesive cardboard strips of equal size (27 x 8 cm), which are suspended vertically in three heights, 10, 30 and 100 cm above the fig mass. On each height, 3 groups of 4 traps were suspended. Within each group, the 4 traps had different colours, which were white, yellow, brown and green, respectively. The reflectance spectra of the colours used as measured by a spectrophotometer were 640, 418, 620 and 418 nm for white, yellow, green and brown, respectively. Each trap was baited with a polyethylene capsule, loaded with 100 µg of TDA [(Z, E)-9,12-tetradecadien-1-yl acetate], the sex attractant for the males of several stored-product Pyralidae species (Levinson and Buchelos 1981, Buchelos and Levinson 1985). The capsule was placed in a hole made at the centre of the sticky surface, in order to have sufficient pheromone emission from both sides of the trap. The distance between two adjacent traps was >2 m.

The traps were inspected for captured insects at 15-day intervals, until September 2001, by replacing the traps with new ones. The old traps were taken to the laboratory for counting and identification of the individuals found. Also, during trap inspection on each trap-check date, the traps within each replicate were rotated clockwise in order to minimize the influence of the individual trapping location, as recommended by Buchelos and Levinson (1993). The replacement of the pheromone capsules was made every two trap-check dates. No insecticidal treatments were applied during the trapping period. Air temperature and relative humidity were recorded indoors by using a digital thermohygrograph (Hobo H8, Onset Computer Co., USA).

During insect counting, the location of each trap in the warehouse (close to the windows or in dark areas of the storage room) was recorded, in order to compare captures between more illuminated or less illuminated areas of the warehouse. Also, the number of individuals found in the figurual outlines (edges) of the strips was counted separately.

Before the analysis the insect counts (for the most numerous species) were transformed to log(x+1) scale, to homogenize variances and standardize means. Data were analyzed,
separately for each species, by using the GLM Procedure of SAS (SAS Institute 1995) with number of captured individuals as the response variable and trap height and trap colour as main effects. Trap-check dates on which no adults were found in the traps, were not included in the analysis. Means were separated by using the Tukey-Kramer HSD test at p=0.05 (Sokal and Rohlf 1995). Additional analyses were conducted, separately for each species, to examine the influence of trapping location (percentage of captures on illuminated or less illuminated areas) and the within trap distribution of captures (percentage of captures on the internal part of the sticky surfaces or on the figural outlines of the traps). These analyses were carried out using a chi-square test (in all cases $df=2$).

Results

The seasonal fluctuation of the temperature and relative humidity indoors is shown in Fig. 1. During the entire monitoring period, 51221 individuals were found and classified in 27 insect taxa. Most of them belonged to Coleoptera, Lepidoptera and Hymenoptera, but also small numbers of Diptera and Hemiptera were recorded (Table 1). Most of beetle and moth species were known stored-product pests (Nitidulidae, Cucujidae, Curculionidae, Silvanidae, Pyralidae), while others are mainly fungus feeders (Cryptophagidae, Mycetophagidae). On the other hand, all Hymenoptera species found were parasitoids known to attack beetles or moths.

The most abundant insect species was *P. interpunctella*, given that more than one quarter of the total number of individuals found belonged to this species. On the contrary, all other pyralid moths were found in relatively low numbers, with the exception of *E. cautella*. Most of *P. interpunctella* adults were found during summer months (late June-late August) where adult numbers were >50/trap (Fig. 2). However, this species was also found in high numbers until early October, while no adults were recorded from early November until late March.

In the case of Coleoptera, the most numerous species was *C. hemipterus*. Adults of this species were found during the entire monitoring period, even during winter months, with the exception of two trap-check dates (late December and early February) (Fig. 2). The seasonal abundance of this species indicated two peaks, during late June and late August, with more than 25 adults/trap. In addition, this species was found in relatively high numbers during spring.

More than 90% of the total number of Hymenoptera individuals found belonged to *H. hebetor* and *C. tarsalis*. These two species indicated similar population trends (Fig. 3). Thus, *H. hebetor* was highly active from late August until late October (approx. 80% of the total number of adults of this species was recorded during this interval), while during the remaining trapping period, captures were rather low. Similarly, *C. tarsalis* was highly abundant during late summer and autumn (approx. 75% of the total number of individuals was found during this interval). Its densities were extremely high during late October 2000 (>30 adults/trap), fifteen days later that the highest density of *H. hebetor*. Finally, it must be noted that, for both species, adults were continuously recorded in the traps during the entire monitoring period, though in small numbers during winter and spring.

In the case of *P. interpunctella*, the main effects trap height and trap colour significantly affected captures ($df=2.564$, $F=3.08$, $P<0.0001$ for trap height, $df=3.564$, $F=8.10$, $P<0.0001$ for trap colour). On the contrary, the interaction trap height X trap colour was not significant ($df=6.564$, $F=0.29$, $P=0.9410$). Significantly more adults were found in the traps that were suspended 10 and 30 cm above the fig mass, in comparison with traps at 100 cm (Fig. 4). Also, white traps caught significantly more adults than brown ones. In contrast, yellow and green traps were statistically equivalent with the other two trap colours (Fig. 4).
Table 1. Insect taxa and percentage of the total number of individuals found, caught in traps during the entire trapping period in the fig warehouse.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>% of the total</th>
<th>Taxa</th>
<th>% of the total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coleoptera</td>
<td></td>
<td>Silvanidae</td>
<td></td>
</tr>
<tr>
<td>Cleridae</td>
<td></td>
<td>Oryzaephilus mercator</td>
<td>0.8</td>
</tr>
<tr>
<td>Necrobia rufipes (Degeer)</td>
<td>&lt;0.1</td>
<td>Oryzaephilus surinamensis</td>
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</tr>
<tr>
<td>Cucujidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptolestes ferrugineus</td>
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<td></td>
</tr>
<tr>
<td>Cryptolestes spp.</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curculionidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sitophilus oryzae (L.)</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptophagidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptophagus spp.</td>
<td>&lt;0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermestidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthrenus spp.</td>
<td>&lt;0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attagenus unicolor Brahms</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trogoderma sp.</td>
<td>&lt;0.1</td>
<td></td>
<td></td>
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<td>Other (Diptera, Hemiptera)</td>
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Fig. 1. Temperature (°C) and relative humidity (%) in the dried fig warehouse during the monitoring period.

As above, in the case of *C. hemipterus* trap height and trap colour had a significant effect on captures ($df=2.780$, $F=13.62$, $P<0.0001$ for trap height, $df=3.564$, $F=36.20$, $P<0.0001$ for trap colour), while the interaction of these two effects was not significant ($df=6.780$, $F=1.47$, $P>0.05$).
Significantly, more *C. hemipterus* adults were found in the traps that were suspended 10 and 30 cm above the fig mass, in comparison with traps that were suspended higher (Fig. 5). As far as the colours of the trapping surfaces concerned, white traps were significantly more efficient than yellow, green, and brown traps.

**Fig. 2.** *Plodia interpunctella* and *Carpophilus hemipterus* adults found per trap (±SE), during the entire monitoring period.

**Fig. 3.** *Habrobracon hebetor* and *Cephalonomia tarsalis* adults found per trap (±SE), during the entire monitoring period.
Fig. 4. Mean number of *Plodia interpunctella* adults/trap (±SE), on traps suspended at various heights (top) and traps with different colours (bottom) (within each diagram, means followed by the same letter are not significantly different; Tukey-Kramer HDS test, at $p=0.05$).

Similarly, trap height and trap colour significantly affected trap performance in the case of *H. hebetor* ($df=2.852$, $F=8.96$, $P<0.0002$ for trap height, $df=3.852$, $F=17.82$, $P<0.0001$ for trap colour) and *C. tarsalis* ($df=2.852$, $F=9.38$, $P<0.0001$ for trap height, $df=3.852$, $F=23.45$, $P<0.0001$ for trap colour). But the interaction between trap height and trap colour was not significant ($df=6.852$, $F=0.49$, $P=0.8148$ for *H. hebetor*, $df=6.852$, $F=0.38$, $P=0.8867$ for *C. tarsalis*). Significantly, more *H. hebetor* individuals were recorded in the two lower trap heights as compared to the traps that were hung 100 cm above the product mass (Fig. 6). On the contrary, for *C. tarsalis* significant differences were noted only between traps at 10 and 100 cm above the product (Fig. 7). White and yellow traps captured significantly more *H. hebetor* adults than green and brown traps, while green traps were more attractive than brown ones (Fig. 6). Finally, white, yellow, and green traps were significantly more effective on the capture of *C. tarsalis* than brown traps (Fig. 7).

Trap location (illuminated or dark areas of the warehouse) did not affect captures of *P. interpunctella* ($\chi^2=0.12$, $P=0.7275$) and *C. hemipterus* ($\chi^2=2.91$, $P=0.0879$), but had a significant effect on the capture of *H. hebetor* ($\chi^2=8.38$, $P=0.0038$) and *C. tarsalis* ($\chi^2=9.61$, $P=0.0019$). Thus, significantly more adults of these two wasp species were found in traps which were suspended at the more illuminated areas of the warehouse, in comparison with
traps at less illuminated areas (Fig. 8). Also, significantly more adults were found in the internal part of the trapping surface as compared to the figural outlines of the cardboard strips, for all the aforementioned species ($\chi^2=175.38$, $P<0.0001$ for *P. interpunctella*, $\chi^2=81.23$, $P<0.0001$ for *C. hemipterus*, $\chi^2=352.72$, $P<0.0001$ for *H. hebetor* and $\chi^2=386.77$, $P<0.0001$ for *C. tarsalis*) (Fig. 9). However, considerable variations were recorded in the «edge» preference among species. Hence, in the case of *C. hemipterus* approx. 40 % of the total number of adults caught was found in the figural outlines of the trap, while the respective figure for the other three species did not exceed 13 %.

![Fig. 5. Mean number of Carpophilus hemipterus adults/trap (±SE), on traps suspended at various heights (top) and traps with different colours (bottom) (within each diagram, means followed by the same letter are not significantly different; Tukey-Kramer HDS test, at p=0.05).](image)

**Discussion**

Aerial sticky traps have been used successfully in Greece for monitoring the seasonal activity of several species occurring in stored products (Buchelos 1980, Levinson and Buchelos 1981, Buchelos and Levinson 1985, 1993, Athanassiou et al. 2002, Eliopoulos et al. 2002b, Athanassiou and Eliopoulos 2003). Baited or unbaited, these traps are capable of detecting a wide number of species that occur in currant stores (Athanassiou and Eliopoulos 2003), providing reliable information about the species’ spectra and their seasonal trends. According
to the results of our study, despite the fact that >70% of the individuals found belonged to four species, an extremely large number of species can be found in stored figs, during a long-term monitoring period.

![Graph showing mean number of Habrobracon hebetor adults/trap on traps suspended at various heights and traps with different colours.](image)

**Fig. 6.** Mean number of *Habrobracon hebetor* adults/trap (± SE), on traps suspended at various heights (top) and traps with different colours (bottom) (within each diagram, means followed by the same letter are not significantly different; Tukey-Kramer HDS test, at p=0.05).

The large number of Lepidoptera found during the present study is attributed to the addition of TDA in the trapping surfaces, which acts as a multi-species male attractant in the case of stored-product Pyralidae (Buchelos and Levinson 1985). On the contrary, unbaited traps provide poor information, and are often non-indicative for the population of adult moths (Mullen et al. 1998, Athanassiou and Eliopoulos 2003). However, other species are caught in high numbers in aerial sticky surfaces, such as Coleoptera and Hymenoptera. Apparently, in the case of beetles, only species with strong flying activity are caught in sufficient numbers in traps, such as Nitidulidae, Cucujidae or Silvanidae. Thus, it is likely that other species existed in the warehouse, but they were not detected due to poor flying performance. It is well known that other trap designs, such as perforated probe traps or floor traps are more efficient for crawling insects (Athanassiou and Buchelos 2001, Eliopoulos et al. 2002b), but the use of several trap types in the same warehouse may cause serious implications arising for the increased labor needed for monitoring. Aerial sticky surfaces can be used for multi-species detection, and seem to be the most effective trap design for stored-product parasitoids.
(Weston and Barney 1998, Johnson et al. 2000, Eliopoulos et al. 2002b). This is due to the fact that wasps fly much more than beetles, which mainly crawl into the product mass.

![Graph showing mean number of adults/trap at different trap heights (top) and trap colors (bottom).](image)

**Fig. 7.** Mean number of *Cephalonomia tarsalis* adults/trap (±SE), on traps suspended at various heights (top) and traps with different colours (bottom) (within each diagram, means followed by the same letter are not significantly different; Tukey-Kramer HDS test, at $p=0.05$).

The seasonal trends of the most abundant species found were notably varied according to species. Thus, adults of *P. interpunctella* are highly active during the warm months of the year, while no activity occurs during late autumn and winter, when prevailing temperatures are rather low for adult activity. This observation agrees well with other studies regarding the seasonal trends of stored-product Pyralidae in Greece (Buchelos 1980, Levinson and Buchelos 1981, Athanassiou et al. 2002, Athanassiou and Eliopoulos 2003). Furthermore, *C. hemipterus* indicated a noticeable presence in the traps mainly during warm months. Similar observations have been reported in a recent study by Athanassiou and Eliopoulos (2003) in stored currant at the same region. The decrease in *C. hemipterus* presence in traps is mainly due to the reduction caused in flying activity by the decrease of temperatures, and may not be indicative of low infestation level in the product mass.

Very few data are available for the seasonal abundance of parasitic wasps in stored-product commodities. Our study indicates that, based on trap catches, a high number of parasitoid individuals exists in stored figs; in fact, more than one third of the total number of individuals found were parasitic wasps. Among the species found, *H. hebetor* and *V.*
canescens are moth parasitoids, while C. tarsalis and H. sylvanidis are beetle parasitoids, all very common species in Greece (Eliopoulos et al. 2002a, b, Athanassiou and Eliopoulos 2003). The two most abundant wasp species (H. hebetor and C. tarsalis) exhibited peaks during late summer and early fall. Additionally, these two species were active during the entire trapping period, even during the winter months, suggesting that these species can survive winter in southern Greece. Thus, the release of these two wasp species during winter can be used to suppress host populations early in the season.

![Graph](image)

Fig. 8. Percentage (%) of adults of each species caught in traps suspended in more or in less illuminated areas ($\chi^2$ test within each species, ns: there are no significant differences, *: significantly different at $p=0.05$).

Traps placed directly over the fig mass (placed at 10-30 cm) captured more adults than traps which are suspended higher (100 cm), for P. interpunctella, C. hemipterus and H. hebetor. This is obviously, due to the fact that the majority of the individuals caught are within the product, to mate, infest or, in the case of parasitoids, seek for a suitable host. Athanassiou et al. (2002) who used sticky traps to monitor adult male activity of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) in stored grain, found that an increased number of males was captured at the lower part of the sticky surface, indicating that most males came from the grain mass. Johnson et al. (2000), using traps to monitor seasonal fluctuation of insects in a fig warehouse, also found more H. hebetor males in traps placed just above the product. Also, Antonin and Strand (1992) reported that more H. hebetor males are found on the surface of shelled corn. Although no quantitative measurements to record the sex of the captured H. hebetor individuals, counting in some of the traps revealed that most of the H. hebetor individuals caught were males, a fact which is mainly attributed to the mate-seeking behaviour. However, for C. tarsalis, traps placed 30 cm and 100 cm above the fig mass were equally efficient, which may indicate an increased, to some extent, flight activity or dispersal trend. Eliopoulos and al. (2002b) reported a considerable dispersion level of C. tarsalis adults into bulked grain, due to its small body size, which allows it easily to pass through the interstitial spaces of grain seeds. Despite the increased capture potential, it must be noted that practically the placement of adhesive traps directly over the fig mass should be avoided,
because the traps are likely to be destroyed during the removal of the product. Also, high numbers of captured insects may cause rapid saturation of the adhesive surface, or lead to overestimation of insect presence and infestation level.

Fig. 9. Percentage (%) of adults of each species caught in the internal part of the sticky surfaces and in the figural outlines of the traps ($\chi^2$ test within each species, *: significantly different at p=0.05).

One of the most interesting results of the present study is the preferential flight to specific visual stimuli, given that very little information is available so far. Thus, some colours of the strips increased trapping efficacy. In all cases examined, with the exception of *P. interpunctella*, brown was the less attractive colour. Thus, traps with more bright colours should be selected for use in stored figs. However, considerable variations were recorded among species as far as the more attractive colour is concerned. White strips were by far the most attractive for *C. hemipterus* and among the more attractive ones for the other three species examined. Green is equally attractive with white and yellow for *C. tarsalis*, but less attractive than these two colours for *H. hebetor*. Our results are in agreement with those noted by Schöller and Prozell (2003). In these tests, by using different colours of funnel traps in a release-recapture study, the authors found that white/yellow funnels were more attractive to *H. hebetor* adults than green ones. However, in exposed sticky surfaces the flying individuals landed directly onto the visual stimuli, while in the case of funnels insects have to move downwards to enter the trap, and thus, direct comparisons between similar characteristics of dissimilar trap types should be avoided. The green that were used by Schöller and Prozell (2003) and by us in the current work, can be classified as «dark» greens. According to recent tests (Athanassiou and Eliopoulos unpublished data), light green is much more attractive to *H. hebetor* than dark green, suggesting that the reflectance spectra of a given colour may be at least as important than the colour itself. In general, the knowledge of the species that occurs in a warehouse can lead managers to select the appropriate trap design (including colour) and this knowledge is essential when developing a long-term trap-based monitoring system. Thus,
based on the present study, white traps should be selected for monitoring, since white performed equally well for the most abundant species. Light is also an important component that determines trapping efficacy. According to our results, more wasps were found in traps placed at more illuminated (close to windows) trap locations, while light has no effect in the case of *P. interpunctella* and *C. hemipterus*. The reasons for this preference have not been investigated so far, and further experimentation is needed to assess these variables. Other shades of the same colours or a different degree of light intensity are likely to produce different results; hence, generalisations about colour/light influence should be avoided.

Despite the fact that significantly more adults were found in the internal part of the trapping surface for all species examined, a considerable proportion of *C. hemipterus* adults was found at the figural outlines of the trap. Quartey and Coaker (1992) reported a similar «edge» preference for *E. cautella*. Athanassiou et al. (2002), using similar traps, also reported that, for *E. kuehniella*, this behavior is expressed more vigorously in unbaited than in TDA-baited strips. This attraction is considered as a part of the male mate-finding behaviour (Levinson and Hoppe 1983).

Traps are useful tools for insect management in stored products, in order to time control, and avoid unnecessary control treatments. Information about the seasonal occurrence of insect populations in stored figs can be utilized for early detection and prevention of the infestation at its early stages. Furthermore, the incorporation of natural enemies, many of which may already occur in high densities in fig stores, is likely to provide a degree of control, in combination with other IPM practices (extreme temperatures, controlled atmospheres etc.). Parasitoid species that are able to remain active during mild winter, like the study area, can be released during that period. At that stage, traps can be used to know the degree of wasp establishment.

**References**


Development time, fecundity and longevity of *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as host

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Abstract: *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) was found in agricultural storage in Ankara (Turkey), and cultured on mature larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Some biological characters of *V. canescens* were studied on mature larvae of *E. kuehniella* at 20 ± 1 °C, 60-70 % RH, and a photoperiod of 16:8 h (L:D). Oviposition period, development time, fecundity and longevity of *V. canescens* were defined. The pre-oviposition period in the parasitoid was less than 24 h. The oviposition period averaged 15.44 days and the post-oviposition period averaged 7 days. The mean development time from egg to adult lasted 36.29 days. The mean production of progeny per female parasitoid was 162.29. Longevity of *V. canescens* was defined in three different treatments. Mean longevity of the parasitoid was found to be 22.43 days amongst ovipositing wasps fed with honey, 38.09 days amongst non-ovipositing wasps fed with honey, and 4.59 days amongst non-fed and non-ovipositing wasps.

Key words: *Venturia canescens*, development time, fecundity, longevity

Introduction

Mediterranean flour moth *Ephestia kuehniella* causes severe damage of stored agricultural products and raw materials for food production. Chemical treatments are the most frequently used strategies to control *E. kuehniella* and other pyralid moths in agricultural storage. Biological control in food storage is being considered as a promising alternative to chemical control of stored-product insects.

*Venturia canescens* has been identified to be a natural enemy of larvae of Mediterranean flour moth occurring in Turkey, and was reared in culture. The parthenogenetic, solitary, koinobiont larval endoparasitoid *V. canescens* is a parasitoid of the caterpillars of various species of phycitid moths (Salt, 1961; Fisher, 1961). Parasitism is the key factor for the evaluation of the suitability of this parasitoid for biological control. For this reason, this aspect was investigated at 20 ± 1°C, 60-70 % RH, and a photoperiod of 16:8 (L:D) h.

Materials and methods

The stock culture of *E. kuehniella* was obtained from University of Ankara, Faculty of Agriculture, Department of Plant Protection. *Venturia canescens* was collected from agricultural storage in Ankara (Turkey). Both host and parasitoid were reared at 25 ± 1 °C constant temperature with a 16:8 h light and dark photoperiod and 60 - 70 % RH (Ozkan, 1999).

The host *E. kuehniella* was reared in clear plastic containers (27x37x7 cm) on a 2:1 mixture of wheat flour and wheat bran containing approximately 800 g food and 2000 *E. kuehniella* eggs. Plastic containers were covered with clothes. The eggs hatch about 4 days.
The moths completed five instars, larval development averaged 35 days. Pupal period was approximately 8 days and the adult moths live approximately 8 days at the conditions of temperature and nutrition used in this study.

*V. canescens* was reared on mature larvae of *E. kuehniella*. Newly emerged wasps were introduced into glass tubes (3x15 cm), and fed with honey regularly for 4-5 days. These 4-5 day old adult wasps, an age which usually coincides with maximum egg loads, fed with honey were transferred in groups of ten or fifteen into the plastic containers containing on an average 2000, 29 day-old mature host larvae. After 24 hours the wasps were removed from the container in order to prevent superparasitism. Development of the wasps from oviposition to adult emergence takes about 25 days.

Experiments were conducted at 20 ± 1°C, 60-70 RH, and a photoperiod of 16:8 (L:D) h. Thirty last-instar larvae of *E. kuehniella* were exposed to one wasp in glass tubes (3x15 cm) containing approximately 30 g food for 24 h. This process was repeated until the wasps died. Ovipositional periods, development time and fecundity of *V. canescens* was defined. Longevity of the wasp was determined in three different treatments: with honey and host, with only honey, and without honey and host.

**Results and discussion**

Results are shown in Table 1. The pre-oviposition period in *V. canescens* was less than 24 h. The oviposition period averaged 15.43 days and the post-oviposition period averaged 7 days. Many researchers have shown that newly emerged *V. canescens* females were capable of immediate oviposition (Ahmad, 1936; Diamond, 1929; Fisher 1959, 1962; White and Huffaker, 1969).

<table>
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<td>15.43± 1.89 (4-34)</td>
<td>22.43± 2.29 (8-37) b n=22</td>
<td>38.09± 3.72 (13-65) c n=22</td>
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Means ± SE followed by different characters differ significantly (ANOVA; p=0.05 Tukey)

Longevity of the parasitoid was determined using three different treatments; non-feeding and non-ovipositing, feeding with honey and non-ovipositing, and feeding with honey and ovipositing conditions (Table 1). The mean longevity of the parasitoid varied significantly among the three treatments. Minimum longevity of the wasp was defined at non-feeding and non-ovipositing conditions. Mean longevity of *V. canescens* was found to be 4.49 days at this treatment. When the parasitoid was provided with hosts and fed with honey, mean longevity of *V. canescens* increased three times, and was found to be 22.43 days. Maximum longevity of the wasp was defined at feeding with honey and non-ovipositing conditions. Mean longevity of *V. canescens* was found to be 38.09 days at this treatment. These results showed that feeding with honey significantly increased the longevity, on the other hand, oviposition significantly decreased the longevity. Numerous laboratory studies have shown that the life
span of adult parasitoids can be increased up to 10-fold by feeding sugar (Leius, 1961; Heimpel et al., 1997; McDougall and Mills, 1997; Olson et al., 2000).

The wasp was successful in completing development on fifth host instar of *E. kuehniella* at 20°C. The mean production of progeny per female parasitoid was 162.29 (33-287), and the mean development time from egg to adult lasted 36.29 (28-57) days under these conditions. Biotic and abiotic factors affecting development of *V. canescens* were summarized by Harvey and Thompson (1995). Eliopoulos and Stathas (2003) have shown that development of *V. canescens* on *E. kuehniella* changed depending on temperature and host stages. In their study, the average development time of the wasp was 38.36 days on fifth instar of the host at 20 °C.

This study has shown that *V. canescens* may become a valuable biocontrol agent of lepidopterous pests in storage facilities. However, further studies are required under laboratory and natural conditions.

References


Effect of relative humidity on the preimaginal development of
*Blattisocius tarsalis* (Acari: Ascidae)

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**Abstract:** The predatory mite *Blattisocius tarsalis* is a native natural enemy of several stored product pests. It is frequently found in stores under high relative humidity (RH) conditions. However, it is advisable to maintain low RH in stores and to reduce the moisture content levels of stored grain and food products. In this work we studied the preimaginal development time of *B. tarsalis* and the consumption of *Ephestia kuehniella* eggs at three constant RH: 40, 60 and 80%. Mean developmental time ranged from 7.8 to 9.6 days at 60% and 80% RH, respectively. Mean number of *E. kuehniella* eggs killed per day averaged 1.2 and did not differ significantly between 60% and 80% RH. At a relative humidity of 40%, only 8% of the individuals completed their immature development.

**Key words:** *Blattisocius tarsalis*, development, predation, relative humidity, stored product pests

**Introduction**

Biological control involving the conservation and augmentation of natural enemies has proven an effective method of pest control in agriculture and forestry. However, few cases of successful biological control have been reported when predators, parasitoids or microorganisms were deliberately released in stores and food factories (Haines, 1999). Research is needed into the biology of these natural enemies in order to select suitable candidates. Many species of natural enemy of stored product pests have been described. One of these, the mite *Blattisocius tarsalis* (Berlese) (Acari: Ascidae), is a polyphagous predator of eggs of many Lepidoptera and Coleoptera pest species. It is widely distributed in Europe, North Africa, America, Asia, and Australia (Hughes, 1976). In the northeast of Spain, it is frequently found in stored food facilities in association with several different pest species (Riudavets et al., 2002a). Several parameters of the biology of this predatory mite, such as its duration of development, prey consumption and functional response have already been studied (Darst and King, 1969; Haines, 1981, Graham, 1970; Nielsen, 1998; Riudavets et al., 2002b). However, these studies were conducted at high relative humidity (RH). The RH and the moisture content of commodities are of critical importance to the survival and development of insects and mites. In the northeast of Spain RH tends to remain above 60% because of the influence of the Mediterranean Sea (InfoMet, 2003). However, RH may fall in summer at some storage locations.

The present work aims to study the possible use of *B. tarsalis* as a biological agent for controlling mill industry pests. This paper presents data on the development and predation capacity of *B. tarsalis* at three constant RH levels (40%, 60%, and 80% RH).

