IOBC / WPRS

Working Group „Integrated Protection in Stored Products“

OILB / SROP

Groupe de Travail „Protection Intégrée de Denrées Stockées“

Proceedings of the meeting

in

Lisbon, Portugal

3 – 5 September, 2001

Edited by
Cornel Adler, Shlomo Navarro,
Matthias Schöller & Lise Stengard-Hansen

IOBC wprs Bulletin
Bulletin OILB srop Vol. 25 (3) 2002

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Regionale Ouest Paléarctique (OILB/SROP)

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ISBN 92-9067-140-6 Web: http://iobc-wprs.org

Participants of the Lisbon conference, September 5, 2001 (photo: P. Trematerra)

Introduction

In September 2-5, 2001, the IOBC Working Group Integrated Protection of Stored Products met under the auspices of the Instituto Investigação Cientifica Tropical of the Centro de Estudos de Fitossanidade do Armazenamento (CEFA) in Lisbon, Portugal. This meeting was attended by 60 participants from 20 countries.

A total of 43 papers were presented in the scientific sessions covering topics such as pest biology and faunistics, storage technology and losses, biological control, phytochemicals, modified atmospheres, inert dusts and synthetic insecticides. The integration of biological control with other methods of Integrated Pest Management was a topic for lively discussion. The current political discussion on food quality and consumer protection in Europe triggered by the BSE crisis and scandals of food contamination was also reflected, and the question was risen if the term "food" should also occur in the name of our IOBC group. Reports from Brazil, Benin, the Cape Verde Islands, Mozambique, Iran, and Sri Lanka helped to understand some of the stored product protection problems in tropical and subtropical climates.

Excursions to a local tobacco producer and a food storage company were also a part of this conference, and a cruise on river Tejo, as well as joint meals in typical down-town tabernas allowed the participants to enjoy the beauty of Lisbon. The local organisers, especially Paula Pereira, Otilia Carvalho and Antonio Mexia, are thanked for their hospitality and their dedication to make this meeting a memorable event.

With my 5-year term as convenor ending in 2001, I resigned from this position, and Dr. Shlomo Navarro from the Agricultural Research Organisation, Bet Dagan, Israel, was elected as new convenor. I am happy that Shlomo took over this task and wish him the best of success. For me, the organisation of meetings of this working group was a lot of fun and I am glad that I can continue to support this group as co-convenor.

Thanks to Lise Stengard-Hansen, Matthias Schöller, and Shlomo Navarro who helped me to edit these proceedings. I also want to thank Catharina Hild and Horst Bathon for their support.

Berlin, January 2002
Cornel Adler, former convenor

To all members of the IOBC group,

I should like to thank to all the colleagues who encouraged me during the last IOBC Working Group on Integrated Protection of Stored Products meeting in Lisbon, 2-5 September 2001, to undertake the mission of convenor in future IOBC activities.

In a changing world strongly influenced by the introduction of new technologies, the IOBC will play a critical role by contributing to a better understanding of the „Integrated Pest Management" concept in stored products. The conventional approach of using contact pesticides and chemical fumigants in stored products has been challenged. Consequently, our objective will be to report on recent research studies carried out on integrated stored product protection, particularly those avoiding the use of chemical pesticides and those that employ advanced detection methods. As the new convenor, I will be glad to cooperate with members of the IOBC group to bring the future meetings to fruitful scientific gatherings.

The Volcani Center, Bet Dagan, March 2002
Shlomo Navarro, new convenor

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GENERAL TOPICS

Integrated Protection of Stored Products
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Species richness and pest control complexity: Will multispecies infestations always require a „multi-bioagent“ control?

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Abstract: A huge number of pest species is associated with stored commodities. In a particular store one may expect a permanent occurrence of many pest species. Control of multi-species infestations by multi-bioagent control will increase pest control complexity, and decrease the acceptance of this strategy by farmers. Therefore, the aims of this paper were to (i) analyze factors which may affect the complexity of bio-control, and (ii) to explore the pest-species richness and chance for their bio-control in stores in the Czech republic (CZ).
Fifty-six arthropod species, 31 insect and 25 mites, were found during a recent faunistic research of stored product pest (SPP) in CZ. However, only few pest species were present at particular stores. Samples of commodities taken from stores (1 sample = 1 chamber of silo/flat store) were most frequently infested by 1 followed by 2 and 4 pest species, 23 % took uninfested samples. In stored grain we found prevalent (23%) the “single species” infestation of stored grain and 18 % of samples was infested by 2 species.
The multi-species infestation potentially requires more complex management. Nevertheless, it seems that the control of multi-species infestation by several biological agents is necessary only if the Acari-Insects occur together. The “Acaro-complex” may be managed by a single predator *Cheyletus eruditus*. Among insects only the bio-control of Coleoptera is available and necessary. This can be accomplished by an augmentation of one or two species of polyphagous parasitoids *(e.g. Lariophagus distinguendus, Anisopteromalus calandrae* or *Holepyris sylvanidis*). Lepidoptera is now economically unimportant in stored grain of CZ. The bio-control of Psocid-complex is not known.
Introducing biological of control of SPP in CZ is probably less difficult than it may be expected from considering only the species richness of SPP pests.

Key words: biological control, stored pests, beetles, parasitoids, predators.

Introduction

In some areas of agricultural IPM (e.g. in greenhouses) the natural enemies play an important role. However, the current stored-product pest (SPP) management relies mainly on chemical and physical broad-spectrum control measures. There are only two cases of a successful commercial introduction of bio-agents for SPP control. The first is the use of the predatory mite *Cheyletus eruditus* to control pest mites in stored grain and empty stores in the Czech Republic (CZ) (Zdárková, 1998), the second is use of the parasitoid wasp *Trichogramma evanescens* to control pest pyralid-moths in food stores of Germany (Schöller et al., 1996; Prozell & Schöller 1998; Schöller, pers. com.). What are the reasons for the low acceptance of bio-control in the stored product environment? One of them is the lack of unbiased information on possibilities and limits of bio-control in SPP. Farmers and food factory managers are worried about the potential complexity of implementation and management of bio-control programmes. Therefore, the aim of this paper was to (i) analyse factors which may affect the complexity of bio-control, and (ii) to explore whether bio-control is realistic in Czech stores in terms of actual pest-species richness.

Host (H)- enemy (E) interactions.

There are a vast amount of publications on insect parasitoids and predators (Hawkins, 1994; Hodek & Honěk, 1996; Hochberg & & Ives, 2000; Godfray, 1994; Hassel, 2000). In these studies, the typical questions asked by both theoretical and applied biologists are: "Which conditions enable the coexistence of host (H) and enemy (E) and stability of H-E interactions (e.g. interference, distribution, heterogeneity, spatial refugees (Bernstein, 2000; Takagi, 1999)?" "How many enemy-species ("ω") are needed to control the particular phytophagous insect below economic injury level (EIL) (Myers et al., 1989)?" "Are complex H-E interactions and food webs more stable than the simple ones (Hawkins et al., 1999)?" Farmers and store keepers, however, rather ask "how many enemy-species (ə) (beneficials) are needed for the control of the whole complex of pests on particular crop/stored commodity?" Note, that „ə" is in this case an approximate measure of the complexity of pest management of a particular protected system (S). Bio-control programmes with great „ə" may be economically unacceptable, since they require much time, specific knowledge and particular technology. Under such conditions, bio-control can hardly replace simple and cheap broad- spectrum insecticides. It is therefore essential to understand the key factors affecting (i.e. increasing or decreasing) the "ə-parameter" in agricultural crop and storage systems.

Factors influencing ə

" **ə**" can be defined as the number of enemies needed to control the actual pest complex in a particular area or protected system. We have recognized three factors influencing ə - (i) No. of pest species present in the studied system or area (**P**), (ii) No. of enemies needed to control a particular pest species (**ω)** and (iii) No. of pest species controlled by a particular enemy species (**φ** = level of polyphagy). "ə" increases with the increase of the first 2 factors (P, ω) whereas the increase of φ, decreases the value of ə.

This has important practical implications for pest management. The use of polyphagous enemies may decrease the complexity (ə) of bio-control even in multiple pest-species infestation. The advantage of polyphagous enemies is further enhanced by their longer persistence after inundative release due to their switching among various prey species. For example *Venturia cannescens* can develop, with various successes on at least 14 host species (Salt 1975). On the other hand, it should be noted that the host preference (i.e. host biased searching/feeding) of polyphagous enemy might have negative influence on the control efficiency of particular pest species in the host-complex. The use of polyphagous parasitoids/predators with a high **φ** may also decrease the risk of control failure due to wrong identification of the pest.

This is important in pests, which are difficult to identify due to their similarity or microscopic size (mites, pyralid moths). For example, the use of predatory mite *Cheyletus eruditus* to control the complex of stored-product pest mites is simple since it does not require determination of astigmatid mites (Fig. 1). *Cheyletus eruditus* is a polyphagous predator and is able to control almost whole Acaroid-complex infesting stored grain (e.g. *Acarus siro*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *Glycyphagus domesticus*, *Tyroglyphus farinae*, *Aleuroglyphus agilis* (Pulpan & Verner, 1965; Zdarková, 1998). *Cheyletus eruditus* (CE) could prey upon 10-15 species of pest mites (φ_{CE} = 10-15 species). The pyralid pests (*Cadra-Ephestia* spp. complex) occurring in food stores plants and mills are difficult to identify. Safe determination of specimen of pyralid moth (e.g. *Ephestia elutella*, *E. kuehniella*, *Cadra cautella*, *C. figuliella*, *C. calidella)* includes dissection and microscopic preparations of genitalia. Fortunately, the complex of these moths can be managed by a single egg-parasitoid, *Trichogramma evanecescens* (TE) ($\varphi_{TE} \cong$ 5 SPP pyralid species). Thus bio-

control using *T. evanescens* wasp or another species of polyphagous wasps (e.g. *Venturia* spp., *Habrobracon* spp.) may be used even by poorly educated personnel (Fig. 2).

Breaking an IPM paradigm: Species specific control of SPP is not realistic.

IPM theorists were often persuaded that control measures should be species specific. The rationale of the "species specific paradigm" is to avoid non-specific and side effects of insecticide control measures or cross-infestations of non-target species by polyphagous enemies (Pimentel et al., 1984). Single pest/enemy relationships would require creation of complex crop/storage eco-systems and may appear unrealistic.

Sinha & Watters (1985) reported 600 species of insects and Hughes (1977) lists over 100 species of mites in stored product environment. Therefore, the total estimate of all SPP around the world (P_{world}) is ca 700 species.

If we assume species-specific control, by using

$$э = \omega.P \qquad (1)$$

we obtain э = 700 enemies for ω = 1 and even э = 2100 enemies for ω = 3.

The numbers of enemies potentially required for species- specific bio-control of SPP thus appears too high. It is impracticable to *(1)* find specific "natural" enemy and screen efficiency for each species SPP, *(2)* find or select efficient strain of enemy, *(3)* develop method of its mass production, *(4)* methods of its release and maintenance, and *(5)* understand all possible cross-interactions among various pests and enemies in the stored systems. The use of limited number of broadly polyphagous enemies seems thus the only realistic approach.

The above example (eq. 1) shows the importance of pest species richness in stored product environment for biological control. This topic is rarely discussed in the current literature on SPP. It is therefore interesting to explore the influence of "P" on " э".

Pest richness and э.

The overall list of SPP species of the world or particular geographical area is impressive. However, farmers regularly encounter only few pest species since the occurrence and distribution of SPP is limited by specific environmental conditions (a-b) (e.g. food, physical factors).

(a) The influence of food resource on P

Stored-products differ in their biochemical composition and therefore also in their bio-availability for development of various species of SPP. Many pests are food specialised to particular foods, e.g. bruchids (*Acanthoscelides* spp., *Bruchus* spp.) to legumes, and *Sitophilus* spp. to cereals. Some species of bruchids are known to be specialised to single host species. Thus, overall SPP richness is much higher than SPP richness of any particular food commodity:

$$P_{\text{all stored product}} > P_{\text{particular product (e.g. stored wheat grain)}} \qquad (2)$$

(b) The influence of geographical and local conditions on P

Although many SPP are cosmopolitan, geographical scale and type of product may decrease pest species richness (P) dramatically. SPP differ in their climatic and microclimatic optima. Sinha & Watters (1985) have established so called "indexes of climatic plasticity" for most of the stored product pests that indicate potential and limits of their geographical distributions.

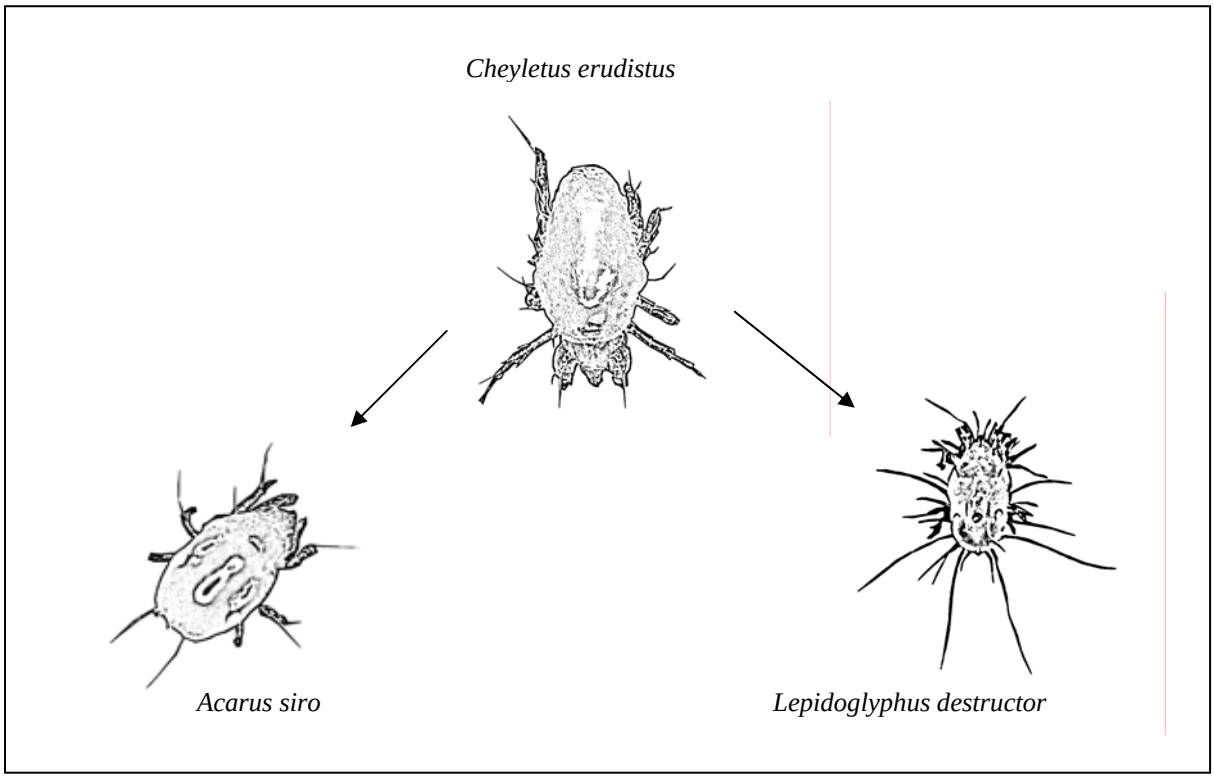

Fig. 1. *Cheyletus eruditus* is polyphagous predator which is able to control almost whole mite Acaroid-complex infesting stored grain

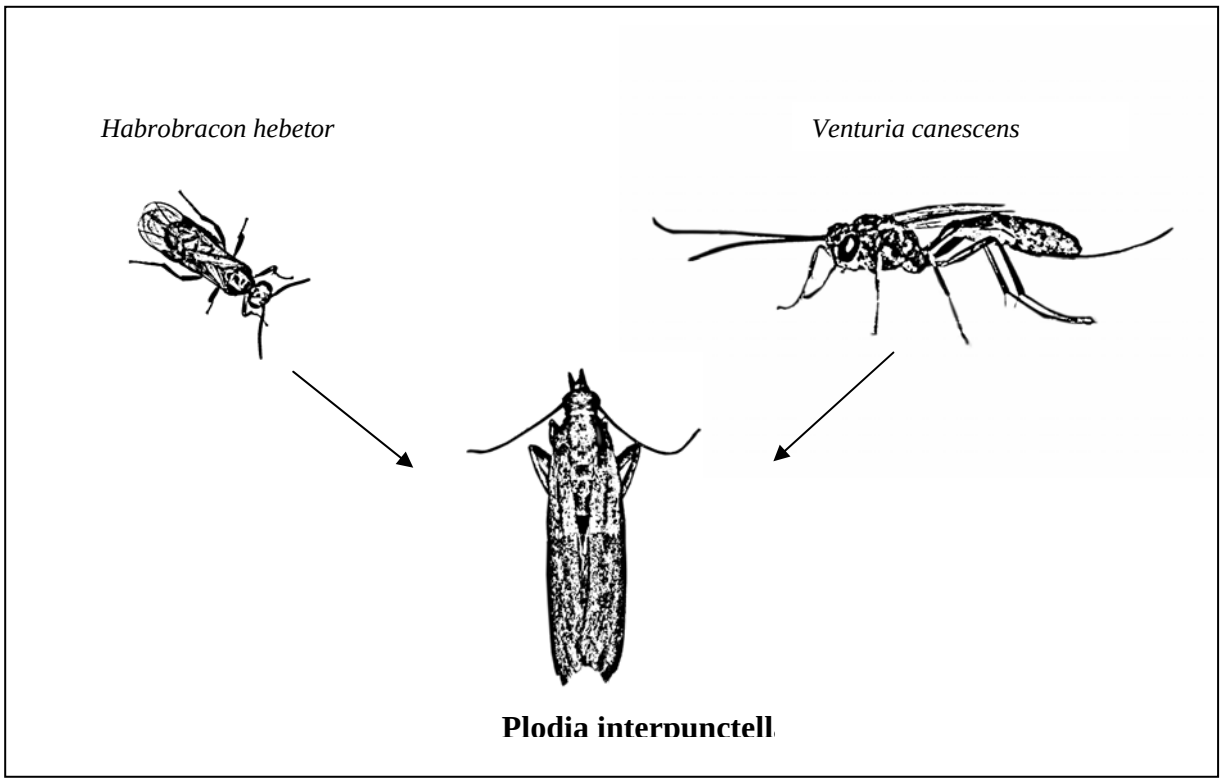

Fig. 2. In addition to *Trichogramma eanescens* pyralid moths complex can be managed by another parasitoids like *Venturia canescens, Habrobracon hebetor*

For example, the moth *Sitotroga cerealella* prefers dry and warm areas of the world, and its occurrence in humid and cold areas of the Central Europe (e.g. Czech Republic) is extremely rare. There are also large differences in pest species richness among farms and stores. The occurrence of a particular pest species on a particular farm is given by chance, actual level of sanitation, technology and local climatic and microclimatic conditions. Based on work of Sinha & Watter (1985), we can conclude that:

$$P_{world} > P_{state/country} > P_{particular\ farm} > P_{store\ chamber\ or\ batch} \quad (3)$$

A large-scale research on fauna of the CZ stores (1996-98) has revealed 56 species of arthropods inhabiting stored grain ($P_{Czech\ Republic\ -\ stored\ grain} = 56$ species). This information, however, tells little about typical number of pest species in a particular grain store ($P_{CZ\ farm\ -\ stored\ grain-\ chamber} = ?$), which is crucial for decision making in pest control. We used the data of this investigation to estimate the typical (most frequent) number of species present in a particular CZ grain store.

What is the most "typical" species richness at Czech farms?

Thirty one insect and 25 mite SPPs have been found during a recent faunistic research of stored grain in Czech Republic (Werner et. al., 1999). However, the most frequent $P_{CZ\ farm-stored\ grain-chamber}$ was low. Although there is $P_{Czech\ Republic\ -stored\ grain} = 56$ pest species, the mean number of species in a store chamber was 2.4 (median 2) and the maximum number was 16. The most frequent infestation was 1 pest species followed by 0, 2 and 4 species per one store chamber (Fig. 3). Thus one–species infestation was the most typical infestation of store chamber in CZ ($P_{CZ\ farm-stored\ grain-chamber} = 1$). The proportion of samples without any pest species or with 1 or 2 pest species among all store chambers was 64 % (Fig. 4). From 2 or 3-species infested chambers was 20 % infested by one group (acari, psocids or beetles) of pest only, and 11 % of chambers had "mixed" species infestation (Fig. 4).

Enemy complexes needed for control of SPP in Czech Republic.

The faunistic data indicated, that the 46 % of grain chambers need no multi-agents control because the chambers were without any pest or infested by only 1 pest species. However, the rest of samples (54 %) would require multi-agents control.

Lepido-complex is economically unimportant in stored grain in CZ. No bio-agent for control of Psocid-complex is known. The control of multi-species infestation by multi-agents strategies is necessary and available only if "Acaro-Beetles" occurs. But the occurrence of this complex is not very frequent. The "Acaro-complex" may be managed by a single predator *Cheyletus eruditus*. The "Coleo-complex" may be managed by one or two species of some polyphagous parasitoids (e.g. *Lariophagus distinguendus, Anisopteromalus calandrae, Holepyris sylvanidis).*

Conclusions

Surprisingly, the complexity of management of biological programs for control of SPP, though certainly higher than for other methods, does not seems too great.

Bio-control of SPP in CZ may be feasible because (i) prevalent pest species richness is generally low in most CZ farms and (ii) many predators and parasitoids are not host specific.

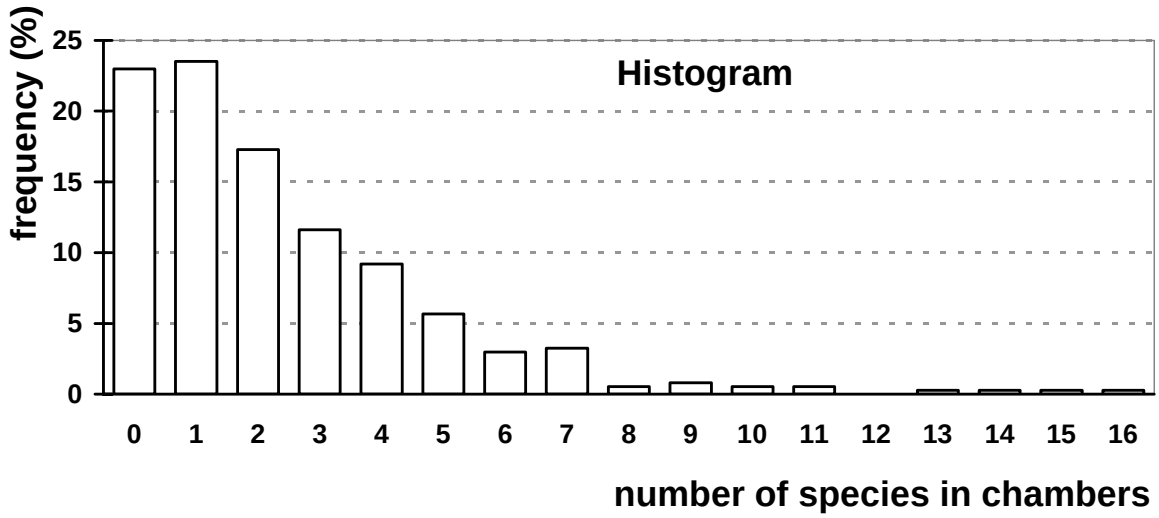

Fig. 3. Histogram of the distribution of number of species found in particular store chambers. Data from faunistic survey (1996-1998) of stored grain in Czech Republic.

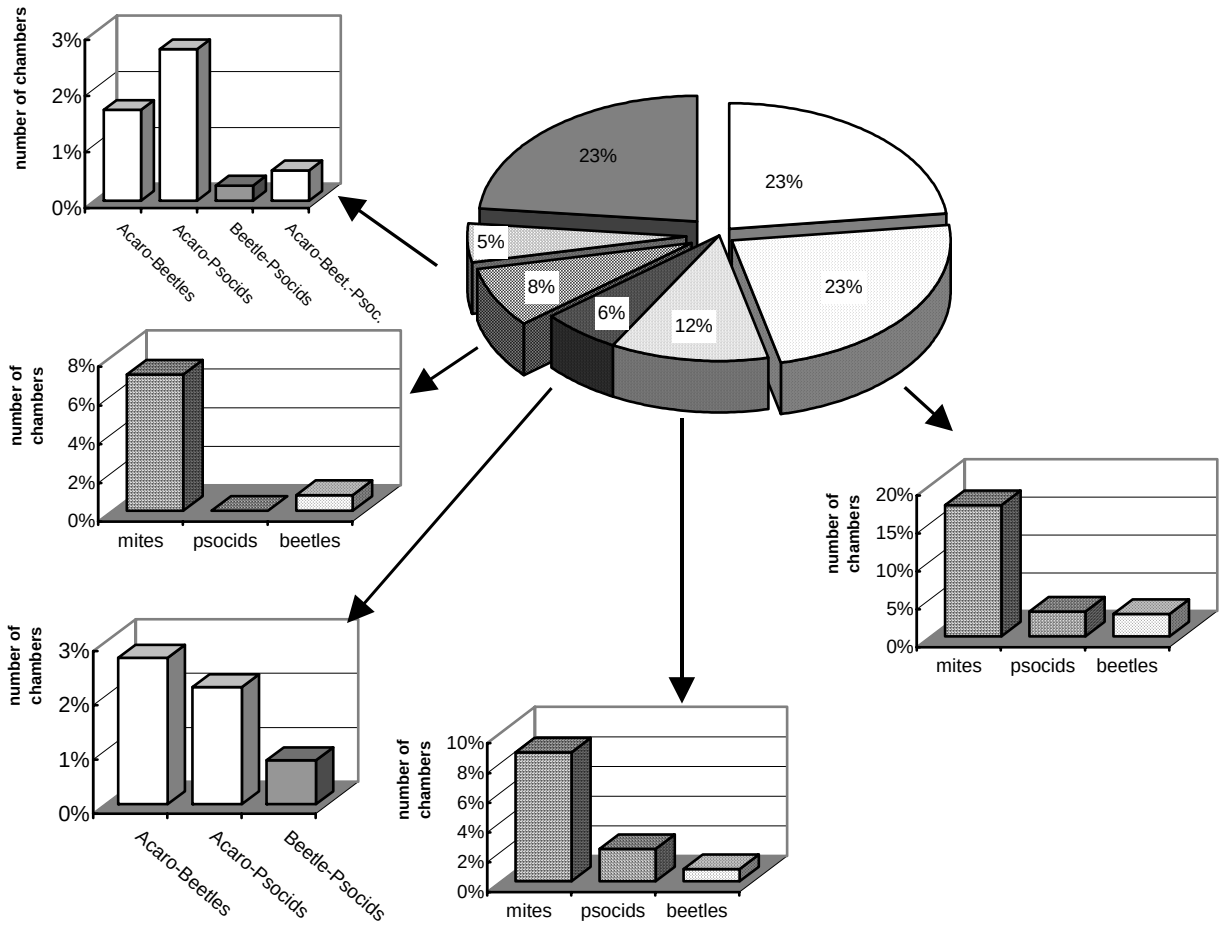

Fig. 4. The proportion of multi-species infestation of stored grain in Czech Republic (1996-1998). Section mean clockwise from above the numbers of pest species present in a chambers: O-species (23%), 1-species (23%), 2 species (18%), 3 species (13%), 4 or more species (23%).

Acknowledgement

We thank A. Honěk and Ing. Jan Janoušek for comments and discussion. This work was supported by the project "Výzkumný záměr- 3335".

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Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
pp. 9-14

Pheromones and Integrated Pest Management in stored products

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Abstract: Crucial factors for Integrated Pest Management (IPM) in stored-products include understanding factors that regulate systems, monitoring insect populations, maintaining good records and using this information to make sound management decisions. The employment of pheromones is one of the most promising techniques aimed at the control of stored-product pests. These substances can lead to a drastic reduction of chemical treatments, thus determining remarkable economic advantages and improvement of product quality, protecting goods from residual insecticides noxious to the consumer. In recent years, considerable progress has been made in the monitoring and control of stored-product insects, Coleoptera and Lepidoptera, by pheromones also used in mass-trapping, attracticide (lure and kill) and mating disruption methods. In stored-product IPM different tolerance thresholds should be established for the various pests depending on their economic impact and on the "filiere place" where they are found. If a limited number of insects can be tolerated at times in a warehouse containing raw materials, in food-processing plants and warehouses containing finished products, the threshold must be necessarily zero. In that context, "*insectistasis"* can be readily achieved by continual supervision of environments by traps in combination with a limited number of preventive and curative measures appropriately timed.

Key words: Pheromones, insects, stored products, IPM.

Introduction

The development of Integrated Pest Management (IPM) programs has been considered by the food industry for both raw and processed commodities (Hagstrum and Flinn, 1996).

The IPM concept emphasizes the integration of disciplines and control measures including biological enemies, cultural management, sanitation, proper temperature utilization and pesticides into a total management system aimed at the prevention of pests from reaching damaging levels. The food industry will need to use IPM programs more extensively in the future to satisfy the increased demands of consumers and regulatory agencies for reduced use of pesticides.

In that context considerable progress has been made in the use of pheromones for monitoring and control (by mass-trapping, attracticide and mating disruption) of stored-product pests (Burkholder, 1990; Phillips, 1997; Trematerra, 1997; Plarre, 1998; Phillips *et al.*, 2000).

Monitoring

Pheromone traps in stored insect management can be used to detect both the presence and the density of pests. They are useful to define areas of pest infestation, particularly where the overall distribution and life cycle are poorly understood. Their purpose is to achieve a more accurate control and to limit the usage of insecticides only when strictly necessary (Table 1).

Table 1. Stored-product insects for which pheromones have been chemically identified

Species	Availability of lures	Sex producing and pheromone type*	References**
Lepidoptera			
Pyralidae			
Corcyra cephalonica	no	M, sexual	Naoshima *et al.* (1991)
Ephestia cautella	yes	F, sexual	Odinokov *et al.* (1991c)
Ephestia elutella	yes	F, sexual	Odinokov *et al.* (1991c)
Ephestia kuehniella	yes	F, sexual	Odinokov *et al.* (1991c)
Plodia interpunctella	yes	F, sexual	Odinokov *et al.* (1991c)
Gelechiidae			
Sitotroga cerealella	yes	F, sexual	Odinokov *et al.* (1991d)
Tineidae			
Tineola bisselliella	yes	F, sexual	Yamaoka *et al.* (1985)
Coleoptera			
Dermestidae			
Anthrenus flavipes	yes	F, sexual	Sharma *et al.* (1991)
Anthrenus verbasci	yes	F, sexual	Kuwahara and Nakamura (1985)
Attagenus brunneus	no	F, sexual	Fukui *et al.* (1977)
Attagenus unicolor	yes	F, sexual	Silverstein *et al.* (1967)
Dermestes maculatus	no	M, aggregation	Levinson *et al.* (1978)
Trogoderma glabrum	yes	F, aggregation-sexual	Mori *et al.* (1985)
Trogoderma granarium	yes	F, aggregation-sexual	Pawar *et al.* (1993)
Trogoderma inclusum	yes	F, aggregation-sexual	Mori *et al.* (1978)
Trogoderma variabile	yes	F, aggregation-sexual	Mori *et al.* (1978)
Anobiidae			
Lasioderma serricorne	yes	F, sexual	Mori and Watanabe (1985)
Stegobium paniceum	yes	F, sexual	Matteson and Mann (1994)
Bostrichidae			
Prostephanus truncatus	yes	M, aggregation	Hodges *et al.* (1984)
Rhyzopertha dominica	yes	M, aggregation	Razkin *et al.* (1996)
Laemophloeidae			
Cryptolestes ferrugineus	yes	M, aggregation	Boden *et al.* (1993)
Cryptolestes pusillus	yes	M, aggregation	Abdukakharov *et al.* (1997)
Cryptolestes turcicus	yes	M, aggregation	Millar *et al.* (1985)
Silvanidae			
Oryzaephilus mercator	no	M, aggregation	Odinokov *et al.* (1993)
Oryzaephilus surinamensis	no	M, aggregation	Boden *et al.* (1993)
Tenebrionidae			
Tribolium castaneum	yes	M, aggregation	Odinokov *et al.* (1991a)
Tribolium confusum	yes	M, aggregation	Odinokov *et al.* (1991b)
Bruchidae			
Acanthoscelides obtectus	no	M, sexual	Mori *et al.* (1981)
Callosobruchus chinensis	no	F, sexual	Mori *et al.* (1983)
Curculionidae			
Sitophilus granarius	yes	M, aggregation	Mori and Ishikura (1989)
Sitophilus oryzae	yes	M, aggregation	Pilli (1993)
Sitophilus zeamais	yes	M, aggregation	Pilli (1993)

(*) M = male; F = female. (**) For chemical synthesis, identification or analysis of pheromones.

Pheromone traps are generally effective when pest numbers are very low and so they can be used qualitatively to provide an early warning of pest incidence. To successfully capture attracted pest insects, a trap has to be escape-proof, this can be achieved by a sticky surface to which the trapped insects become irreversibly attached or by some kind of funnel or pitfall systems (Barak *et al.*, 1990). Designs of traps for moths (*Ephestia* spp., *Plodia interpunctella*, *Sitotroga cereallella*, etc.) and beetles (*Cryptolestes* spp., *Lasioderma*, *Oryzaephilus* spp., *Prostephanus truncatus*, *Rhyzopertha dominica*, *Sitophilus* spp., *Stegobium paniceum*, *Tribolium* spp., *Trogoderma* spp., etc.) infesting stored-products have been developed, generally on an empirical basis.

A list of the factors, known to affect trap catch, that should be addressed during the design, execution and reporting of trapping studies, was reported (Wright and Cogan, 1995). Typical recommendations are provided for the placement of a grid-work of traps and their monitoring for the capture of insects at regular time intervals (Subramanyam and Hagstrum, 1996).

Optimization of traps and lures will allow the realization of new computer-based methods aimed at the organization and interpretation of data and will make it easier to face pest attacks properly (Wileyto *et al.*, 1994).

Mass-trapping

In the case of female-produced sex pheromones only males are trapped. Hence, any attempt to suppress the population by trapping males would require a sufficient number of trapped males so that nearly all females would go unmated.

Theoretical considerations of mass-trapping males take into account the density of males in the population and the potential number of matings a male is able to secure in its lifetime. If a male can mate with ten females in a lifetime, as is the case for *Plodia interpunctella*, then up to 90% of the male population can be trapped without affecting the number of mated females as well as the subsequent larval generation. Under high population levels the rate of female encounters would be high and mass-trapping more difficult to achieve. However, under low population levels males would locate females less frequently and intensive trapping could conceivably reduce male populations to biologically significant levels.

Proper experiments of mass-trapping are not easy to conduct due to inadequate controls or poor replication, although various studies have reported successful in the control of: *Ephestia cautella* in United States; *P. interpunctella* in a storage room for vegetable and flower seeds in France; *Ephestia kuehniella* in some Italian mills; *Lasioderma serricorne* and *P. interpunctella* in two food warehouses in Hawaii; *L.serricorne* in tobacco stores in Greece and in a Hawaiian bakery (Buchelos and Levinson, 1993; Trematerra, 1994; Pierce, 1998; Plarre, 1998).

Mass-trapping both sexes of a population using aggregation pheromones should be more effective than mass-trapping only males. Aggregation pheromones are known from several beetle species that infest stored-products, but few studies have been conducted to suppress populations of these insects.

Attracticide

The attracticide (or lure and kill) concept-based method involves using a pheromone to lure insects to a specific point source or an area whereby they contact a toxicant which causes a rapid kill or contamination with some kind of pathogen.

This method is in someway analogous to mass-trapping, although many more insects are affected because the attracticide is broadcast over a large area and the killing effect is not limited to individual traps.

In the protection of stored-products there are many promising results on the use of the attracticide concept in flour mills and confectionary industries in the control of *E. kuehniella* and *E. cautella*.

In Italian mills Mediterranean flour moth males were successfully lured to laminar dispensers, baited with 2 mg of TDA - (Z,E)-9,12-tetradecadien-1-yl acetate - and treated with 5 mg of cypermethrin, and caused a marked decrease in the population of moths. This technique led to a drastic reduction in chemical treatments with subsequent economic and qualitative advantages (Trematerra, 1995). Another attracticide method utilized pheromones in an inoculation device containing a pathogen (a protozoan in the control of *Trogoderma glabrum*, a granulosis virus against *P. interpunctella*) (Burkholder, 1990; Vail *et al.*, 1993).

Mating disruption

The mechanisms involved in mating disruption may consist of one or a combination of any of the following: the constant exposure of the insect to a relatively high level of pheromone leads to the adaptation of the antennal receptors; a sufficiently high background level of the applied pheromone masks the natural pheromone plumes; the synthetic plume pheromone is applied in a relatively large number of discrete sources.

The limitations and theoretical bases of mating disruption are similar to those for mass-trapping of males.

Several successful experiments have been reported in mating disruption of *E. cautella* and *P. interpunctella* both in the laboratory and in simulated field situation, and *E. kuehniella* in a food industry. Other mating inhibitory compounds are known for Coleoptera *L. serricorne* and *S. paniceum* (Plarre, 1998; Prevet *et al.*, 1989).

However, mating disruption is a potentially effective pheromone-based control method for storage insects and requires further considerations, more data is necessary in order to reduce the quantity of pheromones used and the risk of their residues in food.

Future prospects

In the protection of stored-products different tolerance thresholds should be established for the various pests depending on their economic impact and on the "filiere place" where they are found. For example, a limited number of insects can be tolerated at times in a storehouse containing raw materials, but in food-processing plants and warehouses containing finished products the threshold must be necessarily zero.

The utilization of pheromones and other semiochemicals could lead to a drastic reduction of chemical treatments with consequent economic and qualitative advantages, protecting goods from residual products noxious to the consumer.

Crucial factors for IPM in stored-products include understanding factors that regulate systems, monitoring insect populations, maintaining good records and using this information to make sound management decisions. In that context "*insectistasis*" can be readily achieved by continual supervision of environments by attractant traps in combination with a limited number of curative measures appropriately timed.

New tools have been developed for detecting insects in stored-products, estimating insect population growth, and administering fumigants as well as natural methods of insect control such as grain temperature manipulation. 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 the environmental factors (Arbogast *et al.*, 1998; Shumann and Epsky, 1998; Phillips *et al.*, 2000).

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IOBC Bulletin Vol. 25 (3) 2002
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The use of entomopathogenic fungi for stored product pest control - The "MYCOPEST" project

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Abstract: The need to find alternatives to conventional pesticides for stored product pest control has been recognised by the UK Government and Industry, in a jointly funded 'LINK' project of four years duration. The project focuses on the use of entomopathogenic fungi to control residual infestation in storage structures. Samples of UK sourced, insect specific, fungi have been tested against beetles, psocids, moths and mites in the laboratory. Some strains of fungus have achieved 100% kill of test insects and mites 10 days after initial contact. Results to date have indicated levels of activity that could lead to development of a practical mycopesticide against stored product pests.

Keywords: Entomopathogenic fungi, stored product pests, *Beauveria bassiana, Oryzaephilus surinamensis, Lepinotus patruelis, Ephestia kuehniella, Acarus siro*

Introduction

Stored grain on farms and in commercial stores, and processed grain in mills, is at risk of infestation by insect and mite pests. At present the control of these pests relies heavily on the use of organophosphate (OP) pesticides, supplemented by the fumigant methyl bromide in flour mills. However, the safety of OP pesticides is under review and the use of methyl bromide is being phased out because it is an ozone depleting material. There is, therefore, a need to find alternative control measures.

Some fungi specific to insects have been shown to control some storage pests under tropical conditions and the characteristics of such entomopathogenic fungi could be well suited for use in cooler, maritime climates such as that of the UK. Accordingly, in 1999 the UK Government and Industry funded a four-year research project, which aims to:

- Identify the most effective naturally occurring fungi for the control of UK storage pests.
- Examine their use either alone or as part of an integrated pest management strategy.
- Develop an effective commercial product for use on farms and in mills.

To date, a number of fungal strains have been tested in a primary screen against one storage species each of a beetle, a psocid, a moth and a mite. The fungi have derived from either the existing culture collection at CABI Bioscience or from field material collected in UK grain stores during 2000/01.

Materials and method

Fungi

Fungal isolates of *Beauveria bassiana, B. brongniartii* and *Metarhizium anisopliae*, were prepared by CABI Bioscience from their existing culture collection and one isolate was prepared from a field sample collected in 2001. In total, eight isolates all derived from the UK were tested against three insect species and seven against one mite species. The origins of the eight isolates used are shown in Table 1.

Table 1. Origins of fungal isolates used in primary screen.

	Isolate reference number	Origin
Beauveria bassiana	144	*Sitophilus spp*, Leicester, UK. 2001
	061345	Insect, UK. A.H.S. Brown 1955
	173199	Nasal swab from horse, UK. M. Archer 1973
	173201	Nasal swab from horse, UK. M. Archer 1973
	358840	*Otiorrhynchus sulcatus*, UK. A. Hodges 1973
Beauveria brongniartii	223216	Oats, UK. R. Friman 1978
	303228	Spawned mushroom compost, UK. C. Ottway 1986
Metarhizium anisopliae	177416	*Pemphigus trehernei* on *Aster trifolium* roots, UK. W.A. Foster 1975

The primary screen was carried out according to the methods below:

Insects

The insect species used were *Oryzaephilus surinamensis* (Strain - Tram 9213), *Ephestia kuehniella* (Strain - Lab), and *Lepinotus patruelis* (Strain - GB). Four replicates each of either 15 beetle adults, 15 psocid adults or 15 moth larvae were used for each treatment, together with 4 control replicates. For each treatment, insects were tipped carefully on to the fungal isolate growing on an agar plate. Individual insects were gently rolled over using soft forceps or a fine paintbrush to ensure fungal conidia adhered to all parts of the insect exoskeleton. Control insects were treated in the same way but on plates containing agar without fungus. The insects were then transferred to 9 cm diameter petri dishes containing filter papers moistened with 750 µl sterile distilled water, sealed with 'Parafilm' and kept at 20°C, 70% r.h. After 24 hours the insects were transferred to 120 ml wide necked glass jars containing approx. a 10 mm depth of their culture food (wheatfeed and dried yeast powder mixed with either rolled oats for beetles, glycerol for moths, or skimmed milk powder and wholemeal flour for psocids). Mortality was assessed every few days for 10 days after treatment or until

all the insects had died. Dead insects were removed at each assessment period and surface sterilised by washing in 5% sodium hypochlorite for 5 seconds followed by three rinses in sterile distilled water. The cadavers were then placed in petri dishes on filter papers moistened with sterile distilled water; after 5 days at 20°C, 70% r.h. they were examined for external sporulation of fungus to confirm that death was most likely due to the fungal isolate.

Mites

The mite species used was *Acarus siro* (Strain - 9258/2). To reduce the amount of handling required for the smaller and more delicate mites, freeze-dried fungal spores were mixed with 0.5 ml of sterile distilled water. Then, 50 µl of the suspension was applied to a 4.25 cm diameter Whatman No. 1 filter paper previously moulded in a metal 'former' to produce a shallow depression measuring 33 mm diameter and 2 mm high as described in Thind and Muggleton (1998) (Figure 1). After drying, the treated filter papers were used to prepare mite test cells as described for recovery cells in Thind and Muggleton (1998). A small amount (approx. 3 mg) of mite culture food (wheatgerm and dried yeast powder) was placed in each cell, together with 20 mites. Five replicates were prepared for each treatment, together with five untreated control replicates. The two halves of each cell were clipped together with 'bulldog' clips, and left in a desiccator over water at 20°C. Mortality was assessed every few days for 10 days after treatment or until all the mites were dead. Dead mites were not removed from the cell.

Results and discussion

The mean cumulative percentage mortalities at 7 and 10 days with each of the fungal isolates for *O. surinamensis*, *E. kuehniella*, *L. patruelis* and *A. siro* are shown in Tables 2-5, respectively.

Table 2. *Oryzaephilus surinamensis*
Mean cumulative percentage mortality ± SE at day 7 and day 10 (N=4) (Control responses for all tests are shown in parentheses in each table)

Isolate	Cumulative mean % mortality ± SE day 7	Cumulative mean % mortality ± SE day 10
Beauveria bassiana		
144	81.3 ± 5.0 (1.7 ± 1.7)	91.4 ± 1.9 (3.3 ± 3.3)
061345	0 ± 0 (1.7 ± 1.7)	0 ± 0 (1.7 ± 1.7)
173199	75 ± 9.6 (3 ± 3)	77 ± 8.8 (3 ± 3)
173201	100 ± 0 (1.7 ± 1.7)	100 ± 0 (1.7 ± 1.7)
358840	87 ± 2.7 (3 ± 3)	88.5 ± 1.5 (3 ± 3)
B. brongniartii		
223216	5 ± 1.7 (1.7 ± 1.7)	5 ± 1.7 (1.7 ± 1.7)
303228	5.5 ± 1.8 (10 ± 4.3)	5.5 ± 1.8 (10 ± 4.3)
Metarhizium anisopliae		
177416	2 ± 2 (3 ± 3)	2 ± 2 (3 ± 3)

Table 3. *Ephestia kuehniella*
Mean cumulative percentage mortality ± SE at day 7 and day 10 (N=4)

Isolate	Cumulative mean % mortality ± SE day 7	Cumulative mean % mortality ± SE day 10
Beauveria bassiana		
144	72.4 ± 9.6 (1.8 ± 1.8)	84.8 ± 11 (10.7 ± 4.6)
061345	16.2 ± 9.3 (5.5 ± 3.5)	25 ± 6.8 (10.9 ± 4.7)
173199	68 ± 10.9 (8.5 ± 4.2)	97 ± 3.2 (18.5 ± 6.4)
173201	100 ± 0 (5.5 ± 3.5)	100 ± 0 (10.9 ± 4.7)
358840	91.5 ± 6.4 (8.5 ± 4.2)	100 ± 0 18.5 ± 6.4)
B. brongniartii		
223216	10.2 ± 4.4 (5.5 ± 3.5)	10.2 ± 4.4 (10.9 ± 4.4)
303228	7 ± 4.7 (3 ± 3)	10.5 ± 6.5 (8.5 ± 5.1)
Metarhizium anisopliae		
177416	9 ± 1.4 (8.5 ± 1.4)	20.5 ± 2.5 (18.5 ± 6.4)

Table 4. *Lepinotus patruelis*
Mean cumulative percentage mortality ± SE at day 7 and day 10 (N=4)

Isolate	Cumulative mean % mortality ± SE day 7	Cumulative mean % mortality ± SE day 10
Beauveria bassiana		
144	100 ± 0 (29.1 ± 4.9)	100 ± 0 (30.8 ± 4.1)
061345	61.1 ± 6.4 (30.7 ± 10.4)	63.2 ± 8.0 (34.0 ± 8.9
173199	100 ± 0 (20 ± 10.6)	100 ± 0 (25 ± 7.0)
173201	100 ± 0 (30.7 ± 10.4)	100 ± 0 (34.0 ± 8.9)
358840	100 ± 0 (20 ± 10.6)	100 ± 0 (25 ± 7.0)
B. brongniartii		
223216	36.6 ± 4.8 (30.7 ± 10.4)	38.4 ± 5.0 (34.0 ± 8.9)
303228	38.8 ± 10.3* (44 ± 4.0)	44 ± 9.8 (49 ± 2.1)
Metarhizium anisopliae		
177416	60 ± 39.7 (20 ± 10.6)	62 ± 18.3 (25 ± 7.0)

* Mean cumulative percentage mortality ± SE at day 6

No fungal growth was observed on dead insects from control treatments. The majority of beetles, psocids and moth larvae from treated plates showed signs of fungal growth five days after surface disinfection. In some cases this growth was visible within 48 hours.

The strains of *Beauveria bassiana* outperformed the other entomopathogens and the newly collected strain 144 showed promise.

From these results it would appear that UK isolates 061345, 177416, 223216 and 303228 should not be taken forward to a secondary screen due to the low mortality observed compared to other isolates. The remaining UK isolates will be taken forward to the secondary screen having shown promising results against all four pest species tested to date.

Table 5. *Acarus siro*
Mean cumulative percentage mortality ± SE at day 7 and day 10 (N=5)

Isolate	Cumulative mean % mortality ± SE day 7	Cumulative mean % mortality ± SE day 10
Beauveria bassiana		
144	RESULTS NOT AVAILABLE	RESULTS NOT AVAILABLE
061345	15 ± 7.1 (6 ± 1.9)	44 ± 10.7 (9 ± 2.9)
173199	5 ± 2.7 (5 ± 2.2)	33 ± 3.7 (6 ± 2.9)
173201	50 ± 7.9 (6 ± 1.9)	100 ± 0 (9 ± 2.9)
358840	1 ± 1 (5 ± 2.2)	18 ± 4.1 (6 ± 2.9)
B. brongniartii		
223216	13 ± 3.0 (6 ± 1.9)	24 ± 4.8 (9 ± 2.9)
303228	21 ± 4.3 (5 ± 2.7)	28 ± 6.4 (8 ± 4.1)
Metarhizium anisopliae		
177416	1 ± 1 (5 ± 2.2)	6 ± 2.9 (6 ± 2.9)

Next steps

- Produce full dose response data
- Extend the test species to include a number of other SP pests
- Decide on question of storage v. non storage pathogens
  (From UK stores or CABI collection - still UK)
- Initiate cycling studies
- Study insect/fungal ecology in stores

Reference

Thind, B.B. & Muggleton, J. 1998: A new bioassay method for the detection of resistance to pesticides in the stored product mite *Acarus siro* (Acari: Acaridae). Exp. Appl. Acarol. 22: 543-552.

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IOBC Bulletin Vol. 25 (3) 2002
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Integration of chemical control of cockroaches and biological control of stored-product moths

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Abstract: In many situations, stored-product and urban pests occur simultaneously. Cockroaches like the German cockroach *Blatella germanica* or the Oriental cockroach *Blatta orientalis* are common pests in bakeries, food processing facilities and kitchens. As these cockroaches are potential vectors of diseases, they have to be controlled completely. Traditionally, cockroaches are controlled by spraying contact insecticides. Modern approaches include microencapsulated insecticides formulated within a food-bait. For the biological control of stored-product moths the egg-parasitoid *Trichogramma evanescens* is applied commercially in several countries in Europe. *T. evanescens* is known to be very susceptible to contact insecticides, and no strategies of integration of chemical and biological have been described so far for stored-product protection. Therefore, possible side effects of microencapsulated insecticides on *T. evanescens* have been tested. In Germany, three compounds have been shown to be effective against the German cockroach, Fenitrothione, Hydramethylnone and Fibronil. For testing side effects of these insecticides in the laboratory, the guidelines of the IOBC were applied. However, as these guidelines are designed for sprayed insecticides, a suitable modification for the microencapsulated insecticides had to be found. All three insecticides were found to have no side effects on *T. evanescens*, i.e. parasitism and emergence of progeny was not affected. Therefore, the release of *T. evanescens* and the application of the three mentioned microencapsulated insecticides is a promising strategy to integrate chemical control of cockroaches and biological control of stored-product moths.

Key words: stored product protection, urban pests, *Blatella germanica*, *Trichogramma evanescens*, Integrated Pest Management

Introduction

Egg-parasitoids of the genus *Trichogramma* spp. are the most widely applied beneficial insects against pest Lepidoptera. Side effects of chemical pesticides on *Trichogramma* spp. have been extensively studied (Hassan et al., 1983). The integration of pesticides and the inundative release of *Trichogramma* spp. are necessary, if non-host pest species of economic importance are present simultaneously with the lepidopteran target-pest. For the biological control of stored-product moths the egg-parasitoid *Trichogramma evanescens* is applied commercially in several countries in Europe. Stored-product moths may occur together with other stored-product insect and mite pests as well as urban pests. Urban pests such as cockroaches like the German cockroach *Blatella germanica* or the Oriental cockroach *Blatta orientalis* are frequently encountered in bakeries, food processing facilities and kitchens. As these cockroaches are potential vectors of diseases, they have to be controlled completely. In most cases, control will depend on synthetic chemical insecticides.

Traditionally, cockroaches are controlled by spraying contact insecticides. Modern approaches include microencapsulated insecticides formulated within a food-bait.

Trichogramma spp. are known to be very susceptible to contact insecticides **(**Hassan, 1989). Therefore, possible side effects of microencapsulated insecticides on *T. evanescens* have been tested.

The original IOBC/WPRS bioassay system for the laboratory-based evaluation of pesticides against a standardised group of natural enemies classified compounds as follows: <50% response (% mortality) =„harmless“, 50-79% response=„slightly harmful“, 80-99% response=„moderately harmful“ and >99% response=„harmful“ (Hassan et al., 1985). Subsequent guidelines for the IOBC/WPRS test methods have restricted the „harmless“ category to <30% response, with a median category referred to as the 30-80% response (Hassan, 1992). A „worst case“ sequential (tiered) testing scheme (laboratory: semi-field: field) was recommended. In such schemes, pesticides found to be „harmless“ to a particular natural enemy in the laboratory test are assumed to be harmless to the same organism in the field and further testing (semi-field or field) is not recommended (Hassan, 1989). As the influence of pesticides on the population dynamics of pest-natural enemy interactions is an understudied area and a theoretical basis for an arbitrary lower limit of 50% response for „harmful“ compounds is lacking, a more discriminating five-point rating system was proposed, with the classes 1=0%, 2=<10%, 3=10-30%, 4=31-90% reduction (Theiling & Croft, 1988). These classifications were considered in this study.

Materials and methods

The sequential testing scheme for evaluating side effects of plant protection products on *Trichogramma cacoeciae* (Hassan, 1992) was followed. The normal test procedure was not adequately applicable because it was not possible to spray the products. Three gels were tested: Schwabex gel, fenitrothion microencapsulated (0.8%); Maxforce Schaben-Gel, hydramethylnone (2.0%), and Goliath Schaben-Gel, fipronil (0.05%). These three active ingredients are the only three officially tested in Germany to be effective. The test procedure was adapted according to available information to perform the residual toxicity test. Petri dishes (diameter 9 cm) were provided with 2 drops of gel (0.5g per drop) and an egg-strip of the host *Ephestia kuehniella* Zeller. The gel was covered with fine plastic gauze, mesh wide 0.14 mm. The egg strip was prepared by gluing fresh eggs with „Traganth“ (see appendix 4 in Hassan, 1992) in circular pieces on a strip of thin paper. Ten females of *T. evanescens* were released into Petri dishes covered with plastic strips. The air in the Petri dishes was not exchanged. An untreated control consisted of Petri dishes without gel. The experimental conditions in the climatic chamber were 26°C and 75 % r.h.

The experiment continued for seven days. The number of host eggs parasitised during the course of the experiment was counted not earlier than 9 days after the end of the experiment. At this time, parasitised eggs have turned black and are easily recognized. After the emergence of the *Ephestia* larvae, unparasitized eggs remain yellowish. For each test product, the mean reduction in parasitism was calculated as a percentage of the number of black eggs compared to the number of black eggs in the untreated control. Every trial had three replications.

Moreover, one insecticide commonly used in the organic trade in Germany to control cockroaches was tested, Pistal (pyrethrum + essential oils). This test was conducted according to the standard-test using *Trichogramma cacoeciae* as test organism (Hassan, 1992).

Results

No significant reduction in the number of black eggs compared to the control was found for the tested products ($p>0.05$, Mann-Whitney U-Wilcoxon Rank Sum W Test). The reduction in parasitism of fenitrothion, hydromethylnon and fibronil was 4.41, 10.44 and 6.96 %, respectively (Tab. 1).

Tab. 1: Number of black eggs of *Ephestia kuehniella* per female *Trichogramma evanescens*. Host eggs unlimited, 10 female *T. evanescens* per trial.

Trial no.	Untreated control	Fenitrothion	Hydromethylnon	Fibronil
1	14.3	10.9	15.2	13.9
2	8.3	20.0	9.3	11.8
3	20.5	10.3	14.1	14.4
total	43.1	41.2	38.6	40.1
% black eggs	100	95,59	89,56	93,04
% reduction	0	4.41	10.44	6.96

The rating of these figures is given in Tab. 2. All products were in the IOBC-class 1=„harmless“. According to the five-point rating system, hydromethylnone was found to be more harmful (class 3) than the other two products (class 2).

The initial toxicity for Pistal was rated „4“=harmful (>99%), the persistence was „2“=slightly harmful.

As the effect was below threshold of 30%, no product passed on the next test in the scheme.

Tab. 2: Rating of side effects of three products for cockroach control on *Trichogramma evanescens*.

product active ingredient	Schwabex Gel fenitrothion	Maxforce Schaben-Gel hydromethylnone	Goliath Schaben-Gel fibronil
% reduction	4.41	10.44	6.96
IOBC class	1=„harmless“	1=„harmless“	1=„harmless“
five-point rating system	2	3	2

Discussion

All three gels were found to be „harmless“ according to the IOBC/WPRS classification. The five-point rating system reflected the low reduction in parasitism. However, we consider the bioavailability of the insecticides to be very low. The parasitoids would have to visit the drops of the gel to get exposed. Beside the low reduction in parasitism, sub-lethal impact of the gels may occur. Important areas of sub-lethal pesticide impact on natural enemies are locomotion, consumption, searching, oviposition, handling time, repellency, development time, deformation, longevity, and reproduction, i. e. fecundity, fertility, and sex ratio (Wrigth &

Verkerk, 1995). As the influence of pesticides on natural enemies of stored-product and urban pests is an understudied area, this field needs further studies, too.

One of the active ingredients studied here was tested previously in a liquid preparation, namely fenitrothion 0.1%, trade name Folithion. Initial toxicity was „4“=harmful (>99%) as well as the persistence, „4“=persistent (Hassan, 1994). Consequently, the lower initial toxicity found in this study is due to the preparation as a gel.

Regarding testing the side effects of pesticides on natural enemies, the EU Council Directive 91/414/EEC (15.July 1991) concerning the placing of plant protection products is relevant (Hassan & Forster, 1995). The test guidelines of the IOBC were adapted. Interestingly, the stored product environment is mentioned in Annex II: „Testing is required for all except where exposure does not occur (e.g. food storage in enclosed spaces, wound sealing and healing treatments)“. Obviously, natural enemies of stored-product and urban pests have been forgotten in the directive.

Information on side effects of active ingredients registered for stored-product protection on *Trichogramma* sp. were found for two insecticides only: Actellic 50 (primiphos-methyl), concentration tested 0.2%, and pyrethrum + piperonyl-butoxide, 0.1%. The preparation of the latter was Spruzid-Nova-liquid, however, the active ingredients are used for many insecticides against stored-product and urban pests. Both active ingredients had an initial toxicity rated „4“=harmful (>99%). Persistence was „2“=slightly harmful for pyrethrum + piperonyl-butoxide and „4“=harmful for primiphos-methyl (Hassan et al., 1983). Little additional information was found in the literature. Brower (1984) found *Trichogramma* spp. in peanut-warehouses, which were treated with malathion and phosphine. Eggs of *Corcyra cephalonica* Stainton sprayed with malathion (0.1%) were extremely toxic for *Trichogramma confusum* Viggiani and *T. japonicum* Ashmead (Navarajan et al., 1976). Compared to the untreated control, only 20% of adult *T. japonicum* emerged from host eggs treated wuth DDVP (Dichlorvos), and these surviving adults lived for less than 2 h and were infertile. The LD_{50} of DDVP was 532.5 ppm against larvae and 21.9 ppm against pupae.

Residue-building insecticides for cockroach control are expected to be harmful to beneficial insects, and this seems to be true for preparations intended for spraying, e. g., permethrin 0.02% was rated to be „4“=harmful (>99%) (Hassan et al., 1983). However, no studies with insecticides for urban pest control were conducted so far. The side effects of the product Pistal (pyrethrum + essential oils) was comparable with pyrethrum + the synthetic piperonyl-butoxide. The harmful initial toxicity was expected, but the persistence was not. The persistence may be due to the absence of UV-radiation under the experimental conditions. However, in the urban environment, UV-radiation is frequently absent, too. The present study is a case for which synthetic insecticides have fewer side effects compared to natural products due to their preparation.

Acknowledgements

We would like to thank Dr. Sherif A. Hassan for the possibility to work in his laboratory, and for valuable comments.

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PEST BIOLOGY, FAUNISTICS, STORAGE TECHNOLOGY AND LOSSES

Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
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Stored insect pests in traditional cultivated hulled wheat crop areas of Central-Southern Italy with emphasis on *Sitotroga cerealella* (Olivier)

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Abstract: Results of studies carried out on stored insect pests of hulled wheat (*Triticum dicoccum* Schübl., *T. monococcum* L. and *T. spelta* L.) in traditional crop areas of Molise and Basilicata regions (Central-Southern Italy) were reported. Surveys were carried out from 1993 to 2001 in about 50 warehouses of small farms and food factories. Stored hulled wheat revealed infestations of about 20 insect pests. *Trogium pulsatorium* L. (Psocoptera); *Ephestia elutella* (Hb.), *Plodia interpunctella* (Hb.), *Sitotroga cerealella* (Olivier) (Lepidoptera); *Cryptolestes ferrugineus* (Stph.), *Oryzaephilus surinamensis* (L.), *Rhyzopertha dominica* (F.), *Sitophilus granarius* (L.), *S. oryzae* (L.) and *Tribolium castaneum* (Herbst) (Coleoptera) were more frequently found. Among these species the ecology of *S. cerealella* has been studied. The angoumois grain moth infestations can occur during both preharvest and postharvest storage. Using pheromone traps (delta types) baited with 1 mg of Z7E11-16Ac, observations on adult flights in fields of hulled wheat were carried out. The results obtained showed various levels of infestation, with different population abundance, according to the considered areas. In two fields located in Molise region, *S. cerealella* developed two generations before harvesting; the presence of adult males was recorded also after harvesting, until the beginning of September. Moths' activity suggests adult dispersal from the warehouses to hulled wheat fields.

Key words: Stored insects, *Sitotroga cerealella*, traditional warehouses, Central-Southern Italy.

Introduction

The hulled wheats *Triticum dicoccum* Schübl., *T. monococcum* L. and *T. spelta* L. (emmer, einkorn and spelt), some of the earliest domesticated plants, are at present cultivated in marginal areas of several European countries, including Italy, former Yugoslavia, Spain and Turkey. In recent years, increasing interest has been given to these cereals because of their good potential as underutilized crops that would provide additional income to farmers while contributing to agricultural diversification (Padulosi *et al.*, 1996).

Nowadays, all three species of hulled wheat are grown in Italy, both as animal and human food. The main areas of emmer, einkorn and spelt cultivation are in the Central and Southern part of the country: Garfagnana and Maremma areas in Tuscany region, some valleys of Umbria and Northern Latium regions, the internal territory of Molise region, the Daunian Apennine in Campania and Apulia regions and some hilly and mountainous areas in Basilicata region (Perrino *et al.*, 1996).

In Molise and Basilicata regions, the cultivation of *T. dicoccum*, *T. monococcum* and *T. spelta* landraces occupies niches in peculiar traditional farming systems, with limited resources, usually located in mountain areas, where poor environmental conditions represent a serious constraint for the cultivation of bread and durum wheats (Mariani, 1994).

Hulled wheat species are grown under low-input agriculture, in integrated or ecologically oriented systems (Cicia and D'Ercole, 1995). After harvesting and threshing, the spikelets are commonly stored in traditional storage facilities, generally without particular control

measures against stored product pests. When emmer, einkorn and spelt are used as fodder for animals, the cereals remain in these warehouses for the whole storage period. These granaries often show precarious hygienic conditions. In the case that hulled wheat is destined for human consumption. After few days the harvest is usually taken to industrial warehouses of some food factories, where it will be dehusked and processed (Trematerra *et al.*, 1996).

Most studies of stored product insects have focused on commercial granaries but relatively few have been made on occurrence in hulled wheat warehouses and fields. As in the case of the other cereals, these pests are of considerable economic importance because product quality may be reduced by their presence and activities (Trematerra *et al.*, 1996; Trematerra and Sciarretta, 1998).

This paper presents the results of studies carried out on stored insect pests of *T. dicoccum*, *T. monococcum* and *T. spelta* in traditional crop areas of Molise and Basilicata regions (Central-Southern Italy). Among these insects, the ecology of *Sitotroga cerealella* (Olivier) has been studied. Moth infestations can occur during both preharvest and postharvest storage. As reported by Candura (1950), in Central-Southern Italy *S.cerealella* has four to five generations from March to November: in the field, the moth can develop two generations attacking whole grains, in the warehouses there are usually three generations.

Materials and methods

Surveys were carried out from 1993 to 2001 in about 50 warehouses of small farms and food factories. During different periods of the year, sufficient size samples of stored cereal were taken and examined in the laboratory. There, they were sieved and adult insects were recorded. To determine the presence of immature stages, samples were placed in jars for incubation at 28±1°C temperature and 70% relative humidity (r.h.).

During 2000 and 2001, the observations on *S.cerealella* flight were carried out on 1-2 ha hulled wheat fields situated 300-800 m above sea level, in hilly and mountainous areas of Molise and Basilicata regions.

Pheromone traps for monitoring of the angoumois grain moth adult males consisted of sticky traps (delta type) baited with 1 mg of synthetic sex-attractant Z7E11-16Ac (Novapher, Italy). About 2 traps per hectare, suspended 1.3-1.5 meters above ground, were placed. The adults trapped were counted weekly, while pheromone dispensers and sticky boards were replaced by new ones at intervals of 6 weeks and 2-4 weeks, respectively.

In 2000, surveys were carried out from the beginning of May until the beginning of September in four experimental fields: two in Molise region (Molise A and Molise B), and two in Basilicata region (Basilicata A and Basilicata B). In 2001, surveys were carried out from the second half of April until the beginning of September, in five fields: two in Molise region (Molise C and Molise D), and three in Basilicata region (Basilicata A, Basilicata C and Basilicata D).

In Molise fields, emmer, einkorn and spelt were harvested in the second half of July 2000 and 2001, while in Basilicata fields the harvesting was made in the second half of June 2000 and 2001 (Figures 1-2).

Results

The results of the surveys carried out on insects found in traditional and industrial hulled wheat warehouses are reported in Table 1. The fauna list obtained shows that a total number of about 30 species, belonging to 6 orders, were collected in Molise and Basilicata regions.