**Material and methods**

The *Blattisocius tarsalis* used for the experiments came from colonies maintained at the ‘Institut de Recerca i Tecnologia Agroalimentàries’ (Cabrils, Barcelona). The predatory mite
was originally collected from wheat semolina infested with *Tyrophagus putrescentiae* (Schrank) and *Liposcelis bostrychophila* (Badonnel) in Barcelona, northeast Spain. It was reared on vermiculite and fed throughout the experiment on prey of frozen *Ephestia kuehniella* Zeller eggs. All laboratory studies were conducted in a climatic chamber at 25±1ºC. The relative humidity levels tested were 80%, 60%, and 40%.

We caged individual female mites in 0.67 ml hard gelatin capsules (Acofarma) and regulated humidity by placing individual capsules over solutions of glycerol and water (Braun and Braun, 1958). Three humidity levels (80, 60, and 40% RH) were created within sealed containers (5 L) and the gelatine capsules were placed inside them. After 24 hours, females were removed and 40 to 42 repetitions were made with a single *B. tarsalis* egg. Egg hatch was recorded every 24 hours. After hatch, *E. kuehniella* eggs were added to the capsule in order to maintain a minimum of 10 per capsule: development and prey consumption were checked every 24 hours over a 3-week period.

One-way analysis of variance (ANOVA) and the Tukey test (SAS, 1996) were performed on the data for preimaginal (immature stage) development duration and consumption of *E. kuehniella* eggs to test for significant differences between relative humidity conditions.

**Results and discussion**

The predatory mite was able to complete its preimaginal development at 60% and 80% RH by feeding on *E. kuehniella* eggs. However, the percentage of individuals reaching adulthood was much higher at 80% than 60% RH: 95% and 46% of individuals, respectively (Table 1). At 60% RH the highest mortality occurred during the protonymph stage. At a relative humidity of 40% only 7.9% of individuals completed preimaginal development. At this RH, the majority of individuals died during the egg and protonymph stages, probably as a result of desiccation. Eggs lose water to their surroundings for a large number of reasons, but mainly due to the permeability of their chorion and subchoral membranes depending on their stage of development (Hinton, 1981). At unfavourable levels of humidity, eggs either do not hatch or their incubation period may be considerably extended. As seen in Table 1, the time required for egg development was significantly longer at 40% RH than at 60% (*F*=195.66, df=2;99, *p*<0.001). Egg development time was also longer at 60% than at 80%. Darts and King (1969) and Barker (1967) reported durations of egg development for *B. tarsalis* and *B. keegani* of about 2 days at 25ºC and 75% RH and hatching percentages of ca. 99%. These are somewhat similar to the values obtained in the present study. In comparison, the development time durations required for the larva and nymph stages were similar at all three RH levels (*F*=2.44, df=2;56, *P*>0.05) (Table 1). Nielsen (2001) found a similar mean development time for *B. tarsalis* at 25ºC and 75% RH. However, Haines (1981) found a slightly shorter development time (5.9 days) for *B. tarsalis* when fed on the moth *E. cautella* at 27ºC and 73% RH. Considered together, egg, larva and nymph development were significantly longer at 40% and 60% RH than at 80% (*F*=17.76 df=2;56, *P*<0.001). However, on average, differences in RH were of less than 2 days. Storage insects and mites have the ability to obtain water from food and to use their own metabolic water even at low RH levels (Howe, 1991). As previously stated, RH greatly affected mite survival: it was twice as high at 80% as at 60%.

At all three studied RH levels, the immature stage of *B. tarsalis* consumed more *E. kuehniella* eggs per day at 40% RH than at 60% or 80% RH (*F*=5.44 df=2;29, *P*=0.05) (Table 2). Although it appears that more *E. kuehniella* eggs were consumed at 40% RH than at 60% or 80% RH during total mite preimaginal development, there were no significant differences at the 5% confident level (*F*=2.99 df=2;29, *P*=0.067). Higher consumption at 40% was mainly the result of the longer development time combined with the higher daily predation rate.
Differences in food consumption may be explained by the fact that in the present study food was the only source of water. According to Haines (1981), the total number of *E. cautella* eggs that need to be consumed by *B. tarsalis* to complete its development at 27ºC is 3.8. This is comparable to the values that we found (10 *E. kuehniella* eggs) if we take into account the fact that ca. 35% of the eggs were less than 50% consumed and very few were fully consumed.

Table 1. Preimaginal duration (days) and survival (%) of *B. tarsalis* at 25 ± 1ºC and at three different RH levels.

<table>
<thead>
<tr>
<th>RH (%)</th>
<th>Eggs n</th>
<th>Duration ± SEM</th>
<th>% Hatch</th>
<th>Larvae and nymphs n</th>
<th>Duration ± SEM</th>
<th>Duration ± SEM</th>
<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>40</td>
<td>3.3±0.09a</td>
<td>57.5</td>
<td>21</td>
<td>5.7±0.33a</td>
<td>9.3±0.67a</td>
<td>7.9</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>3.0±0.0b</td>
<td>92.5</td>
<td>36</td>
<td>6.6±0.36a</td>
<td>9.6±0.35a</td>
<td>46.2</td>
</tr>
<tr>
<td>80</td>
<td>42</td>
<td>1.9±0.06c</td>
<td>97.6</td>
<td>38</td>
<td>5.9±0.14a</td>
<td>7.8±0.13b</td>
<td>94.7</td>
</tr>
</tbody>
</table>

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

Table 2. Consumption rate of *B. tarsalis* nymphs on *E. kuehniella* eggs.

<table>
<thead>
<tr>
<th>RH (%)</th>
<th>No. of prey consumed</th>
<th>Total development ± SEM</th>
<th>Per day ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>16.3±1.76a</td>
<td>1.7±0.08a</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>10.9±0.98a</td>
<td>1.1±0.07b</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>10.9±0.81a</td>
<td>1.2±0.11b</td>
<td></td>
</tr>
</tbody>
</table>

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

*Blattisocius tarsalis* seems quite notably affected by a reduction in RH to between 80 and 60%. Although preimaginal development time was not greatly retarded at low (60%) RH, mortality was twice as high as in favourable (80%) RH conditions. At extremely low (40%) RH, *B. tarsalis* invariably failed to complete its development. Future research is needed to improve knowledge of the behaviour of *B. tarsalis* in stores before it can be selected as a candidate for the biological control of stored product pests in the Mediterranean area.

**Acknowledgements**

The authors thank the Ministerio de Ciencia y Tecnología (PETRI PTR1995-0584-OP) and S.E. de Carburos Metálicos S.A. for economic support.

**References**


Occurrence of stored-product pyralid moths and their parasitoids in stored currant and in vineyards

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Abstract: The seasonal occurrence of pyralid moths and their parasitoids was monitored in and around a currant warehouse, as well as in vineyards located near the warehouse and approx. 10 cm from the warehouse. Delta traps were used, baited with TDA, the sex attractant for several pyralid moths. The traps were inspected for captured insects from May until November 2001. The most abundant insect species found were Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), Ephestia cautella (Walker) (Lepidoptera: Pyralidae), and the Hymenoptera parasitoids Cephalonomia tarsalis (Ashmead) (Bethylidae) and Habrobracon hebetor (Say) (Braconidae). Pyralidae were mainly found in and around the warehouse, but they were also recovered in the vineyards, mainly near the warehouse. Most of the adult moths were found during July and August. On the contrary, with the exception of few individuals, all parasitoids were detected in and around the currant warehouse. The highest parasitoid densities, inside and outside of the warehouse, were recorded during August, September and October. On the other hand, early (during May, June and early July) and late in the season (during early November), parasitoid presence was limited, especially out of the warehouse.

Key words: stored currant, Plodia interpunctella, Ephestia cautella, Cephalonomia tarsalis, Habrobracon hebetor, TDA, parasitoids

Introduction

Greece leads the world in the production of currants, given that the annual production in Greece represents more than 80% of the total world production. The majority of these quantities are processed and stored each year in Greek storage facilities. Several insect species infest currant during storage, causing significant quantitative losses and qualitative degradations. The seasonal occurrence of these species has been examined recently by Athanassiou and Eliopoulos (2003), by using adhesive traps. However, there is still inadequate information about the source of infestation, given that, apart from the storage facilities, newly-harvested product is often reported to be infested before it enters the store. Also, the presence of stored-product species is recorded around storage facilities or even in vineyards. The importance of this fact should be substantially evaluated, since it denotes the source and the nature of an infestation. If, for instance, the main source of the infestation comes from insects that occur in the newly-harvested product, current control strategies for the elimination of an infestation at its earliest stages have to be re-examined (Levinson and Buchelos 1981). On the other hand, if the insects that occur outside of a warehouse consist additional sources of infestation, additional measures that restrict insect migration should be taken (Doud and Phillips 2000). Also, several stored-product predators or parasitoids are likely to occur among the insects that are active outdoors and their role should be investigated. Many researchers have reported the occurrence of stored-product moth species in the field, but there are few data concerning predators or parasitoids.
In this study, we used adhesive pheromone-baited traps to monitor the presence of pyralid moths in and around a currant warehouse, as well as in vineyards close or far from the warehouse. Since the adhesive traps are capable of multi-species detection (Athanassiou and Eliopoulos 2003), information was obtained about the occurrence of other stored-product species, including parasitoids.

Materials and methods

The study was carried out in a flat currant warehouse located in Kalamata (Peloponese, Southern Greece), filled with approx. 1200 tons of bulked currant, mainly from the previous years’ harvest (for details about the warehouse see Athanassiou and Eliopoulos 2003). The warehouse had a door and a series of glass windows, usually kept closed. Four adhesive white Delta traps (Agrisense BCS, UK) were suspended in the warehouse, approx. 1 m above the product mass. Four additional traps of the same type were placed around the warehouse (one on each cardinal direction) at a height of 2.2 m above the ground and at a distance less than 15 m from the store.

There were no currant quantities outside of the warehouse, apart from small old product residues, mainly close to the door. Four traps were placed in vineyards in the vicinity of the storage facility (at a distance 100-500 m from the warehouse). At these vineyards, the main cultivated variety was the «Corinth Currant» from which the currants are produced after drying the grapes. In addition, four more traps were suspended in vineyards with the same variety located approx. 10 km from the warehouse. Along this distance (10 km) there were no other stores, but some currant quantities might have been stored in the nearby farm houses. All traps were baited with a polyethylene capsule loaded with 100 µg of TDA [(Z, E)-9,12-tetradecadien-1-yl acetate], the sex attractant of several stored-product Pyralidae species (Levinson and Buchelos 1981, Buchelos and Levinson 1985, Chambers 1990).

The traps were suspended in the above locations on 16 May 2001, and inspected for captured individuals at weekly intervals, until early November of the same year. During each inspection, the cardboard adhesive surface of each trap was replaced and the old cardboard was taken to the laboratory for counting and identification of the insects found. No insecticidal treatments were applied during the monitoring period, but several insecticides were applied by producers in the vineyards, mainly during summer months against Lobesia botrana (Den. & Schiff.) (Lepidoptera: Tortricidae).

Results

A total of 13 taxa were found in traps, which are classified into three orders: Coleoptera, Lepidoptera and Hymenoptera (Table 1). Actually, there was a large number of species belonging to several other orders as well, but their numbers were not included in the results to facilitate counting since a) most of them were not associated with stored products and b) for the majority of these species the identification was not possible at the species or even at the genus level. All taxa presented on Table 1 were found in and around the warehouse, but only 11 and 8 taxa were found in the vineyards close or 10 km from the warehouse, respectively. The relative abundance of each species was notably varied according to the location of the traps. Thus, E. cautella, P. interpunctella, Cryptoestes spp., C. tarsalis and H. hebetor were the most abundant species in the warehouse, corresponding to >70 % of the total number of individuals found. Around the warehouse the species E. cautella, P.
Plodia interpunctella, and C. tarsalis corresponded to >60 % of the total, while the most abundant beetles were the species of the genus Carpophilus. The situation is reversed in the case of traps located in vineyards. Thus, almost 80 % of the total number of individuals found in traps suspended in vineyards located in the vicinity of the warehouse belonged to P. interpunctella and E. cautella. Similarly, in the vineyards located 10 km away from the warehouse, approx. 75 % of the total number of insects belonged to these two species. It must be noted that, in the case of vineyards, a considerable proportion of individuals were classified as «other» species, which are not associated with stored-products (Table 1).

Plodia interpunctella adults were found in and around the warehouse during the entire monitoring period (Fig. 1). In the warehouse, a peak was recorded at mid-June, but the highest numbers were noted on early July and early August. During August, P. interpunctella adult density was high, followed by a gradual decline during late fall. However, an additional peak with more than 25 adults/traps was recorded at mid-September. Around the warehouse, P. interpunctella adults were also found in high numbers, but densities were lower compared to the respective ones in the warehouse. High

Table 1. Insect taxa found during the monitoring period and their relative abundance in the locations examined.

<table>
<thead>
<tr>
<th>Insect taxa</th>
<th>% of the total number of individuals found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In the warehouse</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td></td>
</tr>
<tr>
<td>Pyralidae</td>
<td></td>
</tr>
<tr>
<td>Ephestia cautella (Walker)</td>
<td>11.3</td>
</tr>
<tr>
<td>Ephestia elutella (Hübner)</td>
<td>0.5</td>
</tr>
<tr>
<td>Ephestia kuehniella Zeller</td>
<td>1.1</td>
</tr>
<tr>
<td>Plodia interpunctella (Hübner)</td>
<td>20.4</td>
</tr>
<tr>
<td>Coleoptera</td>
<td></td>
</tr>
<tr>
<td>Cucujidae</td>
<td></td>
</tr>
<tr>
<td>Cryptolestes spp.</td>
<td>11.2</td>
</tr>
<tr>
<td>Mycetophagidae</td>
<td>0.2</td>
</tr>
<tr>
<td>Nitidulidae</td>
<td></td>
</tr>
<tr>
<td>Carpophilus spp.</td>
<td>6.3</td>
</tr>
<tr>
<td>Silvanidae</td>
<td></td>
</tr>
<tr>
<td>Oryzaephilus spp.</td>
<td>3.1</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td></td>
</tr>
<tr>
<td>Bethylidae</td>
<td></td>
</tr>
<tr>
<td>Cephalonomia tarsalis (Ashmead)</td>
<td>12.5</td>
</tr>
<tr>
<td>Hylorhynchus silvanidis (Brèthes)</td>
<td>6.2</td>
</tr>
<tr>
<td>Braconidae</td>
<td></td>
</tr>
<tr>
<td>Habrobracon hebetor Say</td>
<td>16.4</td>
</tr>
<tr>
<td>Ichneumonidae</td>
<td></td>
</tr>
<tr>
<td>Venturia canescens (Gravenhorst)</td>
<td>3.1</td>
</tr>
<tr>
<td>Other taxa (from the orders above)</td>
<td>7.7</td>
</tr>
</tbody>
</table>
Fig. 1. Mean number of *P. interpunctella* adults per trap, in traps located in and around the currant warehouse, during the monitoring period.

Fig. 2. Mean number of *P. interpunctella* adults per trap, in traps located in vineyards around and 10 km from the currant warehouse.
Fig. 3. Mean number of *E. cautella* adults per trap, in traps located in and around the currant warehouse, during the monitoring period.

Fig. 4. Mean number of *E. cautella* adults per trap, in traps located in vineyards around and 10 km from the currant warehouse.
numbers were recorded outdoors during July and August. On late August a peak was recorded (almost 30 adults/trap) and this was the only trap-check date where densities outside reached those inside the warehouse. On the contrary from late-September and until
the end of the monitoring period, a rapid decline was observed, and densities did not exceed 1 adult/trap. In the vineyards, *P. interpunctella* adults were not recorded until mid-June, while captures were notably lower than those inside or outside the warehouse (Fig. 2). In general, traps located in vineyards close or 10 km from the warehouse indicated similar seasonal trends, although moth numbers in the remote fields were continuously low, and did not exceed 3 adults/trap. For both distances, the highest numbers were noted during mid-August. On the other hand, during fall, very few moths were detected in the remote field.

Like *P. interpunctella*, *E. cautella* adults were also present in and around the warehouse during the entire monitoring period (Fig. 3). *Ephestia cautella* adults outside were proportionally few in comparison with the respective captures inside the warehouse, and did not exceed 2.5 adults/trap. In the warehouse, the populations outbursts were recorded during late July and late August. From late September and on, captures were notably reduced, especially outside, where very few moths were detected. Also, *E. cautella* presence was limited in the vineyards, and only in one occasion (late August) captures exceeded 1 adult/trap (Fig. 4). In addition, no adults were detected early (May-early June) or late in the season (late September-November).

No parasitic wasps were detected in the vineyards, with the exception of few individuals of *V. canescens* and *H. hebetor*, which were recorded only in the vineyards around the warehouse (Table 1). *Cephalonomia tarsalis* was found during the entire trapping season, and captures were extremely high during August and especially during October, when *C. tarsalis* presence exceeded 100 adults/trap (Fig. 5). On the contrary, this species was found inside the warehouse in very low numbers during May and July. Out of the warehouse, very few adults were recorded in June, and captures were reduced until mid-August. Later, captures were considerably increased and reached 20 adults/trap on early October. *Habrobracon hebetor* adults were found in high numbers during August, September and October (Fig. 6). As in the case of *C. tarsalis*, in the warehouse this species was found in rather low numbers until the end of July. The majority of *H. hebetor* adults that were found around the warehouse were recorded during July and August, while captures in October were extremely low.

**Discussion**

Our study indicates that stored-currant moths occur in high numbers out of storage facilities, but also in vineyards. On the other hand, stored-product parasitoids, like *C. tarsalis* and *H. hebetor*, also occur in high numbers in and around currant stores, but they are almost absent in vineyards. The significance of the outdoor presence of stored-product moths on the basis of infestation in the field is low, but their presence is highly important as additional sources of infestation. Vick et al. (1987) found substantial numbers of *P. interpunctella* adults out of storage facilities, but not in locations which were far from the warehouses. Doud and Phillips (2000) also reported extremely high numbers of *P. interpunctella* around the facilities of flour mills. These studies suggest that moths that exist outdoors may not feed on outdoor habitats, and this occurrence is mainly due to dispersal patterns (Bowditch and Madden 1996). However, at least for some species, stored-product moths may cause some infestations in the field, at the preharvest stages. Trematerra and Gentile (2003) recorded high numbers of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) in wheat fields in central-southern Italy. In that study, *S. cerealella* densities were high in the fields just before, during but also right after harvest. In light of our findings, *P. interpunctella* and *E. cautella* were found in the vineyards at the preharvest stage or during grape-harvest of Corinth Currant, suggesting that the infestation
in the vineyards may occur at that period. Moreover, captures were continued after harvest and removal of the product from the vineyard, but at this stage, small quantities of overripe grapes still exist. Hence, there is a possibility of newly-harvested product with small moth numbers to enter the store room. The characteristics and the significance of this infestation early at the storage period, must be further evaluated. Nevertheless, newly-harvested product is less likely to be heavily infested (Athanassiou and Buchelos 2001).

*Plodia interpunctella* and *E. cautella* adults are more active close to the warehouses than in more remote locations in adjacent or less adjacent fields. In our study, the presence of this species was negatively affected by the distance from the currant warehouse. This fact stands in accordance with the observations of Doud and Phillips (2000) who found that the presence of *P. interpunctella* was negatively correlated with the distance from flour mill facilities. These data support the above estimation that the invasion of moths form outside (through the windows for instance) may be equally or even more important than the introduction of newly-harvested product containing small numbers of insects. In a flour mill Levinson and Buchelos (1981) concluded that moth infestations were mainly due to the introduction and storage of infested product, rather than the immigration of moths from outdoors. In flour mills, the introduction of infested grains maybe more important then the emigration of insects from outdoor locations, since grains are continuously introduced. In contrast, in currant stores the product is usually introduced once, right after harvest. Males may emigrate from the warehouse interior to outdoor locations around the warehouse for several reasons. Doud and Phillips (2000) noted that significantly more *P. interpunctella* adults were present outdoors of flour mill facilities, in comparison with the respective figures indoors, after fumigations. Thus, moths may disperse to open areas due to an insecticidal treatment and re-enter the store room after the reduction of the insecticidal effect. Similarly, Bowditch and Madden (1996) reported that in a confectionery factory, the application of synergised pyrethroids had little or no effect in distribution of male moth capture rate of *E. cautella*. In our study, the windows of the warehouse were usually closed; and this may be the reason why more adults were found inside the warehouse. Consequently, large openings in the storage facilities, apart from the reduction of chemical control efficacy due to poor sealing and the exposure of insects to sublethal doses, may cause serious emigration of adults moths to outdoor locations, which may expand the infestation, even in the fields. On the other hand, following an insecticidal treatment, small isolated sources of product with poor hygienic conditions (suitable for larval development) or adults escaping to outdoor locations, may cause a considerable reduction of the insecticidal efficacy, and high populations can be easily built up.

The seasonal occurrence of parasitic wasps in the same currant warehouse has been recently reported by Athanassiou and Eliopoulos (2003), but there are no data concerning the existence of these species outdoors. In our study, with the exception of few adults of *H. hebetor* and *V. canescens* which were found in vineyards only in the vicinity of the warehouse, parasitic wasps do not occur in vineyards in the study area. Johnson et al. (2000) found these two species in sentinel traps placed in central California fig orchards, but their numbers are not reported. Although several stored-product parasitoid species have several hosts, including non stored-product species (Brower et al. 1995, Schöller and Flinn 2000), their limited presence in vineyards indicates that the activity of these species is mainly restricted close to warehouses. The traps around the warehouse revealed high densities of parasitoids, especially for *C. tarsalis*, suggesting that hosts were also available outdoors. This fact may be a part of host-seeking behaviour and dispersal characteristics. In cases of high densities, the presence of wasps inside and outside the warehouse presented similar trends. As it was assumed in the case of moth species, the occurrence of wasps out of storage facilities, may act as a reservoir of insect parasitoids. The reduction of
the wasp densities during late fall may be attributed to several factors, such as the reduction on host availability, or the reduction of flying activity due to the negative influence of temperatures prevailing.

In summary, our study documents a high activity level of adult moths and parasitoids in and around stored currant facilities. Generally, this occurrence has three main characteristics. Firstly, insect pests (but not parasitoids as noted in the case study) may enter the store with newly-harvested product, and to some extent, establish an infestation early in the storage season. Secondly, adult moths (but also parasitoids) may easily disperse to outdoor locations and create sources of infestation around the currant store. Finally, moths and wasps may be able to infest and reproduce in small food quantities outdoors, and re-enter the store if sealing is poor. Thus, practices that minimize insect immigration should be applied. Further experimental work is needed to clarify the nature of this outdoor occurrence, emphasizing to the presence of parasitoids.

Acknowledgments

We would like to thank Prof. C. Buchelos for his comments on this work.

References


Stored product psocids as one of the preys of the predatory mite

*Cheyletus eruditus* (Schrank) (Acarina: Cheyletidae)

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**Abstract:** *Cheyletus eruditus* is a predator of stored product mites, but preys occasionally also on other small arthropods such as psocids of the genera *Liposcelis* and *Lepinotus*. Many species of psocids are common stored product pests and they very often occur in grain together with pest mites. The aim of the present research was to study “predator – prey” relations, i.e. *C. eruditus* and psocids. Freshly developed females of *C. eruditus* were fed either by eggs, nymphs, males or females of *Liposcelis decolor* in order to study the predator’s behaviour, sucking time of the prey body and impact on prey population. The results indicate that the predator *C. eruditus* is able to prey on all developmental stages of the psocid *L. decolor*. Mean handling time (manipulation and sucking of psocid prey) varies from 24 to 90 minutes in dependence on the prey body size. Depending on predator–prey ratio (1:2, 1:5) the population size of psocids was depressed to 11 and 12.5 % of the control population size, respectively, during 40 days under laboratory conditions.

**Keywords:** mites, psocids, *Cheyletus eruditus*, *Liposcelis decolor*, predator – prey relations

**Introduction**

*Cheyletus eruditus* (Schrank) is a predatory mite commonly occurring in grain stores. This species is successfully used in biological control against stored product mites, e.g. *Acarus siro* (Žďárková, 1998a, Žďárková, 1998b). It is known from practice, that *C. eruditus* preys occasionally also on other small arthropods such as psocids in the genera *Liposcelis* and *Lipinotus*. Many species of psocids are common stored product pests and they very often occur in grain together with pest mites in the Czech Republic (Werner et al., 1998; Lukáš et al., 2002; Stejskal et al., 2003). Their importance and pest status has been rapidly increasing in the last few years in Europe and worldwide for various reasons (higher abundance, economic, hygienic and allergic impact) (Kučerová 1999, 2002; Mills et al., 1994; Turner, 1999; Turner and Ali, 1996). The relation “predator – prey”, i.e. *C. eruditus* and psocids, have not yet been studied. The aim of the present research is to fill this gap.

**Materials and methods**

**Predator:** *Cheyletus eruditus* (Acarina: Cheyletidae). A laboratory culture of mites was kept in darkness at 20°C and 85 % relative humidity (RH) with *Acarus siro* (reared on lettuce seed) as its prey. Females freshly moulted from quiescent tritonymphs were used in all experiments.

**Prey:** *Liposcelis decolor* (Pearman) (Psocoptera: Liposcelididae). A laboratory culture of the psocids was maintained in darkness at 27°C and 75 % RH on wheat germs, milled oat flakes and dried yeast. Individual instars or adults of psocids (eggs, 1st instar, last instar, males, and females) were used for experiments.

The following topics of the predator – prey relation were studied:
1) Handling time – the total time spent by a female predator to catch, manipulate and suck an individual psocid was measured within an experimental cell (10 × 2 mm) at 27 °C and 85 % RH (n = 10).

2) Predator behaviour – the predator’s manipulation of the prey body during ingestion was studied within an experimental cell (10×2 mm) at 27 °C and 85 % RH (n = 10).

3) Feeding rate – cumulative numbers of psocid prey (1st instar, male, female) ingested by a single female predator during the first ten days of its life within an experimental cell (10 × 2 mm) at 20 °C and 85 % RH, predator – prey ratio 1:10, (n = 10).

4) Population size – suppression of the prey population by the predator in grain samples was evaluated using glass jars (2.5 cm diameter, 3 cm height) loaded with 2 g of wheat at 25 °C and 85% RH, duration 40 days, predator – prey ratio 1:2, 1:5, founded by adults 1 day old. Observations were made under a stereomicroscope (Nikon SMZ 800).

Results and discussion

The mean handling time a C. eruditus female needs for sucking out a psocid varies from 24 min (egg) to 90 min (female) (range 6–145 min) (Fig. 1). The predator manipulates its prey while sucking and changes the place of puncture on the body of the prey (Fig. 3). Mean number of place changes varies from 2 for eggs to 10 for females. Time and number of changes correlated with the size of the prey. Mean size of the eggs (Kučerová, 2002), males and females ( Günther, 1974) is 0.37, 0.9 and 1.25 mm, respectively. The time required for sucking of the stored product mite A. siro is 5 (eggs), 10 (nymphs) and 30 – 40 min (adults) (Boczek, 1959). The size of an A. siro female ranges from 0.35 to 0.65 mm (Hughes, 1976). It corresponds with our results. Psocids (Liposcelis) are about twice as big as mites (Acarus) are.