Table 1. Insect species found in hulled wheat warehouses in Molise and Basilicata regions

Order	Species	Molise region		Basilicata region	
		traditional warehouse	industrial warehouse	traditional warehouse	industrial warehouse
Orthoptera	*Calliptamus barbarus* Costa	●	●		
	Orthoptera spp.			●	
Psocoptera	*Trogium pulsatorium* L.	●	●		
	Psocoptera spp.	●		●	●
Rhynchota	*Aelia rostrata* Boh.	●			
	Carpocoris pudicus Poda		●		
	Eurygaster austriaca (Schrk.)	●	●		
	Lyctocoris campestris (F.)	●	●		
	Lygaeus equestris (L.)		●		
Lepidoptera	*Ephestia cautella* (Walk.)		●		
	Ephestia elutella (Hb.)	●	●	●	
	Plodia interpunctella (Hb.)	●	●	●	●
	Sitotroga cerealella (Olivier)	●	●	●	●
Coleoptera	*Ahasverus advena* (Waltl)	●			
	Carpophilus hemipterus (L.)	●			
	Crisomelidae spp.			●	
	Cryptolestes ferrugineus (Stph.)	●	●		
	Lasioderma serricorne (F.)	●			
	Oryzaephilus surinamensis (L.)	●	●	●	●
	Rhyzopertha dominica (F.)	●		●	
	Sitophilus granarius (L.)	●		●	●
	Sitophilus oryzae (L.)	●	●	●	●
	Stethorus punctillum Weise	●	●		
	Tribolium castaneum (Herbst)	●	●	●	
	Tribolium confusum J. du Val	●			
	Trogoderma granarium Everts			●	
	Typhaea stercorea (L.)	●			
Hymenoptera	*Messor barbarus* L.	●			
	Chalcididae spp.	●			
	Braconidae spp.	●			
	Formicidae spp.				●

The species collected in great part are phytophagous and they are primary and secondary pests, typically associated with stored products. Some insect pests develop and feed inside kernels: *S. cerealella*, *Rhyzopertha dominica* (F.), *Sitophilus granarius* (L.) and *S. oryzae* (L.), consuming the entire seed. Many of the collected species are detritus feeders and they damage broken, cracked, or processed grains: *Ephestia cautella* (Walk.), *Ephestia elutella* (Hb.), *Plodia interpunctella* (Hb.), *Carpophilus hemipterus* (L.), *Cryptolestes ferrugineus* (Stph.), *Lasioderma serricorne* (F.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *Tribolium confusum* J. du Val and *Trogoderma granarium* Everts. Some insects also feed on mold mycelium: *Trogium pulsatorium* L. and Psocoptera species, *Ahasverus advena* (Waltl) and *Typhaea stercorea* (L.). Moreover, other insects collected are predators or parasitoids of phytophagous pests: *Lyctocoris campestris* (F.), *Stethorus punctillum* Weise, *Messor barbarus* L., Calcididae spp. and Braconidae spp. Finally, some species develop and damage cereals exclusively in field: *Calliptamus barbarus* Costa, *Aelia rostrata* Boh., *Carpocoris pudicus* Poda, *Eurygaster austriaca* (Schrk.) and *Lygaeus equestris* (L.).

The results of observations on *S. cerealella* adult flights in hulled wheat fields during 2000 are reported in Figure 1. Captures of males were different according to field locations. In Molise A field, adult males were collected from mid June to the end of July, also after harvesting. The highest numbers of moths were recorded in Molise B field, where *S. cerealella* developed two generations before the grain has been harvested. Captures occurred from the first half of June to the end of July, with flight peaks mid June and in the second half of July. Some adult males were trapped also after harvesting, until the beginning of September. In experimental fields located in Basilicata region the angoumois grain moths trapped were sporadic; they were recorded in the second half of June, before the harvesting of the cereal.

During 2001 (Figure 2), in Molise C field the moths were collected from the beginning of June to the second half of July, before harvesting. The highest numbers of *S. cerealella* males were trapped in Molise D field, with two generations before the grain has been harvested. Males were found from the beginning of June to the end of July. However, some adults were recorded after harvesting, until the end of August. Also during this year, in fields located in Basilicata region moths were sporadic and they were collected before harvesting.

Discussion

The fauna list of insects found in stored emmer, einkorn and spelt revealed that a greater number of species was found in traditional warehouses rather than industrial warehouses. In Molise and Basilicata regions, traditional granaries are generally of poor construction, and farmers are mostly unaware of safe storage practices, such as warehouse preparation for reception of new product, the removal of old product, regular checks of the sanitary state of the warehouse, product control during the storage season and quick intervention with appropriate measures if pests emerge. Consequently these storage facilities are frequent sources of stored pests.

Pest control in stored hulled wheat was most successful in industrial granaries, silos and large floor warehouses, and it is usually based on carrying out both preventive and curative measures, in integrated stored product protection systems. However, in some industrial warehouses the surveys carried out had led to discovery of numerous species of harmful insects that indicates bad storage conditions.

The field occurrence of *S. cerealella* showed various levels of infestation, with different population abundance, according to the considered areas. The highest numbers of angoumois grain moths were trapped in Molise B and Molise D fields, with two generations before the

grain has been harvested. In these locations, the presence of the moth was recorded also after harvesting. These fields are in the same area of Molise region, where there are many traditional warehouses, in which different cereal species, generally used as animal food, are stored. The other fields are near industrial silos or in crop areas where there are no storage facilities.

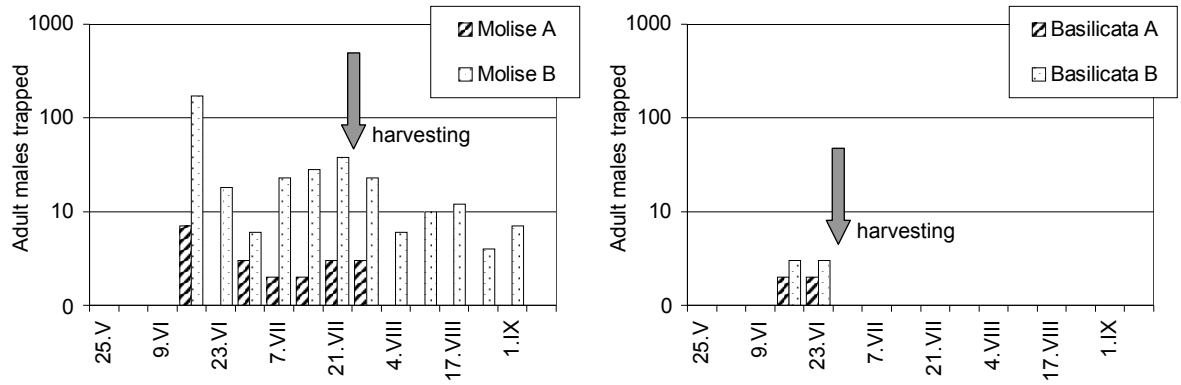

Figure 1. Captures of *S.cerealella* males (log_{10}) in four crop fields during 2000.

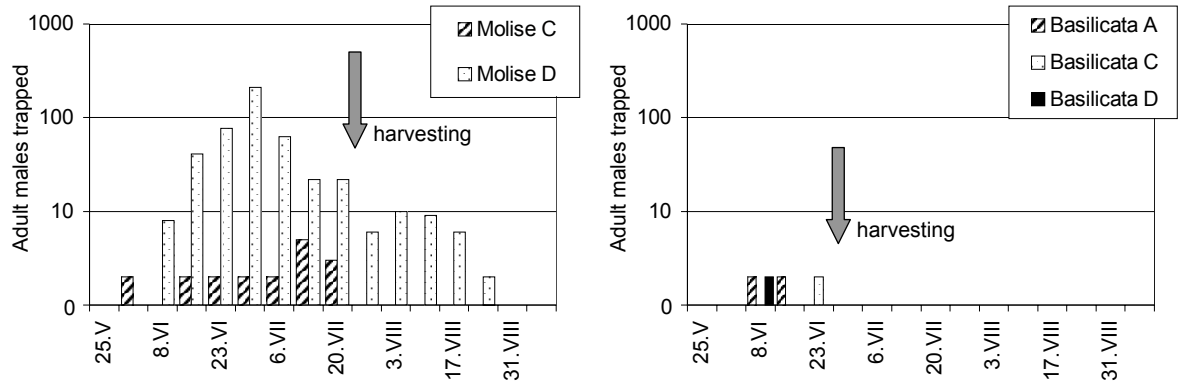

Figure 2. Captures of *S.cerealella* males (log_{10}) in five crop fields during 2001.

Studies carried out to monitor activity and movement of *S. cerealella* in storage and field situations, revealed adult dispersal from the warehouses to cereal plots (Cogburn and Vick, 1981; Vick *et al.*, 1987; Barney and Weston, 1996). Our results suggest that also in hulled wheat crop areas the outdoor activity of the angoumois grain moth is influenced by migration from stored cereals.

Knowledge of the spatial dynamics of *S. cerealella* may contribute to pest management programmes.

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IOBC Bulletin Vol. 25 (3) 2002
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Corcyra cephalonica (Stainton) - an overlooked pest?

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Abstract: One part of the investigation focussed on the development of *Corcyra cephalonica* on cocoa beans and its limitating factors. Four different factors were studied: 2 and 10 first instar larvae were placed on cocoa beans taken from two different lots (from Ivory Coast and from Papua New Guinea), with damaged and with intact testae, conditioned and held at 50 ± 5 % and 87 ± 5 % r.h., at 30 ± 1°C. Infestation rate and number of imagoes hatched per bean were influenced mainly by the condition of the testae and the moisture content. The first instar larvae were hardly capable of infesting infest raw cocoa with intact testae. Testae the damp beans could be attacked more easily. The developmental time depended mainly on the cocoa qualities, ranging from 31 d on damp cocoa beans from Ivory Coast to 144 d on dry beans from Papua New Guinea. Best results of - larval development were reached on cocoa beans from Ivory Coast with damaged testae and by two inserted first instar larvae: 50 % completed development at 87±5% r.h. with a mean of 35.5 d, 80 % at 50 ± 5 % r.h. with a mean of 48.2 d. The cocoa quality did not seem to n the oviposition rate. The mated females laid an average of 231.3 (± 81) eggs, with a maximum of 431. In further experiments it was observed that later instar larvae could easily spread even on dry cocoa beans with intact testae. In a two-choice arena, the females did not prefer damp to dry cocoa beans for oviposition. They strongly preferred crevices for oviposition and avoided laying eggs on smooth surfaces. The chemical stimuli of the cocoa beans seemed to be less important, as a similar oviposition response could be elicited by gauze. There are two aspects of the exhibited behaviour contributing to inconspicuous development of infestations: solitary larvae did not migrate before pupation and females ceased flight activity soon after mating.

Keywords: cocoa beans, *Corcyra cephalonica*, oviposition, pest status, rice moth

Introduction

The rice moth *Corcyra cephalonica* is a serious pest of stored products like cottonseeds, nuts, raw cocoa, rice and maize in areas of tropical climate. After entering West Africa, probably by the import of rice from the Far East in the 1960´s, it is spreading to other important food commodities in the West African sub region (Allotey and Kumar 1985; Allotey 1991). Associated with this emerging problem in the exportating regions is the increasing number of interceptions of *C. cephalonica* on the imports to other climate regions. C. *cephalonica* is second only to *E. cautella´*s (Walker) frequently found on imports to the Port of Hamburg: 1,660 interceptions of *E. cautella* and 851 of *C. cephalonica* were reported during the period 1991-2000. Most of the records by far were on cocoa beans. Howe (1965) stated its maximum rate of increase as tenfold per month without giving information about the rearing conditions. This is surprisingly low, considering the major pest status of this species. There is a lack of information on the distribution of the rice moth after the infested commodities are imported, as the common "moth-traps" are designed for trapping phycitin moth species and no appropriate traps are available for effectively attracting *C. cephalonica*.

The object of this investigation was to provide more information about the life history exhibited by *C. cephalonica* on raw cocoa and the spreading of its infestations.

Material and methods

Insect rearing
The laboratory strain of *C. cephalonica* originated from individuals collected in the warehouses of the Port of Hamburg in the previous two years. The culture was held in 3 L jars, half-filled with raw cocoa, at 21 ± 1°C and 55 ± 5 % r.h. under natural light conditions.

Development of larvae on cocoa beans of different qualities
2 or 10 first instar larvae, respectively, hatched on the same day, were transferred on one singular cocoa bean in a 30 ml glass. Then the glass was closed by a piece of gauze. One out of 4 experimental factors was alternated in each of 16 series: 2 and 10 first instar larvae were set on cocoa beans taken from two different lots (from Ivory Coast and from Papua New Guinea), with damaged and with intact testae, conditioned and held at 50 ± 5 % and 87 ± 5 % r.h., at 30 ± 1°C.

Each series consisted of ten replicates. The cocoa beans studied in the series at 87 ± 5 % r.h. were preconditioned for 2 days. All beans from the Ivory Coast (IC) and all beans from Papua New Guinea (PNG) were taken from the same lot. Both qualities differed in several attributes, such as the lower acidity and the more equilibrated moisture absorption characteristics of the IC cocoa.

The newly hatched imagoes were transferred solitarily or in pairs into 50 ml vials supplied with a small stripe of black wide-meshed gauze and closed with a perforated plastic lid. The imagoes were held at same conditions as the previous larvae. Eggs were removed and counted every 2nd to 3rd day.

Dispersion of the larvae from damaged to intact cocoa beans
Ten first instar larvae, hatched on the same day, were placed on one damaged cocoa bean. This cocoa bean was transferred to a 50 ml glass vial along with 5 intact cocoa beans. The vial was closed with gauze and held at 30 ± 1°C and 50 ± 5 % r.h. Two series of 10 replicates were conducted; one with cocoa beans from the Ivory Coast, the other with beans from Papua New Guinea (taken from the same lots as in the series of the developmental experiment). Observations of adult emergence were made daily or at least every second day and adults were removed from the vials. When no further emergence was observed, the damage done on the cocoa beans was examined.

Oviposition preferences on cocoa beans of different moisture content
The two-choice arena consisted of a plastic petri dish (∅ 9 cm) with 8, resp. 4 in the small version, cocoa beans with intact testae, each of them fixed by a small nail from beneath of the bottom of the dish. Alternately dry and damp beans were arranged concentrically. All cocoa beans used in this experiment were taken from the same lot of Ivory Coast. Damp beans which had been conditioned for one day at 90 ± 5% r.h. and 21 ± 1°C.

One pair of newly emerged *C. cephalonica* was placed in a 1.5 L jar. The jar was placed in the arena with its opening facing down. The arena dish was changed daily in the early afternoon, and the number of eggs laid was counted. Eggs laid on the first day were collected and observed for hatch of larvae. Only oviposition of mated female was taken into account. When the amount of eggs laid went below 20 per day, the arena was changed to the small version with only 4 beans (2 dry and 2 damp). Two control series were conducted; one with an empty petri-dish instead of the arena, and one with an empty petri-dish, covered by black wide-meshed gauze in order to provide facilities for oviposition. Each series consisted of 10 replicates at 28.5 ± 1°C, 55 ± 10 % r.h.

Results

Development of larvae on cocoa beans of different qualities

The condition of the testae was the most important factor for determining the infestation in this experiment: None of the dry and intact beans from Ivory Coast showed signs of feeding. Only 1, resp. 2 of 10 dry PNG beans with intact testae was penetrated by the 2 or 10 inserted first instar larvae, respectively. It was still not evident that the first instar larvae surmounted the undamaged testae of the dry PNG beans, as the testae could have been cracked after insertion of the beans into the vials. The dry IC beans with damaged testae could easily be attacked by the first instar larvae: Nine out of 10 beans were infested after the placement of 2 first instar larvae, and all of the 10 beans after placement of 10 first instar larvae. The infestation rate of the dry PNG beans with damaged testae was lower than the corresponding series of IC beans, but reached the same amount in the series of damp PNG beans with damaged testae. Thus, the second major factor was the moisture content of the beans, as the humidity enabled the first instar larvae to overwhelm the intact testae and raised the infestation rate of the PNG beans with damaged testae. Lastly, not only the moisture content of the testae but also that of the endosperm was decisive for the infestation rate. The number of the inserted first instar larvae did not seem to have any major effect on the infestation rate.

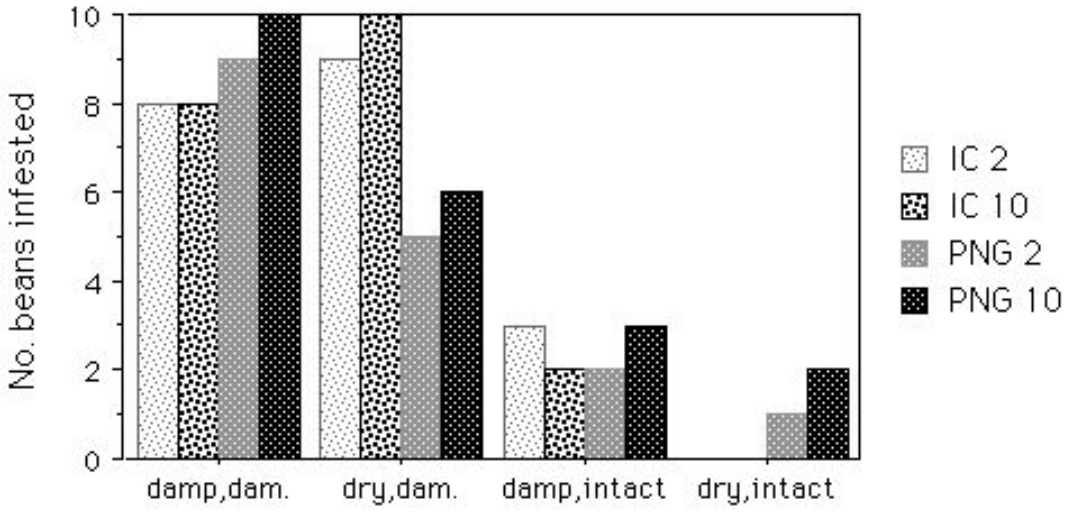

Fig. 1: Number of cocoa beans infested by first instar larvae of *C. cephalonica* (N=10)
"IC": cocoa lot from Ivory Coast, "PNG": cocoa lot from Papua New Guinea, "2" resp. "10": no. first instar larvae inserted, "damp": cocoa beans conditioned and held at 87 ± 5 % r.h., "dry": cocoa beans conditioned and held at 50 ± 5 % r.h., "dam.": damaged testae, "intact": intact testae

The rates of adult emergence per series were similar to the infestation rate of the beans (Fig. 2). More adults emerged in the series with damaged testae. But considering only the successfully infested beans and ignoring the un-infested ones, the mean number of adults emerging per bean did not differ between the corresponding series with damaged and with intact beans (figure not shown). Just like the infestation rate, the rate of imagoes hatched in the series with damaged testae was markedly increased by moisture on the PNG but not on the IC beans.

The occurrence of competition between the larvae can be seen by distinction of the rates between the series with 2 and 10 inserted first instar larvae, particularly on the dry IC beans with damaged testae, the series with the highest number of imagoes hatched: 80 % of the inserted first instar larvae in twos but 44 % of the ones inserted in tens completed their development. Nevertheless, in one replicate of the series with damp IC beans with damaged testae all of the 10 inserted first instar larvae could complete their development on the single cocoa bean.

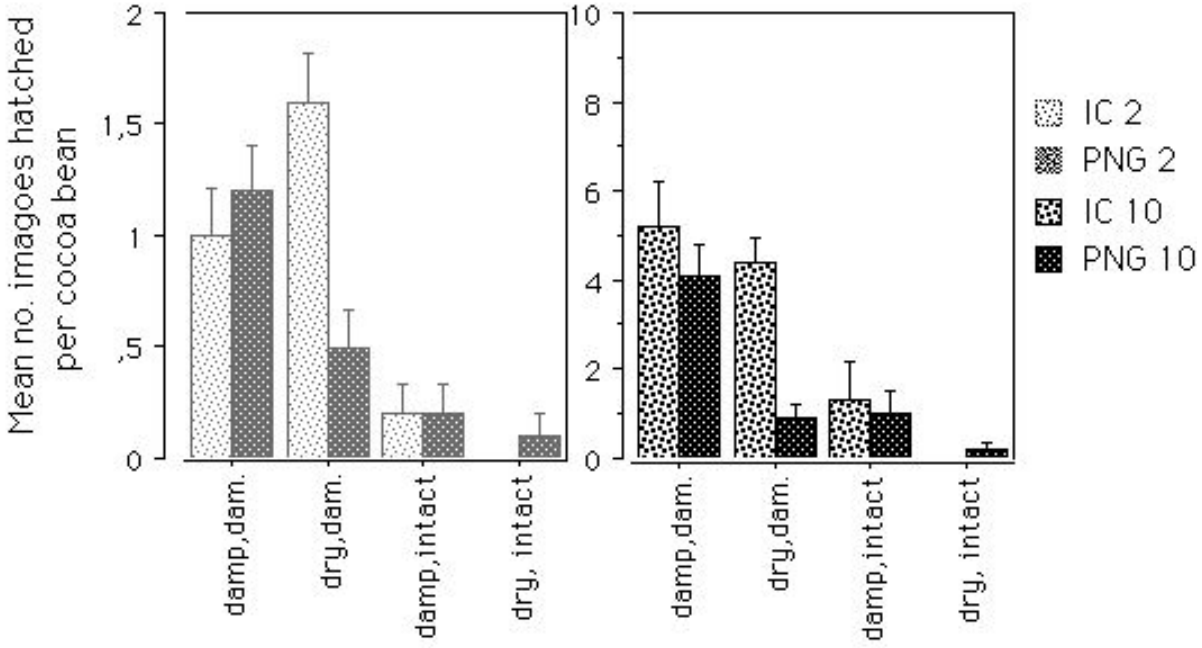

Fig. 2: Mean no. of *C. cephalonica* emerged per cocoa bean (±SE; n=10)
"IC": cocoa lot from Ivory Coast, "PNG": cocoa lot from Papua New Guinea, "2" resp. "10": no. first instar larvae inserted, "damp": cocoa beans conditioned and held at 87 ± 5 % r.h., "dry": cocoa beans conditioned and held at 50 ± 5 % r.h., "dam.": damaged testae, "intact": intact testae

The larval developmental time on the IC beans was much shorter than on the PNG beans of any series (Fig. 3). Again, the moisture content was a more influential factor for the development on the PNG than on the IC beans. The short larval periods of the IC series showed a longer mean developmental time of female larvae with 2.1 d (2 first instar larvae on dry IC beans) to 6.7 d (2 first instar larvae on damp IC beans) difference. An influence of the number of inserted first instar larvae, i.e. a shorter developmental time of the first instar larvae inserted by twos could be observed in the damp beans series, but surprisingly not between the series with dry IC beans with damaged testae, where the competition became evident by differences in the rates of emerged adults.

A clear coherence appeared between crowding and migration behaviour of *C cephalonica*: silk traces left by migrating larvae were found in all replicates of multiple emergence of adults, but in none of the replicates with solitarily developing larvae.

There seemed to be no major influence of the cocoa's quality and condition on initial oviposition. In all cases mated females reached a peak of more than 300 eggs (except on dry PNG beans where only one female was taken into account). The 76 examined unmated

females laid markedly less eggs on the average (170.6 ± 86.2), with some of them refusing to start oviposition for several days, and others laying eggs from first day on with a similar oviposition rate as the mated ones.

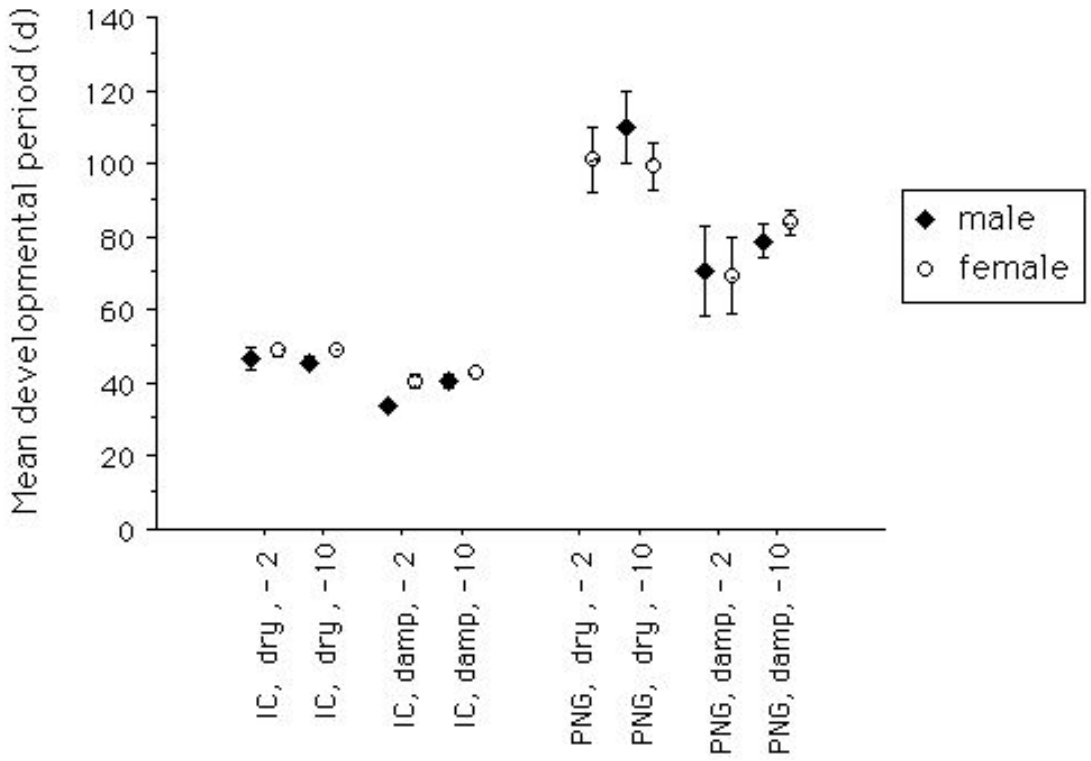

Fig. 3: Mean developmental period of *C. cephalonica* on cocoa beans of different qualities (±SE).
"IC": cocoa lot from Ivory Coast, "PNG": cocoa lot from Papua New Guinea, "2" resp. "10": no. first instar larvae inserted, "damp": cocoa beans conditioned and held at 87 ± 5 % r.h., "dry": cocoa beans conditioned and held at 50 ± 5 % r.h. (N; range):
IC dry -2, M (5; 39 - 57 d), F (11; 40 - 55 d);
IC dry -10, M (18; 40 - 57 d), F (26; 42 - 67 d);
IC damp -2, M (5; 31 - 37 d), F (8; 35 - 48 d);
IC damp -10, M (33; 31 - 80 d), F (32; 34 - 77 d);
PNG dry -2, M (0; -), F (6; 82 - 144 d);
PNG dry -10, M (3; 90 - 120 d), F (7; 83 - 144 d);
PNG damp -2, M (5; 39 - 99 d), F (7; 39 - 113 d);
PNG damp -10, M (18; 46 - 112 d), F (33; 62 - 133 d)

Dispersion of the larvae from damaged to intact cocoa beans
Most feeding by far was done on the originally damaged cocoa bean, but one to two of the former intact beans were attacked in each replicate. In some cases only parts of the testae were devoured, without any feeding done on the endosperm.

Oviposition preferences on cocoa beans of different moisture contents
Females laid slightly more eggs on dry than on damp beans, but this preference was not significant (Tab. 1). The eggs were laid separately, in rows or in batches, sticking in hollows and crevices of the shell or between bean and bottom of the dish. The comparison of the series of the arena experiment with the two control series shows that the availability of oviposition

sites were decisive for the oviposition rate on the first and second day (Fig. 5) and subsequently for the total amount of eggs laid by each female (Tab. 2). Significantly more eggs on the average were laid in the series with gauze compared with the control. The arena series did not differ significantly from the other two sets in the amount of eggs laid.

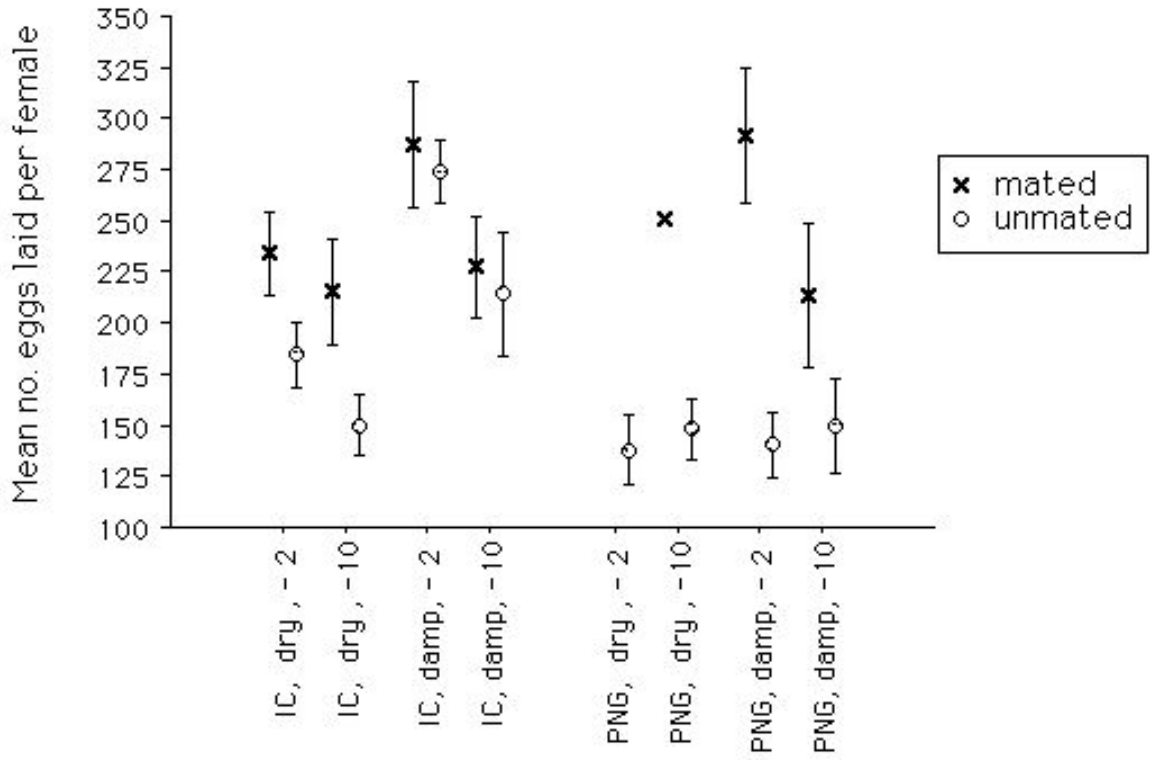

Fig. 4: Mean no. of eggs laid per female of *C. cephalonica* after larval development on cocoa beans of different qualities (±SE)
"IC": cocoa lot from Ivory Coast, "PNG": cocoa lot from Papua New Guinea, "2" resp. "10": no. first instar larvae inserted, "damp": cocoa beans conditioned and held at 87 ± 5 % r.h., "dry": cocoa beans conditioned and held at 50 ± 5 % r.h. (N; range):
IC dry -2, mated (6; 164 - 318), unmated (5; 147 - 227);
IC dry -10, mated (8; 98 - 363), unmated (17; 48 - 289);
IC damp -2, mated (3; 230 - 336), unmated (5; 245 - 319);
IC damp -10, mated (15; 19 - 378), unmated (14; 2 - 375);
PNG dry -2, mated (0; -), unmated (6; 72 - 200);
PNG dry -10, mated (1; 251), unmated (6; 117 - 212);
PNG damp -2, mated (2; 259 - 325), unmated (3; 109 - 164);
PNG damp -10, mated (8; 109 - 431), unmated (20; 2 - 337)

Table 1: Oviposition of *C. cephalonica* in the two-choice arena.

Oviposition site	Mean no. eggs laid per female (SE)
Dry beans	105.7 (14.7) a
Damp beans	93.6 (13.3) a
Dish	9.6 (4,4) b

Means followed by different letters are significantly different ($P<0.05$, t-test, N=10)

Table 2: Oviposition rate of *C. cephalonica* in different arena sets.

Arena set	Mean no. of eggs laid per female (SE)
Cocoa beans (Two-choice arena)	208.9 (23.9) a b
Gauze	217.3 (17.6) a
Control	160.0 (10.4) b

Means followed by different letters are significantly different (P<0.05, t-test, N=10)

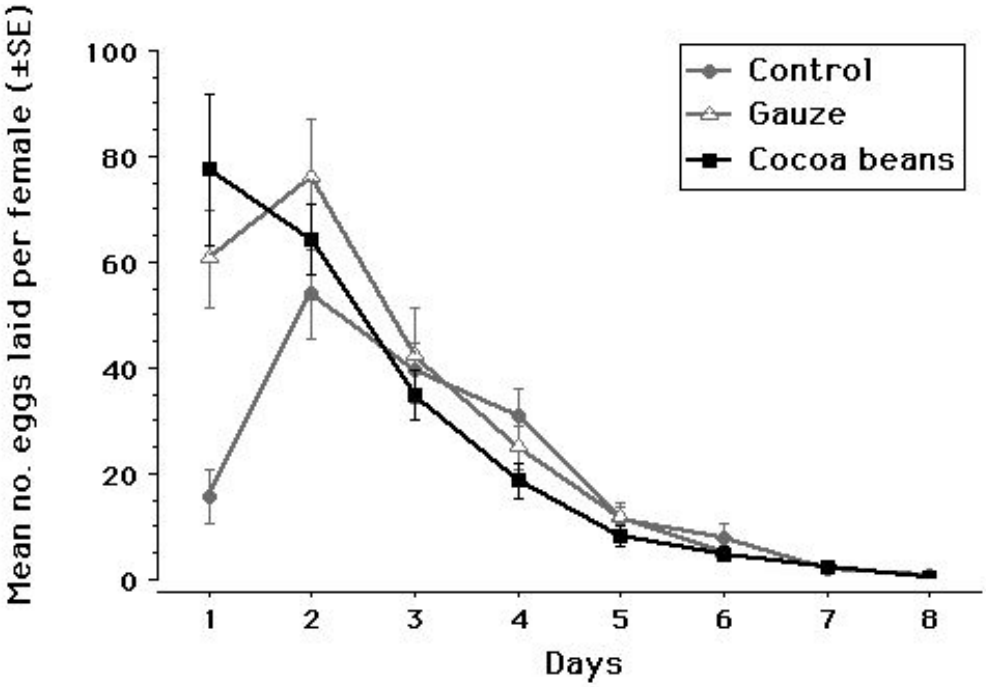

Fig. 5: Oviposition of *C. cephalonica* in an arena with dish containing cocoa beans, gauze-covered empty dish and empty dish (control), (n=10)

On the first day, significantly more eggs were laid on cocoa beans than on the smooth surface of the petri-dish (t-test, P<0.001). The highest number of eggs were laid on cocoa beans on the first day with a steady decline over the following days. The oviposition rate on gauze reached its peak not before the second day. However, oviposition rates in the sets with cocoa beans and with gauze did not differ significantly on any day of experiment (t-test, P>0.05).

Discussion

Cox *et al*., (1981) reported upon the frequent occurrence of *C. cephalonica* on imports to Britain, and conducted rearing experiments on a standard medium under a range of controlled conditions. They reported the most rapid development from egg hatch to adult emergence (23 ± 1.4 d) at 30°C and 90 % r.h. The survival rate was 3 % when a mould inhibitor was added to the food. No larvae could complete development without mould inhibitor. In this investigation, no mould growth was observed on infested cocoa beans, while all un-infested ones started to mould at 87 ± 5 % r.h. within few days. On cocoa beans from Ivory Coast with

damaged testae, the survival rate of the first instar larvae inserted by twos was 50 % with a mean developmental period of 35.5 d at 87 ± 5 % r.h. The survival rate reached 80 % with a mean developmental period of 48.2 d at 50 ± 5 % r.h. Cox et al. (1981) reported a survival rate of 55 % with a mean developmental time of 30±2.5 d at 50 % r.h. and 30°C. Thus, despite the prolonged developmental period, raw cocoa can be a substantial substrate for *C. cephalonica*. The percentage of adult emergence depends on relative humidity and cocoa's quality, both of them factors affecting the moisture content. The developmental time depends strongly on the cocoa's quality, as the development was more rapid on IC beans than on the PNG beans even under condition of high relative humidity, when PNG beans reached a higher moisture content than IC beans. This could be due to the higher acidity of raw cocoa from Papua New Guinea.

The best protection from infestation seems to be the cocoa bean's own shell, as the intact testae can hardly be infested by the first instar larvae. A common damage of the beans is the loss of the germ, which leaves an entrance right into the endosperm. This is caused by over ripeness before fermentation, and its avoidance would be an important contribution to the further protection of the stored raw cocoa.

The 43-mated females examined laid an average of 231.3 (± 81) eggs. This is by far more than cited by Ayyar (1934) and Allotey & Azalekor (2000), where the mean number of eggs is stated between 128 - 157 with a maximum never exceeding 200, but comparable to the record of 210 ± 10 in mean of females reared on groundnuts (Allotey 1991). Maybe that lack of oviposition stimuli in the experimental design was responsible for low fecundity of the rice moth. In the oviposition assay, the chemical stimuli of the cocoa beans with intact testae seemed to be less important for stimulating oviposition, as a similar response could be elicited by gauze. Ayyar (1934) cited that the oviposition of *C. cephalonica* might be stimulated by rough surfaces. He noted that in stores, the eggs are often deposited on food materials, but mostly indiscriminately on walls, bags and other rough surfaces.

Acknowledgements

This work was partially supported by the "Stiftung der deutschen Kakao- und Schokoladenwirtschaft".

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IOBC Bulletin Vol. 25 (3) 2002
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Insects and mites of stored products in the northeast of Spain

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Abstract: Insects and mites associated with stored products were surveyed from 1999 to 2001 in the northeast of Spain. Sixty-two samples from several cereals, dried fruits, legumes, herbs, spices and processed food products were collected in 11 localities. A total of 29 species of arthropods representing six orders and 15 families were found, predominantly Coleoptera and Lepidoptera. Among pests, the rice weevil, *Sitophilus oryzae* (L.) and the lesser grain borer, *Rhyzopertha dominica* (F.) were the most abundant species in stored cereals. The cigarette beetle, *Lasioderma serricorne* (F.), the rust-red flour beetle, *Tribolium castaneum* (Herbst), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) and the Indian meal moth, *Plodia interpunctella* (Hübner) were the most numerous and widely distributed species sampled in residues of food products. One predatory mite and five Hymenoptera species were considered to have potential for biological pest control.

Key words: survey, stored product pests, natural enemies, northeast of Spain

Introduction

The Catalan food industry is one of the most important in Spain. According to the official statistics of the Spanish Ministry of Agriculture, Fisheries and Food (1999), Catalonia (northeast of Spain) represents the first Spanish region in capital investments, sales of products and processed raw material. Most food factories process big quantities of raw materials that they have to keep stored during some period of time. Also, finished or processed products are often stored before being packaged or distributed to the consumers. Consequently, one of the most important problems that affect these industries is insect and mite contamination. The impact of pests is not only economic because of weight losses, expenses on sanitation or discredit by customers, but also indirect because of toxic effects, allergy, or toxicological and environmental problems related to excessive use of chemical control treatments.

The aim of this study was to determine the insect and mite fauna associated with raw material and finished products that are processed by the agrofood industry in Catalonia.

Material and methods

The study was conducted from 1999 to 2001. Surveys for insects and mites were conducted at 24 industrv facilities or warehouses on 10 localities widely distributed within Catalonia. Special attention was paid to two localities with many food processing plants, accounting 30 and 27% of total samples, respectively, and there more than one collection was made per year. Samples were taken from raw materials such as cereals (wheat, oats, rye, maize and rice), dried fruits and nuts (almonds, pistachios, dates, raisins and dried apricots), legumes (haricot beans), herbs and drugs (*Chamomilla recutita, Lavandula angustifolia, Melissa officinalis, Thymus vulgaris, Achillea millefolium, Opuntia ficus-indica, Foeniculum vulgare,*

Kola acuminata), spices (coriander, walnut bark, red pepper, sesame) and finished or processed products such as flour, semolina, pasta, cassava, mushrooms or pet food. Specimens of insects and mites were collected by hand-picking, aspirating or sieving. Samples were taken to the laboratory for closer examination. Specimens collected were preserved, determined or submitted to the appropriate taxonomists for identification. Subsequently, the specimens were deposited in the IRTA-collection.

Results and discussion

In total, 29 species of arthropods representing six orders and 15 families were found on a total of 62 samples in raw material and processed food products (Table 1). Orders with the greatest number of species were Coleoptera and Lepidoptera.

Twenty-one species were collected from cereals. Coleoptera accounted for more than 50% of species of arthropods collected, Hymenoptera 22% and Lepidoptera 10%. Among pests, the rice weevil, *Sitophilus oryzae* (L.), and the lesser grain borer, *Rhyzopertha dominica* (F.) were the most abundant species in stored cereals. In addition, four species of parasitoid Hymenoptera were found in all cereals, among them the pteromalids *Anisopteromalus calandrae* (Howard) and *Lariophagus distinguendus* (Foerster) are known to attack several weevil species (Gordh & Hartman 1991, Brower et al. 1996, Lucas & Riudavets 2001). The predatory mite *Blattisocius tarsalis* (Berlese) was also present in wheat and rice.

The cigarette beetle, *Lasioderma serricorne* (F.) and the drugstore beetle, *Stegobium paniceum* (L.) were the most abundant pests in dried herbs and spices. Since they are polyphagous (Arbogast 1991), they were also found infesting wheat, rye, pasta and pet food.

The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), was the only species recorded in dates, raisins and dried apricots. The moths *Plodia interpunctella* (Hübner) and *Ephestia* sp. were present in almonds and pistachios.

The Indian meal moth, *P. interpunctella*, the rust-red flour beetle, *Tribolium castaneum* (Herbst), *O. surinamensis*, and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) were found in wheat flour. The mold mite, *Tyrophagus putrescentiae* (Schrank) was present in wheat semolina and pet food but always related with high humidity conditions. Psocids, an increasing problem worldwide (Turner 1994), were collected in semolina, pasta and cassava, but also in wheat, herbs and spices. The bean weevil, *Acanthoscelides obtectus* (Say) was only found in haricot beans, where the predatory mite *B. tarsalis* was also present.

Acknowledgements

We thank Marina Blas (Univ. de Barcelona), Paco Ferragut (Univ. Politéc. Valencia), Arturo Baz (Univ. Alcalá de Henares) and M. Jesús Verdú (IVIA, Valencia) for taxonomic identifications, and O. Alomar and C. Castañé for the review of the manuscript. This research was financially supported by the company "S.E. Carburos Metálicos S.A.", by the Ministerio de Ciencia y Tecnologia, project FEDER Nº2FD1997-0736, and by postdoctoral scholarships from the "Comissionat per a Universitats i Recerca of the Generalitat de Catalunya" and from the "Fonds pour la Formation de Chercheurs et l'Aide à la Recherche du Québec" to É. Lucas.

Table 1. Species of insects and mites associated with different stored food products in the Northeast of Spain

Arthropod*[1]	Cereals*[2]	Dried herbs & Spices*[3]	Dried Fruits*[4]	Processed & Stored Food*[5]
ACARINA				
Acaridae				
Tyrophagus putrescentiae (Schrank)				Ws, Pf
Ascidae				
Blattisocius tarsalis (Berlese)	Wh, Ri			Ws, Be
PSOCOPTERA				
Psocidae				
Liposcelis bostrychophilus Badonnel				Ws
Other Psocoptera	Wh	He, Sp		Pa, Ca
THYSANOPTERA				
Tubulifera	Ry			
COLEOPTERA				
Anobiidae				
Lasioderma serricorne (Fab.)		He, Sp		Pa
Stegobium paniceum (L.)	Wh, Ry	He, Sp		Pa, Pf
Bostrichidae				
Rhizopertha dominica (Fab.)	Wh, Oa, Ry, Ma, Ri			Ca
Bruchidae				
Acanthoscelides obtectus Say				Be
Cucujidae				
Cryptolestes ferrugineus (Stephens)	Wh, Ry, Ma			Wf, Ca
Curculionidae				
Sitophilus oryzae (L.)	Wh, Oa, Ry, Ma, Ri			Pa
Sitophilus zeamays Mots.	Oa			
Silvanidae				
Oryzaephilus surinamensis L.	Ry		Fr	Wf
Oryzaephilus mercator (Fauvel)	Ma			
Tenebrionidae				
Gnathocerus cornutus (Fab.)	Wh			
Gnathocerus maxilosus (Fab.)	Ry			
Tribolium castaneum (Herbst)	Wh, Oa, Ry, Ma	Dr		Wf, Ca, Pf
Tribolium confusum Jacquelin du Val	Wh, Ry, Ma			Ca
Alphitobius laevigatus (Fab.)				Pf
Trogossitidae				
Tenebroides mauritanicus (L.)	Wh, Ry			
LEPIDOPTERA				
Tineidae				
Nemapogon granella (L.)				Mu
Pyralidae				
Ephestia sp.	Ma		Al	Pf
Plodia interpunctella (Hübner)	Wh, Ry	Sp	Al, Pi	Wf, Ws, Pa, Pf
Pyralis farinalis L.				Pf
HYMENOPTERA				
Pteromalidae				
Anisopteromalus calandrae (Howard)	Wh, Oa, Ry, Ma, Ri	He, Sp		
Lariophagus distinguendus (Foerster)	Wh, Ry, Ri	He		
Other Hymenoptera	Ry, Ma	He, Dr		

*[1] Other Psocoptera (cf. 1 species); Thysanoptera (cf. 1 species); Other Hymenoptera (cf. 3 species).
*[2] Cereals: Wh = Wheat, Oa = Oats, Ry = Rye, Ma = Maize, Ri = Rice.
*[3] Dried herbs and Spices: He = Herbs, Dr = Drugs, Sp = Spices
*[4] Dried Fruits: Al = Almonds, Pi = Pistachios, Fr = Dates, raisins and dried apricots
*[5] Processed & Stored Food: Wf = Wheat flour, Ws = Wheat semolina, Pa = Pasta, Be = Beans, Ca = Cassava, Pf = Animal feed, Mu = Mushrooms.

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IOBC Bulletin Vol. 25 (3) 2002
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The feeding interactions of astigmatid mites (Acari: Astigmata) and microfungi in stored grain habitats (Mini-Review)

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Abstract: Stored grain represents anthropogenic habitats; consisting of (i) grain, (ii) plant debris, (iii) microfungi and (iv) arthropods. The microfungi growing on the grain may serve as a possible food source for the astigmatid mites. The interaction between the mites and fungi includes 3 main topics. (1) The fungi could serve as possible food sources for astigmatid mites. The mites need specific digestive enzymes to utilize fungal food sources. (2) The fungi differ in their attractiveness and palatability due to the content of mycotoxins and secondary metabolites in fungal cells. There are differences between spores and mycelium in microfungal species. Some microfungal spores are passed through the mites gut without digestion. (3) The mites strongly influence fungal mycelium by grazing. The numbers of microfungal colonies might change after the infestation of the stored grain by mites. The mites also disperse fungal spores on their bodies and in their faeces, however due to their small moving distance the dispersion can not be so important in stored grain habitats. The positive effect of mites feeding on mycelium respiration was reported in the conditions of nutrient rich substrates and patches distribution of microfungi. The knowledge of the interactions of mites and fungi from the field and laboratory observations is reviewed and applied into the stored grain habitats.

Key words: mites, grain, fungi, feeding, digestive enzymes

Introduction

Stored grain represents a special case of anthropogenic habitats, consisting of grain, plant debris and their pests. The organisms growing or feeding on the grain may serve as possible food sources for others. The infestation of stored grain represents, from the ecological point of view, an increase in the complexity of the food chain.

The mites are the most dominant arthropod group in stored grain in the Czech Republic. They were found in 65% of grain samples - out of 374 samples collected, the mean abundance was 308 individuals.kg^{-1} of grain, maximum 25 500 individuals.kg^{-1}. The astigmatid mites (Acari: Astigmata) were eudominant (Žďárková, 1998). Mites often occur in association with microfungi, and their succession is related to the degree of kernel damage, temperature of the air, and water content of the grain during harvesting, shipping and storage of the grain (Sinha, 1964 and 1979; White et al., 1979).

Both the astigmatid mites and microfungi are considered to be economically important pests, decreasing the quality of stored grain when present in large numbers. The mites as well as microfungi are important producers of allergens or mycotoxins, respectively (Hage-Hamsten and Johansson, 1992; Tee, 1994; Miller, 1995).

Ancestrally, the astigmatid mites were probably fungivorous (O´Conor, 1979). The indices of possible dispersion of fungi by mites have been brought since the 1930s (Jacot, 1931). The stored grain habitats are simpler associations than the soil and their conditions are much easier to simulate in the laboratory. Therefore, they may serve as a very suitable object for mite – fungi interaction studies (Franzolin et al., 1999).

The aim of the present paper is to summarize the up-to-date knowledge on the interactions of microarthopods and fungi from the field and laboratory observations and to apply them into the stored grain habitats.

Methodical approaches

The feeding interaction of astigmatid mites and microfungi may be studied from various aspects. The use of several methodical approaches simultaneously is necessary for understanding the interactions of mites and microfungi. Following methods are available and following aspects should be taken in account:

- The cultivation of microfungi from the gut content or the body surface of mites (Williams et al., 1998, Hubert et al. 2000) indicates the dispersion of fungal spores by the mites.
- The detection of substrate-specific digestive enzymes (particularly the carbohydrases) indicates, if the mites are able to digest the compounds of the fungal cell walls and cell-contents. However, the detected activity of a substrate-specific enzyme indicates but does not proof the substrate utilisation (Hubert et al., 2001a).
- Microanatomical observation of the contents of digestive tract indicates the actual digestion and utilisation of food in the gut (Smrž and Čatská, 1989, Hubert et al., 2001b).
- Food preference tests indicate the attractiveness and palatability of microfungal diets. The tests include: (a) food choice tests, indicating the attractiveness of diets; (b) counting of faecal pellets, indicating the intensity of food ingestion; (c) rearing of the mites on different diets and determination of their fecundity and development (d) determination of searching time; (e) measuring of the distance to which the mites are able to perceive the food sources; (f) determination of the olfactory discrimination of the food by the mites (Žďárková, 1979).
- The microcosm experiments. The microcosms include usually mites, grain and micromycets. The changes in colony forming units (CFU) after some days of incubation are compared, or the respiration rate of the microcosm is recorded, indicating the metabolic activity (Griffiths et al., 1959, Hanlon, 1981; Visser et al., 1981).

Are the microfungi a food source for astigmatid mites in stored grain habitats?

The food sources in stored grain habitats may be classified as stored grain, plant debris, microfungi and bacteria (Figure 1). These food sources differ in the predominant storage and structural oligo/polysaccharides. The main storage polysaccharide of plants is starch, while the disaccharide trehalose has this function in fungal cells. Among the structural carbohydrates, cellulose predominates in plants, while chitin is the most common in fungal cell walls. The bacterial cell wall polysaccharide is usually made from N-acetyl-glucosamine and N- acetylmuramate. Lipids and proteins represent other important food sources for mites, however, they are distributed in all the compared food sources.

The fungal mycelium could be divided into cell walls and cell contents. The easiest way for mites to feed on cell contents is to pierce the cell wall with needle-shaped chelicerae. Another way is to chew and ingest complete cells, which would require the presence of enzymes to help digest cell walls so that the cell contents are available for uptake (Siepel, 1990). The presence of chitinase and trehalase among mites enables to distinguish between grazers or browsers (e.g. fungivorous grazers or feeders). The grazers are able to digest both cell walls and cell contents, the browsers only cell contents (Siepel and Ruiter-Dijkman, 1993). The data concerning the trehalase activity are rare or lacking in astigmatid mites. Likewise the adaptation to fungal diets based on digestive enzymes is puzzling (see table 1).

The histological observation of gut contents of mites, indicates fragmentation of mycelimum by chelate chelicerae. A part of mycelium is usually cut and fungal cell walls are mechanically destroyed (see Smrž and Čatská, 1987). The enzymatic destruction of cell walls in order to digest the cell contents, is not necessary. The digestion of fungal cells might be associated to the enzymes splitting fungal storage compounds more than fungal cell walls (Hubert et al. 2001b).

The enzymes decomposing cellulose and hemicelluloses might also be produced by the micro-organisms living in plants debris or plants cell walls (external-rumen hypothesis of Marraun et al., 1999). The enzymes of the animals (beta-glycosidase) are able to compete with the microbial enzymes for the degradation products of plant debris (Nielsen, 1962). The mites could be able to consume the grain cells damaged by fungal exoenzymes.

The quantitative and qualitative measurement of digestive enzymes in the future observations seems to be necessary for evaluation of microfungi digestion.

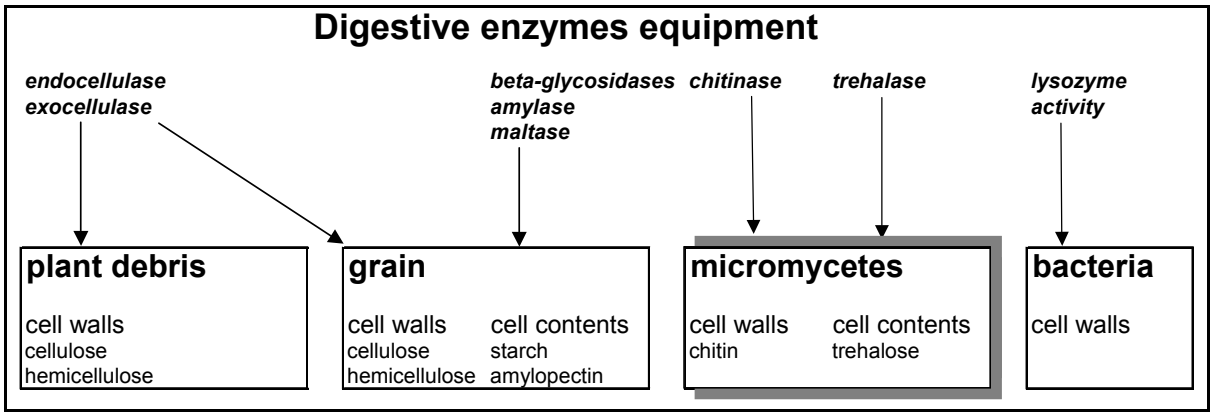

Figure 1. Hypothetical using of food sources based on mites digestive enzymes.

Does the selective feeding in mites on microfungi exist in stored grain habitats?

It is difficult to evaluate the energetical contribution of diets, directly. Thus, the population growth is often used as an indicator of food suitability. Generally, some microfungi represent a food source and are attractive for mites, while others are toxic (Griffiths at al., 1959; Solomon, 1964; Sinha, 1964; Pankiewicz-Nowicka et al., 1986; Smrž and Čatská, 1987). The differences in palatability were reported among the fungal species and even among the different strains of a species. The specimens reared on fungi with different palatability showed differences in several microanatomical features, e. g. amount of guanine deposites in the body parenchyma or presence of the extra-intestinal microflora (Smrž and Čatská, 1989). The spores were less favourable than were the mycelium pellets (Parkinson et al., 1991b), similarly some spores were passed through the gut undigested (Smrž and Čatská, 1987). The viability of spores after gut passing depends on microfungal species (Griffiths, 1959). Saichue et al. (1972) observed changes in attractiveness of one microfungal species over time while the culture was aging: The senescent culture was less attractive for mites than the young one. The contact of adults of *Tyrophagus putrescentiae* with the fungi did not effect eggs hatching generally, however eggs or juveniles were sensitive and did not develop on some micro-fungi (Pankiewicz-Nowicka, 1984). These experiments can be explained by the fact, that a single fungal species may have strains with different levels of toxins or secondary metabolite varying during fungal growth (Rodriguez et al., 1984).

Table. 1. A review of the presence of digestive enzymes in astigmatid mites

species	trypsin	chymotrypsin	cysteine	carboxypeptidases	lipase	esterase	Amylase	glucoamylase	alpha-glucosidase	alpha galctosidase	beta-glucosidase	beta-glucuronidase	beta galctosidase	Cellulase	trehalase	Lysozyme	Chitinase	Reference
Acarus siro	1	1	1		1	1	1		1	1	1	1	1	0		1	1	Bowman and Child 1982, Akimov and Barbanova 1976a
Aleuroglyphus ovatus			1				1							1			1	Edwards et al. 1992
Caloglyphus berlesei							1							1			1	Akimov and Barbanova 1976a
Carpoglyphus lactis							1							0			0	Akimov and Barbanova 1976a
Coleochaeta molitor							1							1			1	Akimov and Barbanova 1976b
Cosmoglyphus absoloni							1							1			1	Akimov and Barbanova 1976b
Dermatophagoides farinae	1	1					1	1								1		Stewart et al. 1998
Dermatophagoides pteronyssius	1	1					1	1								1		Stewart et al. 1998
Glycyphagus domesticus	1	1	1		1	1	1		1	0	1	1	1	0		1	1	Bowman and Child 1982 Bowman 1984
Histiogaster bacchus							1							1			1	Akimov and Barbanova 1978
Lepidoglyphus destructor	1	1	1		1	1	1		1	1	1	1	1	0		1	1	Bowman and Child 1982 Stewart et al. 1998; Bowman 1984
Rhizoglyphus callaea	1	1	1		1	1	1		1	1	1	1	1	1		1	1	Bowman and Child 1982
Rhizoglyphus echinopus							1											Bowman 1984 Barabanova (1972, 1976)
Rhizoglyphus robini	1	1	1		1	1	1		1	1	1	1	1	1		1	1	Bowman and Child 1982
Tyreophagus entomophagus							1							1			1	Bowman 1984 Akimov and Barbanova 1978
Tyrophagus longior	1	1	1		1	1	1		1	1	1	1	1	1		1	1	Bowman and Child 1982
Tyrophagus putrescentiae	1	1		1			1											Akimov and Barbanova 1978 Ortego et al. 2000
Tyrophagus similis							1							0	1		0	Matsumoto 1965 Siepel and Ruiter-Dijkman 1993

Some astigmatid species are able to feed on microfungi exlusively and complete their life cycle on the fungal diet. Usually, the feeding on microfungi is variable among astigmatid species, which may be explained by different digestive enzymes. But wheat germs had higher attractiveness suitability for mites development than in the tested microfungal species (Pankiewicz-Nowicka and Boczek, 1984, Parkinson et al., 1991a; 1991b).

How does feeding by mites influence the microfungal community in stored grain habitats?

It is well known that the mites strongly influence the growth of microfungi by grazing and spore dispersion (White et al., 1979; Armitage and George 1986; Williams et al., 1998; Franzolin et al., 1999). The effect of astigmatid mites on fungal growth has not been observed, but it is well documented for other microarthropods such as springtails and oribatid mites in soil.

The mites are able to disperse the spores by carrying them along on the surface of their bodies and by passage through their intestinal tract (Griffiths et al., 1959). The main question concerning the spore dispersion is: to what distances are the spores transported by the mites? The mites are able to move several centimetres per minute on a smooth surface, but their

movement inside the grain is much more complicated and some further observations are necessary.

The effect of mite grazing depends on their feeding habits. (Siepel and Maaskamp, 1994). Digestion of fungal cell walls next to contents (grazing) leads to stimulation, digestion of cell contents only (browsing) leads to inhibition of the fungal growth, respectively, at equal densities of mites (Siepel and Maaskamp, 1994). Grazing may induce the development of a fast-growing hyphal morphotype and promote production of extra-cellular enzymes, such as proteases and amylases followed by nutrient release in the close neighbourhood of the hyphae (Hedlund et al., 1991). The fitness gained by attractive fungi from spore dispersal by mites may well compensate for losses due to grazing, especially if the fungi are patchily distributed (Bengtsson et al. 1993).

The fungi attract their grazers by volatile compounds (Bengtsson et al., 1988; 1991). From an evolutionary point of view it is not clear why the fungi should develop certain odours, depending on being grazed, to achieve their higher metabolism and growth (Bengtsson et al.; 1993). Patches in fungal distribution on the substrate lead to alternating high and low grazing pressure of micro-arthropods and may eventually result in an overall stimulating effect on micro-fungal respiration (Bengtsson et al., 1993).

High densities of micro-arthropods tend to overgraze the fungal mycelium, but high substrate quality permits a strong growth of fungi that is not easily overgrazed by micro-arthropods (Hanlon and Anderson, 1979; Hanlon, 1981). High microarthropod numbers lead to an increased fungal destruction and thus to lower respiration. A high nutrient availability leads to a high fungal growth rate and consequently a more rapid recovery from grazing damage and thus a higher respiration (Siepel and Maaskamp, 1994). The balance between grazing and spore dispersion and mineral releasing on another hand form the final effect on microfungi growth.

Concluding remarks

The mites and fungi multi-infestation of stored grain brinks the following aspect would into account: The mites species with trehalase in their enzymatic equipment are able to use the fungi as food source. This feeding behaviour could eliminate the effect of inhibitors of the mites digestive enzymes in genetically modified plants, used for the pest control (cf. Sanchez-Monge et al., 1996, Ortego et al., 2000). The selective feeding of mites on various fungal species indicates the possibility of selective fungal dispersion on mite bodies and in their digestive tract via faeces. The selective dispersion increases the importance of the mites as vectors of fungi. The feeding interactions of mites and fungi could lead to reduction of micromycetes growth due to the overgrazing by mites, or higher microfungi diversity in the grain due to positive effect of selective grazing. The effect of interaction depends on the species composition (mites and microfungi) and the system heterogeneity.

Acknowledgements

This project was supported by NAZV -EP 9138.

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Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
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Survey and estimate of moth population density in a flour mill in Cape Verde Islands

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Abstract: A survey was conducted in a flour mill in Cape Verde Islands and a total of 11 species of insects and mites were found. The most common species were *Tribolium confusum* and *Corcyra cephalonica*. Pheromone traps were used to estimate moth population density at different points within the flour mill in Cape Verde. *Ephestia cautella* was the dominant species on wheat silos while *E. kuehniella* was a significant pest throughout the milling process. Adults of *E. kuehniella* occurred within the flour mill during the entire year. The index of dispersion indicated that the adults might follow the aggregate pattern, at high relative densities. With the decrease of mean trap catches the adult males' populations seemed to be randomly or uniformly dispersed.

Key words: pheromone trap, *Ephestia cautella, Ephestia kuehniella,* flour mill, spatial pattern

Introduction

Insects found in flour mills are common contaminates of grain and mill products and may cause important losses in the food industry (Mills & Pederson, 1992). Studies on infestations in the milling machinery in the United Kingdom (Dyte, 1965) on insect infestations in mills at Mindelo, Cape Verde (Carvalho,1984) and in Portugal (Pereira, 1999) had been conducted in the flour mills environment. *Ephestia kuehniella* Zeller has been a frequent pest of flour mills and has been reported in the United Kingdom (Jacob & Cox, 1977), Italy (Trematerra, 1990) and Portugal (Pereira, 1998). Direct damage to the product, live or dead insects, larval and pupal exuviae together with dense webbing produced by the moth larvae may block the milling machinery and can be a serious problem in flour mills.

Environmental conditions in Cape Verde Islands with a dry season and low temperatures during November to June, and a rainy season with high temperatures during July to October, favour insect development and problems caused by insect infestation may occur.

Additionally, with the increasing use of insecticides for control of stored pests in flour mills, data on sampling and on trapping are needed to enable early detection of changes in insect population that may increase to economically damaging levels.

In the present study a survey was conducted to determine the arthropods species present in a flour mill, in Cape Verde Islands, that may be accountable for infestations and deterioration of food products. In addition, infesting moth species, namely, *Ephestia cautella* (Walker) and *E. kuehniella* were monitored over 18 months period and distribution patterns of *E. kuehniella* were studied.

Material and methods

The flour mill, built in 1974, used 80 tonnes of wheat per day and produced 62 tonnes of wheat flour per day for human consumption and by-products of wheat flour milling, as bran and germ.

The flour mill has vertical silos with a capacity of 4000 tonnes, used for incoming wheat, usually stored at 13% moisture content. Remote temperature sensors were installed at four different points as warning devices and wheat is ventilated to minimize moisture pockets and temperature differences. Insecticides are applied to wheat if storage is prolonged more than three months.

Before milling, wheat is transferred from the silos to the wheat cleaning section and then prepared for milling by being added water to permit moisture distribution throughout the wheat kernels. The tempered wheat is then sent to the milling process milled. The flour on its way to storage passes through centrifugal impact machines where live insects and insect eggs that might have survived the entire milling process are killed by severe impact. The final products are stored in silos for one or two weeks and then packed in bags.

A survey was conducted, in December 1999, and a total of 38 samples were collected from different places in the various sections of the flour mill. The 38 samples included six samples taken from the storage of wheat area, nine samples from the wheat cleaning section, 10 samples from the milling section, 13 samples from the flour storage and packing area. From different locations a total of 15 samples of product and 23 samples of food residues over machinery and floor residues were taken. The samples were kept separately and examined after sieving at the laboratory for signs of infestation. Those without insect activity were incubated at 25ºC and 70% r.h. until the emergence of adult insects. From visual search in the different sections, some insects were also collected.

Pheromone traps were used for monitoring *E. cautella* and *E. kuehniella* inside this facility. Six delta pheromone traps were installed, one in the wheat storage area, three in the milling section and two in the flour storage area, from early December 1999 to late July 2000. From August 2000 to mid June 2001, nine funnel pheromone traps were used, one in the storage wheat area, three in the milling section and five in the flour storage area, including packing area.

Delta traps (AgriSense-BCS, UK) consisted of a corrugated plastic (280 mm high x 200 mm x 120 mm) using a replaceable sticky insert, renewed whenever they showed dust accumulation. The funnel traps (AgriSense-BCS, UK) consisted of rigid plastic and the insects caught were collected inside the trap. The pheromone dispensers for both traps, contained 2 mg of *(Z,E)*-9,12-tetradecadienyl acetate and were replaced every six weeks. Pheromone traps were suspended 2 m above the floor level and the number of moths caught was recorded weekly. The temperature and relative humidity were recorded outside the factory.

Spatial distribution of *E. kuehniella* was based between average and variance by calculating the index of dispersion (Southwood, 1978).

The dispersion pattern of adult *E. kuehniella* was tested using the index of dispersion

$$I_D = (N-1)s^2 / \bar{x},$$

where N is the number of samples, s^2 is the sample variance and $\bar{x}$ is the sample mean. I_D is approximately distributed as χ^2, with $n-1$ degrees of freedom. If values were between the confidence intervals of 0.05 and 0.95 a random distribution is indicated; outside the confidence intervals of 0.05 or 0.95, the spatial pattern may be classified, respectively, as regular or aggregated (Davis, 1994).