The most frequently sucked places were on the abdomen and legs (male, female), or head and abdomen (1st instar) (Fig. 2). Mean cumulative number of prey captured and consumed by one female of C. eruditus was 8.5 for males, 7 for 1st instars and 6 for females of L. decolor during the first ten days of the predator’s life (Fig. 4). The number of prey captured depends not only on prey size, but also on other circumstances (e.g., rate of movement of the prey, searching time, etc.). A C. eruditus female consumes for example 1- 3 adults of A. siro per day (Boczek, 1959). The population size of the psocids was suppressed to 11 – 12.5 % of the control population size depending on the predator-prey ratio (Fig. 5).

Fig. 1. Cheyletus eruditus – handling time.
Preliminary results indicate that the predator *C. eruditus* is able to prey on all developmental stages of the psocid *L. decolor* and partially suppress their population growth under laboratory conditions. Further research of these problems will concentrate on the whole development of *C. eruditus* on psocid prey and detailed studies of behavioural and ecological predator – prey relations. The interaction of *C. eruditus* and psocids and mites as prey are particularly promising.

![Fig. 2. Sucking frequency of *C. eruditus* female at various body parts of prey *L. decolour*.](image)

![Fig. 3. Manipulation of prey by female *C. eruditus* - changes of sucking place per captured individual.](image)
Fig. 4. Consumption of *L. decolor* per individual female of *C. eruditus* in the first ten days of life.

Fig. 5. Decrease of psocid population caused by the predator *Cheyletus eruditus* in grain samples.
Acknowledgements

I wish to thank to Dr. Eva Žďárková for valuable comments. The work was supported by the grant COST OC 842.10.

References

The influence of temperature on the sequence of biological control of stored food mites

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Abstract: It is known that temperature influences development of mites more than relative humidity. We investigated the effects of high temperature (30°C) on the course of biological control which is commonly used in grain stores against acaroid mites using the predatory mite *C. eruditus*. The results show that the course of biological control is twice as fast at 30°C than at 22°C. Whereas the biological control after 6 weeks was almost over at 30°C, the prey was not influenced by the predator at 22°C. Developmental time of the predator at 22°C is 30 days, at 30°C 10 days (Boczek, 1959). Biological control of storage mites using *C. eruditus* can thus be recommended for temperatures up to 30°C.

Keywords: temperature, biological control, storage mites

Introduction

The biological control of stored food mites by using the predator *Cheyletus eruditus* has been studied from different aspects for many years (Pulpán, Verner 1965; Žďárková & Horák, 1990; Žďárková, 1994; Žďárková, 1995; Žďárková, 1996; Žďárková, 1998a; Žďárková & Fejt, 1999). Instructions for use of biological control in stored grain and seeds were also prepared (Žďárková, 1998b). It is known that temperature influences development of mites (Fejt & Žďárková, 2001) more than relative humidity. Both participants in biological control, *C. eruditus* and *Acarus siro*, have almost the same optimum temperature range for development (25–30°C), but the low temperature threshold is different. In the predator it is 12°C and the development takes 164 days (Žďárková & Horák, 1999). In its most common prey *A. siro* the threshold is 5°C (Cunnington, 1976) and the development takes 140 days. The above data may suggest that biological control cannot be successful, especially considering the fact that the temperature in stores in the Czech Republic is usually 10-15°C, i.e. in a range in which reproduction of the predator is difficult. But in summer, the temperature fluctuates and can go up to 20°C. Effective biological control depends also on other factors, such as commodities and its moisture content, infestation, etc. Actually sometimes biological control goes on in the stores spontaneously without any regulation. (Pulpán & Verner, 1965). However, the course of biological control in southern countries with warmer climate would be much faster. Therefore, we investigated the effects of high temperature (30°C) on the course of biological control that is commonly used in the grain stores against acaroid mites using the predator *C. eruditus*.

Material and methods

Forty plastic boxes (15 x 20 x 8 cm) filled with clean and sterile wheat (500 g) and lettuce seed (250 g) were used (20 of each substrate). The boxes were covered by meshed lids and 20 of them kept at 22°C and 20 at 30°C and 85 % RH for a week for balancing the moisture
content of the seeds. Ten boxes in each temperature served as controls and 10 were experimentals. All boxes were infested by 100 *Acarus siro* and left for a week. Than 2 females of *Cheyletus eruditus* were added to each experimental box.

The experiment lasted 2-3 months, samples (50-100 g) were taken after one month and than every 1-2 weeks. Berlese funnels were used for the extraction of mites from the samples. The mites and their predators originated from cultures reared in the laboratory for over twenty years at 22°C and 85 % RH on wheat germs. The predators were reared on lettuce seed with *A. siro* as prey.

**Results and discussion**

**Temperature of 22°C**

*A. siro* was developing fast on both substrates. The population in the control boxes reached 14,500 (wheat) and 18,000 (lettuce) specimens after 6 weeks. The effect of predators did not appear during the first 6 weeks; the population of *A. siro* in the experimental boxes grew as quickly as in the controls reaching 17,000 (wheat) and 20,000 (lettuce) specimens. The first decline of *A. siro* was apparent on lettuce seeds after 9 weeks. At that time, on lettuce the 830 *A. siro* and 4400 *C. eruditus* were found, respectively, and on wheat 2000 *A. siro* and 2100 *C. eruditus*. The population of *A. siro* the in control boxes also declined to 9,900 on lettuce and 4,200 on wheat (Fig. 1).

![Fig. 1. Biological control of *Acarus siro* by *Cheyletus eruditus* on wheat and lettuce seed at 22°C. contr w = *Acarus siro* on wheat in untreated controls, contr ls = *A. siro* on lettuce seed in untreated controls, wheat ac = *A. siro* on wheat in experimental boxes with *Cheyletus eruditus*, wheat ch = *C. eruditus* on wheat in experimental boxes, ls ac = *A. siro* on lettuce seed in experimental boxes, ls ch = *C. eruditus* on lettuce seed in experimental boxes.](image)

**Temperature of 30°C (Fig. 2)**

The predatory mites influenced the prey population from the very beginning, both the populations of *A. siro* and *C. eruditus* were developing fast on both substrates. The population of *A. siro* in the untreated control boxes reached about 2300 specimens on wheat and lettuce after one month, but in the experimental boxes where the population of the predator had already 1400 (wheat) and 2100 (lettuce) specimens, it was lower – 1200 and 1050 *A. siro* on wheat and lettuce, respectively. Six weeks after the experiment started, the biological control
was almost over. There were 100 *A. siro* and 500 *C. eruditus* specimens on wheat and 16 *A. siro* and 1700 *C. eruditus* specimens on lettuce, whereas the *A. siro*-population in the control boxes was still increasing.

The results show that the course of biological control is twice as fast at a temperature of 30°C than at 22°C. Whereas the biological control was almost over after 6 weeks at 30°C, the prey was not influenced by the predator at 22°C. Developmental time of the predator at 22°C is 30 days, and at 30°C 10 days (Boczek, 1959). Biological control of storage mites using *C. eruditus* can thus be recommended for temperatures up to 30°C.

![Graph](image.png)

**Fig. 2.** Biological control of *Acarus siro* by *Cheyletus eruditus* on wheat and lettuce seed at 30°C. contr w = *A. siro* on wheat in untreated controls, contr ls = *A. siro* on lettuce seed in controls, wheat ac = *A. siro* on wheat in experimental boxes with *C. eruditus*, wheat ch = *C. eruditus* on wheat in experimental boxes, ls ac = *A. siro* on lettuce seed in experimental boxes, ls ch = *C. eruditus* on lettuce seed in experimental boxes.

### The effect of temperature on predatory mites

The effect of temperature on the ability of *Amblyseius longispinosus* (Evans) was studied by Nakagawa (1991). When the initial ratio of predators to prey (*Tetranychus kanzawai* Kishida) was 1:30, the predator suppressed the prey population within 8.5 days at 30°C, 12 days at 25°C and 18.6 days at 20°C.

Interspecific predation and cannibalism and associated rates of oviposition were assessed for adult females of *Metaseiulus occidentalis* and *Typhlodromus pyri* when provided with unlimited amounts of eggs, larvae, protonymphs or deutonymphs of *Tetranychus urticae* (MacRae & Croft, 1993). Predation by *T. pyri* was higher than that by *M. occidentalis* at 12.5 and 15°C but similar at 25°C. *Metaseiulus occidentalis* did not feed appreciably on phytoseiid larvae at 15 and 12.5°C. Neither phytoseiid oviposited at 12.5°C when fed phytoseiid larvae, but *T. pyri* did at 15°C. It was concluded that *T. pyri* was a more active predator at low temperatures.

The development and reproduction of *Typhlodromus talbii* (= *Paraseiulus talbii*) was studied in the laboratory by rearing the predator on different kinds of food at 20 and 27°C. The warmer temperature affected the duration of development and oviposition rates positively, but total fecundity was similar at both temperatures. Predators reared at 27°C consumed more prey than those reared at 20°C (Camporese & Duso, 1995).

As apparent from the above literature data, predatory mites may have different requirements for temperature, which can influence their efficiency.
Acknowledgement

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References

Physical control and modified atmospheres
Impact of fumigants applied to control storage pests on fruit quality of dried figs

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Abstract: Dried fig production is an important activity in the western part of Turkey. Sarlılop (=Calimyrna) variety is the standard variety for commercial drying. It is known world-wide as the supreme variety due to its big size, light colour and soft texture. Storage pests namely *Ephesia cautella*, and *Carpoglyphus lactis* may cause significant losses in dried fig trade. Currently, methyl bromide (MeBr) fumigation is the common way to control storage pests except for the organic products. Due its ozone depleting effect, safe alternatives are being searched to replace MeBr. These alternatives must be economically feasible, easy to apply, with no negative effects on the commodity, and an efficient control of pests. A project that will lead to the phase-out of MeBr as a stored product treatment in Turkish dried fig sector is being carried out since 2000. It evaluates the economic and technical feasibility of two alternative technologies: 1) CO₂ at elevated temperatures and/or CO₂ in combination with pressure, and 2) magnesium phosphate in combination with heat in gas tight chambers. This paper summarizes the effect of MeBr, phosphine, and carbon dioxide used to control storage pests on dried fig quality during storage period. Quality parameters such as total soluble solids (%), titratable acidity (%), pH, dry matter (%), water activity (w_a), colour (L, a, and b values by Minolta chromometer), and sugaring (%) are assessed in treated and non-treated samples.

Keywords: fig, *Ficus carica* L., fruit quality, phosphine, carbon dioxide, storage pests, *Ephesia cautella*, *Carpoglyphus lactis*

Introduction

Turkey is known as the major producer and exporter of dried fruits and nuts, namely dried figs, raisins, dried apricots, hazelnut, pistachio nut and pine nut. A common problem faced in the trade of these commodities is the damage caused by storage pests. The degree of the problem caused by storage pests may vary according to the product. Besides, different countries may have varying tolerance limits for these commodities. Turkey provides 55 to 60 % of the world dried fig crop, and the production comes from the western part of Turkey from a single variety ‘Sarlılop’ (=Calimyrna) known all over the world as the best variety for commercial sun-drying (Anonymous, 2001).

An important constituent affecting the durability of food is its water content. The dried fig fruits, after sun-drying seem to be safe for microbial activity with an intermediate moisture content. The growth of bacteria, fungi and yeast are inhibited below certain levels of water activity. The levels are below 0.90 for bacteria, 0.75 for halophytic bacteria, 0.60 for osmophyllic yeast, and 0.65-0.70 for fungi. Lower water activity levels exert an impact on enzyme activity also and especially amylase, peroxidase and phenoxoydase become inactive at water activity levels below 0.85 (Cemeroğlu and Acar, 1986). Nearly 60 % of the dry matter in figs is composed of sugars (Holland, et. al., 1992). Dried fruits exchange water with the environment until they reach to an equilibrium. This exchange is quite rapid due their high sugar content. The loss in fruit moisture content of dried figs result in the accumulation of...
sugars as crystals first on the surface and then within the fruit tissue. Extreme sugaring also affects the texture and increases firmness (Aksoy and Dokuzoğuz, 1983). Piga et. al. (2003) determined quality changes that occurred in dried fruits of San Pietro and Verde fig varieties during a six months storage period. They report a decrease from 1.24 to 1.12 % in titratable acidity in San Pietro variety but an increase from 0.92 to 1.00 in Verde. The pH values remained stable. The colour determined as Hunter L, a, b and tan a/b values was different in the two varieties however, the changes during the storage period was not statistically significant in both of the varieties. Temperature and oxygen content are stated as the two major factors affecting fruit colour (Cemeroğlu and Acar, 1986).

Being free from insects or insect damage is accepted as an important quality parameter in dried figs. Various studies report *Ephestia cautella* (Walker), and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), *Carpophilus hemipterus* L., *C. obsoletus* Er., *C. mutilatus* Er. and *C. bipustulatus* Heer (Coleoptera: Nitidulidae) as the major fig pests present at the drying yards or storage facilities in fig drying areas (İyriboz, 1940; Ülkümen & Özbek, 1948; Guimaraes, 1967; Tunçyürek, 1972; Simmons & Nelson, 1975; Erakay & Özal, 1979; Özar et al., 1986). The almond moth, *E. cautella*, the major pest in the Aegean Region may cause quality losses up to 5.20 % and reduce the yield by 1.2 % (Damarlı et al., 1998). In a recent study carried out in the Aegean Region in 2001-2002, the almond moth, *E. cautella*, the Indian meal moth, *P. interpunctella* and the dried fruit beetles, *Carpophilus* spp. were determined as the most important pests in Aydın and Izmir provinces, where all the commercial dried fig is produced in Turkey. The highest infestation level of *E. cautella* was found in Tire (İzmir) as 16.8 % followed by Kuyucak (Aydın) with 15.4 %. *P. interpunctella* displayed the highest infestation levels in Aydın, Kuyucak (3.4%) being the first, followed by İncirliova (3.2%) township (Turanlı, 2003). Currently, dried fig fruits are all, except those organically grown, fumigated with MeBr in order to control these storage pests. However, MeBr is going to be phased out in 2007 according to the Methyl Bromide Action Plan of the Turkish Government (Anonymous, 2004). In order to prepare Turkish dried fig sector for the phase out period and to find out technically and economically feasible alternatives to MeBr, a project entitled “Project to Phase-Out Methyl Bromide in the Dried Fig Sector in Turkey” is carried out with funding provided by the World Bank through the Multilateral Funds for the Implementation of the Montreal Protocol on Substances that Deplete the Ozone Layer. This paper evaluates the effect of tested MeBr alternatives on quality parameters of dried figs.

**Materials and methods**

**Figs used in the experiments**
Sun-dried fruits of intermediate moisture Sarılop (=syn. Calimyrna) fig variety were used in tests. The treatments and quality analysis were performed on fig fruits classified as Class A.

**Fumigation methods**
Phosphine and carbon dioxide fumigation trials were carried out in flexible PVC storage units (36 tonnes capacity Volcani Cube storage unit). The flexible units were loaded 15 tonnes of dried figs in the perforated plastic boxes. Phosphine application was performed as 1 g.m⁻³ gas for four days (22-25 September 2002). Carbon dioxide was applied at a concentration of 98 % between September 25 and October 2, 2002. Fumigation with MeBr was performed for 24 hours on October 8-9, 2002 (a dosage of 60 g/m² was applied) in fumigation chambers. The tested exposure times aimed to get complete mortality for all life stages of *E. cautella* and *Carpoglyphus lactis*. 
Fruit quality parameters
After short-term application of MeBr, phosphine and carbon dioxide, treated and non-treated fruits were stored at ambient conditions and quality assessment was performed after the treatment and after 5 months of storage. The temperature and relative humidity values were recorded in the storage room by data loggers. The range of the mean temperature during storage was 6.3-28.8 °C, and the relative humidity varied between 40-65.1 %. Dried fig quality was assessed by testing the following parameters: average fruit weight (g), moisture content (%), by electrical conductivity/Dried Fruit Moisture Tester and Oven), total soluble solids (% Effegi refractometer), water activity (a_w) (at 25 °C by Novasina water activity meter), firmness (penetrometer/Nippon), titratable acidity (% as citric acid), pH and skin (external), and flesh (internal) colour (L, a, b, a/b values/Minolta chromometer). The ratio of total soluble solids (TSS) to titratable acidity (TA) content is calculated as an indication of taste. Sugar formation on the fruit surface was determined visually according to the intensity of the white deposition, and an average class value was calculated by multiplying the number of fruits in each class with the class value (5 = no sugaring and 1 = intensive sugar formation) and dividing the sum by the total number of fruits in the sample.

The data obtained is analyzed statistically by using SPSS 7.5 for Windows.

Results and discussion
Dried fig lots prepared at commercial scale were treated with MeBr, phosphine and carbon dioxide to control storage pests and the effect of the treatments on fruit quality was assessed. The major parameters that determine the quality of dried fig fruits were checked in samples taken from treated lots after fumigation and compared with the non-treated fruits. Table 1 shows marked differences between the examined lots regarding the initial average fruit weight, titratable acidity, water activity, colour, and degree of sugaring. Statistical analysis revealed no significant differences between non-treated and treated lots except few parameters as the moisture content in Phosphine treatment and titratable acidity, total soluble solids/titratable acidity, colour (Hunter a and b values) and sugaring in carbon dioxide application. The significant differences seem to appear mainly because of the variation among individual fig fruits within the sampled lots rather than the effect of the treatment.

During storage at ambient conditions between September/October to January/February, a general weight loss was observed in all of the tested variables. This change is due to water loss which consequently increases the fruit firmness and enhances the formation of sugar crystals on the fruit surface (Aksoy and Dokuzoöz, 1983). The three tested fumigants exerted no significant effect on weight or on water loss (Tables 1 and 2). Among the tested parameters only the pH was significantly affected by phosphine and carbon dioxide application however, the pH value was increased by phosphine whereas decreased by carbon dioxide treatment. The effect of carbon dioxide application on pH was confirmed by the increased titratable acidity. Carbon dioxide applications seemed to enhance sugaring more than the other two fumigants after 5 months of storage (Table 2).

As a conclusion, short term application of the three fumigants, MeBr, phosphine and carbon dioxide, used to control storage pests in dried fig sector, seem to exert similar effect on fruit quality even after 5 months of storage under ambient conditions. Taking into consideration these findings, the recommendations that can be made to the dried fig packing companies as an alternative to MeBr after the phase out will depend on the investment and operational cost and on the time required to have complete control over storage pests.
Table 1. Fruit quality parameters of dried figs treated with methyl bromide, phosphine, and carbon dioxide compared with non-treated controls shortly after fumigation.

<table>
<thead>
<tr>
<th>Quality Parameters</th>
<th>Methyl bromide</th>
<th>Phosphine</th>
<th>Carbon dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated F</td>
<td>Control</td>
</tr>
<tr>
<td>Average fruit weight (g)</td>
<td>19.73</td>
<td>20.30</td>
<td>Ns</td>
</tr>
<tr>
<td>Firmness</td>
<td>0.77</td>
<td>0.74</td>
<td>Ns</td>
</tr>
<tr>
<td>Total soluble solids (%)</td>
<td>60.00</td>
<td>58.70</td>
<td>Ns</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>0.48</td>
<td>0.50</td>
<td>Ns</td>
</tr>
<tr>
<td>TSS/TA</td>
<td>125.73</td>
<td>122.90</td>
<td>Ns</td>
</tr>
<tr>
<td>Ph</td>
<td>5.14</td>
<td>5.12</td>
<td>Ns</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>24.35</td>
<td>24.20</td>
<td>Ns</td>
</tr>
<tr>
<td>aw</td>
<td>0.46</td>
<td>0.45</td>
<td>Ns</td>
</tr>
<tr>
<td>L</td>
<td>57.10</td>
<td>61.46</td>
<td>Ns</td>
</tr>
<tr>
<td>A</td>
<td>7.80</td>
<td>7.49</td>
<td>Ns</td>
</tr>
<tr>
<td>B</td>
<td>22.76</td>
<td>23.95</td>
<td>Ns</td>
</tr>
<tr>
<td>A/B</td>
<td>0.34</td>
<td>0.31</td>
<td>*</td>
</tr>
<tr>
<td>Sugaring</td>
<td>2.41</td>
<td>2.29</td>
<td>Ns</td>
</tr>
</tbody>
</table>

Ns = non significant; ** Difference significant at 5% level; *** Difference significant at 1% level

Table 2. Fruit quality parameters of dried figs treated with methyl bromide, phosphine, and carbon dioxide compared with non-treated controls stored under ambient conditions for five months after fumigation.

<table>
<thead>
<tr>
<th>Quality Parameters</th>
<th>Methyl bromide</th>
<th>Phosphine</th>
<th>Carbon dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated F</td>
<td>Control</td>
</tr>
<tr>
<td>Average fruit weight (g)</td>
<td>19.47</td>
<td>20.43</td>
<td>Ns</td>
</tr>
<tr>
<td>Firmness</td>
<td>0.84</td>
<td>0.83</td>
<td>Ns</td>
</tr>
<tr>
<td>Total soluble solids (%)</td>
<td>60.87</td>
<td>60.00</td>
<td>Ns</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>0.53</td>
<td>0.48</td>
<td>Ns</td>
</tr>
<tr>
<td>TSS/TA</td>
<td>115.07</td>
<td>125.67</td>
<td>Ns</td>
</tr>
<tr>
<td>Ph</td>
<td>4.66</td>
<td>4.66</td>
<td>Ns</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>20.15</td>
<td>20.49</td>
<td>Ns</td>
</tr>
<tr>
<td>aw</td>
<td>0.48</td>
<td>0.50</td>
<td>**</td>
</tr>
<tr>
<td>L</td>
<td>58.28</td>
<td>59.54</td>
<td>Ns</td>
</tr>
<tr>
<td>A</td>
<td>4.45</td>
<td>4.00</td>
<td>Ns</td>
</tr>
<tr>
<td>B</td>
<td>15.07</td>
<td>14.25</td>
<td>Ns</td>
</tr>
<tr>
<td>A/B</td>
<td>0.30</td>
<td>0.28</td>
<td>**</td>
</tr>
<tr>
<td>Sugaring</td>
<td>1.42</td>
<td>1.33</td>
<td>Ns</td>
</tr>
</tbody>
</table>

Ns = non significant; ** Difference significant at 5% level; *** Difference significant at 1% level
Acknowledgement

Partially supported through the project (TTGV-P2/30m) entitled “Project to Phase-Out Methyl Bromide in the Dried Fig Sector in Turkey” funded by the World Bank through the Multilateral Funds for the Implementation of the Montreal Protocol on Substances that Deplete the Ozone Layer.

References


Control of *Cryptolestes pusillus* (Coleoptera, Cucujidae) and *Tribolium castaneum* (Coleoptera, Tenebrionidae) at high temperatures

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Abstract: Experiments were carried out under laboratory conditions to test the efficacy of 45°C, 50°C and 55°C against the flat grain beetle *Cryptolestes pusillus* (Schönh.) and the red flour beetle *Tribolium castaneum* (Herbst). Four developmental stages of defined age and adults were exposed to these temperatures by placing them together with 10 ml of substrate into pre-heated glass tubes submerged in a temperature-controlled water bath. At 45°C, 35 h exposure time were needed to control all stages of *C. pusillus* and 38 h to control *T. castaneum*. At 50°C, lethal exposure times were reduced to 115 min for *C. pusillus* and *T. castaneum*, and at 55°C both species could be controlled within 25 min of exposure. The results are compared with data on other species.

Key words: heat treatments, red flour beetle, flat grain beetle

Introduction

Heat treatments may gain importance as a fast and residue-free disinfestation method of storage structures and premises in food-industry after the loss of methyl bromide. These treatments have been studied or used in various countries like Australia (Banks and Fields 1995, Wright et al. 2002), Germany (Rassmann 1995, Gaigl 2002, Hofmeir 2002), Japan and Korea (Hofmeir 2002), the United States of America (Heaps 1996, Dowdy and Subramanyam 1999), as well as Austria and Switzerland. An overview on the efficacy of extreme temperatures for disinfestation is given in Fields (1992) and Burks et al. (2000). However, for many stored product insect species, systematic studies on the efficacy of different temperatures against the various developmental stages are still lacking. It is the purpose of this and earlier studies (Adler and Rassman, 2000; Adler 2002, 2003) to fill this gap.

In the present study, the flat grain beetle *Cryptolestes pusillus*, as a small pest in grain and grain products and the red flour beetle *Tribolium castaneum* as a typical pest of flour mills were exposed to different high temperatures.

Material and methods

All developmental stages and adults of both species were tested. Cultures and substrates were kept at 25±1°C and 65±5% r.h. To obtain the developmental stages of defined age, 500 adult *C. pusillus* were placed onto a mixture of 150 ml wheat bran and 20 ml rolled oats each week. After 7 d the beetles were removed and 350 ml wheat bran as well as 30 ml of rolled oats were added as additional feed substrate. A cellulose tissue paper was also added for providing hiding place for the insects. In case of *T. castaneum* 150 young adult beetles were placed onto 150 ml of wheat bran for 7d and 350 ml of wheat bran and a tissue paper was added after adults were removed.

Before each experiment, the culture substrate was mixed to secure an even distribution of individuals. Then portions of 10 ml of this substrate (wheat bran and rolled oats or wheat
bran) were separated and exposed to 45°C, 50°C, and 55±0.3°C in preheated glass tubes in a water bath (Huber HS 40, Germany). Five exposure times were tested at a time and experiments were carried out in at least three replicates. Further details on materials and methods may be found in Adler (2002, 2003).

At 45°C, exposure times of 5, 10, 15, 20, 25, 30, 35, 40, and 80 hours were tested. Whereas, at 50°C the tested exposure times were 5, 10, 15, 25, 35, 45, 55, 65, 75, 95, 135 and 175 min and at 55°C the exposure times were of 3, 5, 10, 20 and 40 min.

Results and discussion

At 45°C, 35 h exposure was required to control the flat grain beetle and 38 h to control the red flour beetle. As depicted in Fig. 1 and Fig. 2, similar exposure times of 115 min were needed to control all stages of *C. pusillus* and *T. castaneum* at 50°C. At 55°C, 25 min of exposure was sufficient to control all stages of both tested species.