Results and discussion

Five species of Coleoptera, three species of Lepidoptera, one of Psocoptera, one of Dictyoptera, and one of mites were found in the product and the residue samples collected from the flour mill (Table 1). *E. cautella* was caught by visual search and by pheromone traps in the wheat storage area. *E. kuehniella* was captured only by pheromone traps in the milling and in the flour storage sections. *Corcyra cephalonica* (Stainton) was collected in residue samples, product samples and by visual search in the milling and in the flour storage sections. *Periplaneta americana* (L.) was found by visual search in the wheat storage area.

Table 1: Insect and mite species found in the flour mill.

Arachnida **Prostigmata** Cheyletus eruditus *Hughes* **Insecta** **Coleoptera** *Rhyzopertha dominica* (F.) *Sitophilus* spp. *Tribolium confusum* J. Du Val *Tribolium castaneum* (Herbst) *Gnatocerus cornutus* (F.)	**Lepidoptera** *Ephestia cautella* (Walker) *Ephestia kuehniella* Zeller *Corcyra cephalonica* (Stainton) **Psocoptera** *Liposcelis* spp. **Dictyoptera** *Periplaneta americana* (L.)

In the wheat storage area *Rhyzopertha dominica* (F.)(one adult) and *Sitophilus* spp. (one adult) were recorded in a wheat sample. *Tribolium castaneum* (Herbst)(3 adults) was collected in one food residue sample and Psocoptera was found in two residue samples taken from the wheat storage section (Table 2).

In the cleaning section one product sample was infested with *Sitophilus* spp. (one adult) and in two residue samples over machinery, *Tribolium confusum* J. Du Val (3 adults) *T. castaneum* (8 adults) and Psocoptera were found (Table 2).

In the milling section, *T. confusum* (16 adults) and *Gnatocerus cornutus* (F.) (one adult) were found in a residue sample taken from the machinery. In a food residue sample collected from the purifiers floor *T. confusum* (11 adults), *R. dominica* (one adult) and *C. cephalonica* (4 adults) were found. A residue sample collected over machinery, after incubation revealed the presence of *C. cephalonica* (27 adults) and *T. confusum* (one adult).

In the flour storage area, three product samples were infested with *C. cephalonica* (4 adults), *T. confusum* (one adult), *T. castaneum* (one adult) and *G. cornutus* (one adult). In five residue samples *C. cephalonica* (5 adults), *T. confusum* (11 adults) and *G. cornutus* (5 adults) were recorded. After incubation, three residue samples collected from the flour storage section were infested with *C. cephalonica* (46 adults), *T. confusum* (29 adults), *R. dominica* (3 adults) and *G. cornutus* (15 adults). After incubation, *Sitophilus*, *R. dominica*, *T. confusum*, *G. cornutus*, and *C. cephalonica* were found in the samples.

The most common species found in the samples was that of *T. confusum* recorded in six residue samples and in one product sample. Most of the insect species were collected mainly from the wheat storage section, which raises the hypothesis that they were brought from outside. The coleopteran species *T. confusum*, *T. castaneum*, *G. cornutus*, *Sitophilus* spp. and *R. dominica* and the Lepidoptera *C. cephalonica* found is this survey were also recorded by Carvalho (1984).

Table 2: List of species, location and total of individuals found in samples taken in a flour mill, before and after incubation.

Species	Location	Total individuals	Total individuals after incubation
Arachnida			
Prostigmata			
Cheyletus eruditus Hughes	wheat storage (residue sample)	1	0
Insecta			
Coleoptera			0
	wheat storage (product sample)	1	0
Rhyzopertha dominica (F.)	cleaning section (product sample)	0	1
	flour storage (residue sample)	0	3
Sitophilus spp	wheat storage (product sample)	1	1
	cleaning section (product sample)	1	0
	cleaning section (residue sample)	3	0
Tribolium confusum	milling section (residue sample)	16	1
J. du Val	flour storage (residue sample)	11	29
	flour storage (product sample)	1	0
Tribolium castaneum	wheat storage (residue sample)	3	0
(Herbst)	cleaning section (residue sample)	8	0
	flour storage (product sample)	1	0
	milling section (residue sample)	1	0
Gnatocerus cornutus (F.)	flour storage (residue sample)	5	15
	flour storage (product sample	1	0
Lepidoptera			
Corcyra cephalonica (Stainton)	milling section (residue sample)	4	27
	flour storage (residue sample)	5	46
	flour storage (product sample	4	34
Psocoptera			
Liposcelis spp.	wheat storage(residue sample)	11	0
	cleaning section (residue sample)	2	0

From the data collected it was possible to identify certain insect pest species which were associated with particular materials and locations within a flour mill. Insects of stored products survive in debris when residual material is not removed, so a threat to product quality remains. Some stored-product insects develop and feed inside cereal grains. These include *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) found in one wheat sample taken from the storage section.

Corcyra cephalonica (Stainton) is an important secondary pest of cereal grains and their products in many tropical countries (Hill, 1990). *Ephestia cautella* (Walker) with a wider distribution, is a major pest of stored products and seeds, the larvae eat the germ and consume much of the endosperm, leaving most of the bran uneaten (Madrid & Sinha, 1982).

Psocoptera recorded on food residue samples are a group of small insects that feed on fungi or stored flours and cereal products (Hill, 1990).

P. americana is a major pest in tropical parts of Africa and all types of foodstuffs are eaten, and rubbish dumps in the tropics as well as sewers are infested (Hill, 1990).

In this flour mill, mean density referred to the mean number of insects found in each sample (Table 3), reached 3.7 insects/sample in the residue samples taken from the milling section with 33.3% of infested samples, followed by residues samples taken from the wheat storage section (3.5 insects/sample).

Table 3: Mean density of insects found in different places inside the flour mill.

Location	Number of samples	Infested samples (%)	Total individuals	Mean density
Wheat storage				
Product	2	50.0	2	1.0
Residues	4	75.0	14	3.5
Cleaning sectior				
Product	4	25.0	1	0.3
Residues	5	40.0	13	2.6
Milling section				
Product	4	0.0	0	0.0
Residues	6	33.3	22	3.7
Flour storage				
Product	5	60.0	7	1.4
Residues	8	62.5	21	2.6

From pheromone traps it was evident that *E. cautella* was captured only in the wheat storage area. In a delta pheromone trap (Fig. 1) placed in the wheat silos during 34 weeks, from December 1999 to July 2000, the total number of *E. cautella* adults collected was 167 adults, and captures ranged from 0 to 25 adults per week. Higher catches were recorded on mid-August with 120 *E. cautella* adults caught per week on a single funnel trap (Fig. 2) located in the wheat silos section.

A total of 1046 and 872 males *E. kuehniella* were captured, on three and two delta pheromone traps installed, respectively, in the milling and in the flour storage section, from December 1999 to 25 July 2000, during 34 weeks. Maximal trap catches occurred on 4 July 2000 in the milling section (38.3 mean insects/trap/week) (Fig. 3) and on 6 June 2000 in the flour storage section (39.0 mean insects/trap/week) (Fig. 4).

In the milling section a total of 2185 male *E. kuehniella* was caught in three funnel pheromone traps during 46 weeks, from August to mid June 2001. During the same period of time, in the flour storage section, five funnel traps collected 4307 male *E. kuehniella*. The highest trap catches were recorded on 28 August 2000 with 43.3 mean insects/trap/week, and on 22 August 2000 with 57.0 mean insects/trap/week, in the milling section and in the flour storage section, respectively. The number of *E. kuehniella* males caught per week in the flour storage section was larger from mid August to mid October 2000 when mean temperatures ranged from 25.5-27.1ºC.

Climatic conditions in this region, with mean temperatures around 21ºC were recorded during January and February. Mean temperatures of 27ºC, during August and September were favourable for *E. kuehniella* growth and reproduction as shown by the number of males captured during the entire year, inside this flour mill (Figs. 3 and 4).

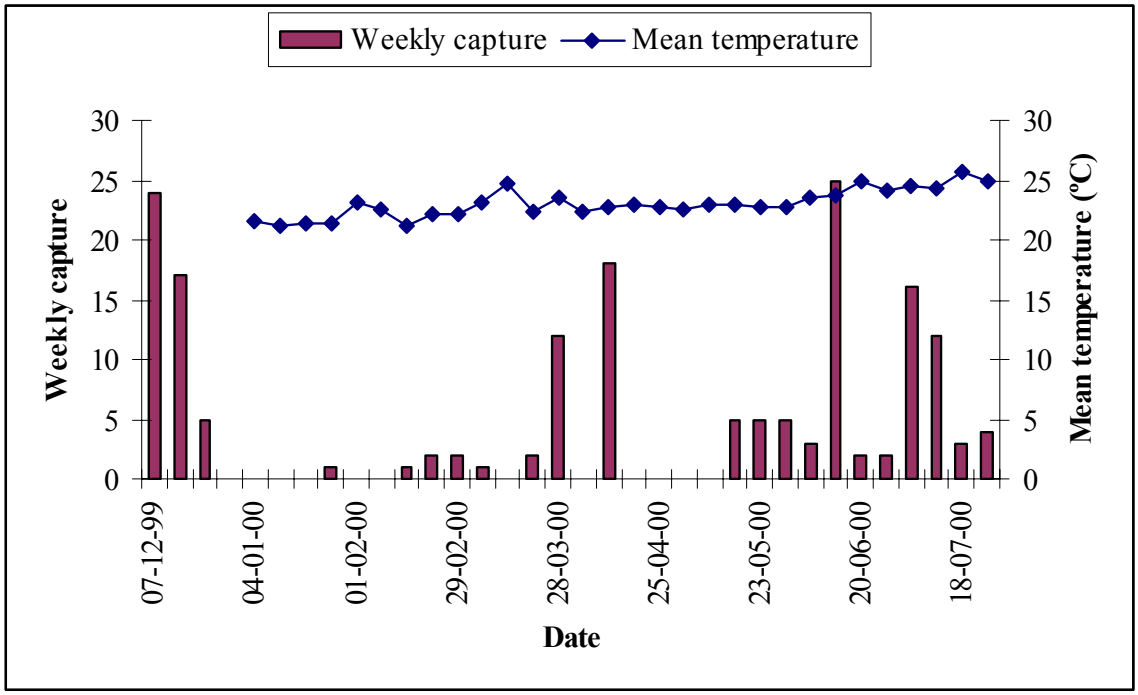

Fig. 1: Weekly capture of *Ephestia cautella* in the wheat storage area with delta traps

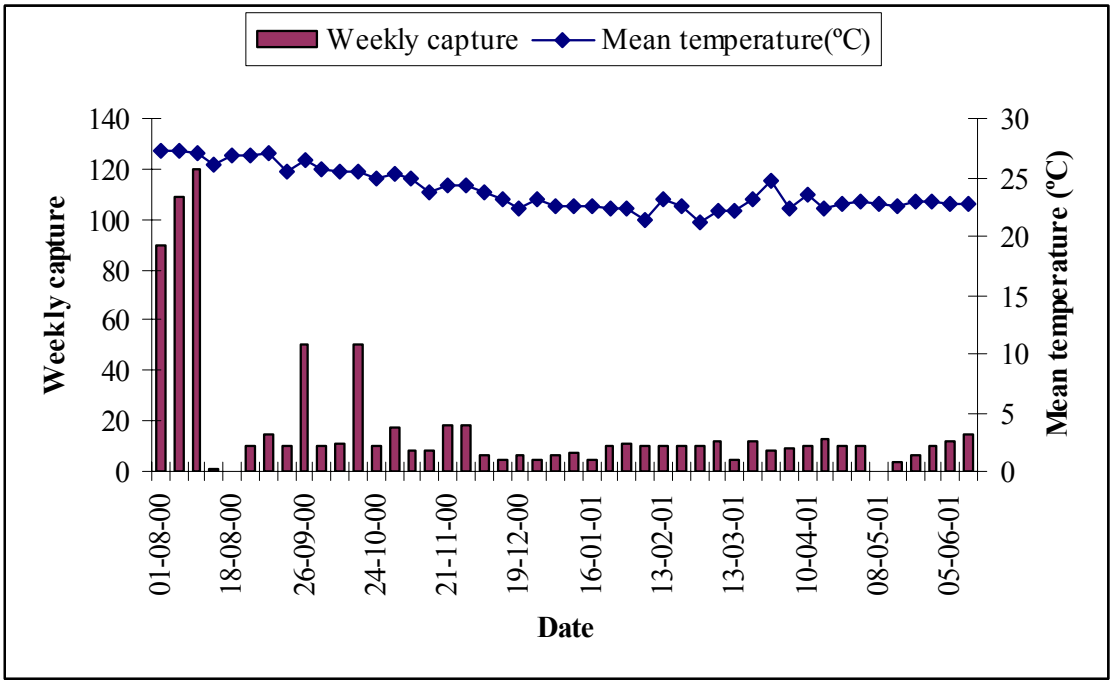

Fig. 2: Weekly capture of *Ephestia cautella* in the wheat storage area with funnel traps

Mean adult populations of *E. kuehniella*, as determined by pheromone trapping, were dissimilar between the two designs of traps. From December 1999 to July 2000, mean pheromone delta trap catches fluctuated from 0 to 38 moths per week (Fig. 5). Differences between individual trap catches over the same period ranged from 0 to 78 males per trap. While mean pheromone funnel trap catches fluctuated from 3 to 50 moths per week (Fig. 6),

differences between individual trap catches from August 2000 to June 2001 ranged from 1 to 180 males per trap.

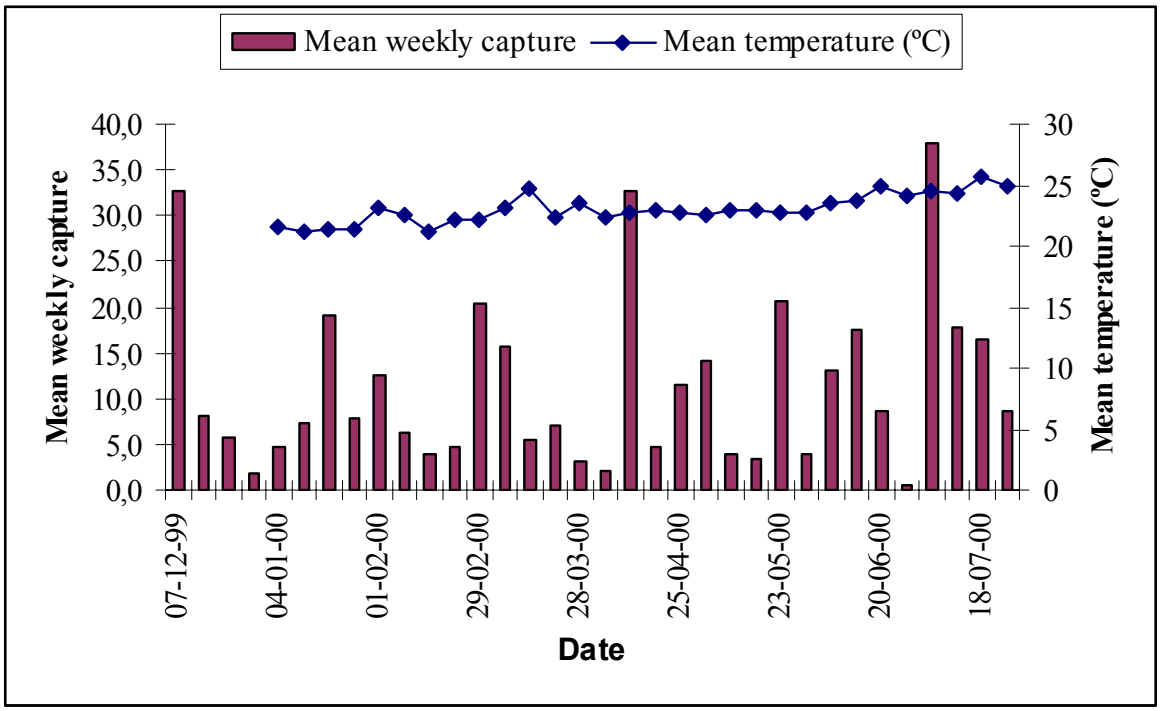

Fig. 3: Mean weekly catch of *Ephestia kuehniella* in the milling section with delta and funnel traps

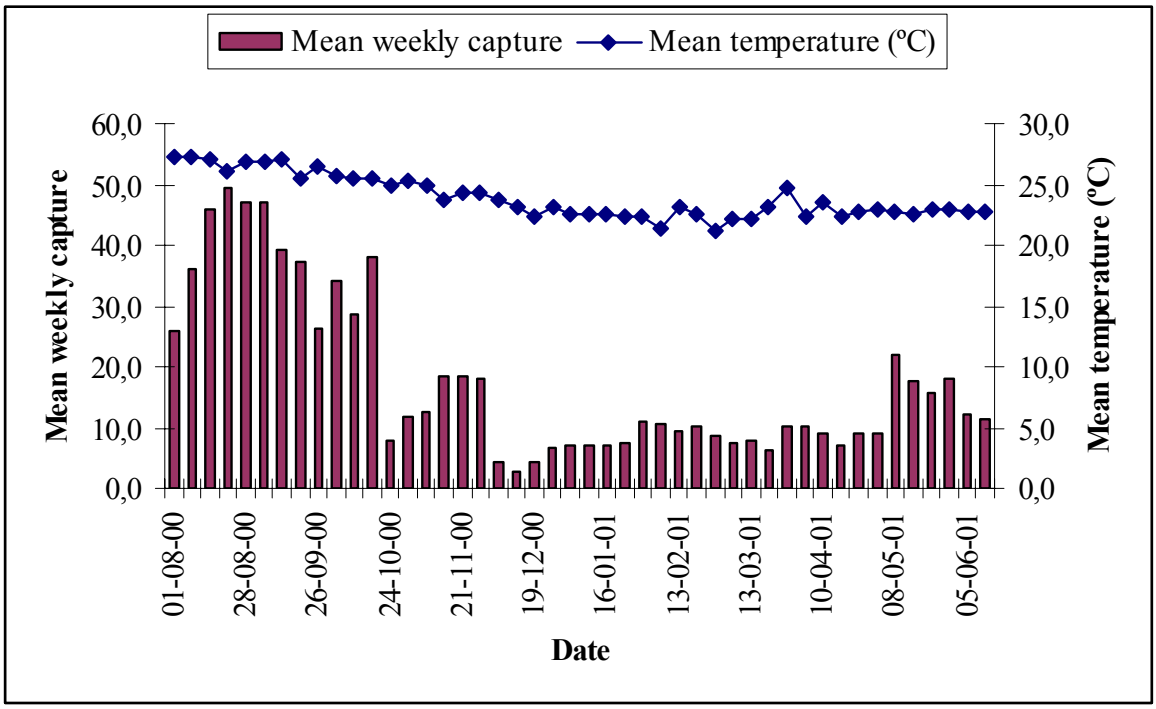

Fig. 4: Mean weekly catch of *Ephestia kuehniella* in the flour storage section with delta and funnel traps

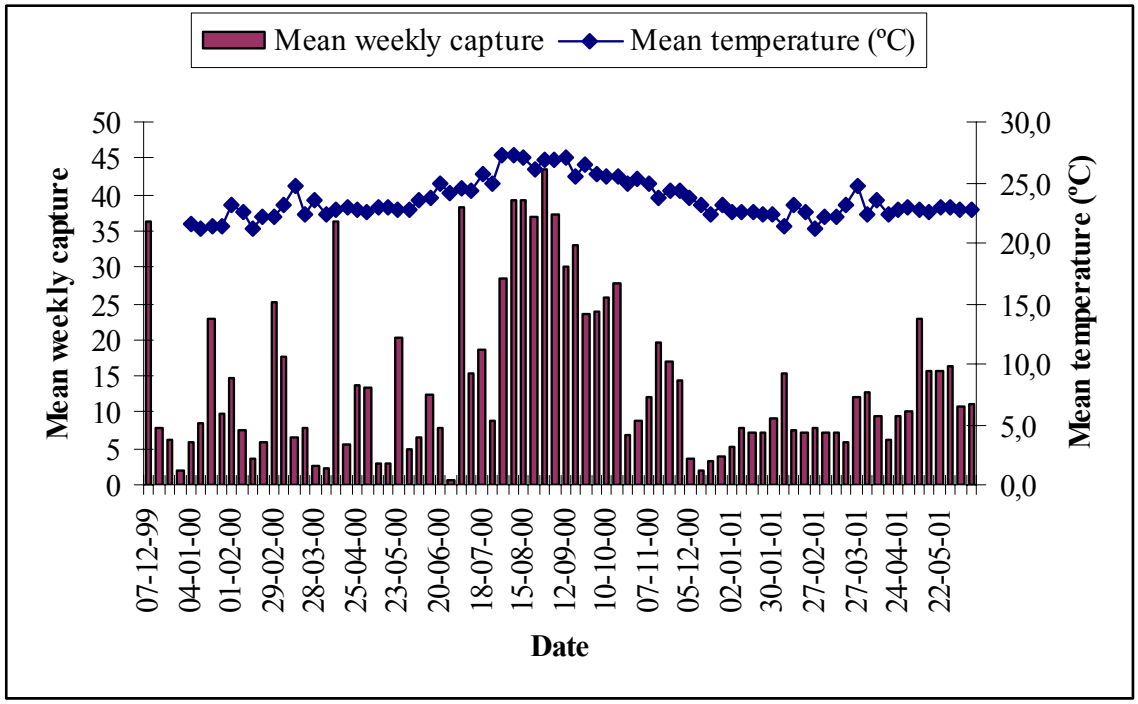

Fig. 5: Mean weekly catches of *Ephestia kuehniella* in five delta traps in flour mill during 34 weeks

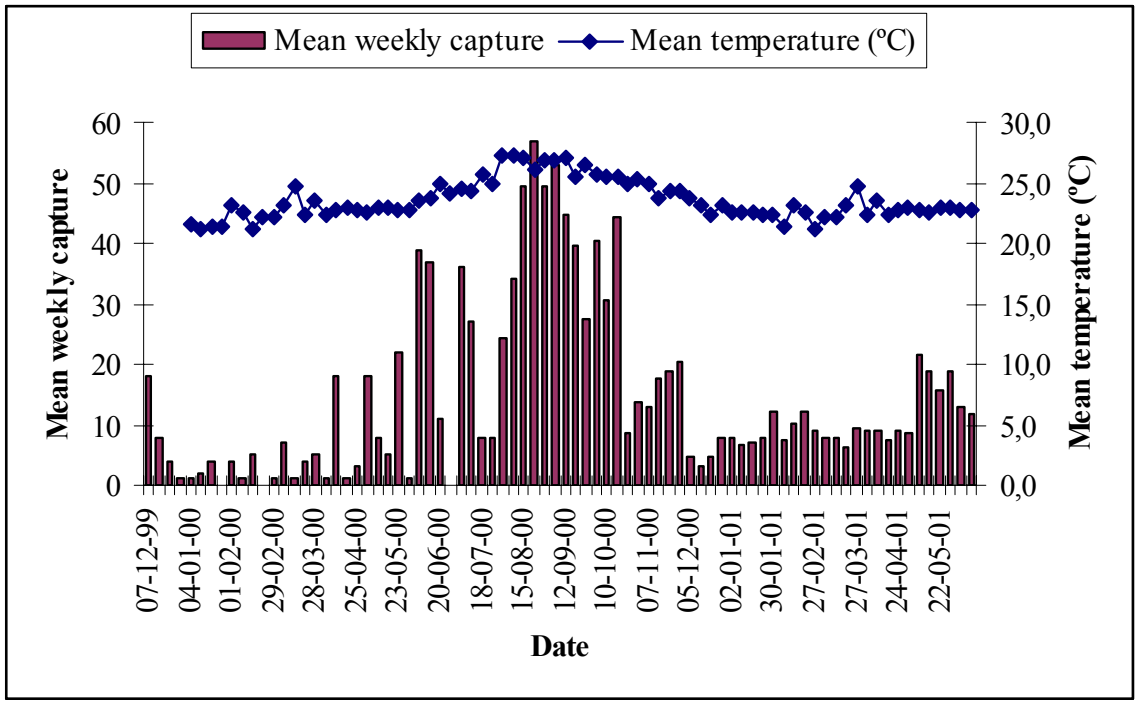

Fig. 6: Mean weekly catches of *Ephestia kuehniella* in eight funnel-traps in flour mill during 46 weeks.

Although *E. kuehniella* was not found in the samples this species turned out relatively abundant during the monitoring period and it showed activity throughout the year with peaks achieved in mid-August, in the rainy season (July to October) with temperatures of 24.4-28.3ºC and 64.0-79.5% r.h.. For both *E. cautella* and *E. kuehniella* their activity peaks are probably related to high environment temperatures. The environmental conditions observed in

Cape Verde were favourable for the development of these insect populations. Therefore, problems caused by insect infestations may occur and be responsible for loss of quality and nutritive value in flour.

Table 4: Index of dispersion for adult males of *Ephestia kuehniella* caught in pheromone traps ([U] Uniform distribution; [R] Random distribution; [A] Aggregate distribution)

N= 5 Delta traps					N= 8 Funnel traps				
Date	Mean	Variance	ID		Date	Mean	Variance	ID	
07-12-99	32.6	473.8	58.1	A	01-08-00	25.9	618.1	167.2	A
14-12-99	8.0	8.5	4.3	R	08-08-00	36.0	1040.6	202.3	A
21-12-99	5.8	4.7	3.2	R	15-08-00	45.8	2693.9	412.2	A
28-12-99	1.8	3.7	8.2	R	22-08-00	49.5	3472.6	491.1	A
04-01-00	4.8	24.2	20.2	A	28-08-00	47.1	2164.4	321.54	A
11-01-00	7.2	59.7	33.2	A	05-09-00	47.0	3181.7	473.9	A
18-01-00	19.2	161.2	33.6	A	12-09-00	39.1	822.7	147.2	A
25-01-00	7.8	19.7	10.1	A	19-09-00	37.1	568.7	107.2	A
01-02-00	12.6	62.3	19.8	A	26-09-00	26.1	513.8	137.7	A
08-02-00	6.2	64.7	41.7	A	03-10-00	34.3	582.8	119.11	A
15-02-00	4.0	6. 0	6.0	R	10-10-00	28.8	347.1	84.5	A
22-02-00	4.8	72.7	60.6	A	17-10-00	38.1	793.8	145.8	A
29-02-00	20.4	494.8	97.0	A	24-10-00	8.0	3.7	3.3	R
07-03-00	15.6	92.8	23.8	A	31-10-00	11.8	27.4	16.3	A
14-03-00	5.4	21.3	15.78	A	07-11-00	12.6	21.7	12.0	R
21-03-00	7.0	17.0	9.7	A	14-11-00	18.5	6.0	2.3	R
28-03-00	3.2	6.7	8.4	R	21-11-00	18.3	14.5	5.6	R
04-04-00	2.0	15.5	31.0	A	28-11-00	18.1	20.1	7.8	R
11-04-00	32.6	583.3	71.6	A	05-12-00	4.3	1.1	1.8	U
18-04-00	4.6	21.3	18.5	A	12-12-00	2.6	0.8	2.2	R
25-04-00	11.6	52.8	18.2	A	19-12-00	4.1	1.3	2.2	U
02-05-00	14.2	71.2	20.1	A	26-12-00	6.5	7.7	8.3	R
09-05-00	4.0	8.0	8.0	R	02-01-01	6.9	5.0	5.1	R
16-05-00	3.4	22.3	26.2	A	09-01-01	7.1	5.0	4.9	R
23-05-00	20.6	272.3	52.9	A	16-01-01	7.3	3.4	3.2	R
30-05-00	4.0	4.0	4.0	R	23-01-01	7.6	2.0	1.8	U
06-06-00	13.0	228.0	70.2	A	30-01-01	11.0	17.7	11.3	R
13-06-00	17.4	373.3	85.8	A	06-02-01	10.5	25.1	16.8	A
20-06-00	8.6	30.3	14.1	A	13-02-01	9.3	7.9	6.0	R
27-06-00	0.4	0.3	3.0	R	20-02-01	10.4	22.3	15.0	A
04-07-00	37.8	565.7	59.9	A	27-02-01	8.6	4.0	3.2	R
11-07-00	17.8	77.7	17.5	A	06-03-01	7.6	6.3	5.8	R
18-07-00	16.6	360.8	87.0	A	13-03-01	7.8	5.4	4.8	R
25-07-00	8.6	3.8	1.8	R	20-03-01	6.3	2.2	2.5	R
					27-03-01	10.4	16.3	11.0	R
					03-04-01	10.45	16.8	11.4	R
					10-04-01	9.1	2.1	1.6	U
					17-04-01	7.0	4.0	4.0	R
					24-04-01	9.1	1.6	1.2	U
					01-05-01	9.1	4.7	3.6	R
					08-05-01	22.0	32.6	10.4	R
					15-05-01	17.6	22.0	8.7	R
					22-05-01	15.8	10.8	4.8	R
					29-05-01	17.9	22.7	8.9	R
					05-06-01	12.0	37.1	21.7	R
					12-06-01	11.4	6.3	3.9	R

Spatial distribution

The Index of dispersion for adult males of *E. kuehniella* is presented in Table 4.

From December 1999 to July 2000, five delta traps were used to monitor males of *E. kuehniella* and they followed mainly the aggregate pattern. From August 2000 to June 2001 the delta traps were changed to funnel traps and the number of sampling units increased from five to eight traps. From August to October 2000 the mean trap catches increased and the males followed the aggregated pattern. From October 2000 to June 2001 both mean sample and sample's variance decreased and the males seemed to follow the random distribution.

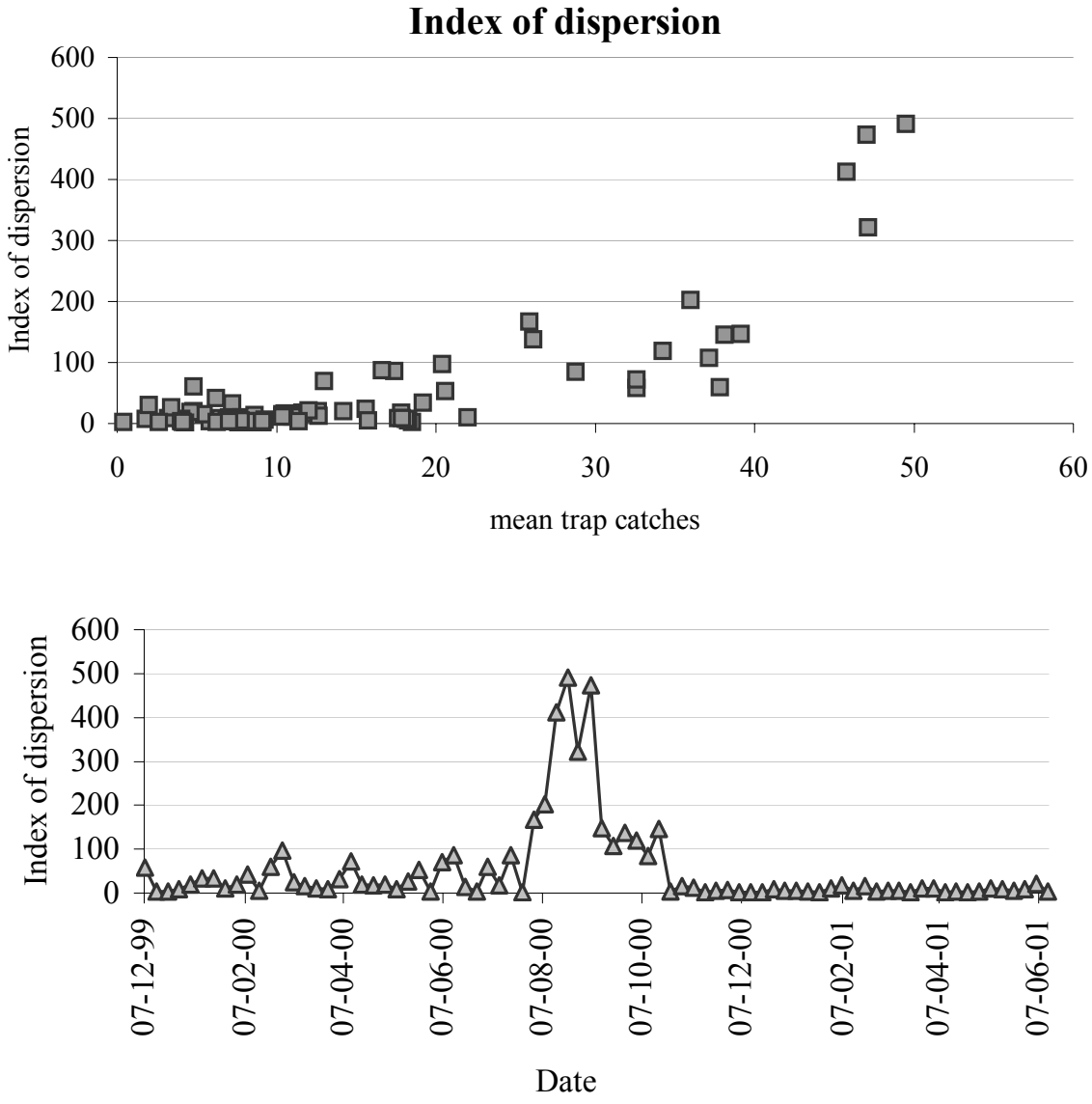

Fig. 7: Index of dispersion for adult males of *Ephestia kuehniella* related to sample mean and sample date.

Fig. 7 shows the Index of dispersion for adult males of *E. kuehniella* related to sample mean and sample date.The Index of dispersion increased with the increase of the mean trap

catches. With the growth of the relative density of *E. kuehniella* males, the insects tended to follow the aggregate pattern.

The change from delta traps to funnel traps and the increase on the number of sample units may work as mass trapping, since the degree of aggregation increased significantly (as the mean trap catches) from July to October 2000. The main spatial pattern of males of *E. kuehniella* changed to random pattern and the mean trap catches and the sample variance decreased, from October 2000 to June 2001.

Acknowledgments

The authors would like to thank the flour mill management for allowing the use of their facilities for these studies and to staff whom assisted in the collection of data.

This work was supported in part by a grant of the Fundação para a Ciência e Tecnologia.

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Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
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Density and spatial pattern of cigarette beetles and tobacco moths in Cape Verde Islands

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Abstract: Pheromone traps were used over a 2-year period to study the density and spatial pattern of *Lasioderma serricorne* and *Ephestia elutella* in a cigarette factory located in Mindelo, Cape Verde Islands. Under such subtropical conditions, *L. serricorne* was considered to be a major pest of tobacco while *E. elutella* was a minor pest. *E. elutella* was recorded occasionally in the regular monitoring and trap catches were very low. The relative population density of *L. serricorne* was high during these trials and the spatial pattern tends to be aggregated.

Key words: pheromone traps, *Lasioderma serricorne, Ephestia elutella*, stored tobacco, population density, spatial pattern

Introduction

The cigarette beetle (*Lasioderma serricorne* F.) and the tobacco moth [*Ephestia elutella* (Hb.)] are the two most common insect species found in stored tobacco and both species feed on cured tobacco leaves. *L. serricorne* is known to infest and consume all stages of cured tobacco and has a worldwide distribution being considered of economic importance (USDA, 1972). *E. elutella* is mainly confined to temperate zones due to its readiness to enter diapause at lower temperatures or in short photoperiods and it is almost excluded from the tropics due to intolerance of long exposures to high temperatures (Cox & Bell, 1991).

Pheromone traps provide an easy, efficient and sensitive means to detect insects in stores and facilities, and managers can use this information to locate infestations and make management decisions (Philips, 1997).

In 1997, when the cigarette factory of Cape Verde Islands was reactivated, the managers were faced with high infestations of the cigarette beetle and some cigarette packs were rejected at that time. It was necessary to implement a monitoring program that started in September 1998, using pheromone traps either for cigarette beetles and tobacco moths.

The main objectives of these trials were to detect sources of infestation, to implement efficient cleaning actions and to provide an empirical economic threshold for taking action for chemical control methods.

Materials and methods

The experiments were carried out over a period of about 110 weeks, from 28 September 1998 to 6 February 2001, in the cigarette factory and related stores (two tobacco stores and a distribution store), situated in S. Vincent Island, in Cape Verde.

Two types of pheromone traps provided by AgriSense-BCS, UK, were used to monitor and to study the spatial pattern of both species:

Mini Delta, for cigarette beetles, is a triangular trap with three exposed adhesive surfaces (130 mm x 110 mm) and presents a pattern of black and white vertical stripes. The traps were supplied with 2 mg of pheromone lure specific for *L. serricorne* (4S,6S,7S)-4,6-Dimethyl-7-hydroxynonan-3-one (Serricornin), enclosed in a small plastic vial and placed in the middle of the trap. These traps were placed about 1.5 m above the floor.

Nine Mini Delta traps were used until July 2000. Due to the introduction of highly infested tobacco in the two tobacco stores, the number of traps was increased to one more trap in each place: one in the factory, one in each tobacco store and one in the distribution store, which contained packs of cigarettes. From July 2000 to February 2001 a total of 13 Mini Delta traps was used.

Delta traps, for tobacco moths, consisted of corrugated plastic (280 mm high x 200 mm x 120 mm) using a replaceable sticky insert and polythene vials. The pheromone dispensers contained 2 mg of (*Z,E*)-9,12-tetradecadienyl acetate. Delta traps were placed about 2 m above the floor level.

Nine Delta traps were used from September 1998 to July 2000, except in October and November 1999, and the number of traps was reduced to five, from August 2000 to February 2001.

The Mini Delta traps, the sticky inserts of the Delta traps and the pheromone lures were replaced every six weeks and the number of insects caught was recorded weekly and identified. The temperature and relative humidity were recorded every week, outside the factory and related stores, from September 1998 to September 1999, and every ten days during 2000 and during January 2001.

Cigarette beetles and tobacco moths caught in each week were used to calculate the mean crowding ($\overset{*}{x}$) according the equation created by Lloyd (1967). The mean crowding is used to describe the mean number of other individuals per individual in an average sample unit and these indices express the level of "crowding" in a given unit of habitat.

The patchiness linear regression examines the relationship between Lloyd's mean crowding and the mean insects caught per week: $\overset{*}{x} = \alpha + \beta\bar{x}$ (α = y-intercept and β = regression slope).

The intercept, α, is termed as the index of basic contagion and has been interpreted as the average number of other individuals living in the same quadrate per individual. When $\alpha=0$ the basic component is a single individual; if $\alpha<0$ there is repulsion among individuals; for $\alpha>0$ the basic component is a colony. $\alpha+1$ gives a measure of clump size.

The slope, β, is defined as the density contagiousness coefficient and is a measure of the spatial distribution of the clumps: when $\beta=1$, the clumps are distributed in a random way; when $\beta<1$ the distribution is uniform, and when $\beta>1$ the distribution is aggregated. SE_α and SE_β are the standard errors of the estimates α and β (Iwao, 1968; Southwood, 1978). r^2 represents the proportion of the $\overset{*}{x}$ variability explained by the linear relation with $\bar{x}$ (Bhattacharyya & Johnson, 1977).

Results and discussion

Environmental conditions

The temperature and relative humidity conditions are shown in Fig. 1.

From September to December 1998, the mean temperature ranged from 22.7ºC (last week of December) to 28.3ºC (in the first week of October) and the mean relative humidity varied from 63% r.h. (last week of December) to 76.5% r.h. (in the third week of October). From January to September 1999, the mean temperature ranged from 19.8ºC (last week of February) to 27.6ºC (in the third week of September) and the mean relative humidity ranged

from 57.5% r.h. (in the third week of January) to 79.5% r.h. (in the third week of September). During 2000, the mean temperature varied from 21.1ºC (in the last ten days of February) to 27.4ºC (in the beginning of August) and the mean relative humidity ranged from 55.5% r.h. (in mid March) to 77.5% r.h. (in the beginning of October). Two seasons were present: the dry season (November to June) with lower temperatures of 19.8-27.3ºC and 55.5-76.5% r.h. and the rainy season (July to October) with higher temperatures of 24.4-28.3ºC and 64.0-79.5% r.h..

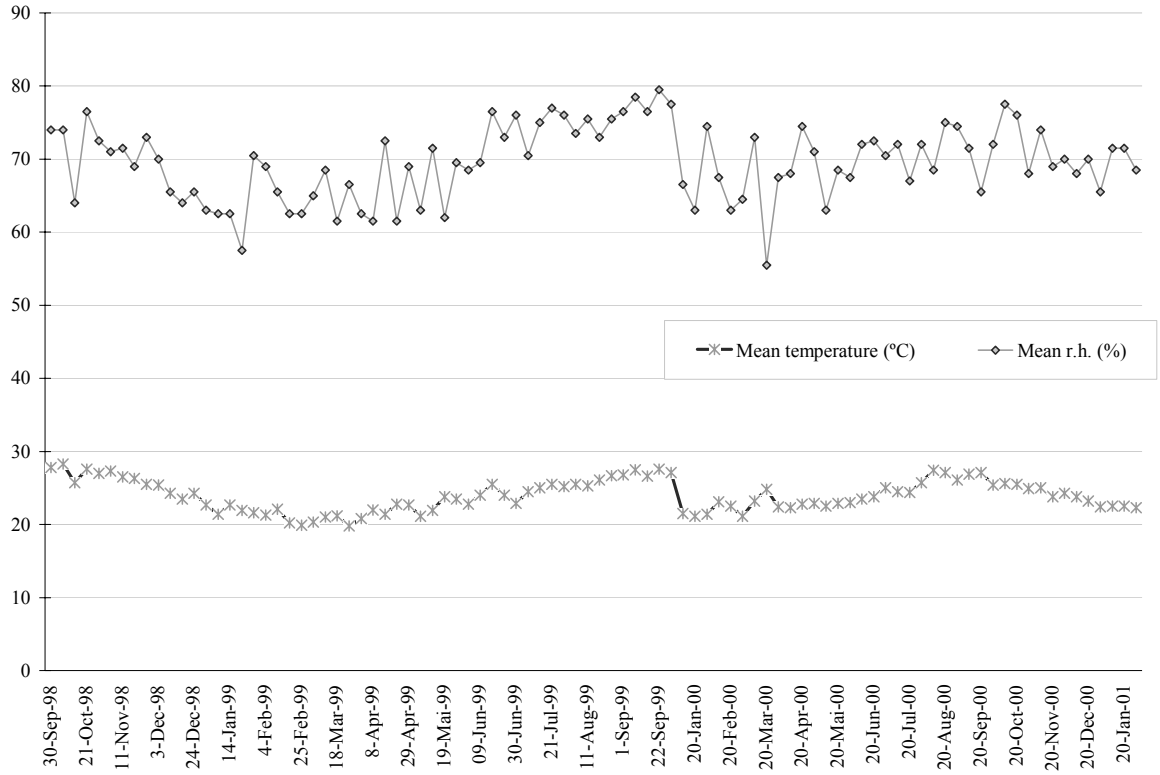

Fig. 1: Environmental conditions outside the cigarette factory and related stores in S. Vincent Island, Cape Verde.

Considering that the larval activity of the cigarette beetle ceases when temperature falls between 19.5ºC and 15.5ºC (Runner, 1919) the environmental conditions were favourable for pest development during the trial periods and hibernation was not likely to occur. Although development of the tobacco moth can be completed from about 15-30ºC, at 30ºC infertility prevents the establishment of a second generation (Bell, 1975) and development and multiplication is optimal near 25ºC and 70% r.h. (Howe, 1965). Mean temperature and mean relative humidity registered in this region throughout the year were considered favourable for *E. elutella* development.

Total of insects caught

From 28 September 1998 to 6 February 2001, a total of 4595 adult males of cigarette beetles was captured: 2537 individuals in the tobacco stores, 1394 in the factory and 664 in the distribution store. During the same period a total of 171 adult males of *E. elutella* was recorded: 34 moths in the tobacco stores, 101 in the factory and 36 in the distribution store.

Relative density

Lasioderma serricorne: The relative densities (mean catch per trap per week) of adult males of cigarette beetles in the tobacco stores, in the factory and in the distribution store, and the mean temperature outside the facility are presented in Fig. 2.

In the tobacco stores, from September 1998 to September 1999 two major peaks occurred: the first, during the second week of December 1998, with 42 insects/trap/week and the second, during the second week of February 1999, with 26 insects/trap/week. From December 1999 to February 2001 the major peak occurred in the last week of July with 62.5 insects/trap/week. During May and June 2000, the number of cigarette beetles caught increased due to the introduction of highly infested tobacco into the tobacco stores.

In the factory, the three major peaks occurred in mid December 1998, with 17.3 insects/trap/week, 17.2 insects/trap/week in the first week of January 1999 and 32 insects/trap/week in the last week of August 2000.

In the distribution store the highest trap catches occurred during the first week of the trial (October 1998) with 48 insects/trap/week, in the first week of April 1999 with 20 insects/trap/week and 25 insects/trap/week in the last week of January 2000.

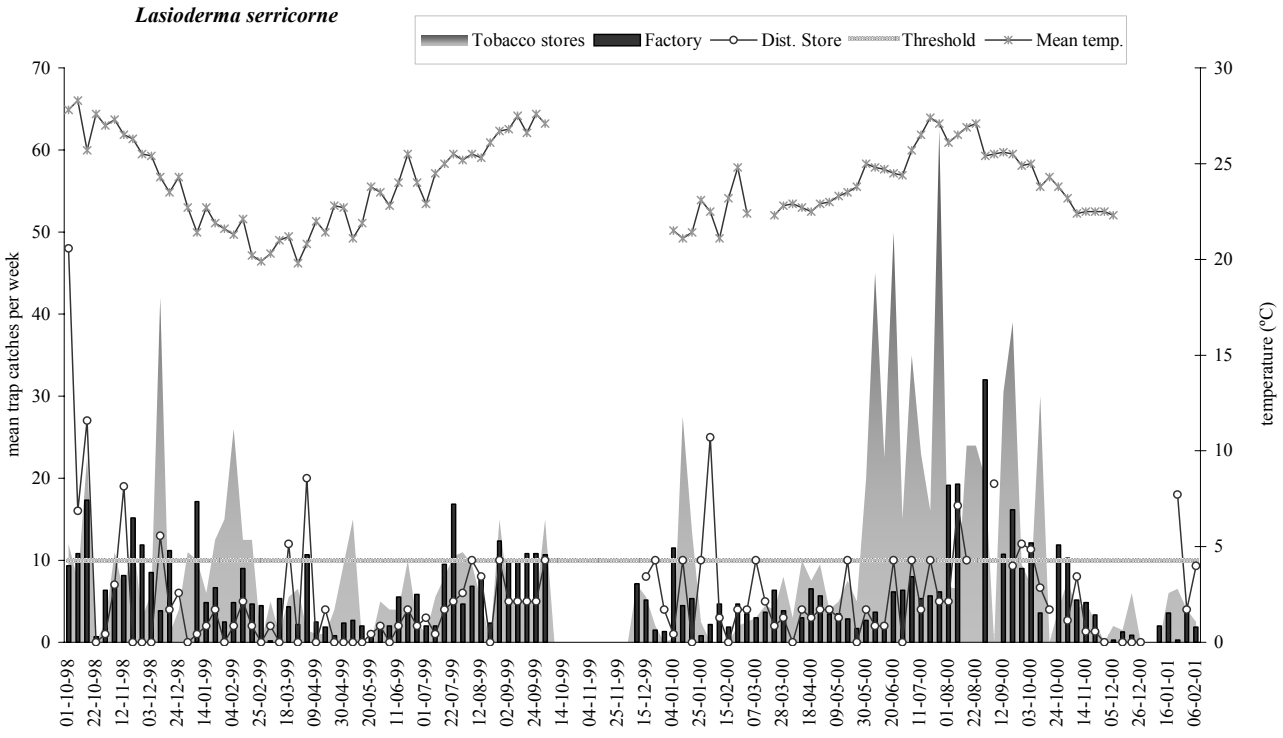

Fig. 2: The relative density of males of *Lasioderma serricorne* trapped in the factory and stores and the mean temperature outside the factory and the threshold level.

The relative density of cigarette beetle males may increase considerably during the dry season but these results could be strongly influenced by the introduction of infested product the tobacco stores, as seen in the results obtained during the year 2000.

The monitoring programme allowed the manager to detect the source of infestation and to improve the efficacy of control methods, such as fumigation, and cleaning techniques. Since the initiation of the monitoring program the manager established an empirical threshold

level of 10 insects/trap/week to initiate sanitation and chemical control actions at the appropriate time. As Fig. 2 shows, these decision tools resulted in a decrease in the pest populations during the trials until May 2000 when highly infested tobacco was introduced in the tobacco stores. After this period it was necessary to increase the number of traps and to improve the sanitation program and the chemical control strategy.

Ephestia elutella: The relative density of adult males of tobacco moths in the factory and related stores and the mean temperature outside the facility are presented in Fig. 3. It was found that the mean number of adult moths caught in the nine traps was very low and the possibility of damage by *E. elutella* was negligible. The number of *E. elutella* recorded from Delta traps remained low from September 1998 to December 1999, declined during 2000 and after August 2000 no records were obtained. The highest relative density of moth population was 2.3 insects/trap/week, observed only in the beginning of the trials. The occurrence of the tobacco moth seemed more frequent during the dry season and this species was absent after August 2000 shown by the absence of captures until February 2001.

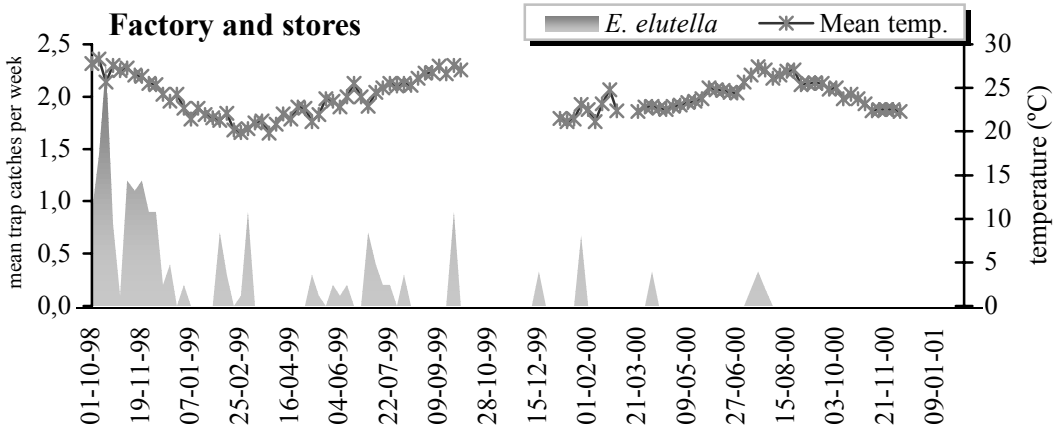

Fig. 3: The relative density of males of *Ephestia elutella* trapped in the factory and related stores and the mean temperature outside the factory.

Spatial distribution

Lasioderma serricorne: Table 1 shows Iwao's patchiness regression estimates for males of cigarette beetle captured in the pheromone traps. The linear regression model provided a good description of almost the whole data set (r^2= 0.66), but the fit of this regression improved as the trials progressed [r^2 increased from 0.39 (from October to December 98) to 0.76 (from 2000 to February 2001)]. The slope β suggested a strong tendency of aggregation in the dispersion pattern related to density (β=2.1) and the intercept α indicates with a probability of 0.90 that the basic component is an individual.

During the first 14 weeks (n) of the trial, the relative density of males was very high and only few pairs $\overset{*}{x}-\bar{x}$ fit the linear regression (r^2=0.39). The results show that the males were distributed in overdispersed colonies with a mean size (α+1) of 7.2 males, but with a probability of 0.36 that the basic component is an individual, distributed in an aggregated pattern (β=1.79).

Between January and September and during December 1998 (43 weeks) more pairs $\overset{*}{x}-\bar{x}$ fit the linear regression (r^2=0.48) compared with the beginning of trials, but these data

still fit this linear regression poorly. During this period the colony size decreased to a mean of 3.6 males and the degree of aggregation also decreased (β=1.3).

During 2000 and until February 2001, the data fit the linear regression well (r^2=0.76). The slope β indicates a stronger tendency of aggregation compared with previous data, and the basic component was an individual (p=0.94) with some degree of repulsion between individuals.

Ephestia elutella: For tobacco moths, Iwao's patchiness regression did not provide good fit to the whole data set (r^2=0.21) (Table 1). Few pairs used in the regression seemed to follow the aggregated pattern (β=1.96) and males seem to form small colonies with a mean size (α+1) of 1.9 individuals.

Table 1: Iwao's patchiness regression estimates for males adults of *Lasioderma serricorne* and *Ephestia elutella* associated to cigarette facility and related stores sampled with pheromone traps

Data	n[a]	$\alpha \pm SE_\alpha$	$t = \alpha / SE_\alpha$ *	$\beta \pm SE_\beta$	$t = (\beta - 1) / SE_\beta$ #	r^2
Lasioderma serricorne						
Whole trial	110	0.1524±1.2750	0.1196 (p=0.90)	2.1082±0.1444	7.6724 (p=0)	0.66
Oct-Dec. 98	14	6.1591±6.4319	0.9576 (p=0.36)	1.7940±0.6416	0.7940 (p=0)	0.39
Jan-Sep. and Dec. 1999	43	2.5844±1.4035	1.8414 (p=0.07)	1.2799+0.2095	1.3364 (p=0)	0.48
2000 and Jan-Feb. 01	53	-0.1252±1.7697	-0.0708 (p=0.94)	2.2683±0.1781	7.1223 (p=0)	0.76
Ephestia elutella						
Whole trial	34	0.8565±0.4378	1.9556 (p=0.06)	1.7351±0.5869	1.2525 p=0.01)	0.21

[a] number of pairs $\overset{*}{x} - \bar{x}$ used in the regression

*: Student test for α=1

#: Student test for β=1

Discussion

L. serricorne was the main pest of stored tobacco in the cigarette factory of Cape Verde Islands. The environmental conditions were favourable all year for the development of this species and it seems likely that hibernation does not occur. Larvae prefer lower humidity when the temperature falls between 25ºC and 28ºC (Fletcher *et al.,* 1973); this may explain one of the two peaks of trap catches that was registered during the dry season.

The relative density of cigarette beetle males did not vary significantly during the monitoring period, when compared with the populations registered under temperate conditions (Genève *et al.,* 1987; Levinson & Buchelos, 1988; Carvalho & Amaro, 1995; Buchelos & Trematerra, 1998). Considering that a species distributed worldwide needs special adaptations in order to minimise restrictions due to environmental conditions, temperature variability is very important from the ecological point of view. Although environmental conditions in tropical regions may be more favourable to development of

cigarette beetles, the higher temperature variability registered in temperate regions may have a more stimulating effect on the increase rate of cigarette beetle populations (Odum, 2000).

Concerning spatial patterns it seems that males of cigarette beetles are distributed in an aggregated pattern; when the relative population of cigarette beetles was very high the individuals may form colonies and compete for the same female.

Kohno *et al.,* (1986) and Howlader & Ambadkar (1995) reported that females mark oviposition sites with an oviposition deterring chemical/pheromone so that conspecific females can recognise those sites and refrain from oviposition in the same site or vicinity. Isolation of this chemical has revealed that it is serricorone, a minor component of the sex pheromone of *L. serricorne* (Imai *et al.* 1990). This natural adaptation ensures dispersal of the species and increases the availability of food for the larvae upon hatching.

The spatial pattern of female *L. serricorne* is not very well known because pheromone traps only catch males. When a strong infestation occurs females are able to disperse from infested to uninfested product. Males may not follow the same behaviour, because mated females may refuse additional copulation and produce some pheromone antagonist (Levinson & Levinson, 1987). Males remain in the infested areas and form colonies with many or few individuals to compete for the increasingly fewer virgin females. In the next generation, due to the dispersed behaviour of the mated females, either the degree of aggregation or the mean size of the male colonies may decrease. The relative population density of *E. elutella* was very low and proved to be economically unimportant in the cigarette factory of Cape Verde Islands. Although the environmental conditions seemed to be favourable to its development, several authors suggest that this species prefers temperate regions and is better adapted to feed on the whole leaf of flue-cured and Oriental tobaccos than finished tobacco ready for processing cigarettes (Ashworth, 1993; USDA, 1972). Iwao's patchiness regression did not provide good fit to the data. Only a few pairs of tobacco moth data used in the regression seemed to follow the aggregate pattern and males may form small colonies.

Acknowledgements

The authors would like to thank the company management, especially Dr. Jorge Benchimol Duarte, for allowing the use of their facilities for these trials and the production staff who assisted in the collection of data. We are also indebted to Prof. Dr. Laura Torres that assisted us in the statistical studies. The research reported in this paper was supported in part by Fundação da Ciência e Tecnologia in the "Projecto no Âmbito da Cooperação Directa com os Países de Língua Oficial Portuguesa".

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IOBC Bulletin Vol. 25 (3) 2002
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Population fluctuations of *Lasioderma serricorne* and *Ephestia elutella* in stored tobacco

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Abstract: Fluctuations in the population densities of *Lasioderma serricorne* and *Ephestia elutella* in stored tobacco were recorded from pheromone trap catches. Both species occurred together and feed on stored tobacco. Seasonal changes in population size are discussed and may result from variations in the external physical environmental factors.

Key words: pheromone trap, *Lasioderma serricorne*, *Ephestia elutella*

Introduction

Lasioderma serricorne F. (cigarette beetle) and *Ephestia elutella* (Hb.) (tobacco moth), both feed on cured tobacco and are pests of economic interest.

Price (1975) suggested for insect species which have similar niche exploitation the need for some modifications or adaptations to coexist such as: character displacement, habitat selection, microhabitat differences, temporal differences and diet. But when two species with very similar needs coexist for long periods, sooner or later they will compete for food and may allow the local extinction or displacement at least of one of the two species. This interspecific competition for the same resources may be not drastic if each species is adapted to different physical environmental conditions.

The main purpose of the present work was to evaluate seasonal fluctuations of cigarette beetle and tobacco moth populations in three stores with different types of tobacco (green Virginia and Burley and processed tobacco) and at different environmental conditions.

Material and methods

Trials were conducted, during 116 weeks, from 12 October 1998 to 30 December 2000, in three tobacco stores belonging to a processing tobacco factory in Portugal:

- Store-1 – with green Virginia
- Store-2 - with green Virginia and Burley
- Store-3 - with processed tobacco

Two types of pheromone traps, New Serrico (from Fuji Flavor Co., Ltd., Japan), and Pherocon® II (from Trécé, Inc., Salinas, USA) were used for monitoring *L. serricorne* and *E. elutella,* respectively.

New Serrico was designed as a slim box (~180mm x 80mm x 7mm) to protect and enclose the two adhesive surfaces. Each trap was supplied with both pheromone lure and food attractant. The pheromone lure, (4S,6S,7S)4,6-Dimethyl-7-hydroxynonan-3-one (serricornin), lasted four to six weeks. The food attractant was obtained by steam distillation of a few kinds of herbs, in order to attract *L. serricorne* females, and lasted two weeks.

The traps were fixed in a position of 1-1,5m height according to a distance which varied from 20m to 25m. A total of 28 pheromone traps were observed weekly and the insects were identified and registered.

Pherocon® II trap consisted of two glued surfaces and had two openings (14 x 5cm) and were provided with a rubber pheromone lure that lasted six months. Traps were renewed every six weeks or whenever they showed dust accumulation. The traps were set at a height of 2-3 meters at a density of one trap per 30 x 30m, approximately. A total of 20 Pherocon® II traps were observed weekly and the insects were registered and identified.

The number of traps used in each store and store areas are presented in Table 1.

The temperature and relative humidity were recorded on thermohygrographs installed in the three stores.

Table 1: Tobacco stores area and number of New Serrico and Pherocon®II pheromone traps in each store

Local	Area (m^2)	New Serrico	Pherocon®II
Store-1	6480	9	8
Store-2	3645	5	4
Store-3	5400	14	8

Results

Environmental conditions in tobacco stores

In Store-1, mean temperatures during 1999 ranged from 10.5ºC (mid January) to 32ºC (mid July) and during 2000 varied from 11ºC (first week of January) to 35ºC (mid July). The mean relative humidity varied from 25.5% (mid July 2000) to 83% (first week of February 2000).

In Store-2, during 1999 the mean temperature registered ranged from 13ºC (third week of February 1999 and last week of April 2000) to 26.5ºC (mid July 1999 and first week August 2000) and the mean relative humidity from 38.5% (last week of August 1999) to 61.5% (third week of February 1999).

In Store-3, the mean temperatures observed in 1999 ranged from 12ºC (last two weeks of February) to 36ºC (mid July) and during 2000 from 11ºC (first week of February) to 34ºC (last week of July). The mean relative humidity, during 1999, ranged from 48% (third week of June) to 84% (mid December) and during 2000 from 30.5% (first week of June) to 72% (second week of February).

Howe (1957) reported that eggs of *L. serricorne* developed and hatched between 20ºC and 34ºC, whilst larvae developed between 20ºC and 37ºC at various humidities, ceases activity when temperature falls 19.5ºC and hibernates at 15ºC (Runner, 1919). Below 15ºC development does not occur and cigarette beetles require 781.9 day-degrees accumulated, above the 15ºC threshold, to complete development from egg to adult, reared on breadcrumbs (Niiho, 1985). Cigarette beetles, between 35% and 95% r.h., showed the same survival period (Khan, 1983) and the optimal temperature for its development ranges from 32ºC to 35ºC (Howe, 1965).

The cigarette beetle records in the traps occurred during the periods with registered mean temperature of 19.5ºC at least and the populations developed well according to increasing temperatures, since the highest values observed reached the optimal *L. serricorne* environmental conditions.

Optimal development conditions for *E. elutella* are 25ºC and 70% r.h. (Howe, 1965) and can complete its development, from egg to adult between 15º-30ºC (Bell, 1975).

Relative density of cigarette beetles and tobacco moths

In Store-1 (Fig. 1), a total of 888 males of cigarette beetles and 682 males of tobacco moths were caught.

During 1999, the first adult emergence occurred, at the end of April for *L. serricorne*, and in mid April for *E. elutella*, and the trap catches did stop, respectively, in the second week of November and in the third week of December.

For cigarette beetles two clear emergence peaks were observed, one in the last week of May, with 11.8-mean insects/trap/week, and other in mid October, with 11.1-mean insects/trap/week.

Tobacco moths were not collected during August and two emergence peaks occurred one during the last week of May, with 4.5-mean insects/trap/week, and other in the first week of November, with 2.25-mean insects/trap/week.

During 2000, the first trap catches occurred in mid May for cigarette beetles and during the third week of March for tobacco moths, although males were trapped in the second week of January (0.25-mean insects/trap/week) and in the first week of February (0.13-mean insects/trap/week).

For *L. serricorne*, two peaks were registered, the first, with 7-mean insects/trap/week, in mid July and the second, with 1-mean insect /trap/week, in the third week of August.

E. elutella was not caught during August and the highest catches occurred in the fourth week of May, with 4.25-mean insects/trap/week, and in mid September, with 1.75-mean insects/trap/week.

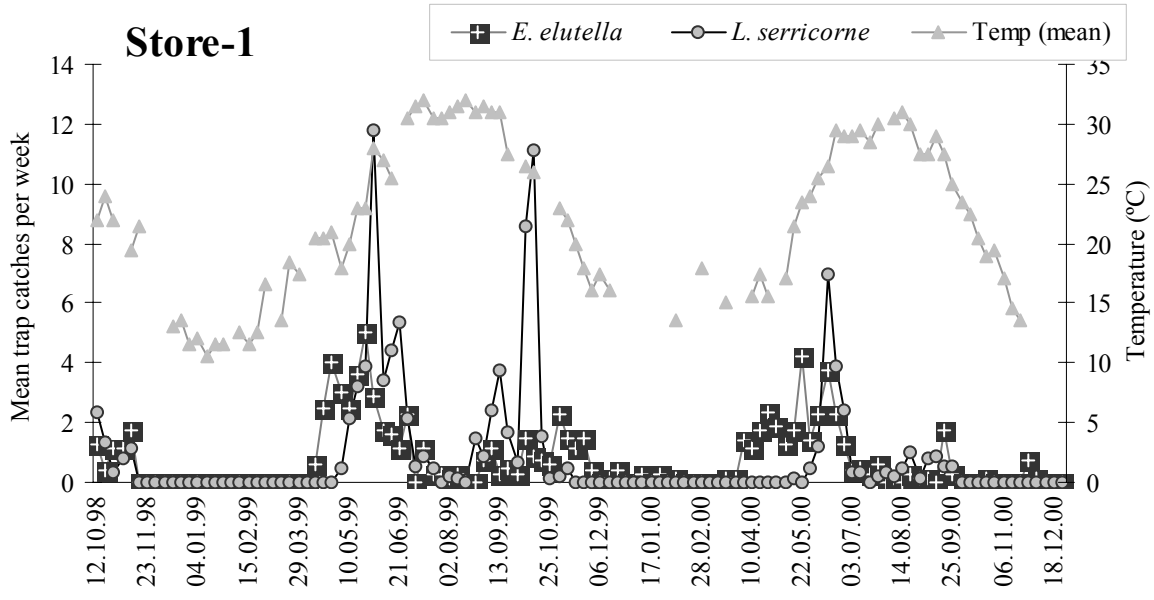

Fig. 1: Relative density of cigarette beetles and tobacco moths and mean temperatures observed in Store-1

In Store-2 (Fig. 2), a total of 44 males of cigarette beetles and 275 males of tobacco moths were collected in the traps.

During 1999, cigarette beetles were recorded from mid June to the final of October. A peak of 1.4-insects/trap was registered in mid July. Tobacco moths were presented from the last week of April to mid November.

During 2000, the capture rate of *L. serricorne* was very low and only five adults were collected during four weeks, between June and July.

The tobacco moths recorded in the traps occurred from mid April to the first week of December and two peaks were registered, the first during the fourth week of May, with 4.5-mean insects/trap, and the second during the fourth week of July, with 2.75-mean insects/trap. From August to December the mean trap catches ranged from 0 to 0.75-insects.