![Fig. 1. Efficacy of heat at 50°C against *C. pusillus*. Trend lines are drawn for the most tolerant (bold) and the most sensitive stage (broken line).](image)

Most tolerant in both species appeared pupae and grown larvae (stage 4). Eggs and young larvae were rather sensitive to heat. At 45°C, the adult beetles of *C. pusillus* were as tolerant to the treatment as stage 4. A similar effect occurred for *T. castaneum* at 55°C, while in all other cases beetles were less tolerant than pupal stages. However, in comparison to the lesser grain borer *Rhyzopertha dominica* and the tobacco beetle *Lasioderma serricorne* (Adler 2002, 2003) these differences among the different developmental stages appeared much less pronounced. These results may indicate the minimum treatment times required for the control of these two species in flour mills or other buildings that were cleaned to contain substrate layers of max. 6 mm. When data are compared with earlier studies on the lesser grain borer
*Rhyzopertha dominica* and the tobacco beetle *Lasioderma serricorne* (Adler 2002, 2003) it is obvious that these latter species are much more heat tolerant than *C. pusillus* and *T. castaneum*. Under similar conditions, 80 h were needed to control *R. dominica* in whole wheat grains at 45°C, 370 min at 50°C and 30 min at 55°C. For *L. serricorne* in wheat bran, 65 h, 420 min and 55 min were necessary at the respective temperatures. In these experiments the exposure time was calculated from the introduction of the substrate together with the insects into the preheated glass tubes (time for heating of substrate was not deducted from the total exposure time). At 50°C, the egg stage of the tobacco beetle was most tolerant (Adler 2003), while at other temperatures late larvae and or pupae survived the longest.

![Efficacy of heat at 50°C against *T. castaneum*. Trend lines are drawn for the most tolerant (bold) and the most sensitive stage (broken line).](image)

From the data available it may be deducted that heat treatments in tobacco factories should be carried out at 45°C for at least 3 d, at 50°C for at least 7 h, and at 55°C for at least 1 h. In flour mills at respective temperatures, exposure times of 2 d, 120 min and 30 min could be sufficient. Nevertheless, this latter value may be true only for *T. castaneum*, possibly only for the tested strain. It is thus recommendable to continue systematic testing of stored product pest species for their relative tolerance to heat treatments in order to establish effective exposure times.

**References**


Utilization of freezing temperatures to control
*Callosobruchus maculatus* Fabr. (Coleoptera, Bruchidae)

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Abstract: Pulses are one of the most important crop groups cultivated in Turkey. Among pulses, chickpeas are the leading crop with a production amounting to some 700,000 -750,000 tonnes annually. Chickpeas are, however, susceptible in storage to different species of beetles belonging to the family Bruchidae. A serious postharvest pest of chickpeas is the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). Chickpeas produced conventionally are fumigated with phosphine if a cowpea weevil infestation is found. However, disinfection of chickpeas produced organically poses a problem. Among disinfections of chickpeas by non-chemical means, the use of extreme temperature and modified atmospheres are the leading techniques in practice. Among the extreme temperatures, the use of extremely low temperatures is one of the most reliable methods. The purpose of this research was to determine the duration of exposure needed for total mortality of *C. maculatus* at -18°C in the laboratory. For this purpose a series of experiments were conducted. Total mortality for 0-24, 24-48, and 48-72 h eggs from daily infested culture, and for the other developmental stages, 10-14, 20-24, 32-36, 42-46 days after infestation total mortality were determined on a 20 minute interval tests. We found that exposure time for total mortality of egg stage were 2 h. No adult emerged from the other aged cultures when exposed 3 h.

Key words: *Callosobruchus maculatus*, low temperature, insect control, organic chickpeas.

Introduction

Because of problems related to the toxic residues in food, the development of resistance and adverse environmental effects, conventional chemicals have been or may be restricted in the stored product insect control programs. Thus, ecologically safe methods to control insect pest of stored products have been extensively investigated. Of these, the storage of food at low temperature is one of the well known alternative methods.

Fortunately, insects are sensitive to temperature changes within their environment and the postharvest environment is sufficiently insulated allowing the manipulation of temperature. There are two basic effects of low temperature: reducing the development rate, feeding, and fecundity; and decreasing survival (Banks and Fields, 1995; Fields and Muir, 1995). Therefore, the use of extreme temperatures to restrict pest population is an ideal tool for organic food industry. The lethal temperature zones for insects are those above or below the suboptimum which will eventually kill the organisms. The susceptibility of insects to lethal cold temperatures varies greatly between species and the life stages, and is often dependent upon factors such as temperature, length of exposure, sex, and ambient air relative humidity.

The aim of the present work was to determine the effects of the low temperature at -18°C on the mortality of the *Callosobruchus maculatus* infested cowpeas in the laboratory.
Material and methods

Cultures of the cowpea weevil *C. maculatus* were raised on chickpeas at 25 ± 1°C and 65 ± 5% RH. Before each trial, chickpeas were frozen for at least two days to exclude contamination by insects. For this study, at least hundred pairs of *C. maculatus* adults were released for 24 h into plastic boxes with perforated lids (20 by 12 by 10 cm) containing 500 g seeds. The adults were removed after 24 h, and then seeds bearing eggs were kept in the rearing room for obtaining 0-24, 24-48, and 48-72 h old eggs. Individual bioassays consisted of 15 g seeds held inside 30 ml plastic vials, which were capped with a screened lid.

Complete development from egg to adult took approximately 50-55 days at 25 ± 1°C and 65 ± 5% RH. For the immature stages (larvae and pupae) in the experiment, at least hundred pairs of *C. maculatus* adults were kept for 4 days on 500 g seeds in rearing jars with perforated lids. After 4 days, the adults were removed and then seeds infected with insects were kept in the rearing room until 10-14, 20-24, 32-36, 42-46 day old stages were attained. Individual bioassays consisted of 15 g infected seeds held inside 30 ml plastic vials, which were capped with a screened lid. All experiments were carried out at -18°C. Temperature inside the cold room was monitored using HOBO T type thermocouple recorders (Onset Computer, Pocasset, MA).

Eggs, larvae, and adults were exposed to -18°C for 20, 40, 60, 80, 100, 120, 140, 160, and 180 minutes, while untreated controls were kept in the insect rearing room at 25 ± 1°C and 65 ± 5% RH. After exposure, infested seeds contained in 30 ml plastic test vials were transferred to insect rearing room at 25 ± 1°C and 65 ± 5% RH. All experiments were carried out in three replicates. Adult emergence was recorded weekly from eggs and other immature stages in the experiments. Results were given according to the percentage reduction of adult emergence compared with that of control.

Results and discussion

Reduction of adult emergence from eggs and the other immature stages becomes pronounced with the length of the exposure time. Fig. 1 presents the results of exposure of egg stages to -18°C. According to the results, there was no difference between ages of eggs for complete reduction of adult emergence, and 120 minute exposure yielded no adult emergence. Whereas, Cline (1970) reported that mortality greatly varies with the age of the egg, with young and old eggs being the least cold hardy. Adler (1960) stated that eggs of *Plodia interpunctella*, *Sitotroga cerealella* and *Tribolium confusum* were killed rapidly by low temperatures. His data show complete mortality of egg stage of both *P. interpunctella* and *S. cerealella* after 4 h exposure to -16.7°C; whereas complete mortality of the egg stage of *T. confusum* was achieved after 5 h exposure to that temperature. Mullen and Arbogast (1979) showed that survival time of egg stages of five important stored product pests including *C. maculatus* decreased rapidly as temperature dropped below freezing. At -5°C, egg stage of *C. maculatus* survived an exposure over 14 h, and at -20°C, LT₀₅ were found to be in excess of 1 h. Fields (1992) stated that egg stages of insects tend to be more susceptible to low temperatures than other life stages, and age of the egg may affect response.

Fig. 2 shows the percentage of reduction of adult emergence from *C. maculatus* infested seeds when 10-14 day old (young larvae), 20-24 day old (mid stage larvae), 32-36 day old (mature larva, 42-46 day old (pupae and un-emerged adults) were exposed to -18°C in comparison to the controls of the same age. Our tests showed that the order to cold tolerance increased with the age of immature stages of 10-14, 20-24, 32-36, 42-46 day old. Adult emergence from 10-14 day old stages was completely inhibited by 100-minutes exposure.
Fig. 1. Percent reduction of adult emergence of *Callosobruchus maculatus* from 0-24, 24-48, and 48-72 h old eggs exposed to -18°C.

Fig. 2. Percent reduction of adult emergence of *Callosobruchus maculatus* from 10-14, 20-24, 32-36, and 42-46 days old immature stage exposed to -18°C.
However, 120 minute exposure was necessary for the 20-24 and the 32-36 day old stages. For the oldest immature stages (42-46 day old), 180 minute exposure was needed for complete inhibition. Johnson and Valero (2000) reported that recently emerged adults of *C. maculatus* were completely dead or moribund after 50 minutes exposure to -18°C. For the immature stages, they found that the youngest stage (1-3 day old eggs) was the most cold tolerant, and adult emergence was reduced by more than 99% for all treated ages after 180 minutes of exposure to -18°C. Donahaye *et al.*, (1991) found that all stages of *Carpophilus hemipterus* and *C. mutilatus* were killed within 2.25 h when exposed to -18°C.

Its of our study showed that all stages of *C. maculatus* may be easily controlled at -18°C in 180 minute exposure {Guray is that correct? Shlomo= actually all stages except 42-46 days old stage can be controlled in 120 minute exposure. But 42-46 days old stage (more tolerant than the other stages) can be controlled in 180 minutes exposure period. So, 180 minutes exposure is sufficient to control all stages in this test }. Donahaye *et al.*, (1995) reported that less than 3 h exposure at -18°C were required to kill (using the upper confidence limit of the LT99) all stages of *Tribolium castaneum*, *Oryzaephilus surinamensis* and *Ephestia cautella*.

Fields (1992) pointed out that there is considerable variability in response to low temperatures by geographical strains of stored product insects, and between field and laboratory populations. In commercial freezers gradual cooling may allow pest insects to acclimate to freezing temperatures, extending time for disinfestations (Johnson and Valero, 2000; Donahaye *et al.*, 1991 and 1995; Adler and Rassmann, 2000). Thus, additional studies in commercial freezers with different cooling rates should be considered for studying low temperature exposure schedules.

**Acknowledgement**

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**Reference**


Emigration and control of nitidulid beetles from dates using heat

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Abstract: Dates are subject to infestation by nitidulid beetles during and after harvest. Fumigation of dried fruits with methyl bromide (MB) upon arrival at the packing plant effectively controls infestation and causes a high proportion of larvae and adults to emigrate from the fruit before they succumb. This work was undertaken to investigate the effectiveness of heat treatment as an alternative to MB, which would remove insects from the dates, prevent insect development, and preserve fruit quality. Dates grown in Israel served as a model for development of the technology designed to be integrated into the pre-storage drying process. The test insects were Carpophilus hemipterus larvae reared on a synthetic food medium and held at 26°C and 75% relative humidity. Artificial feeding sites destined to simulate the dates were prepared consisting of cardboard rectangles placed on food medium contained in Petri dishes. Exposure to different treatments was carried out in 2.54 L desiccators. For each treatment, exposure times of 2 h after the feeding sites reached the target temperature were employed. Temperatures of 40°, 45°, 50° and 55°C were tested. The ratio of the number of larvae found outside the feeding sites to the total number of insects was used to describe the term "percent disinfestation". Disinfestation was greatest (92.3%) at 50°C and the difference was highly significant from exposure at 40° and 55°C. At 50° and 55°C 100% mortality was obtained. Conventional drying temperatures for most date varieties are in the range of 50° to 55°C. Since percent disinfestation and control was most effective at 50°C, application of heat appears an encouraging solution for the treatment of dates as a replacement to MB. The laboratory findings served as basis for two field trials carried out at a date drying station. This consisted of a hot-house holding pallets of stacked crated dates arranged in rows and covered by plastic liners to form drying ducts. One extremity of each duct was connected to a thermostatically control chamber supplying solar heated air, and the other end appended to large fans set to extract air from the ducts. Crates with artificially infested dates were positioned at strategic sites and the drying pass of 45°C was preceded by a 2 hour pass at a target temperature of 50°C. Results showed that although mortality after 2 h was incomplete at some sites, disinfestations was very high, and over the normal drying period of up to 72 h mortality would have been complete.

Key words: dried fruit, dates, disinfestation, Nitidulid beetles, Carpophilus spp., heat treatment, methyl bromide alternatives, IPM, storage pest control

Introduction

The problem of infestation in dried fruit is two-fold: Insects can cause serious damage to the fruit rendering it unfit for human consumption, but they also contaminate it, rendering it unacceptable for marketing in international trade. Furthermore, the stored-product insects commonly infesting dried fruit, particularly moths of the family Phycitidae and beetles of the family Nitidulidae, are also field pests. The initial source of infestation is frequently on ripening fruit on the tree, with infestation continuing in the packing-houses and during storage. Consequently it is an accepted practice both in the date and fig industries, as well as with other fruits and nuts, to fumigate the harvested fruit immediately upon arrival at the packing-houses, in order to break the chain of insect infestation. Both in Turkey (figs) and in Israel (dates), fumigation using methyl bromide (MB) in fumigation chambers is the accepted
practice. Among the many advantages of MB is the fact that not only does the fumigant kill the insects rapidly, thereby permitting short turnover times, but has the additional effect of causing the adults and larvae to abandon the fruit before they die (Donahaye et al. 1991; Navarro et al. 1989). This decontamination effect is invaluable in actually decreasing the infestation levels of dead insects remaining in the fruit. Phosphine (PH₃), the only other widely used fumigant still acceptable under international legislation, has neither the rapid kill produced by MB, nor the all-important decontamination effect. Unfortunately MB is now targeted as an ozone depleting chemical, and under the Montreal Protocol of the UNEP (2002) its use must be phased out by 2005 for Non-Article 5 countries, and by 2015 for Article 5 countries. Although there are exemptions for quarantine and pre-shipment purposes, as well as the possibility to apply for exemptions where no existing alternative exists, the onus is on the applicant to demonstrate that every effort to is being made to research alternative treatments (TEAP & MBTOC 2003).

There is some information in the literature on the sensitivity of stored-product insects to high temperatures (Evans 1987, Fields 1992, Gonen 1977a, b, and Howe 1965), though all these studies were made on grain pests. With regard to dried fruits, laboratory work of Lindgren and Vincent (1953) showed that to obtain 90% mortality of adult nitidulids, exposure to 49°C for 4 to 20 minutes was needed, depending upon the relative humidity. Al-Azawi et al., (1984) showed that under laboratory conditions, adults of the dried fruit beetle, *Carpophilus hemipterus* (L.) is relatively tolerant to heat with exposures of from 25 to 60 minutes required to achieve complete mortality at 50°C. For complete mortality of all stages of the Tropical Warehouse moth *Cadra cautella* infesting stored dates, 33 minutes were required at exposure to 60°C (Al-Azawi et al., 1983). However, for dates, the technologically feasible temperature range lies between 45 and 55°C since lower temperatures only produce mortality after prolonged periods whereas temperatures of 60°C or above exert an undesirable drying effect on the fruit, or even biochemical alteration.

The study reported here, summarizes the laboratory work undertaken to determine the parameters of temperature and time required to obtain optimum emigration and optimum kill of the beetle *Carpophilus hemipterus* this being a typical representative of the other closely related species of *Carpophilus* that attack dried fruit in the region.

The second part of the study relates to field trials undertaken to apply the laboratory findings to a commercial scale date drying installation using an electrically assisted solar energy installation expressly designed for this purpose.

**Materials and methods**

**Laboratory studies**

Laboratory cultures of the test insect *Carpophilus hemipterus* originated from infested dates. They were reared under standard conditions of 26°C and 75% rh on an artificial diet. To obtain larvae of uniform age, cultures were established by placing adults in 200 ml rearing jars containing the culture medium. After 2 days oviposition, the adults were removed and the insects were reared until they reached the required age. Larvae used in the experiments were 6-8 days old. The experiments to determine percent disinfections and mortality levels of the *C. hemipterus* adults and larvae were carried out at four temperatures of 45°, 50°, 55° and 55°C.

Artificial feeding sites were prepared to simulate the dates. They consisted of cardboard rectangles placed on food medium contained in Petri dishes. Larvae were placed in the Petri dishes, and penetrated beneath the cardboard rectangles. After 24 h, while the larvae inside, the feeding sites were placed in the exposure chambers. Exposure was carried out in 2.54 L
desiccators. Temperatures within the artificial feeding sites were always 1° to 2°C lower than the nominal test temperature in the desiccators and it took approximately 60 min to reach to the test temperature from 26°. For each treatment, an exposure time of 2 h, after the feeding sites reached the test temperature, were employed. The ratio of the number of insects found outside the feeding sites to the total number of insects was used to describe the term "percent disinfestation". After each treatment, the number of survivors was examined and percentage of mortality calculated.

Field trials
Field trials were carried out in the commercial drying facilities of the Timura Company located at the agricultural cooperative Moshav Mehola in the north of the Jordan Valley. The drier takes advantage of solar heat supplemented by an LPG (liquid petroleum gas) heater, used to compensate for temperature drop at the cooler times of the day. Tests were carried out on dates of the Madjoul variety. Crates containing infested dates were exposed in strategic locations of the drier to verify if the heated air caused emigration of larvae as occurred in the laboratory. Below each crate, a second empty crate containing a liner was spread to collect the larvae that emigrated from the dates. In each bioassay, about 500 larvae per crate were used to test for emigration and mortality. Three subsequent trials were carried out. In each trial four crates were placed in strategic points; two crates on top and two crates at the lower extreme sections. Reported results are average of these three trials.

Results

Laboratory studies
The disinfestation value was greatest at exposure to 50°C (92.3%) this level being highly significant and different from disinfestations at 40° and 55°C (Fig. 1). Previous data that reported on disinfestation levels using MB indicated that the highest disinfestation did not exceeded 90% (Donahaye et al., 1991; 1992). The highest mortality reached was 100% and was obtained at 50° and 55°C (Fig. 2).

![Fig. 1. Percent disinfestation of C. hemipterus larvae from artificial feeding sites at various temperatures for 2 hours of exposure after the test temperature was reached.](image-url)
Field trials

The drying facility consisted of a hot-house (Fig. 3) that accommodated rows of pallets covered by a plastic liner to convey the heated air through the boxes containing dates (Figures 4 and 5). The drier thermostat was set to 50°C and laboratory-infested dates were used as bioassay (Fig. 6). After treatment, the infested dates were analyzed for survival in the laboratory (Figures 7 and 8). The data indicate that the target temperature of 50°C could be achieved within one hour after the introduction of the dates into the drier. An additional two hours were necessary to achieve emigration and mortality of the insects. Of the dates used for bioassay that were placed at strategic locations near the lower periphery of the drying duct (where air circulation was suspected to be restricted), a temperature drop of between 2° and 3°C was found from the target temperature. These slight differences allowed larval survival at the end of two hours exposure. In the top layers of the drying rows, the bioassays of infested dates revealed total emigration and mortality of larvae. The slight temperature difference at the lower layer of the drying duct allowed insect survival of up to 60% while emigration was 70% of the total number of insects. However, it should be mentioned that the entire drying period at a temperature of 45°C could last as long as 72 h and even 96 h to cause insect mortality. These aspects of the heat treatment are currently under investigation.

Discussion

Post-harvest quarantine treatments using high temperatures have been studied on various commodities, but the present study is the first on dates to determine the emigration of nitidulid beetles. There is a wide range of insect pests that could be the target of heat treatments. To make heat treatments effective against insect pests, the effects of high temperatures on insect physiology must be understood. Insects, being cold blooded, are particularly sensitive to heat. Studies on the effects of heat in insect metabolism demonstrate some adaptability to thermally challenging environments. In our laboratory study, a heating
time of about 60 minutes was necessary until the temperature reached its target level. This heating time may also affect the emigration rate of the larvae. Respiration, as to be expected, is also affected by heat. As body temperature of the insect increases, there are concomitant increases in both metabolism and respiration up to an upper critical thermal limit. The effects
of heat on the nervous and endocrine systems are another area where elevated temperature damage needs further investigation. Among the most studied responses of insects to heat is the elicitation of heat shock proteins. The impact of these proteins on thermo-tolerance needs investigation.

The heat treatment is intended to replace the conventional fumigation with MB, within the framework of studies to find MB alternatives. Since dates are first disinfested using MB and then dried when necessary, it is expected that this treatment will be most suitable for dates that are subject to drying before storage. As for dates that are already at their moisture content suitable for storage (such as the Deglet-Nur variety), exposure to heat will last no more than 2 hours after the dates reach the target temperature. Preliminary experiments have shown that at a short exposure of 2 hours an insignificant moisture reduction of dates is incurred.

Conclusions

Disinfestation from dates in the laboratory was greatest at 50°C and reached 92.3%. Complete mortality was obtained at 50° and 55°C. Since conventional drying temperatures for most date varieties are in the range of 45° to 55°C, application of heat appears an encouraging solution for the treatment of dates as a replacement to MB. This approach was tested at a commercial scale date drying facility and was shown to be feasible with no modification needed to the actual installation. Since at present, dates are first disinfested using MB and then dried when necessary, it is expected that this treatment will be most suitable for dates that are subject to drying before storage. As for dates that are already at their moisture content suitable for storage, exposure to heat will last no more than 2 hours after the dates reach the desired temperature.

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References

Gonen, M., 1977a: Susceptibility of *Sitophilus granarius* and *S. oryzae* (Coleoptera: Curculionidae) to high temperature after exposure to supra-optimal temperature. – Ent. exp. Appl. 21: 243-248.


The efficacy of modified atmosphere applications against dried fruit pests in Turkey

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Abstract: Dried fruits consisting mainly raisins, figs and apricots play an important role in Turkish agricultural export out of a total production of approximately 350,000 tonnes/year with a value of about $US 250 million. Methyl bromide, deep freezing, carbon dioxide at normal atmospheric pressure, high pressure carbon dioxide, cold storage, and phosphine applications are on the list of the currently used techniques for the control of main storage dried fruits pests, namely *Ephestia cautella* Walk., *Plodia interpunctella* Hbn. (Pyralidae:Lepidoptera) *Carpophilus* spp. (Nitidulidae:Coleoptera) *Oryzaephilus surinamensis* L. (Silvanidae: Coleoptera), and *Carpoglyphus lactis* (L.) (Carpoglyphidae: Acari). Recent field studies carried out at several dried fruits processing facilities has clearly shown that treatment of the product with high CO₂ concentration in the gas-tight, flexible PVC envelopes gave a complete mortality, in less than five days, for all life stages of *E. cautella* and *C. lactis*, the main postharvest pests of the dried fruits. The similarities between the cost of CO₂ and MB applications, which is approximately 1 €/tonne of dried fig, makes the modified atmosphere technique a feasible alternative to MB for the disinfestation of dried fruits. Application of CO₂ under high pressure is another method in use for replacing MB but is still expensive (up to10 €/tonne of dried figs) for the Turkish dried fruit sector in comparing with other methods..

Key words: modified atmospheres, carbon dioxide, PVC cocoon, dried fruits, *Ephestia cautella*, *Carpoglyphus lactis*, insect control

Introduction

Turkey, one of the leading dried fruits trading countries, produces approximately 350,000 tonnes of dried fruits, annually. Turkey ranks first in world raisin production (about 250,000 tonnnes/year). In addition, 60-70 % of the world dried apricot and hazelnut production is produced in Turkey. Hazelnut (ca 400,000 tonnnes, walnut 120,000 tonnnes, pistachio nuts 70,000 tonnnes and almond 35,000 tonnnes are amongst the other nuts produced in Turkey. Most of the dried fruit exports are realized from the Aegean Region through the Aegean Exporters’ Union.

Climatic conditions prevailing in Turkey provide a suitable environment for storage pests, including *Ephestia cautella*, *Plodia interpunctella*, *Oryzaephilus surinamensis*, *Carpophilus hemipterus*, and *Carpoglyphus lactis* (Anonymous, 1995; Özer et al., 1989).

Dried fruits are subject to infestation by storage pests during and after harvest. To control storage pests in the processing and storage, methyl bromide (MB) is the unique agent in use in Turkey. According to Turkish authorities, the phase-out of MB in storage will be by 2004. Thus, environmentally sound, development of user-friendly, effective, and economic alternatives to MB is urgently needed for the dried fruit sector in Turkey.

Modified atmospheres (MAs) applications offer safe, economic, and practical solutions and seem to be among the most promising alternatives to MB for dried fruits. This study was
carried out to investigate the applicability of MA technique in flexible units to achieve complete control of dried fruit pests.

**Materials and methods**

**MA application**
Flexible PVC storage units termed GrainPro cocoons™ (GrainPro Inc, USA) of 36 m³ in volume were loaded with about 15 tonnes dried figs contained in perforated plastic boxes of 25-30 kg in capacity. Test species were placed at different levels of the cocoon prior the sealing. CO₂ was flushed from steel cylinders equipped with a siphon that conveyed the gas in liquid phase into the cocoons. When the desired gas concentrations were reached, gas inlet and air outlet openings were tightly closed (Fig. 1).

**Monitoring equipment**
CO₂ and O₂ concentrations at the bottom and top levels of the cocoons were daily monitored by an analyzer equipped with a thermal conductivity detector (Gow Mac CO₂ analyzer Model 20-600), and an electrochemical detector (David Bishop Inst. O₂ analyzer Model OxyCheck 2) for the entire exposure periods, respectively. The cocoons were also equipped with temperature and air relative humidity (RH) data loggers (Onset Co., model: Hobo® H8 Pro Series) (Fig. 1).

**Test insects**
Test insects shown in Table 1 were contained in 100 ml perforated plastic containers including food. In each experiment, five groups of insects were placed in different locations inside the cocoon (Fig. 1). After each exposure, test insects were transferred to the laboratory where they kept at 25 °C and %65 RH in a controlled room. Mortality of the active stages was determined 14 days after the end of the trials, while eggs and pupal mortality were determined as a failure of hatch 10 days after the end of each exposure.

**Results and discussion**
The purging system caused a slight inflation of the cocoon which provided an initial CO₂ concentration of >90% which was maintained throughout the experiments without significant reduction in the gas concentration (Table 2). Monitoring gas concentrations over the experiments revealed that the cocoon used in the tests had enough tightness to use for short-term disinfection practices of dried fruits by means of high CO₂. The temperature and the RH values did not significantly change during the experiments (Table 3).

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Age (days)</th>
<th>Number per replicate</th>
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<tr>
<td><em>Ephestia cautella</em></td>
<td>eggs</td>
<td>1-3</td>
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<tr>
<td></td>
<td>mature larvae</td>
<td>18-20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>pupae</td>
<td>1-3</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>adults</td>
<td>1-2</td>
<td>50</td>
</tr>
<tr>
<td><em>Carpoglyphus lactis</em></td>
<td>mixed</td>
<td>mixed</td>
<td>Uncounted</td>
</tr>
</tbody>
</table>
The mortality results of the tests were given in Table 4. From the table, it can be seen that exposure of all stages of *Ephestia cautella* and *Carpoglyphus lactis* to high carbon dioxide (>90%) containing atmosphere resulted in a complete mortality in 3, 4 and 5 days exposures, respectively. Similar results were found by Hashem and Reichmuth (1994), who exposed *Ephestia elutella* to 90% carbon dioxide atmosphere at 25 °C. They reported that egg mortality of *Ephestia elutella* was 100% after 3 days of exposure. Markze et al. (1970) reported that a complete failure of the emergence of *Plodia interpunctella* pupae treated with an atmosphere composed of 97.2% CO$_2$+0.8% O$_2$+2.0%N$_2$ at 27 °C and 61% RH was obtained within 3 days. Navarro and Calderon (1974) found that in an atmosphere composed of 86% CO$_2$, 10% O$_2$ and 4% N$_2$, the time required for a complete mortality of pupal stage of *Ephestia cautella* was 4 days at 54% RH and 26 °C. Navarro et al. (1985) found that two days exposure of the adult stage of *Acarus siro* in 20, 30 and 40% CO$_2$ and 21% O$_2$ in N$_2$ or an atmosphere containing 2% O$_2$ in N$_2$ caused total mortality.

**Fig. 1.** A schematic diagram of the cocoon showing the gas purging system including the gas inlet and outlet ports and the location of the data loggers of the experimental design.

**Table 2.** Minimum and maximum gas levels measured at bottom and top of the cocoons over the exposure periods.

<table>
<thead>
<tr>
<th>Exposure period (hours)</th>
<th>O$_2$ (%) bottom</th>
<th>O$_2$ (%) top</th>
<th>CO$_2$ (%) bottom</th>
<th>CO$_2$ (%) top</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>0.4-0.6</td>
<td>0.7-0.9</td>
<td>97-98</td>
<td>95-96</td>
</tr>
<tr>
<td>96</td>
<td>0.5-0.9</td>
<td>0.5-1.2</td>
<td>95-97</td>
<td>92-97</td>
</tr>
<tr>
<td>120</td>
<td>0.7-0.9</td>
<td>0.9-1.2</td>
<td>95-97</td>
<td>93-95</td>
</tr>
</tbody>
</table>
Conclusions

The results in this trial showed that high carbon dioxide applications in the flexible, gas-tight, PVC cocoons could be effective for dried fruits in comparatively short exposure periods. The similarities between the cost of CO₂ and MB applications, which is approximately 1 €/tonne of dried fig, makes the modified atmosphere technique a feasible alternative to MB for the dried fruits disinfections. Carbon dioxide application under high pressure is another method in use for replacing MB but is still expensive (up to 10 €/tonne of dried figs) for the Turkish dried fruit sector. Further studies are needed to get additional data on other pests infesting dried fruits for determining the exposure times needed for the control of the most resistant developmental stages.

Table 3. Mean temperatures and relative humidity over the exposure periods

<table>
<thead>
<tr>
<th>Exposure period (hour)</th>
<th>Temperature (°C)</th>
<th>Relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean range</td>
<td>mean range</td>
</tr>
<tr>
<td>72</td>
<td>25.71 25.01 - 26.34</td>
<td>57.46 56.42 - 63.44</td>
</tr>
<tr>
<td>96</td>
<td>28.04 27.12 - 28.39</td>
<td>52.68 50.94 - 53.82</td>
</tr>
<tr>
<td>120</td>
<td>26.97 26.34 - 27.84</td>
<td>53.62 48.36 - 57.88</td>
</tr>
</tbody>
</table>

Table 4. Mortality records of test individuals over three exposure periods.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Age (days)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (days)</td>
<td></td>
<td>Exposure time (h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72  96  120</td>
</tr>
<tr>
<td>Ephhestia cautella</td>
<td>eggs</td>
<td>1-3</td>
<td>100 100 100</td>
</tr>
<tr>
<td></td>
<td>mature larvae</td>
<td>18-20</td>
<td>100 100 100</td>
</tr>
<tr>
<td></td>
<td>pupae</td>
<td>1-3</td>
<td>100 100 100</td>
</tr>
<tr>
<td></td>
<td>adults</td>
<td>1-2</td>
<td>100 100 100</td>
</tr>
<tr>
<td>Carpoglyphus lactis*</td>
<td>mixed</td>
<td>mixed</td>
<td>100 100 100</td>
</tr>
</tbody>
</table>

* Heavily mite infested figs were used.

Acknowledgment

This work was carried out through the projects entitled “Project to Phase-Out Methyl Bromide in the Dried Fig Sector in Turkey (TTGV-P2/30m)” funded by the World Bank through the Multilateral Funds for the Implementation of the Montreal Protocol on Substances that Deplete the Ozone Layer; “Mites infesting stored products in Diyarbakir and Sanliurfa provinces and their control (TARP 1848)” funded by The Scientific and Technical Research Council of Turkey (TUBITAK). We wish to express our appreciation to TARIS and Aegean Exporters’ Union for their valuable assistance during the course of the experiments.

References


The use of portable systems to control insect pests by low pressures

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Abstract: The possibilities of using low pressure as a method to control insects have long been suggested and studied. Until now the ability to implement the full potential of this method has been limited, but in recent years flexible mobile chambers made of welded PVC liners have been introduced. Under vacuum, these chambers shrink over the periphery of the commodity and hold it fast. The system is sealed by an air-tight zipper and is able to retain a vacuum or different compositions of modified atmospheres. At the base of the chamber, an inlet and hosing enable connection to a vacuum pump that maintains the prerequisite low pressure. Previous laboratory studies have revealed the effect of 50 ± 5 mm Hg on six important stored-product pests: *Trogoderma granarium* (Everts), *Lasioderma serricorne* (F.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst) *Ephestia cautella* (Walker), and *Plodia interpunctella* (Hübner). At 30°C and a relative humidity of 55% the egg is the most resistant stage in all species, the times needed to obtain 99% mortality being 46 h, 91 h, 32 h, 22 h, 45 h, and 49 h respectively. Additional results indicated that at lower temperatures or at higher relative humidities, the times needed to achieve mortality were prolonged. Three parameters are most important in the determination of treatment time required for a given commodity. To control all pests, the range of insect species likely to infest the specific commodity must be drawn up. Treatment time must be based on the sensitivity of the most resistant stage of the most resistant species in this list, which is obtained from knowledge of previous infestations. However, no less important are temperature and the relative humidity in the chamber, both these parameters being determined by the condition of the commodity. Consequently, it is the temperature and moisture content of the commodity, and its insect fauna that determine the duration of treatment. To demonstrate this principle, a range of commodities containing natural and artificial infestations was subjected to low pressure for 5 days exposure, under ambient conditions (Mediterranean summer climatic) and 100% mortality was recorded in all cases. In conclusion, the use of low pressure is now a promising option for insect control in stored commodities without the requirement of potentially harmful chemicals.

Key Words: vacuum, flexible treatment chambers, stored-products, insects

Introduction

The use of low pressures to control insects in post-harvest storage has been studies by Back and Cotton (1925), Bare (1948), and Calderon et al. (1966). It was shown that mortality is caused mainly by the low partial pressure of oxygen that results in hypoxia (Adler et al., 2000; Navarro and Calderon, 1979) and also dehydration due to removal of water vapor under vacuum (Jay et al., 1971; Navarro, 1978).

Several studies were conducted on the effect of low pressures on the mortality of storage insects under various trial regimes (Calderon et al. 1966; Finkelman et al. 2003a), but in order to compare sensitivities under uniform treatment conditions and make these findings available for use by commercial companies we have studied the effect of 50 ± 5 mm Hg on six important stored-product pests: *Trogoderma granarium* (Everts), *Lasioderma serricorne* (F.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst) *Ephestia cautella* (Walker),
and *Plodia interpunctella* (Hübner) at 30°C and relative humidity of 55%. The egg was found to be the most resistant stage in all species, the times needed to obtain 99% mortality being 46 h, 91 h, 32 h, 22 h, 45 h, and 49 h respectively (Finkelman et al. 2004a, b).

However, it should be emphasized that all the above experiments were carried out under laboratory conditions.

In the past, the possibility of using low pressures at the large scale commercial level was abandoned due to the requirements for massive, rigid and expensive structures needed to withstand the low-pressures.

Recently a new and innovative technology was introduced for the hermetic storage of durable commodities, and is now in use on an industrial scale (Navarro et al., 1988; 1990; 1994; Silberstein et al., 1998). The structures consist of flexible plastic chambers with manufacture specifications to a level of gas tightness that also enables treatments with modified atmospheres or fumigants without significant gas loss over short exposure times (Navarro et al., 1995). The structures are termed “GrainPro Cocoons™”.

In order to adapt this storage system to the application of low-pressure conditions it was necessary to develop an initial commercial product and find solutions to the problematic of air extraction, leak sealing and commodity loading. It was suspected from the outset that certain commodities, when treated with prolonged vacuum, could present a potential problem because they release corrosive out-gassing of vapors liable to contaminate the oils used in most commercially available vacuum pumps. Accordingly, it was found necessary to select and apply oil type filter/pump configurations that would most economically meet the parameters identified for the successful operation of the system. Navarro et al. (2001) was the first to report on a trial set up with an experimental prototype that was conducted in 1999 in Foxboro MA, USA for treatment of cocoa bean pests, and later in Israel (Navarro at el. 2001). Later, Finkelman et al. (2002, 2003b) reported on additional experimental work conducted in Israel and Ivory Coast. In these later trial 15 m³ capacity cube was used with pressure maintained between 23 to 75 mm Hg for three days and five sets of bioassay tubes were inserted. Again, complete mortality of test insects (All developmental stages of *E. cautella* and *T. castaneum*) was observed after the 3-days exposure to vacuum. Until the study report on here, the commodity used was cocoa beans due to the high added value of this commodity and support from the industry. However, the potential of vacuum for insect control in durable stored products is not limited to the chocolate industry, and the paper reports on its application to a wide range of commodities.

**Materials and methods**

**Previously established data**

Several laboratory studies undertaken on the effect of 50 mm Hg on different stored product insects at different temperatures have been published (Finkelman el al. 2002; 2003b) and Table 1 is a partial summary of previous findings. These findings were used to plan and execute a series of semi-commercial field trials, where to determine exposure time, the following three parameters were taken into consideration:

- **Insect species present.** To control all pests, the range of insect species likely to infest the specific commodity must be drawn up.
- **Sensitivities of species and instars:** Treatment time must be based on the sensitivity of the most resistant stage of the most resistant species in this list.
- **Temperature and moisture content of the commodity:** These abiotic micro-environmental conditions also influence the rate at which mortality takes place under low pressure.
Field trials
The field trials were conducted during 2001 at different food manufacturing factories in Israel. Both vacuum cubes of 7.5 m³ and V-HF (Vacuum-Hermetic Fumigation) cubes of 34 m³ capacity, adapted to facilitate low pressure, were used. The low pressure in the cubes was established using a rotary-vane, oil-lubricated vacuum pump (3 hp Becker model U 4.70, Germany) to within the range of 23 and 75 mm Hg for a duration of 5 days. A variety of durable commodities was used as shown in Table 3.

Table 1. The effect of 50 mm Hg on egg mortality at 55% r. h. and 30°C.

<table>
<thead>
<tr>
<th>Test insects</th>
<th>LT99 values (hours to obtain 99% mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trogoderma granarium</td>
<td>46 h</td>
</tr>
<tr>
<td>Lasioderma serricorne</td>
<td>91 h</td>
</tr>
<tr>
<td>Oryzaephilus surinamensis</td>
<td>32 h</td>
</tr>
<tr>
<td>Tribolium castaneum</td>
<td>22 h</td>
</tr>
<tr>
<td>Ephestia cautella</td>
<td>45 h</td>
</tr>
<tr>
<td>Plodia interpunctella</td>
<td>49 h</td>
</tr>
</tbody>
</table>

As mentioned above, mortality under low pressure is dependent upon the partial pressure of oxygen available for insect respiration. Table 2 indicates the relationship between these two variables.

Table 2. Units used to express atmospheric pressure and their equivalent partial pressure of oxygen expressed in mm Hg and in percentage.

<table>
<thead>
<tr>
<th>mm Hg (torr)</th>
<th>atmosphere</th>
<th>kg/cm²</th>
<th>inches Hg</th>
<th>kPa</th>
<th>mbar</th>
<th>mm Hg Oxygen</th>
<th>% Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>760</td>
<td>1.00</td>
<td>1.03</td>
<td>29.92</td>
<td>101,325</td>
<td>1,013</td>
<td>159</td>
<td>20.9</td>
</tr>
<tr>
<td>600</td>
<td>0.79</td>
<td>0.82</td>
<td>23.62</td>
<td>79,993</td>
<td>800</td>
<td>125</td>
<td>16.5</td>
</tr>
<tr>
<td>500</td>
<td>0.66</td>
<td>0.68</td>
<td>19.68</td>
<td>66,661</td>
<td>667</td>
<td>105</td>
<td>13.8</td>
</tr>
<tr>
<td>400</td>
<td>0.53</td>
<td>0.54</td>
<td>15.75</td>
<td>53,329</td>
<td>533</td>
<td>84</td>
<td>11.0</td>
</tr>
<tr>
<td>300</td>
<td>0.39</td>
<td>0.41</td>
<td>11.81</td>
<td>39,997</td>
<td>400</td>
<td>63</td>
<td>8.3</td>
</tr>
<tr>
<td>200</td>
<td>0.26</td>
<td>0.27</td>
<td>7.87</td>
<td>26,664</td>
<td>267</td>
<td>42</td>
<td>5.5</td>
</tr>
<tr>
<td>100</td>
<td>0.13</td>
<td>0.14</td>
<td>3.94</td>
<td>13,332</td>
<td>133</td>
<td>21</td>
<td>2.8</td>
</tr>
<tr>
<td>50</td>
<td>0.07</td>
<td>0.07</td>
<td>1.97</td>
<td>6,666</td>
<td>67</td>
<td>11</td>
<td>1.4</td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The vacuum cube system
In order to adapt the standard cubes to low pressure use, a quick-release hose and one-directional valve were incorporated. In addition, the system was connected to the pump using flexible 1.5" connecting tubes. The system was designed to be modular enabling the user to connect several cubes to the same vacuum pump, or to disconnect one of the cubes without changing the pressure in the other connected cubes.
The V-HF system
A specially constructed V-HF module of 5.5 m long, 2.6 m wide and 2.4 m high was used to accommodate pallets containing the commodities. The V-HF module consisted of two sections; the upper section that was destined to cover 1.4 m from the top and the bottom section that had a wall of 1 m high. The commodities were loaded over the bottom section of the module on pallets using a forklift. Then, the top and the bottom sections were zipped together to obtain a sealed structure. At start of the trial, a slight vacuum of 100 Pa was applied to adhere the V-HF liner to the bagged commodities, thus minimizing the free space within the V-HF system. Data loggers to measure temperature and air relative humidity were also placed in each trial on top and bottom of inside the V-HF module.

Fig. 1. The Vacuum cube system.

Fig. 2. The V-HF (Vacuum-Hermetic Fumigation) system.
Bioassay

Five sets of bioassay replicates were placed in each of cubes, each set containing all life stages of either *E. cautella*, *T. castaneum*, *O. surinamensis* or *P. interpunctella*. Four of the bioassay sets were located, one on each side of the four cube walls at mid-center height, and one at the top-center. The control bioassay was placed on the top, above the liner of the cube in an open plastic container filled with the commodity being tested. Temperatures at the top and at the four side faces of the cubes were recorded during the trials using data-loggers (HOBO Pro Series).

Results

The results of the semi commercial trials are given in Table 3. Under the experimental conditions no living insects were found in any of the commodities or test vials after the 5 day exposure period. These trials clearly demonstrate that the use of low pressure is now a promising option for insect control in stored commodities without the requirement of potentially harmful chemicals.

Table 3. Semi-commercial field tests that produced 100% mortality of adult insects under low pressure (23 – 75 mm Hg) for 5 days.

<table>
<thead>
<tr>
<th>Treated Commodity</th>
<th>Infestation found in the treated commodity</th>
<th>Test insects used in the trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oats</td>
<td><em>T. castaneum, O. surinamensis</em></td>
<td><em>E. cautella</em></td>
</tr>
<tr>
<td>Corn chips</td>
<td><em>E. cautella</em></td>
<td><em>T. castaneum, E. cautella, O. surinamensis</em></td>
</tr>
<tr>
<td>Cocoa beans</td>
<td></td>
<td><em>E. cautella, O. surinamensis, T. castaneum, P. interpunctella</em></td>
</tr>
<tr>
<td>Wheat</td>
<td><em>S. oryzae, O. surinamensis, T. castaneum</em></td>
<td><em>O. surinamensis</em></td>
</tr>
<tr>
<td>Wheat flour</td>
<td><em>R. dominica O. surinamensis, T. castaneum</em></td>
<td><em>T. castaneum, O. surinamensis, E. cautella</em></td>
</tr>
<tr>
<td>Semolina</td>
<td></td>
<td><em>T. castaneum, O. surinamensis, E. cautella</em></td>
</tr>
<tr>
<td>Almonds</td>
<td></td>
<td><em>O. surinamensis, L. serricorne, E. cautella</em></td>
</tr>
<tr>
<td>Garden peas</td>
<td></td>
<td>*C. chinensis, <em>S. oryzae, T. castaneum</em></td>
</tr>
<tr>
<td>Chick peas</td>
<td>*S. oryzae, *C. chinensis, T. castaneum, <em>R. dominica</em></td>
<td>*C. chinensis, <em>S. oryzae, T. castaneum</em></td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td></td>
<td><em>T. castaneum, L. serricorne, E. cautella</em></td>
</tr>
<tr>
<td>Semolina</td>
<td></td>
<td><em>T. castaneum, O. surinamensis, E. cautella</em></td>
</tr>
<tr>
<td>Rice</td>
<td>*T. castaneum, <em>S. oryzae, O. surinamensis</em></td>
<td>*O. surinamensis, E. cautella, <em>S. oryzae</em></td>
</tr>
</tbody>
</table>

*Sitophilus oryzae, Callosobruchus chinensis and Rhyzopertha dominica* adults have not yet been subjected to laboratory exposures under 50 mm Hg and at 30ºC.
Acknowledgement

This research was a collaborative project with GrainPro and Haogenplast, supported by a grant from the United States-Israel Science and Technology Foundation (USISTF), ARO Project No. 417-0384-02

References


The lethal effects of high carbon dioxide on various life stages of
_Ephestia cautella_ (Wlk.) (Pyralidae: Lepidoptera)

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*Corresponding author e-mail: suletutuncu@hotmail.com

Abstract: Almond moth, _Ephestia cautella_ is the main pest of dried figs and shelled hazelnuts in the Aegean and the Black Sea Region of Turkey, respectively. In this paper, the effects of high CO2 levels on the mortality of _Ephestia cautella_ were reported. Eggs, larvae, pupae and adults of different ages of _Ephestia cautella_ were exposed to 90% and 96% CO2 in air at two temperatures of 27.5°C and 32.5°C at 65%±5 relative humidity for different exposure periods. Complete mortality of eggs was achieved within 48 hours of exposures at both CO2 levels. Although, 8 hours of exposure at both CO2 levels was found to be sufficient to achieve a complete mortality for the young larvae, complete mortality of old larvae was achieved after 36 hours of exposure at the same conditions. Complete mortality of pupae at both CO2 levels was obtained at 36 hours of exposure. Adults were controlled after 24 h of exposure at both CO2 levels.

Key Words: _Ephestia cautella_, dry fruit, carbon dioxide, mortality

Introduction

Almond moth, _Ephestia cautella_ is the main pest of dried figs in the Aegean Region and shelled hazelnuts in the Black Sea Region of Turkey (Anonymous, 1995). Control of the almond moth in Turkey depends mainly on methyl bromide (MB) fumigation in the Aegean Region, in particular. The phase out of MB by the end of 2004 in Turkey, is currently imposing a bottle-neck for the Turkish dried fruit export unless acceptable alternative control methods are developed. Thus, the objective of this paper was to provide basic data on the effects of high CO2 on the various developmental stages of _Ephestia cautella_.

Materials and methods

Insect culture techniques

Laboratory cultures of _Ephestia cautella_ maintained in a rearing room at 25°C and 65% relative humidity (RH) were used for these experiments. Eggs were collected daily from 2.5-L PVC jars in which 50 to 100 young adults were confined. Larvae of the almond moth were separated from the cultures when desired age interval was reached. To obtain _E. cautella_ pupae, pieces of polyethylene transparent tubes of 2.0–2.5mm i.d. X 7mm long were placed in the rearing jar when the larvae begin to wander. Wandering larvae tend to enter and pupate inside these tubes (Navarro and Gonen, 1970). Daily checks of the larvae enabled separation of 1–3 day-old pupae which were collected and placed with the tubes in the gas exposure bottles.
Modified atmospheres (MA)
Mortality of *Ephestia cautella* was determined after exposure to atmospheres containing 90% and 96% CO₂ in air at 27.5±1°C and 32.5±1°C. Normal air served as a control. Gas mixtures were obtained from pre-mixed pressurized steel cylinders prepared by Karbogaz Inc. (Gebze/Turkey), and maintained at a rate of 100 ml/min at 65% RH. Table 1 shows the different developmental stages, the number of individuals and age groups of *Ephestia cautella* exposed to high CO₂ concentrations.

Exposure flasks
The flasks consisted of 1–L gas-washing bottles supplied with an inlet and outlet. Known numbers of insects (Table 1) in special vials were transferred to the experimental bottles, which were then connected to the exposure apparatus. An analyzer equipped with a thermal conductivity detector (Gow Mac CO₂ analyzer Model 20-600), and an electrochemical detector (David Bishop Inst. O₂ analyzer Model OxyCheck 2) for the flushing period were attached to the outlet needle of each bottle to check the CO₂ and O₂ concentrations. After flushing into the gas analyzers, the inlet and outlet ports of the bottles were then blocked tightly.

Results
Among the eggs, 24-48 h-old ones were the most resistant to high CO₂ concentrations and the maximum complete mortality time was obtained in 48 h of exposure at 90% CO₂ at 27.5°C. Old larvae were more resistant than young ones to tested CO₂ levels and the longest exposure time for total mortality was obtained in 32 h at 90% CO₂ at 27.5 °C. The most resistant stage was the pupae for which the longest exposure period needed for complete mortality was 36h at 90% CO₂ at 27.5 °C. Complete mortality of adults was achieved within 24 h of exposure.

Table 1. Numbers and the ages of *Ephestia cautella* at the stages used for testing their mortality.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Age of insects (days)</th>
<th>Numbers per replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>0–24</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>24-48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48-72</td>
<td></td>
</tr>
<tr>
<td>Young larvae</td>
<td>7–10</td>
<td>50</td>
</tr>
<tr>
<td>Old larvae</td>
<td>18–22</td>
<td>50</td>
</tr>
<tr>
<td>Pupae</td>
<td>0–1</td>
<td>50</td>
</tr>
<tr>
<td>Adults</td>
<td>10–14</td>
<td>25</td>
</tr>
</tbody>
</table>

*Age for larval stages from egg stage; pupae from pupation; and adults from emergence

Discussion
Navarro and Donahaye (1990) reported that high insect mortality could be obtained in comparatively short exposure times at high CO₂ conditions than at low oxygen atmospheres. Numerous reports on various insect species also confirm that high mortalities under MAs could be obtained in short exposures are in accordance with our finding presented in this communication. In accordance with our results presented here, Jay (1984) reported that a complete mortality in all developmental stages of *Ephestia cautella* exposed to an atmosphere
composed of 63% CO₂ in air was attained in an exposure period of 2-5 days at 27°C and 66% RH. Finkelman et al., (2004) reported that in a reduced atmospheric partial pressure equivalent to an oxygen content of 1.3–1.8% at 30°C and at 55% RH, the times needed to achieve LT₉₉ for eggs, larvae, pupae and adults of *E. cautella* were 44.8 h, 10.3 h, 6.6 h and 6 h, respectively. The results of Finkelman et al., (2004) are concurrent with our results, which indicate that the most resistant stage against the modified atmospheres is eggs.

Due to the lack of detailed information on lethal effects of high CO₂ atmospheres on the developmental stages of *Ephestia cautella*, our findings were compared to available studies carried out with other insect pests of stored products. Tunc (1983) reported that complete eggs mortality in *Plodia interpunctella* at high 44.3% CO₂ in air at 20 °C and 60% RH were attained in 4 days. Similarly, complete mortality of *T. castaneum* eggs was obtained after 4 days of exposure to 2–4% O₂ (Calderon and Navarro, 1980; Tunc and Navarro, 1983). Donahaye et al., (1996) revealed that 99% mortality in all developmental stages of *Tribolium castaneum* exposed to 1% O₂, 85% N₂, 14% CO₂ at two temperatures of 26°C and 30°C were achieved in exposure periods ranging between 49.2 -165 h. Annis and Morton (1997) reported that LT₉₉ for eggs, larvae, and young adults of *Sitophilus oryzae* exposed to 95% CO₂ in air at 25°C and at 60% RH was between 1.43-5.82 days, while in the same conditions, LT₉₉ time for pupae was up to 15.31 days. According to Ofuya and Reichmuth (1993), the time required for the complete mortality of all developmental stages of *Callosobruchus maculatus* in an atmosphere composed of 88% CO₂ in air at two temperatures of 25 °C and 32 °C at 70% RH were in the range of 1-5 days. In case of *Callosobruchus subinnotatus*, LT₉₉ times for all life stages was reported as 30 h to 6 days in an atmosphere composed of 90% CO₂ in air at 30 °C and 55% RH (Mbata and Reichmuth, 1996). Jay and Cuff (1981) reported that upon exposure to ~1% O₂ at 27°C and 60% RH for 24 h, mortality for 2–14 day-old adults of *T. castaneum* was 99.2%. Navarro (1978) obtained a high mortality (95%) in *T. castaneum* adults at 1% O₂ in N₂ after 96 h at 26 °C and 54% RH  Rameshbabu et al. (1991) reported that a complete mortality of *Cryptoles ferrugineus* adults at 90% CO₂ was attained after 96 h of exposure at 20°C.

In conclusion, MA applications provide an inexpensive and practical method for dried fruit disinfections as an alternative to MB. It does not require a sophisticated technology which is usually not available to small scale dried fruit processing plants in Turkey. The most important advantage of this technology is its safety to operators and to consumers. Amongst the other MA options, the application of CO₂ is also the only one that brings a total mortality in comparatively short exposure times. These results should encourage the dried fruit processors in Turkey towards the use of MA techniques as an alternative to MB. Additional studies must be done to evaluate further the applicability of MAs against other dried fruits pest at the temperatures and relative humidities practically encountered in dried fruit processing plants in Aegean Region throughout the year.

Acknowledgements

This work was carried out through the projects entitled “*Project to Phase-Out Methyl Bromide in the Dried Fig Sector in Turkey* (TTGV-P2/30m)” funded by the World Bank through the Multilateral Funds for the Implementation of the Montreal Protocol on Substances that Deplete the Ozone Layer; “*Mites infesting stored products in Diyarbakir and Sanliurfa provinces and their control* (TARP 1848)” funded by The Scientific and Technical Research Council of Turkey (TUBITAK). We also wish to express our appreciation to TARIS and Aegean Exporters’ Union for their valuable assistance during the course of the experiments.
References


Results of a practical test with the ThermoNox® heat treatment procedure, an alternative possibility for control of stored product pests by thermal eradication

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2 Austria Tabakwerke AG, Porzellangasse 51, A-1090 Wien, Austria
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Abstract: During a practical test with the ThermoNox®, a heat treatment procedure was essayed and control of all stages of the tobacco beetle (Lasioderma serricorne) was achieved at temperatures of 50-60°C. Almost all test organisms, even those in the middle of a tobacco bale were killed. Although the temperature there did not rise above 20°C, the reason for the death of these beetles and larvae far below a lethal level cannot be explained satisfactorily.