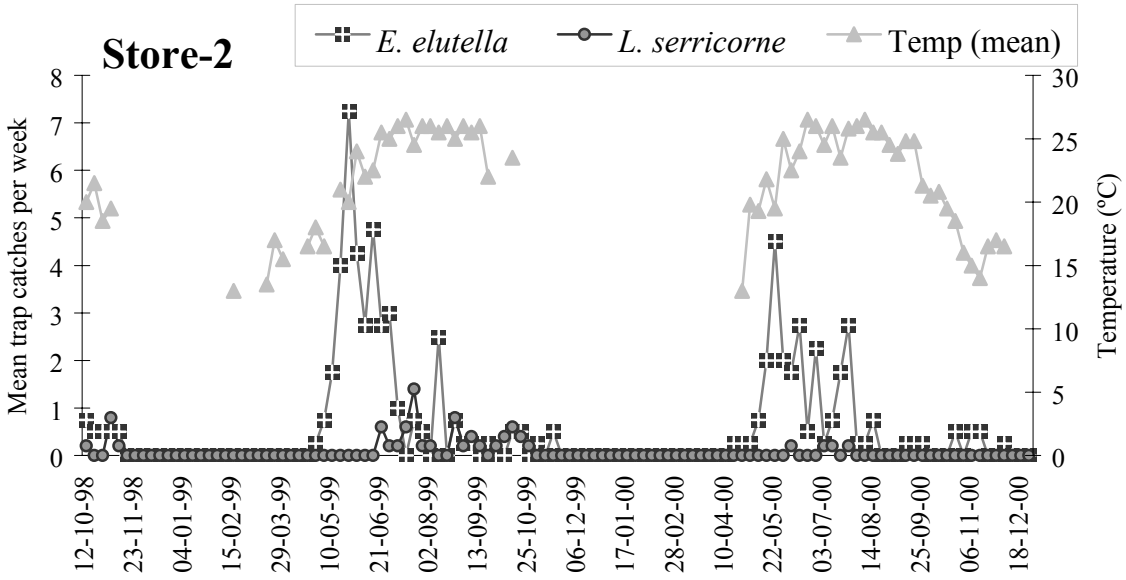

Fig. 2: Relative density of cigarette beetles and tobacco moths and mean temperatures observed in Store-2

In Store-3 (Fig. 3), a total of 1300 males of cigarette beetles and 120 males of tobacco moths were trapped.

During 1999, the first captures occurred in mid April for both species and the last ones were observed during last week of November and in the first week of December, respectively for cigarette beetles and tobacco moths.

For cigarette beetles two peaks occurred, one in the final of June, with 37.3-insects/trap, and the other in the first week of October, with 6.6-insects/trap.

For tobacco moths the two peaks, both with 1.75-insects/trap, were registered in mid July and in mid October. During August and September the rate of captures was very low and ranged from 0-insects to 0.25-insects.

During 2000, cigarette beetles were recorded from last week of May to the fourth week of October, although in mid March and mid April few males were caught. The highest mean trap catches occurred in mid July, with 1.6-insects/trap, and at the end of July, with 2.8-insects/trap.

Tobacco moths were trapped from the beginning of July to the fourth week of October, but the males relative density was very low and ranged from no insects, during nine weeks, to 0.9-insects in the third week of August.

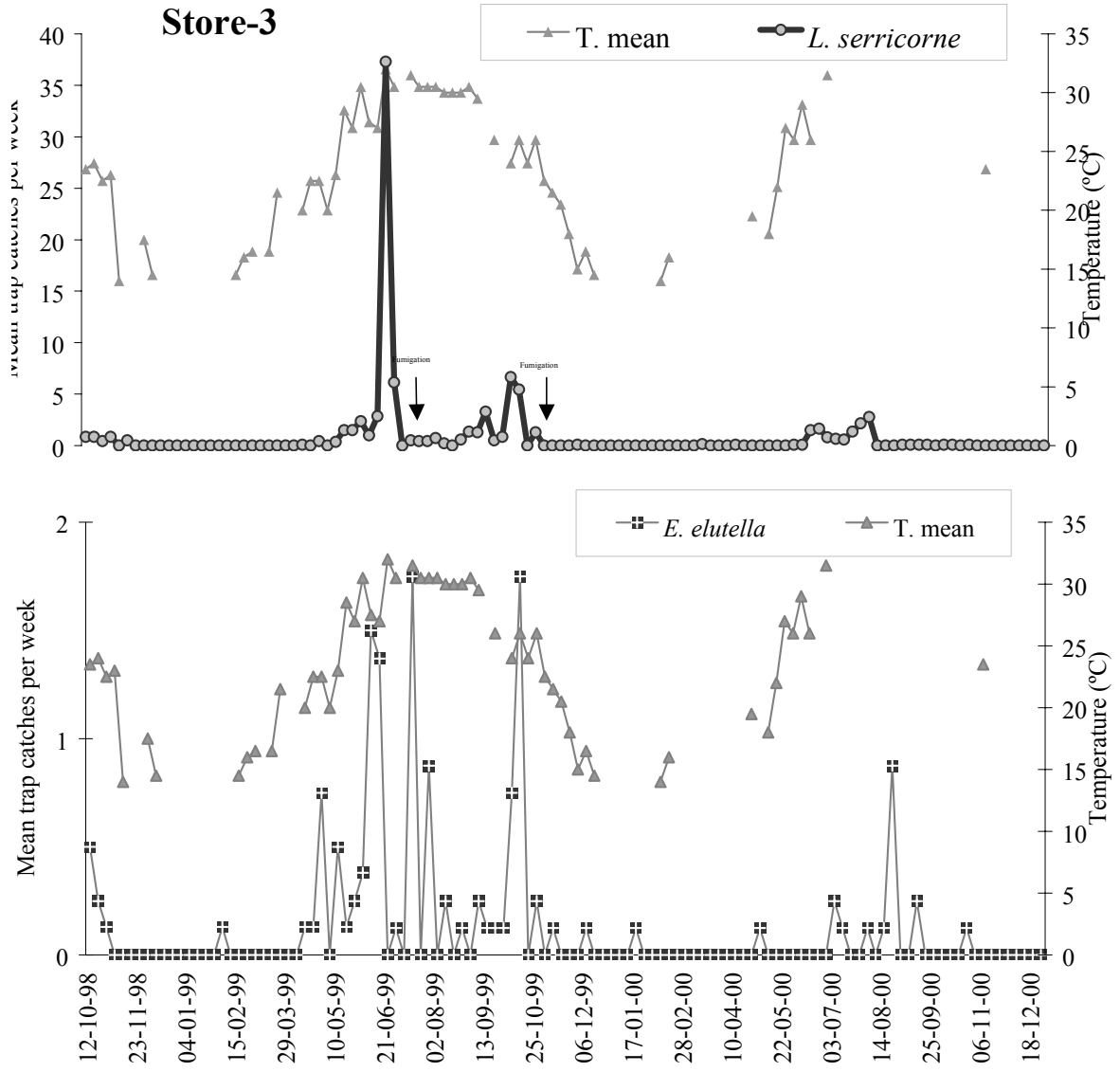

Fig. 3: Relative density of cigarette beetles and tobacco moths and mean temperatures observed in Store-3

Discussion

From 12 October 1998 to 26 December 2000, *L. serricorne* and *E. elutella* were recorded from pheromone traps in the three tobacco stores. *L. serricorne* was the most abundant with a total of 2232 males and *E. elutella* reached a total of 1077 males and, in Store-1, with green Virginia tobacco, and Store-3, with processed tobaccos, the dominant species was *L. serricorne*. In Store-2, containing green Virginia and Burley tobaccos, *E. elutella* was more serious with a total of 275 adults registered while *L. serricorne* reached 44 adults, over the same period of time.

The adult emergence of cigarette beetles occurred as temperature reached the minimal mean value of 19.5ºC, and their relative population grew until the temperature reached the optimal conditions. At least, two generations per year may occur, in stores populations, and larvae of the third generation seem to cease their activity and hibernate when temperature

conditions fall below 19.5ºC. The first adults emerged mainly in May and reached the highest values during June and the peaks of the second generation, which seemed smaller, occurred either in August (2000) or October (1999).

In Store-1 and Store-3 mean temperature reached 32ºC during summer, which are the optimal conditions for *L. serricorne* development. In Store-3 the maximum trap catches occurred when temperatures reached this value and due to fumigation treatment cigarette beetle populations did not keep on growing. In Store-2 cigarette beetle populations were strongly reduced due to lower temperatures and/or the Burley stored tobacco, which is not a favourable foodstuff for its development.

The temperature range over which *E. elutella* development can be completed is about 15-30ºC (Bell, 1975). At 30ºC, the upper limiting temperature for development of *E. elutella*, adults can be infertile (Bell, 1975) and they cannot tolerate long exposures to high temperatures (Cox & Bell, 1991). The temperature thresholds and critical photoperiods of 14hr or less (Bell, 1976) for diapause give support to the view that *E. elutella* is a temperate species.

In this trial, it seemed that summer temperatures above 30ºC resulted in moth populations decrease probably due to the emergence of infertile adults in the beginning of the summer. During summer weekly mean temperatures were 28-32ºC in Store-1, 22-27ºC in Store-2, and 26-32ºC in Store-3, but daily maximum temperatures often reached, 35ºC, 32ºC, and 36ºC, respectively. Environmental conditions registered in 1999 seemed more favourable to *E. elutella* development although its relative population density remained low when compared to *L. serricorne* captures registered in the other stores.

Generally, *E. elutella* overwinters as last-instar larvae, in diapause, in cool climates (Bell, 1976). The number of generations of *E. elutella* in natural conditions is two (Richards & Waloff, 1946) with the first adults emergence at the end of May, a maximum at the end of June and a smaller second generation in September-October; the resulting last instar larvae from the September generation, enter diapause and survive winter in temperate regions (Bell, 1975). In this trial, it seems that emergence of *E. elutella* occurred since the end of April and reached a peak at the end of May and a second smaller emergence was registered in August, September.

Since cigarette beetles and tobacco moths are influenced differently by environmental conditions, it might be possible to have the two species coexisting all time by finding the proper combinations of temperature and relative humidity. *L. serricorne* is more favoured by warmer temperatures (20-34ºC) while *E. elutella* is better adapted to cooler temperatures between 15-30ºC. *E. elutella* showed a wider range of tolerance for physical factors and a lesser degree of dominance than *L. serricorne*.

Acknowledgements

The authors would like to express their appreciation to the company management for allowing the use of their stores and providing the traps for these trials, and to the production staff, particularly Maria Antónia Fernandes, who helped in the data collection.

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IOBC Bulletin Vol. 25 (3) 2002
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A survey of mycological, entomological and storage conditions in agricultural stored products in São Tomé e Príncipe

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Abstract: São Tomé e Príncipe is a country located off the West coast of Africa, in the Guinea Golf. Agriculture is dominated by cocoa production, which constitutes the main export product. A survey of mycological, entomological and storage conditions was conducted in cocoa stores and in stores with other agricultural products (rice, lentil, yam flour, maize flour, milk powder, pepper and coffee). Fungi found were *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Geotrichum* sp. In relation to insects, from a total of 25 identified species, 20 were Coleoptera, and the others were Lepidoptera, Hymenoptera and Heteroptera. Psocoptera and Acarina were also detected but not identified. Most of the stores need to undergo a general rehabilitation and a more careful application of general rules of handling and good storage practice.

Key words: fungi, insects, storage conditions, S. Tomé e Príncipe

Introduction

São Tomé e Príncipe, one of Africa's smallest countries, is located off the West coast of Africa, in the Guinea Golf. The country has two main islands and several small islets. The islands are of volcanic origin, with fertile soil and rich vegetation. Lying on the equator, the climate is tropical humid with a relatively dry season, during June, July and August. The annual average air temperature in the town of S. Tomé is 25.4ºC, with an average minimum of 21.7ºC and maximum of 28.9ºC. The annual average relative humidity of the air is 82%.

The Growth Domestic Product (GDP) composition was, in 1997, by sector: 58% services; 23% agriculture and 19% industry. The islands' agriculture is dominated by cocoa production which constitutes the main export product, followed by copra, coconuts and coffee (Oliveira, 1993). The fall in the world price for cocoa, especially during the 1980s, saw a decrease in the production with an obvious negative impact in its economy.

The annual average production of commercial cocoa during the last five years ranged between 4000 and 5000 t, according to official data.

Domestic food-crop production is insufficient to meet local consumption demands, so the country imports some of its food and also receives substantial help in donations (Carvalho, 1995), especially through the World Food Programme, but also from other organizations (Carvalho, 1995; Espírito Santo, 1999). Efforts are being made in order to increase and diversify food production, so the country is not so dependent on food from the exterior.

In 1966 a team of researchers from CEFA (Storage Pest Management Centre, at that time with a different name) began an in-depth study of storage conditions in S. Tomé e Príncipe. The main objective was the protection of stored cocoa (Gouveia & Sousa, 1967). Pests and fungi in stored products, but especially in cocoa, were identified and the environmental and storage conditions were evaluated (Gouveia & Sousa, 1967; 1968a; 1968b; 1970; Barbosa, 1968; Baeta-Neves *et al*., 1970; 1971a; 1971b; Gouveia, 1976). This work continued until the independence of this country, from Portugal, in 1975.

In 2000 a joint project between CEFA and CIAT-STP (Technological and Agronomical Research Centre of S. Tomé e Príncipe) re-established the connection to storage in S. Tomé e Príncipe.

This paper presents the data obtained during a technical visit of CEFA researchers to this country in September 2000. A mycological and entomological survey was conducted in different stores in S. Tomé island and the storage conditions were evaluated in S. Tomé e Príncipe islands.

Mycological and entomological survey

Material and methods

The experimental procedure is summarised in fig.1.

Results

The results of the mycological and entomological survey conducted in different stores in S. Tomé island are presented in table 1.

In rice, a few *Xylocoris flavipes* (Reuter) individuals (Heteroptera: Anthocoridae), *Cephalonomia tarsalis* (Ash.) (Hymenoptera: Bethylidae) and *Theocolax elegans* Westwood (Hymenoptera: Pteromalidae) were also observed. Psocoptera and Acarina were detected in different food products but were not identified to species level. In store 1 several food products showed rodent excrements.

Storage conditions survey

The survey was conducted in cocoa stores and in stores with other agricultural products. The first group included the stores of cocoa farm enterprises, Água-Izé, Bela Vista, Diogo Vaz, Porto Real and CGI. The second group included the farm enterprises Monte Café (coffee) and Porto Real (copra). A third group included the stores of GGA (Gabinete de Gestão Ajudas – Office for the Management of Food Donations), PAM (Programa Alimentar Mundial –World Food Programme) and Red Cross.

The commercial cocoa storage period is generally short, less than three months. There are, nevertheless, situations where cocoa is stored for periods of up to five months. These longer periods occur more frequently during the lower production season when stocks are built up to achieve required quantities for export.

With the exception in the PAM store, which was recently built, in 1990, it is a common point to the stores surveyed that they need to undergo general rehabilitation. A more careful application of the general rules of handling and good storage practice is strongly recommended as well as a strict follow-up of cleanliness and hygiene rules in storage.

The co-operation between governmental and non-governmental organisations may result in a change from the routine situations to a better usage of the existing storage facilities.

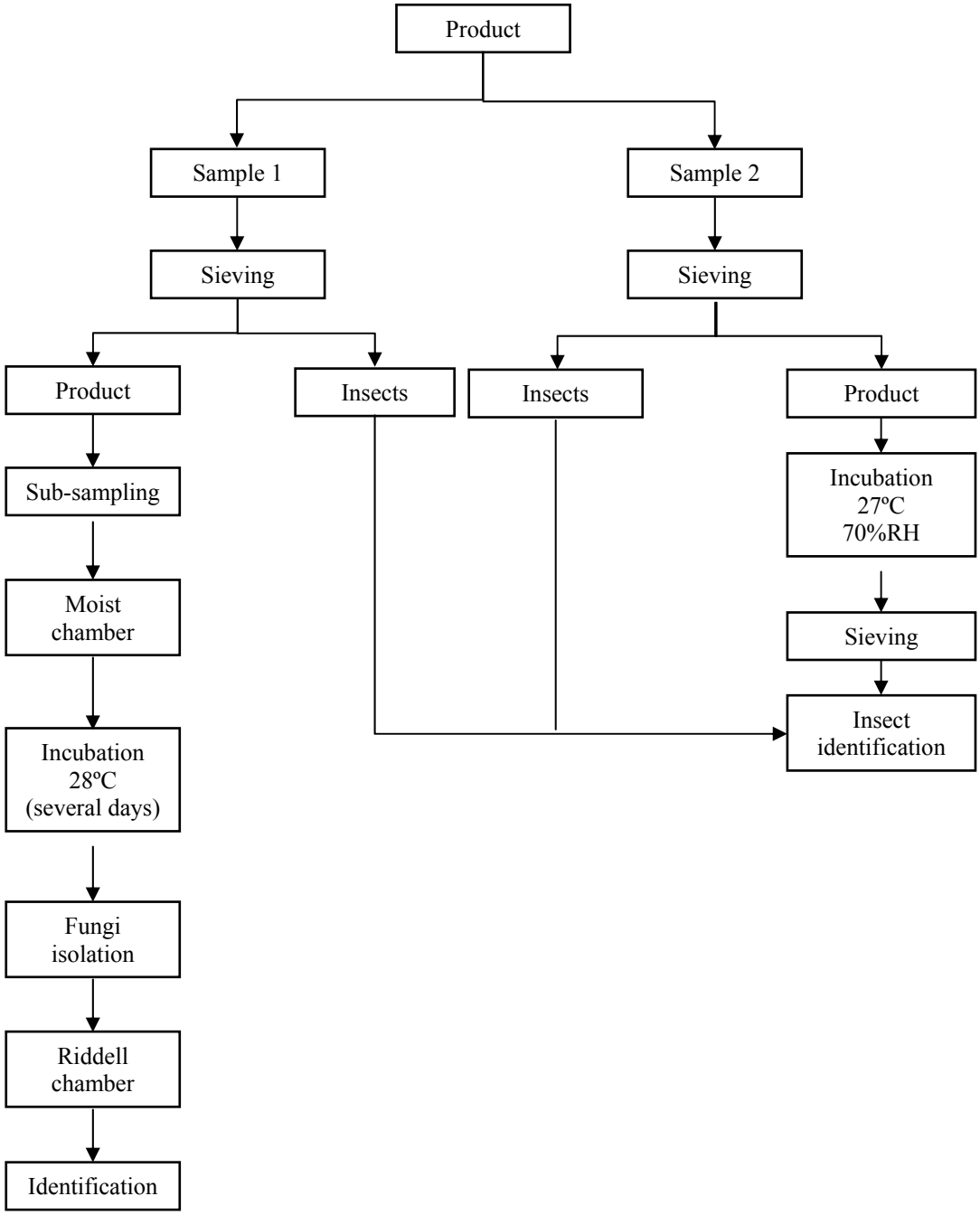

Fig. 1. Experimental procedure in the mycological and entomological survey.

Discussion

The climate in S. Tomé is highly favourable for fungal development. Cocoa is the main export product and levels of mycotoxins are evaluated by the importing countries. So, the country's economy depends on the protection of cocoa against fungal infestation. For this reason, cocoa storage conditions must be reviewed. Losses due to fungi in damp tropical climate countries with less highly developed technology are staggering; an estimate of 5 to 10% of all food production is not unrealistic (Pitt & Hocking, 1997).

In relation to human health it is essential that fungi and pests (insects, mites and rodents) are controlled. Apart from chemical control, local traditional control methods should be surveyed and evaluated for future utilization.

Table 1. Fungi and insect pests (Coleoptera and Lepidoptera) present in samples of agricultural products stored in S. Tomé island.

Product	**Stores**	**Fungi**	**Insects**
Rice	1,2,3	*Aspergillus candidus* *A. flavus* *A. niger* *Aspergillus* sp. *Penicillium islandicum* *Penicillium* sp.	*Sitophilus oryzae* (46%) *Oryzaephilus mercator* (20%) *Cryptolestes pusillus* (14%) *Oryzaephilus surinamensis* (5%) *Silvanus inarmatus* *Cryptolestes capensis* *Carpophilus bipustulatus* *Ahasverus advena* *Silvanus unidentatus* *Tenebrio molitor* *Sitophilus zeamais* *Carpophilus hemipterus* *Attagenus piceus* *Tribolium castaneum* *Tribolium confusum* *Haptoncus luteolus* (n= 470)
Maize flour	1	*Aspergillus candidus* *A. flavus* *A. niger* *Aspergillus* sp. *Penicillium* sp.	*Tenebrio molitor* (79%) *Anobium punctatum* (12%) *Stegobium paniceum* *Corcyra cephalonica* * (n= 58)
Milk (powder)	1	-	*Tenebrio molitor* (n=1)
Lentil	3	-	*Stegobium paniceum* (60%) *Attagenus piceus* (n=33)
Yam flour	3	*Aspergillus candidus* *A. niger* *Aspergillus* sp.	*Carpophilus hemipterus* (86%) *Tribolium castaneum* (5%) *Dinoderes minutus* *Tenebrio molitor* *Cryptolestes pusillus* *Tribolium confusum* (n= 179)
Cocoa	4,5	*Fusarium* sp. *Geotrichum* sp.	*Tinea pellionella* * (53%) *Corcyra cephalonica* * (20%) *Carpophilus hemipterus* *Ahasverus advena* (n=15)
Pepper	6	-	*Ahasverus advena* (n=1)
Coffee	7	*Aspergillus candidus* *A. niger*	*Dinoderes bifoveolatus* (n=7)

Note: Insect species are presented, in each product, by descending order of abundance; they all belong to Coleoptera except those marked with* which are Lepidoptera. Samples were taken in seven stores.

Acknowledgments

The authors thank Engº Ílidio Lucas Tomás for his help in the identification of fungi and Dr. Andrew Polaszek for identification of Hymenoptera. This work was financed by Fundação para a Ciência e a Tecnologia.

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Current status of temperature control in port terminal silos in the Cape Verde Islands

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Abstract: Cape Verde is an archipelago of ten islands located in the Atlantic Ocean, 750 km west of the Senegal coast. The country is largely dependent on cereals, importing around 70-90% of its needs, with maize being the most important with 60-70% of all cereals imported.

The storage system comprises both bulk and bag storage. Two port terminal elevators in two distinct islands guarantee the receiving in bulk from ships and grain storage in bulk for variable periods. Stores along all islands guarantee an additional storage capacity as well as grain handling in bags. One of the port terminal elevators is located in Praia – the capital of Cape Verde – in the Santiago Island, and the other in Mindelo in the S. Vicente Island.

Analysis of data from 1998 to 2000 of the Praia port terminal elevator showed that maize for human consumption, mostly white dent, was stored for longer periods than maize for animal feed, mostly yellow. The respective upper limits of allowed initial moisture content, for storage, of 12.5 %, $^{w}/_{w}$ and 14 %, $^{w}/_{w}$ were largely determinant on the storage length. Although the temperatures were recorded only daily, in some aerations it was possible to follow the cooling/heating front. In some situations, fumigation, bin transfer and aeration made it possible to extend the periods of storage in safe conditions.

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Insects and mites associated with stored products and their arthropod parasites and predators in Khuzestan province (Iran)

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Abstract During 1999–2000, a faunistic survey was conducted in Khuzestan province, Iran to collect and identify insects and mites, including pests and their natural enemies associated with stored products. The faunal studies provided a fundamental knowledge for a better understanding of ecological aspects. The identified species are listed in 3 categories: I) Destructive insects and mites, a total of 29 species from 20 families belonging to 8 orders were collected and identified; II) Insect predators and parasitoids, *Xylocoris* sp., *Habrobracon hebetor*, *Habrobracon brevicornis, Choetospila elegans*, *Pteromalus* spp., *Anisopteromalus* spp. *Cephalonomia tarsalis*, *Laelius anthrenivorus*; III) Predatory mites: *Hypoaspis (Geolaelaps) aculeifer*, *H. (Pneumolaelaps) sclerotarsa*, *Proctolaelaps pygmaeus*, *Macrocheles* sp., *Acarapsis docta*, *Cheyletus malaccensis*.

Key words: stored products pests, parasitoids, predatory mites, Iran

Introduction

Iran produces annually over one million tons of nuts and dates. The average storage losses caused by insects and mites are reported to range from 30 to 35 percent (Freeman, 1958). A study on stored products pests was undertaken at Shahid-Chamran University in Khuzestan province with particular emphasis on entomophagous insects and predatory mites. Khuzestan province is neighburing Iraq, Oman sea, Saudi Arabia, Kuwait, Oman, Bahrain, Qatar, and the United Arab Emirates. As it is now evident that Methyl Bromide is a significant stratospheric ozon depleting chemical, the aim of the present faunistic study is to evaluate the species present for biological control as a potential substitute for chemical methods. Finally, using a biological method can improve the quality of the products and make them more acceptable in international markets.

Materials and methods

To collect insects and mites associated with stored products, 0.5-1 kg samples of feeds, dates, nuts, cereals and beans were taken and carefully checked for pest infestation. Insects and mites were collected by using a Berlese funnel or various sizes of sieves. In addition a vaccum system (aspirator) and different sizes of brushes (such as camel hair brush) were found to be useful tools for collecting, manipulating and handling different insects and mites. Mites were kept in Lactophenol and mounted with Hoyer's fluid. Microscopic slide mounts of mites were prepared and compared with pertinent references for identification such as "Insect and Mite Pests in Food" (Gorham, 1987).

Results and discussion

As a result of our studies we present the following list of species organized in 3 categories:

I) Destructive insects and mites:
A total of 29 species out of 20 families belonging to 8 orders were collected and identified.

II) Insect predators and parasitoids

No.	Genus / species	Order / Family	Sample and / or Host
1	*Xylocoris* sp.	(Hemiptera: Anthocoridae)	Cereals
2	*Habrobracon hebetor* (Say)	(Hymenoptera: Braconidae)	Larvae of *Ephestia* spp.
3	*Habrobracon brevicornis* (Wesmael)	(Hymenoptera: Braconidae)	Larvae of *Ephestia* spp.
4	*Choetospila elegans* Westwood	(Hymenoptera: Pteromalidae)	*Sitophilus* spp.
5	*Pteromalus* spp.	(Hymenoptera: Pteromalidae)	Coleoptera
6	*Anisopteromalus* spp.	(Hymenoptera: Pteromalidae)	Coleoptera
7	*Cephalonomia tarsalis* (Ashmead)	(Hymenoptera: Bethylidae)	*Oryzaephilus surinamensis* (L.)
8	*Laelius anthrenivorus* Trani	(Hymenoptera: Bethylidae)	*Anthrenus* spp.

III) Predatory mites

No.	Genus / species	Order / Family	Sample and / or Host
1	*Hypoaspis (Geolaelaps) aculeifer* (Canestrini)	Mesostigmata: Laelapidae	Barley and Pea
2	*Hypoaspis (Pneumolaelaps) sclerotarsa* Costa	Mesostigmata: Laelapidae	Stored Products
3	*Proctolaelaps pygmaeus* (Müller)	(Mesostigmata: Ascidae)	Barley and Lentils
4	*Macrocheles* sp.	(Mesostigmata: Macrochelidae)	Wheat and Chicken feeds
5	*Acarapsis docta*	(Prostigmata: Cheyletidae)	Acaridae
6	*Cheyletus malaccensis* Oudeman	(Prostigmata: Cheyletidae)	*Acarus siro* L.

Kamali (1989) in his checklist of stored products pests, recorded 191 species of useful and destructive arthropods and rodents from Iran. Freeman (1958) in his report on stored products pests in Iran, recorded 9 species of mites belonging to 6 families.

It could be concluded that wasps and predatory mites are promising candidates for the biological control of stored products pests in the climatic conditions of Khuzestan province.

The most active of all the natural enemies found were in the families Braconidae and Bethylidae.

Most species of Bethylids live a concealed life and are hard to find.

Therefore, little is known about their biology. However, parasitic wasps have been proposed as insecticide alternatives.

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Modular design of "Standard Pest-Monitoring Procedure"

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Abstract: IPM is based on cost benefit analyse. Full costs of monitoring therefore should be included into decision-making process. In this paper is proposed a modular design of "standard pest-monitoring procedure (SPMP)" which enables the flexible economic evaluation of pest monitoring. Economical optimisation of pest monitoring and sampling in stored product environment is discussed.

Introduction

Agricultural IPM is a decision making process (DMP) based on cost-benefit analyse (Mumford, Knight 1997). In other words, IPM is based on comparison of costs of pest damage and costs of negative internalities and externalities of various pest control interventions. Way and van Emden (2000) recommended to define IPM as " the best mix of tactics for a given pest problem in comparison with yield, profit and safety of alternative mixes". To create economically realistic DMP, all types of costs and benefits of pest control mixes should be taken into account, including the full costs of monitoring. Many scientists claim the monitoring a keystone of IPM. However, Way and van Emden (2000) warn that "selective controls demand more monitoring than "supervised" use of broad spectrum chemical controls, and the costs of monitoring in England were once (apple orchards) estimated to be greater than savings when compared with schedules spraying (Fenemore, Norton, 1985)". This alarming message breaks traditional field-IPM paradigm. Do we know enough about cost and benefits of pest monitoring in stored product environment? Hagstrum and Subramanyam (2000) recently concluded: "information available on the cost of monitoring (of stored product pests) is very limited". However, even the fragmentary information now available may have limited value and may resemble something like a "black-box". For example Hagstrum and Flinn (1995) reported " trier sampling costs 0.07 USD/t". It is not clear (i) what type of costs are included (e.g. travelling costs ?), and (ii) how general this information is, since sampling price depends on specific conditions that vary substantially from place to place (e.g. actual local price of labour, price of equipment etc.).

Therefore, I think, farmers and pest control operators need a general decision making-tool to flexibly estimate their "site specific" costs of monitoring. Modular or "brick-work" model, enabling each farmer to build his/her specific "monitoring construction", may be useful. In addition, the modular structure of decision making tool facilitates its incorporation into the future expert systems. This paper is a first attempt at propose a modular design of the "standard pest-monitoring procedure (SPMP)" which could serve as a general framework for economic evaluation of pest monitoring. Economical optimisation of pest monitoring and sampling in stored product environment is also discussed.

Standard pest-monitoring procedure: terms

"Standard pest-monitoring procedure" (SPMP) can be defined as a set of specific activities that are necessary to collect information on changes of population (or injury) on

certain site (field/store) and crop/stored commodity during period of crop planting or storage. Each "site specific" SPMP is composed from various number of monitoring steps (MSs).

Monitoring step (MS) is one inspection of particular field/store and crop/commodity. Each "MS" consists of three (i.e. logistic, sampling and determination) modules:

(i) Logistic module (LM) - Includes return travel activities to the particular store + samples delivery (mail shipment, farmer/messenger trip) to the laboratory.

(ii) Sampling module (SM) -Cover all sampling, detection and travel activities in the store. Samples are taken from, or traps are inserted into so-called "sampling points". No. of sampling points depends on sampling precision level, pest population density and a total amount of the sampled commodity (Hagstrum, Subramanyam, 2000).

(iii) Determination module (DM)- Includes determination activities in the store and laboratory.

Each module may contain various number (n) of sub-modules (SBM_n). E.g. the structure of DM for stored product mites will certainly be more complex than the one for beetles. Also LM in "mite SPMP" may be much more complex: in UK the use "BT trap" for monitoring mites requires the shipment of BT traps by farmers/ food-plant employees to the Central Science Laboratory for pest separation and determination (B. Thind, pers. comm.).

The analysis of key sub-modules in various agriculture environments and particular pest groups remains to be specified.

Estimation of cost of "SPMP"

Cost (C) of the particular project is estimated (e.g. Frank, 1995) as a sum of cost of capital (K) and of labour (L):

$$C = K + L \qquad (1)$$

Then cost of "SPMP" can be estimated:

$$C_{SPMP} = C_{MS} \cdot n = (K_{MS} + L_{MS})\, n \qquad (2)$$

where

n – is No. of monitoring steps (MS) during the period of storage (e.g. n=12 for monthly visits of store during 1 year period)

K_{MS} – capital per one MS (cost of equipment amortisation + expendable supplies)

L_{MS} – labour per one MS (cost of human labour per hour, multiplied by No. of man-hours per MS)

Cost of one monitoring step (C_{MS}) is calculated:

$$C_{MS} = C_{LM} + C_{SM} + C_{DM} \qquad (3)$$

where

C_{LM} - cost of logistic module

C_{SM} - cost of sampling module

C_{DM} - cost of determination module

Measuring the time budget in SPMP

The price of monitoring equipment is relatively stable across various geographical regions while the cost of labour varies considerably. However, time-consumption of particular sampling procedures may be relatively constant. It is therefore useful to understand the

general sampling "time budget". Some (original and literature data on "handling time" for selected SPP sampling and detection techniques are summarised in the Tab. 1. Tab. 2 shows the time required for walking (speed) of a human moving on (i) stored grain surface (wheat kernels) and (ii) concrete floor (empty flat store). The latter information is needed for calculation of the travelling time between sampling points (i.e. trapping/sampling sites) in grain store or food processing plant.

Illustrative example: Data of the Table 1 and Table 2 may provide an illustrative estimate of a sampling time in a sampling module ("SM-time"). In a small flat store with the 40x 50 m area, 12 traps arranged in a 10 x 10 m grid, and an average infestation of 5 beetles per trap, the total SM-time is 1528,7 seconds (0. 43 hour).

The calculation of the above SM-time consumption divided into 4 sub-modules /SBM_{1-4}/ is as follows:

SBM_1 - travel time among 12 sampling points /130 m/ = 206.7 s. ;
SMB_2 - handling time of 12 traps = 252 s;
SBM_3 - looking content and count No. of insects in 12 traps = 936 s;
SBM_4 - caught of each (n= 60) insect in all traps = 136 s.

Table 1. Handling time in various SPP sampling/detection methods

Sampling / Detection Method	**Time**	**Reference (OM - original measurement)**
Grain trier + sieves (200 g wheat infested by 10 adults of *Sitophilus granarius)*	28 s / sample	OM
Grain probe (Storgard WBII) (take out, remove content, insert into grain)	21 s / trap	OM
PC trap (Agrisense-Igrox) (take out, remove content, insert into grain)	16 s / trap	OM
Look content and count No. of insects in one probe trap	1.3 min. / trap (78 s)	Subramanyam et al. (1989)
Each insect caught	0.038 min / specimen (2.28 s)	Subramanyam et al. (1989)
Filth flotation test (CSL)	15 min / sample 30-35 min / 4 samples	B. Thind (in press)

Table 2. Human walking speed on the two store surfaces (Average time consumption ± SE, N = 10)

	1m	10 m (recalculated)
Grain surface	1.59 s ± 0.1	15.9 s
Flour concrete surface	1.04 s ± 0.06	10.4 s

Optimisation of "SMPP" in developed and developing countries may be different.
It is not easy to estimate the optimal number of monitoring steps or sampling points (samples, traps) per particular grain store or food plant. Dent (2000) points out that "too few samples will reduce the value of the estimate (e.g. Vlug and Paul, 1986) and too many will increase cost of the programme, where cost may be measured in terms of time, labour, equipment or financial outlay." Hagstrum and Subramanym (2000) recently reviewed the sampling optimisation in silo/flat stores. It is accomplished by using sequential sampling plan. Generally, with increasing demands on "detection probability" or decreasing population density increases No. of samples, human labour and monitoring costs. Hagstrum and Subramanyam (2000) suggest that "the labour needed to detect insect infestations in grain can be reduced trough automation" - e.g. using acoustical sensors (Hagstrum et. al., 1996), electronic noses (Ridgway et al., 1999), NIR imaging (Ridgway, Chambers, 1998) or molecular technology (Hidayat, et al. 1996). This statement, however, may not be universally valid. Robert Frank (1995), a prominent socio-economists, point out that the economical feasibility to introduce some new technology is not given by the availability of capital investment but rather by the ratio of cost of capital and cost of labour in the particular country. Generally, the point of minimal costs represents the point of contact of iso-quantum curve (Q) of outputs (e.g. products, services) with iso-cost line of inputs (amount of capital & labour), which slope equals to $-w/r$ (w- cost of labour, r - cost of capital) (Frank, 1995). As mentioned above, the cost of monitoring equipment (*capital*) is approximately equal in all countries, while the cost of labour differs dramatically. (For example, Hedges /1998/ refers that a pest control operator's "man-hour rate" may reach 60-100 USD in USA, while in developing countries may be an average "man-hour rate" far bellow 1 USD /Frank, 1995/). Thus the slope of iso-cost line for rich countries $(-w_{RICH}/r)$ differ from the one in poor countries $(-w_{POOR}/r)$. As a consequence, the optimal mix of inputs of capital (K^*) and labour (L^*) differ notably in both types of economies. From Figure 1 it is apparent that in developed countries the most economically optimal "monitoring mix" contains higher proportion of labour than capital. The reverse is true for the developed countries.

What pest controllers can learn from the "retail attraction model"?
Generally, the large district silos are sampled and controlled in a more systematic way than the small stores at farms. Therefore, the relative costs of labour and capital are probably not the only factors that influence farmers DMPs. Human DMP is extensively studied and modelled by micro-economists and socio-economists. I think that their studies may be a valuable inspiration also for the understanding of DMP in pest control. Micro-economists are especially interested in DMP of " buying behaviour" (Hawkins et al. 1989). Beside others, they establish so called "retail attraction model" (or "retail gravitation model") which summaries all key factors influencing a consumer choice of store (retail outlet):

$$MSi = \frac{\frac{Si}{Ti^{\lambda}}}{\sum_{i=1}^{n} \frac{Si}{Ti^{\lambda}}} \qquad (4)$$

where,

MS_i = market share of store i
S_i = size of store i
T_i = travel time
λ = attraction factor for particular product category

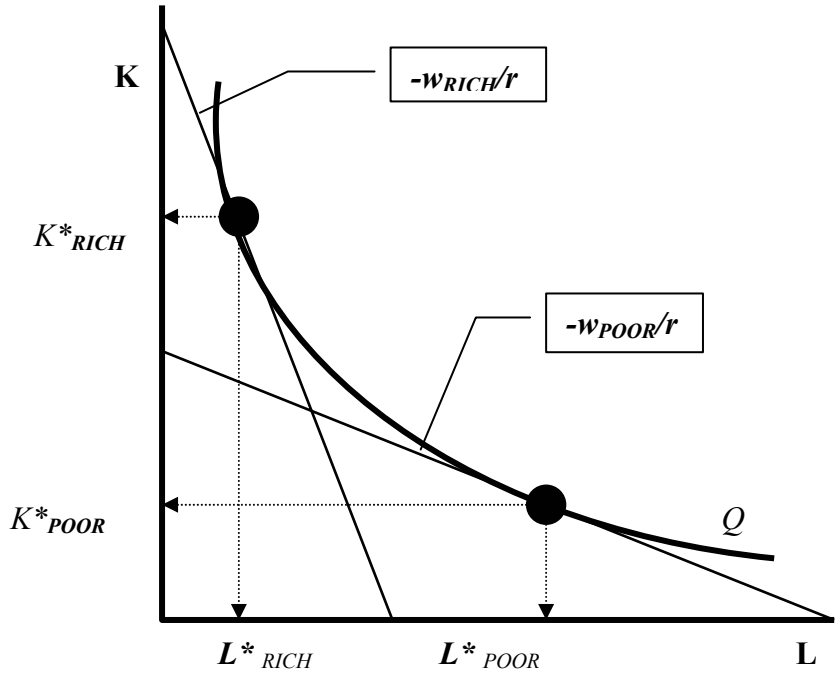

Fig. 1. Optimal mix of capital (K*) and labour differ (L*) in developing (POOR) and developed (RICH) countries (for details see text).

Economical "retail attraction model" demonstrates that the choice of the store is influenced by the following factors: attraction and price of product, size of the store and a distance of the store from the customer. If all other things are approximately equal in a store selection decision, the consumer generally will select the closest store. However, the size of the store, value and attraction of products changes the decision maker behaviour dramatically. Even remote big hypermarkets attract more customers and acquire large market share than the proximate small markets or shops. In various products was estimated various "attraction coefficients" (λ) (Hawkins et al. 1989). For a convenience (low-involvement) item or minor shopping good (e.g. newspapers), the λ is quite large (product "attractivity" is low, however) since shoppers are unwilling to travel very far for such items. However, major high high-involvement purchases such cars or speciality items generate greater willingness to travel distant trading areas.

The above model (4) may seem useless for pest monitoring. However, important similarities can be traced. "Monitoring intensity a frequency " in various stores and commodities is condition dependent. These conditions may include the size of the store, volume of commodity, distance of store from office, and "attractivity" and price of the commodity. These factors are analogical to those, which are recognized as key components of the "retail attraction model (4)". We can imagine that each store, or field of protected crop, "competes" for a "share" of farmer's/pest controller's attention and time, which are limited and expensive. If we assume similar economical behaviour of human in both types of decision making processes, than the "retail attraction model" can generate serious predictions also for pest control. It can be predicted that low price commodities (e.g. grain) in small stores, which are remote from the office, receive very low monitoring and control attention (e.g. low number of inspection during season and limited extent sampling, if any). By contrast, low volume high-price and quality-sensitive stored commodities (seeds, spices, sunflower, poppy seed, rasins, nuts, coffee beans etc.) or high volumes (district grain silos) of low price

commodities (grain) receive large attention and time investment even when dislocated. Many food industry enterprises hire external pest control service ensured by professional pest control operators (PCOs). Similarly in large food production plants can be expected an extensive monitoring programmes, while in small food shops/stores and restaurants will be the pest monitoring, provided by PCOs, rather rare. The frequent pest monitoring of a large number of small enterprises ("low price and revenue pest control contracts"), dispersed across the large town area, is economically very little attractive to PCOs. In these cases the prophylactic or calendar pest control, without monitoring, is much more profitable and competitive.

Acknowledgement

This work was supported by a project MZe ČR-NAZV-EP 9138, and KONTAKT -ME 326/1999.

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Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
99-104

The use of light traps for monitoring flies in a cheese industry in Sicily

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Abstract: Species composition and population dynamics of Diptera infesting a dairy in Sicily were monitored weekly using light traps from November 1998 to October 1999. During the year, mean captures of Diptera were highest in November (2185.5) at the beginning of the study. From December to March, population of flies were strongly depressed. In April and May the mean captures increased rapidly (from 321 in March to 876 in May), maintaining these values until October (1538). The highest number of pests were recorded in the production area of cheese. A brief comment is given for each species recovered more frequently.

Key words: Diptera, Muscidae, Phoridae, Sphaeroceridae, Psychodidae, Fannidae, Milichidae, IPM.

Introduction

The need of better hygienic standard in food production, as a consequence of the European Commission Directive on the H.A.C.C.P. (Codex Alimentarius Commission, 1995) gave further opportunities to develop IPM programs to control pests, with the aim to optimise the production techniques in the food industry.

Dairy production is attractive for arthropods, especially for Diptera (Ottogalli *et al.*, 1979; Trentini *et al*., 1997; Süss & Locatelli, 2001). Favourable environmental conditions (both temperature and moisture) and unlimited pabulum permit the insects to wholly express their biological potential (Domenichini, 1976; Hegazi *et al*., 1978). The presence of flies in dairy could represent a serious hygienic-sanitary risk (Greenberg, 1973; Süss, 1997). Knowledge about the species composition of flies infesting dairies is a fundamental step to improve hygiene standards (Santini, 1995) and to apply IPM programs (Trentini *et al*., 1997; Süss & Locatelli, 2001).

In Sicily, the temperate weather conditions throughout the year, represent an additional favourable factor for the development of flies.

In the present study, the objectives were to investigate the species of flies present, the level of infestation at a given time period, the seasonal changes in fly populations, and to determine the effectiveness of fly traps as monitoring devices. Moreover, information was gathered from literature for each species recovered in higher percentage are given, in the perspective to better understand the role of flies on diary in Sicily.

Material and methods

Observations were made from November 1998 to October 1999 in a dairy recently built, located in Zafferana Etnea (Catania). In the neighbourhood a sheepfold and a poultry house for commercial egg production are located. Each entrance to the dairy building is provided with double doors and anterooms. Monitoring of flies was carried out using 10 Turbo 3000 light traps (TRAPPENS®, Milan). The device consists of a special 32 Watt ultra-violet

lamp that attracts flying insects, and a turbo-suction system with an axial-radial profile fluorescent propeller that sucks them towards a pull-out collection drawer. Inside the drawer the insects die by dehydration. With the aim to determine the location in the building with the highest risk of infestation, traps were hung 2 m above the ground in three different areas: two in the chemical and microbiological laboratory (A), four in the production area of cheese (B), and four in the packaging area (C). All traps were checked and emptied weekly. In the laboratory, all collected insects were counted and identified with a stereoscope microscope. Species determination of flies was carried out by specialists.

During the experiment the management staff carried out weekly treatments with chemical formulations (permethrin and piperonil butoxide) inside the building. Moreover, bioallethrin and bioresmethrin were applied daily using an automatic timer distributor. During the peak of infestation, a larvicide (ciromazine 2%**)** was sprayed two times in the surrounding area of the building.

Trap captures were expressed as the mean numbers of flies caught per trap per week. Differences in the capture among the three areas were determined using analysis of variance (ANOVA). Significant ANOVAs were followed by the Tukey mean separation tests ($P = 0.05$), using Statistica V.5.1. (Statsoft Inc., 1996).

Results and discussion

The mean numbers of captured Diptera throughout the year with the traps located in the different areas (A, B and C) are summarized in Table 1, and annual fluctuation are graphically given in Figure 1. Data show that November was the month with the highest captures of flies. In this month the mean number of collected Diptera significantly differed in the location ($P < 0.05$), being higher in locations A and B (529.5 and 1576, respectively) than in C (80). The numbers of flies caught strongly decreased in December, and reached a low level in February and March, probably caused by the low temperatures in these months. Beginning in April the captures increased constantly , especially in locations B and C. In these latter, the captures recorded from August to October were always significantly different with those of A ($P < 0.05$) reaching a second peak in October (512.7 in B and 900 in C). Data on the mean total captures show that location B was significantly ($P < 0.05$) more infested (1796.3) than locations A and C (841.3 and 1045.1, respectively).

Fly species and mean numbers of Diptera families collected are reported in table 2 and 3. Largest numbers of flies caught were of the family Muscidae.. Their presence was almost negligible from November to February, but increased significantly ($P < 0.05$) from the beginning of March and persisting at high level until October (Table 3). Muscidae were significantly greater in local C, especially during the period July-October (300.6). Our data are in agreement with those of Trentini *et al*. (1997) who reported that the frequency of Muscidae in dairies was higher from July to November. Within this family, the species mostly recorded (Table 2) was *Musca domestica* L., a pest commonly found in the food industry and dairies (Trentini *et al*., 1997; Süss & Locatelli, 2001) and a primary object of most fly management and control programs (Axell & Arends, 1990). It is probable that the neighbourhood with sheepfold and poultry strongly increased the presence of this pest. The false stable fly, *Muscina stabulans* (Fallén) and *Morellia hortorum* Fallén were also recorded even if in small number. The first species has habits similar to those of the house fly with which it frequently coexists, and it is typically present in poultry houses for commercial egg production (Axtell & Arends, 1990). *M. hortorum* is a fly normally associated with cattle farming (Hillerton, 1988).

Table 1. Mean number ± standard error and range of Diptera caught in light traps in chemical and micro biological laboratory (A), production area (B) and packaging area (C). Means within a row followed by the same lowercase letter are not significantly different Tuckey'test, P>0.05).

	A	B	C
Nov.	529.5 ± 233.5 a (296-763)	1576.0 ± 635.0 b (941-2211)	80.0 ± 36.0 c (44-116)
Dec.	272.2 ± 65.8 a (142-449)	342.5 ± 139.4 a (125-731)	53.5 ± 36.7 b (0-159)
Jan.	235.5 ± 97.8 a (90-537)	243.2 ±7.0 a (225-259)	1.0 ± 0.6 b (0-2)
Feb.	91.5 ±24.4 a (30-148)	98.5 ± 23.3 a (57-163)	11.5 ± 2.2 b (6-17)
Mar.	133.4 ±17.9 a (84-180)	126.0 ± 20.9 a (73-172)	62.4 ± 9.6 b (32-89)
Apr.	205.0 ±36.1 a (114-287)	373.0 ± 77.1 a (237-589)	176.2 ± 70.8 b (5-336)
May	281.4 ± 45.5 a (192-424)	348.8 ± 88.0 a (116-650)	246.4 ± 67.6 a (225-371)
Jun.	141.0 ±36.8 a (33-197)	394.2 ± 81.5 ab (263-625)	604.0 ± 223.2 b (67-1004)
Jul.	158.7 ±95.5 a (39-444)	352.2 ± 99.7 a (118-605)	406.0 ± 56.6 a (239-481)
Aug.	94.8 ± 24.0 a (46-179)	311.8 ± 51.3 b (152-458)	432.4 ± 101.7 b (221-783)
Sep.	109.5 ± 15.0 a (79-150)	513.2 ± 30.2 b (450-568)	476.5 ±91.5 b (273-665)
Oct.	126.2 ± 13.7 a (93-160)	512.7 ± 81.2 b (293-666)	900.0 ± 77.5 b (247-606)
Tot.	841.3 ± 146.4 a (364-2018)	1796.3 ±435.1 b (394-6034)	1045.1 ± 253.7 b (4-2416)

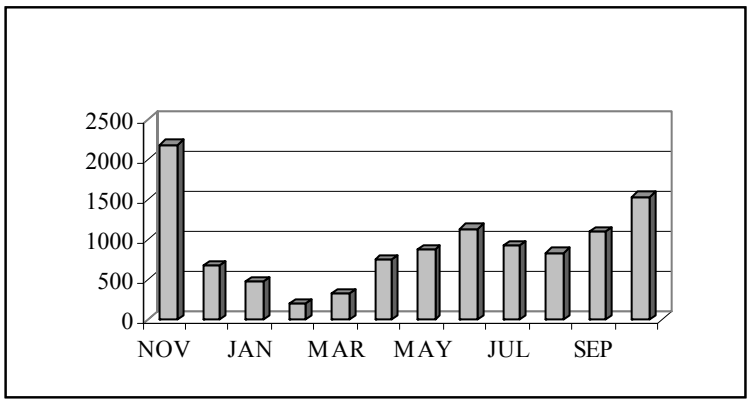

Fig. 1. Mean numbers of Diptera collected from November 1998 to October 1999.

The Phoridae flies were present in high number from November to February (Table 2) and significantly decreased ($P < 0.05$) to a low level in the successive months. They were prevalently concentrated in locations A and B. In A, their presence was consistent throughout the year. Phoridae are the flies recorded in the highest percentage next to Muscidae, (30% and 37.9%, respectively). The most recovered species of the family Phoridae was the filth-inhabiting humpbacked fly *Dohrniphora cornuta* (Bigot), one of the most conspicuous and widespread species, that develops in almost any organic material and human filths (Barnes, 1990). Another largely collected species was *Megaselia scalaris* (Loew), a fly of tropical and subtropical origin that recently became cosmopolitan (Disney, 1991) with a large variety of food preferences (Disney, 1994; Santini, 1998). Unlike *D. cornuta*, this species has been reported several times as causing intestinal and urogenital myiasis in man and mammals (Sherman, 2000). Noteworthy is also *M. rufipes* (Meig.) a cosmopolitan species, recovered in a rather low percentage in this study.

Table 2. Mean numbers (± standard error) of Diptera families caught throughout the year in the chemical and microbiological laboratory (A), the production area (B) and the packaging area (C). Means within a row followed by the same lowercase letter are not significantly different (Turkey' test, P>0.05). *: Syrphidae, Calliphoridae, Sarcophagidae.

	Muscidae	**Psychodidae**	**Fannidae**	**Drosophilidae**
A				
Nov./Feb.	2.5 ± 1.0 aA	9.9 ±2.1 aA	0.5 ± 0.2 aA	0.7 ±0.3 aA
Mar./Jun.	44.4 ± 11.9 bA	23.3 ± 3.1 bA	0.9 ± 0.3 aA	22.5 ± 11.5 bA
Jul./Oct.	44.1± 8.4 bA	5.6 ±1.2 aA	1.2 ± 0.9 aA	1.8 ± 0.6 abA
B				
Nov./Feb.	2.5 ± 1.3 aA	15.1 ±3.2 aA	1.9 ± 0.5 aB	1.6 ± 0.8 aA
Mar./Jun.	69.3 ±18.2 bAB	25.5 ± 5.0 aA	17.1 ± 4.6 bB	18.3 ± 11.0 bA
Jul./Oct.	44.1 ± 8.4 bA	51.5 ± 11.1 bB	9.7 ± 6.3 bAB	8.1 ± 2.7 abA
C				
Nov./Feb.	3.3 ± 1.4 aA	0.6 ± 0.3 aA	1.5 ± 0.4 aB	7.1 ± 4.2 aA
Mar./Jun.	141.7 ± 56.9 bB	4.0 ± 1.9 aB	19.7 ± 4.8 bB	9.6 ± 2.5 abA
Jul./Oct.	300.6 ± 44.1 cB	20.5 ± 7.1 bC	15.8 ± 7.4 bB	15.8 ± 3.9 bB
	Phoridae	**Milichidae**	**Sphaeroceridae**	**Others***
A				
Nov./Feb.	121.5 ± 46.7 aA	-	107.8 ± 19.8 aA	-
Mar./Jun.	22.8 ± 2.5 bA	1.3 ± 1.3 a	67.6 ± 13.6 aA	0.4 ± 0.2 aA
Jul./Oct.	9.6± 1.3 cA	46.5 ± 8.1 bA	9.2 ± 4.9 bA	0.5 ± 0.2 aA
B				
Nov./Feb.	353.9 ± 155.3 aA	-	16.8 ± 11.1 aB	-
Mar./Jun.	78.4 ±10.4 bB	-	80.3 ± 26.9 bA	1.3 ± 0.4 aA
Jul./Oct.	70.7 ± 12.5 bB	12.7 ± 3.9 B	8.4 ± 5.0 aA	1.6 ± 0.4 aB
C				
Nov./Feb.	4.5 ± 3.0 aB	-	1.3 ± 1.1 aB	0.6 ± 0.3 a
Mar./Jun.	7.7 ± 4.1 aC	-	65.5 ± 17.6 bA	0.5 ± 0.2 aA
Jul./Oct.	23.2 ± 3.6 bC	13.2 ±5.1 B	28.1 ± 11.5 bA	2.4 ± 0.6 bB

Coproica hirtula (Rondani) and *Leptocera caenosa* (Rondani) are species belonging to the family of Sphaeroceridae, recovered in a remarkable percentage (16%) (Table 3) from November to June, to decrease consistently in summer and the beginning of autumn (Table 2). Both species are cosmopolitan and usually abundant wherever there are decomposing organic materials (Greenberg, 1972; Papp & Rohàcek, 1987). As their common name "small dung flies" suggests, they are associated with faeces (Greenberg, 1972) and could be vectors for pathogenic microrganisms.

Species belonging to other families were found in smaller numbers (Tables 2 and 3). Among these are the "moth flies", *Psychoda* (= *Tinerea*) *alternata* Say and *Clogmia albipunctata* (Willistone) (Psychodidae), two cosmopolitan species with larvae that breed on decaying organic matter and in sewage fields (Redborg *et al.*, 1983). Their presence was more considerable from July to October in location B.

Table 3. Main collected species of Diptera.

Family	Species	% of capture
Muscidae	*Musca domestica* L.	34.5
	Muscina stabulans (Fallén)	3.1
	Morellia hortorum Fallén	0.1
Fannidae	*Fannia canicularis* (L.)	3.0
Calliphoridae	*Lucilia sericata* (Meig.)	0.1
	Calliphora vicina Rob.-Desv.	0.1
Sarcophagidae	*Sarcophaga carnaria* (L.)	0.1
Drosophilidae	*Drosophila melanogaster* Meig.	2.5
	D. hydei Sturtevant	0.5
Milichidae	*Desmometopa m-nigrum* (Zett.)	3.0
Phoridae	*Megaselia rufipes* (Meig.)	1.0
	M.scalaris (Loew)	11.0
	Dohrniphora cornuta (Bigot)	18.0
Sphaeroceridae	*Coproica hirtula* (Rondani)	10.0
	Leptocera carnosa (Rondani)	6.0
Psychodidae	*Psychoda alternata* Say	5.0
	Clogmia albipunctata (Willistone)	2.0

The little house fly *Fannia canicularis* (L.) (Fannidae) and *Desmometopa m-nigrum* (Zett.) (Milichidae) were found from March to October, with *F. canicularis* prevalently recorded in locations B and C, whereas *D. m-nigrum* was more abundant in location A ($P < 0.05$) (Table 2). Trentini *et al*., (1997) mentioned that the fly was abundant in dairies only in April and May.

The fruit flies *Drosophila melanogaster* Meigen and *D. hydei* Sturtevant (Drosophilidae), are normally recovered in food industry (Süss & Locatelli, 2001). In the present study they were found in low numbers (Table 3) in the period from March to June (Table 2).

During the experiment, other species of Diptera were caught occasionally. Among these, noteworthy were the sheep blowfly *Lucilia sericata* (Meig.), the bluebottle *Calliphora vicina* Robineau-Desvoidy (Calliphoridae), and the fleshfly *Sarcophaga carnaria* (L.) (Sarcophagidae).

The data analysis reveals the presence of several species of Diptera that are indicative of an inappropriate sanitary management. The introduction of these insects into the locations within the diary are probably caused by an improper pest management in the areas around the main production sites (A, B and C) and by the sliding doors. In particular, in this study a high number of Diptera was recorded in the room of microbiological analysis, an area that should be totally isolated and aseptic. The detection of Phoridae and Sphaeroceridae in the production area, with a high probability of larvae being present in the dump system, causes a certain worry as well as the capture of some species of Muscidae that must be linked to the nearby bovine and avicultural stock farms. It may thus be concluded that there is a need to improve IPM of Diptera in Sicilian diaries and in this task light traps were confirmed to be a useful tool for monitoring also in the practical environment .

Acknowledgments

The authors wish to thank Prof. Giovanni Salamanna for the identification of Psychodidae, Dr. Gerhard Baechli for identification of Drosophilidae, Dr. Mauro Gori for the identification of Phoridae and Milichidae and Dr. Lorenzo Munari for identification of Sphaeroceridae.

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BIOLOGICAL CONTROL

Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
105-108

Trichogramma turkestanica against *Ephestia kuehniella* in flour mills: extent of host-feeding and initial results of a field trial

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Abstract: The Mediterranean flour moth *Ephestia kuehniella* is a major pest in European flour mills. The possibilities for biological control of *E. kuehniella* have been investigated. The egg parasitoid *Trichogramma turkestanica* has been subjected to studies concerning the relationship between temperature and parasitism and host-feeding. *T. turkestanica* females killed between 1.7 and 9 host eggs by host-feeding per day, the number increasing with temperature in the range 15°C and 30°C. A field trail was conducted with this natural enemy in an industrial flour; parasitoids were released in flour rooms in two silo buildings. The results varied in the four rooms. Data are being analysed to find an explanation to the different results.

Key words: flour mills, biological control, *Ephestia kuehniella*, *Trichogramma turkestanica*, egg parasitoid

Introduction

The Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is a major pest in European flour mills. In Denmark the major fumigant for control of pests in flour mills, methyl bromide, a suspected ozone depleting substance, was phased out in 1998. In the rest of the European Union a complete phase out in 2004 has been agreed. The possibilities for biological control of *E. kuehniella* have been investigated within the auspices of a national project. The egg parasitoid *Trichogramma turkestanica* Meyer (Hymenoptera: Trichogrammatidae) was selected for further studies of its potential for this purpose. Temperatures in Danish flour mills are relatively low during spring (Hansen, 1998) and the natural enemy must be active and effective at these temperatures. The relationship between temperature and different biological parameters of *T. turkestanica* have thus been investigated (Hansen, 2000a; 2000b; Hansen & Jensen, 2001). The magnitude of host-feeding is reported below in addition to the initial results of a field trial with *T. turkestanica*.

Materials and methods

Insects

The *T. turkestanica* strain had originally been collected from eggs of *Heliothis armigera* Hübner in Egypt in 1981 and named *T. evanecens* "Lager" strain. It had been subjected to studies that were published under this name in Germany (e.g. Schöller, 1996; 2001) and in Denmark (Hansen 1998; 2001a). Specimens of this strain were obtained from AMW Nützlinge, Pfungstadt, Germany to initiate a laboratory colony at the Danish Pest Infestation Laboratory and later for direct introductions in the field trail. The laboratory colony was reared on UV-sterilised eggs of *E. kuehniella* at 27±1°C, 75±5% R.H. and a photoperiod of 16:8 (L:D).

Host-feeding

The studies of host-feeding were part of a major experimental series on the relationship between temperature and parasitism and longevity, described in detail in Hansen & Jensen (2001). Single parasitoids were given 50 fresh *E. kuehniella* eggs per day. After exposure to the parasitoids, the *E. kuehniella* eggs were kept individually and incubated until eclosion. The investigations were conducted at 15, 20, 25 and 30°C (±0.5°C) and 75±5% R.H. Dead unparasitised host eggs were considered to have been killed by host-feeding. The means were adjusted for maximal control mortality (mean control mortality plus SD) to avoid overestimation of the effect of the parasitoid when introduction rates are to be calculated.

Field trial

The trials were conducted in an industrial flour mill, built in 1950 and with a production capacity of 300 tonnes of flour per day. Introductions of parasitoids took place in two concrete silo buildings which had a history of constant infestation with *E. kuehniella*. Building A contains six flour silos, 130 tonnes each. Parasitoids (40,000 weekly) were released in the rooms above and below the silos, ground area 62 m². Building B contains 12 700-t grain silos. Parasitoids (80,000 weekly) were released in the rooms above and below the silos, ground area 284 m². Parasitoid introductions took place from April 12 and until July 5, after which date the effect of the parasitoids would not be seen on the adult flour moth densities until the following year. The pest populations were monitored by the means of pheromone traps (funnel trap with pheromone dispenser (AgriSense-BCS, U.K.: Z-9, E-12-tetradecadien-1-yl acetate)). Due to the small volume of the rooms only one trap was used in each room. Parasitism was monitored on sentinel egg cards replaced weekly. Temperature and relative humidity was recorded by dataloggers.

Results and discussion

Host-feeding

T. turkestanica is a destructive host-feeder, as it kills the host during this process. The average daily host-feeding rate (adjusted for control mortality plus SD) is shown in figure 1. *Trichogramma turkestanica* females killed between 1.7 and 9 host eggs by host-feeding per day. Over the total life-span of the female host-feeding amounted to 43 to 55 host eggs per female at 15 to 25°C and 17 hosts per female at 30°C.

Table 1. Daily host-feeding in *Trichogramma turkestanica* on *Ephestia kuehniella* in relation to temperature.

	temperature			
	15°C	20°C	25°C	30°C
hosts per female per day (adjusted mean ± SD)	1.7±0.1a	5.6±4.3b	5.2±2.2b	9.0±5.4c

Means followed by different letters are significantly different in pairs at $P<0.05$ identified by a Wilcoxon two-sample test on the medians.

Host-feeding, even with this very conservative estimate, was unexpectedly high. At 20° and 25°C it was almost equal to the daily parasitism (Hansen & Jensen, 2001). At 15°C host-feeding was much greater than daily parasitism (0.2 hosts per female per day). This can be a

result of increased nutritional requirements needed to maintain the long life span (32 d) at these low temperatures.

For other *Trichogramma* species the proportion of direct induced mortality (host-feeding and host suppression) varied between 24 and 70% of the total parasitoid impact at 25°C (Vásquez *et al*., 1997). This is in line with the current study where the proportion of host-feeding at 25°C was 41%. Host-feeding is considered to support egg production, while nonhost foods are mostly necessary for maintenance (Jervis & Kidd, 1986). In *T. turkestanica* oviposition and host-feeding both occurred throughout the total female life-span. The parallel course of host-feeding in comparison to parasitism observed in *T. turkestanica* suggests that host-feeding is a prerequisite for oviposition.

Field trial

The flour moth population levels as reflected in trap catches from the four rooms during the trial are shown in fig. 1. For comparison trap catches from three previous years were used. During this period weekly aerosol treatments with pyrethrin were conducted supplemented with residual treatments when necessary.

During the years 1996, 1997 and 1999 (data from 1998 not available) trap catches in three of the rooms remained below 10 moths per trap per day. In room ”B top” peak trap catches of 25 and 28 moths per trap per day were seen in 1997 and 1999, respectively.

In 2000 when *T. turkestanica* were released, flour moth trap catches remained at 10 moths per trap per day or below in two of the four rooms. In a third room, trap catches were almost as low until the end of August after which the population increased to a peak of 50 moths per trap per day and subsequently decreased again. In the fourth room, trap catches exceeded 30 moths per trap per day in July and peaked at 50 moths per trap per day in the beginning of September.

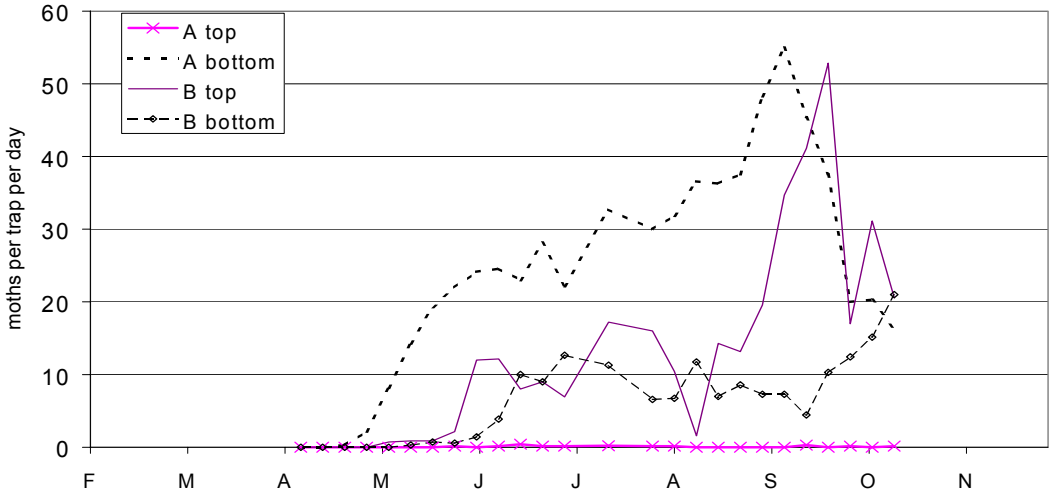

Fig. 1. Trap catches of *Ephestia kuehniella* in four locations in a flour mill where *Trichogramma turkestanica* was released.

Parasitism of sentinel eggs was highest in ”A top”, reaching 100% during most of the introduction period. In the other rooms the maximum parasitism observed was 25%, 45% and 70% for rooms A bottom, B bottom and B top, respectively. Parasitism of sentinel eggs

ceased completely 3 weeks after parasitoid introductions were terminated. Thus it seems that the parasitoids did not establish and reproduce in the mill.

As a preliminary conclusion of the trials: introductions of parasitoids led to the same level of control as weekly pyrethrin applications in two of the four rooms, to partial control in one room and complete failure in the fourth room. The data are being analysed to find factors (temperature, food sources, physical environment) to explain the different results in the four rooms. Observation of parasitism of sentinel eggs supports the assumption that utilisation of this natural enemy must be based on inundative releases.

Acknowledgements

The technical assistance of L. Damberg and U. Cold (Danish Pest Infestation Laboratory) is gratefully acknowledged. This project was supported by the Danish Ministry of Food, Agriculture and Fisheries, grant no. BIO96-SSL-14.

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Oviposition of *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) parasitizing the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

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Abstract: The Indian meal moth *Plodia interpunctella* causes severe damage to stored agricultural products and raw materials for food production like nuts, dried fruits, cocoa beans and even cereal grain. Residue-building contact insecticides or fumigants like phosphine serve as chemicals of choice for the control of this pest. The parasitic wasp *Venturia canescens* has been identified as a natural enemy of larvae of the Indian meal moth and other pyralid moths. Parasitism is a key factor for the evaluation of the suitability of this wasp for biological control. This aspect was investigated at different temperatures and feeding conditions. Ten last-instar larvae of *P. interpunctella* were exposed to one wasp for 4 h. This was repeated at 24 h-intervals until the wasps died. Of the larvae exposed, 76, 56 and 76% were parasitised at 20°C without honey, 20°C with honey and 25°C with honey, respectively. The wasps deposited a maximum of six and eight eggs per host at 20°C and 25°C, respectively. The maximum number of eggs laid per day was 23, 30 and 31 at 20°C without honey, 20°C with honey and 25°C with honey, respectively. The maximum number of progeny per female, 159, was recorded at 20°C with a honey-fed parasitoid. Increasing the temperature from 20°C to 25°C led to deposition of twice as many eggs. Honey as additional diet increased parasitism. Interestingly, the development of the larvae was adjusted to the speed of development, which led to survival of the wasp during winter in diapausing host larvae.

Key words: stored product protection, biological control, endoparasitoid, diapause, oviposition.

Introduction

The parthenogenetic ichneumonid wasp *Venturia canescens* (Gravenhorst, 1829) parasitises the larvae of stored-product moths (Salt, 1964; Harvey *et al*., 1996) including the Indian meal moth *Plodia interpunctella* (Hübner) (Harvey et al., 1994). Ten moth species were recorded as hosts. *V. canescens* is among the best studied natural enemies of stored-product pests. At least 144 publications have been published on *V. canescens* (Schöller, 1998). However, this wealth is mainly due to the fact that it was used as a model organism in basic biology. Few data are available on age-specific fecundity, longevity, percentage of parasitism, intrinsic rate of natural increase and other life-history data relevant for biological control.

The Indian meal moth *Plodia interpunctella* causes severe damage to stored agricultural products and raw materials for food production like nuts, dried fruits, cocoa beans and even cereal grain. At present, this insect is the most important pest in the confectionery industry around the world. Residue-building contact insecticides or fumigants like phosphine serve as chemicals of choice for the control of this pest. Provided that a pronounced reduction of the

infestation can be achieved, biological control is a more attractive solution as it is less toxic to applicators and the environment.

The aim of this study was to determine the parasitism capacity, longevity and preimaginal mortality of *V. canescens* with *P. interpunctella* as host.

Materials and methods

Wasps 2 ± 2h after adult emergence were studied. Ten last-instar larvae of *P. interpunctella* (sex-ratio 1:1) were exposed to one wasp for 4 h in constant darkness. This was repeated at 24 hour intervals until the wasps died. Therefore, it was possible to link every host larva to every individual parasitoid. The experimental vials (diameter 7.5 cm, height 9.5 cm) contained a layer of 20 ml wheat bran and ten last-instar larvae. After exposure, the wasps were transferred to empty vials.

Five out of the ten larvae of *P. interpunctella* were transferred to petri-dishes together with the wheat bran and kept in a climatic chamber at 25°C and 65-75% RH in constant darkness. The petri-dishes were checked daily for emergence of adult wasps or moths. Pupae of *P. interpunctella* from which no adults emerged were dissected after four weeks. The following information was recorded: adult *V. canescens* or *P. interpunctella* (no parasitism), dead larvae of *P. interpunctella*, remnants of larvae of *V. canescens* within pupae of *P. interpunctella*, and missing individuals. This trial was conducted at constant temperatures of 20°C, 25°C and 30°C with honey and without honey and 42 to 45% RH.

The remaining five larvae of *P. interpunctella* were prepared for dissection by keeping them in petri-dishes without food for 24 h and 21°C in constant darkness. During this time, eggs of *V. canescens* within their host increase in volume up to two-fold (Corbet & Rotheram, 1965). The increase in volume eased the detection of eggs during dissection. The weight and sex of the larvae was determined as well as the width of the head capsule. Until dissection, the larvae were kept in a deep-freezer at -18°C. The larvae were dissected in toluol and KOH (0.17 molar) and eggs counted. This trial was conducted at 20 and 25°C.

The preimaginal mortality of *V. canescens* was determined by exposing four last-instar larvae of *P. interpunctella* (sex-ratio 1:1) to one honey-fed *V. canescens* 36±12h after adult emergence. If a larva was attacked by the parasitoid, this larva was removed and replaced by another larva of the same sex. It was recorded if a larva was merely attacked, or parasitised, i.e. the attack was followed by cocking behaviour. Following the exposure, the host larvae were kept in a vial (diameter 2 cm, height 3 cm) with 1 g wheat bran at 25°C and 70% RH for five weeks and checked daily for adult emergence. The ratio of parasitised host larvae and emerged adults of *V. canescens* or *P. interpunctella*, respectively was used for determination of the preimaginal mortality of *V. canescens*. The trial had 11 replicates.

Results

Oviposition of Venturia canescens

On the first day of exposure to host-larvae, all *V. canescens* oviposited less eggs compared to the following two days (table 1), a minimum of 3 and a maximum of 9 eggs. Within the first three days, five unfed females deposited a mean of 42 eggs at 20°C. Provision of honey did not increase the number of eggs laid during first three days at 20°C. However, during the whole life-span the honey-fed females laid twice as many eggs. At 25°C and with provision of honey, the number of eggs laid during day one to three was increased by 65% compared to 20°C.

Table 1: Number of eggs oviposited by *V. canescens* within four hours daily into 10 larvae of *Plodia interpunctella* at two temperatures, unfed or honey-fed.

	20°C / unfed	20°C / honey	25°C / honey
day 1	6	7	13
day 2	17	22	31
day 3	19	14	27
total days 1-3	42	43	71
total life-span	69	129	107

Parasitism during the first three days averaged 73.6%, ranging from 36 to 90%. Of the larvae exposed during the whole life span of the parasitoids, 76, 56 and 76% were parasitised at 20°C without honey, 20°C with honey and 25°C with honey, respectively. Superparasitism ranged from 31 to 44% of all larvae parasitised. A maximum of 6 and 8 eggs per host were recorded at 20°C and 25°C, respectively. The maximum number of eggs laid per day was 23, 30 and 31 at 20°C without honey, 20°C with honey and 25°C with honey, respectively. Maximum oviposition occurred within the first six days. At 20°C, honey fed females parasitised until day 23. During the last three days no more larvae were parasitised. In all trials, some host larvae were superparasitised and some larvae were unparasitised. This was also true in cases where a single female laid more than 10 eggs per day. Parasitisation of all the 10 host larvae exposed per day rarely occurred.

The weights of male and female moth larvae were 11.7±1.9 (mean±SD) and 15.3±2.5 mg, respectively (n=385). This difference in weight was highly significant ($p<0.001$, t-test). At both 20 and 25°C almost half of the parasitised larvae were male, so no sex differentiation by the parasitoids was found.

Progeny of Venturia canescens

The Kruskal-Wallis 1-Way Anova showed a significant impact of temperature and feeding conditions on the number of progeny ($p<0.05$). The maximum number of progeny per female, 159, was recorded at 20°C with a honey-fed parasitoid. Under these conditions, the average number of progeny was about five times that of all other conditions tested (fig. 1). The number of progeny was lowest in unfed females at 30°C. In all trials, significantly more progeny was produced by fed females compared to unfed females (Mann-Whitney U - Wilcoxon Rank Sum W Test, ($p<0.05$).

No significant difference in the number of progeny was found between unfed females at 20 and 25°C, and honey-fed females at 25 and 30°C, respectively. The percentage of emerging adults of moths and parasitoids was comparable for the progeny of unfed and honey-fed parasitoids. Generally, the percentage of emerging parasitoids increased with increasing temperature.

Preimaginal mortality of Venturia canescens

In this trial, 74 *P. interpunctella*-larvae were attacked, 69 of which were parasitised (93.2%). Out of the 69 parasitised larvae, 64 *V. canescens* emerged (92.7%) (Tab. 2). Consequently, the preimaginal mortality of *V. canescens* was 7.3%. From every parasitised larva of *P. interpunctella*, an adult insect emerged, either *P. interpunctella* or *V. canescens*. Again, almost the same number of male and female larvae of *P. interpunctella* were parasitised.

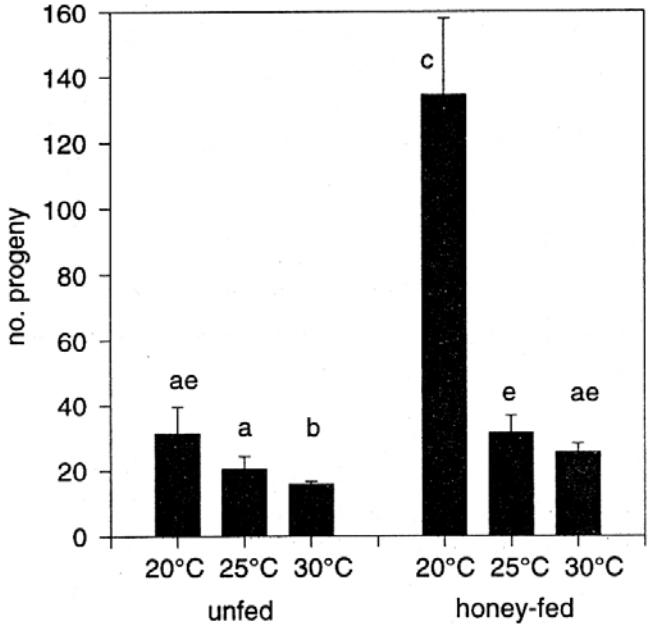

Fig. 1: Total number of progeny of unfed or honey-fed *V. canescens* at three temperatures.

Discussion

The mean of 134 adult progeny per female suggests that *P. interpunctella* is a very suitable host for *V. canescens*. A significant effect of temperature and feeding conditions was shown: parasitism was favoured by higher temperatures and the availability of honey. With *Ephestia kuehniella* (Zeller) as host, Ahmad (1936) found a developmental threshold of the moth between 8°C and 10°C, and for *V. canescens* between 12 and 15°C. At 23°C and higher, the population of *V. canescens* grew faster than that of its host. In this case, high temperature favours the parasitoid, and low temperatures the host.

In the trials conducted to determine the natural mortality of *V. canescens*, either adult *V. canescens* or *P. interpunctella* emerged from parasitised hosts. This result suggests that preimaginal mortality of *V. canescens* occurred in the egg stage, as the feeding activity of the parasitoid larva would have increased the mortality of *P. interpunctella* (Salt, 1964). Moreover, in the trials to determine the number of progeny of *V. canescens*, only 2.9% of the host larvae contained dead larvae of *V. canescens*. These results support the hypothesis that superparasitism increases the fitness of *V. canescens*. If several eggs are laid into one host, the probability that parasitoid progeny develops increases. Rogers (1972) was the first to suggest that *V. canescens* can discriminate between parasitised and unparasitised hosts. A significant discrimination was shown 12 h after the previous oviposition. Information about the status of the host is thought to be obtained by receptors on the ovipositor. We must point out that single individuals were tested, so no effects of interaction between different individuals were considered as in other studies (e.g. Marris *et al*., 1996). Comparing biological data on *V. canescens* is complicated by the fact that *V. canescens* is not a biological species (see e. g. Ziegler et al., 1997). Recent studies have detected significant differences between iso-female lines.

Large and heavy host larvae were shown to increase the size and the progeny of adult *V. canescens* (Harvey et al., 1995). Female larvae of *E. kuehniella* are heavier than male larvae. Based on the study of Kraaijeveld *et al.* (1999) we expected *V. canescens* to prefer the larger female host larvae, but no sex differentiation by the parasitoids was found.

Table 2: Number of progeny and natural mortality of *V. canescens* parasitising *Plodia interpunctella*. m=male host larva; f=female host larva; F1=adult filiar generation; n.d.=sex not determined.

Imagines *V. canescens* (parental generation)		**Behaviour *V. canescens* (parental generation)**		**Sex of parasitised host larvae**		**Number of emerged adults (F1) and sex of host larvae**				**Mortality [%] *V. canescens* (F1)**
						parasitism		**no parasitism**		
No. wasp	**no. hosts attacked**	**cocking**	**no cocking**	**m**	**f**	***V.c.***	***P.i.***	***V.c.***	***P.i.***	
1	6	6	-	5	1	5	1 f	-	-	16.7
2	6	6	-	1	5	5	1 f	-	-	16.7
3	7	5	2	2	3	4	1 f	-	2 m	20
4	12	11	1	5	6	10	1 m	-	1 f	9.1
5	15	14	1	7	7	14	-	-	1 f	0
6	1	1	-	-	1	1	0	-	-	0
7	5	5	-	3	2	4	1 m	-	-	20
8	1	1	-	-	1	1	0	-	-	0
9	10	10	-	n.d.	n.d.	10	0	-	-	0
10	4	3	1	1	2	3		-	1 m	0
11	7	7	-	5	2	7	0	-	-	0
total	**74**	**69**	**5**	**29**	**30**	**64**	**5**	**0**	**5**	**mean 7,3**

Obviously, new eggs are produced during the adult life (synovigenic species). Feeding of wasps prior to release or during the biological control process is highly recommended. Field studies in Berlin suggested that *V. canescens* is very abundant (Rassmann *et al.,* 1999). The development of the *V. canescens*-larvae is adjusted to the speed of development of the hosts, leading to survival of the wasp during winter in diapausing host larvae. This feature favours the establishment of both naturally occurring and laboratory-reared *V. canescens* after release for biological control. It still has to be shown as to how this parasitoid can be implemented into a concept of integrated moth pest control in stored product protection.

Acknowledgements

The authors would like to thank Prof. Dr. Monika Hilker and PD Dr. Stefan Scheurer for valuable comments on the original Diploma-study. Sabine Berger, BBA Berlin, supported us in rearing *P. interpunctella*.

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Effectiveness of the wasp *Lariophagus distinguendus* Förster (Hymenoptera: Pteromalidae) in biological control of the weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae) in stored grain

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Abstract: Reproductive capacity and longevity of *Lariophagus distinguendus* Förster (Pteromalidae) was examined under different conditions in the laboratory with larvae of the granary weevil *Sitophilus granarius* L. (Curculionidae) as hosts. Reproductive capacity varied largely between *L. distinguendus* strains of different origin. Longevity was increased in the presence of hosts, probably due to host feeding by the parasitoids. Different host-parasitoid-ratios were investigated in order to promote the determination of the number of parasitoids necessary to be released for a sucessful use in practice. The results were discussed with respect to the potential use of *L. distinguendus* for the biological control of the granary weevil.

Key words: *Lariophagus distinguendus*, *Sitophilus granarius*, Biological control, Stored grain, Fecundity, Longevity

Introduction

The use of parasitoids in biological pest control is already common in different agricultural and horticultural fields. However, only a few alternatives are given for biological protection of stored products. According to the current knowledge the wasp *L. distinguendus* seems to be a promising candidate for biological pest control in stored grain (Steidle & Schöller, 2001).

The suitability of a potential candidate for biological pest control can get evaluated with a whole string of criterions (i.e. Alebeek, 1996; Kidd & Jervis, 1996). Already numerous investigations were made to most of the criterions (Steidle, 1998). Following, own investigations will be presented in the course of which the reproductive capacity of the wasp as well as the ability of adaptation to different storage conditions were examined in order to check the suitability of *L. distinguendus* for a biological control of *S. granarius*. In all experiments the granary weevil *S. granarius* was used as host.

Material and Methods

All insect cultures were kept at 25 ± 5 °C, 70 ± 5 % r.h. and a photoperiod of 16:8 (L:D).

For the investigation of the reproductive capacity of *L. distinguendus* isolated pairs of wasps were kept in petri-dishes, each filled with 4g infested grain. The experiment was carried out in a climatic chamber at 20°C and 60 ± 18 % r.h.. The wasps were daily transferred to petri-dishes with new hosts. Also vitality was observed daily. After ca. 6 weeks

of development, the progeny were isolated and counted. Three weeks later, the experiment was stopped.

In an additional experiment, isolated pairs of wasps were kept in petri-dishes, each filled with healthy grain to investigate the impact of hostfeeding on the longevity of *L. distinguendus*. In this experiment, vitality was also observed daily.

Five different host-parasitoid-ratios and a control were tested to find out the most effective ratio. The experiment was carried out in a climatic chamber at 20°C. Starting populations of 10 female granary weevils per 3l-jars, filled with 2 kg of wheat, were set. The granary weevils used were about 14 days old at the beginning of the experiment. Different host-parasitoid-ratios were produced by a weekly release of different numbers of *L. distinguendus*-females for a period of five weeks, beginning one week after the populations of *S. granarius* were set. The wasps were released in the host-parasitoid-ratios 1:0,2 (1 female weevil to 0,2 female wasps, or 10 weevils to 2 wasps), 1:0,5 – 1:1 – 1:2 and 1:5. The experiment was carried out in 15 repetitions – to an extent of 90 jars. After the F1-generation of the granary weevils had started to emerge, 3 jars per variant were examined every four weeks. Only the adult progeny were counted, developmental stages in the grain were disregarded.

Results

Reproductive capacity of L. distinguendus

Reproductive capacity depends on temperature, host, and substratum – but there are also considerable variations between the different origins of *L. distinguendus* (Steidle, 1998). Four different *L. distinguendus*-strains were examined (table 1). The British, the Swiss and the Berlin strain were investigated at 25°C. They showed highly significant differences in fecundity. The extent of variation reaches from an average of 92,5 descendants per female of the Britisch strain to an average of only 1,3 descendants per female of the Berlin strain (Steidle & Schöller, 2001). The fecundity of the FU strain was investigated at 20°C. A female of this strain produced 62,65 descendants in average, which shows a high fecundity of this strain in comparison to the others. Wasps of this FU strain were used for all the other experiments.

Table 1: Fecundity of geographically different origins of *L. distinguendus* (host: larvae of *S. granarius*)

Strain	Descendants per female [1]	Temperature [°C]
Slough, GB	92,5 ± 23,6 (n=13)	25
Berlin, D	1,3 ± 2,4 (n=25)	25
FU, D	62,6 ± 21,4 (n=20)	20
Uzwil, CH	10,9 ± 7,1 (n=17)	25

[1] mean ± SD (n).

Impact of hostfeeding on the longevity of L. distinguendus

Longevity of isolated pairs of wasps kept in petri-dishes with infested grain was compared with the longevity of wasps kept in petri-dishes with healthy grain. The analysis with a Mann-Whitney U-test showed a significantly shorter longevity of female wasps in the variant

„without hosts“ in comparison to the longevity of female wasps in the variant „with hosts“ (table 2). It implies that hostfeeding has a clear impact on longevity of female *L. distinguendus*.

Table 2: Impact of the presence of hosts on the longevity of adult *L. distinguendus*-females

Substratum	Longevity E [d][1]
Without hosts	14,8 ± 2,8a[2] (n=10)
With hosts	27,5 ± 10,59b (n=20)

[1]mean ± SD (n); [2]means followed by the same letter are not significantly different (Mann-Whitney U-test, $p \leq 0,01$).

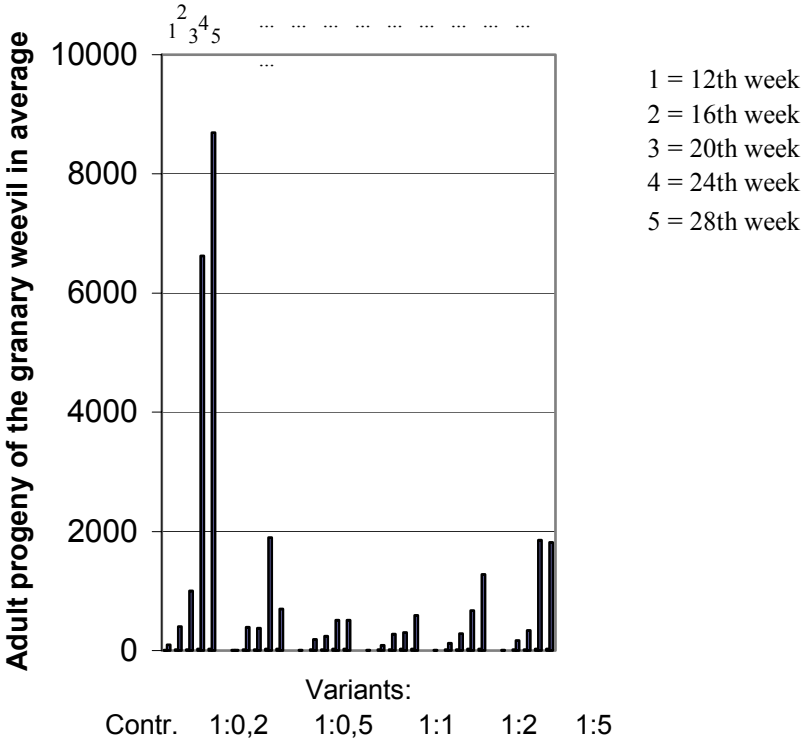

Fig. 1: Effectiveness of *L. distinguendus* in the suppression of the reproduction of *S. granarius* at 20°C, investigating different host-parasitoid-ratios

Effectiveness of different host-parasitoid-ratios

After about 12 weeks of development, the first weevils of the F1-generation emerged. The first jars were therefore examined in the 12th week of the experiment. The analysis with a One-Way ANOVA and LSD-test showed a high significant difference in the population-development between the control jar and the other variants on a level of significance of $p < 0,001$.

The next 3 jars per variant were examined after 16 weeks. The statistical analysis showed a small difference in the population-development of the granary weevil between the control variant and the host-parasitoid-ratio 1:1 as well as between the ratios 1:0,2 and 1:1 on a level of significance of $p < 0,05$.

Four weeks later the next 3 jars per variant were examined. There was no statistically significant difference in population-development between the variants. However in tendency the population of *S. granarius* developed better in the control variant than in the other variants.

After 24 weeks of experiment, there was a highly significant difference in the population-development between the control and the other variants on a level of significance of $p < 0,01$.

The last jars were examined after 28 weeks. The statistical analysis showed a highly significant difference in the population development between the control and the other variants on a level of significance of $p < 0,001$.

In summary, there was always a clear, general suppression of the reproduction of *S. granarius* by the parasitoids with the host-parasitoid-ratio 1:1 showing the best effectiveness (fig. 1).

Conclusions

The presented data are of importance for the evaluation of the suitability of *L. distinguendus* for the biological control of *S. granarius.* The assumption that the wasp *L. distinguendus* is a promising candidate for biological pest control in stored grain could be supported by the following conclusions:

- there are strains of *L. distinguendus* with a very high reproductive capacity - for the use of *L. distinguendus* in biological control of *S.granarius* a strain with a high fecundity should be selected
- besides the parasitic way of life of *L. distinguendus* there is an additional reduction of the *S. granarius*-population by hostfeeding
- there is a general suppression of the reproduction of *S. granarius* by the parasitoids, with the host-parasitoid-ratio 1:1 showing the best effectiveness

Further studies are required at lower temperatures, and also to improve the strategy of releasing the parasitoids.

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Biological control potential with native *Dinarmus* wasp species in grain legumes stored on farm

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Abstract: Grain legumes stored on-farm in the tropical belt are prone to infestation by bruchid weevils, often even before harvest. When left untreated, weevil populations can grow exponentially and can completely destroy crops within a few months. Non-toxic means of control offer particular promise as they are safe for handling and for the consumer (Dorn, 1998). Efficacy, reliability and environmental safety may, however, be critical issues.

We identified native *Dinarmus* wasp species (Hymenoptera: Pteromalidae) as effective natural antagonists of bruchid weevils both in Columbia and India, thus did not have to introduce exotic species with their inherent risk for non-target insects. *Dinarmus* spp. destroy their host by both oviposition and host feeding (Schmale *et al.*, 2001). Host feeding adds an interesting aspect to biological control as it can lead to a rapid elimination of the pest population, and to an eradication of any insect in the stored good within one generation. To increase reliability of the control under variable conditions, the combination of biological control with host plant resistance has been examined. The promise of such integrated control programs is discussed.

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Predation by *Blattisocius tarsalis* (Acari: Ascidae) on stored product pests

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Abstract: Studies were conducted on the predation of *Blattisocius tarsalis* (Berlese) on several stored product pests under controlled laboratory conditions. Adults of *B. tarsalis* were able to prey on eggs of the mould mite (*Tyrophagus putrescentiae* (Schrank)), the Indian meal moth (*Plodia interpunctella* (Hübner)), the Mediterranean flour moth (*Ephestia kuehniella* Zeller), the cigarette beetle (*Lasioderma serricorne* (Fabricius)), the rusty grain beetle (*Cryptolestes ferrugineus* (Stephens)), the rust-red flour beetle (*Tribolium castaneum* (Herbst)), the bean weevil (*Acanthoscelides obtectus* (Say)), and first instar nymphs of the booklouse (*Liposcelis bostrychophila* (Badonnel)). Functional responses of *B. tarsalis* females to eggs of *P. interpunctella* and *L. serricorne* were compared. Results suggested a type II functional response. Attack coefficients of *B. tarsalis* on the two prey species were not significantly different. As opposed to this, *B. tarsalis* spent less time handling eggs of *L. serricorne* than eggs of *P. interpunctella*.

Key words: Predation, functional response, biological control.

Introduction

Stored products are infested by a wide range of pest species, some of them inhabiting the same facility simultaneously. *Blattisocius tarsalis* (Berlese) is a polyphagous predatory mite that has been recorded world-wide in association with insect and mites infesting stored products (Graham, 1970; Haines, 1981). Its predatory capacity and biology has been studied in the laboratory when feeding on *Ephestia*, *Plodia* and *Tribolium* species (Darst and King, 1969; Haines, 1981; Nielsen, 1998). Recently, this predatory mite has been observed in stored food facilities in which no insecticides were applied in the North East of Spain (Riudavets *et al*., 2001). Therefore, the objective of our research was to assess the ability of *B. tarsalis* to predate on different stages of several insect and mites commonly found in storage facilities. According to the results of the first set of experiments, *Plodia interpunctella* (Hübner) and *Lasioderma serricorne* (Fabricius) eggs were chosen for a study of the functional response of the predatory mite. The potential capacity of *B. tarsalis* as a biological control agent of pests of stored products is discussed.

Materials and methods

Insects

Stock colonies of the predatory mite *B. tarsalis* were started with individuals collected on wheat semolina infested with *Tyrophagus putrescentiae* (Schrank) and *Liposcelis bostrychophila* (Badonnel) in Barcelona, North East of Spain, and reared on vermiculite and *Ephestia kueniella* (Zeller) eggs as prey (Nielsen 1998). The prey species tested in this study were obtained from stock colonies maintained at the Institut de Recerca i Tecnologia Agroalimentàries (Cabrils, Barcelona). All laboratory studies were conducted in a climatic

chamber at 25±1ºC, 75% RH and 16:8 h L:D. Transparent plastic cages (4 ml, 26 mm diameter) were used as experimental arenas.

Prey consumption

To determine *B. tarsalis'* consumption on different pest species, female predatory mites were isolated individually either with ten eggs, five adults or five immature stages of each of the ten pest species tested (see Table 1). The number of individuals consumed was recorded after 24 hours. There were 3 replicates per prey species and stage of development.

Functional response experiments

According to the results obtained in the prey consumption experiments and the relevance of the species as pests on stored products, *P. interpunctella* and *L. serricorne* were chosen for a study of the functional response of *B. tarsalis* when exposed to eggs of the two species. Prior to the experiments, *B. tarsalis* females were fed the tested prey for 24 h and then starved for 24 h more. The densities tested were 1, 2, 3, 4, 6, 9 and 12, and an additional density of 15 with *L. serricorne*. We tested one individual predator and one density of prey species per arena, and the number of completely or partially deflated eggs was recorded 24 h after predator release. Every combination of prey type and prey density was replicated 15 times. Eggs were not replaced during the experiment. Fifteen replicates per prey species with 12 or 15 eggs of *P. interpunctella* and *L. serricorne* respectively, were conducted without predators as control.

Data Analysis. To determine the shape of the functional response data were fitted to a polynomial function that describes the relationship between the proportion of prey eaten (Ne/N_0) and the prey offered (N_0), by using the logistic regression (Juliano 1993):

$$\frac{Ne}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}, \qquad [1]$$

with P_0, P_1, P_2 and P_3 being the intercept, linear, quadratic, and cubic coefficients, respectively. These parameters were estimated using the method of maximum likelihood (PROC CATMOD, SAS Ins. 1989). If $P_1<0$, the proportion of prey eaten declines monotonically with the initial number of prey offered, thus describing a type II functional response. If $P_1>0$ and $P_2<0$, the proportion of prey eaten is initially positively density-dependent, thus describing a type III functional response.

Logistic regression results for *B. tarsalis* with the two prey tested suggested a type II functional response. Thus handling times (T_h) and attack coefficients (a) were estimated using the Holling 'disc equation' (Holling 1966):

$$Ne = \frac{aN_0T}{1 + aN_0T_h} \qquad [2]$$

where T = total time available (i.e. 24 h). Parameters were obtained by fitting observed data to equation 2 using non-linear least-square regression (PROC NLIN, SAS Ins. 1989).

To compare the functional responses of *B. tarsalis* between the two prey tested, two new parameters were included in the Holling 'disc equation' (Juliano 1993):

$$Ne = \frac{[a + Da(z)]N_0T}{1 + [a + Da(z)]N_0[T_h + DT_h(z)]} \qquad [3]$$

where z is an indicator variable of each population. The parameters D_a and D_{Th} estimate the differences between the populations in the values of the parameters a and T_h respectively. If these parameters are significantly different from 0 then the two populations differ

significantly in the corresponding parameters. Non-linear least-square regressions were used to obtain parameter estimates (PROC NLIN, SAS Ins. 1989).

Results and discussion

B. tarsalis is well known as a predator of Lepidoptera (Haines, 1981; Nielsen, 1998). Nevertheless, this predatory mite is able to feed on other insect and mite species (Haines, 1981). Indeed, in this study females of *B. tarsalis* were able to prey on the eggs of mites, psocoptera, coleoptera and lepidoptera. Nevertheless, they were not able to prey on the other developmental stages tested, except for the first nymphal instar of the psocid *L. bostrychophila* (Table 1). The objective of this experiment was not to compare consumption rates of *B. tarsalis* among prey species, but to exclude species or stages as its prey. On this basis, *B. tarsalis* was able to consume a similar number of eggs (*i.e.* around 2 eggs/24h) of most of the species tested, and a higher number of eggs of *L. serricorne* (3.3±0.88) and *P. interpunctella* (frozen eggs, 3.0±0.0) (Table 1). In other experiments reported in the literature similar values were obtained for *B. tarsalis*' predation on *E. kuehniella* eggs (≅3.5 eggs per day at 25°C (Nielsen, 1998)) and on *T. castaneum* eggs (1.2 eggs per day (Haines, 1981)). By contrast, White and Huffaker (1969) obtained a higher consumption on eggs of *E. kuehniella* (5.5 eggs/day) and Darst and King (1969) a lower consumption on eggs of *P. interpunctella* (1.2 eggs/day) than those obtained in the present study.

Table 1. Consumption rate (24 h) of *B tarsalis* on several stored product pest species. Each predatory female was supplied either with 10 eggs, 5 nymphs, 5 larvae or 5 adults of each of the different prey species tested (N =3). (25±1ºC, 75% HR, 16:8 h L:D).

Species	**no. prey killed (Means±SEM)**		
	eggs	**larvae/nymphs**	**adults**
Acaridae			
Tyrophagus putrescentiae	2.3±0.33	0	0
Psocoptera			
Liposcelis bostrychophila	-	2.0±0	0
Coleoptera			
Tribolium castaneum	2.0±0	0	0
Lasioderma serricorne	3.3±0.88	0	0
Cryptolestes ferrugineus	1.6±0.33	0	0
Acanthoscelides obtectus	2.0±0.58	-	0
Sitophilus oryzae	-	-	0
Rhyzopertha dominica	-	-	0
Lepidoptera			
Plodia interpunctella	2.3±0.88 (3.0±0*)	0	-
Ephestia kuehniella	2.6±1.20*	-	-

*Frozen eggs

The logistic regression for data of *B. tarsalis* consumption of the eggs of *P. interpunctella* and *L. serricorne* had a significant linear parameter $P_1<0$ (Table 2), suggesting a type II functional response for both types of prey (Fig. 1). Table 3 shows the

estimates of attack coefficients and handling times for both prey species and their significance levels. Attack coefficients of *B. tarsalis* with eggs of *P. interpunctella* and *L. serricorne* were not significantly different from one another (D_a = -0.031). Therefore, the average number of eggs of both prey that the predatory mite is able to attack per searching time unit should be similar. Indeed, at low prey densities *B. tarsalis* consumed a similar number of eggs of the two prey species. By contrast, *B. tarsalis* spends significantly less time handling the eggs of *L. serricorne* than the eggs of *P. interpunctella* (D_{Th} = -0.421, $P < 0.001$). This would explain differences in consumption found between prey types at high prey density ranges. However, it has to be taken into account that the eggs of *P. interpunctella* are twice as large as the eggs *of L. serricorne* (0.101 mm^3 and 0.048 mm^3 respectively). Since handling time decreases exponentially as predator-prey weight ratio increases (Cohen and Tang 1997), differences on the size of the eggs may be the main explanation for differences in handling times found in our experiments.

Table 2. Maximum-likelihood estimates from logistic regression of proportion of prey eaten as a function of initial prey densities by *B. tarsalis*.

Prey	Parameter	Estimate	SE	χ^2	*P*
P. interpunctella	Intercept (P_0)	1.2452	0.3392	13.48	0.0002
	Linear (P_1)	-0.5552	0.1461	14.44	0.0001
L. serricorne	Intercept (P_0)	2.1832	0.4705	21.53	0.0000
	Linear (P_1)	-0.1998	0.0851	5.51	0.0189

Table 3. Attack coefficients (*a*) ($hours^{-1}$) and handling times (T_h) (hours) from non-linear regression of the number of prey eaten by B. *tarsalis* as a function of initial prey density.

Prey	R^2	*a* (Probability) (Asym. 95% CI)	T_h (Probability) (Asym. 95% CI)
P. interpunctella	0.80	0.074 ($P < 0.01$) (0.030-0.119)	8.321 ($P < 0.001$) (6.535-10.113)
L. serricorne	0.88	0.043 ($P < 0.001$) (0.031-0.056)	3.903 ($P < 0.001$) (3.090-4.717)

df (*P. interpunctella*) = 68, df (*L. serricorne*) = 78. R^2 are the coefficients of determination obtained from R^2 = 1 - (sum of squares of residuals/total sum of squares).

As shown in Figure 2, the proportion of partially deflated eggs of *P. interpunctella* increased with increasing prey density, whereas it was the opposite for *L. serricorne*. According to Haines (1981) and Darst and King (1969), *B. tarsalis* completely deflates most of the moth eggs when feeding. In contrast, Nielsen (1998) reported that most of the eggs of *E. kuehniella* consumed by *B. tarsalis* were partially consumed. Predatory mites have been observed to return to a dead prey several times to feed on it when prey density was low (Sandness and McMurtry, 1972). At high prey densities, however, predatory mites tend to consume less of each prey item because satiation is reached, and the amount of food needed to compensate the gut deficit is smaller than the food content of the prey (Sabelis, 1992). Apparently, *B. tarsalis* may have reached satiation when feeding on *P. interpunctella* eggs, since the density at which the plateau of the functional response occurs coincides with the

density at which the proportion of partially deflated eggs increases. Small egg size (thus small food content) could explain why for *L. serricorne* almost all the eggs were totally consumed.

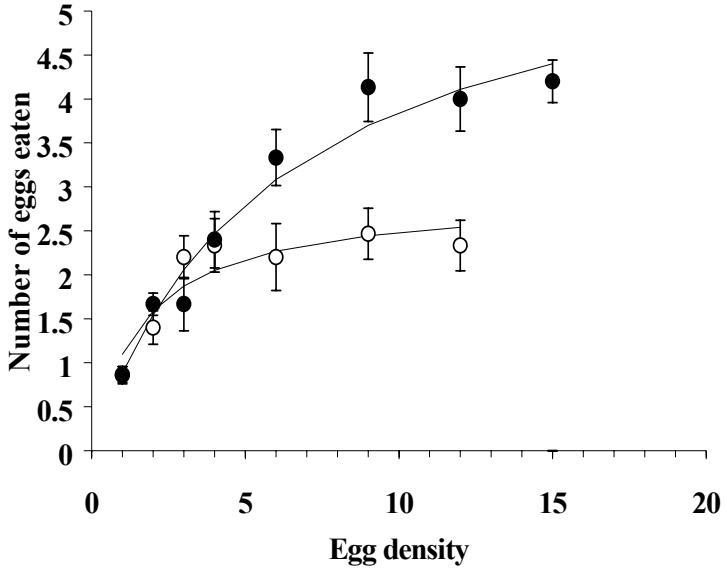

Fig 1. Functional responses of *B. tarsalis* when exposed to different densities of *P. interpunctella* (○) and *L. serricorne* (●) eggs. Points are means±SE ($N = 15$, $T = 24$ h).

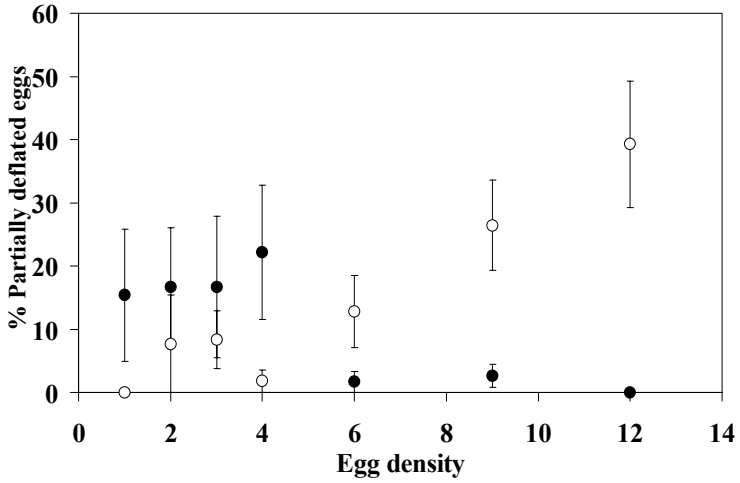

Fig. 2. Proportion of partially deflated eggs consumed by *B. tarsalis* at different densities of eggs of *P. interpunctella* (○) and *L. serricorne* (●).

Due to *B. tarsalis*' capacity for increasing the number of prey eaten as prey density increases and the wide range of prey species that it is able to consume, this predator appears to be a suitable candidate for the biological control of stored product pests. However, the present study cannot be used to infer that *B. tarsalis* would prey on these pest species in a food storage facility. An array of different factors such spatial coincidence, prey preference, predator experience and other factors involved in predator-prey interactions may be relevant

when determining host suitability. Future research is needed on these aspects for the accurate determination of the efficiency of *B. tarsalis* as a biocontrol agent of stored product pests.

Acknowledgements

This research was financially supported by the company "S.E. Carburos Metálicos S.A." and by the Ministerio de Ciencia y Tecnologia, project FEDER Nº2FD1997-0736

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Occurrence of Hymeopterous parasitoids of stored product pests in Greece

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Abstract: During a two-year survey on the insect fauna of stored products in Greece, sixteen species of hymenopterous parasitoids were collected on various stored products from different localities. The survey was conducted on grain, flour, legumes, tobacco and dried fruits stored in varying quantities and different types of storage facilities, flourmills and household stores. All parasitoid species collected, are recorded for the first time in Greece. The highest percentage of parasitoid presence was recorded in dried fruits, tobacco and flour. Eight parasitoids attacked coleopterous hosts, six attacked lepidopterous ones and two species attacked both. The most dominant wasps were, in decreasing order, *Holepyris sylvanidis* (Bréthes) (Bethylidae), *Anisopteromalus calandrae* (Howard) (Pteromalidae), *Venturia canescens* (Gravenhorst) (Ichneumonidae), *Cephalonomia tarsalis* (Ashmead) (Bethylidae) and *Theocolax* (*Choetospila*) *elegans* (Westwood) (Pteromalidae). The most frequent parasitoid was *A. calandrae* followed by *H. sylvanidis*, *Th. elegans*, *V. canescens*, *Habrobracon (Bracon) hebetor* Say (Braconidae) and *C. tarsalis*. The phenomenon of co-occurrence of two or more parasitoids was observed in many cases. The most common pairs of coexisting wasps competing for the same host were: *A. calandrae* - *Th. elegans*, parasitizing *S. oryzae* and *R. dominica*, *H. sylvanidis* - *C. tarsalis*, parasitising *Tribolium* sp., *Oryzaephilus* sp. and *Cryptolestes* sp. and *V. canescens* - *H. hebetor*, parasitising moth larvae.

Key words: stored products, parasitoids, biological control, dominance, frequency, co-occurrence, Greece

Introduction

Insect pests of stored products are attacked by a variety of natural enemies, such as predatory insects and mites, hymenopterous parasitoids, other vertebrates and pathogenic microorganisms. Although the use of beneficial insects to control pests is well established in agriculture and horticulture, their use against post-harvest pests was very limited until recently. During the last decade, however, biological control gradually occupies a significant part in stored product IPM due to many reasons, such as its many advantages over traditional chemical methods, pest resistance to conventional pesticides, the phase-out of methyl bromide, the favourable for beneficial insects conditions of the stored-product environment (protected from wind, rain, extreme temperature fluctuations, preventing insects from leaving, high concentration of habitat and host within a limited area) and its compatibility with other IPM methods (Arbogast, 1984; Haines, 1984; Gordh & Hartman, 1991; Brower *et al*., 1996; Schöller *et al*., 1997; Reichmuth, 2000; Schöller & Flinn, 2000).

The majority of the parasitoids found in stored products belong to the order Hymenoptera (Gorham, 1987; Gordh & Hartman, 1991; Haines, 1991). These parasitic wasps are natural components of storage ecosystems, some being of potential value as biocontrol agents for storage pests. Until recently, 58 species of predators and parasitoids of 79 stored product pests have been studied and nearly 900 publications have dealt with biological control in storage facilities during the last 80 years (Schöller, 1998).

Recording, collecting and mass rearing of indigenous beneficial insects is the main approach to the implementation of biological control in storage facilities. Introduction and establishment of exotic natural enemies (classical biological control), however, is very limited in storages as most natural enemies of storage pests have been widely distributed through world trade of stored products and are now cosmopolitan (Arbogast, 1984; Schöller *et al*., 1997; Schöller & Flinn, 2000). Moreover, native natural enemies are preferable to imported ones due to their perfect acclimatization with the ecosystem (Metcalf, & Flint, 1962; DeBach, 1964; Delucchi, 1976; Ridgway & Vinson, 1977). Nevertheless, importing of exotic species requires special licence and is subjected to different legal constraints in European and other countries, with the exception of those considered as endemic (Brower *et al*., 1996; Cox & Wilkin, 1998; Schöller & Flinn, 2000).

Therefore, as very few faunistic data on these beneficial insects exists in many countries, their recording and identification is necessary (Schöller & Flinn, 2000). This has not been carried out in Greece so far. The present survey is the first in Greece undertaken exclusively on these parasitoids and is a part of a larger study on implementation of IPM methods on stored commodities. The objectives of this study are a) to record the parasitoid fauna in Greek stores and b) to examine host and habitat preference, co-occurrence, dominance and frequency of the species found.

Materials and methods

The survey lasted for two years (1999-2000) and included a wide range of products stored in varying quantities and different types of storage facilities. The materials sampled, included grains (wheat, maize, oats, barley), flour (wheat, maize), legumes, tobacco and dried fruits (figs, raisins, sultanas). The storage facilities examined included large concrete silos, warehouses, flat granaries, farm stores, flourmills and household stores.

A total of 587 samples, weighing approx. 300 g each, were obtained from stored products in several localities. Samples were kept in cylindrical 2-lt glass jars at 30^{o}C, 60-70% R.H. and 16L:8D light regime. Collection and counting of insects (host and parasitoid adults) was conducted by the time the sample had been carried to the laboratory. The procedure was repeated at daily intervals for three weeks, in order to collect newly emerged adults. In case no insects were found the sample was discarded and not included in the results. Identification of most of the host and parasitoid species was carried out by the authors, using relevant keys. In cases where this was not possible, specimens were sent to specialists.

The parasitoids recorded were associated with the appropriate hosts found in the same sample. This association was made not only by hypothesis based on references but also by rearing the parasitoids on the hosts in the laboratory for several generations. This procedure was carried out successfully for the most frequent species. Association with the habitat, which the parasitoid had been collected on, was also noticed.

For the quantitative categorisation of the species found, the criteria “Dominance” and “Frequency” were used, as suggested by Curry (1973). “Dominance” indicates the percentage of individuals of a given taxon compared to the individuals of all taxa found. Thus, a given species (or taxon) can be classified as “Dominant” (>5%), “Influent” (2-5%), or “Recedent” (<2%). “Frequency” is the percentage of samples in which the particular taxon was detected. Thus, a species can be classified as “Constant” (>50%), “Accessory” (25-50%), or “Accidental” (<25%). Finally, the frequency of co-existence of parasitoid species in the same sampling unit was also recorded.

Results

The majority of the samples originated from Western and Central Greece. From the 587 samples collected in total, 422 were infested, 103 of which (24,4%) contained parasitoids. Almost half of the infested samples (44%) were grains (53,2% wheat, 36,9% maize, 6,5% oats and 3,4% barley), followed by dried fruits [(16%) (35,9% sultanas, 32,8% figs and 31,3% raisins)], flour [(15%) (60% wheat and 40% maize)], legumes (14%) and tobacco (11%) (Fig. 1). The highest percentage of parasitoid presence was recorded in dried fruits (35,8%), tobacco (27,1%) and flour (24,6%) (Fig. 2).

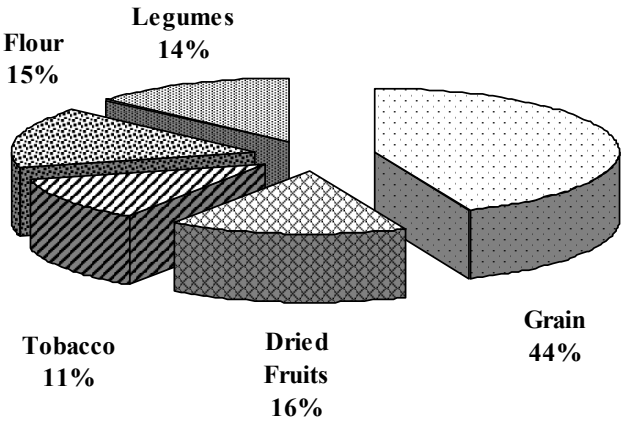

Fig. 1. Collected samples : Product categories

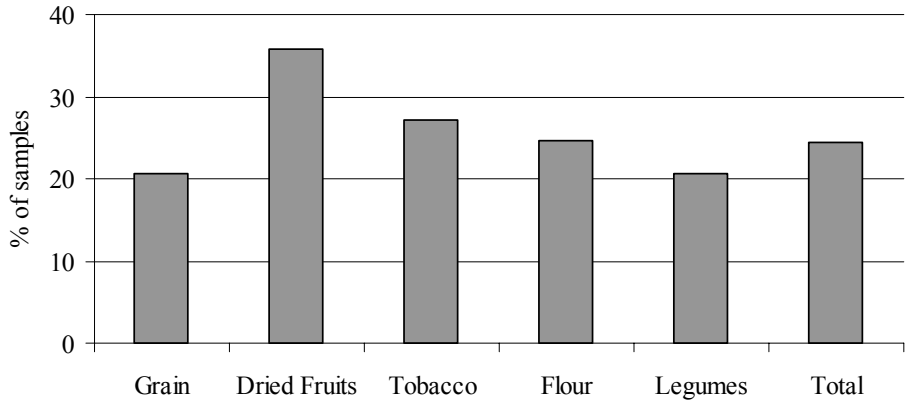

Fig. 2. Percentage of parasitoid-containing samples

Sixteen species of parasitic wasps were recorded. All these species are recorded for the first time in Greece. These wasps were associated with the presence of the most numerous coleopterous and lepidopterous hosts found in the same sample (Tables 1 & 2). It should be noted that only a few individuals from other pest species, mainly fungus–feeders (Mycetophagidae, Cryptophagidae, Lathrididae etc) were collected and their role as possible host was not investigated. Eight parasitoids attacked coleopterous hosts, six lepidopterous and two species, *A. calandrae* and *L. distinguendus*, both.

Table 1. Hymenopterous parasitoids associated with coleopterous pests collected on various stored products in Greece.

	Parasitoid	Coleopterous Host													
		Sitophilus oryae	*Sitophilus granarium*	*Rhyzopertha dominica*	*Cryptolestes ferrugineus*	*Cryptolestes* spp.	*Oryzaephilus surinamensis*	*Oryzaephilus mercator*	*Tribolium castaneum*	*Tribolium confusum*	*Stegobium paniceum*	*Lasioderma serricorne*	*Acanthoscelides obtectus*	*Bruchus* sp.	*Dermestidae*
1	*Anisopteromalus calandrae* (Howard) Pteromalidae	+	+	+							+	+	+		
2	*Holepyris sylvanidis* (Bréthes) Bethylidae				+		+	+	+	+					
3	*Theocolax elegans* (Westwood) Pteromalidae	+		+								+			
4	*Cephalonomia tarsalis* (Ashmead) Bethylidae	+		+	+		+	+	+	+					
5	*Lariophagus distinguendus* Förster Pteromalidae		+								+	+			
6	*Dinarmus* sp. Pteromalidae												+	+	
7	*Cephalonomia waterstoni* Gahan Bethylidae				+	+	+		+						
8	*Heterospilus prosopidis* (Viereck) Braconidae												+		
9	*Laelius* sp. Bethylidae														+
10	*Cephalonomia* sp. Bethylidae											+			

Table 2. Hymenopterous parasitoids associated with lepidopterous pests collected on various stored products in Greece.

	Parasitoid	Lepidopterous Host						
		Ephestia kuehniella	*Cadra cautella*	*Ephestia elutella*	*Plodia interpunctella*	*Sitoroga cerealella*	*Epestia figuliella*	*Tinea granella*
1	*Anisopteromalus calandrae* (Howard) Pteromalidae			+	+	+		
2	*Venturia canescens* (Gravenhorst) Ichneumonidae	+	+	+	+		+	+
3	*Trichogramma* sp. Trichogrammatidae	+	+	+	+	+	+	
4	*Habrobracon* (*Bracon*) *hebetor* Say Braconidae	+	+	+	+		+	
5	*Lariophagus distinguendus* Förster Pteromalidae					+		
6	*Pteromalus cerealellae* (Ashmead) Pteromalidae					+		
7	*Holepyris hawaiensis* (Ashmead) Bethylidae	+			+			
8	*Dibrachys* sp. Pteromalidae	+			+			+

All parasitoids were classified as "Accidental" as no species was present in more than 25% of the total number of samples (Fig. 3). *Anisopteromalus calandrae* was present in

11,8% of the total number of samples, followed by *H. sylvanidis* (8,5%), *Th. elegans* (6,9%), *V. canescens* (6,6%), *H. hebetor* (6,6%), *C. tarsalis* (5,7%) and *Trichogramma* sp. (3,8%). For all other wasp species, frequency did not reach 2%. As far as dominance is concerned, none of the collected parasitoid species was classified as "Dominant", given that none of them amounted to more than 5% of the total number of collected insects. The most abundant species was *H. sylvanidis* representing 2,3 % of collected insects (Fig. 3). The other most abundant wasps were, in descending order, *A. calandrae* (2,1%), *V. canescens* (1,4%), *C. tarsalis* (1,4%), *Th. elegans* (1,3%), *H. hebetor* (1,2%) and *Trichogramma* sp. (0,9%). All other parasitoids did not exceed 0,2 % of the total number of collected insects.

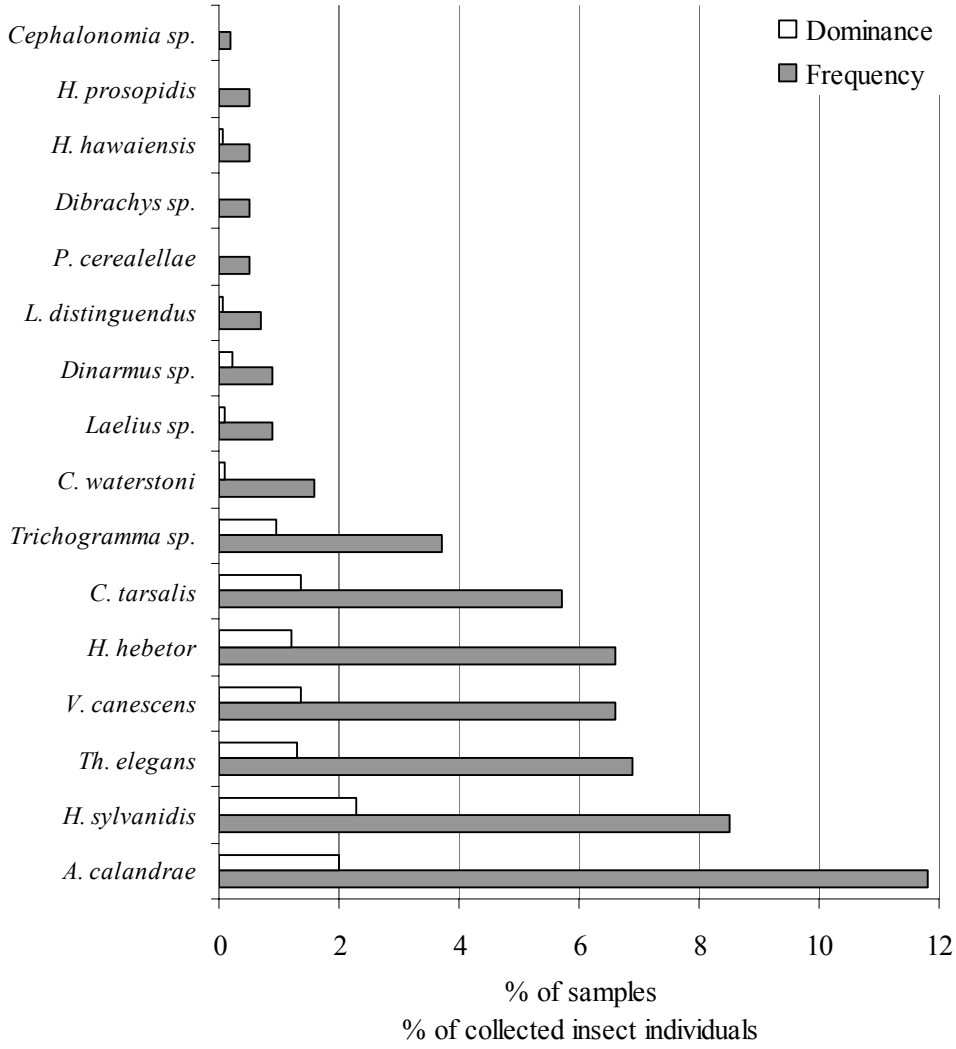

Fig. 3. Dominance and frequency of collected parasitoids

The only wasp which was noted in all types of products, was *A. calandrae* (Table 3). This species was the most frequent in grain (17,4%), followed by *H. sylvanidis*, *Th. elegans* and *C. tarsalis*. *V. canescens* was its counterpart in flour, occurring in more than 18% of flour samples, followed by *Trichogramma* sp., *H. sylvanidis*, *H. hebetor* and *C. tarsalis*. *A. calandrae* was also present in most samples of legumes and tobacco (10,3% and 14,6%, respectively). Individuals of the genus *Dinarmus* had also a significant presence in legumes

(6,9%). *H. hebetor* occurred in most samples of dried fruits (14,9%). The presence of *V. canescens* and *H. sylvanidis* was also noteworthy.

Accordingly, the most abundant parasitoids in grain were *A. calandrae*, *Th. elegans*, *H. sylvanidis* and *C. tarsalis*. In flour samples the majority of wasps consisted of *H. sylvanidis*, *Trichogramma* sp. and *V. canescens*. Individuals of the genus *Dinarmus* and *A. calandrae* were the most numerous wasps found in legumes, representing 2,7% and 1,8% of the collected insects in these samples, respectively. *A. calandrae* was by far the most abundant parasitoid in tobacco samples (4,7%), whereas no other wasp exceeded 0,7%. Two parasitoids of lepidopterous larvae, *H. hebetor* and *V. canescens* were the most numerous in dried fruits samples, representing 4,3% and 3,8%, respectively, of the number of insects found in this type of product. *H. sylvanidis* (3,4%) and *Trichogramma* sp. (1,4%), also appeared in noticeable numbers.

Table 3. Dominance, Frequency and number of samples in which each parasitoid was detected (D: Dominant, In: Influent, R: Recedent, C: Constant, A: Accessory and Ac: Accidental).

Parasitoid	**Grain** (n=184)		**Flour** (n=65)		**Legumes** (n=58)		**Tobacco** (n=48)		**Dried Fruits** (n=67)		**No of samples**
	Dom.	Freq.	Dom.	Freq.	Dom.	Freq.	Dom.	Freq.	Dom.	Freq.	(n=422)
A. calandrae	3,2 **In**	17,4 **Ac**	0,1 **R**	3,1 **Ac**	1,8 **R**	10,3 **Ac**	4,7 **In**	14,6 **Ac**	0,2 **R**	4,5 **Ac**	50
H. sylvanidis	2,2 **In**	11,4 **Ac**	3,5 **In**	12,3 **Ac**					3,4 **In**	10,4 **Ac**	36
Th. elegans	2,4 **In**	10,9 **Ac**	0,5 **R**	6,2 **Ac**	0,5 **R**	5,2 **Ac**	0,6 **R**	4,2 **Ac**			29
V. canescens	0,1 **R**	3,8 **Ac**	3,1 **In**	18,5 **Ac**			0,5 **R**	2,1 **Ac**	3,8 **In**	11,9 **Ac**	28
H. hebetor	0,7 **R**	4,3 **Ac**	0,7 **R**	12,3 **Ac**			0,6 **R**	4,2 **Ac**	4,3 **In**	14,9 **Ac**	28
C. tarsalis	2,0 **In**	8,2 **Ac**	1,9 **R**	7,7 **Ac**					0,3 **R**	6,0 **Ac**	24
Trichogramma sp.	0,1 **R**	0,5 **Ac**	3,2 **In**	13,8 **Ac**			0,3 **R**	4,2 **Ac**	1,4 **R**	6,0 **Ac**	16
C. waterstoni	0,1 **R**	1,6 **Ac**	0,1 **R**	3,1 **Ac**					0,2 **R**	3,0 **Ac**	7
Laelius sp.			0,1 **R**	1,5 **Ac**			0,7 **R**	4,2 **Ac**	0,2 **R**	1,5 **Ac**	4
Dinarmus sp.					2,7 **In**	6,9 **Ac**					4
L. distinguendus	0,1 **R**	0,5 **Ac**							0,2 **R**	3,0 **Ac**	3
P. cerealellae	0,1 **R**	1,1 **Ac**									2
Dibrachys sp.	0,1 **R**	1,1 **Ac**									2
H. hawaiensis	0,1 **R**	0,5 **Ac**							0,2 **R**	1,5 **Ac**	2
H. prosopidis					0,4 **R**	3,4 **Ac**					2
Cephalonomia sp.							0,2 **R**	2,1 **Ac**			1

The highest number of parasitoid species was noted in grain (12 species) and dried fruits (10), followed by flour (9), tobacco (7) and legumes (4). In most cases more than one wasp species were recorded in the same sample. One species alone was found in a mere 13,6% of the samples containing parasitoids (Fig. 4). The majority of samples contained 2 or 3 species (25,2% and 28,2%, respectively) attacking the same or different host.

The phenomenon of co-occurrence among parasitoids was observed in many cases (Table 4). The most common combinations of coexisting wasps competing for the same host were : a) *A. calandrae – Th. elegans – C. tarsalis* in grain parasitising internal feeding beetles (*S. oryzae* and *R. dominica*), b) *H. sylvanidis – C. tarsalis* in grain, flour and dried fruits, attacking external feeding beetles (*Tribolium* sp., *Oryzaephilus* sp. and *Cryptolestes* sp.), and

c) *V. canescens – H. hebetor* - *Trichogramma* spp. in grain, flour and dried fruits parasitising lepidopterous pests.

A. calandrae and *Th. elegans* coexisted in 15 grain samples (9 wheat and 6 maize) (Table 4). The former was the dominant species representing 66% of the total collected adults of two wasps in these samples. The rate of coexistence was also high in the case of *A. calandrae* and *C. tarsalis* (4 wheat samples), as well as between the latter and *Th. elegans* (4 wheat samples). In both cases, the numbers of collected adults of the competing wasps were identical.

H. sylvanidis and *C. tarsalis* were another pair of competing parasitoids. They were recorded in 7 wheat and 4 flour samples, competing for larvae of external-feeding beetles, such as *Tribolium* sp., *Cryptolestes* sp. and *Oryzaephilus* sp. *H. sylvanidis* developed higher populations in both habitats, reaching 59% and 61% of collected adults of these two species in grain and flour, respectively.

The most frequent case of coexistence was among parasitoids of lepidopterous pests in grain, flour and dried fruits. *V. canescens* and *H. hebetor* were competing for lepidopterous larvae in all of the aforementioned products. Despite the superiority of *H. hebetor* in grain (25:75) and dried fruits (22:78), the two wasps were found in similar numbers in flour (44:56). Egg parasitoids of the genus *Trichogramma* were found to coexist with *V. canescens* or *H. hebetor* in very few samples.

Table 4. Frequency of co-occurrence of most frequent parasitoids (No of samples) competing for the same host in the same sample (in parenthesis: % of adults)

	Parasitoid	**Grain**	**Flour**	**Dried Fruits**
parasitising internal feeding beetles	*A. calandrae*	12	2	1
	Th. elegans	1	2	-
	C. tarsalis	5	2	2
	A. calandrae + *Th. elegans*	15 (66, 34)	-	-
	A. calandrae + *C. tarsalis*	4 (52, 48)	-	*
	Th. elegans + *C. tarsalis*	4 (53, 47)	*	-
	A. calandrae + *Th. elegans*+ *C. tarsalis*	*	-	-
parasitising external feeding beetles	*H. sylvanidis*	14	4	6
	C. tarsalis	8	1	3
	H. sylvanidis + *C. tarsalis*	7 (59, 41)	4 (61, 39)	*
parasitising lepidopterous pests	*V. canescens*	1	3	0
	H. hebetor	2	3	2
	Trichogramma sp.	1	4	0
	V. canescens + *H. hebetor*	6 (25, 75)	4 (44, 56)	6 (22, 78)
	V. canescens + *Trichogramma* sp.	-	4 (47, 53)	*
	H. hebetor + *Trichogramma* sp.	-	*	*
	V. canescens + *H. hebetor* + *Trichogramma* sp.	-	-	*

* co-occurrence observed in very few samples (<4) or very few wasps collected

Discussion

The highest percentage of parasitoid presence was recorded in dried fruits, tobacco and flour. This can be attributed to the fact that many of these products were heavily infested due to poor storage conditions such as high temperature, poor hygienic conditions, quantities of old product remaining in the store, long-term storage etc. As opposed to this, grain and legumes were stored mainly in large silos and warehouses, respectively, where storage conditions were much more appropriate, as these stores were rather clean and storage period was short (usually less than three months). Hence, in most cases the infestation level was relatively low. This could account for the more frequent presence of parasitoids in dried fruits, tobacco and flour as all natural enemies, in general, are more likely to occur in heavy infestations (Metcalf & Flint, 1962; Ridgway & Vinson, 1977; Arbogast, 1984).

Apart from the separate presence of each species, the most important findings of the present survey are the noticeable differences in the composition of parasitoid fauna among the various products examined. The main reasons for this variation are a) presence of different hosts in every product, b) some special features of each product (e.g. interstitial spaces of seeds, tobacco toxins etc) c) various types of storage (bulk, packaged etc.) (Brower *et al*., 1996; Schöller & Flinn, 2000). Nevertheless, some wasps were present in many product categories, such as *A. calandrae* which proved to be not only the most frequent parasitoid but also the only wasp which was present in all product categories. This is due to its wide host and habitat range as it can parasitise both coleopterous and lepidopterous pests of various stored products (Thompson, 1954; Ghani & Sweetman, 1955; Gordh & Hartman, 1991; Brower *et al*., 1996; Schöller & Flinn, 2000).

The most frequent and numerous parasitoids in grain were the beetle parasitoids *A. calandrae*, *H. sylvanidis*, *Th. elegans* and *C. tarsalis*, as they represented more than 88% of the adult wasps collected on grain samples. This is due to the fact that most grain samples were infested by coleopterous pests, whereas only a few moths were observed. All aforementioned species have been repeatedly recorded on stored grain (Khan & Anwarullah, 1970; Abdul-Rahman *et al*., 1977; Awadallah *et al*., 1985; Sedlacek, 1998, and others). In contrast to this, flour was mainly infested by lepidopterous pests with *Tribolium* sp. and *Cryptolestes* sp. being the only beetles of noteworthy presence. This is the reason why the most frequent wasps were *V. canescens*, *Trichogramma* sp., *H. hebetor* (parasitoids of lepidopterous pests), *H. sylvanidis* and *C. tarsalis* (parasitoids of *Tribolium* sp. and *Cryptolestes* sp.) (Thompson, 1954; Krombein, 1979; Brower *et al*., 1996; Schöller, 1998; Schöller & Flinn, 2000; Reichmuth, 2000). The presence of these wasps in stored flour has been noted in other recent studies as well (Awadallah *et al*., 1983; Prozell & Schöller, 1998; Pereira, 1998).

The prominent presence of *A. calandrae* in tobacco (63% of collected adult parasitoids) is due to the infestations by *L. serricorne* and *E. elutella*, which are the major pests of stored tobacco. This wasp can effectively parasitise both these pests and is one of the most important biocontrol agents in tobacco stores (Bare 1942; Ahmed & Khatun, 1988; Brower *et al*., 1996; Schöller & Flinn, 2000). In legume samples the main pests were *A. obtectus* and *Bruchus* sp. Consequently, the only parasitoids of notable presence were *A. calandrae* and unidentified species of the genus *Dinarmus*, constituting 83% of adult wasps in this product category. Both of them are important larval and pupal parasitoids of several bruchid species (Monge & Huignard, 1991; van Alebeek *et al*., 1993; Brower *et al*., 1996; Schöller & Flinn, 2000). Dried fruit samples were found to be infested mainly by *P. interpunctella*, *E. figuliella*, *C. cautella* moths and *O. surinamensis* and *O. mercator* beetles. These infestations may be the reason for the intense presence of the parasitoids *H. hebetor*, *V. canescens*, *Trichogramma* sp. (moth

parasitoids) and *H. sylvanidis* (parasitoid of *Oryzaephilus* sp.). It should be mentioned that these wasps constituted 90,8% of the total number of collected adult parasitoids on dried fruit samples. The noticeable presence of these wasps in dried fruits warehouses has been reported in other studies as well (Donohoe & Barner, 1934; Johnson *et al*., 2000).