Key words: heat treatment, tobacco, Lasioderma serricorne, stored product, pests, control

Introduction

Infestation of plant products during storage is a common occurrence in spite of comprehensive hygienic measures during storage and control treatments. Infestation reduces the quality of the manufactured food products through feeding and contamination (insect fragment, excrements and exuviae). Contaminated stores can give rise to allergies in man and animals and even cause serious health problems. The control of storage pests is often achieved by fumigation using one of the common fumigants such as phosphine or methyl bromide. Due to technical and structural limitations, the use of these fumigants is often difficult or even impossible in many situations.

The HACCP (Hazard Analysis Critical Control Point) concept of the food-hygiene regulation as well as the principles of integrated plant protection require that a combination of pest control methods, taking into consideration of biological knowledge and monitoring systems, be implemented to reduce the use of chemical pest control products and minimize the contamination of stored products with pesticides.

The company Hofmeir GesmbH in Fahlenbach, Germany, developed a thermal eradication process within the last few years, which is marketed under the name ThermoNox®. In cases where other pest control methods are impracticable, a thermal eradication using the ThermoNox® procedure, with its slight environmental effects, is a good alternative for efficient pest control (Müller, 1999). To test this process the Austria Tabak AG placed suitable premises at the disposal of the authors. As test organism, adults and larvae of the tobacco beetle, Lasioderma serricorne (Fabricius) was used.

Operation and technical data

In the ThermoNox® process, a special thermal circulation heaters of the type WEO 9/18 (Fig. 1) slowly heats the air and all equipment in the room to a lethal temperature of 50-60°C. The strong blast apparatus blows the heated air throughout the treated space and permits penetration of heated air even in small cracks in floor, ceiling, and wall constructions as well
as inside hollow cavities in machines. Temperature of the heated air is recorded by integrated thermostats, which switch off at approximately 50°C, thus preventing heat damage of the treated equipment at higher temperatures.

Fig. 1. Heater of the type WEO 9/18 (H. Klapal)

Before beginning of the heat treatment, doors, windows, and all other openings of the premises should be sealed and the treated premises should be cleaned from dust deposits, all combustibles should be removed. Operation of all production equipment and electronic devices should be turned off. All machinery should be left in a stopped state for better heat transmission. According to the manufacturing company, ThermoNox® heaters have been tested for fire and explosion safety as well as concerning effects on structures, equipment, machinery, and the quality of stored products. They correspond to the EU regulation 94/9EG and have obtained the GS sign for safety certification.

The heaters can be used at 16 or 32 amperes with a heating capacity of 9 or 18 kW. Energy consumption is given at 3-4 kWh/m³ in summer and 4-5 kWh/m³ in winter, calculated for a heat treatment period of approximately 50 h.

The heat treatment process consists of 3 phases: heating, holding the temperature and cooling. ThermoNox® heaters are placed in the premises in a way that insures uniform distribution of temperature throughout the treated space, in the air as well as in floor, ceiling, walls, and equipment. At the end of the heating phase air temperature is regulated to reach 50-60°C and must be held for a period of 24-50 hours, so that the heated air penetrates into the cracks and other inaccessible places for an exposure time necessary to control the pests. The heated areas can be inspected during the treatment, and the position and direction of the heaters can be adjusted to achieve optimal heat distribution. In the cooling phase the heaters are shut off, and the treated premises slowly achieve their former temperature. During heating
and cooling, temperature changes of more than 6°C per hour should be avoided in order to prevent damage to the structure and the machinery. Further information on the ThermoNox® procedure can be obtained on the website www.thermonox.de or at the e-mail address info@thermonox.de.

**Experiment design**

To test the ThermoNox® procedure under practical conditions the Austria Tabakwerke AG and the BFL conducted a joint experiment in a 5000 m³ large production hall of a cigarette factory in Linz, Austria, from 21st to 23rd December, 2001. The trial was conducted under extreme due to the building structure being metal-concrete walls, ceilings and floors, large metal window areas, as well as by ambient temperatures around freezing.

To prevent heat losses, windows, doors, and wall openings were sealed with construction wood, foils, and adhesive tape. All the machinery in the premises was emptied and cleaned and the floors were broom-swept to remove dust. All machinery openings, doors, pipes, sluices, and screw conveyors were sealed. All combustibles were removed and electronic elements turned off. To test the effects of the heat treatment on tobacco, a 55 kg bale (50 x 50 x 35 cm) was placed in the hall. These preparations as well as installation and cabling of 16 ThermoNox® heaters of the type 9/18, 9.75-18.75 watts, took approximately 4 hours.

The heating phase began on 21st December at 13:00. Room temperature was 22°C, and the relative humidity 50-55%. Air temperature near the floor was measured using a calibrated data logger of type EBI-125A, which recorded the temperature every 15 minutes throughout the experiment. Furthermore, two infrared pistols of the type Raytek Minitemp TM MT4 were used to take temperature measurements of different parts of the hall and equipment at the end of the heating phase. Temperature measurements in the tobacco bale were made with a calibrated spear thermometer.

Twenty-four hours after the start of the heating phase air temperatures had reached 44.4°C. The heaters were kept running until 23rd December, 13:00, before they were turned off. Because of the low ambient temperatures, the entire cooling phase took approximately 2 days.

To test the biological efficacy, 20 wooden tubes (12 x 12 x 100 mm, diameter 10 mm, closed with cotton), and four glass tubes (2.5 ml, metal capsule) (Fig. 2) were filled with 20 adult tobacco beetles each. These tubes were placed in different parts of the hall, including relatively inaccessible areas such as cracks in the walls, floors and ceilings, machine cavities and cable shafts. Furthermore, 16 plastic tubes with an uncounted number of larvae and adults were distributed in the hall in the same manner. Two plastic cups with 5 beetles and 5 larvae were also placed in the middle of the tobacco bale. As a control batch four wooden and four glass tubes with 5 beetles each as well as a plastic cup with different developmental stages of the pest were left in an untreated office in the same building.

**Results**

**Results of temperature measurements**

Temperatures of 50-60°C were achieved in almost all areas of the treated production hall. In the middle of the tobacco bale only a minimal temperature increase was noticed. The results of the temperature measurements are given in Table 1. The heating process reduced the relative air humidity in the hall from 50-55% to 18.2%.
Results on the efficacy tests
All tobacco beetle stages distributed within the production were eradicated to 100%. All of the beetles and larvae in the tobacco bale were killed with the exception of one larva that showed unstable activity. This result is inexplicable because the temperature in the tobacco bale did not achieve lethal values. Results are summarized in Table 2.

![Fig. 2. Wooden and glass tubes with tobacco beetles (F. Rappl)](image)

Table 1. Temperature measurements in different areas of the hall at the end of the heating phase (temperature in °C).

<table>
<thead>
<tr>
<th>Area of measurement</th>
<th>1st measurement</th>
<th>2nd measurement</th>
<th>3rd measurement</th>
<th>mean</th>
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<tr>
<td>outer wall</td>
<td>56</td>
<td>59</td>
<td>57</td>
<td>57.3</td>
</tr>
<tr>
<td>ceiling</td>
<td>52</td>
<td>51</td>
<td>55</td>
<td>52.7</td>
</tr>
<tr>
<td>floor</td>
<td>53</td>
<td>50.5</td>
<td>58</td>
<td>53.8</td>
</tr>
<tr>
<td>corrugated paper</td>
<td>55</td>
<td>50</td>
<td>52</td>
<td>52.3</td>
</tr>
<tr>
<td>jig</td>
<td>46</td>
<td>52</td>
<td>50.5</td>
<td>49.5</td>
</tr>
<tr>
<td>metal machinery parts</td>
<td>55</td>
<td>58</td>
<td>–</td>
<td>56.5</td>
</tr>
<tr>
<td>condensed water collector</td>
<td>53</td>
<td>–</td>
<td>–</td>
<td>53</td>
</tr>
<tr>
<td>middle of tobacco bale</td>
<td>18</td>
<td>22</td>
<td>–</td>
<td>20</td>
</tr>
</tbody>
</table>

Compilation of costs

Personnel and heater rental € 5,240.74
Freight costs € 679.71
Energy costs (industrial tax), approximately 22,500 kW/h x € 0.09 € 2,025.00
Total costs € 7,946.45
Summary

Heat treatment using the ThermoNox® pest eradication process achieved temperatures of 50-60°C in the production hall of a cigarette factory in Austria. Despite the adverse construction conditions and low outside temperatures, mortality of 100% of all test organisms was achieved in almost all cases. The process is well-suited for pest eradication under conditions which do not allow the use of phosphine or methyl bromide. No damage to construction, machinery, or electronic equipment was noticed. Inexplicable is the mortality of the test organisms in the middle of a tobacco bale, where temperatures did not reach lethal values. Further experiments will be necessary to evaluate the efficacy of the process in other bulk stored products, e.g. cereal grain stored in large silos, ships and other storages.

Table 2: Results on the efficacy tests

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Number of beetles or larvae</th>
<th>Treated or untreated</th>
<th>Sample location</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>alive</td>
</tr>
<tr>
<td>24</td>
<td>480 beetles</td>
<td>treated</td>
<td>production hall</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>unknown</td>
<td>treated</td>
<td>production hall</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>5 beetles</td>
<td>treated</td>
<td>tobacco bale</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>160 beetles</td>
<td>untreated</td>
<td>office</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>unknown</td>
<td>untreated</td>
<td>office</td>
<td>100</td>
</tr>
</tbody>
</table>

References

Novel quarantine treatments of narcissus flies using vacuum, CO2 or hermetic conditions

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Abstract: Infested narcissus bulbs with maggots of the large narcissus fly exposed in the laboratory to treatments of 50 mm Hg (vacuum), 90% CO2, or hermetic conditions resulted in LT99 values (hours to obtain 99% mortality) of 23, 24 and 34 h, respectively. Respiration of the bulbs under hermetic conditions in the laboratory trials caused a decrease of O2 within 18 h to 0.1% and an increase in CO2 concentration to 24% within 34 h at 30ºC. Three field tests were conducted at a packinghouse, using a prototype of the newly developed V-HF (vacuum hermetic fumigation) system to demonstrate its commercial effectiveness to control the large narcissus fly under hermetic conditions for 48 h exposure.

Keywords: methyl bromide alternatives, quarantine treatment, vacuum, CO2, hermetic, large narcissus fly, Merodon equestris, narcissus bulb

Introduction

The large narcissus fly Merodon equestris F. (Fig. 1) is a quarantine insect species that attacks narcissus bulbs (Fig. 2) as well as bulbs of other geophytes (Fig. 3). Fumigation with methyl bromide is the only rapid treatment available for handling infested bulbs (Donahaye et al. 1997; Navarro et al. 1997). However, the most recent international resolution under terms of the Montreal Protocol, ending the use and production of methyl bromide, as well as the phytotoxic effects caused by methyl bromide required renewed studies to find alternative fumigation methods (UNEP 1998).

The objectives of this work were to develop best method that will not damage the bulbs and will be effective in controlling the narcissus fly using gaseous treatments: low pressures, carbon dioxide (CO2), or hermetic sealing.

Materials and methods

For the laboratory experiments, maggots of the species were obtained from field infested narcissus bulbs and exposed to treatments. Because the Merodon develops inside the bulb, maggots infested bulbs were placed inside the treatment chambers in an incubator of 30ºC (Fig. 4). Three gaseous treatments were tested: low pressures, CO2, or hermetic conditions on the survival of the large narcissus fly maggots within their living habitat - the flower bulbs. Insect mortality was determined by examining the state of each infested bulb containing the maggots. They were removed from the bulbs and exposed to a gentle heat of about 40ºC. Insects capable of moving were considered alive.
To implement the laboratory results and to determine their commercial value, three field trials were conducted in Israel. A prototype of the newly developed V-HF system was used to demonstrate its effectiveness to control the large narcissus fly under hermetic conditions.

The V-HF system has two main components: a sealed chamber and a supporting light metal frame (Villers 2001). This system consists of an 18.75 m³ sleeve-shaped chamber made of flexible liner sheeting that can hold vacuum or modified atmospheric gas compositions, with a front opening that is sealed by an air-tight zipper. The supporting portable light metal-frame can be assembled in the designated location and used to hold the chamber in shape for easy loading and unloading of the commodity.

In the field test the bulbs were placed in the V-HF system on their original shipping pallets using a forklift (Fig. 5). Each pallet consisted of 920 kg of narcissus bulbs stored in 40 crates stacked in 8 rows (Fig. 6). In each experiment, 3 pallets of the commodity were arranged inside the V-HF system. At start of the experiment, a slight vacuum of 10 Pa was applied to adhere the V-HF liner to the crates, thus minimizing the free space within the V-HF system (Fig. 7).

Results and discussion

Laboratory tests of 50 mm Hg vacuum, 90% CO₂ and at hermetic conditions showed that exposure times required to obtain 99% mortality were 23, 24, and 34 h, respectively (Table 1). Germination of the bulbs obtained from the laboratory tests and planted in October, showed excellent results with vacuum treatment or hermetic conditions, and acceptable results under CO₂.

Table 1. Effect of vacuum, CO₂, or hermetic conditions on LT₉₉ values (hours to obtain 99% mortality) of the large narcissus fly, Merodon equestris maggots in narcissus bulbs stored at 30°C.
In the field tests, the desired modified atmosphere was obtained by the respiration of the narcissus bulbs, using the V-HF system. In all of the three trials, the O\textsubscript{2} concentration decreased to 0.1\% within 18 h, while the CO\textsubscript{2} concentration after 48 h increased up to 21\% at 30\textdegree C (Fig. 8).

Fig. 4. Treatment chambers in the laboratory experiments

Fig. 5. Loading the V-HF system with pallets containing narcissus bulbs

Fig. 6. Narcissus bulbs stored in V-HF system before sealing

Fig. 7. V-HF system under slight negative pressure

Narcissus bulbs respiration in the V-HF system

Fig. 8. Oxygen and carbon dioxide concentration measured inside the V-HF system during the hermetic conditions. (Gas concentrations given in \%, and time in hours).
Acknowledgements

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References


Thermonox-heat treatments in a flour mill – model and reality

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Abstract: Two heat treatments using electric Thermonox blow-heaters were carried out in the flour mill Rosenmuehle, located in Landshut, Germany, in 2002 and 2003. In the first treatment, electrical energy was taken from the local energy provider, in the second experiment, part of the energy needed was provided by mobile generators to test the feasibility of this technique. Required energy was calculated according to the volume and specific weight of materials treated and their respective thermal conductivity. This is compared to the energy actually needed in both treatments. Fossil fuel generators produced electricity at 0.0775 EUR/kWh which amounted to almost double the cost of the electricity provided by the local energy provider (0.04 EUR/kWh) excluding the rent of the generator, supervision and maintenance costs. Thus, the use of generators may be feasible only in the absence of a reliable energy provider or in case of incompatible combinations of voltage and alternating currents (e.g. 110V, 60 Hz) as may be found in some Asian countries to avoid the costly installation of high-power transformers.

Key words: flour mill, heat treatment, insect control
**Efficacy of vacuum and saturated water steam on stored-product moths and beetles**

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**Abstract:** The effect of a combination of vacuum and saturated water steam used commercially for pasteurization on two stored product pests was tested. The pasteurization process, consisting of three phases, is described in detail. The warehouse moth *Ephestia elutella* and the tobacco beetle *Lasioderma serricorne* were exposed to various combinations of temperatures (50, 60 and 70°C) and vacuum conditions (150 and 400 mbar). The duration of the exposure to the high temperature ranged between 0.1 and 1 min., and the duration of the exposure to the vacuum between 5 and 7.8 min. Complete control of *E. elutella* was achieved in all treatments. The only test were surviving *L. serricorne* were found two weeks after the trial was the exposure to 50°C for 0.1 minute combined with a vacuum of 150 mbar for 5 minutes. The potential of the pasteurization process for stored-product insect pest control is discussed.

**Key words:** *Ephestia elutella*, *Lasioderma serricorne*, heat, vacuum

**Introduction**

Despite their low moisture content, durable stored products may be contaminated by micro-organisms. If they are used e.g. for pharmaceutical and cosmetics industry products, the stored products have to be decontaminated by pasteurization. In Germany, a combination of vacuum and saturated water steam is used commercially for this purpose under the brand name SteamLab-System. The products are treated within a pressure chamber. During the treatment cycle, the products are exposed to temperatures ranging between 70 and 125°C within a pressure chamber. The pasteurization time ranges from 20 to 180 seconds, depending on the product to be treated.

The process consists of three phases (Fig. 1): (1) Conditioning: evacuation of the vacuum chamber; the air, which has an isolating effect, is removed to allow the steam to reach directly the surface of every particle of the product and to condensate on its surface. The product surface is warmed up to the specified temperature during several cycles of strong vacuum and vapour injection. The cycle sequence ensures that condensate immediately evaporates and that surface pores are opened and widened. To limit the condensation to the surface of the products and not on the surface of the walls of the treatment chamber, the walls are heated. (2) Pasteurization: The micro-organisms are inactivated in a saturated steam environment at the given temperature on the surface of the product. This phase lasts 1 to 5 minutes depending on the product, but typically does not exceed 180 seconds. The target treatment temperature is reached quickly through rapid heating and the short exposure to heat prevents the penetration of the heat into the product preserving the product qualities. Treatment temperatures typically range from 80° to 120°C depending on the product and its specific treatment recipe. (3) Cooling/drying: During the following cooling and drying phase, cycles of injection and evacuation of preheated filtered compressed air (at 30 mbar) are applied. The air functions as a conveying gas whose evacuation carries with it the moisture from the treatment chamber.
The pasteurized product is cooled off. The duration of this phase and the number of cycles is a function of the product characteristics.

![Graph illustrating the process of the SteamLab-System](image)

Fig. 1. Illustration of the process of the SteamLab-System.

Stored products treated with the SteamLab-System comprise typically spices, herbs and medical teas, nuts, dried fruits, vegetables, mushrooms and seeds of high-value. Entire seeds may be treated as well as flakes, milled products or powder. The system is constrained by the fact that a thin layer only can be treated, consequently bags have to be opened and the product has to be transferred to special boxes for treatment. Specific combinations of heat, vacuum and duration of the treatment are chosen to ensure the quality of the products is not altered.

Obviously, this pasteurization process should also affect stored-product pests, too. In this study, we exposed two stored product insects which commonly occur in spices, herbs and nuts to the pasteurization process, namely the warehouse moth *Ephestia elutella* (Hübner, 1796) and the tobacco beetle *Lasioderma serricorne* (F., 1792).

**Material and methods**

The test insects were cultivated at 26°C and 75% r.h. Every week, approximately 500 adults of the tobacco beetle were placed onto 500 ml wheat bran and 50 ml of broken tobacco leaves. After 7 days, the adults were removed. Complete development from egg to adult took approximately 9 weeks. Age cohorts were mixed by combining 1 to 8 week old cultures plus adults. Every week, approximately 500 eggs of the warehouse moth were placed onto 500 ml wheat bran. Complete development from egg to adult took approximately 5 weeks. Age cohorts were mixed by combining 1 to 4 week old cultures plus eggs. The mixed cultures were filled into small metal-gauze cages (7.8 x 1.4 cm), closed with a rubber plug.
The treatment chamber, a stainless steel autoclave equipped with a single door, had a volume of ca. 0.5 m³. A two-speed fan mounted in the chamber ceiling provides the effective heat transfer and the uniform temperature distribution during the conditioning and pasteurization phase. The chambers are equipped with sensors, special armature and a sterile aeration filter of 0.2 μm. There is a standard control panel with a 2-row LCD display and a connection to an operating console for controlling the plant. The vacuum system is composed of one or several liquid ring vacuum pumps. With 2 runs per hour, ca. 400 kg/h of a product with a bulk density of 0.4 could be pasteurized in this chamber. We tested the following temperatures: 50, 60 and 70°C, and the following vacuum conditions: 150 and 400 mbar. The duration of the exposure to the high temperature ranged between 0.1 and 1 min., and the duration of the exposure to the vacuum between 5 and 7.8 min. For the combinations of vacuum and temperature tested, see Tab. 1. The gauze cages were placed on the trays, below the surface of the products. For the products used, see Tab. 1.

Results

Complete control of *E. elutella* was achieved in all treatments. The results for *L. serricorne* are given in Table 1. The only test were surviving beetles were found two weeks after the trial was the exposure to 50°C for 0.1 minute combined with a vacuum of 150 mbar for 5 minutes.

Table 1. Effectiveness of a commercially applied pasteurization process on the tobacco beetle.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregano powder</td>
<td>50</td>
<td>0,1</td>
<td>150</td>
<td>200</td>
<td>1</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
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<td>0,5</td>
<td>150</td>
<td>200</td>
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<td>Majoran powder</td>
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<td>100</td>
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<tr>
<td>Gravel sand</td>
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<td>90</td>
<td>150</td>
<td>8</td>
<td>8,3</td>
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<tr>
<td>Dates</td>
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<td>2</td>
<td>70</td>
<td>150</td>
<td>5</td>
<td>13,3</td>
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</table>

Discussion

Both the application of heat (Burks et al., 2000) and vacuum (Finkelman et al., 2003) are well-known methods to control stored-product insect pests. Heat causes injury at the cellular level in insects, including injury to DNA and denaturation of enzymes (Denlinger & Yocum, 1998). The low partial pressure of oxygen, resulting in hypoxia, has been shown to be the main cause of death of moths under low pressures (Navarro & Calderon, 1979). The
combination or integration of methods can be used especially to solve complex situations of infestation (Brower, 1999). In the example presented here, control of microorganisms and macroorganisms infesting sensitive products of high value was achieved. The data suggests that control was achieved mainly by heat, because the tobacco beetle survived a vacuum of 150 mbar if it was exposed to 50°C for 0.1 min only, but was controlled completely with 400 mbar if it was exposed to 50°C for 1 min. The conditions to control the tobacco beetle could have been determined more accurately, however, we decided to test conditions which are typically used for pasteurization rather than using the treatment chamber for the purpose of insect pest control alone.

The SteamLab-System is a suitable method to control the tested stored-product pests. The tobacco beetle was chosen because it is known to tolerate high temperatures (Kirkpatrik & Tilton, 1972). The control of the drugstore beetle should also be achieved with the conditions identified to control the tobacco beetle, as the drugstore beetle is less tolerant to high temperatures (Weidner, 1983). Similar results as those found for the warehouse moth are expected for the Indian meal moth and the tropical warehouse moth, other phycitid moths frequently infesting herbs and spices (Schöller & Prozell, 2004). However, this has to be verified in future studies. The control of stored-product pests is an extra-effect added to the pasteurization. The SteamLab-System can be also used for the purpose of pest control, especially with lower temperatures (50-60°C), e.g. for sensitive products of high value.

References


EcO₂ B.V. – non-toxic pest control in stored products as the alternative for methyl bromide and phosphine

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Abstract: Stressing the importance of non-toxic methods of pest prevention, detection, and control in stored products, this presentation will focus on the environmentally friendly alternatives of the company EcO₂ B.V., which control pest in stored products.

The company is specialized in environmentally friendly pest control in food supplies, spaces where food supplies are stored, buildings, monumental buildings and wood packaging material. As opposed to many other companies, EcO₂ B.V. does not use any toxic gasses. The techniques are environmentally friendly, 100% mortality rate, and safe for people. Besides that, no resistance will influence the pests and after treatment, no residues will leave on the product.

EcO₂ B.V. is established in Numansdorp, the Netherlands and has been specialized in pest control for years. The techniques, are all developed in co-operation with Ministry of Housing, Spatial Planning and the Environment and the Organization for Applied Scientific Research. The EcO₂ techniques are all developed by their own specialists, which have experience in the climate control, food preservation, gas technology and traditional pest control.

EcO₂ B.V. controls pests in food supplies, buildings and spaces in an ecologically way with own developed techniques. These techniques are all applied in service terminals as well as with a mobile installation. Every terminal has the disposal of an online connection with the head office to control the temperature, humidity and the level of oxygen. Every product, object or building that has been treated with one of our techniques will be certified by a treatment certificate and control marks on the products. The use of our ecological techniques does not require any permission or legislation and are so timesaving.

Key words: controlled atmosphere, modified atmosphere, heat treatment, combined treatments

The techniques we are using are:

- **Controlled Atmosphere Technique**
  To control insects, rats, and mice in foods, and related products, and antique in our Service Terminals.

- **Heat treatment Technique**
  To control insects in warehouses, monumental buildings, wood and packaging material and antique in our Service Terminals or on location.

- **Modified Atmosphere Technique**
  To control rats and mice, exclusively on location.

- **Combination of Heat treatment and Modified Atmosphere Techniques**
  To control insects, rats and mice, exclusively on location.

The techniques of EcO₂ B.V. are safe for people and products. They do not contain any toxic gasses, which also do not leave any residue on the products.
Chemical control
The efficacy of phosphine fumigation against dried fruit pests in Turkey

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Abstract: Phosphine fumigation trials were carried out both in flexible storage units (36 tonnes capacity Volcani Cube storage unit) and in stacks under tarp. The flexible units were loaded with 15 tonnes of dried figs in perforated plastic boxes, while the stacks with perforated plastic boxes contained about 50 tonnes of dried figs. The tarps were sealed with sand-snakes to the concrete floor. The following test insects were introduced in perforated plastic containers for bioassay: *Ephemis cautella* (eggs, larvae, pupae), *Carpophilus* spp. (larvae), *Oryzaephilus surinamensis* (eggs, larvae, pupae and adults), and *Carpoglyphus lactis* (mixed stages). The fumigant formulation used in this study was from Degesch FUMI-CEL, a magnesium phosphide formulation in a solid plate form. Fumigations in the flexible storage units continued for three, four and five days’ exposure, while stacks were fumigated for only three days. During the trials the gas concentrations were monitored by an analyzer equipped with an electrochemical detector. All test insects from the bio-assays were kept for two weeks at 25°C and 65% relative humidity. Insect mortality of 100% within bio-assays was achieved.

Key words: phosphine; fumigation; dried fruit pests, mortality.

Introduction

Turkish edible nut and dried fruit production is very high and dominates the world market. Turkey is one of the most important dried fig producing and exporting country, with a production amounting to some 50,000 tonnes annually, comprising from 50 to 55% of the international market. Beside dried figs, Turkey is recognized as the largest exporter of hazelnuts, supplying about 85% of the world hazelnut exports. In addition, more than 50% of the world dried apricot and raisin production is supplied by Turkey.