The increased number of parasitoid species, which was noted in grain samples, could be attributed to the very large amount of samples from this product in comparison with the others (44% of collected samples). The proportion of approx. 2:1 between coexisting adults of *A. calandrae* and *Th. elegans* collected on 15 grain samples, suggested the dominance of the former species over the latter. Similar suppression has been verified in laboratory tests, where *A. calandrae* was more efficient than *Th. elegans* in parasitising *S. zeamais* in maize and *S. oryzae* in wheat, whereas under interspecific competition, the emergence of *Th. elegans* was significantly reduced by the presence of *A. calandrae* but not vice versa (Williams & Floyd, 1971; Wen *et al*., 1994; Wen & Brower, 1995). However, Press (1992), reported reduced effectiveness of *A. calandrae* in wheat infested by *S. oryzae* in comparison to *Th. elegans*, and attributed this to the large body size of *A. calandrae*, which prevented the parasitoid from moving through the small interstitial spaces of the grain.

The co-occurrence of *H. sylvanidis* and *C. tarsalis* in grain and flour resulted in the suppression of the latter wasp. Very few studies have been made on the biological and ecological characteristics of *H. sylvanidis* (Abdella *et al*., 1985; Ahmed *et al*., 1997; Ahmed & Islam, 1998) and *C. tarsalis* (Gahan, 1931; Powell, 1938; Howard *et al*., 1998), while there is no record of them under conditions of interspecific competition. The much larger-sized *H. sylvanidis* seems to suppress its competitor. This may be attributed to two factors, a) its capability to disperse more easily inside the store, due to its larger wings, and reaching the host more easily, b) its wider host range as it can develop on many external-feeding coleopterous pests such as *Tribolium* sp., *Oryzaephilus* sp. and *Cryptolestes* sp., whereas *C. tarsalis* parasitizes almost exclusively the saw-toothed beetle *O. surinamensis* and occasionally the rice weevil *S. oryzae* (Powell, 1938; Krombein, 1979; Howard *et al*., 1998; Evans, 1977). An additional indication thereof is the fact that the said hosts were the only ones, on which *C. tarsalis* has been successfully reared in the laboratory during present study. Contrarily, *H. sylvanidis* has developed sufficiently on *T. confusum*, *T. castaneum, C. ferrugineus*, *O. mercator* and *O. surinamensis*.

Unlike the above-mentioned interactions, these among moth parasitoids have been thoroughly studied. Competition between *H. hebetor* and *V. canescens* for moth larvae has been examined in the laboratory (Press *et al*., 1977; Ridout, 1978; Petters & Stefanelli, 1983; Wool *et al*., 1987; Kamin-Belsky *et al*., 1987), as well as in storage facilities (Donohoe & Barnes, 1934; Bare, 1942; Prozell & Schöller, 1998; Johnson *et al.*, 2000). In the laboratory tests it was obvious that larvae of *V. canescens* could not survive in hosts that were afterwards attacked by *H. hebetor*, whereas host larvae previously parasitised by *H. hebetor* were not attacked by *V. canescens*. This fact explains the suppression of *V. canescens* in grain (1:3) and dried fruits (1:3,5).

However, this was not observed in flour (approx.1:1), possibly due to the different product type. Schöller & Flinn (2000) note that the type of stored product often determines whether the pest is situated within reach of the parasitoid or not. *V. canescens* has a rather long ovipositor with which it can sting a host larva situated under a 1-cm thick layer of flour (Corbet & Rotheram, 1965). There are many pyralid larvae which develop at that depth in flour stored in mills and other facilities. On the other hand, *H. hebetor* is unable to reach the host at such a depth, as its ovipositor is very short.

Interaction of these larval parasitoids with the egg parasitoids of the *Trichogramma* genus cannot be regarded as direct competition as they attack different stages of the host.

Thus, species of this genus have been used as complementary parts of a biological control programme in storage facilities with *H. hebetor* (Brower & Press, 1990) and the predator of various stored product pests *Xylocoris flavipes* (Brower & Press, 1998). In these cases the proportion of coexisting adults collected on flour and dried fruits do not have the same significance as in the other cases of coexistence.

It is clear that this study is of descriptive nature. However, it reveals the importance of parasitoids as control agents for stored product pests and supplies useful data on the presence of hymenopterous fauna in different types of storage facilities and stored products. Further research on the life history and biology of some parasitoids, which have not been studied in detail, is required. Apart from that, further experimentation is required on rearing, quality control, transport and release methods of beneficial insects. Compatibility with other control methods, cost/benefit analysis, legal constraints on the use of biocontrol in storages, commercial availability of natural enemies, their effectiveness under natural storage conditions in a variety of facilities and products should also be thoroughly examined. Undoubtedly, a lot of research is still required in order to render biological control a successful and practically important component of stored product IPM.

Acknowledgements

The authors are indebted to Dr. Eric Grissell and Dr. Robert Carlson, Systematic Entomology Laboratory, Agricultural Research Service, US Department of Agriculture for the identification of *A. calandrae* and *V. canescens*, respectively. We like also to thank Prof. Howard Evans, Colorado State University, and Dr. Mamoru Terayama, expert on hymenoptera systematics, for the identification of *H. sylvanidis*, *C. tarsalis* and *Th. elegans*. Finally, we thank all our colleagues and personnel of local Agricultural Stations for their valuable help in collecting samples.

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Bugs in space: aspects of the system *Callosobruchus maculatus* (Col.: Bruchidae) and *Uscana lariophaga* (Hym.: Trichogrammatidae) in stored cowpea

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Abstract: We have studied and described the following spatial aspects of the interaction between the bruchid *Callosobruchus maculatus* and its natural enemy *Uscana lariophaga* in stored cowpea: the three-dimensional spatial oviposition pattern of individual *C. maculatus* females; foraging behaviour of *U. lariophaga*; and host finding behaviour of *U. lariophaga*. The results are discussed from the perspective of the foraging behaviour of *U. lariophaga* in stored cowpea.

Key words: cowpea, *Callosobruchus maculatus, Uscana lariophaga,* spatial oviposition pattern, foraging behaviour, host finding

Introduction

Cowpea is an important crop for subsistence farmers in West Africa (Nwokolo, 1996). The grains or pods are stored during the dry season for home consumption, for trade, and to provide sowing-seed for the next growing season. Stored cowpea is often infested with *Callosobruchus maculatus* L. (Col.: Bruchidae), which may result in heavy qualitative and quantitative losses (Jackai & Daoust, 1986; Caswell, 1981; Adams, 1977; Schulten, 1982). We have studied the indigenous egg parasitoid *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae) as a candidate for a conservation strategy of biological control (Van Huis *et al.*, 1991). Here we focus on spatial aspects of this system. Spatial processes, such as dispersal of beetles and foraging behaviour of the parasitoids, can have a profound impact on population dynamics and consequently on biological control. In three experiments, described below, we have studied the spatial distribution of *C. maculatus* eggs in stored cowpea, and the foraging and host finding behaviour of *U. lariophaga*.

Materials and methods

General

In the experiments and in the rearings we used cowpea (*Vigna unguiculata*) seeds of the variety 'Black Eyes'. The *C. maculatus* culture originated from the Niamey region in Niger. Beetles were reared in petridishes on cowpea seeds at 12L:12D and at 35±1°C during the photophase and at 25±1°C during the scotophase. *U. lariophaga* was reared on *C. maculatus* eggs at 30±1°C and 12L:12D. All experiments were carried out at 30±1°C.

Experiment 1. Spatial oviposition pattern of Callosobruchus maculatus
The three-dimensional distribution of eggs by *C. maculatus* females was studied in 1-liter glass beakers filled with cowpea. A 1 h old female was released at the centre of the cowpea mass in each beaker. The female was allowed to oviposit for seven days, after which the beaker was emptied layer by layer, using a sticky disk. Each seed was carefully inspected for eggs and the position of each bean with eggs was recorded using a set of coordinates. The experiment was replicated 19 times. The data were analysed using spatial point pattern analysis techniques. Kernel estimation was used to calculate the volumes of the egg clusters, expressed as the volume which would incorporate 90-95% of the eggs.

Experiment 2. Foraging behaviour of Uscana lariophaga
Foraging behaviour of *U. lariophaga* females was observed in arenas consisting of a petridish (13.6 cm) filled with one layer of cowpea seeds. The arena contained either a small host patch (3-6 fresh *C. maculatus* eggs on 2-4 beans), a large host patch (18-22 eggs on 7-10 beans), or no host patch. A 2-23 h old female wasp was released at the centre of the arena. A mirror, placed underneath the arena, allowed the observer to monitor the wasp continuously. Both the behaviour and the location of the wasp were recorded. Behaviour was monitored using a hand-held computer (Psion Workabout, Psion PLC, London, UK) and the computer program "The Observer for Windows 5.0" (Noldus Information Technology, Wageningen, the Netherlands). The coordinates of the wasp were recorded at a resolution of single beans, using a detailed map of the arena. Each wasp was observed for a period of up to 1.5 hour. An observation was terminated if the wasp did not leave the release site (a gelatine capsule) within five minutes, if the wasp did not move for 30 minutes, if the wasp walked more than about 4 cm on the petridish, or if the wasp flew away and did not return to the seed layer within about 20 seconds. A total of 37 wasps was released.

Experiment 3. Host patch finding by Uscana lariophaga
(a) Host patch finding over long distances: Host patch finding over distances of up to 75 cm was investigated in 95×20×20 cm boxes, filled with cowpea and placed with the long side horizontal. A host patch consisting of 20 beans carrying 565±146 (SD) fresh *C. maculatus* eggs was placed at the centre of the square cross section of the box and at 10 cm from one of the ends of each box. Beans containing altogether fifteen parasitized eggs that were about to emerge were placed at a horizontal distance of 5, 15, 25, 40 or 75 cm from the host patch. After three days the host patches were taken out of the boxes and any wasp inside the host patch was removed. The host patches were incubated for another three days, after which the numbers of parasitized and unparasitized eggs were counted. Each distance was replicated five times, except 5 cm, which had four replicates.

(b) Host patch finding over short distances: Host patch finding over distances of up to 10 cm was investigated in cylindrical containers with a height of 14.5 cm and a diameter of 11 cm. The cylinders were filled with cowpea and placed with the long side horizontal. A host patch containing 26±4 (SD) eggs on 10 beans was placed 2.3 cm from one end of the container. At 2.5, 5 or 10 cm from the host patch a 2-17 h old female wasp was released. After 2, 4 or 8 hours the host patch was removed and incubated as described for experiment 3a. The numbers of parasitized and unparasitized eggs were counted. Each treatment combination was replicated 20 times.

Results

Experiment 1. Spatial oviposition pattern of Callosobruchus maculatus
Oviposition by individual *C. maculatus* occurred in single clusters of 70 eggs on average. The egg density at the centre of the clusters was on average 0.6 eggs per bean. 90-95% of the eggs fitted within a volume of 19.1 cm^3.

Experiment 2. Foraging behaviour of Uscana lariophaga
We did not detect any influence of the presence or size of the host patch on the behaviour of the wasp. The most important factor which influenced the behaviour of *U. lariophaga* was an encounter with a host egg: this changed the walking trajectory from 'straight' to 'tortuous' and it increased the residence time per bean. The average residence time of *U. lariophaga* on a bean was 39 s per visit (parasitizations not included).

From a distance of about 4-6 beans from the host patch, the wasps moved directedly towards the host patch. Upon encountering a bean with an egg, the probability that *U. lariophaga* would step onto that bean was twice as high than would be expected if she would have randomly chosen a bean to move to. Once on a bean with an egg, however, she failed to find the egg during one visit in about 60-70% of the cases.

Experiment 3. Host patch finding by Uscana lariophaga
(a) Host patch finding over long distances: The distance between the host patch and the introduction site did not have an effect on the probability that the host patch was found, but it did have an effect on the number of parasitized eggs in the host patch. The equation $y = 431 \cdot \exp(-0.057 \cdot x)$, with x = the distance between the host patch and the introduction site and y = the number of parasitized eggs, described the data with a coefficient of determination (R^2) of 0.67.

(b) Host patch finding over short distances:
Both distance and time had an effect on the probability of finding the host patch and on the number of parasitized eggs. The fraction of host patches that was found varied between 0.2 for the '10 cm, 2 h' treatment and 0.9 for the '2.5 cm, 8 h' treatment. The average number of parasitized eggs varied between 1 and 22 for the same respective treatments.

Discussion

The fact that *C. maculatus* oviposits in clusters with a density of 0.6 eggs·$bean^{-1}$ implies that random search would be an effective search strategy for *U. lariophaga* within host clusters. She would probably encounter a host even after randomly moving across a few beans. Experiment 2, however, shows that *U. lariophaga* does not move about randomly within a host patch; instead, she preferably chooses to move to beans with eggs. This, in conjunction with the strong arrestment response as was observed in Experiment 2, makes searching within clusters even more effective.

Another implication of oviposition by *C. maculatus* in clusters is the fact that *U. lariophaga* needs to find those clusters, possibly over somewhat longer distances. This was investigated in Experiment 2 and 3. Experiment 2 showed that movement was random until the wasp came as close as 4-6 beans from the host patch, after which movement became directed towards the host patch. Experiment 3a shows that *U. lariophaga* is able to find host patches over distances of up to 75 cm. The number of eggs that was parasitized, however, decreased with distance. This was probably a result of fewer wasps that found the host patch, and those that did reach the host patch probably arrived late, leaving little opportunity for them to parasitize eggs.

The results of Experiment 3b allow us to estimate the arrival times for those wasps that reached the host patch. The arrival times can be estimated based on the total amount of time that was available (2, 4, or 8 h), the number of eggs that were parasitized, and the estimated amount of time that was needed for those parasitizations. The latter can be derived from Experiment 2. Based on these arrival times (data not shown) we can calculate the median net displacement rate in the direction of the host patch, which appears to be 1.4 cm/h or two beans per hour. Van Alebeek (1996) reported median net displacement rates of 2.1-3.3 cm/h in the upward direction, but *U. lariophaga* has a strong negative geotactic response and upward displacement is faster than horizontal displacement.

Our findings give insight into the foraging environment of *U. lariophaga* and the decisions she is facing while searching for hosts. They will be used to build and validate a spatially explicit computer model which simulates the foraging behaviour of this wasp.

Note: This is a selection of results that will be reported in more detail in the following forthcoming papers:

Stolk, C., A. Stein, S.B. Slumpa, S.K. Tiase & A. van Huis. (in press) Exploring the foraging environment of a natural enemy of *Callosobruchus maculatus*: Spatial egg distribution in stored cowpea. Entomologia experimentalis & applicata.

Stolk, C., W. van der Werf & A. van Huis. Foraging behavior of *Uscana lariophaga* (Hym.: Trichogrammatidae) in stored cowpea. In preparation.

Stolk, C., M.N. Ghimire, S. Souquié, W. van der Werf and A. van Huis. Host finding by *Uscana lariophaga* in stored cowpea: the effect of distance, time interval, host patch size and spatial orientation. In preparation.

Acknowledgements

We thank Simon B. Slumpa and Frans van Alebeek, who carried out Experiment 1, and Sandrine Souquié and Mukti Ghimire, who carried out Experiment 3a and b, respectively. Leo Koopman, Frans van Aggelen, André Gidding, Gerard Pesch and Mariët Kools reared the insects that we used in the experiments. Alfred Stein and Evert-Jan Bakker gave advice on the statistical analysis of Experiment 1 and 3, respectively.

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Van Huis, A., Kaashoek, N.K. & Maes, H.M. 1991: Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hym.: Trichogrammatidae): a review. In: F. Fleurat-Lessard & P. Ducom (eds.), Proceedings of the Fifth International Working Conference on Stored-product Protection, Bordeaux, France, September 9-14, 1990. pp. 99-108.

PHYTOCHEMICALS

Determination of stability of essential oil constituents as repellents

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Abstract: Chemicals that exert repellent activity offer two advantages in stored product environments: Contamination of stored food by material applied is minimized as it may take place only by repellent particles suspended in air, and food, presumably, is protected without contamination by pests. Repellents are, however, desired to show a long lasting activity for long term protection of food in closed spaces of storages. A system that allows to test activity of known concentrations of repellents in air at given intervals of time with minimum loss of material during run of tests was designed in order to determine stability of repellent activity. The device consists of a glass Y-tube with arms, one connected to a repellent containing jar and the other one to a control jar. A low rate air movement was created by sucking the air in jars through arms of the Y-tube with a peristaltic pump connected to the stem (main arm). The lowest air flow rate that gave the highest differrence between numbers of test insects preferring one of the arms was 0.33 ml/s in main arm of the olfactometer and the shortest period needed for the highest number of insects to make a preference between the arms of olfactometer was 90 seconds in tests conducted on *Tribolium confusum* du Val adults using essential oil constituents. Repellency of 8 essential oil constituents anethole, carvacrol, 1,8 cineole, *p*-cymene, menthol, γ-terpinene, terpinen-4-ol and thymol was tested 5 times within a period of 8 weeks, at the beginning, at the end of 1^{th}, 2^{th}, 4^{th} and 8^{th} weeks. Approximate total loss of material during run of tests was estimated 10% after 8 weeks. Loss of material can be reduced further by using jars of larger capacity. All compounds showed repellency in varying degrees against *T. confusum*. Repellency of all compounds gradually decreased as the time passed.

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Potentials of cinnamaldehyde and methylchavicol as grain protectants against four insect pests of stored products

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Abstract The efficacy of cinnamaldehyde (zimtaldehyde) and methylchavicol (4-allyl-anisol) as repellents, toxicants and grain protectants against infestation by *Callosobruchus chinensis, Oryzaephilus surinamensis, Sitophilus granaries* and *Sitophilus oryzae* was investigated under laboratory conditions (25±1°C and 60±5 % r.h.). The experiments carried out include contact and fumigant toxicity assays, treatment of grains with test compounds as admixtures and repellency assays. Both cinnamaldehyde and methylchavicol showed a high efficacy as contact insecticides on the experimental insects. They were more potent against *C. chinensis* and *O. surinamensis* (≥ 15 µl/ml of the test compounds induced 100 % insect mortality) than against *S. granaries* and fumigant toxicity assays, treatment of grains with test compounds ad admixtures and repellency assays. Both cinnamaldehyde and methylchavicol showed strong efficacy as contact insecticides towards the experimental insects. They were more potent against *C. chinensis* and *O. surinamensis* (> 15 µl/ml of the test compounds induce 100 % insect mortality) than against *S. granaries* and *S. oryzae*. Increasing the exposure time to 48 hours effectively) increased the mortality rate of *S. granarius*. Cinnamaldehyde was more effective as a fumigant than methylchavicol. It also showed stronger repellency than methylchavicol. Both compounds showed strong contact toxicity against the stored product insect pests used for experiments.

Key words: phytochemical, *Callosobruchus, Oryzaephilus, Sitophilus,* repellent, toxic

Introduction

Higher plants are promising new sources of natural pesticides and antifeedants against insect pests of stored products. Many substances derived from plant affect insect pests of stored produce in various ways (Su, 1983; Jacobson, 1989; Shaaya et al., 1991).

Acetone extracts and essential oils of several herbs, spices and medicinal plant have been found to posses various effects on insects. Some are attractants, others are components of pheromones and insect alarm systems. Some may be repellent or toxic to insect pests (Inscoe, 1982; Mayer and McLaughlin, 1991; Ho et al., 1994).

Cinnamaldehyde is the main constituent of the essential oil of *Cinnamon aromaticum*. It is also found in appreciable quantities in Cassia oil. Up to 98 % of this compound exists in the trans form. It is resistant to alkali and extensively used in the perfume industry. Methylchavicol (4-allyl-anisol) is commonly found in *Ocimum* species of plants as an essential oil component.. Whole leaves of the French tarragon (*Artemisia drunculus)* contains 86.4 % methylchavicol. *Ocimum kenyensis* contains 12.8 % of this compound.

The present research work was designed to investigate the potentials of cinnamaldehyde and methylchavicol as protectants, antifeedants and repellents against four insect pests of stored products.

Materials and methods

Test insects: Test insects were bred in the dark at 65-70 % r.h. and temperatures of 20-21°C for *S. granarius* and *S. oryzae* were cultured on wheat grains (containing broken grains). *O. surinamensis* was cultured on oats to which glycerine and yeast had been added. *C. chinensis* was cultured on green peas.

Test compounds: test compounds (cinnamaldehyde and methylchavicol) were commercially obtained (Sigma chemicals). The physical properties of these compounds have been described in Table 1. They were dilute with acetone and used for bioassays.

Dose-response contact toxicity bioassays

Contact toxicity bioassays were conducted according to the method of Huang et al., 1997. Whatman No 1 filter-paper of the appropriate sizes was fitted into 50 mm (diameter) corning glass petri-dishes. 0.5 ml of the appropriate dilution of test compounds in acetone (0-400 µl/ml) were used to impregnate the filter papers. Each filter paper was left to dry for 10 min. Pure acetone solvent was used as control. 20 insects were introduced to the filter paper in each petri-dish. Glass rings were used to confine the insects to the top of the filter papers. Mortality counts were made after 23 h and 48 h by observing the insects using a hand lens. Moribund insects (those ambulating weakly) were not counted as dead. Each experimental unit (consisting of various dosage levels) was replicated 3 times. Experiments were conducted at 55-65 % r.h. in the dark. LD_{50} and LD_{95} values (where applicable) were calculated using Tablecurve 2D x 4 software programme (Jandel Scientific software, 1996).

Table 1: Properties of pure substances

Name of compound	**Source**	**Molecular weight**	**Melting Point (°C)**	**Boiling Point (°C)**	**Oral Toxicity (mg/kg)**
Methylchavico (4-allyl-anisol)	*Ocimum* species	148.21		215-216	LD_{50} (oral for rats) 1230
Cinnamaldehyde (zimtaldehyde). Trans-3-phenyi-2-propanol	Cassia oil (about 75%)	132.16	–8 to 7.5	127	2220

Repellency bioassay

Repellency properties of cinnamaldehyde and methylchavicol were determined using the area preference method as described by McDonald et al., 1970. Test areas were made up by filter paper discs cut in half.

Solutions of the compounds used for repellency experiments were made in acetone (0-400 µl/ml). Each solution was uniformly applied to a half-filter paper disc using a micro-pipette. The other half of the paper was treated with acetone alone. Treated and control half-discs were air-dried for 10 min to allow complete evaporation of acetone solvent. Full discs were subsequently prepared by attaching treated halves to untreated halves with a sellotape. Each filter paper was then placed into 50 mm petri-dish. Ten (10) adult beetles of mixed sex were released at the centre of each filter disc and subsequently the petri dish was covered. Each treatment was replicated 5 times. The number of insects present on control (N_C) and

treated (N_T) half discs were recorded after 30 min of exposure. % Repellency (PR) values were calculated as follows

$PR = (N_C-N_T)/(N_C+N_T)x100$

PR values were analysed using analyses of variance in a one-way classified multiple comparison test, where the different dosage level were only variable. Mean repellency values were assigned to repellency classes according to the method of Juliana and Su (1983). Graphs of % repellency versus dosage levels were plotted for each species.

Experiment to distinguish between contact and fumigant toxicity effects

Whatman No 1 filter papers of 50 mm diameter were impregnated with appropriate concentrations of pure compounds selected to cover LD_{50} and LD_{95} values for mortality (determined from contact toxicity studies). The solvent was allowed to evaporate from the filter paper (10 min). A nylon mesh of 0.11 mm pore size, cut to fit the diameter of the petri-dishes was fitted 1 mm above the impregnated dried filter paper. 20 adult beetles were placed on this nylon mesh and the petri-dishes were covered. Mortality counts were made after 24 h and 48 h, respectively. Each experiment was replicated 5 times. Control contained filter papers that had been impregnated in the same fashion with acetone alone.

Toxicity on grain

Portions of 100 g substrate were mixed with various amounts of the chemicals in a mechanical shaker (Multifix GmbH Germany) for 15 min. Green peas were used as substrate for *C. chinensis* while wheat grains were used as substrate for *O. surinamensis, S. granarius* and *S. oryzae*. Three replicates were set up for each treatment and for the controls. The number of dead insects was counted after 1, 2, 4 and 8 days. After the 8th day, both the living and dead insects were removed. The grains were subsequently stored under the same experimental conditions (55-65 % r.h. and 25±1°C) for 4 months and observed for the emergency of F1 progeny.

Table 2: Contact toxicity of cinnamaldehyde and methylchavicol to four insect pests

Compound	**Experimental Insects**							
	Callosobruchus chinensis		***Oryzaephilus surinamensis***		***Sitophilus granarius***		***Sitophilus oryzae***	
Cinnamaldehyde	***24 h***	***48 h***	***24 h***	***48 h***	***24 h***	***48 h***	***24 h***	***48 h***
LD_{50} mg/cm²	5.00	5.00	7.53	7.66	22.64	14.37	59.65	35.24
LD_{95} mg/cm²	5.01	5.81	7.76	7.60	NA	NA	60.17	47.66
R^2	1.00	1.00	1.00	1.00	0.97	0.98	0.99	1.00
Fit standard error	0.24	0.48	0.39	0.38	1.25	1.23	1.10	0.80
Mean mortality (%)	83.15	83.25	70.55	72.80	30.55	39.05	41.45	42.25
Residuals	1.31	1.02	0.03	0.09	0.01	0.02	0.00	0.00
Methylchavicol	***24 h***	***48 h***	***24 h***	***48 h***	***24 h***	***48 h***	***24 h***	***48 h***
LD_{50} mg/cm²	8.34	7.50	13.00	12.20	39.72	14.39	16.58	14.96
LD_{95} mg/cm²	10.95	10.83	14.09	13.42	NA	17.71	17.29	15.73
R^2	0.98	0.96	0.99	1.00	1.00	1.00	1.00	1.00
Fit standard error	1.30	1.35	0.96	0.82	0.17	0.62	0.40	0.65
Mean mortality (%)	71.50	80.20	49.25	60.05	17.05	41.30	34.45	40.35
Residuals	2.49	0.13	2.41	0.35	0.01	0.05	0.48	0.05

Results

Cinnamaldehyde and methylchavicol were more effective as contact toxicant to *C. chinensis* and *O. surinamensis* than towards *S. granarius* and *S. oryzae* (see Table 2). Efficacy of the two compounds towards *S. oryzae* were comparable. Methylchavicol was less potent than cinnamaldehyde against four insect pests of stored products. Comparative mean mortality values are also shown in Table 2. Toxicity caused by the pure compounds when used as admixtures to grains is shown in Table 3. LD_{50} and LD_{95} values decreased with an increase in exposure period. Cinnamaldehyde was also more effective in grain admixture than methylchavicol.

Table 4 shows the fumigant toxicity of cinnamaldehyde towards *C. chinensis* and *O. surinamensis, S. granarius* and *S. oryzae*. Cinnamaldehyde was a better fumigant, showing as much toxicity as it showed in contact toxicity studies. Methylchavicol exhibited poor fumigant effect. Table 5 contains the mean repellency values as well as the repellency classes assigned according to the method of Juliana and Su (1983). Cinnamaldehyde also showed high repellency towards *S. oryzae*. 15 µl/ml caused 100 % repellency. Mean repellency values (calculated over a range of dosage levels used for experiments) were highest for *C. chinensis* and *S. granarius* (93.83 % and 0 %, respectively). Methylchavicol was most repellent to *S. granarius* (88.89 %). Cinnamaldehyde possessed better residual toxicity for the test insects than methylchavicol. Experiments to determine the volatility of test compounds in open petri-dishes under the same experimental conditions had indicated that 1 h exposure (at 55-65 % r.h. and 25°C), 8 % cinnamaldehyde and 7.18 % of methylchavicol still remained on the filter paper discs.

Table 3: Toxicity of methylchavicol and cinnamaldehyde applied on grain with insect exposure for 1, 2, 4, and 8 days

Parameter	***Callosobruchus chinensis***				***Oryzaephilus surinamensis***				***Sitophilus granarius***				***Sitophilus oryzae***			
	Methylchavicol															
Exp.time (d)	**1**	**2**	**4**	**8**	**1**	**2**	**4**	**8**	**1**	**2**	**4**	**8**	**1**	**2**	**4**	**8**
R2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	0.97	1.00	1.00	1.00	1.00	1.00	1.00
Fit Se	0.46	0.38	0.00	0.00	0.22	0.22	0.22	0.22	0.58	0.62	0.62	0.87	0.69	0.20	0.89	0.69
Residuals	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.48	0.00	0.00	0.00	0.02	0.00	0.00
LD_{50}	0.08	0.05	0.02	0.02	0.01	0.01	0.01	0.45	0.45	0.25	0.11	0.10	0.09	0.07	0.05	0.05
LD_{95}	0.14	0.05	0.01	0.01	0.04	0.04	0.04	0.04	0.46	0.43	0.20	0.20	0.13	0.11	0.07	0.06
mortality (%)	94.8	100	100	100	99.3	99.4	100	100	57.8	68.9	72.6	77.2	80.2	83.9	89.8	91.7
	Cinnamaldehyde															
Exp.time (d)	**1**	**2**	**4**	**8**	**1**	**2**	**4**	**8**	**1**	**2**	**4**	**8**	**1**	**2**	**4**	**8**
R2	0.99	1.00	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fit Se	1.55	0.75	0.62	0.81	0.85	0.50	0.00	0.00	0.42	0.35	0.69	0.69	0.00	0.38	0.00	0.00
Residuals	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.35	0.00	0.00	0.00
LD_{50}	1.50	0.05	0.04	0.03	0.05	0.01	0.01	0.01	0.05	0.05	0.05	0.05	0.01	0.01	0.01	0.01
LD_{95}	0.01	0.01	0.01	0.01	0.07	0.01	0.005	0.005	0.005	0.06	0.05	0.05	0.04	0.005	0.005	0.005
mortality (%)	82.5	100	100	100	97.3	98.9	100	100	91.9	92.4	94.8	96.8	98.0	99.2	100	100

LD-values in mg/100g of substrate (green peas or wheat grain)

Discussion

Although structural similarities exist between cinnamaldehyde and methylchavicol, their various toxic effects towards experimental insects were not similar. Both of them have the benzene ring structure but different attached groups. This would lead to variations in the electronic, rotational and vibrational frequencies in energy levels around the molecules and would ultimately affect their chemical potencies against the test insects. Cinnamaldehyde was a more potent repellent against test insects than methylchavicol. However, its toxic effect was comparable to that of methylchavicol particularly against *S. granarius* and *S. oryzae* in contact toxicity studies.

Table 4: Fumigant toxicity of cinnamadehyde

	R^2	Fit Se	Residuals	LD_{50}	LD_{95}
Callosobruchus chinensis					
24 h	1.00	0.014	0.0013	0.108	0.393
48 h	1.00	0.14	0.0013	0.108	0.397
Oryzaephilus surinamensis					
24 h	1.00	0.14	0.0013	0.108	0.397
48 h	1.00	0.14	0.0013	0.108	0.397
Sitophilus granarius					
24 h	0.99	0.60	0.00	3.830	64.114
48 h	1.00	0.0003	0.00	0.002	0.099
Sitophilus oryzae					
24 h	1.00	0.014	0.0013	0.108	0.397
48 h	1.00	0.014	0.0013	0.108	0.397

Table 5: Mean repellency of cinnamaldehyde and methylchavicol

	% Mean repellency		Repellency class	
Callosobruchus chinensis	93.33±1.15	58.15±3.01	**V**	**III**
Oryzaephilus surinamensis	87.41±1.18	37.41±2.16	**V**	**II**
Sitophilus granarius	100±0.0	88.89±1.89	**V**	**V**
Sitophilus oryzae	90.37±1.42	72.60±1.27	**V**	**IV**

Repellency classes assigned according to the method of Juliana and Su (1983).
0 = < 0.1; I = 0.1-20; II = 20.1-40; III = 40.1-60; IV = 60.1-80; V = 80.1-100

Acetone extracts and essential oils of several herbs spices and medicinal plants have been found to possess insecticidal properties. Some are attractants. Others are components of pheromones and insect alarms system. Some possess antifeedant and repellent properties or may be toxic to insect pests (Inscoe, 1982; Mayer and McLaughlin, 1991; Ho et al., 1994, 1995; Ho and Ma, 1995; Obeng-Ofori and Reichmuth, 1997). Cinnamaldehyde is the main component of the essential oil of *Cinnamon aromaticum*. It is also found in appreciable quantities in cassia oil. Up to 98 % of this compound exists in the trans form. It is resistant to alkali and is extensively used in the perfume industry. Methylchavicol is commonly found in *Ocimum* species of plants, as an essential oil component. Cinnamaldehyde has been shown to posses toxic and antifeedant properties against *Tribolium castaneum* and *Sitophilus zeamais*

(Huang and Ho, 1998). Cinnamaldehyde is quite volatile, therefore its contact toxicity might actually be a combination of contact and fumigant effects. It would be difficult to eliminate the effects of fumigant toxicity while studying contact toxicity effects. The fluid and oily nature of cinnamaldehyde and methylchavicol suggest that they may act as physical poisons, exerting an asphyxiant effect and slowly killing the insect by exclusion of air.

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Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
153-164

Ability of products derived from the leaves of *Clausena anisata* to protect stored legumes from attack by *Callosobruchus maculatus* and *C. chinensis* (Coleoptera, Bruchidae)

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Abstract Dried ground leaves of *Clausena anisata*, essential oil extracted from leaves and anethole, identified as the principal constituent of this oil, were tested under laboratory conditions for their ability to protect stored legumes from attack by *Callosobruchus maculatus* L. and *C. chinensis* on mung bean (*Vigna mungo*) and green peas, respectively. Contact toxicity and repellency was tested on filter paper, contact toxicity also by a treatment of beans. Beans treated with dried ground leaves or essential oil extract, respectively, caused significant reductions in the survival rate and number of progeny production. There was more than 90% adult mortality and reduction in adult emergence in grain treated either with one of the two materials at a dose of 5g / 50g grain for dried ground leaves or with 8µl / 40g grain for the essential oil.

Essential oil and anethole impregnated on filter paper discs or coated on beans were found to be highly toxic to both insect species with anethole evoking the highest toxicity especially when coated on grains. The LD_{50} of the crude oil extract to *C. maculatus* and *C. chinensis* were 5,97 and 4.30µl/40g grain, whereas for anethole they were 0.37 and 0.65µl/40g grain respectively. Moreover essential oil and anethole produced a stronger repellent activity against the test insects. The results are discussed in terms of the efficacy of products derived from *C. anisata* for protection against insect infestation in traditional storage of legumes in Africa.

Key words: Essential oil, anethole, insects, bruchid, contact toxicity, repellent

Introduction

In most developing countries, grains are subject to attack by many insect species especially at small scale farmer levels where storage conditions are usually inadequate to prevent or reduce insect attack.

Intensified efforts to develop insecticide-based techniques for protecting grains in small traditional farm stores have only been partially successful because of problems such as high cost of synthetic insecticides and erratic supply due to foreign exchange constraints. There are also health risks to farmers and other hazards to the environment as a result of misuse of insecticides.

The plant based insecticides (PBIs) including allelochemical compounds such as essential oils could be an useful complementary or alternative method to the heavy use of classical insecticides. This could increase the biodegradability of insecticide treatment and therefore decrease the quantity of toxic insecticide residues, increase insecticide selectivity and develop a better respect for the environment.

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 (Hassanali *et al*., 1990; Poswal and Akpa, 1991; Parh *et al*., 1998). We have started a modest survey of ethnobotanical materials used traditionally for stored product protection in the Western highlands of Cameroon with the objective of evaluating their active constituents. One plant material form the subject of this paper: the leaves of *Clausena anisata* Will. Ex. Benth. (Rutaceae). *C. anisata* is a small tree which grows in the savanna regions of West Africa (Yensu, 1978). Its uses in traditional medicine against many diseases or as an insect repellent in some parts of Africa and the Philippines has been reviewed (Yensu, 1978).

This study was carried out to evaluate the ability of dried ground *C. anisata* leaves, essential oil extracted from the leaves and anethole identified as the main constituent of this oil to protect stored beans from infestation by *Callosobruchus chinensis* and *C. maculatus.*

Materials and methods

Collection and preparation of the plant materials.

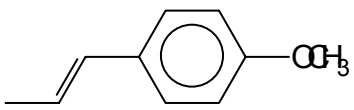
The leaves of *Clausena anisata* Will. Ex. Benth were collected from the Bafou village located in the Menoua Division of the Western highlands of Cameroon in April 1999. The identity of the plant was confirmed at the Camerounian National Herbarium where a voucher specimen was deposited. The plant leaves were dried naturally on laboratory benches at room temperature (26-28°C) for 5 days by which time they were crisp dried. The dried material was ground in a coffee grinder (Jankle and Kundel KG, Typ A10, N5614, Germany) and then passed through a sieve with a mesh size of 0.5mm. The resulting fine powder was used as direct admixture to the grains at 4 different doses.

Extraction Procedure and characterisation of essential oil

The dried plant leaves were subjected to hydrodistillation using a modified Clevenger type apparatus for 6h. Oil collected was dried over anhydrous sodium sulfate filtered and weighed yielding 2% (w/w) of a strong sweet smelling and a brownish oil which was stored in the refrigerator until used.

Analyses of the essential oil was carried out by GC-MS on a HP 5890 II gas chromatograph coupled to a HP 5972 mass selective spectrometer using DB wax fused silica capillary column (60m x 0.25mm i.d,0.25µm film thickness). GC conditions: the oven was programmed at 60-220°C at a rate of 5°C/min using helium as carrier gas, flow rate 0.9ml/min, injector temperature 230°C, interphase temperature 240°C and mass range up to 650a.m.u. The constituents were identified by their retention index and mass spectrum in comparison with those of standard synthetic compounds. A total of six compounds representing 96.3% of the oil were identified as the main constituents with the most important being trans-anethole (80.7%) and trans-isoeugenol methyl ether (11.5%) (table 1).

Anethole (99 % purity) used for bioassays was purchased from Aldrich Ltd., Germany.



anethole

Culturing of insects

Callosobruchus 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 culture maintained at the Federal Biological Research Centre for Agriculture and Forestry, Institute for Stored Product Protection, Berlin, Germany. The food media used were green peas for *C. chinensis* and mung beans (*Vigna mungo*) for *C. maculatus* procured in Berlin.

Contact toxicity of dried ground leaves on grains.

The fine powder obtained from dried ground leaves of *C. anisata* was mixed with 50g samples of grain in 380ml glass jar at four different doses of 1.3, 2.5, 5 and 10% (w/w) for the two insect species. The plant product / grain admixtures were thoroughly mixed with a rotary shaker (Multifix GmbH, Germany) for 30 min. Control in each set of treatment consisted of grain containing no plant materials and each dose was replicated four times. 25 unsexed 1-2 days old adults of each type of insects were introduced into each jar. The glass jars were covered with cotton cloths held with rubber bands. In order to estimate mortality, the number of dead insects in each jar was counted daily up to 6 days. Percent insect mortality was calculated by using the Abbott's correction formula for natural mortality in untreated controls (Abbott, 1925):

$$\frac{\text{percent of dead insects in treated jar- percent of dead insects in control}}{\text{100 - percent of dead insects in control}} \text{ x } 100$$

Contact toxicity of essential oil and anethole on filter paper

The contact effect was evaluated by treating a 7cm diameter (38.5cm^2) Whatman N°1 filter paper dics with the substances dissolved in acetone. The filter paper was placed in a glass Petri dish (7cm diameter). An aliquot of 1, 2, 4 and 8µl of essential oil and 0.125, 0.250, 0.500 and 1.00µl of anethole dissolved in 1ml of acetone was applied to filter paper dics corresponding to the following doses of 0.026, 0.052, 0.10 and 0.20µl/cm^2 for essential oil and 0.0032, 0.0065, 0.013 and 0.026µl/cm^2 of anethole. The control dishes were only treated with acetone. Thereafter the acetone in all dishes was allowed to evaporate for 10 min prior to the introduction of twenty 1-2 day-old adults of each insect species separately into each dish. Each set of treatment was replicated four times. Insect mortality was recorded daily up to 4 days and percent mortality calculated by using the Abbott formula.

Contact toxicity of essential oil and anethole on grain

The effect of essential oil and anethole treated legumes on adult mortality of the two beetle species was studied in the laboratory at 25-27°C and 70-75 % RH in the dark. The legumes were separately treated with solutions made up of 0, 1, 2, 4 and 8µl of essential oil and 0, 0.25, 0.50, 1.00 and 2.00µl of anethole in 1ml acetone. Test solutions were mixed with 40g samples of beans in 380ml glass jars and stirred continuously for 20min to ensure even spread of the material over the surface of the beans and kept for 30min to allow the solvent to evaporate completely. The beans were then infested with twenty 1-2 days old of each insect species separately per jar and each jar was covered with a nylon mesh held in place with rubber bands. Each treatment was replicated four times. The number of dead insects in each jar was counted daily and percent mortality calculated by using the Abbott formula.

Progeny production in beans treated with ground dried leaves and essential oil.
The effect of dried ground leaves and essential oil on F_1 progeny produced by *C. chinensis* and *C. maculatus* was investigated in beans treated with the above different doses of ground leaves and essential oil. After counting the mortality for the last day in each set of treatment, the remaining living adults were removed and the jars were kept under the same experimental conditions until the emergence of F_1 progeny adults occurred. The counting period of F_1 progeny was established so as to avoid an overlap of population generation. The percent reduction in adult emergence or inhibition rates (IR %) were calculated by the following formula:

$$IR(\%) = (Cn-Tn) / Cn \times 100$$

where Cn = number of insects in the control jar and
Tn = number of insects in the treated jar

Repellency bioassay
The repellent action of essential oil and anethole against the two insect pests was assessed by the area preference test described by McDonald et al.(1970). Test areas consisted of 7cm Whatman N° 1 filter papers cut in half. Different test solutions were prepared by dissolving 1, 2, 4 and 8µl of essential oil and 0.5, 1, 2 and 4µl of anethole in 0.5ml acetone. Each solution was applied to a half filter paper disc (19.25cm^2) as uniformly as possible with a micropipette and corresponding to the dosages of 0.052, 0.104, 0.208 and 0.416µl/cm^2 for essential oil and 0.026, 0.052, 0.104 and 0.208 µl/cm^2 for anethole. The other filter paper halves were treated with acetone alone and both were air-dried for 10min to evaporate the solvent completely. Each treated half disc was then attached lengthwise, edge-to-edge, to a control half-disc with sellotape. The remade disc was placed in a Petri dish and 20 unsexed beetles of each species were released separately at the centre of each filter paper disc and then covered. Each treatment was replicated 5 times. The number of insects present on control (Nc) and treated (Nt) were recorded after 2h exposure. Percent repellency (PR) values were computed as:

$$PR = [(Nc-Nt/Nc+Nt)] \times 100$$

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=01-20%); class II (PR=20.1-40%); class III (PR=40.1-60%); class IV (PR=60.1-80%); class V (PR=80.1-100%).

Results

Contact toxicity of dried ground leaves on beans
Figures 1 and 2 show the variation of mortality of the two insect species on beans treated with dried ground leaves of *C. anisata*. The mortality was related to the dose and exposure period but the former was more potent than the latter. The highest dose (10%) induced respectively 100% and 91% mortality of *C. chinensis* and *C. maculatus* after 6 days of exposure. The doses of 1.3 and 2.6% induced no mortality of *C. chinensis* after 2 days of exposure contrary to *C. maculatus* where these doses induced 20 and 52% after the same period exposure, respectively. The probit analysis of the second day data revealed that *C. maculatus* was more susceptible (LD_{50}=6.3%) than *C. chinensis* (LD_{50}=12.5%) to the dried ground leaves of *C. anisata.*

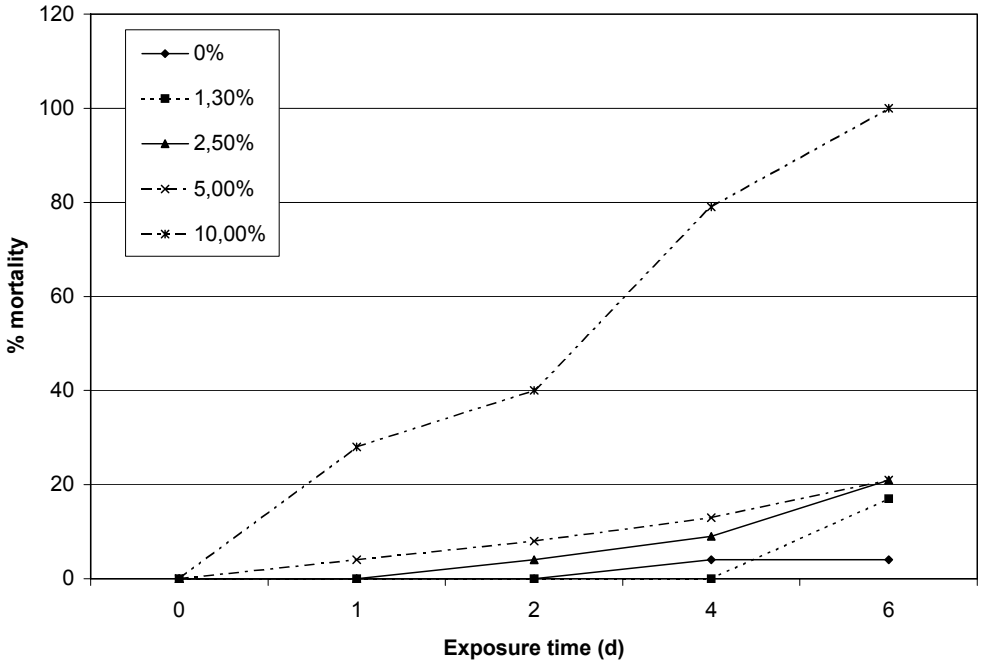


Fig. 1. Toxicity of beans treated with dried ground leaves of *Clausena anisata* to *C. chinensis*

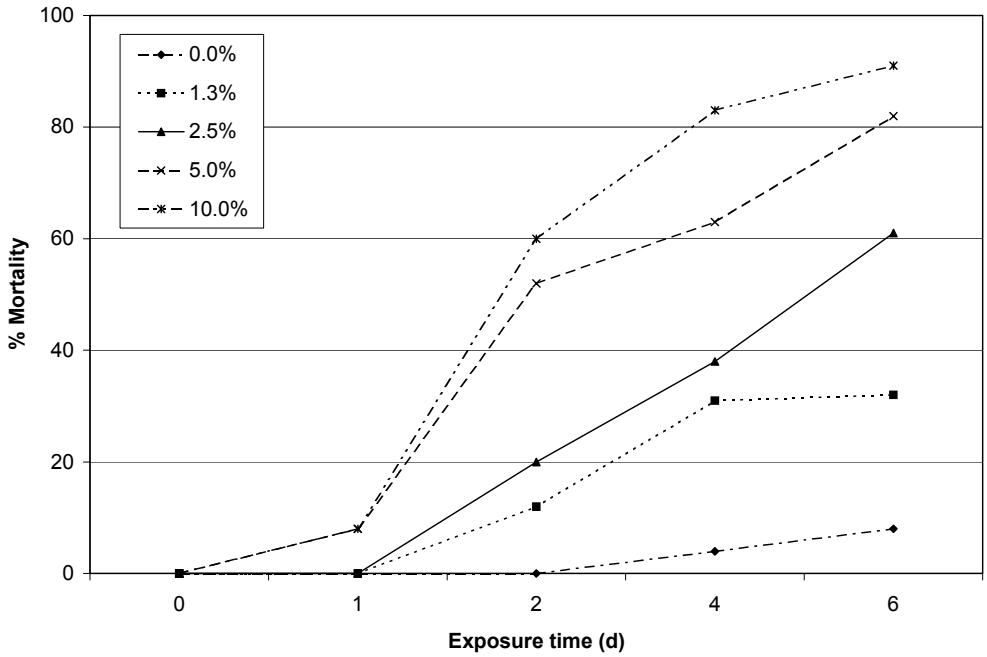


Fig. 2. Toxicity of beans treated with dried ground leaves of *Clausena anisata* to *C. maculatus*

Contact toxicity of essential oil and anethole on filter paper

Percent mortality of each insect species after 24-h exposure to increasing doses of essential oil and anethole on filter paper discs are shown in figs. 3 and 4. All doses of the chemicals significantly killed more insects than the control and insect mortality was dose-dependent.

These figures revealed that *C. chinensis* was more susceptible than *C. maculatus* both to anethole and crude essential oil extract impregnated on filter paper discs. It is also evident that the crude oil extract was less toxic than anethole against both insects species.

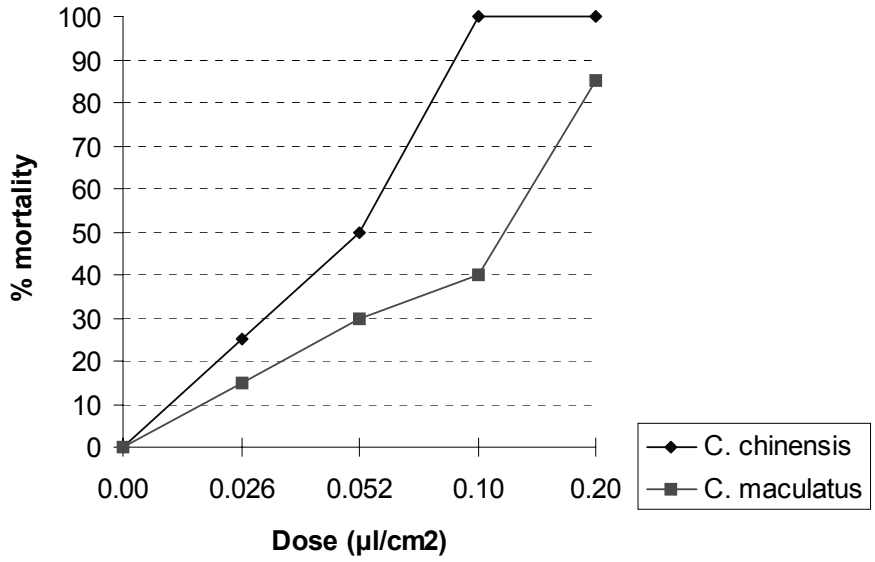


Fig. 3. Percent mortality of *C. chinensis* and *C. maculatus* on filter paper discs impregnated with essential oil of *Clausena anisata*

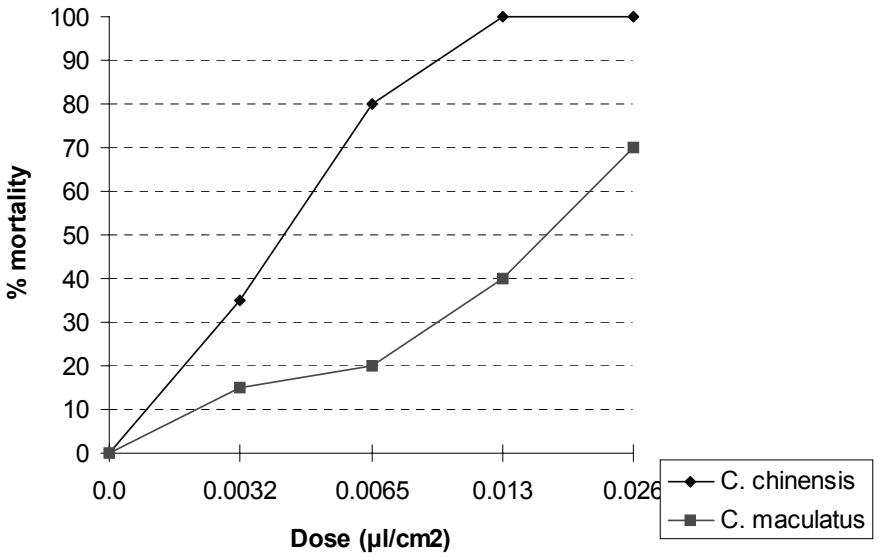


Fig. 4. Percent mortality of *C. chinensis* and *C. maculatus* on filter paper discs impregnated with anethole

Contact toxicity of essential oil and anethole on beans

Figures 5-8 show the percentage mortality of the two insect species in beans treated with different doses of essential oil and anethole. The experiments showed that both the essential oil extract and anethole-coated beans were toxic to both the insect species. Mortality was

dose-dependent. The highest dose of essential oil (8µl) induced 100% mortality of *C. chinensis* within 2 days of exposure and 85% mortality of *C. maculatus* within the same period. On the other hand, the dose of 2µl of anethole was able to induce 100% mortality of *C. chinensis* and 85% of *C. maculatus* within 1 day of exposure. The probit analysis of the first day data revealed that the beans treated with anethole were more toxic than those treated with crude essential oil extract to both test insects (table 2).

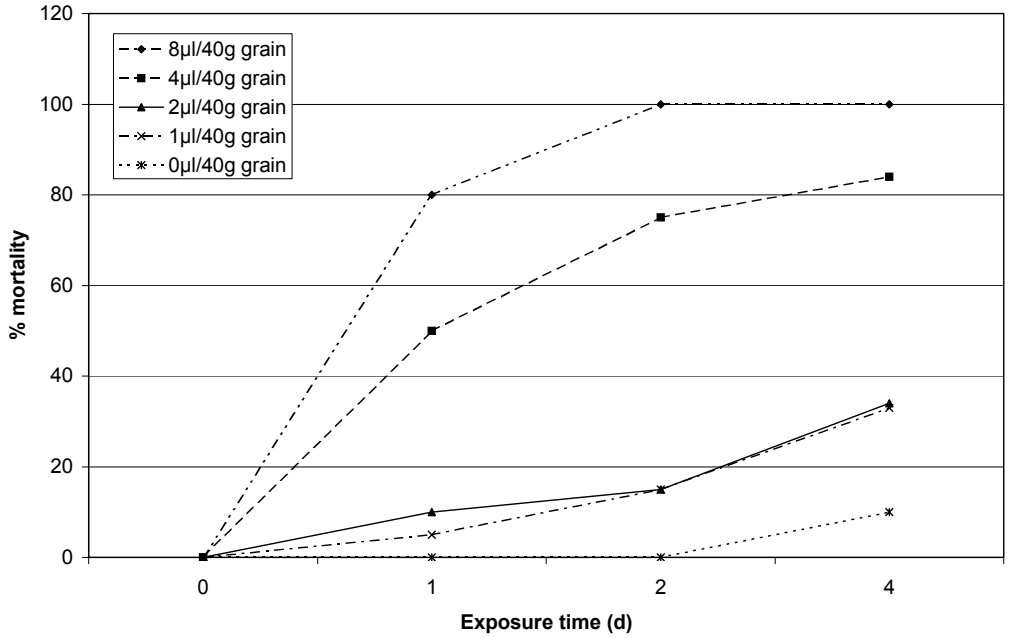


Fig. 5. Mortality of *C. chinensis* exposed on beans treated with essential oil from *Clausena anisata*

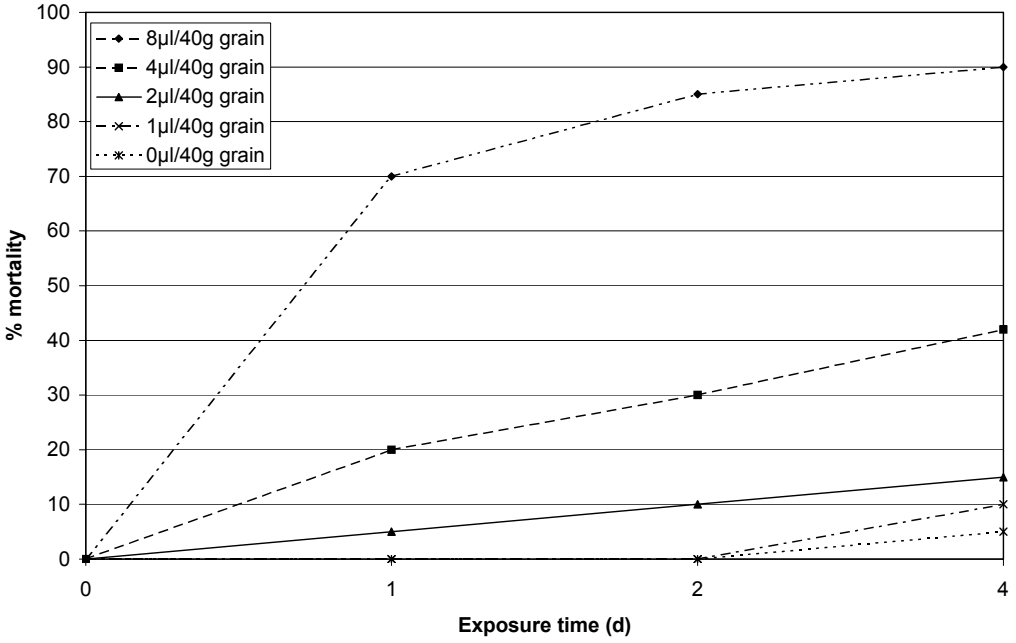


Fig. 6. Mortality of *C. maculatus* on beans treated with essential oil from *Clausena anisata*

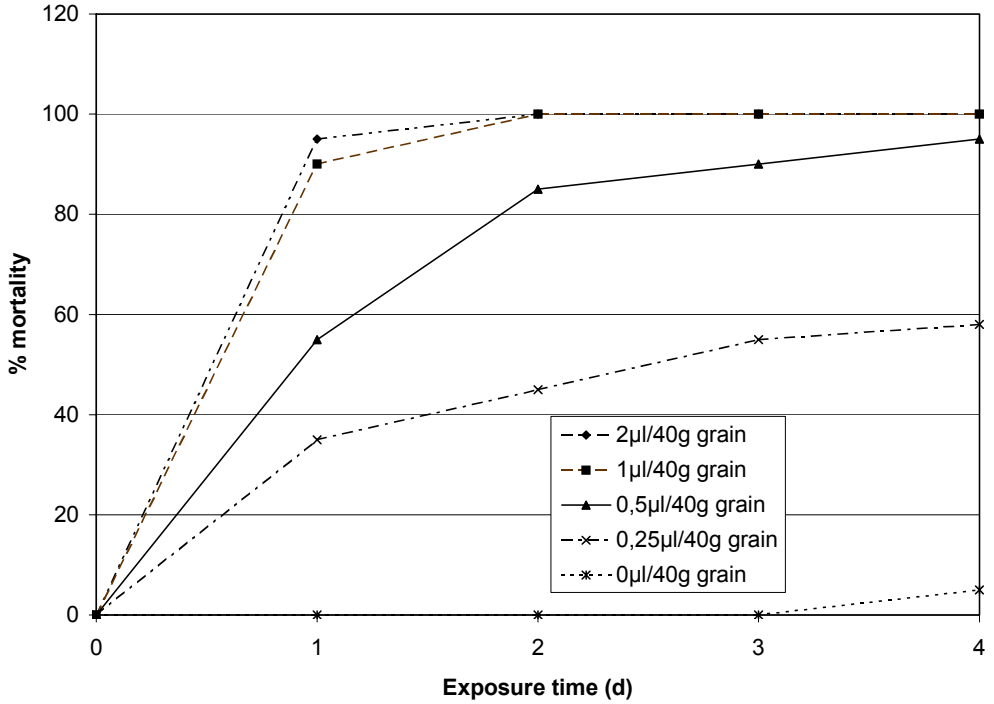


Fig. 7. Variation of mortality of *C. maculatus* on beans treated with anethole

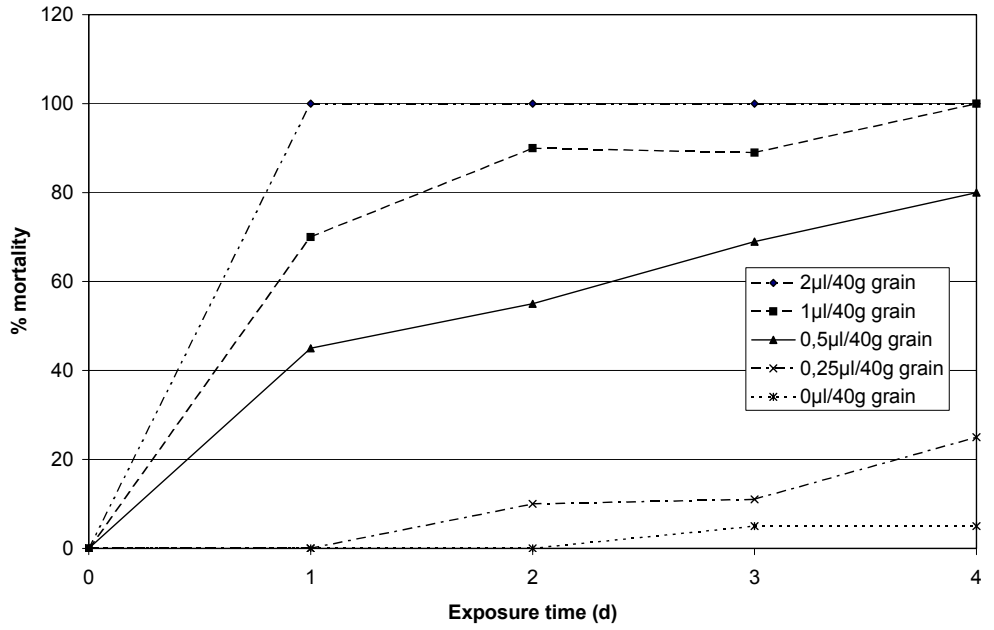


Fig. 8. Percent mortality of *C. chinensis* on beans treated with anethole

Progeny production in beans treated with dried ground leaves and essential oil extract

The number of F_1 progeny produced by the two insect species in untreated beans and beans treated with different doses of dried ground leaves and essential oil are shown in table 3.

All doses of dried ground leaves totally suppressed the F_1 progeny emergence of *C. maculatus* whereas for *C. chinensis* only few insects emerged from the jars treated with the lowest doses (1.3 and 2.5%) and nearly nothing from those of the highest doses (5 and 10%).

On the other hand, essential oil extract caused significant reduction in the number of progeny produced by both the insect species. No progeny of *C. maculatus* was produced in beans treated with the highest dose of essential oil (8µl) and only 4 adults of *C. chinensis* emerged from the jars treated at this dose (table 3).

Table 1. Main volatile components of *C. anisata* leaves in the Western highlands of Cameroon.

Common name	Formula	% composition
Sabinen	$C_{10}H_{16}$	0.53
Myrcen	$C_{10}H_{16}$	0.72
γ-terpinen	$C_{10}H_{16}$	1.76
germacren-D	$C_{14}H_{24}$	1.02
trans-anethole	$C_{10}H_{12}O$	80.77
trans-isoeugenol methyl ether	$C_{11}H_{14}O_2$	11.47

Table 2. LD_{50} calculated for mortality within one day of exposure of *C. maculatus* and *C. chinensis* on beans treated with crude essential extract and anethole

	LD50 (µl /40g beans)	
	Crude essential oil	anethole
C. maculatus	5.97	0.37
C. chinensis	4.30	0.65

Repellency

Table 4 gives the mean repellency values of the essential oil and anethole at different doses for each insect species. Both the essential oil and anethole were repellent to *C. chinensis* and *C. maculatus* with the essential oil evoking the highest repellent action. Percent repellency of anethole to *C. chinensis* was not dose-dependent, it decreased with the increasing doses of anethole.

Discussion

The essential oil extracted from *C. anisata* leaves and anethole identified as its principal constituent provided the greatest protection of green peas and mung beans beans respectively against attack by *C. chinensis* and *C. maculatus*. Exposure of adults of the two insect species to higher doses of dried ground leaves of this plant induced high mortality after 4 days of exposure (figs. 1 and 2). Although the lowest doses of ground leaves and essential oil did not induce considerable mortality of the two insect species fed on beans during the exposure period, they caused significant reduction in the number of F_1 progeny production comparatively to the controls (table 3).

Table 3. Number of F_1 progeny produced by *C. maculatus* and *C. chinensis* fed on beans treated with dried ground leaves and essential oil from *Clausena anisata.*

Treatments	*C. chinensis*		*C. maculatus*	
Dried ground leaves (g / 50g bean)	mean number of F_1 progeny	% reduction in adult emergence	mean number of F_1 progeny	% reduction in adult emergence
0.625	25	86.5	0	100
1.25	12	93.5	0	100
2.50	04	98	0	100
5.00	0	100	0	100
Control	185	0	114	0
Essential oil (µl / 40g beans)				
1	126	49.0	28	90.6
2	78	68.4	25	91.6
4	12	95.1	18	94.0
8	04	98.3	0	100
Control	247	300	0	100

Data are averages of four replicates

Table 4. Mean percentage repellency values of the essential oil extract and anethole at different doses for *C. maculatus* and *C. chinensis.*

Treatments (dose in µl / cm^2 of filter paper)	Mean % repellency (PR)±SEM	
	C. chinensis	*C. maculatus*
Essential oil		
0.052	90 ±12	78±16
0.104	90 ±12	83±11
0.208	95 ± 5	90±10
0.416	95 ± 9	95±9
Overall mean PR	92.5±2.5	86.5±6.5
Repellency class	V	V
Anethole		
0.026	90 ±7	75±5
0.052	73 ± 19	83±13
0.104	73± 24	85±9
0.208	55± 11	90±7
Overall mean PR	72.8±12	83.3±5.4
Repellency class	V	V

Emergence of adult insects from all control samples indicated that test insects were capable of effective oviposition during the different periods used for mortality studies and that prevention of progeny emergence was exclusively due to treatment. It is also possible to suppose that the shorter survival period of the insects on beans treated with high doses of ground leaves and essential oil and which induced 100% mortality within 1 or 2 days after treatment did not allow enough time for oviposition and thus directly affecting the emergence of progeny. In the cases where low doses were used for treatment, the insects survived for few days on beans before dead but still the number of F_1 progeny produced was very less. This indicated that chemicals either suppressed oviposition or killed the eggs laid on beans. In addition, the essential oil extract and anethole were highly repellent to the two insect species (table 4). This repellent action increase the potential practical value of the leaves of *C. anisata* in protecting beans from attack by stored product insect pests.

In all cases, when the activity of the crude essential oil extract is compare to that of anethole (figs. 5-8, table 2), it is established that anethole is more effective on both insect species than did the crude essential oil. This suggests that anethole which is the main component of this oil may be responsible of its high toxicity.

On the other hand, recent studies revealed some interesting insecticidal activities of anethole against several insect pests. Bazzoni *et al*. (1997) showed that anethole was highly toxic and totally inhibited the reproductive activity of adult *Ceratitis capitata* Wied (Diptera, Tephritidae) which causes serious damage to fruit crops. Similar studies also revealed its potent toxicity against many other stored product insect pests (Marcus and Lichtenstein, 1979; Regnault and Hamraoui, 1995; Ho *et al*., 1997).

The efficacy of *C. anisata* as protectant against insect damage may then be attributed to the high concentration of anethole in the leaves, although other toxic minor components such as isoeugenol methyl ether (11.5%) and germacren D (1.0%) could be important.

The results of this study indicated good potential for the use of the leaves of *C. anisata* in storage pest management and demonstrated a scientific rationale for the incorporation of the leaves of this plant into stored product protection practices of communities in the Western highlands of Cameroon.

Future work would focus on more investigations of the effects of the derivatives of this plant materials against other local stored product pests and mammals fed on treated materials.

Acknowledgements

This research project was supported by the German Academic Exchange program (DAAD) and the Third World Academy of Science (TWAS). The Federal Biological Research Centre for Agriculture and Forestry, Institute for Stored Product Protection, Berlin and all its staff is thanked for providing laboratories facilities and equipments for this work. Dr. C. Bauermann, Institut für Getreideverarbeitung GmbH, Potsdam, is gratefully acknowledged for helping in the chemical analysis of essential oil.

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Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
165-169

Plants as insecticides for the protection of stored cowpea – Back to basics

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Abstract The possibility of protecting stored cowpea seeds with local plant material is investigated. In rich countries of the developed world, plants as insecticides may seem dangerous and not very effective, although purified plant compounds are often used. For subsistence farmers in third world countries plants could be a cheap and relatively safe alternative for chemical insecticides.

Key words: Storage, bruchid, *Callosobruchus*, *Vigna unguiculata*, botanical insecticides.

Cowpea and its main storage insect pest

Cowpea (*Vigna unguiculata* Walp.) is an important crop in tropical regions, particularly in West Africa. The seeds are rich in protein and B-vitamins (Phillips & McWatters, 1991) and are therefore important in the diet of many low-resource subsistence farmers as 'the meat of the poor'.

In the field, several seed beetle species lay their eggs on the surface of maturing pods or on ripening seeds. The most important species in cowpea is the cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae). This beetle is responsible for more than 90% of all insect damage to cowpea seeds (Caswell, 1981). The larvae develop inside the bean, destroying its contents and after a few weeks, new adults emerge, ready to mate and oviposit on the available beans again. With the harvested beans, beetle eggs are taken from the cowpea field to the storage room where infestation may reach 100 % within 3 to 5 months (Singh, 1977). Due to this insect infestation, the germination of the seeds decreases, and incidence of fungal infection increases (Charjan & Tarar, 1994).

In the field

There are many environmentally sound ways to keep insect infestation in stored cowpea seeds at low levels even in the tropics (see Van Huis, 1991). The infestation level of freshly harvested beans should be as low as possible. Growing resistant varieties, if they are available, is one option. However, in West Africa, availability is often limited. Moreover, new bean varieties could have different seed colours, textures and sizes, and would therefore be unsuitable for cultural practices such as religious ceremonies.

Inter-cropping could help to confuse the beetles and thus prevent infestation of the seeds. The other crop could serve as a refuge or a source of food for parasitoids and predatory insects (Khan *et al.*, 1997). Weeding the field, to remove reservoirs of insects in possible alternative host-plants is another measure that could be taken to keep initial infestation at a low level.

Altering harvest time, getting the crop off the field before the ripe seeds attract insects could also prevent severe infestation. If the beans are then stored in the pod in clean storage structures, the infestation rate will be low (Van Huis, 1991).

From the field to storage

After harvest, infestation will still be present but in low numbers. At least two percent of the beans will show traces of beetle infestation (Prevett, 1961). Some of the infested beans could be picked out but never will one find all infested beans. Therefore, control measures should be taken against further development of a beetle population.

Proper drying of the beans before they are put into store lowers the beetles' reproductive success (El-Sawaf, 1956). Moreover, dry seeds are less susceptible to mould and fungi that often come with beetle infestation.

Raising the temperature in the storage room to at least 47 °C for more than four hours kills the adult beetles present between the beans (Iloba & Osuji, 1986). If the temperature reaches over 57 °C for at least one hour, all developmental stages of the infesting beetle are killed (Kitch *et al.*, 1992). In tropical countries, these temperatures could be reached if the beans are either hung over a fire or exposed to the midday sun in plastic bags or on a black sheet of plastic covered by a transparent one. If plastic is available, and if the treatment is repeated when needed, this could be a good method to reduce infestation.

The beans could also be disinfested by freezing them, irradiating them with gamma rays, or keeping the stored beans under a controlled atmosphere containing high carbon dioxide or nitrogen concentrations (Boeke *et al*, 2001). However, for most subsistence farmers these methods, due to a lack of equipment, are not applicable.

Storing beans in airtight structures, bottles, plastic bags, oil drums etc. that are filled to the rim with seeds would cause the developing insects to use up all the oxygen within two weeks and to suffocate before they can do serious damage (Caswell, 1973).

In storage

When the beans are stored, they could be treated in different ways. The most obvious seems the treatment or fumigation with synthetic pesticides. Many of these chemicals have proven to be very effective against bruchids when applied at the right time, in the right quantities, with the right application method, etc. For low income families in villages, however, the availability and costs of such chemicals can bring about great problems whereas a lack of knowledge about the application may reduce the efficacy of the pesticide and can cause hazardous situations for consumers of the beans. Abuse of pesticides, determined over two months in only a part of Benin, led to 24 lethal accidents and 241 cases of acute poisoning (Tovignan *et al.*, 2001). Moreover, resistance of the beetles to some pesticides has already been reported (Ayad & Alyousef, 1986; Evans, 1985). Another disadvantage is that these insecticides kill all insects, including beneficial ones such as the natural enemies of the beetles.

If the beans are left untreated, many of the developing beetles will be parasitised by specialised parasitic wasps. Parasitisation by different parasitoïd species can happen in all developmental stages of the beetle: as eggs, larvae or pupae. Under optimised laboratory conditions, parasitisation can control the bruchid infestation up to 82% (Cortsero *et al.*, 1997). In the field and normal, untreated stores, the parasitoids can suppress the build up of beetle populations, but the control is never 100%.

Fine sand or ash can be mixed with stored beans to make a barrier which prevents emerged adult beetles from finding each other for mating or from reaching a next bean to oviposit on. These dusts can effectively suffocate the adults, larvae and possibly eggs (Chinwada & Giga, 1997). The large quantities of the protective material needed make this method of protection less practical, especially for considerable quantities of stored beans.

Traditionally, plants were used to treat stored products. These could be applied in many different forms: as whole plants in layers between pods or seeds, as powders, extracts or oils mixed with seeds or as volatile oils or extracts acting as fumigants (Boeke *et al.*, 2001).

Plants as insecticides

Selecting plants with insecticidal properties could be easy. Certain plants are not attacked by insects because they obviously have some built-in direct defence mechanism. This mechanism can consist of external structures such as nettle hairs or thorns, or of secondary plant metabolites that are toxic, repellent or invoke an antifeedant effect to non-specialised insects (Schoonhoven *et al.*, 1998). The insecticidal compounds from one plant, when applied to another can retain their effect and thus protect the treated plant. If such insecticidal compounds or plant products containing them would be applied to stored beans, they could (and have been shown to) effectively protect stored cowpeas against bruchid infestation (Boeke *et al.*, 2001).

Of course, insects do not avoid these plants alone for no reason. Very bitter or nasty tasting compounds may have antifeedant effects on insects and on mammals, as well. Moreover, plants originally are the producers of most toxic compounds as used in medicine or drugs. The fact that insects do not attack certain plants could be due to compounds that are toxic for mammals, too. The toxicity of plant products should be investigated profoundly before use on food products.

Availability of the plants as insecticides should be taken into account, as well. If the plant is used to protect a stored product, it should be available in large enough quantities at the time of harvest or shortly afterwards. Even in the tropics, plants are not available the whole year since they depend on rainy periods for their seasonal cycle. Moreover, once a plant has been discovered as a potent protective agent, it might become rare if it is not grown deliberately.

In the developed world

In developed countries, rules for food safety and quality management are usually made and checked to protect consumers. Stored seeds in large warehouses are treated with approved methods such as insecticides of which the effect on man is known.

The use of pesticides is dynamic and trends do exist. Agents that are used successfully now might be prohibited in a few years, as was the case with DDT. Resistance of insects against single-component pesticides might develop, or the residual effect of the agent may be unacceptable. Plants could then serve as the source of inspiration in the search for new insecticides.

Usually, money and equipment is available for research to identify, extract or even to synthesise pure insecticidal compounds. Active compounds can mostly be isolated from a plant and these can then be used in quantified, mixtures as insecticides. Investigations concerning toxicity and effects on organisms are more often done on such pure compounds than on biologically variable mixtures such as complete plants or extracts.

The concentration of secondary plant compounds is often low or very low in the plant and changes with the plant part, plant age, growing situation etc. (Schoonhoven *et al.*, 1998). Extraction of the active compounds will often result in insecticides that are more effective, since the ineffective bulk of primary and secondary plant compounds are removed and only the compounds sought for are recovered. With the right equipment the variations can be overcome to produce mixtures of known composition.

In developing countries

Traditionally in tropical countries, plants were used as insecticides but with the introduction of subsidised chemical insecticides, much of this traditional knowledge was forgotten. When the subsidies stopped, the chemicals were no longer affordable for most of the low resource producers of cowpea. Now people would want to store their products, but most of the cowpea is sold immediately after harvest for a low price because insect damage will make it worthless within a few weeks.

If the traditional knowledge could somehow be restored, the storage of cowpea with plant materials could become general practise again. The technology, money, means and the legal need to look for the active ingredients may not be present, but in these situations, sources of insecticidal compounds, the plants, might be available.

Many plants have been used traditionally for many generations. Therefore, the toxicity of these plants utilised in the traditional way could be considered acceptably low against human consumers of the beans.

Advantages of the application of nearby growing plants as insecticides would be that, effective ones could offer a cheap alternative for synthetic insecticides. They would be relatively easily obtainable and application should not bring about serious health risks for the person handling them. The method would be looked upon as environmentally sound, since no new residues are brought into the environment. An additional advantage would be that the development of resistance would take longer if a mixture of compounds is used instead of one pure effective agent.

An example: The neem tree

Very few insects feed on the neem tree, *Azadirachta indica* Juss. Meliaceae. This tree grows everywhere in the tropics. It does not have thorns or any external defence structures, but it contains among others a compound azadirachtin. This compound has a strong antifeedant effect on all kinds of insects and it has effects on oviposition behaviour, metamorphosis, fecundity and fitness of insects on treated substances. The highest yield of pure azadirachtin is 10 g/kg from the kernels of the fruits of the tree (Schmutterer, 1990). The oil pressed from the kernels, when applied to cowpea seeds completely inhibits the development of a bruchid beetle population. Normal numbers of eggs are laid, but they do not hatch and no emerging adults are found (Boeke, unpublished results).

The effective compound is not (very) toxic to humans. The use of azadirachtin in mixtures is allowed in many first world countries, whereas the crude neem oil cannot be used. For the use of neem oil in the third world, some state that it could easily, safely and effectively be applied to stored seeds. Oil from neem kernels can be easily extracted, even by hand. The material is present everywhere and would not be used for anything else but the extraction of the oil. Others would say that due to its very bitter taste, and the possibility of aflatoxins in the neem seeds due to fungal growth, the use of neem oil on stored seeds for consumption should be advised against.

Conclusion

Plant products could act as insecticides. If money allows it, or if laws and rules oblige it, pure effective compounds could be used. In other situations, the whole plant or easily obtained extracts could offer a solution for the problems of availability, health risks, costs, and resistance against synthetic pesticides. Especially low resource farmers in the tropics would benefit from cheap ways to protect their stored seeds.

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IOBC Bulletin Vol. 25 (3) 2002
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Efficacy of burnt plant material smoke for protection of stored paddy against infestation of *Sitophilus oryzae* (L.)

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Abstract: In Sri Lanka, up to 60% of the total rice production is produced on the village level and 4% to 6% of paddy is lost during the storage. The treatment with smoke from burnt plant materials is used as a method of insect pest control by many rural farmers. Plant smoke was generated by flameless heating (about 300°C under controlled air supplement near to pyrolysis) of plant leaves. Four different plant species, *Azadirachta indica, Lantana camara, Ocimum sanctum* and *Oryza sativa* (paddy straw), were used to generate the smoke. Each generated smoke was conditioned to 75% rh by a saturated NaCl solution, and CO_2 was removed by KOH solution for 12 h before the testing. Rice weevils were reared on freshly harvested paddy and one week old adult insects were used for the experiment. Bioassays were carried out by 2 h intervals up to 48 h at 30-32°C. After 48 h of exposure time, 100% adult insect mortality was observed in *Azadirachta indica, Lantana camara, Ocimum sanctum* except in the smoke generated by *Oryza sativa*. According to the results of LT_{50} and LT_{90}, *Lantana camara* smoke showed highest efficacy among all plant species. Efficacy of smoke generated by *Azadirachta indica* and *Oryza sativa* did not show significant differences in LT_{50} and LT_{90} values. Effectiveness of smokes against adult rice weevils were observed in the following order: *Lantana camara> Ocimum sanctum>Azadirachta indica* and *Oryza sativa* (paddy straw).

Key words: burnt plant materials, flameless heating, smoke, *Azadirachta indica, Lantana camara, Ocimum sanctum*, *Oryza sativa,* rice weevils

Introduction

Annual paddy production in Sri Lanka is approximately 2.66 million metric tons. Approximately 60% of the production storage in the village level and the rest of 40% is available as marketable surplus soon after harvest A major loss of grain in the post harvest system occurs during the storage particularly at farm level due to improper and inadequate storage facilities. It has been revealed that approximately 4 to 6% of paddy is lost during the farm level storage in Sri Lanka (Palipane, 1978). Many farmers today adapted to bag storage system rather than use of traditional storage. The high level of insect infestation was observed in the many village level storage probably because of greater surface area of the grain bulk is exposed to the atmosphere, thus providing favourable conditions for insects to multiply in the surface layers of the grain mass. *Sitophilus spp* were identified as one of a major insect pest problem of stored paddy and rice.

Today most of the farmers adopt chemical insect control practices such as mixing of contact insecticide dusts with the stored grain. However most of the village level (small scale growers) farmers still adopted traditional methods of insect control such as adding plant material, inert dust, hermetically storage and smoking (De Lima, 1987, Poswal and Akpa, 1991) of stored grains by burned plant materials. Burning of plant products on hot coconut charcoal could easily develop the smoke composed of many volatile organic constituent as

well as various gasses. Efficacy of application of such a method to control stored insect pest not known. Therefore investigation of traditional grain smoking method in effective way could help to develop more appropriate technology for stored grain protection. The objective of this study was evaluated the efficacy of plant material burned smoke against *Sitophlios oryza*.

Materials and methods

Insect culture

About 5kg of freshly harvested long grain paddy sample was obtained from the farmers field and storage under -18°C about 14 days to prevent from any previous insect infestation. Then the sample was stored in a closed glass jar at room temperature (30 -32°C) about 3 months for complete the ageing process. *Sitophlis oriza* (rice weevil) was obtained from the Rice Processing Research and Development Centre, Anuradhapura, Sri Lanka. The culture was maintained at 30± 2°C and 70-75% RH conditions.

Plant material

Leaves from *Azadirachta indica, Lantana camara, Ocimum sanctum* were harvested from the farmers fields and six months old *Oryza sativa* (paddy straw) was obtained from paddy fields. All the plant materials were dried under the room temperature (32°C) about 10 days and stored at refrigerator prior to analysis.

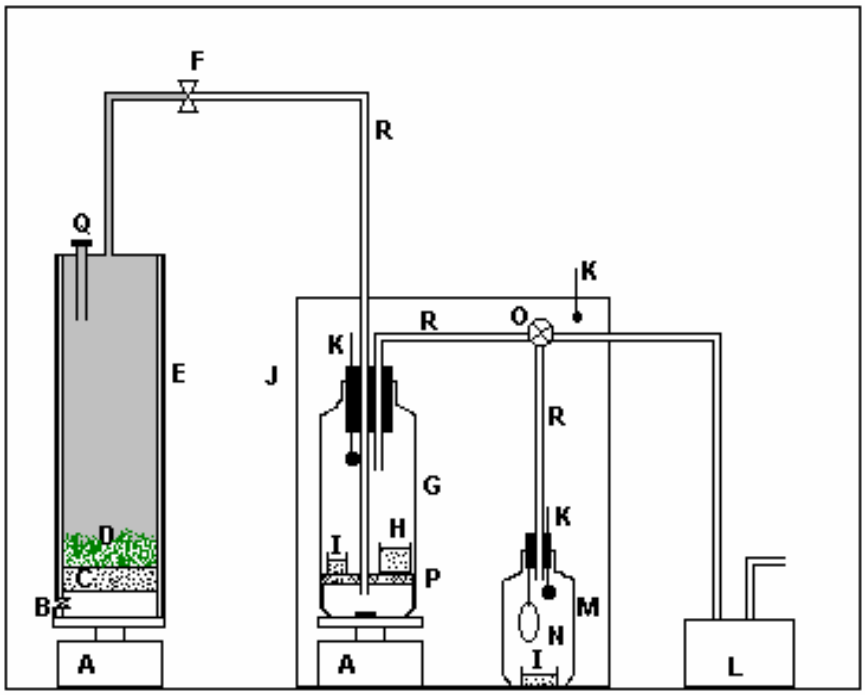

Fig. 1. Instrumental set-up for generation of plant material burned smoke (A: laboratory hot plate; B: air flow control value; C: fine sand layer; D: plant material; E: metal and clay cylinder; F: value; G: smoke collecting glass jar; H: KOH solution; I: saturated NaCl; J: incubator; K: thermometers L: vacuum pump; M: test jar; N: wire cage O: two way value; P: glass wool layer; Q:cylinder orifice; R: polystyrene tubes).

Smoke generation

Plant materials were burnt inside the 60 cm height (∅ 13.5 cm) steel cylinder (surrounded with another clay cylinder) under control air flow system (Fig. 1). It was contained fine sand layer of 3 cm in height and 2.5 cm above from the cylinder bottom. Whole set-up was fixed on a laboratory hot plate. Five litre glass bottle (smoke collector) and the cylinder were coupled through metal and heat resistance polystyrene tubes (∅ 1 cm). Inside the glass bottle was contained a saturated NaCl solution and 200 ml of 10 M KOH solution. Another glass jar of 750 ml volume was used as a test jar. Both glass jars and vacuum pump were connected through a two way value. Very limited length (20 cm) of polystyrene tube (∅ 1.5 cm) was used to connect the both jars. Three hours before start the experiment hot plate was set to 300°C and it was help to built up the high temperature (>300°C) inside the sand layer. Fifty grams of plant material was added into the hot sand layer and the generating smoke was collected into the 5 litre glass jar through the vacuum pump about 2 minutes. The collected smoke was let inside the jar about 12h to absorb the generated CO_2 by KOH solution. Fifty unsexed, two week old adult of rice weevils and 20 g of rice were enclosed in wire screen and fix into a test jar. It was maintained at 31±1°C and 75% rh prior to the experiment. Test jar was treated with pre-treated smoke while stirring the gases inside the smoke collecting jar. Insect mortalities were counted 2, 4, 6, 8, 10, 12, 14, 16, 18, 24,30, 36 and 48 hours after treatments. Adults were considered dead if appendages did not move when prodded to an insect pin. All treatments and the control were replicated 4 times.

Statistical analysis

Abbot corrected (Abbott, 1925) percentage of mortality data were subjected to analysis of LT_{50} and LT_{90} values by using Table Curve 2D software (Anonymous , 1994). LT_{50} and LD_{90} values were analysed using analysis of variance (ANOVA) and the least significant difference (LSD) test was used to separate the means.

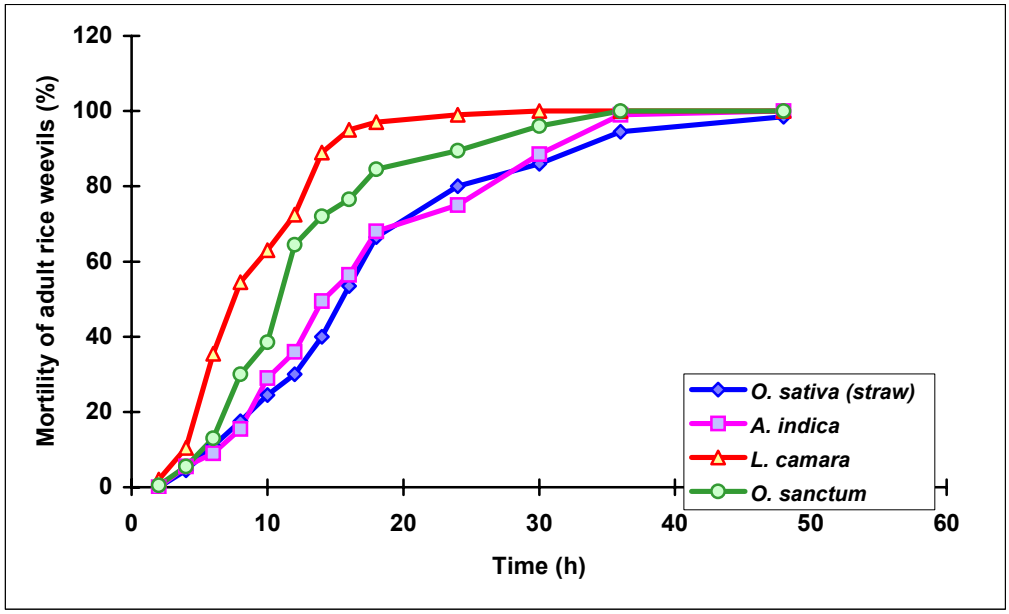

Fig. 2. Mortality of adult *Sitophilus oryzae* caused by smoke generated form four different plant materials.

Results

The effectiveness of plant materials smoke is illustrated in Fig. 2. The experiments showed that the smoke drive from all the plant materials were toxic to the adult beetles. Smoke of *L. camara, O. sanctum* and *A. indica* caused the highest mortality (i.e 100%) within 48 hours after treatments except paddy straw (*O. sativa).* Among all the tested plant burned smoke, *L. camara* showed highest efficacy.

The analysis of variance of LT_{50} and LT_{90} values showed that insect mortality differed significantly ($p<0.05$) among smokes (Table 1.) those from *L. camara* and *O. sanctum.* The Smoke generated from burned *L. camara* were showed significantly lowest values of LT_{50} (7.81±0.08 h, $p<0.001$) and LT_{90} (15.47±0.29 h, $p<0.001$) compare to other three smokes. *O. sanctum* also showed the significant effectiveness next to the smoke of *L. camara*. However there was no significant difference of smoke effectiveness between *A. indica* and paddy straw.

Table 1. The response of *Sitophilus oryzae* adults to four different plant material burnt smokes

Plant species	**LT_{50}(±SE) (hours)**	**LT_{90} (±SE) (hours)**
Lantana camara	7.81(±0.08)***a***	15.47(±0.29)***a***
Ocimum sanctum	10.73(±0.26)***b***	21.20(±1.02)***b***
Azadirachta indica	14.52(±0.52)***c***	30.14(±0.87)***c***
Oryza sativa (straw)	15.47(±0.57)***c***	27.74(±0.88)***c***

Means in a column followed by the same letters are not significantly different, $p>0.05$ by LSD. Means of four replicates.

Considering all the LT_{50} and LT_{90} data attest an order of effectiveness of smokes against adult rice weevils were observed in the following order: *Lantana camara> Ocimum sanctum> Azadirachta indica* and *Oryza sativa* (paddy straw).

Discussion

Many plants produce volatile organic compounds (VOC) even those compounds can release into atmosphere as products of their metabolism. These VOC generally consist of isoprene (C_5H_8), monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$); some aldehydes and carboxylic acids (Darmais et. al., 2000). It is well known that fumigants from various plant extracts act as insect repellent of many species of grain pests. In addition, some of plant extract of VOC act as insect feeding deterrent and inhibit their development.

The results indicate that there were high significant differences between the activity of smoke *L. camara* and *O. sanctum* compare to *A. indica* and paddy straw. *L. camara* leaves contain lantadene A, lantaden B, icterogenin and essential oil as major chemical constituents (Duke, 1985; Oliver-Berver, 1986). Application of *L.camara* leaf extract caused 40% of mortality in adult *C. chinensis* within seven days (Saxena, et.al.,1992). Major chemical composition of *O. sanctum* are eugenol, eugenol methylether, carvacrol and caryophyllene (Jogia, 1984; Lal et.al., 1978). Ahn et. al., (1998) reported that the carvacrol has broad insecticidal activity even act as a fumigant against agricultural, stored product and medical

insect pests. Azadirachtin is considered the most important active principle chemical compound present in *Azadirachta indica.* It is regarded as the most effective insect growth regulator. Adding of 5% of oil seed cake with the maize or 5%ground seed powder with the wheat significantly reducing damage of *Sitophilus oryzae* by inhibiting oviposition (Bowry, et.al. 1984; Rout, 1986).

Any bio-product burns above 300°C with low oxygen level produces the acrid smoke, which composed of various gas, liquid, oil and char; the yield of the different products are strongly dependent on the pyrolysis process conditions. Plant material brunt under control atmosphere above 300°C is more similar to the pyrolysis condition. During partial burning (under controlled air supplement) of a plant material, produces smoke which contents various chemicals. Even at such higher temperature many chemical constituents subject to the thermal degradation (Madorsky, 1964 ; Ohtani and Tsuge, 1995) but many VOC can easily escape in to the smoke. It is obvious that *L. camara* and *O. sanctum* contain high essential oils than *A. indica* and paddy straw. *A. indica* more popular as contact insect growth regulator than it is an effective fumigant (Schmutterer, 1990). In this experiment we used paddy straw as treatment assuming that straw contains much less VOCs than other three plant materials. Therefore this could be the reason for paddy straw and *A. indica* showed similar result. However, high yield of azadirachtin has been obtained by using pyrolysis of *Azadirachta indica* (Ley et. al., 1988). Selvaraj et. al., (1995) confirmed that the smoke produced by mix leaves powder of *Adhatoda vasica*, *Azadirachta indica* and *Ocimum sanctum* on burning charcoal which can repelled the *Armigeres subalbatus* and *Culex quinquefasciatus* biting activity for 6-8h and it was highly toxic to these adult mosquitoes. *L. camara* contains toxic compounds (Roth et. al., 1994) than other three plant materials which we have tested. The biggest problem encounters with smoking method is production of polycyclic aromatic hydrocarbons (PAHs) due to incomplete burning and smoke comes into direct contact with product (Guillen et.al., 2000).

In conclusion, the plant materials derived smoke might be useful products for protect the stored rice and paddy from insect pests, but further research and development is necessary to identify the active compounds in smokes and reduce the PAHs.

Acknowledgement

I am very grateful to Mr. Asoka Peries for his technical support during this study. I also like to extend my gratitude to Dr. Reichmuth and Dr. Cornel Ardler at Federal Biological Research Centre for Agriculture and Forestry, Institute for Stored Product Protection, Germany. My travelling to Lisbon was supported by GFF and IOBC.

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IOBC Bulletin Vol. 25 (3) 2002
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Activity of chilli, *Capsicum annuum* L. var. *acuminatum*, on stored product insects *Oryzaephilus surinamensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst)

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Abstract: Fruits, extracts and metabolites of chilli, *Capsicum annuum* L. var. *acuminatum*, typical of the geographic area of the Molise region (Central Italy), were tested in an arena for their attractive/repellent activity against adults of saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), rice weevil, *Sitophilus oryzae* (L.) and rust-red flour beetle, *Tribolium castaneum* (Herbst). The biological activity of chilli fruits were investigated comparing: whole fruits; cut fruits with seeds; cut fruits without seeds; whole seeds; split seeds. According to the results obtained in the arena tests, whole fruits were attractive for all three insect species; cut fruits with seeds, cut fruits without seeds and split seeds were attractive for *O. surinamensis* adults; cut fruits with seeds were repellent against *T. castaneum*; whole seeds and split seeds revealed a repellent activity against *S. oryzae* and *T. castaneum*. The extracts of chilli fruits, *n*-butanol, watery and *n*-hexane, were active in modifying the behaviour of *S. oryzae* and *T. castaneum* adults: a repellent effect against *S. oryzae* adults was showed by all extracts; watery extract was attractive for *T. castaneum*. In the tests with metabolites isolated from chilli fruits, capsaicin and dihydrocapsaicin were not active against *O. surinamensis* and *S. oryzae*, instead dihydrocapsaicin resulted repellent against *T. castaneum* adults.

Key words: *Capsicum annuum*, stored products Coleoptera, attractive and repellent activity.

Introduction

Plants of the genus *Capsicum* (Solanaceae) have numerous species and varieties and have been used for many centuries as spice, vegetable and external medicine. Nutritional, metabolic and pharmacological studies have been performed, and organic compounds in fruits and seeds have been shown to be important in cancer chemo-prevention.

With regard to the chemical constituents in *Capsicum* plants, many studies exist in the literature about less polar compounds such as carotenoids, fatty acids and capsaicins, while the polar ones have not been sufficiently examined (Iorizzi *et al.*, 2000).

Effects of powders or extracts from *Capsicum* species on the biology and the behaviour of stored product insects, and pest control properties as toxicity, repellency or antifeedant activity, were investigated mainly in developing countries.

A repellent effect of chilli powders or extracts was observed against *Callosobruchus maculatus* (F.), *Rhyzopertha dominica* (F.), *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst); a toxic effect was revealed against *C. maculatus*, *R. dominica*, *Sitophilus oryzae* (L.) and *Tribolium confusum* J. du Val (Morallo-Rejesus, 1987; Williams and Mansingh, 1993; Onu and Aliyu, 1995; Gakuru and Foua-Bi, 1996; El-Lakwah *et al.*, 1997). Also, *Capsicum* fruits, ground, sliced or left whole, were found effective in controlling *C. maculatus* infestations (Ofuya, 1986; Zibokere, 1994).

In our investigation we tested fruits and some extracts and metabolites of chilli, *Capsicum annuum* L. var. *acuminatum*, typical of the geographic area of the Molise region (Central Italy), for their attractive/repellent biological activity against adults of saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), rice weevil, *S. oryzae* and rust-red flour beetle, *T. castaneum*.

Material and methods

Plant material and compounds extraction

Samples of *C. annuum* var. *acuminatum* were frozen immediately after collection and kept frozen until extraction.

The collected plant samples were cut, separated from seeds and extracted in MeOH solvent at room temperature; the MeOH extract was concentrated and extracted, according to Kupchan *et al.* (1973), with the following solvents: *n*-hexane, chloroform ($CHCl_3$) and *n*-butanol (*n*-BuOH). Watery residue after butanolic extraction also was included in the biological assay. Analysis of CHCl3 extracts was undertaken by a combination of MPLC and HPLC techniques and led to the isolation of capsaicin and dihydrocapsaicin as major components of the mixture.

Experimental insects

Test insects, *O. surinamensis*, *T. castaneum*, and *S. oryzae* were collected from farm-stored wheat in the Molise region and cultured for two generation at 25±1°C temperature and 70±5% relative humidity (r.h.) prior to the experiments. The feed medium used was *Triticum durum* Desf. with 13% moisture content.

Test conditions and mathematical tools

All experiments were performed in the laboratory using established colonies of insects in complete darkness at 25±1°C and 70±5% r.h. Release and recapture of *O. surinamensis*, *S. oryzae* and *T. castaneum* adults by three modified active devices from Flit-Trak M2 traps (Mullen, 1994) were carried out in a circular plastic arena (45 cm diam. x 30 cm high); teflon paint was used to prevent beetle escape.

T. durum grown in the Molise region was used as a bait in the experiments. In each trap of the different tests, about 5 g of kernels were used: cracked kernels in *O. surinamensis* and *T. castaneum* tests, intact kernels in *S. oryzae* tests. For each experimental run, a trap containing only *T. durum* kernels was used as control.

In all experiments 100 adult beetles of mixed sex and age were released. The number of trapped insects was checked after 12 h and the percentage frequency of detection was then computed. Five replicates were performed for each type of comparison using a total of 500 insects.

Since one trap is connected to each choice, each insect is deemed to have made that choice either by remaining trapped or by lying 'near' (under/on top) of the trap. The number of individuals making the given choice among a group of possible choices during the given experimental run represent the data to be analysed. Several different combinations of choices were analysed, and t-tests were performed to establish the significance of differences between individual comparisons (for a full explanation refer to Trematerra *et al.*, 1996 and 2000).

Biological assay

The bioactive fruits, extracts and metabolites were tested for their attractive/repellent activity against *O. surinamensis*, *S. oryzae* and *T. castaneum*.

The sets of comparisons with chilli fruits were effected as follow: whole fruits, cut fruits with seeds, control; cut fruits with seeds, cut fruits without seeds, control; cut fruits without seeds, seeds, control; seeds, split seeds, control. The sets of comparisons with extracts and metabolites were effected as follow: CHCl3 extract, *n*-BuOH extract, control; *n*-hexane extract, watery extract, control; capsaicin, dihydrocapsaicin, control.

Results and discussion

The results obtained in the biological tests, statistically analysed, are reported in tables 1-4. According to our experiments, parts of fruit, extracts (*n*-butanolic extract, watery extract and *n*-hexane extract) and metabolites (dihydrocapsaicin) by *Capsicum* were active in modifying the behaviour of *O. surinamensis*, *S. oryzae* and *T. castaneum* adults in the arena.

The tests with chilli fruits gave a clear biological response in the following cases (table 1): whole fruits were attractive against all three insect species; cut fruits with seeds, cut fruits without seeds and split seeds were attractive against *O. surinamensis* adults; cut fruits with seeds were repellent against *T. castaneum*; whole seeds and split seeds revealed a repellent activity against *S. oryzae* and *T. castaneum*. Otherwise, only in one test out of two an attractive activity of cut fruits without seeds against *S. oryzae* and *T. castaneum*, and a repellent activity of whole seeds against *O. surinamensis* and *S. oryzae* was observed.

Table 1. Responses of *Oryzaephilus surinamensis*, *Sitophilus oryzae* and *Tribolium castaneum* to different parts of chilli fruits: whole fruits, cut fruits with seeds, cut fruits without seeds, whole seeds; split seeds (normalized % of individuals; ± normalized SD)

Case	**Species**	**Whole fruits**	**Cut fruits with seeds**	**Control**
A	*O. surinamensis*	40 ± 2.8 **A**	40 ± 2.8 **A**	19 ± 2.0 **B**
	S. oryzae	38 ± 2.8 **A**	38 ± 2.7 **A**	24 ± 2.2 **B**
	T. castaneum	42 ± 2.9 **A**	20 ± 2.0 **B**	33 ± 2.6 **C**
Case	**Species**	**Cut fruits with seeds**	**Cut fruits without seeds**	**Control**
B	*O. surinamensis*	41 ± 2.9 **A**	32 ± 2.5 **B**	26 ± 2.3 **C**
	S. oryzae	27 ± 2.3 **A**	37 ± 2.7 **B**	36 ± 2.7 **B**
	T. castaneum	26 ± 2.3 **A**	31 ± 2.5 **B**	36 ± 2.7 **B**
Case	**Species**	**Cut fruits without seeds**	**Whole seeds**	**Control**
C	*O. surinamensis*	49 ± 3.1 **A**	23 ± 2.2 **B**	29 ± 2.4 **C**
	S. oryzae	43 ± 2.9 **A**	22 ± 2.1 **B**	36 ± 2.7 **C**
	T. castaneum	43 ± 2.9 **A**	26 ± 2.3 **B**	31 ± 2.5 **C**
Case	**Species**	**Whole seeds**	**Split seeds**	**Control**
D	*O. surinamensis*	26 ± 2.3 **A**	48 ± 3.1 **B**	27 ± 2.3 **A**
	S. oryzae	33 ± 2.6 **AB**	29 ± 2.4 **A**	38 ± 2.8 **B**
	T. castaneum	21 ± 2.1 **A**	25 ± 2.2 **A**	49 ± 3.1 **B**

Means values in a row followed by a different letter are significantly different (P> 0.05)

On the basis of the results observed for *S. oryzae* and *T. castaneum*, substances contained in various parts of chilli fruits can exercise a different biological activity, in fact hexocarp

layers seem to be attractive, whereas seeds are repellent. *O. surinamensis* adults exhibit behavioural responses very dissimilar from the other two pest species tested.

A repellent effect against *S. oryzae* adults was observed using *n*-butanolic extract, with statistically significant differences (table 2).

Table 3 shows a clear preference for *n*-hexane extract by *O. surinamensis* and a repellent activity against adults of *S. oryzae* and *T. castaneum*. Watery extract was repellent against *S. oryzae* and attractive for *T. castaneum*, with statistically significant differences.

In the tests with *Capsicum* metabolites, capsaicin and dihydrocapsaicin, isolated from chilli fruits, were not active against *O. surinamensis* and *S. oryzae*, instead dihydrocapsaicin resulted repellent against *T. castaneum* adults (table 4).

The changing biological activity observed for various extracts and metabolites, probably depends on the part of the fruit in which they are contained (endocarp, mesocarp, hexocarp). Also, new investigations are needed to evaluate attractive/repellent activity of compounds extracted from seeds.

Table 2. Responses of *Oryzaephilus surinamensis*, *Sitophilus oryzae* and *Tribolium castaneum* to CHCl3 extract and *n*-butanolic extract (normalized % of individuals; ± normalized SD)

Species	**$CHCl_3$ extract**	***n*-butanolic extract**	**Control**
O. surinamensis	33 ± 2.6 **A**	35 ± 2.6 **A**	32 ± 2.5 **A**
S. oryzae	35 ± 2.7 **A**	29 ± 2.4 **B**	36 ± 2.7 **A**
T. castaneum	30 ± 2.4 **A**	31 ± 2.5 **A**	35 ± 2.6 **A**

Means values in a row followed by different letter are significantly different (P> 0.05).

Table 3. Responses of *Oryzaephilus surinamensis*, *Sitophilus oryzae* and *Tribolium castaneum* to watery extract and *n*-hexane extract (normalized % of individuals; ± normalized SD)

Species	**Watery extract**	***n*-hexane extract**	**Control**
O. surinamensis	33 ± 2.6 **AB**	38 ± 2.8 **A**	30 ± 2.4 **B**
S. oryzae	30 ± 2.4 **A**	32 ± 2.5 **A**	40 ± 2.8 **B**
T. castaneum	38 ± 2.8 **A**	26 ± 2.3 **B**	30 ± 2.4 **B**

Means values in a row followed by different letter are significantly different (P> 0.05)

Table 4. Responses of *Oryzaephilus surinamensis*, *Sitophilus oryzae* and *Tribolium castaneum* to capsaicin and dihydrocapsaicin (normalized % of individuals; ± normalized SD)

Species	**Capsaicin**	**Dihydrocapsaicin**	**Control**
O. surinamensis	33 ± 2.6 **A**	35 ± 2.7 **A**	32 ± 2.5 **A**
S. oryzae	34 ± 2.6 **AB**	36 ± 2.7 **A**	30 ± 2.5 **B**
T. castaneum	39 ± 2.8 **A**	24 ± 2.2 **B**	37 ± 2.7 **A**

Means values in a row followed by different letter are significantly different (P> 0.05)

The three pest species *O. surinamensis*, *S. oryzae* and *T. castaneum* showed different responses in the various experimental tests.

In the case of *O. surinamensis*, compounds with a biological activity were mainly attractive: whole fruits, cut fruits with seeds, cut fruits without seeds, split seeds, *n*-hexane extract.

In *S. oryzae* tests, an attractive activity for whole fruits and cut fruits without seeds and a repellent activity for whole seeds, split seeds, *n*-hexane and watery extract was revealed.

Similar results were obtained for *T. castaneum*, that showed attractive response for whole fruits, cut fruits without seeds and watery extract and repellent response for cut fruits with seeds, whole seeds, split seeds, *n*-hexane extract and dihydrocapsaicin.

With limitation on the use of pesticides in stored products growing, and increasing public demands for wholesome and pest-free food products, the need for developing biorational pest management technologies in stored-products, such as those using attractants, repellents and natural toxicants, is greater than ever before (Trematerra and Lanzotti, 1999).

At this regard, the attractant/repellent compounds from *Capsicum* could be employed in preventing, or reducing, damages caused by insect pests. Also, a correct use of chilli fruits in traditional agriculture, especially in developmental countries (i.e., cutting or cracking the whole fruits to activate a repellent action) should be evaluated to assure a reliable protection against stored product insects.

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PHYSICAL CONTROL

Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
183-185

ThermoNox - Heat treatment as a non-toxic pest control

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Not only human beings but also a considerable number of insects regard grain and products made from it as important basic food. The most common insects threatening this kind of stored products are the grain weevil, *Sitophilus granarius*, the Mediterranean flour moth, *Ephestia kuehniella* and the rust-red flour beetle, *Tribolium castaneum*.

It is generally believed that the optimal temperature for almost all of these insects is somewhere between 15° and 38°C. At temperatures beyond 45°C both the insects and their eggs are killed within a few hours. Most insects cannot reduce their body temperature by perspiring or breathing. They will simply die due to coagulation of their body proteins.

Description of the ThermoNox system

Heat treatment has been practised for many years in variable forms. Until the ThermoNox procedure was developed most of the equipment used consisted of a heating device based on an oil- or a gas-burning-unit situated outside the building to be treated. The heated air was led into the building by tubes or fixed installed distribution pipes. The energy consumption was immense because all the outdoor air had to be heated and blown into the building. Sporadically, problems occurred because the high air temperatures used by this method caused damage on heat sensitive components.

In contrast, the ThermoNox procedure is based on the principle that only the air inside the building has to be heated. High air temperatures are not necessary. The heater causes a circulating airflow inside of the building and leads to a very low energy consumption.

Description of the heater-technical and function

The ThermoNox heater is equipped with an axial fan of 0.75 kW, two heating registers of each 9 kW, a thermostat to monitor the temperature, a safety device to avoid too high temperatures and a switch panel containing the electrical equipment. The three-phase power supply (380-400 Volt) is provided by a 15 m long cable with a mounted CEE plug. In addition, the CEE plug is equipped with a switch function to change two of the phases. This could be needed to change the revolving direction of the fan. The measures of the housing are 430 to 610 mm and a height of 1,040 mm, energy-consumption is 9.75/ 18.75 kW, the weight of the heater is 75 kg, including the 15 m cable.

An axial fan sucks the air at the floor level and blows it through the heating register. The heated air leaves the heater at the top horizontally. Due to the fact that only the air inside the room to be treated has to be heated, the thermal efficiency is optimised and the energy consumption is as low as possible.

The temperature of the circulated air is monitored by the integrated thermostats.

At a room temperature at about 50°C the thermostat switches off the heating elements. As soon as the temperature decreases the heating register is reactivated to keep the lethal temperature in the room constantly at about 50 to 55°C. In case of a malfunction, e.g. when

the air temperature in the heater rises to more than 140°C, the STB (safety-temperature-switch) automatically switches the main energy supply off. The light construction of the heater in combination with two wheels and a handle makes it easy to rearrange the position of the heaters during the heating period. So it is possible to direct the heated air upon massive equipment with low heat conductivity.

Description of the safety precaution

The heater was primarily developed to be used in the flour milling industry and similar production facilities to avoid the use of methyl bromide and other toxic agents due to pest control. In these factories the hazard caused by combustible dust has to be regarded. In Europe the EU-regulation 94/9EG (also known as ATEX 100 a) concerning equipment used in an explosive atmosphere has to be considered. Because of this the heater was constructed to fulfil the classification ex II 3 D according to the 94/9/EG which means it may be operated in the Zone 22. The heater was finally checked by the Berufsgenossenschaft NG in Mannheim and obtained the GS-sign, indicating proved and certified safety.

Practice

For an efficient heat treatment six basic rules have to be regarded:

1. Shut down the entire production equipment.
2. Clean all the rooms carefully, remove dust deposits, wrapping paper, bags, packaged product and other removable, inflammable devices in the rooms which are to be heated.
3. Remove gas containers (like spraycans) from the area to be heated.
4. Switch off compressors and ventilate the pressure tanks.
5. Remove combustible fluids.
6. Switch off all electronic components. (Temperatures could reach 60°C!)

The heating period in production buildings with several floors can last up to 48 h.
A single-room heat treatment, e.g. hotel rooms, normally take 24 h.
During the heating period, the inspection of the heated areas has to be carried out at regular intervals, in order to check that the heat distribution is sufficient and to recognize possible disturbances.

Use

Normally, at least 2 (but often more) WEO are used at each floor. They are positioned in a way that they produce a heat accumulation. To treat a single room, one WEO should be enough. The heaters are connected to existing CEE-plugs. At a heating power of 18 kW you need a 32 A fuse. If the energy supply is only equipped with a 16 A fuse heaters can be used at half power (9 kW) as well.

In case you do not have any or not enough CEE plugs we can provide one or more distributors with a maximum of 15 pieces of 32A plugs. This distributor must be connected with your main energy supply.

Since last year, we have started producing modified units:

Firstly, the WEO 4.5/9. It is operated at 4.5 or 9 kW, has a very quiet fan and can be run with a 16 A fuse. It is mainly used in private rooms or hotels.

The second variation are variable volt-types (220V / 440V) with 60 Hz offered to several Asian countries and Mexico.

Power consumption

The power consumption using a ThermoNox® heat treatment is only about 3 to 4 kWh per m³ volume. Within the last six years the ThermoNox heater has been used successfully in the food industry (mills, bakeries, restaurants, pasta factories), pet-food industry, hotels, warehouses (clothing) and as protection of wood constructions in old buildings et-cetera-et-cetera... .

Until now we have carried out the treatment in facilities up to 100,000 m³ with best results. The method is being practised in Europe and in several Asian countries and has been receiving a steady growing appreciation.

Conclusion

Using the ThermoNox method brings you one dozen of advantages:

1. Absolutely non-toxic pest control
2. Due to air circulation, low cost alternative
3. Machinery and conveying systems do not have to be deassembled - just emptied
4. No residue in product, machinery or rooms
5. Authority permission is not needed
6. No insect resistance
7. Even the eggs of the insects will be killed
8. The treatment can be carried out by any trained person
9. A ThermoNox heat treatment is an internal procedure - poison-warning signs outside of the building are not required
10. The rooms can be inspected during the heating period without protection equipment
11. Using a non-toxic alternative could be a subject in your advertising
12. Avoiding the use of methyl bromide is your active part in saving the stratospheric ozone layer.

Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
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Efficacy of high temperatures to control *Lasioderma serricorne* and *Rhyzopertha dominica*

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Abstract The application of heat could be an alternative to fumigating premises of food industry like flour mills. High temperatures above 45°C are lethal to stored product arthropods and lethal exposure times decrease with increasing temperature. In literature, the tobacco beetle *Lasioderma serricorne* and the lesser grain borer *Rhyzopertha dominica* were reported more heat tolerant than other stored product pests, but there exist few sound data. In the laboratory, probes with *L. serricorne* in wheat bran and *R. dominica* in whole wheat grains were tested for their tolerance to heat. Probes consisted of 10 ml of substrate with one developmental stage (eggs, young larvae, old larvae, pupae) or 50 adults and were filled into glass tubes already preheated in a hot water bath at 45°C or 50°±0.1°C for exposure periods between 2.5 h to 40 h at 45°C and for 20 min to 60 min at 50°C.

First results indicate that *L. serricorne* could be controlled at 45°C within 40 h but not *R. dominica.* At 50°C the longest exposure time tested (60 min) proved far too short, complete control may be achieved in the range of 3-4 h. Pupae and late larval stages were more heat tolerant in both species compared to adults, eggs and young larvae. The presence of substrate was essential for the survival of adults that were killed at 45°C in an empty glass tube within 10 h exposure but survived much longer exposure times when tested in 10g substrate.

Keywords: Heat, tobacco beetle, lesser grain borer, disinfestation.

Introduction

The phase out of the production and use of methyl bromide as a fumigant for space treatment, that will be completed in industrialised countries by the year 2005, forces many European flour mills and other food processing facilities to look for different means of disinfestation. An alternative is needed that will allow complete disinfestation in a comparably short treatment time. Moreover, the consumer demands control measures that leave no residues, and workers unions, as well as authorities responsible for workers safety prefer treatments that pose less risk to the staff involved. Heat could be an alternative fulfilling all these demands while still being economically feasible. A comprehensive overview on the effects of temperature and thermal treatments for stored product protection is given by Burks et al. (2000). The application of heat does not involve any chemical agent and thus does not require authorisation as a plant protection treatment for stored product protection in Germany. According to Denlinger and Yocum (1998) there are three main hypothesis for the injury caused by heat:

1. Disruption of ionic balance across cell membranes
2. Disruption of the cell protein biosynthesis by injury to the desoxyribonucleic acid
3. Denaturation of enzymes and disruption of metabolism.

Heat treatments involving electrical heaters or fossil fuel burners and fans to distribute hot air in a building are commercially available in the United Kingdom and Germany, as well as in the United States.

Under the light that heat may be applied on broader terms in the future, it is important to study the heat tolerance of certain pest species. Kirkpatrik and Tilton (1971) compared various adult stored product pest insects in an infrared heat treatment of 150 g samples of soft winter wheat at 49°C regarding the mortality produced and gave the following order of decreasing tolerance:

Most tolerant *Lasioderma serricorne*
Rhyzopertha dominica & Cryptolestes pusillus
Sitophilus oryzae & Tribolium castaneum & Trogoderma variabile
Sitophilus gran. & Gibbium psylloides
Cathartus quadricollis & Oryzaephilus mercator
Least tolerant *Tribolium confusum & Oryzaephilus surinamensis*

Data on lethal exposure times for certain species at certain temperatures, however, are quite scarce. Wilkin & Nelson (1987) found that 15 min exposure to 60°C killed all stages of *T. castaneum, O. surinamensis*, *Cadra cautella* and *Plodia interpunctella*. Al-Azawi et al. (1984) mention that adults of the dried fruit beetle *Carpophilus hemipterus* were quite heat tolerant and required up to 60 min exposure to 50°C for complete control. It has been stated that much research is still needed to better define the temperature-time-mortality relations for stored-product insects (Burks et al. 2000). Grossmann (1931) found that *Tribolium castaneum* of mixed age from egg to adult could be controlled at 45°C within 11 h and at 50°C within 15 min, *S. oryzae* within 3 h or 10 min, respectively, when exposed to various temperatures in test tubes in a water bath.

From the data available, Norstein (1996) postulated that a minimum of 22 h exposure at 45°C and of 40 min at 50°C should be sufficient to control all stored product pests. The present study was carried out to check if this assumption was correct for *L. serricorne* and *R. dominica*.

Material and methods

Both *L. serricorne* and *R. dominica* were cultivated at 25±1°C and 65±5% r.h. Every week, 600 young adults of the tobacco beetle were placed onto 500 ml wheat bran and approximately 50 ml of broken tobacco leaves. After 7 days the adults were removed. The lesser grain borer was reared on whole winter wheat grains. Weekly, 100 adults were released on 200g of grain to oviposit for 7 days.

Complete development from egg to adult took approximately 9 weeks for both species. Four age cohorts were formed by combining 1 and 2 week old cultures, 3 and 4 week, 5 and 6 week, 7 and 8 week old cultures. Probes for heat experiments were prepared by mixing substrate and juvenile stages of two weeks and by filling 10 ml into a small flask. Additionally, 50 young adults were counted and placed together with 10 ml uninfested substrate into similar flasks.

The heat experiments were carried out in glass tubes submerged in a heated water bath (Huber HS 40, Germany) equipped with a strong whirl pump to keep temperatures constant (figure 1). The desired temperature was adjusted and the glass tubes were preheated prior to filling in insects and substrate. The time necessary to heat the substrate and insect probes to the desired temperature (± 0.3°C) was determined with both electric and a mercury thermometers. This time period was added to the exposure time tested. Product moisture contents in both wheat grains and wheat bran were approx. 14 % corresponding to a relative humidity of 65-70 %.

After filling in the substrate with insect probes the glass tubes were closed with a cork stopper to avoid cooling by convection. A cotton plug was inserted into glass tubes containing adult insects to prevent beetles from climbing up to a cooler zone above the water level as had been observed in preliminary tests at 45°C.

Water bath temperatures and temperatures within an empty glass tube were continuously recorded using an electric thermometer (PME Temperatur Multimeter, Germany; Jumo TDAt-74/1, Germany) and a plotter (Kipp & Zonen, BD 112, Holland, figure 1).

Results and discussion

It took approx. 10 min to heat 10 g of wheat grains until the desired temperature (± 0.3°C) could be measured in the center of the glass tube and approx. 15 min to heat the mixture of wheat bran and tobacco leaves.

Figure 1. Water-bath with heated insect probes and temperature monitoring equipment

The results of the heat treatments at 45°C are given in figure 2 and figure 3. According to the logarithmic trend lines, the order of growing heat tolerance of different developmental stages of *L. serricorne* was beetles, stage 1, stage 2, stage 3, stage4. In *R. dominica* the order was stage 1, stage 2, beetles, stage 3, stage 4. The sensitivity of egg and early larval stages seems remarkably similar in both species (figure 2 and figure 3). During larval development and up to pupation heat tolerance seems to increase in variable degrees with *R. dominica* ending up obviously much more heat tolerant than *L. serricorne* pupae. And in both species adults were less heat tolerant than late developmental stages which may be due to the larger body surface to volume-ratio. *L. serricorne* adults, however, were less heat tolerant than eggs and first larval stages of the same species.

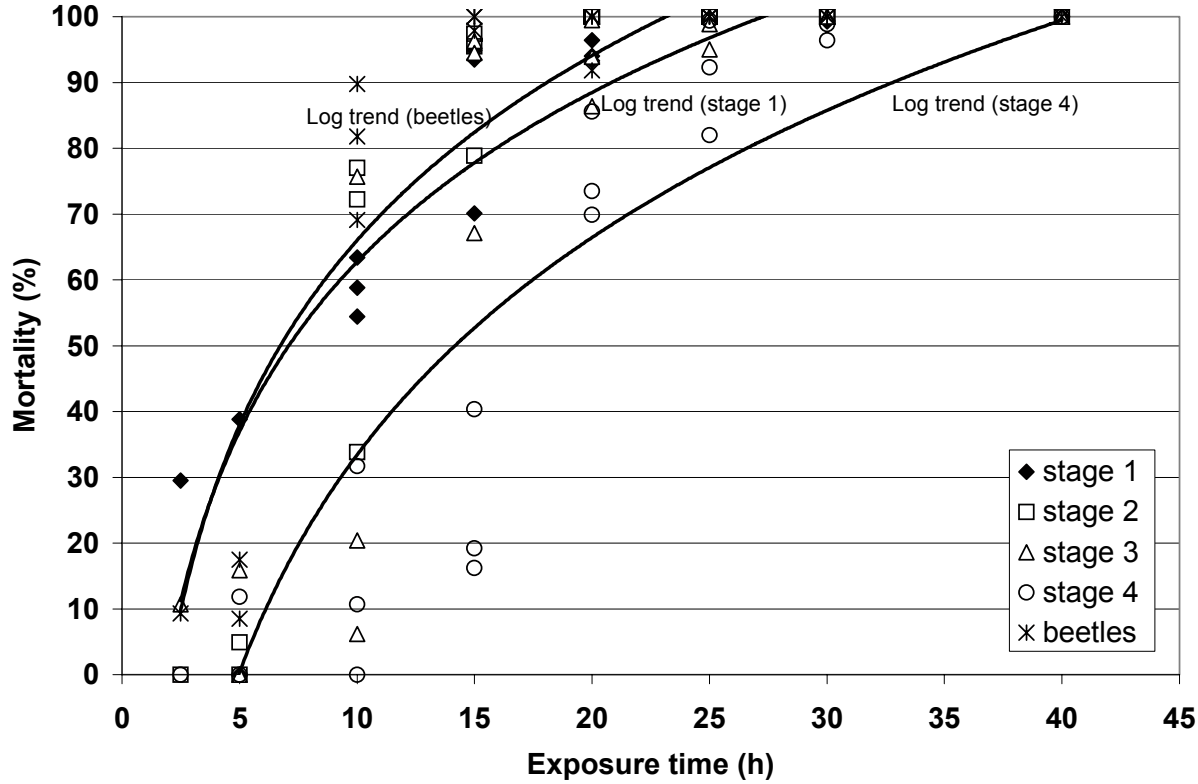

Figure 2. Efficacy of heat at 45°C against *Lasioderma serricorne* in wheat bran

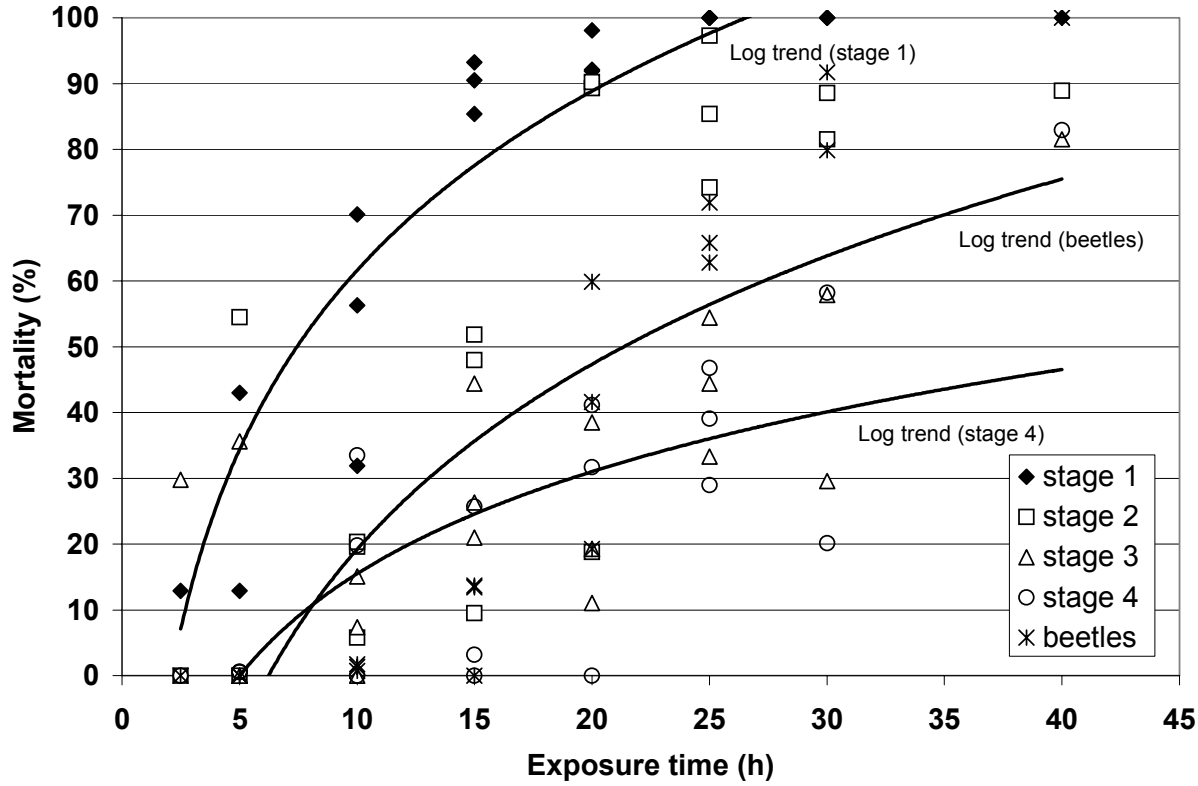

Figure 3. Efficacy of heat at 45°C against *Rhizopertha dominica* in wheat grains

At 50°C, the tested exposure times of up to 40 min were not sufficient to control either of the two species. After this time, mortalities of stage 4 were in the range of 10-30 % and control could be possible after exposure times of some 3-4 h. This seems to indicate how variable different stored product pests react to high temperatures and how dangerous it may be to generalise from available data. All results clearly contradict the statement of Norstein (1996) that stored product pests may be controlled by minimum exposure times of 22 h at 45°C and 40 min at 50°C. From the data gained it may be concluded that 40 h at 45°C are the minimum to control *L. serricorne*. For control of *R. dominica* double this time may be expected.

Contrary to the findings of Kirkpatrick and Tilton (1971) *R. dominica* proved more heat tolerant in the present study than *L. serricorne*. This may be due to the fact that Kirkpatrick and Tilton used infrared radiation in larger quantities of soft winter wheat and that they tested adult insects only. Infrared radiation may convey heat to insects containing water in their hemolymph more thoroughly. It could also be that the *R. dominica* strain of their study was genetically less tolerant than the one described in this paper.

The presence of substrate was essential for the survival of adults that were killed at 45°C in an empty glass tube within 10 h exposure but survived much longer exposure times when tested in 10g substrate. This could be due to the fact that being submerged in a mass of struggling con-specifics is an additional stress to beetles.

It may be concluded that it is dangerous to generalise on lethal exposure times of stored product insects regarding heat treatments from the data available. Contrary to widespread expectations still present among stored product entomologists, mortality seems not to be a simple function of temperature and time but differs a lot between species and developmental stage. More research is needed to elucidate the effect of different high temperatures on various species, to compare strains within a species and to determine the potential of heat tolerance that may be aquired by a population exposed to sub-lethal heat treatments.

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MODIFIED ATMOSPHERES

Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
193-197

Nitrogen as a major component of a controlled atmosphere to manage stored product insect pests in large vertical storage

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Introduction

Considerable research has been conducted using controlled atmospheres to manage stored product insect pests of cereals, and this type of grain quality management is becoming more attractive with increased market demand for grain that is free from chemical residues (Bailey and Banks, 1980). Controlled atmosphere grain storage consists of generating and maintaining a single or multiple component of atmospheric gases so that the gas(es) become the most significant component of the atmosphere within the storage. This is typically accomplished by reducing the concentration of O_2 and increasing the concentration of N_2 and/or CO_2 . The intergranular atmospheric composition affects grain deterioration, as insects, mites and aerobic fungi and bacteria require certain levels of oxygen to respire and thrive.

Controlled atmospheres have been generated by a number of methods. In low oxygen, high nitrogen environments, liquid nitrogen, cylinderized gaseous nitrogen, pressure swing absorption and membrane technologies have been the most commonly used. Liquid and cylinderized nitrogen are mechanisms to obtain the highest purity but the supplies can be finite and the costs very high. Pressure swing absorption is economical for high purity and flow rates but the technology is older and the production cannot be changed without changing the entire system. With membrane systems, costs are relatively low, they are almost maintenance-free and the production with these types of units is easily upgradable.

Supplemental to the effective control of the target insect pests, controlled atmospheric storage is also known to suppress mould growth, preserve grain quality and maintain the germination capacity of the grain in storage (Banks, 1981). However, while the benefits of using inert atmospheres to manage grain quality are many and useful, there are some limitations to how effective these techniques can be in an applied situation. Banks and Annis (1990) state that different concentrations of particular controlled atmospheres are required to be effective against different species or even different life stages of the same species. It is also known that different controlled atmospheres have varying effectiveness on different species. In applied situations, while it is common that one species may dominate the pest population within the commodity, it can be very common that more than one species exists. In the commercial handling of bulk commodities such as grain, the managers of these commodities expect single applications of disinfestation products to be all-inclusive. It is therefore important that much research be conducted to establish mortalities for different species and their associated life stages.

Structures, sealing and an appropriate monitoring mechanism are important factors to consider for controlled atmospheres to be successful. The structure being used for containing grains for atmospheric storage is probably the most limiting factor. In most cases, grain storage was not designed to hold gas and this results in a great deal of time being spent on sealing and monitoring. Therefore, results of fumigation and/or quality maintenance can be

quite variable. Sealing techniques usually include testing the gas holding capacity of the bin and using effective materials to secure openings, valves and ventilation ducts. Monitoring must also include well-timed measurements of gas concentrations both inside and outside of bins in order to maintain the concentrations of the controlled atmosphere. From an operations perspective, this can be difficult due to other operational issues and resource commitments.

Other factors impacting the effectiveness of an atmospheric control program are also important. These include abiotic (gas composition, grain moisture/relative humidity and temperature) and biotic (target organism, population levels and distribution) and can have an affect on the time requirements for insect control.

Given the factors associated with using controlled atmospheres as described above, it is the purpose of this study to examine the commercial application of a controlled atmosphere to a large concrete commercial storage to monitor efficacy and application from a membrane based generator. Data are provided here on the response of the Red flour beetle adults, *Tibolium castaneum* (Herbst) to atmospheres low in oxygen and high in nitrogen.

Materials and methods

The bin used for the experiment is within a concrete storage facility capable of handling over 250,000 metric tonnes of grain. The bins used are called star bins as they are the triangular or diamond shaped spaces between the conventional cylindrical storage. These are typically used for fumigation in Canada as they are approximately 210 m^3 and have capacity for approximately 120 metric tonnes of wheat. The bins are constructed of reinforced concrete that is 20 cm in thickness. The bin used was 29 m in height. Openings are typically on top and bottom. The top opening and bottom valve are constructed of 1 cm steel plate and are typically 3600 cm^2 in size.

The controlled atmosphere was produced using a membrane based non-cryogenic generator. The unit that was used for these experiments was a cabinet style system capable of producing up to 140 m^3/hr. Installation of the generator was simple. A compressed air line was attached to the 'in' line on the generator. The compressor used was capable of drying atmospheric air prior to sending it through the generator. This allows for an increase in efficiency. As the atmospheric air moves through the membrane, fast molecules such as H_2O, O_2 and CO_2 permeate the membrane while the slower N_2 molecules move directly through at 99.5% purity and at about the same pressure that they were introduced. The nitrogen generated from the membrane sieving flows through an outlet to the bin that is to contain the controlled atmosphere. Purging of the bin was completed in 24 hours.

The concentration of the atmosphere was maintained within the storage by creating a positive pressure. This was accomplished by creating a slight vacuum on the bin by activating the dust removal system from the top while adding the nitrogen at the bottom of the bin at a slightly greater rate than is being removed. The vacuum removed gas at approximately 3 cubic feet per minute (cfu) while the addition of gas was at 5 cfu. After purging, oxygen concentrations can be measured at the top of the bin to ensure that concentrations are maintained at the desired level. This was eventually replaced with detection tubes measuring gas levels at 2 and 15 meters from the bottom of the bin. Bin seals were tested with soapy water to determine if leaks were present.