To control storage pests which infest these commodities in the processing and storage, methyl bromide (MBr) is the fumigant of choice and it has been used for disinfestations purposes for many years in Turkey. Thus, Turkey’s dried fruit industry relies heavily on methyl bromide fumigation to meet market requirements for infestation free products. The phase-out schedule of methyl bromide in Turkey has increased the urgency to find alternatives. Phosphine is a proven major tool in the protection of food, seed and feed stocks against insect pests in storage, including commodities, structure, containers, chambers, and stacks under tarpaulins world-wide. However, fumigation studies involving phosphine on dried fruits in Turkey are limited. The present trials were carried out to obtain basic data on the effectiveness of fumigation of dried fruits with FUMI-CEL™, a magnesium phosphide formulation in flexible storage units (36 tonnes capacity Volcani Cube™ or GrainPro Cocoon™) and stacks under tarps.
Materials and methods

The fumigation trials were carried out on dried figs. In the first trial, three flexible units were loaded with 15 tonnes of dried figs in perforated plastic boxes each holding 25-30 kg. The flexible units were placed in the open at the dried fig processing and storage facility of TARIS (Izmir, Turkey). The volume of each unit was 36 m$^3$ having 200 cm high, 500 cm long, 360 cm wide, and composed of a lower and upper section. These structures are portable, highly gas-tight, and suitable for use at village level. Each flexible storage unit was equipped with T type thermocouples (Onset Co. USA, model: Hobo), PVC gas sampling lines, and test insects in perforated plastic containers at two different levels (marked as A and C at top, B and D at bottom layers). Each flexible unit was treated with FUMI-CEL$^\text{Tm}$, a magnesium phosphide formulation at a dosage of 1 g phosphine/tonne for 3, 4 and 5 days of exposure. The required amount of plates (FUMI-CEL$^\text{Tm}$) were weighed and placed on the bottom layer of the storage units. Immediately after dosing, the upper section of the flexible unit was attached to the lower section and sealed using a gas-tight zipper. Gas concentrations in the stacks were monitored using a Bedfont phosphine monitor (measuring range 0–2000 ppm). At the end of the exposure periods, fumigation was terminated and the test insects in perforated plastic containers retrieved. In addition, a sample of dried figs (25-30 kg) was taken for observations on natural insect survivors. Adult emergence in the samples was checked for live adults after two weeks. All laboratory insects in the perforated plastic containers were kept for two weeks at 25ºC and 65% relative humidity and then checked for mortality. Infestation on untreated dried figs served as control.

In the second trial, stacks of dried figs in perforated plastic boxes, were established at the same storage facility of TARIS as in the first trial. Each stack contained approximately 55 tonnes of dried figs. Three FUMI-CEL$^\text{Tm}$ plates were inserted into a gap between the boxes, so fumigation was carried out at the dosage of 1.8 g phosphine/tonne for only 3 days of exposure. Immediately after dosing, the stack was covered with tarpaulins. The covers were checked before use for damage, pinholes and other defects. Any holes if present were sealed with adhesive tape. The tarpaulin cover was sealed to the concrete floor using sand-snakes. Gas monitoring from different levels of the stack and other observations were similar to those in the first trial. After the fumigation, the stacks under tarpaulin and under the flexible units were aerated until the level of phosphine gas was 0.3 ppm or below. Then, the test insects in plastic boxes and dried fig samples were taken for observation similar to those in the first trial.

The effectiveness of phosphine was determined against a laboratory strain of *Ephestia cautella* (eggs, larvae, and pupae), *Carpoglyphus lactis* (mixed stages), *Oryzaephilus surinamensis* (eggs, larvae, pupae, and adults), and *Carpophilus* spp. (larvae (natural population)).

Results and discussion

Upon exposure to air, the magnesium phosphide formulation begins to react with atmospheric moisture to release phosphine gas. This reaction starts slowly, then accelerates gradually and then tapers off again as the magnesium phosphide is spent. The rate of decomposition will vary depending upon the moisture and the temperature conditions. Magnesium phosphide formulations release phosphine more completely and more rapidly at temperatures below 20ºC compared to aluminium phosphide. The temperatures during the trials ranged from 25 to 28ºC. In the present study, recorded gas concentration values were plotted against the exposure period for both flexible storage units and stack tests. The moisture content of the
dried figs was in the range of 20 and 23%, both in the flexible units and the stacks. The peak phosphine concentration was noticed only at the end of the first day of exposure (Figs 1 and 2). Similar to magnesium phosphide, the evolution of phosphine from aluminium phosphide tablet preparations is dependent on temperature and humidity in the enclosure or the moisture content of the commodity (Banks, 1991; Ducom and Bourges, 1993). Rajendran and Muralidharan (2001) stated that retention of phosphine during bag-stack treatment of paddy rice is influenced by the moisture content of the product. In our trials, the distribution of phosphine gas was generally not a problem. Concentrations of phosphine at different sampling points in the flexible units were not markedly different during the fumigation; therefore, the average values were presented in Fig. 1. In the fumigation under tarpaulin, marked differences in concentration were recorded in the values obtained at top (A and C with a maximum of 1300 ppm), and bottom (B and D with a maximum of 1200 ppm) layers (Fig. 2).

The decay in gas concentration in the flexible storage units and in the tarp covered stack was slow which is clearly shown in the graphs (Fig. 1 and 2). This slow decay apparently enabled retention of gas during the treatment for an effective fumigation in both trials. In fumigation of stored products, the decay in gas concentration has been attributed predominantly to (i) sorption of the fumigant by the commodity and (ii) leakage of the gas. The degree of sorption is determined by the chemical composition of the commodity, water activity and particle size (Banks, 1993; Annis, 1990). In the present study gas loss can be attributed predominantly to sorption of the fumigant by the commodity, but further research is needed concerning sorption of gas by dried fruits.

![Graph showing phosphine concentration during 3, 4 and 5 days of fumigation](image)

**Fig. 1.** Phosphine concentration during 3, 4 and 5 days of fumigations of dried figs in flexible storage units, initial dosage was 1 g phosphine/tonne.
Fig. 2. Phosphine concentration during 3 days of fumigation of dried figs under tarpaulin, initial dosage was 1.8 g phosphine/tonne.

In the current trials, no live test insects were found in any of the exposure periods. Furthermore, no active insects were found in the sampled dried figs after the two fumigation trials. Although, 100% mortality of insects was observed at the end of 3 days of exposure in flexible storage units and in stacks under tarpaulin, further work will be necessary to support these findings. Because of the danger of developing resistance to phosphine, the three days of exposure may not be sufficient for dried fruit pests. Storage of grain in bag-stacks fumigated with phosphine under gas-proof covers or sheets is commonly practiced in the tropics (Taylor and Gudrups, 1996; van S. Graver, 1990). Phosphine fumigation of dried fruits under tarpaulins may be easiest way for processors, but flexible storage units can be used at a farmer levels both for fumigation and for storage.

Acknowledgements

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References


Effect of phosphine concentration and exposure period on the mortality of *Tribolium castaneum* (Hbst.) collected from Sheikhupura district, Punjab - Pakistan

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Abstract: Different strains of *Tribolium castaneum* (Hbst.) collected from various locations of Sheikhupura district were reared in laboratory for getting stocks of individuals of uniform age under controlled conditions of temperature and humidity. These insects were exposed to 200, 300 and 400 ppm concentrations of phosphine gas for 1, 3, 5, 7 and 14 days. The results showed that 200 ppm concentration of phosphine gas with 14 days exposure period, gave almost 100 percent mortality of *T. castaneum* whereas higher concentration of 300 and 400 ppm gave 100 percent mortality after an exposure period of 7 days.

Key words: phosphine, concentration, exposure period, mortality, *Tribolium castaneum*

Introduction


The aim of the present study was to evaluate the most effective combination of phosphine gas combinations and exposure period against various strains of *Tribolium castaneum* (Hbst.), one of the most commonly met with insect pests of stored grain, collected from Sheikhupura district, Punjab - Pakistan.

Materials and methods

The present experiment was carried out in the department of Agricultural Entomology, University of Agriculture, Faisalabad (Pakistan) in order to determine the most effective combinations of concentration (200, 300 and 400 ppm) and exposure period (1, 3, 5, 7 and 14 days), which could give maximum mortality of *Tribolium castaneum* (Hbst.) adults.

Collection of specimen for rearing

The adults of *T. castaneum* were collected from different localities viz. Sheikhupura, Shahkot, Nankana, Sangla Hill, Mananwala and Faroqabad of Sheikhupura district. The specimens collected from each particular locality were pooled together and designated as one strain. Each strain was labeled and reared separately in the laboratory for one generation to obtain
homogeneous progeny. Adults were placed in an anhydrous incubator at 30 ± 2°C and 70 ± 5% R.H. Rearing media was prepared by sterilizing the infested wheat grains at 60°C for five hours. Powdered yeast was added to the breeding jars. The mouths of jars were covered with muslin cloth properly. Adults of *T. castaneum* appeared 23 days after liberation and then again peak emergence was seen after another two days i.e. at 25th day. Progeny removed from these stocks was supposed to be of equal age. These stocks were further held for a period of two weeks before exposure to phosphine gas.

**Phosphine gas generation**
Phosphine gas was generated following F.A.O.’s method (Anonymous, 1975). A funnel tied with thread was hung over a cylinder with H₂SO₄ solution. A pallet of Aluminum Phosphide wrapped in muslin cloth was dropped in the solution. Another cylinder of low volume was inverted on the solution and the air present in it was sucked with the help of syringe until the solution rose into the inverted cylinder up to the mark. With the release of gas from under the funnel, the level of solution in the inverted cylinder went down slowly and gradually. When the cylinder became filled with gas, 5 ml gas was sucked out of it with the help of syringe and injected into desiccators of known volume. Again 50 ml of gas taken out from the desiccators was injected into the Harris meter (Harris *et al*., 1981) for recording the concentration.

**Test procedure**
Adults 150 in number of each strain were placed in 50 ml glass beakers in batches of 50 each. These beakers were properly labeled and covered with muslin cloth. For each individual test, each strain of *T. castaneum* (50 insects in one beaker) in three repeats, were placed in a desiccators along with 200 grams infested grains for treatment with different concentrations of phosphine gas i.e. (200, 300 and 400 ppm, respectively) for different exposure periods of (1, 3, 5, 7 and 14 days). Mortality data were obtained for each combination of concentration and exposure period. One treatment was kept as control. The amount of phosphine gas required for each test was calculated using the formula recommended by the FAO (Anonymous, 1975).

A 500 micro liter syringe was used to inject phosphine gas into the desiccators. The concentration of phosphine gas C2, C3 and C4 (200, 300 and 400 ppm) were checked and maintained periodically for each exposure periods T1, T2, T3, T4 and T5 (1, 3, 5, 7 and 14 days respectively). Observations on insect mortality were recorded 1, 3, 5, 7, and 14 days after each treatment.

**Statistical analysis**
The statistical analysis of mortality data was carried out following two factors CRD analysis of variance. The means of all the treatments were compared by Duncan’s Multiple Range Test at P 0.05 (Steel and Torrie, 1984).

**Results and discussion**
Observations on percent mortality with different concentrations at 14 days exposure period was observed to be the maximum in case of each strain. These results are supported by Price and Mills (1988) who reported that exposure period of phosphine was more critical factor than concentration. At shorter exposure periods even at higher concentration the mortality was low. It is clear from the data given in Table-1a that mortality with concentration of 200 ppm at 7 and 14 days exposure periods did not differ significantly at Sheikhupura and Shahkot while it differed significantly in case of rest of the exposure periods. Whereas, Nankana,
Table 1. Comparison of Percent Mortality in Different Strains of *Tribolium castaneum* in the Control Treatment

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<td>1.33g 2.67</td>
<td>1.67j 3.33</td>
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<td>1.67j 3.33</td>
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<td>1.67j 3.33</td>
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<td>2.67j 5.33</td>
<td>3.33j 6.67</td>
<td>5.33h 10.67</td>
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Table 1a. Percent Mortality in Different Strains of *Tribolium castaneum* with 200 ppm at Different Exposure Time

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<td>42.33d 84.67</td>
<td>43.00de 86.00</td>
<td>41.33e 82.67</td>
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Table 1b. Percent Mortality in Different Strains of *Tribolium castaneum* with 300 ppm Phosphine at Different Exposure Time

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Any two means sharing similar letter(s) in a row do not differ significantly from each other at P= 0.05

Table 1c. Percent Mortality in Different Strains of *Tribolium castaneum* with 400 ppm Phosphine at Different Exposure Time

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<td>Nankana</td>
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</tbody>
</table>

Any two means sharing similar letter (s) in a row do not differ significantly from each other at P= 0.05

C = Concentration, T = Exposure Time and Mort. = Mortality.
Sangla Hill, Manawala and Farooqabad strains have a significant difference at 7 and 14 days exposure periods with the same concentration (200 ppm).

In case of 300 ppm concentration the exposure periods of 7 and 14 days did not differ statistically only slight differences in percent mortality were seen (Table-1b). Data given in Table-1c showed that 400 ppm gave almost 100 percent mortality at 7 and 14 days exposure periods. This is a clear indication that increase in exposure time with low concentration up to 14 days gave 100 percent mortality while with 300 ppm 100 percent mortality was achieved at 7 days exposure time.

The data lead to the conclusion that at extended exposure period all concentrations showed increased mortality. Kashi (1982) also found that with increase in concentration or by extending exposure period the level of mortality increased. Rajendran (1994) observed that lower CT products caused reduced insect mortality while at higher no significant change in the mortality response was observed. In CT product, the exposure period played a vital role for the control of stored grain insect pests. These results are supported by Othman (1990) who observed that concentration and exposure period could interact to influence the rate of mortality. Similar results were reported by Ahmad (1991). The present findings revealed that \textit{T. castaneum} (Hbst.) could be controlled effectively with 200 ppm concentration of phosphine gas but at 14 days exposure period. This gave almost 100 percent mortality of the pest. Ahmad and Mahmood (1996) reported that 200 ppm concentration of phosphine retained at least for 12 days in enclosure, was essential for complete control of insect pest. In Pakistan the storage facilities are inadequate and grains are stored in old sacks or earthen pitchers in the rooms or in bins in the rural areas. The storage structures are not properly built, and can not be fumigated as per requirement. The maintenance of 200 ppm concentration for 14 days is not possible. That is why we need to use higher dose for short exposure period. The results have also shown that the 100 percent mortality can be achieved with 400 ppm at 7 days exposure period. These results are comparable with those of Winks (1984), Chaudhry and Price (1990) and Rajendran (1994).

References


Propylene oxide: a fumigant for quarantine purposes as a potential alternative to methyl bromide

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Abstract: In this study, PPO at low pressure (100 mm Hg) was tested in his study for rapid disinfestation of durable stored products as replacement for methyl bromide by evaluating its toxicity to confused flour beetle (Tribolium castaneum (Herbst)) in present of various commodities, its sorption and residue on various commodities. It required a dosage of 19.9, 24.1, 47.4 and 83.4 mg/l to kill 99% of the pupae of T. castaneum when fumigated in empty space and in presence of wheat, cacao beans and corn, respectively. Thus, much higher dose of PPO required for PPO fumigation in presence of corn and cacao beans to obtain the complete mortality of the pupa of T. castaneum. Sorption of PPO by corn, wheat and cacao bean after 4-h exposure time was relatively high, varying from 57% to 79% of initial concentration. The greatest sorption of PPO for 4 h exposure period was observed by corn (79%). The PPO residue in corn, cocoa bean and wheat were a maximum average of 157, 117 and 133 ppm respectively at 0-1 day after termination of aeration, which all were below the 300 ppm maximum tolerance. Based on its high and rapid toxicity to insects, its reasonable sorption by the commodities and its rapid desorption from the commodities, the combination of PPO with low pressure can be a potential as fumigant for replacing alternative methyl bromide for quarantine purposes.

Key words: Propylene oxide, quarantine fumigation, toxicity, stored product insects, sorption

Introduction

Propylene oxide (PPO) is a liquid fumigant under normal temperature pressure (NTP) with a low boiling point (35°C) and a noticeable ether odour. It is also not an ozone depleter and therefore is environmentally benign. It is a safe fumigant for use on food as a sterilant because they are quickly converted to non-toxic glycols in the stomach. Therefore, it has been used for food sterilization since 1958 and is an FDA approved fumigant to control microbial contamination on certain dry food product such as dry and shelled walnuts, cocoa powder and spices. It was also found to have potential as soil fumigants to control some soil born pathogens, nematodes and weeds. PPO has been recently demonstrated by preliminary tests to have insecticidal properties under vacuum conditions and to show potential as fumigant by killing all stages of the confused flour beetle, the Indian meal moth and the warehouse beetle. PPO is flammable from 3% to 37% volume in air and therefore, to avoid flammability it should be applied under low pressure or CO2-enriched atmospheres. PPO effectiveness is enhanced at low pressure or in a combination with CO2.

Some preliminary studies reported by Griffith (1999), Isikber et al. (2001) and Navarro et al. (2004) PPO has insecticidal properties under vacuum conditions as a fumigant by killing all stages of various stored product insects within a short exposure time. Zettler et al. (2002) also showed that the 8%-92% mixture of PPO and CO2 was effective in controlling...
postharvest insect pests and a dose of 45 mg/l PPO at 38°C for 48 h produced complete control of mixed life stages of various stored product insects. Methyl bromide (MB) is apparently the only fumigant available for quarantine treatment of commodities where rapid disinfestation techniques are essential. The loss of MB could have a significant negative impact on world agriculture, particularly because no available alternatives to either fumigant currently exist for rapid disinfestation of commodities. Thus, there is a critical need to develop new fumigants for quarantine purposes.

A non-ozon depletion fumigant, propylene oxide (PPO) has been considered for rapid disinfestation of durable stored products as replacement for methyl bromide (Griffith, 1999; Isikber et al., 2001; Zettler et al., 2002 and Navarro et al., 2004). In this study, PPO at low pressure (100 mm Hg) was tested for rapid disinfestation of durable stored products as replacement for methyl bromide by evaluating its toxicity to confused flour beetle (*Tribolium castaneum* (Herbst)) in present of various commodities, its sorption and residue on various commodities.

**Materials and methods**

**Test insects**
Tests were carried out on only pupal stage of *T. castaneum*, which is known the most resistance stage to PPO. The pupae were obtained from laboratory cultures reared at 26 ± 1°C and 70 ± 5% relative humidity (r.h.) on standard cultures (Donahaye, 1990). Two days old pupae were obtained by daily separation from culture jars and held in wheat flour for 24-h before the exposure.

**Commodities**
Hard red winter wheat (*Triticum sp.*.) at a moisture content (m.c.) of 11.2%±0.2, Grade no.2 yellow U.S.A. corn for feed (*Zea mays* L.) with m.c. of 11.8%±0.1 and Ivory Coast cocoa beans (*Theobroma cacao* L.) at m.c. of 6.3%±0.3 were used in the tests.

**Fumigation chambers**
Test chambers consisted of 2.64-L desiccators, each capped with a ground-glass 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 fumigant. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps. The desiccators were sealed with silicone vacuum grease.

**The fumigant**
The fumigant was 99 + % pure liquid PPO that was withdrawn from a sealed vial fitted with a rubber septum, using a gas-tight syringe. CO₂ was supplied from a cylinder and was 99 + % pure.

**Dosing and fumigation procedures**
Propylene oxide was introduced as a liquid into the desiccators using 50 or 250 µL gas-tight syringes. Pressure in each desiccator was measured using a 0 to 800 mm Hg Celesco, U.S.A. vacuum gauge (model SE-2000). The 100 mm Hg measure referred to herein is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Prior to each test, each desiccator was filled up with one kg of wheat, corn and cacao beans and then 50 pupae confined, separately, inside 3 cm diameter by 8 cm long wire-mesh cages were placed into the commodity. For fumigations at low pressure, prior to the introduction of the required PPO concentration, 100 mm Hg was obtained by evacuating air. PPO at 100 mm Hg was tested at four to five dosages varying from 5 mg/liter to 82 mg/liter. Each test was replicated at least twice. The 4-h exposure was used throughout all the experiments. The gas mixtures in the
desiccators were stirred for at least 20 min. For all fumigations, r.h. and temperature were maintained at 65 ± 5% at atmospheric pressure and 30 ± 1°C respectively.

**Measurement of sorption and residue in the commodity**

Each commodity weighing 1.0±0.01 kg placed into the fumigation chamber. Thereafter, the lids of each fumigation chamber were tightly closed using high vacuum silicone grease. The fumigation chambers before treatment were held for two or three hours for preconditioning of the commodities at 30°C. Sorption profiles of PPO were determined for each commodity at a dose of 82 mg/l applied over a 4 h period. The calculated volumes of PPO were introduced as a liquid into the desiccators containing the commodities by using 50 gas-tight syringes. Controls consisting of sealed, empty fumigation chambers were also dosed to determine the “chamber effect” on fumigation concentrations. All exposures were conducted at 30±2°C and 60%±10 relative humidity, ambient conditions. PPO was sampled from the free-space of each chamber to determine the decrease in fumigant concentration due to sorption.

The gas concentration of PPO was measured using a Shimadzu 17A GC fitted with an FID (Flame Ionization Detector) and an EC™–WAX capillary column (30 m length x 0.25 mm ID x 0.25 μm Film Thickness) run at 170°C isothermal. During the operation, gas flow rates were 30 mLmin⁻¹, 50 mLmin⁻¹, 500 mLmin⁻¹ for helium, hydrogen and air, respectively. Temperatures were 170°C, 250°C and 260°C for column oven, injector port and detector, respectively. With these conditions, the retention time of PPO was ca. 2.65 min. The PPO residues in wheat, corn and cocoa beans were measured after 4-h fumigation at 30°C at the sole dose of 112 mg/l PPO. The levels of PPO residue on each commodity were determined at the end of the fumigation and following a 3-day aeration period. The levels of PPO residue in the commodities were determined by a commercial analytical laboratory service (Aminolab Ltd. Israel) following the analytical method that was a modification of the ASTA analytical method of the Official Methods of Analysis of the AOAC (Anonymous, 2000).

**Data processing and analysis**

After each treatment, the pupae were transferred to 200-mL jars containing food medium and were held at 26 ± 1°C and 70 ± 5% r.h. until examined for mortality. Mortality counts for pupae were made 9 d after exposure based on those pupae that failed to produce adults. Zero dose control and dose-mortality responses were subjected to probit analysis by the POLO-PC computer program (LeOra Software 1987) to determine LD₃₀'s, LD₉₀'s and their respective 95% confidence intervals.

**Results and discussions**

The LD₃₀ and LD₉₀ levels for PPO at 100 mm Hg against pupal stage of *T. castaneum* resulting from 4-h laboratory fumigations in empty space and presence of wheat, corn and cocoa beans are presented in Table 1. There was a significant difference in toxicity of PPO at 100 mm Hg against the pupae fumigated in empty space and presence of wheat, corn and cocoa beans. It required a dosage of 19.9, 24.1, 47.4 and 83.4 mg/l to kill 99% of the pupae of *T. castaneum* when fumigated in empty space and in presence of wheat, cacao beans and corn, respectively (Table 1.). The results indicated that a four-fold increase in LD₉₀ value of PPO at low pressure took place when the pupae were fumigated in presence of corn as compared to those fumigated in empty space. Whereas, there was two and half-folds increase in LD₉₀ value of PPO at low pressure fumigated in cacao beans as compared to those fumigated in empty space. On the other hand, there was not much difference in LD₉₀ values for PPO fumigation in presence of wheat as compared with empty space PPO fumigation. Present study indicated that much higher dose of PPO required for PPO fumigation in presence of corn and cacao...
beans to obtain the complete mortality of the pupa of *T. castaneum*. This could be due to a higher sorption of PPO by these corn and cacao beans. It is a well-recognized fact that a much higher dose of fumigants is required to kill an insect in a container filled with a commodity than in an empty one, owing to the sorption of the gas by the product. Thus, Punj (1969) reported that LD$_{50}$ value of different fumigants against *T. castaneum* in presence of paddy and groundnut kernels varied from 2.7 to 7.5 times as in empty space.

Table 1. Probit analysis data for propylene oxide at low pressure of 100 mm Hg for the pupae of *T. castaneum* P resulting from 4-h laboratory fumigations at 30°C in empty space and in presence of wheat, cacao beans and corn.

<table>
<thead>
<tr>
<th>Tested insect species</th>
<th>n$^a$</th>
<th>Slope±SE</th>
<th>LD$_{50}$ (Fiducial limit)$^b$ (mg/L)</th>
<th>LD$_{99}$ (Fiducial limit)$^b$ (mg/L)</th>
<th>H$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty</td>
<td>200</td>
<td>19.8±3.94</td>
<td>16.2 (14.33 – 17.33)</td>
<td>21.2 (19.75 – 23.76)</td>
<td>0.79</td>
</tr>
<tr>
<td>Wheat</td>
<td>200</td>
<td>8.4±0.87</td>
<td>12.8 (11.72 – 13.81)</td>
<td>24.2 (21.75 – 27.99)</td>
<td>0.20</td>
</tr>
<tr>
<td>Corn</td>
<td>200</td>
<td>10.3±1.03</td>
<td>47.2 (44.01 – 50.08)</td>
<td>79.5 (72.99 – 89.55)</td>
<td>0.75</td>
</tr>
<tr>
<td>Cacao Beans</td>
<td>200</td>
<td>6.5±0.82</td>
<td>20.2 (18.57 – 21.70)</td>
<td>45.9 (39.36 – 58.96)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

$^a$ Number treated, excluding controls.
$^b$ Numbers in brackets give the 95% confidence range.
$^c$ Heterogeneity factor, chi-square/degrees of freedom (chi-square is significant, *P* < 0.01)

Concentrations of PPO (mg/l) in fumigation chamber of 2.64 l during four hours of exposure after the application of PPO dose of 82 mg/l to 1 kg of wheat, corn and cacao beans at 30°C and 60±5% relative humidity are presented in Figure 1. In the corn and cacao beans cases, it is clear that there is an initial rapid decrease in concentrations of PPO during first 1 h exposure followed by a more gradual subsequent drop. This indicates that PPO is initially taken up faster by corn and cacao beans than by wheat. Sorption of PPO by corn, wheat and cacao bean after 4-h exposure time was relatively high, varying from 57% to 79% of initial concentration. The greatest sorption of PPO for 4 h exposure period was observed by corn (79%), whereas the wheat displayed the lowest sorption of PPO (57%), which indicates a reasonable sorption of PPO by the commodities tested at short exposure time (4-h). These findings may be compared with several studies on sorption of other fumigants by wheat, although sorption varies in extent according to the type of commodity fumigated, as well as with other factors such as the temperature during and following fumigation, moisture content, fumigation concentration, and dosage time (Banks, 1986). The results obtained by Berck (1965) indicated that at 27°C, sorption of ethylene dibromide, ethylene dichloride and carbon tetrachloride by wheat at 13.5% m.c. after 4 h exposure was less than 40%, 20% and 5% respectively. Cherif et al. (1985) reported that at 26°C, sorption of methyl bromide by wheat at 12 m.c. after 6 h exposure was less than 70%. It appears therefore that PPO tends to be more highly sorbed by wheat than ethylene dibromide, ethylene dichloride and carbon tetrachloride, whilst it is sorbed slightly higher than methyl bromide.

The PPO residue in corn, cocoa bean and wheat were a maximum average of 157, 117 and 133 ppm respectively at 0-1 day after termination of aeration, which all was below the 300 ppm maximum tolerance determined by US FDA. A very low PPO residues ranging from 6 to 14 ppm was detected at 3 days after termination of aeration (Table 2). This data indicate that the PPO rapidly desorbs from the commodity at conditions of NAP and 30-35°C. Very low levels of PPO residue in the commodities indicate that PPO was physically bound to the
commodity and the fumigation chamber, and did not chemically react with components of the commodity. Thus, it is clear that most of the sorption of PPO by the commodity was physical. These data also support those of Zettler et al. (2002) who showed that the PPO residues among almonds, pecans and walnuts immediately after 4-h fumigations were well below the 300 ppm tolerance and that residues could not be detected following three days aeration.

![Graph showing PPO concentrations](image)

Fig. 1. Concentrations of PPO (mg/l) in fumigation chamber of 2.64 l during four hours of exposure after the application of PPO dose of 82 mg/l to 1 kg of wheat, corn and cacao beans at 30°C and 60±5% relative humidity.

Based on its high and rapid toxicity to insects, its reasonable sorption by the commodities and its rapid desorption from the commodities, the combination of PPO with low pressure can be a potential as fumigant for replacing alternative methyl bromide for quarantine purposes where rapid disinfection of commodities is essential. However, more research is needed to obtain data on its penetration through the mass of commodities and its phytotoxicity of PPO.

Table 2. PPO residues ppm on wheat, corn and cocoa beans after 4-h fumigation at 30°C and atmospheric pressure with a dose of 112 mg/l PPO.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Average PPO Residue (ppm) in sample during aeration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
</tr>
<tr>
<td>Wheat</td>
<td>133</td>
</tr>
<tr>
<td>Corn</td>
<td>157</td>
</tr>
<tr>
<td>Cocoa beans</td>
<td>117</td>
</tr>
</tbody>
</table>
Acknowledgements

We thank the Ministry of Foreign Affairs of Israel (MASHAV) and the University of Kahramanmaras Sutcu Imam Turkey, for providing the funds that enabled Dr Ali A. Isikber to participate in this study during a post-doctorate fellowship undertaken at the Agricultural Research Organization Volcani Center. This work was funded in part by a grant from the United States – Israel Science and Technology Foundation (USISTF), ARO project number 5288.

References


Alternative fumigants to methyl bromide, for the control of stored product insect pests

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Abstract: A study was conducted to evaluate the potential of methyl iodide (CH$_3$I), carbon disulfide (CS$_2$), benzaldehyde (C$_7$H$_6$O), some essential oils and several isothiocyanates for the control of the main stored product insects. The toxicity of the various fumigants was assessed against adults, pupae and larvae of six major stored-product insects.

Key words: methyl iodide, carbon disulfide, benzaldehyde, essential oils, isothiocyanates, control, stored product insects

Introduction

Methyl bromide and phosphine are the most widely used fumigants for controlling pest infestation in grain, dry food products and quarantine insects in cut flowers. Some stored-product insects have developed resistance to phosphine and with the proposed phase out of methyl bromide in the near future, there is an urgent need to search for suitable alternatives with the potential to replace these fumigants.

This paper reports the results of studies on the fumigant toxicity of some common isothiocyanates (ITCs) and of three fumigants: carbon disulfide (CS$_2$), methyl iodide (CH$_3$I) and benzaldehyde (C$_7$H$_6$O). We also report on the isolation from the seeds of *Eruca sativa* (salad rocket) an isothiocyanate not yet fully identified (alkil-thio-alkil-isothiocyanate) which is highly active against stored product insects. We also isolated another yet an unidentified active isothiocyanate from *Diplotaxis tenuifolia*.

Materials and methods

The stored-product insects against which the various compounds were tested were laboratory strains of *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (E.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *Trogoderma granarium* Everts, *Plodia interpunctella* (Hübner) and *Ephestia cautella* (Walker). The isothiocyanates (ITCs) were obtained by putting 100g of ground seeds into a round bottom flask with buffer solution (1% ascorbic acid). The flask was held in a water bath (t = 70°C) for 2 hours to facilitate the hydrolysis of sinigrin to ITC by the enzyme myrosinase which is present in the seeds. The second step was steam distillation using the Dean-Stark apparatus (Leoni et al., 1997). The yellow, upper layer which separated was then extracted with petroleum ether. Finally the petroleum ether was evaporated under a steam of air. GC, NMR and IR were used for the chemical identification of the unknown ITCs. The essential oil obtained from the seeds of *Eruca sativa* is partially identified as alkil-thio-alkil isothiocyanate. CS$_2$, CH$_3$I and C$_7$H$_6$O were purchased from Sigma USA.
Bioassays
Three types of tests were performed to evaluate the activity of the fumigants. The first screening of the fumigants, was space fumigation in glass chambers of 3.4-L capacity (Shaaya et al. 1991). The highly active oils were then assayed in 600 ml glass chambers filled to 70% by volume with wheat (11% m.c). Pilot tests were carried out in simulation glass columns 10 cm in diameter x 120 cm in height, filled to 70% by volume with wheat.

The insects were introduced in cages each holding 20 insects of the same species together with food. Groups of four cages were suspended by a steel wire at different heights from the bottom of the column.

Results and discussion

Studies with isothiocyanates
Four most active ITCs were tested; allyl, methyl, butyl and ethyl against adults of four stored-product insects. In the case of allyl ITC, a concentration of 1 µL/L air and exposure time of 3 h was enough to kill all the adult insects studied. Methyl ITC at a concentration of 1.5 µL/L air for 2.5 h caused 100% mortality (Table 1). The fumigant activity of the essential oil extracted from the seeds of *Diplotaxis tenuifolia* and the oil from *Eruca sativa* were found to be very active against the stored-product insects tested. Their activities were comparable to that of allyl-ITC, except for *T. castaneum* which was much more susceptible to allyl-ITC (Table 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. µL/L</th>
<th>Exposure time (h)</th>
<th>% Mortality, 7 days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S.o*</td>
</tr>
<tr>
<td>Allyl Isothiocyanate</td>
<td>1.0</td>
<td>1.5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3.0</td>
<td>100</td>
</tr>
<tr>
<td>Ethyl Isothiocyanate</td>
<td>1.0</td>
<td>1.5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Butyl Isothiocyanate</td>
<td>1.5</td>
<td>3.0</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>3.0</td>
<td>100</td>
</tr>
<tr>
<td>Methyl Isothiocyanate</td>
<td>1.0</td>
<td>1.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.5</td>
<td>100</td>
</tr>
</tbody>
</table>

* S.o - *Sitophilus oryzae*; R.d - *Rhyzopertha dominica*; O.s - *Oryzaephilus surinamensis*; T.c - *Tribolium castaneum*

In pilot tests using high columns filled with 70% wheat with and without CO₂ and circulation. Allyl ITC at a concentration of 20 µL/L air and exposure time of one day, the addition of 20% CO₂ was not enough to cause the penetration of the fumigant. Insects mortality was obtained only in the upper part of the column (Table 3). When circulation was applied, total mortality was obtained with all the adults tested except for *T. castaneum*. Total mortality of *T. castaneum* was achieved, when circulation and CO₂ were applied (Table 3). It should be mentioned however that by increasing the exposure time to 4 days allyl ITC penetrated by gravity only and caused 100% mortality of all the adult insects except for *Tribolium* in 90 and 120 cm height (results not shown).
Table 2. Fumigant activity of isothiocyanates isolated from two crucifera seeds against adults of stored product insects, space fumigation.

<table>
<thead>
<tr>
<th>Seeds</th>
<th>Conc. µL/L</th>
<th>Exposure time (h)</th>
<th>% Mortality 7 days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S.o*</td>
</tr>
<tr>
<td><em>Diplotaxis tenuifolia</em></td>
<td>1</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td><em>Eruca sativa</em></td>
<td>1</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>Allyl-Isothiocyanate</td>
<td>1</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.5</td>
<td>100</td>
</tr>
</tbody>
</table>

* S.o - Sitophilus oryzae; R.d - Rhyzopertha dominica; O.s - Oryzaephilus surinamensis; T.c - Tribolium castaneum

Table 3. Toxicity of allyl-isothiocyanate against stored product insects using high columns filled with 70% wheat, with and without CO2 and forced circulation.

<table>
<thead>
<tr>
<th>Insect</th>
<th>Insects positioned at shown distances from top (cm)</th>
<th>% Mortality 7 days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Exposure time 1 day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 µL/L + 20% CO2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 µL/L +Circulation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 µL/L +Circulation +20% CO2</td>
</tr>
<tr>
<td>Tribolium castaneum</td>
<td>10 + 20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0</td>
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<td></td>
<td></td>
<td>15</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>120</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>10 + 20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Sitophilus oryzae</td>
<td>10 + 20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td>100</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Rhizopertha dominica</td>
<td>10 + 20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Oryzaephilus surinamensis</td>
<td>10 + 20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>100</td>
</tr>
</tbody>
</table>

Studies with CH3I and CS2
In space fumigation, CH3I was very effective against all insect stages tested. Exposure to a concentration of 3-5 µL/L air for 3 h was enough to obtain 100% mortality of all the stages of the test insects, except pupae of T. granarium (Table 4). CS2 was less effective and needed a concentration of 6-10 µL/L air for 24h to achieve total mortality of all test insects except T. granarium larvae (Table 4). The relative toxicities of the two fumigants to the test insects varied as follows.

- For CH3I, adults of S. oryzae were found to be most susceptible followed by R. dominica, O. surinamensis and T. castaneum. In the case of larvae, P. interpunctella were most susceptible followed by T. castaneum and T. granarium. The pupae of T. granarium were most resistant than those of T. castaneum and P. interpunctella (Table 4).
Table 4. Activity of CH₃I and CS₂ against stored product insects, space fumigation.

**CH₃I (exposure time 3 h)**

<table>
<thead>
<tr>
<th>Conc. µL/L</th>
<th>R.d* Adult</th>
<th>O.s* Adult</th>
<th>S.o* Adult</th>
<th>T.c Larvae</th>
<th>T.g* Larvae</th>
<th>P.i* Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>41</td>
<td>10</td>
<td>55</td>
<td>7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>85</td>
<td>100</td>
<td>65</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>100</td>
<td>–</td>
<td>95</td>
<td>77</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>–</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**CS₂ (Exposure time 24h)**

<table>
<thead>
<tr>
<th>Conc. µL/L</th>
<th>R.d* Adults</th>
<th>O.s* Adults</th>
<th>S.o* Adults</th>
<th>T.c* Larvae</th>
<th>T.g* Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>72</td>
<td>53</td>
<td>23</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>90</td>
<td>30</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>100</td>
<td>74</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>–</td>
<td>93</td>
<td>70</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>–</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* S.o - Sitophilus oryzae; R.d - Rhyzopertha dominica; O.s - Oryzaephilus surinamensis; T.c - Tribolium castaneum; T.g - Trogoderma granarium; P.i - Plodia interpunctella

Table 5. Fumigant activity of CH₃I and CS₂ against stored product insects, on winter wheat at 70% filling ratio, in 600 ml chambers.

**CH₃I**

<table>
<thead>
<tr>
<th>Conc. µL/L</th>
<th>Exposure Time Hours</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R.d* Adults</td>
<td>O.s* Adults</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>88</td>
</tr>
<tr>
<td>3.5</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>5.0</td>
<td>3</td>
<td>–</td>
</tr>
</tbody>
</table>

CH₃I-Sp. gravity = 2.28

**CS₂**

<table>
<thead>
<tr>
<th>Conc. µL/L</th>
<th>Exposure Time (Days)</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R.d* Adults</td>
<td>O.s* Adults</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100</td>
</tr>
</tbody>
</table>

CS₂-Sp.-gravity 1.26; *S.o - Sitophilus oryzae; R.d - Rhyzopertha dominica; O.s - Oryzaephilus surinamensis; T.c - Tribolium castaneum; T.g - Trogoderma granarium

- For CS₂, adults of R. dominica were found to be most susceptible, followed by O. surinamensis, S. oryzae and T. castaneum. Larvae of T. granarium were most resistant than those of T. castaneum (Table 4).

In the experiments with 600-mL glass chambers filled with wheat, CH₃I also showed higher activity than CS₂. The concentrations of CH₃I and exposure times needed to obtain a
total mortality of the test insects were similar to those in the space fumigation tests, whereas with CS$_2$, much higher concentrations were needed to obtain a total adult mortality. These large differences between the two fumigants were probably due to higher sorption rate of CS$_2$ in wheat compared with that of CH$_3$I (Table 5).

Table 6. Fumigant activity of benzaldehyde against various developmental stages of stored product insects, space fumigation.

<table>
<thead>
<tr>
<th>Conc. µL/L</th>
<th>Exposure Time (Days)</th>
<th>Insects*</th>
<th>% Adult Mortality 7 days after treatment</th>
<th>% Eggs hatched</th>
<th>% Pupae developed to adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>1</td>
<td>S.o</td>
<td>39</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.d</td>
<td>71</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O.s</td>
<td>16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T.c</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>S.o</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.d</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O.s</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T.c</td>
<td>65</td>
<td>0(63)</td>
<td>45(55)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E.c</td>
<td>–</td>
<td>0(85)</td>
<td>73(81)</td>
</tr>
</tbody>
</table>

*S.o - Sitophilus oryzae; R.d - Rhyzopertha dominica; O.s - Oryzaephilus surinamensis; T.c - Tribolium castaneum; E.c - Ephesia cautella

- Adult mortality in control 0-5%. - Numbers in brackets are values of control.
- No mortality was recorded from larvae of T.c, T.g and E.c fumigated with 5 µL/L for 3 days

Table 7. Fumigant toxicity of benzaldehyde against stored product insects using 600 ml fumigation chambers filled with 70% wheat.

<table>
<thead>
<tr>
<th>Conc. (µL/L)</th>
<th>Exposure Time Days</th>
<th>Insects*</th>
<th>% Mortality, 7 days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adults</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>S.o</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.d</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O.s</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>7</td>
<td>T.c</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.o</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.d</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T.g</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O.s</td>
<td>100</td>
</tr>
</tbody>
</table>

Mortality in control was 0-5%. Third instar larvae were used. – *S.o - Sitophilus oryzae; R.d - Rhyzopertha dominica; O.s - Oryzaephilus surinamensis; T.c - Tribolium castaneum; T.g - Trogoderma granarium

Studies with Benzaldehyde

In space fumigation, benzaldehyde was less active than the other fumigants tested. A concentration of 3 µL/L air and exposure time of 1 day caused 100% mortality of adults of Sitophilus, Rhyzopertha and Oryzaephilus, but only 65% mortality of Tribolium. There was also 100% mortality of eggs of Tribolium and Ephesia, but no effect on the pupae of these
insects (Table 6). In the studies with 600-mL fumigation chambers filled with 70% wheat by volume, a concentration of 50 µL/L air and an exposure time of 7 days caused 100% mortality of adults of *Sitophilus*, *Rhyzopertha* and *Oryzaephilus*. Increasing the concentration to 100 µL yielded very low mortality of adults and larvae of *Tribolium* and also the larvae of *Trogoderma* (Table 7).

**Studies with essential oils**
A large number of essential oils extracted from aromatic plants were screened for biological activity against stored product insects. Two essential oils, ZP51 and SEM, were found most potent against the insects tested. In space fumigation enough a concentration of 0.5 µL/L air and exposure time of 24h to obtain 100% adult mortality. For larvae, a higher concentration was needed (Table 8). Using high columns filled with 70% wheat, a concentration of 50µL/L air of ZP51 (= 50 g/m³) and exposure time of 7 days was enough to cause 100% mortality of *S. oryzae* and *T. castaneum* and 94% and 99% of *O. surinamensis* and *R. dominica* (Table 9).

**Table 8. Toxicity of a number of active essential oils against developmental stages of stored products insects after exposure for 24 h to the essential oil.**

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Conc. µL/L</th>
<th>% Mortality, 7 days after treatment</th>
<th>Adults</th>
<th>Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S.o*</td>
<td>R.d*</td>
</tr>
<tr>
<td>SEM</td>
<td>0.5</td>
<td>100</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>ZP-51</td>
<td>0.5</td>
<td>97</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>Lemonen</td>
<td>0.5</td>
<td>27</td>
<td>(40-14)</td>
<td>27</td>
</tr>
<tr>
<td>Control</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The numbers in brackets are the mortality range
*S.o* - *Sitophilus oryzae*; *R.d* - *Rhyzopertha dominica*; *O.s* - *Oryzaephilus surinamensis*; *T.c* - *Tribolium castaneum*; *T.g* - *Trogoderma granarium*; *P.i* - *Plodia interpunctella*; *E.c* - *Ephestia cautella*

**Table 9. Fumigant activity of ZP51 against stored product insects on winter wheat 70% filling ratio, using columns 1.2 m high.**

<table>
<thead>
<tr>
<th>Conc. µL/L</th>
<th>Exposure Time (Days)</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.o*</td>
<td>T.c*</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>94</td>
</tr>
<tr>
<td>70</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>97</td>
</tr>
</tbody>
</table>

*S.o* - *Sitophilus oryzae*; *R.d* - *Rhyzopertha dominica*; *O.s* - *Oryzaephilus surinamensis*; *T.c* - *Tribolium castaneum*
Conclusions

In this study we reported on the isolation of two ITCs from cruciferae seeds. These ITCs showed high activity against stored-product insects, comparable to that of allyl-ITC. The ITC isolated from *Eruca sativa* was partially identified as alkil-thio-alkil ITC.

Comparison of the three fumigants tested, CH₃I, CS₂ and C₇H₆O:
- CH₃I was found to be the most toxic to stored-product insects, followed by CS₂ and C₇H₆O.
- CH₃I was found to be less sorptive than CS₂ and to be less penetrative in wheat. It should be mentioned that CH₃I is very toxic and its use as a fumigant is restricted.
- CS₂ is flammable and is used mainly as a supplement to other compounds. Mixture of trichloroethylene, carbon disulfide, and carbon tetrachloride in a ratio of 64:26:10 was developed by us and was found effective against stored-product insects (Polacek et al., 1960).
- In this work we reported also on two essential oils which are active as fumigants against stored-product insects. The low concentration of 50g oil/ m³ wheat which is needed to obtain effective control as compared with the recommended concentration of methyl bromide of 30g/m³, make these oils potential substituted for methyl bromide.

Acknowledgement

Thanks to Miss Esther Ndumi Ngumbi, for her great help in the preparation of this manuscript.

References


Report about a deltamethrin-resistant strain of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) in Italy

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**Abstract:** The toxic effect of deltamethrin on two strains of *Rhyzopertha dominica* (F.) was tested: the susceptible strain was reared in the Istituto di Entomologia agraria of Milan, while the other one was obtained from cereals treated with hydrogen phosphide (phosphine), coming from a Southern Italian warehouse. The insects belonging to the presumably resistant strain were assayed at the sixth generation. Both populations were exposed to grain treated with 22 mg/Kg of deltamethrin at 2.4%. After 10 days, 100% mortality was achieved in insects belonging to the susceptible strain. The presumably resistant strain instead showed 78.8% mortality after 42 days with subsequent development of a F1 progeny.

**Key words:** lesser grain borer, phosphine resistance, cross resistance

**Introduction**

Genes able to give resistance to an insecticide can often favour the development of cross-resistance also towards other active ingredients (a.i.) (Perez-Mendoza, 1999). In fact it was observed that the level of tolerance to the different a.i. tends to increase more rapidly in susceptible strains, compared to the resistant ones, generation after generation, under the selective pressure exerted by the insecticide (Lorini & Galley, 2000).

In resistant strains the survival is strictly related to the activation of particular enzymes able to create real detoxification. In most cases it’s a matter of mixed-function oxidase, transferase, esterase and carboxylesterase (Rossi, 1989). Insects use enzymes not only to control their homeostasis, but also to protect themselves from the presence of potential toxic substances in the environment (Ribeiro *et al*., 2003).

As to insects infesting foodstuffs, there are many cases of resistance developed towards pyrethroids, such as deltamethrin, cypermethrin and permethrin, in various areas of the world, but particularly in Brazil, Australia and India. They are mostly Coleoptera, such as *Sitophilus zeamais* Motsch. (Ribeiro *et al*., 2003; Perez-Mendoza, 1999), *Rhyzopertha dominica* (F.) (Lorini & Galley, 1999, 2000) and *Tribolium castaneum* (Herbst.) (Collins, 1998).

In Italy, a case of reduced susceptibility of *R. dominica* towards an organophosphate (metacriphos) was reported by Arcozzi & Contessi (1987): none of the tested formulations was able to eliminate the infestation. Treatments with other a.i., such as pirimiphos-methyl and permethrin also proved to have limited success even at the highest concentrations (Arcozzi *et al*., 1987).

There are many methods available to evaluate the levels of resistance developed by insects: results can be affected by numerous variables such as typology, speed of the adopted test and behaviour of the insect placed in contact with the treated material. Such factors could contribute to increase the risk of selecting potentially resistant strains in addition to genetic predisposition of the individuals (Lorini & Galley, 1998).
Insecticide toxicity can be tested by topical application, a filter paper test or by treating the cereal, which afterwards will be used as substrate for the insects. A detailed description of the above mentioned methods is reported in Anon. (1969, 1974), Champ (1968), Samson & Parker (1989), Samson et al. (1989). The direct treatment of cereals is the method in which conditions of the test are most similar to those in the field (Samson et al. 1989).

The toxic effect of deltamethrin against two strains of R. dominica (F.) was tested: a susceptible strain, reared at the Istituto di Entomologia agraria of Milano, and a strain collected in a Southern Italian warehouse with grain previously treated with phosphine. Deltamethrin is commonly used in cereal disinfestation in Italy.

Materials and methods

Both strains of Rhyzopertha dominica (F.) were reared under controlled conditions (27±1°C and 70±5% r.h.). The individuals belonging to the strain characterised by a presumed resistance were reared up to the sixth generation. 2 tests were carried out:

Test I
A sample of 1 kg of wheat was treated with 10 ml of a solution containing 22 mg of deltamethrin at 2.4%. After the treatment, the sample was left to dry for about 24 hr. Afterwards 25 g of wheat and 20 adults of R. dominica belonging to the resistant and susceptible strains were placed in polyethylene containers (⌀: 11.5 cm; h: 6 cm). An identical procedure with no insecticide applied was carried out for the untreated controls. Four replicates were prepared for both tests. Observations of the prepared material were made after 5, 10, 20 and 42 days from the beginning of the tests. At the end of the controls, the samples were kept in a thermo-controlled cell for 6 weeks in order to determin adult emergence.

Test II
The toxic effect of deltamethrin, when placed in contact, was tested by using glass Petri dishes (⌀: 13.8 cm; h: 2 cm). The treated surface for each dish was of 150 cm²: 1.5 ml of insecticide solution (0.184 mg a.i.) was sprayed on each of these dishes. 3 replicates were used. The controls were made after 1.5, 17, 24, 96 and 121 hr from the beginning of the treatment. For each test 20 adults were exposed. Mean and standard deviation were calculated.

Results and discussion

Test I
According to the results, mortality differed considerably between the two strains of R. dominica used in this test (Tables 1 and 2).

In the susceptible strain, the mean number of dead adults, after 5 days of contact with the wheat treated with deltamethrin, was 6 times higher than in the resistant strain. 100 % mortality were achieved after 10 days of exposure. No frass was observed in the jars containing treated cereals and no adult R. dominica emerged within 3 months.. On the contrary, in the case of the resistant strain, a higher agility in insects movements was noticed. Some specimens survived 42 days of exposure even though showing evident symptoms of poisoning. A scarce quantity of frass debris, indication of insect activity, was found in the experimental jars. The observation of debris under the stereomicroscope revealed the presence of a high number of eggs, even though some of them showed evident anomalies in shape and dimension.

The mean number of emerged adults found in treated samples and untreated controls is reported in tables 3 and 4.
Table 1. Mean numbers (± SD) of dead adults of *Rhyzopertha dominica* (resistant strain) on cereal in the treated and in the controls after 5, 10, 20 and 42 days from the beginning of the test (N=20).

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>Mean number (± SD) of dead adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Treated</td>
<td>2.2±0.43</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.0±0.00</td>
</tr>
</tbody>
</table>

Table 2. Mean numbers (± SD) of dead adults of *Rhyzopertha dominica* (susceptible strain) on cereal in the treated and in the controls after 5, 10, 20 and 42 days from the beginning of the test (N=20).

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>Mean number (± SD) of dead adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Treated</td>
<td>12.7±1.89</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.0±0.00</td>
</tr>
</tbody>
</table>

Table 3. Mean number (± SD) of emerged individuals of *Rhyzopertha dominica* (resistant strain) in the treated and control samples.

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>Mean number (± SD) of dead adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
</tr>
<tr>
<td>Treated</td>
<td>0.0 ± 0.43</td>
</tr>
<tr>
<td>Untreated</td>
<td>28.7 ± 2.22</td>
</tr>
</tbody>
</table>

Table 4. Mean number (± SD) of emerged individuals of *Rhyzopertha dominica* (susceptible strain) in the treated and control samples.

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>Mean number (± SD) of dead adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
</tr>
<tr>
<td>Treated</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>Untreated</td>
<td>75.0 ± 14.17</td>
</tr>
</tbody>
</table>

In the susceptible strain, a total of 493 individuals of the F1 generation developed in the untreated cereals, while no individuals emerged from the treated samples. In the presumably resistant strain, however, a total of 593 individuals were found in the untreated cereal and a total of 93 emerged from the treated samples.

**Test II**

After 2 hr of exposure, the insects belonging to the susceptible strain showed evident symptoms of poisoning with significantly slowed down movement. In this strain the highest mean number of dead individuals was observed after 96 hr of contact with the treated surface.
On the contrary, in the resistant strain, adult mobility was higher after 24, 96 and 121 hr of exposure and the highest mean number of dead individuals was found after 121 hr of contact (table 5).

Table 5. Mean number (± SD) of dead adult individuals observed in the dishes treated with deltamethrin (N=20).

<table>
<thead>
<tr>
<th>Observation after hr</th>
<th>Mean number (± SD) of dead individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>1.5</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>17</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>24</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>96</td>
<td>0.3 ± 0.47</td>
</tr>
<tr>
<td>121</td>
<td>1.0 ± 0.82</td>
</tr>
</tbody>
</table>

* Significantly slowed down movements

Moreover, it was observed that most insects could not assume a normal body posture keeping their tarsi in contact with the surface. In order to verify if this observation could be related to the toxicity of deltamethrin, the individuals with difficulties in movement were transferred onto filter paper. 52.5% of individuals belonging to the resistant strain managed to assume a normal posture after about 3 min of contact, showing, however, a certain difficulty to co-ordinate their movements. No individuals of the susceptible strain showed this ability, while it took about 30 sec for untreated individuals to assume the correct body posture.

According to the results, deltamethrin was toxic for both strains even if to a varying degree: 100% of mortality of all individuals placed in contact with the cereal treated with deltamethrin was reached after 10 days from the beginning of the test in the case of laboratory strain, while the percentage of mortality was 78.8% in the resistant strain.

After 6 weeks in a thermostatic cell, adult F₁ individuals only emerged from the treated cereal only in the case of the field strain. Therefore, it seems that the strain in question has developed a certain degree of resistance towards deltamethrin.

As to the alterations of movements and of deambulation observed in the susceptible and resistant strains of *R. dominica* placed in contact with surfaces treated with deltamethrin, Lorini & Galley (1998) state that slowed down movements could reduce the assimilation of the a.i. with consequent probable selection of a strain able to resist to the toxic activity of the insecticide.

**References**