The insects used for the study were *T. castaneum*. All test insects originated from cultures obtained from the stored product protection section at the Cereal Research Centre in Winnipeg. Twenty adults of mixed age and sex were placed into probe pitfall traps. Insects were placed into the traps with cracked grain and were store at 25°C for 72 hours prior to exposing them to the controlled atmosphere. Three traps were attached to a steel cable at three

locations at 10 meter intervals so the there were three traps within one meter from the top of the bin and three located 10 and 20 meters below this respectively. Traps were placed into the bin and upon filling with grain, nitrogen was introduced. The bin was filled with approximately 90 tonnes (one railcar) of wheat. Control insects were placed in traps and were left in a container located above the bin for the duration of the exposure. Traps were exposed to the nitrogen atmosphere in the bin for 7, 14 or 21 days. After the exposure, the bin was aerated and the grain was removed. The traps were removed and adults were separated from the cracked grain and assessed for mortality. Cracked grain from the traps at each level were placed into jars containing 500 g of cracked grain at 15% moisture content and they were stored at 25°C and 70% r.h. for 40 days.

Wheat used in the study was of various classes including: Canadian Western Amber Durum, Canadian Western Hard Red Spring and Red ad White Canadian Prairie Spring. All are hard wheats designed for producing bread or noodles. Moisture contents and temperatures were varied. Moisture varied from 11.8% to 13.2% and temperature ranged from 11°C to 22°C. This resulted in a relative humidity between 48% and 57%.

Indices used to make determinations of effectiveness were adult mortality and survival of the F1 generation. Material in the traps was sieved and introduced adults were assessed for mortality. Adults were removed and the grain was incubated to determine if there was survival of any offspring.

Results and discussion

Part of the trouble with attempting research trials in an applied setting is the lack of control of intangible variables. Reasons for the lack of mortality in the 2nd and 3rd trails could have been from leakage. While the gas readings showed low oxygen content there may have been a problem with the input and output of gas within the bin. Modification to the input and exhaust were made for the 3rd and subsequent trials and lack of mortality has not been observed since that time.

Mortality of the adult *T. castaneum* exposed to the controlled atmosphere was variable. Greater than 99% mortality was achieved in trials 1, 4, 5, and 6 while essentially no mortality occurred in trials 2 and 3. It is felt that this is likely due to leakage or gas mixture problems within the bin as the lack of mortality does not seem to be related to temperature or exposure time. Three trials were conducted for 7 days with 2 of the trials having high mortality and one having minimal mortality. Two of these trials were conducted at temperatures between 14°C and 17°C. Banks and Fields (1995) site work illustrating that >95% mortality of *Tribolium castaneum* can be achieved in >14 days. Jay et al. (1971) demonstrated that mortality of *T. castaneum* in a high N_2 / low O_2 environment is inversely related to relative humidity at constant temperatures. There were similar findings for insects exposed to the controlled atmosphere for 21 days. Adler (1996) found a very strong relationship between mortality of *Sitophilus* and temperature when exposed to >98% N2. Jayas et al (1991) state that mortality of insects may depend, not only upon the abiotic conditions but on the state of the insect and how quickly it is exposed to the controlled atmosphere. Storey (1975) found very high mortality of pyralid moths after 72 hours although temperature was 27°C. In Adlers work, *Sitophilus* species required over 20 days of exposure to a similar controlled atmosphere to cause 100% mortality at 25°C.

As it seems that species and life stage can effect the rate of mortality, a great deal more work and many more carefully conducted trails with this type of generator in a commercial setting will be needed to develop a clearer picture of how well this type of technology works.

Developing matrices for different species in different stages of development would be useful to those managing grain stocks to enable them to use this type of technology effectively.

As mentioned, a high nitrogen atmosphere has the potential to reduce damage caused by heating. This is caused by reducing the metabolic processes which maintain it. Stored grains at appropriate moisture and temperature levels are in a suspended or dormant state. Changing (generally increasing) these abiotic factors causes the resumption of the metabolic processes and the potential for the deterioration.

Reducing the levels of oxygen halts the amount of respiration from the grain resulting in a quick cessation of the heating cycle, which in turn stops the rapid deterioration of the stored commodity. The complex of grain and the associated microorganisms can be sites of intense respiration. This is an exothermic phenomenon and is related to the presence of oxygen. In airtight or controlled atmosphere storage, the respiration is blocked and the degradation of the grain into CO_2, H_2O and heat is eliminated. Atmospheres high in nitrogen and low in oxygen are also said to reduce aflatoxin formation in grains with higher moisture contents as well as reducing the total mold and bacterial count within that particular storage.

Safety is another advantage of using systems of this type. Other gases used in controlled atmospheric storage are know to concentrate in certain areas (e.g. low lying) within a calm environment and can build up to lethal potential. While the high N_2 environment is also lethal, it recombines in to the atmosphere readily. For example, gas meter readings close (within 10 cm) to a leak will sound an alarm. However, when the meter was moved to approximately 60 cm of the leak the meter showed normal O_2 levels.

In summary, the utilizing of N_2 produced with molecular filter technology as a controlled atmosphere shows potential to be an effective method to preserve grain quality attributes. The molecular filter technology is a quick, easy and cost effective means to achieve the desired controlled atmosphere. The devices are relatively simple and can be compact and portable enough to meet user needs if locations are relatively remote.

Table 1. Mortality of *Tribolium castaneum* subjected to a nitrogen based controlled atmosphere of greater than 98%.

Trial	Wheat Class*	Exposure Period (days)	Temp. (°C)	Moist. (%)	Mortality (%)**				F1 (test) (p/a) ***	F1 (control) (p/a)
					L1	L2	L3	control		
1	cwad	7	22	12.5	98.3	98.3	Lost	0	a	p
2	cps	7	16	13.5	3.3	0	1.7	5	p	p
5	cps	7	15	13.2	100	100	100	1.7	a	p
3	cwhrs	14	19	12.8	3.3	3.3	5.0	0	p	p
6	cwhrs	14	17	13.0	100	100	100	0	a	p
4	cwhrs	21	11	11.8	100	100	95.0	0	a	p

* cwad – Canadian Western Amber Durum, cps – Canadian Prairie Spring, cwhrs – Canadian Western Hard Red Spring

** L1 – 10 meters from top of bin, L2 – 20 meters from top, L3 – 29 meters from top

*** p – present, a - absent

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Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
199-206

Controlling insect pests of stored medicinal plants by controlled atmospheres

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Abstract: Various stages of *Trupanea stellata* and *Lasioderma serricorne* were exposed to four gas mixtures differing in their CO_2 content (20%, 40%, 60% and 80% CO_2). In general, increase in carbon dioxide combined with decrease in oxygen resulted in increasing mortality. The gas mixture containing 80% CO_2 was the most effective to control the different stages of *T. stellata* (more tolerant than the different stages of *L. serricorne* insects). The use of this gas mixture to disinfest chamomile for 7day exposure in 31.4 m^3 PVC sheets (tunnel shape) and 30 m^3 fumigation chamber under temperature range between 28 to 35^oC, resulted in complete control. The use of CO_2 for creating an atmosphere lethal to stored-product insects in sealed container vans during shipment of medicinal plants was promising as a residue free control measure. Also studied was the rate of infestation of chamomile flowers by *T. stellata* under field conditions.

Key words: Carbon dioxide; medicinal plants; insect control, modified atmosphere; population dynamics, *Trupanea stellata; Lasioderma serricorne,* chamomile.

Introduction

Medicinal plants such as Chamomile (*Matricaria chamomilla* L.); Fennel (*Foeniculum vulgare* Miller) and Anise (*Pimpinella anisum* L.) are produced in Egypt using the organic farming system according to European Regulations in about 2500 Feddan (1 Feddan = 4200 m^2) (Council Regulation (EEC), 1991). In this system, neither chemical fertilizers nor synthetic pesticides are used. Most of these products are for export to the European and American markets, in which the major constraints for exportation are the detection of either insect infestation or chemical residues..

Chamomile is one of the most sensitive medicinal plant to insect infestation either in the field or during storage. Chamomile is exposed during flowering in the field to attack by the chrysanthemum fly *Trupanea stellata* (F.). The development of this insect continues during drying and the first two to three weeks of storage. During drying, processing and storage the chamomile is also exposed to attack by the cigarette beetle *Lasioderma serricorne* F.

The classic way to control these insects has been by the use of fumigants such as methyl bromide (CH_3Br) and phosphine (PH_3), which are not allowed for treatment of organic products. Recent work in some countries has focused on the possibility of using the inert gases (CO_2 or N_2) as an alternative to chemical fumigants. This method of treatment is commonly termed modified atmosphere (MA) or controlled atmosphere (CA) (Jay and Pearman, 1971; Jay *et al*., 1971; Jay, 1980; Reichmuth, 1988; 1993; Conyers and Bell, 1996; Hashem and Reichmuth, 1994; 1996; Hashem, 2000).

This work reports on the population dynamics of *T. stellata* under field conditions and tests of susceptibility of various stages of *L. serricorne* and *T. stellata* to different mixtures of carbon dioxide, nitrogen and oxygen. It also presents two procedures for applying the efficient

mixture resulting from the laboratory experiments to disinfest stored products of the above mentioned medicinal plants.

Material and methods

Population dynamics of the chrysanthemum fly T. stellata infesting chamomile flowers

The population dynamics study of *T. stellata* was carried out in Sakaran farm, Fayoum region from Dec. 7th, 1999 to May 27th, 2000. Representative samples of 400 chamomile flowers were collected weekly, randomly and transported to the laboratory for examination on the same day. To examine the different stages found inside the flowers, each flower was examined under stereomicroscope following dissection. The number of larvae, pupae and infested flowers were recorded.

Susceptibility of different stages of L. serricorne and T. stellata to alterations of atmospheric gas concentration

Stock culture and preparation of different stages to exposure

The parental insects of *L. serricorne* were obtained from infested chamomile and reared on chamomile powder at 30°C ± 2°C and 70% ± 5% r.h. Adult and pupal stages were selected for treatments because the adult is the most susceptible stage and the pupa is the most tolerant (Keever, 1989). Trials were carried out on one week old adults, 2-3 days old pupae and 3rd instar larvae of *L. serricorne*. The experimental unit for *L. serricorne* was 50 adults; 50 pupae; 50 larvae and 50 dried chamomile flowers infested by *T. stellata*. Each unit of *L. serricorne* was prepared in a cylindrical cage (6 cm high and 1.5 cm diameter) made from 40 mesh stainless-steel wire gauze closed with rubber foam. Cages containing adults was supplied with food. Each 50 dried chamomile flowers was placed in small paper bag.

These cages, each containing one stage of adults, pupae and larvae of *L. serricorne,* and small paper bags containing infested chamomile flowers were introduced into 30 L plastic container (Hashem and Risha, 2000; Hashem et al., 1994)

Gas-mixtures

The tested atmospheres were prepared from ternary mixtures CO_2, O_2, and N_2. This component was monitored using a paramagnetic oxygen analyzer (SERVOMEX/ England). To improve distribution of the components, the cylinders with gas mixtures were kept at room temperature for two days before starting the experiment (Reichmuth, 1987). The gas mixtures tested were:

Gas-mixture 1: 20% CO_2, 64% N_2 and 16% O_2;
Gas-mixture 2: 40% CO_2, 48% N_2 and 12% O_2;
Gas-mixture 3: 60% CO_2, 32% N_2 and 8% O_2 and
Gas-mixture 4: 80% CO_2 , 16% N_2 and 4% O_2.

Exposure procedure

Different stages of insects were exposed to gas mixtures in dressel flasks kept at room temperature (30°C±2). The gas mixtures were supplied from gas-mixture cylinder through copper tubes purged trough a humidifying unit containing saturated $NaCl/H_2O$ solution in flasks, to create 70% r.h.

At the outlet of the containers the O_2 content was determined continuously by an oxygen analyzer. After about 15 min. (time for about 10 replacements of total container volume by gas mixture) the outlet concentration became identical with the inlet concentration. Following different exposure periods ranging from 1 to 4 days, each container was aerated and the insects were transferred from the cages to individual Petri-dishes (6 and 18 cm diameter) and

held at 30°C ± 2°C and 70% ± 5% r.h. Adults were examined for mortality 48 hours after exposure and the pupae and larvae of *L. serricorne* were examined for adult emergence. The chamomile flowers were examined for adult emergence of *T. stellata*. Each sample was accompanied by an untreated control. Experiments were repeated three times.

Experimental design and statistical analysis

The experiments were designed to provide time-mortality regression lines for the different stages in various combinations of atmospheric gases. For each case, tests were carried out at different exposure periods as mentioned above.

Moralities of *L. serricorne* adults in treated batches were corrected by Abbotts formula (Abbott, 1925). Data were subjected to probit analysis (Finney, 1971) to calculate the regression equation, slopes of regression lines and the values of LT_{50} and LT_{99}, with the help of the computer program of Noack and Reichmuth (1978).

Large scale application of the efficient CO_2 -concentration for controlling stored medicinal plants

Using 31.4 m^3 PVC sheet (tunnel shape)

A PVC sheet to provide a tunnel shape volume of 31.4 m^3 (10 m long and 2 m diameter) was used for treatment with controlled atmospheres. The sides of this tunnel had two valves; an input and output valve. The input valve was attached to the CO_2 cylinder with a rubber tube and fitted inside with another rubber tube extending to the bottom to distribute CO_2 in the whole volume of the fumatorium. The output valve was used to evacuate the air and to attach to the oxygen analyzer to measure the oxygen rate during and after treatment.

A quantity of infested 120 boxes (20 kg each) of medicinal product was treated in each test. Insects were exposed in 18 cages of adult, pupal and larval stages (50 individuals/ cage/stage/ species) of *L. serricorne* and 6 paper bags of infested chamomile flowers (50 flowers each) placed in the fumatorium. Additional 18 cages containing insects and 5 paper bags (prepared as described above) were inserted in untreated products to serve as controls. The fumatorium was closed tightly using silicon around the openings to avoid gas leakage. The applied gas mixture was 80% CO_2, 16% N_2 and 4% O_2 and the exposure period was 7 days.

Using 30 m^3 fumigation chamber

A large scale fumigation chamber (2.5 m high x 3 m width x 4 m long) was built in SEKEM Co. for biological products at Belbeis, Sharkia Governorate. It was considered a relatively tightly sealed structure. The roof, walls and the interior side of the door were lined with aluminum sheets (150 μ thick) and the floor was covered with stainless-steel sheet (1 mm thick). The door of the chamber had two openings; a lower opening for gas input and an upper opening for gas output. To ensure the air tightness of the chamber, all fill spouts, door margins and manholes, were sealed with duct tape (obtainable from refrigeration or air-conditioning supply houses). The pressure test to determine the efficacy of fumigation in the buildings, chambers and stores against stored product insect pests was applied before introducing CO_2 (Reichmuth, 1993). Application of the gas mixture creates a positive pressure in the chamber that must be released through the output opening.

A quantity of infested 1500 kg chamomile (in boxes) was put in the chamber. Twelve cages of adult, pupal and larval stages (50 individuals/cage/stage) of *L. serricorne* were placed in wire cages to monitor insect mortality within the treated product. The same number of additional cages (prepared as described above) was inserted in untreated products to serve as controls. Small paper bags of 50 infested chamomile flowers with *T. stellata* each were placed also within the treated product to monitor the insect mortality after treatment. To

measure the temperature and relative humidity inside the chamber throughout the test, a thermohygrograph was installed in its center.

After exposure, each stage of the two insects was transferred from the cages to Petri dishes and held at 30°C ± 2°C and 70% ± 5% R.H. After 48 hours exposure, the adults were examined for mortality. Insects not responding to gentle stimulation were considered dead. The criterion of dead pupae and larvae was based on failure to develop to adults. At the same time, the infested samples from chamomile were examined to evaluate the efficacy of the gas mixture used in the treatment through the survived and killed stages of both insect species.

Results and discussion

Population dynamics of the chrysanthemum fly T. stellata infesting chamomile flowers

Data illustrated in Fig. 1 show the population fluctuations of *T. stellata* larvae and pupae per 400 chamomile flowers as percentage of infestation. The obtained results show that the variations in the population density of larvae and pupae of *T. stellata* fluctuated from one date to another.

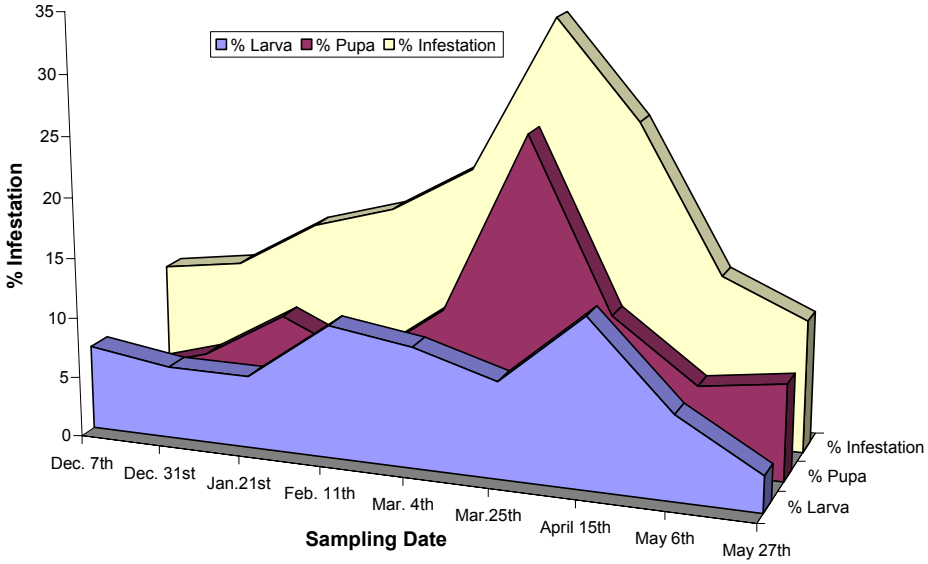

Fig. 1. The percentages of infested chamomile flowers by larvae and pupae of chrysanthemum fly *Trupanea stellata*

As indicated in Fig. 1, two peaks were obvious for the larval stage in Feb. and April, but in the case of pupal stage three peaks of abundance were obvious. The first peak of the pupal population was recorded on January 21st, after that of larvae with nearly three weeks and almost equal to it. The second peak of pupae was recorded in the third week of March, whereas the population density of larvae represented by the first peak on Feb. 11th. This can be explained in that the present larvae transferred to pupae representing the end of a generation with a decrease in oviposition during this time (Barakat, 1996). The infestation rates were high during the season especially on March 25th (34%) of the experimental season. Knowing that import countries reject any product if the rate of infestation reaches 5%, explains the importance of this pest attacking chamomile.

Susceptibility of adult and pupal stages of T. stellata and L. serricorne to alterations of atmospheric gas concentration

Results of the laboratory experiments to achieve LT_{50} and LT_{99} levels appear in Figs. 2 and 3. At all gas mixtures, the stages of *T. stellata*, were more tolerant than the various stages of *L. serricorne*. The LT_{99} values for larval stage (more tolerant than other stages) of *L. serricorne* were 4.80; 4.30; 2.82 and 0.6 days exposed to gas mixtures 1, 2, 3, and 4, respectively. The LT_{99} values of *T. stellata* were 6.40; 5.60; 4.70 and 4.00 days, respectively. The LT_{99} values for pupal stage of *L. serricorne* were 3.50; 3.00; 1.60 and 0.51 days, respectively, and those for adult stage were 2.81; 2.00; and 1.00 days, respectively.

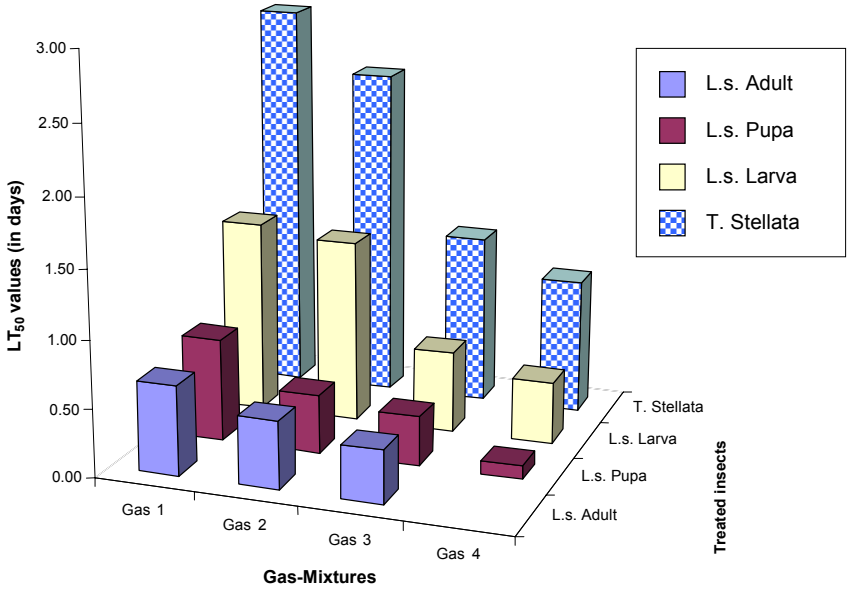

Fig. 2. LT_{50} values (in days) of treated stages of *Lasioderma serricorne* and *Trupanea stellata* exposed to four different gas mixtures

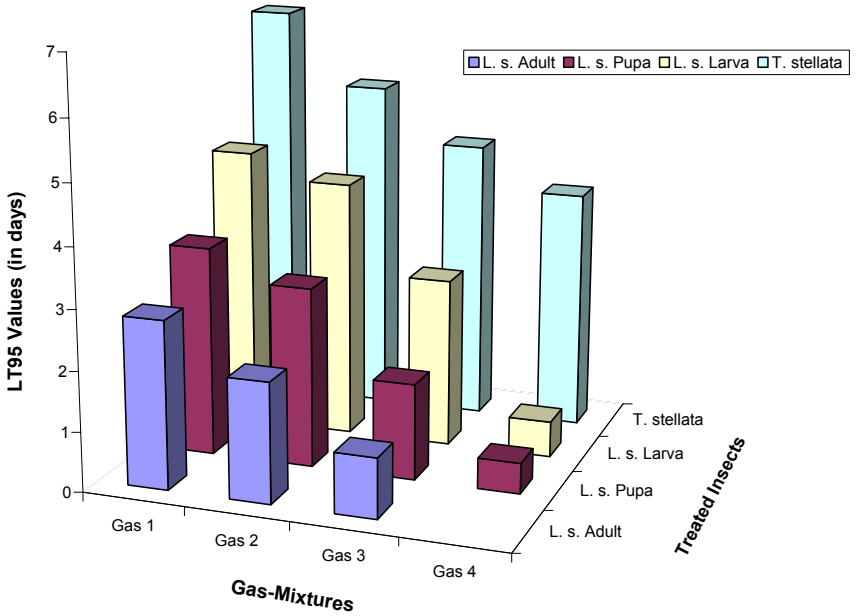

Fig. 3. LT_{99} values (in days) of treated stages of *Lasioderma serricorne* and *Trupanea stellata* exposed to four different gas mixtures

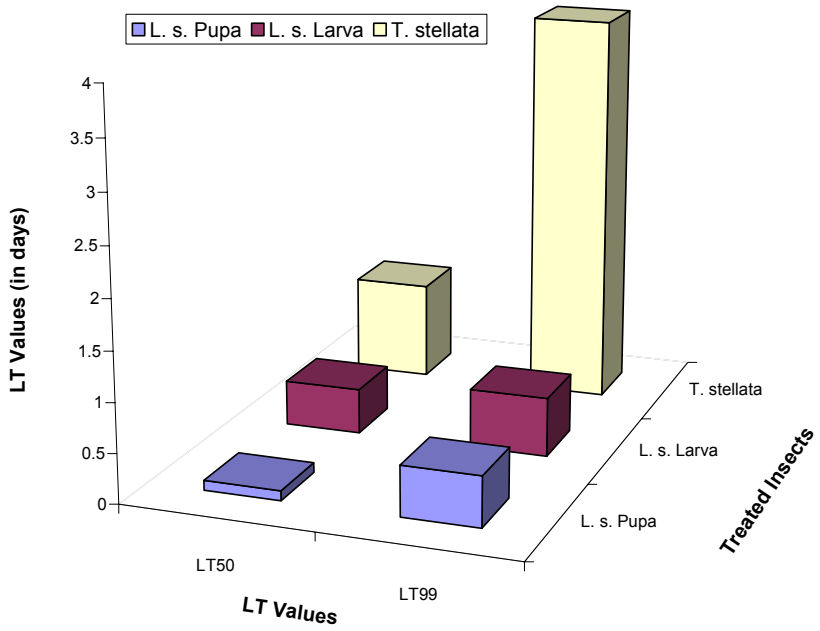

Fig. 4. LT_{50} and LT_{99} values (in days) for different stages of *Lasioderma serricorne* and *Trupanea stellata* exposed to 80% CO_2 in air

Mortality level of insects exposed to the mixture containing 80% CO_2, were higher than those of the insects exposed to mixtures containing 20%, 40% and 60% CO_2 at all exposure periods ranging from 1 to 4 days (Fig. 4). When, using the mixture containing 80% CO_2, mortality reached 100% after 1 to 2 days for adults and after 5 to 7 days for the more tolerant stages of both insects. Hashem and Reichmuth (1996) have shown that decreasing the oxygen content in the mixture increases the mortality in shorter exposure period. The descending order of the treated stages according to the LT_{50} and LT_{99} values was as follows:

Stages of *T. stellata* > Larva of *L. serricorne* > Pupa of *L. serricorne* > Adult of *L. serricorne*

These results agree with those obtained by Jay and Pearman (1971); Childs and Overby (1983); Reichmuth (1988); Soderstrom *et al*., (1991); Hashem (1993) and Hashem (2000).

Large scale application of the efficient CO_2 concentration for controlling stored medicinal plants

Based on the results of the above mentioned tests (Figs. 2-4), the upper confidence limit of the $LT_{99.999}$ at 95% of the gas mixture containing 80% CO_2 against the different stages of *T. stellata* was applied in the stainless-steel fumatorium and in the fumigation chamber. Free space temperature throughout the application time ranged from 26.2 to 34.7°C in the fumatorium and from 28.7 to 30.9°C in the chamber. The mean relative humidity in the fumigation chamber was 65.6%.

All treated stages of both insects were killed after 7 days exposure and mortality in untreated adults was very low (not more than 7%). Mortality of untreated pupae was higher (22%) than those of the adults, but still much less than the treated pupae. Keever (1989) indicated that the pupae of the cigarette beetle are often more adversely affected during tests than the other stages (probably because they are more sensitive to handling).

Medicinal plants are shipped overseas inside container vans. The present findings indicate that using carbon dioxide in such vans and sealing them may be a method for disinfecting these products as long as the transit period is not less than 5 days and the temperature is not less than 28°C. At lower temperatures the exposure period should be extended as indicated by Bailey and Banks (1980). Different preventive measures should be implemented in the field to minimize the infestation rate by the chrysanthemum fly *T. stellata.*

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IOBC Bulletin Vol. 25 (3) 2002
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Integrated storage pest control methods using vacuum or CO_2 in transportable systems

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Abstract: The suggested potential alternatives to MB (methyl bromide) for disinfestation of durable commodities are likely to be costly compared to the use of MB. In addition, very few of the suggested treatments have the effectiveness of short exposure time comparable to MB. The objective of our investigation was to identify the combinations that enhance the effectiveness of the treatments based on vacuum or a combination of heat and CO_2.

Tests of the influence of CO_2 at 45ºC on reducing the exposure time expressed as LT_{99} values for diapausing larvae of *Trogoderma granarium* showed that by increasing the CO_2 concentration to 90% the exposure time decreased to about 10 h, whereas at 35ºC the LT_{99} value was 29 h. *Ephestia cautella* pupae were shown to be the most resistant stage to the same treatment with an LT_{99} value of only 3 h. For *Oryzaephilus surinamensis* under the same conditions, it was 9 h for the most resistant egg stage.

In laboratory studies with *Lasioderma serricorne* exposed to low pressures at 30ºC, LT_{99} value for adults was 15 h when exposed to 25 mm Hg. *Trogoderma granarium* larvae were the most resistant species, whereby 172 h exposure was necessary under the same conditions.

These encouraging reports led to the idea of developing a transportable system to render the technology a practical tool for the control of insect pests. Experiments were carried out using a 15-m^3 capacity plastic container termed the "Volcani Cube™" or "GrainPro Cocoon®". The pressure was maintained between 25 to 29 mm Hg for 17 days. Bioassay in field trials demonstrated that complete mortality of test insects composed of mixed ages of *E. cautella Plodia interpunctella*, and larvae of *T. castaneum* was observed on the 3-days exposure to vacuum.

Key words: IPM, storage pests control, vacuum, CO_2, transportable systems, methyl bromide alternatives, *Oryzaephilus surinamensis, Trogoderma granarium, Ephestia cautella, Plodia interpunctella, Tribolium castaneum, Lasioderma serricorne*

Introduction

There are only two universally available fumigants remaining for disinfestation of durable commodities, namely, methyl bromide (MB) and phosphine (PH_3). Each of these has its own limitations. MB is facing a phase-out in developed countries by the year 2005 and worldwide by the year 2020 under the terms of the Montreal Protocol (UNEP, 1998). Under present agreements of the Montreal Protocol, there are exemptions for all countries from controls on MB when used for quarantine and pre-shipment fumigations, and for some critical agricultural uses, yet to be defined. PH_3 is a very useful fumigant but it is slow acting and insects in various countries have developed resistance (Winks, 1987; Zettler, 1993).

Since the announcement that MB would be phased out due to its role as an atmospheric ozone-depleter, research has focused on finding alternatives that can be registered as effective and safe. Although there is a large number of suggested potential chemical and non-chemical alternatives to MB, each has limitations that prevent it from being a direct replacement for

methyl bromide in all its current uses (Bell *et al*., 1996). Controlled atmospheres (CAs) have been one of the considered alternatives to MB. This technology can fulfill a specific niche where use of other fumigants is unacceptable such as treating organic foods. The use of controlled atmospheres as alternative to MB at normal ambient temperatures is limited by the long exposure times required to produce complete mortality (Navarro and Jay, 1987). These periods are similar to those required for PH_3 fumigations (Navarro and Donahaye, 1990). Insect development and metabolism are positively correlated with temperature (Donahaye *et al*., 1996), and it has long been recognized that insecticide treatments, particularly those affecting the respiratory system are more pronounced at higher temperatures. In cases where rapid disinfestation of commodities is required, the possibility of using CO_2 at temperatures raised to levels that will not adversely affect the commodity should be considered.

The possibility of using low pressures in post-harvest storage was first explored by Back and Cotton (1925), Bare (1948), and later on by Calderon *et al*. (1966), and Navarro and Calderon (1969; 1972a; 1972b). Recently Mbata and Phillips (2001) investigated the effects of temperature and exposure time on mortality of three stored product insects exposed to low pressure. Insect mortality under low pressure is predominantly a result of oxygen deficit and not due to physical pressure effects (Navarro and Calderon 1979). The effects of vacuum in combination with elevated temperatures were not considered in some studies (Back and Cotton 1925; Bare 1948; Calderon *et al*., 1966; Calderon and Navarro 1968).

To achieve the extremely low pressures for insect mortality a prohibitively expensive investment might be required in massive vacuum chambers. In a first attempt to use low pressures to store cacao beans, Challot and Vincent (1977) used polyethylene bags to apply and maintain a low pressure of 600 mmHg in order to preserve cacao beans quality. Although 600 mmHg may be effective in maintaining the product quality, and prevent ingress of insects, storage insects can tolerate this pressure. For mortality of storage insects, low pressures below 100 mm Hg are required.

Gas tight flexible structures using the hermetic storage method have been developed and are in use on an industrial scale (Navarro *et al.,* 1988; 1994; Navarro *et al.,* 1990; Silberstein *et al.,* 1998). These structures consist of plastic chambers with manufacturers specifications to a gas tightness level that will enable treatment without significant modified atmosphere or fumigant gas loss and within exposure times of no longer than 24 hours (Navarro *et al.,* 1995). They are termed ”Volcani Cube™” or ”GrainPro Cocoon®” (Navarro *et al.,* 1999) and have potential for use in small-scale applications, particularly for high-value crops such as cocoa, coffee, and spices. The use of these flexible storage facilities to maintain low pressures was reported in two recent works (Phillips *et al*., 2000; Navarro *et al*., 2001). In these structures, low pressures of 25-30 mmHg were achieved and cacao beans were preserved for over two months.

The objective of this paper was to report on the effects of exposure time and treatment temperature on mortality of different life stages of stored product insect pests exposed to elevated temperatures and a CO_2 enriched atmosphere or under a constant low pressure.

Materials and methods

Temperature, CO_2, and low-pressure combinations

For CO_2 treatments, concentrations varying from 60% to 90% of CO_2 in air at temperatures varying from 30° to 45°C were tested. For low pressure treatments, absolute pressures of 25, 50 and 100 mm Hg at temperatures varying from 18° to 35ºC were tested..

Test insects

Diapausing larvae of Khapra beetle *(Trogoderma granarium)* were obtained by removing active larvae from cultures and placing them in groups of several hundred without food for one month at 28ºC (Lindgren and Vincent, 1960). Adults of *Oryzaephilus surinamensis, E. cautella* and *Lasioderma serricorne* were taken from laboratory cultures maintained at the Department of Stored Products, Volcani Center, Agricultural Research Organization, Bet Dagan, and mass reared on standard artificial diet. Eggs, pupae and adults (1-2 days old) and larvae (4-15 days old) were taken from the same batch.

Eggs of tested species were used within 0-2 days of oviposition. The eggs were obtained by placing 500-1000 adults beetles in 500 g of wheat flour containing 5 g of brewers' yeasts. To obtain eggs from moths, *E. cautella* were placed on a mesh covered inverted jar, overnight and the females laid the eggs in a Petri dish. Two Perspex slides each with 50-drilled "wells" were used to individually place 100 eggs from each of the studied species. The slides were then covered with a cover glass to retain the eggs (Navarro and Gonen, 1970).

Exposure of insects to low pressures

For all treatments, the r.h. in the exposure chamber consisting of vacuum flasks of 0.5-L was maintained at above 70% using a wad of folded filter paper imbibed with a saturated solution of sodium nitrite. Exposure temperatures were 25, 30 and 35ºC. For exposure, sets of 50 insects were confined in cages of 15-mm diameter and 50 mm length made of 100 mesh stainless steel.

Post fumigation procedures

Following treatment, larvae, pupae and adults were transferred to small jars (50 ml) and maintained at 28±1°C and 65±5% R.H. The larvae were provided with food. The eggs were transferred to watch glasses and incubated under the same conditions as the other developmental stages. Mortality counts for larvae were carried out after two weeks of exposure; for pupae after one week, for adults after one day, and for eggs after 4-5 days of exposure. Mortality for larvae was based on those that failed to pupate, for pupae, those that failed to emerge as adults, for adults, those that were dead or moribund, and for eggs, those that failed completely to hatch.

Statistical analysis

To determine the lethal time to obtain 99% mortality (LT_{99}) data were subjected to probit analysis (Daum, 1979). Results in this paper are presented without detailed statistical analysis to show the ranges of exposure times needed to control the test insects.

Results and discussion

Effects of CO_2 and temperature

Table 1 shows the influence of CO_2 concentrations at different temperatures as expressed in LT_{99} mortality values for diapausing larvae of *T. granarium*. At 45^{o}C, increasing the CO_2 concentration to 90% the LT_{99} value decreased to 10 h, whereas at 35ºC the LT_{99} value was 29 h.

T. granarium is one of the most serious pests of stored cereal grains and oil seeds, and is subject to strict quarantine regulations in the US, Australia and several other countries. It is a member of the dermestid family and is a voracious feeder of grain products. The larvae can hide in cracks of the storage structure in a state of facultative diapause and can remain in this condition for years. It is particularly difficult to control with insecticides. Consequently, many quarantine treatments are mandatory when products such as rugs, spices and cereal products are imported from infested countries. In such situations, MB is still the only effective

fumigant against this pest. Present distribution of *T. granarium* includes Western Africa through the Northern Indian subcontinent (Cuperus *et. al.,* 1992). Results shown in Table 1 may serve as guidelines to the possibility of applying slightly elevated temperatures for the control of the most resistant diapausing larvae of *T. granarium.*

Table 1. Influence of CO_2 concentrations expressed in LT_{99} (hours to obtain 99% mortality) values for *Trogoderma granarium* diapausing larvae at three different temperatures.

Temperature (^oC)	CO_2 concentration (%)			
	60	**70**	**80**	**90**
35	38	29	–	29
40	24	28	20	–
45	15	17	15	10

A similar approach of applying various CO_2 concentrations at different temperatures was investigated for four developmental stages of *E. cautella*. Results in Table 2 summarize the effectiveness of the combination of CO_2 at temperatures in the range of 35ºC to 45ºC. Tests with *Ephestia cautella* showed that the pupa was the most resistant stage when exposed to 90% CO_2 with an LT_{99} value of 17 h at 35ºC, and only 3 h when exposed at 45ºC. The adult was the most sensitive stage of E. cautella requiring only 4 h of exposure to 90% CO_2 at 35ºC.

Table 2. Influence of CO_2 concentrations expressed in LT_{99} (hours to obtain 99% mortality) values for *Ephestia cautella* various development stages exposed to CO_2 concentrations in air at three different temperatures.

Temp. (°C)	**35**				**40**				**45**			
CO_2 (%)	**60**	**70**	**80**	**90**	**60**	**70**	**80**	**90**	**60**	**70**	**80**	**90**
Eggs	23	23	17	9	16	12	8	5	9	5	3	2
Larvae	60	27	20	12	17	9	6	6	5	4	2	2
Pupae	56	37	17	17	36	10	8	4	7	4	4	3
Adults	20	14	6	4	6	5	3	2	3	2	2	2

Results on the influence of various CO_2 concentrations at different temperatures on *O. surinamensis* development stages are shown in Table 3. For this species as well, increasing the CO_2 concentration resulted in decreasing the LT_{99} value. Generally, the eggs were the most resistant stage; at 40ºC and 90% CO_2 a six h exposure was required for an LT_{99} value.

Effects of low pressures and temperature

Table 4 shows partial results obtained on three developmental stages of *L. serricorne* exposed to low absolute pressures of 25, 50 and 100 mmHg at 18^0, 25^0, and 30^0C. Although the LT_{99} value for *L. serricorne* adults exposed to 25 mm Hg at 30ºC was 15 h, there is an apparent resistance of this species to low pressures. For example, eggs exposed to 25 mmHg even at 30^0C needed 75 h exposure to attain LT_{99} value. Bare (1948) also observed greater tolerance of *L. serricorne* eggs compared with other stages exposed to low pressure. More work is required to reveal the resistance of this species to low pressures.

Table 3. Influence of CO_2 concentrations expressed in LT_{99} (hours to obtain 99% mortality) values for *Oryzaephilus surinamensis* various development stages exposed to CO_2 concentrations in air at three different temperatures.

Temp. (°C)	Life Stage	CO_2 concentrations (%)			
		60	**70**	**80**	**90**
30	Eggs	–	–	38	22
	Adults	21		22	9
35	Eggs	29	25	21	9
	Adults	26	11	8	4
40	Eggs	15	7	6	6
	Larvae	8		2	2
	Pupae				5
	Adults	12	11	6	3

Table 4. Effects of temperature and low-pressures on LT_{99} (hours to obtain 99% mortality) of *Lasioderma serricorne* various development stages.

Pressure (mmHg)	Temp.(ºC)	Eggs	Larvae	Adults
25	**18**	–	–	47
	25	–		26
	30	75		15
50	**18**	–		157
	25	–	191	43
	30	–	49	15
100	**18**	136	–	–
	25	75	–	75
	30	40	–	–

The influence of low pressures at different exposure times and temperatures as expressed in LT_{99} mortality values for diapausing larvae of *Trogoderma granarium* is shown in Table 5. When the pressure was decreased to 25 mm Hg and the temperature raised to 35°C, the LT_{99} value was 146 h; at 30°C under the same pressure, it was 172 h. These lengthy exposures are comparable with 6 and 7-day exposures required for phosphine fumigation (Navarro and Donahaye, 1990). These findings may also be compared to those of Calderon and Navarro (1968), on non-diapausing larvae at 25ºC and 65% r.h., where complete mortality was obtained within 120 h exposure to 20 mm Hg. Clearly, not all fumigation treatments are addressed to the control of *T. granarium*. Many commodities may be infested with species less resistant than *T. granarium*.

Rigid metal chambers have been in use for the implementation of vacuum fumigation in agricultural commodities (Bond, 1984). A recently developed technology based on high-pressure CO_2 treatments also makes use of rigid chambers (Adler *et al*, 2000). These

structures are expensive and lack of transportability, which restricts the implementation of environmentally sound methods. Based on the encouraging data herein obtained, in order to render the technology a practical tool, the possibility was recently investigated of using CO_2 or low pressures to control storage insects in a transportable system (Phillips *et al*., 2000). The transportable system was made of flexible PVC, which has been in use commercially for hermetic storage of grain and other commodities to control insect disinfestation by naturally obtained modified atmospheres (Navarro *et al.,* 1999). Experiments were carried out using a 15-m^3 capacity plastic container termed the "Volcani Cube™" or "GrainPro Cocoon®". The pressure was maintained between 25 to 29 mm Hg for 17 days. Bioassay in field trials demonstrated that complete mortality of test insects composed of mixed ages of *E. cautella Plodia interpunctella*, and larvae of *T. castaneum* was observed following the 3-days exposure to vacuum (Phillips et al. 2000). For the disinfestation of durable commodities, these flexible storage containers can be considered as an alternative to treatments with methyl bromide and other toxic fumigants.

Table 5. The influence of reduced pressures on exposure time expressed in LT_{99} (hours to obtain 99% mortality) values for *Trogoderma granarium* diapause larvae at three different temperatures.

Temperature (°C)	Pressures in mmHg		
	25	**50**	**100**
25	>360	>360	>360
30	172	260	>360
35	146	153	>360

Acknowledgements

We thank Dr. S. Angel, and Dr. P. Villers, president of GrainPro Inc. for reading the manuscript and making useful suggestions. This work was funded in part by a grant from the United States-Israel Science and Technology Foundation, Israel Agricultural Research Organization Project No. 5288.

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Control of *Sitophilus oryzae* (L.) and *Oryzaephilus surinamensis* (L.) in rice by CO_2 under increased pressure

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Abstract: Cereals have an important nutritional value and they are one of the principal alimentary resources worldwide, among them rice has a remarkable position. During storage, products may be infested by microorganisms and/or arthropods causing deterioration of the grain. This makes a treatment necessary for their control. Carbon dioxide (CO_2) is an alternative treatment as effective as conventional chemical control but minimizing the contaminant effects. *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is responsible for most of the insect-related damage of stored rice. This insect is called primary insect pest, or internal feeder, because the adults attack whole kernels and larvae feed and develop entirely within the kernels. *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) is a secondary pest that feeds on damaged kernels. The aim of this paper is to establish the conditions and efficacy of control of *S. oryzae* and *O. surinamensis* in contaminated stored rice using CO2 under increased pressure.

Key words: *Sitophilus oryzae; Oryzaephilus surinamensis*; pest control; stored product; rice; CO_2; high pressure.

Introduction

From harvest to consumption, cereals go through different stages: crop, threshing and ventilating, drying, storage, preliminary methods of processing and final processing. When these stages are carried out in the wrong way, cereals can be attacked by microorganisms and insects. Insects can attack the product in the field and accompain it through the different stages of processing, or they can reach it during processing, or even when the final product is stored. Storage conditions contribute to the deterioration of the grains since high temperatures and humidity favour the growth of fungi and offer favourable conditions for the growth of insects. These insects favour the development of other insects and microorganisms which degrade the product. This is an important risk for human health and cause serious economic losses.

For all the above considerations, disinsectation and disinfection of the food during or after processing is necessary. The use of chemical products, now a quick and effective usual practice, has become a problem owing to its toxicity and the possible residues, which added to the growing concern about environmental problems and led to the search of alternative methods equally effective which guarantee the quality of the products.

Stahl *et al*. (1985 a, b) described a new process using CO_2 under high pressure for pest control. Supercritical CO_2 had already been used to extract different compounds in the alimentary industry and nowadays has numerous applications. Its lethal effect on insects

seems to be due, on the one hand, physiological stress on their cells (owing to the quick pressure build up and subsequent decrease of the pressure) since as a result of the lower viscosities of the SC-CO_2, this invades the inner part of the cells giving rise, in the phase of depressurisation, to the split of the cells causing the death of insects and microorganisms. On the other hand, the lethal effect could be due to the lack of O_2 in the environment becoming anaerobic, and also to the changes in the pH of cells caused by absorption of CO_2 (Ulrichs *et al*., 1996).

According to follow up studies, the quality of the treated products was not affected detrimentally. Furthermore, using CO_2 under high pressure has the added practical advantage of requiring extremely short lethal exposure times, ranging from minutes to only few hours (Reichmuth and Wohlgemuth, 1994; Prozell and Reichmuth, 1996). The CO_2 also seems to have a beneficial secondary effect since upon displacing the O_2 present in the pores of the food to treat, it preserves them during longer time from undesirable oxidation reaction.

Material and method

All experiments were conducted with the SFC-500 (AINIA) equipment with a 300 ml of useful volume capacity able to reach a pressure up to 300 bar.

Experiments were undertaken with adults and eggs of *Sitophilus oryzae* (L.) (Coleoptera, Curculionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae). Both species originated from samples of contaminated rice and were reared in a climatic chamber at 26.5ºC and 60% RH.

The product chosen was white rice, variety "bahía". For each trial the size of the sample was 200 g. The methodology of infestation for the two species was the same one: Each sample of 200 g of rice was infested with 50 adults, kept 5 days in the chamber to allow oviposition before exposing it to the treatment with CO_2. We will evaluate the efficacy of the processing upon adults and eggs of each of the species.

A large number of tests have been made for each species. They consisted of three repetitions of 36 tests each, combining pressures of 1, 25, 60 and 100 bar; temperatures of 20, 40 and 60ºC and times of 5, 30 and 60 min., and control samples (Table 1).

Table 1. Tests with *Sitophilus oryzae* and *Oryzaephilus surinamensis*

T (ºC)					
20		40		60	
P (bar)	t (min)	P (bar)	t (min)	P (bar)	t (min)
	5		5		5
1	30	1	30	1	30
	60		60		60
	5		5		5
25	30	25	30	25	30
	60		60		60
	5		5		5
60	30	60	30	60	30
	60		60		60
	5		5		5
100	30	100	30	100	30
	60		60		60

Results and discussion

The efficacy of the treatments of adults and eggs of the two species is established from the results obtained (Table 2 and 3) through an analysis of variance (ANOVA) with an unique variable of classification and with a significance level of 95%.

Table 2. Results of the preliminary tests with *S. oryzae.*

T ºC	P (bar)	t (min)	% M	nº h	T ºC	P (bar)	t (min)	% M	nº h	T ºC	P (bar)	t (min)	% M	nº h
			0	283				6	56				4	175
		5	2	171			5	4	238			5	4	248
			0	189				0	138				0	262
			2	396				4	259				16	39
	1	30	6	224	40	1	30	8	125	60	1	30	2	150
			0	152				0	144				13	89
20			4	256				100	18				0	103
		60	8	248		25	5	100	18				0	250
			0	186				100	21				0	262
			100	111				0	184				0	194
	25	5	100	143				0	292		Con-trol		0	163
			100	41		Con-trol		0	108				0	279
			0	180				0	123				0	93
	Con-trol		0	194				0	110				0	195
			0	277				0	177				0	157

Other combinations of the parameters tested 100% mortality for adult and eggs
(% M= % adult mortality; nº h= survival eggs)

The treatment is effective for adults of *Sitophilus oryzae* and *Oryzaephilus surinamensis* at 60ºC, 1 bar and 60 minutes, as well as 25 bar and 5 minutes for the three temperatures tested.

At 20 and 40ºC, 25 bar and 30 minutes, 100% mortality of adults and eggs of *S. oryzae* was obtained as well as at 60ºC, 1 bar and 60 minutes. With these results the conditions were limited and new series of tests are proposed.

The possible contamination of the first series of tests made with *O. surinamensis* mase a repetition necessary.

Ulrichs (1994), and Nakakita and Kawashima (1994) indicated the importance of the decompression speed on mortality. Owing to the need for evaluation of this parameter, new research is carried forward at present. Moreover, it´s necessary to make quality studies of the rice treated and make new experiments with real samples at great scale to evaluate the possible industrial application of the method.

Table 3. Results of the tests with *O. surinamensis*

T ºC	P (bar)	t (min)	% M	nº h	T ºC	P (bar)	t (min)	% M	nº h	T ºC	P (bar)	t (min)	% M	nº h
			4	46				0	41				0	73
		5	8	27			5	0	27			5	0	72
			6	36				1	76				0	94
			0	38				0	30				0	12
	1	30	18	34		1	30	4	34		1	30	0	64
			4	47				2	173				0	1
			0	3				0	60				100	1
		60	6	34			60	34	59			60	100	0
			0	68				28	69				100	0
			100	12				100	9				100	2
		30	100	9			5	100	13			30	100	5
	25		100	0				100	0		25		100	0
			100	4				100	1				100	2
		60	100	6		25	30	100	28			60	100	5
			100	0				100	0				100	0
			100	10				100	1				100	1
		5	100	7			60	100	0		60	30	100	1
20			100	0	40			100	0	60			100	0
			100	5				100	0				100	5
	60	30	100	3			5	100	0			5	100	1
			100	0				100	0		100		100	0
			100	1				100	0				100	0
		60	100	7		60	30	100	33			30	100	0
			100	0				100	0				100	0
			100	9				100	3				0	52
		5	100	0			60	100	3				0	30
			100	0				100	0				0	41
	100		100	24				100	9				0	61
		30	100	2		100	30	100	8		Con-trol		0	11
			100	0				100	0				0	30
			0	11				0	17				0	57
	Con-trol		0	38		Con-trol		0	40				0	17
			0	24				0	28				0	37
			0	17				0	106				0	60

Other combinations of the parameters tested 100% mortality for adults and eggs
(% M= % adult mortality; nº h= survival eggs)

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INTERT DUSTS AND SYNTHETIC INSECTICIDES

Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
221-229

Integrated pest management for stored grain in the U.K. incorporating diatomaceous earths to prevent surface infestations of insects and mites.

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Abstract: Laboratory work recommended a dose of 3 g/kg diatomaceous earth (DE) as a top-dressing for prophylactic control of invertebrate pests, as part of a UK integrated grain storage strategy, based on cooling. Both this and a reduced dose of 1 g/kg were compared under field conditions. Two 20t bins of feed wheat were top-dressed with 3 g/kg "Protect-it", two at 1 g/kg and two left untreated. The grain had initial moisture contents (MC) of 15-16% and had been infested with 1.2 insects /kg (*Oryzaephilus surinamensis* and *Sitophilus granarius*) and 3 mites /kg (*Acarus siro* and *Lepidoglyphus destructor*). The bins were cooled from 20-28°C to 5°C by winter using 6°C differential fan control. Mite numbers at the surface peaked after 12 weeks, coinciding with surface MCs of 18-19% when numbers of *A. siro* in the untreated bins were 2,600 and 19,000 / kg compared to less than 1 / kg in all the treated bins. Numbers of insects in the surface traps fell in all bins throughout the experiment but after 17 weeks both species were reduced by >90% for both doses compared to the untreated bins. These results validate the use of DEs as part of an IPM strategy and indicate that the lower dose could be commercially effective.

Key words: Diatomaceous earth, Inert dust, Grain storage, Integrated Pest Management, *Acarus siro*, *Lepidoglyphus destructor*, *Sitophilus granarius, Oryzaephilus surinamensis*

Introduction

Research has demonstrated the effectiveness of cooling alone in the British climate, to prevent the development of pests and to remedy outbreaks in stored grain (Burrell, 1967). However, there are also shortcomings of such a non-chemical method. The surface fluctuates in temperature sufficiently with ambient to permit the survival of insect populations (Armitage and Llewellin, 1987) and in storage for 2 seasons, the breeding of certain species (Armitage *et al.*, 1994). The grain surface also picks up atmospheric water in the winter and may permit the development of mite populations (Armitage, 1984).

A UK Integrated Pest Management (IPM) Strategy for stored grain, incorporating physical control with pest monitoring and surface treatment, has solved some of these problems (Armitage *et al.*, 1994). Formerly, a top-dressing of a conventional organophosphorus based (OP) pesticide was sufficient to remedy the shortcomings of cooling alone but reliance on OPs is unwise, as their market life at present seems limited. Some candidate replacements which have received a lot of attention around the world are inert or silicaceous dusts. They have the advantage that they are naturally occurring and harmless to ingest but they are less effective at high humidities, require high doses that inhibit grain flow and are a respiratory nuisance (Golob, 1997). Diatomaceous earths (DEs) have been registered as a grain protectant for treating grain and storage structures in Australia, Canada, China, Croatia, Denmark, Germany and USA and in the UK are marketed for treatment against poultry mite and domestic pests.

Laboratory work on a 50 g scale, indicated that the Australian DE, 'Dryacide' was more effective against mites at temperatures as low as 10°C but that high doses of 3-5 g/kg were required to control all species at moisture contents (MCs) close to 17% which is likely to be the MC of surface grain in the UK during the winter (Cook et al., 1999). There were only minor differences in the efficacy of different DEs towards mites (Armitage et al., 1999). Against stored product insects, mortality was less at 10°C compared to 25°C with the DE 'Protect-it' (Collins *et al.*, 2001). This was not unexpected, with reduced insect activity probably resulting in decreased dust pick-up. Work on 20 kg bins of grain simulating bulk surface conditions, indicated that at 15°C, 3 g/kg required 5 weeks for total mite suppression and 13 weeks to control adults of the most DE tolerant insect, *Sitophilus granarius* (Cook and Armitage, 2000).

The experiments described here were the first practical-scale experiments to be carried out in the UK, which successfully showed the effectiveness of integrating a top-dressing of DE in conjunction with cooling to suppress both mite and insect populations. Pairs of 20 t open-topped bins were treated at 3 g/kg, the recommended UK dose from laboratory studies and a "fractional" dose of 1 g/kg, which is the recommended dose in warmer/dryer countries such as Australia.

Materials and method

The experiment compared mite and insect numbers in six cooled 3 m deep bins of feed wheat at about 15% MC. Two of the silos were top-dressed with diatomaceous earth at 3 g/kg, raked in to 0.3 m, two at 1 g/kg and two left untreated to act as controls. The inert dust was raked in to a depth of 0.3 m.

Grain

Each bin contained 20 t of feed wheat from the UK 1999 harvest at 20-30°C and 15-15.5% MC on intake. It is important to note that safe storage of grain is normally based on storing at MCs lower than 15% to prevent mites infesting the bulk and that the MCs here were deliberately high to allow seeded populations to establish for the purpose of the experiment.

Introduced infestation

Two weeks before treatment, initial populations of 1.2 /kg of the insects *S. granarius*, *Oryzaephilus surinamensis* and 3 /kg of the mites *A. siro and L. destructor* were achieved by introduction into each bin at 3 depths and 9 columns via a narrow-bore plastic pipe, emptied of grain by vacuum.

Treatment

The bins were treated with 'Protect-it', a dust based on DE of fresh-water origin. Four of the six bins were treated, two at 3 g/kg and two at 1 g/kg. The bin surface area was $9m^2$. Doses were calculated based on 600 g/m^2 = 3 g/kg. Sufficient inert dust was weighed into plastic bags and sprinkled on to the surface of the treated replicates. This was then raked in to a depth of 0.3 m.

Aeration

The grain was cooled using low-volume fans at 9 $m^3/h/t \pm 1.5$, switched on and off by a differential thermostat set at 6°C. Cooling commenced immediately after treatment. The airflows were measured with a hot-wire anemometer.

Temperatures
Temperatures were measured in each bin by a central row of thermocouples at the surface, 1m, and 2 m attached to squirrel data loggers.

Insect populations
Each month, 9 Pitfall Cone (PC) traps (Cogan and Pinniger, 1989) were placed equidistantly in a 3 x 3 grid at the surface of each bin and 5 probe traps inserted at each of 1m and 2m depth in the centre and corner positions. These were left in place for a week before being withdrawn and the contents recorded.

Spear samples
Each month, five samples were withdrawn from each bin at the surface, 1m and 2m corresponding to the centre and corner trap positions. Mite numbers were estimated by sieving through 1.7mm mesh test sieves and examining the dust under a binocular microscope. Where mite numbers were very high, a disc divided into areas was used (Solomon, 1962). The MC of the samples was determined by BS4317, grinding 5 grammes of grain, drying in a ventilated oven at 130°C for 2 hours and comparing dry and wet weight.

Data analysis
Comparisons were made using ANOVA at the 95% confidence limit. Equilibrium relative humidities (ERH) were calculated from temperature and MC using *Grain Pest Adviser* decision support software (Denne, 1988), available as *Integrated Grain Storage Manager* (*IGSM*) (© Central Science Laboratory & Imperial College) and were based on the work of Pixton and Warburton (1972;1982). To determine population inhibition, control numbers from both bins were pooled and the mean calculated, unless otherwise stated. Mean percentage inhibition for each treatment was calculated as a proportion of the mean control, expressed as:

$$100 - \left[\frac{\text{mean number of live mites per treatment x 100}}{\text{mean number of live control mites}}\right] = \%\ \text{inhibition}$$

Results and discussion

Physical conditions
The fans were switched on immediately after treatment at week 0. Mean bulk temperatures for all 6 bins followed a typical cooling pattern for automatically controlled fans as recommended by Armitage *et al.* (1993), cooling to 15°C within 2 weeks to prevent *O. surinamensis* breeding, to below 10°C within a further 2 months to prevent *S. granarius* and to below 5°C to prevent mites and to kill insects (Figure 1.) Surface temperatures followed the same pattern as ambient, with surface warming occurring during the Spring. There was no significant difference between surface temperatures for all bins when compared over time ($p=0.987$).

Mean bulk MCs were around 15% throughout the experiment (Figure 2.). The surface steadily adsorbed moisture and had already risen to 17% (ERH 78%) after only 4 weeks. The surface MC peaked at 19% (ERH 81%) after 12 weeks and then gradually desorbed for the remainder of the experiment. There was no significant difference between surface MCs for all bins when compared over time ($p=0.821$).

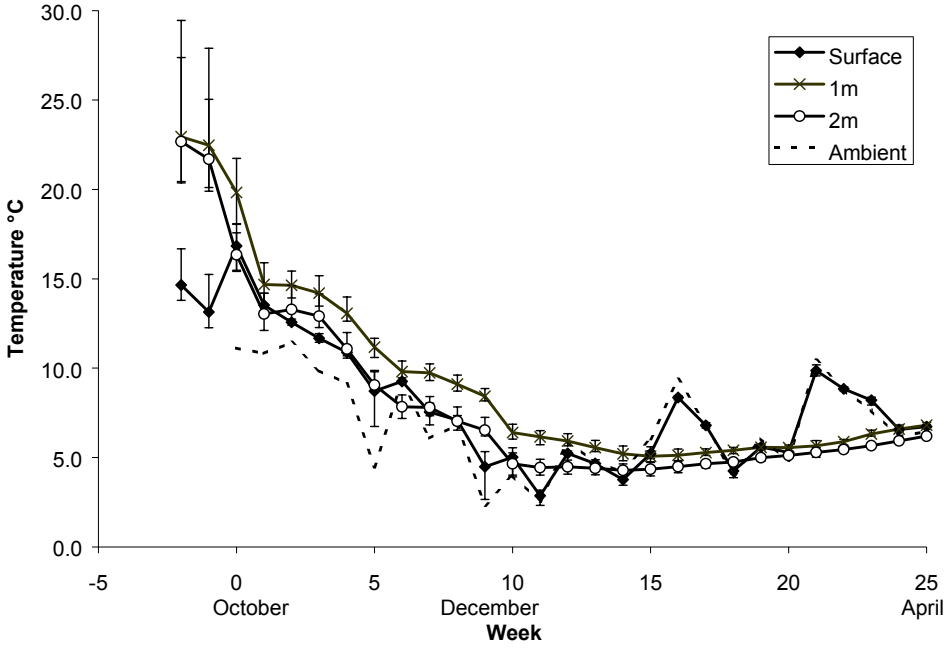

Fig. 1. Mean temperature of treated and untreated 20 t bins of wheat at 3 depths (+/-range).

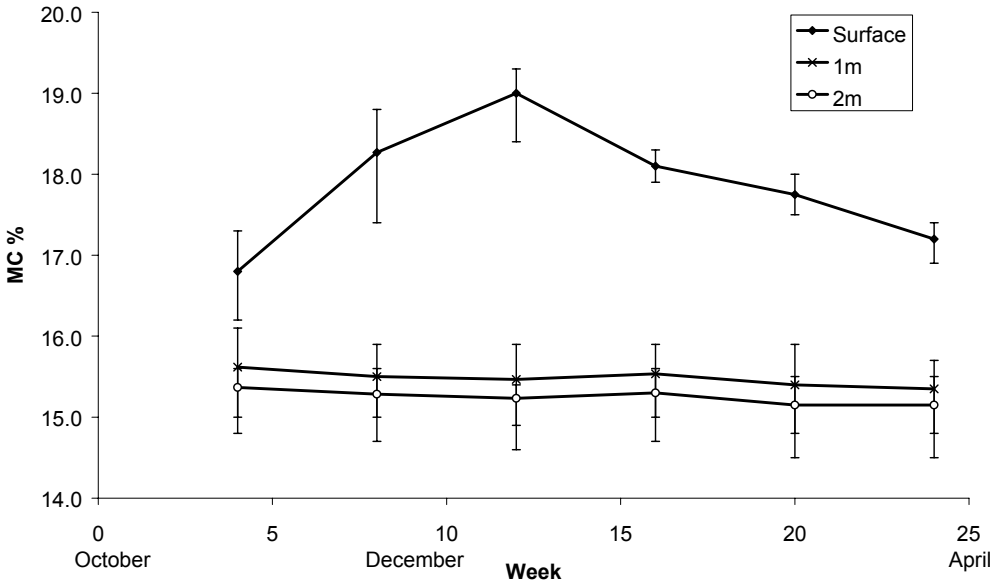

Fig. 2. Mean percentage moisture content of treated and untreated 20 t bins of wheat at 3 depths (+/-range).

Mites

Surface numbers of *A. siro* in the untreated bins responded to the changes in moisture, peaking at 19273 (bin a) & 2616 (bin b) /kg on week 12 and then gently declining to 3261 (a) & 962 (b) by the end of the experiment (Figure 3.). The larger dose of 3 g/kg achieved 100%

control after only 4 weeks, agreeing with previous laboratory work. The lower dose of 1 g/kg was also highly effective, giving 100% control in one of the bins after 16 weeks and having inhibited the population of the other by 99.9% compared to the least vigorous control by the end.

A. siro numbers within the bulk followed a similar trend. However, control numbers peaked at a fraction of those at the surface (<5%). One reason for this was because bulk temperatures decreased to and then remained below the breeding threshold. The other reason was that ERH's declined to just above the breeding threshold, also slowing the rate of development. Survivors were found within the bulk for all treated bins, which was not unexpected, since the DE would not have percolated down to any great depth.

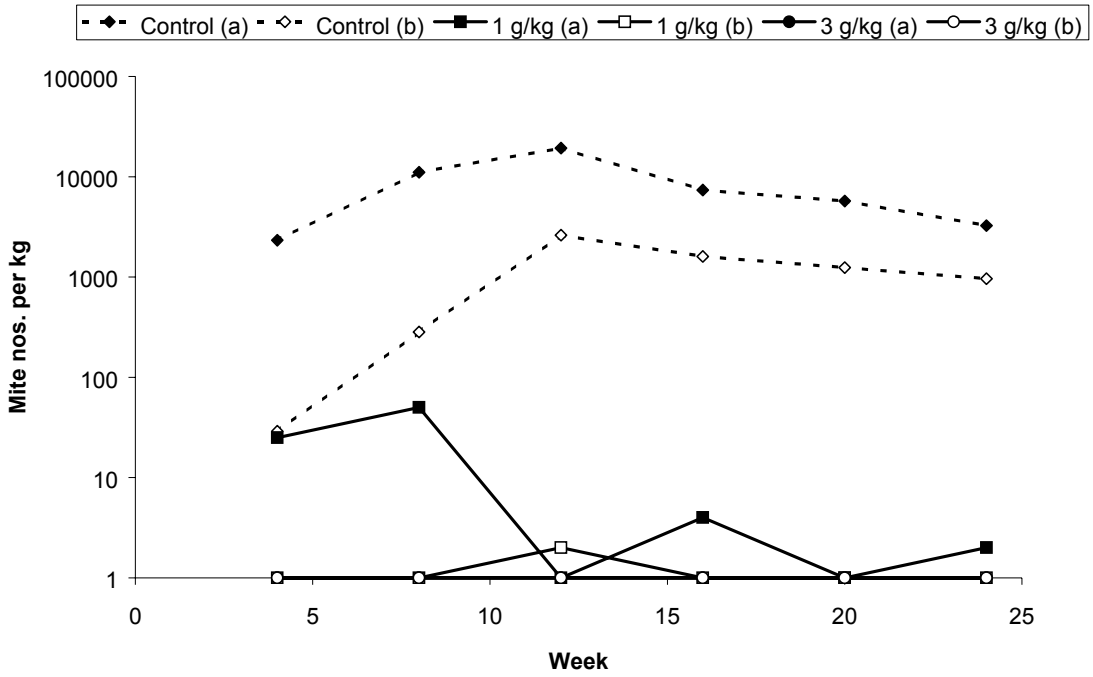

Fig. 3. Surface numbers per kg of *Acarus siro* in 20 t bins of wheat after surface treatment with DE at 0, 1 and 3 g/kg (log scale).

The slower breeding *L. destructor* peaked at 944 per kg after 8 weeks in control bin (a) and 475 per kg after 16 weeks in bin (b). Populations were at 144 and 196 /kg respectively at the end of the trial. Again complete control was achieved after 4 weeks at 3 g/kg, although a slight resurgence occurred at week 12 in one of the bins. Complete control was observed for both doses by the end of the experiment. Bulk numbers failed to exceed 137 /kg and again a similar pattern was observed as *A. siro*.

Naturally occurring predatory mites, *Cheyletus eruditus* were found in all bins (Figure 4.). The higher number found in control bin (b) may have accounted for the lower number of astigmatid mites. It is uncertain whether the decline in the treated bins was an effect of the DE or a response to decline in prey. The latter is more likely and it is interesting that a few of these predators were still present in one of the lower dosed bins, with 92% inhibition compared to the least vigorous control.

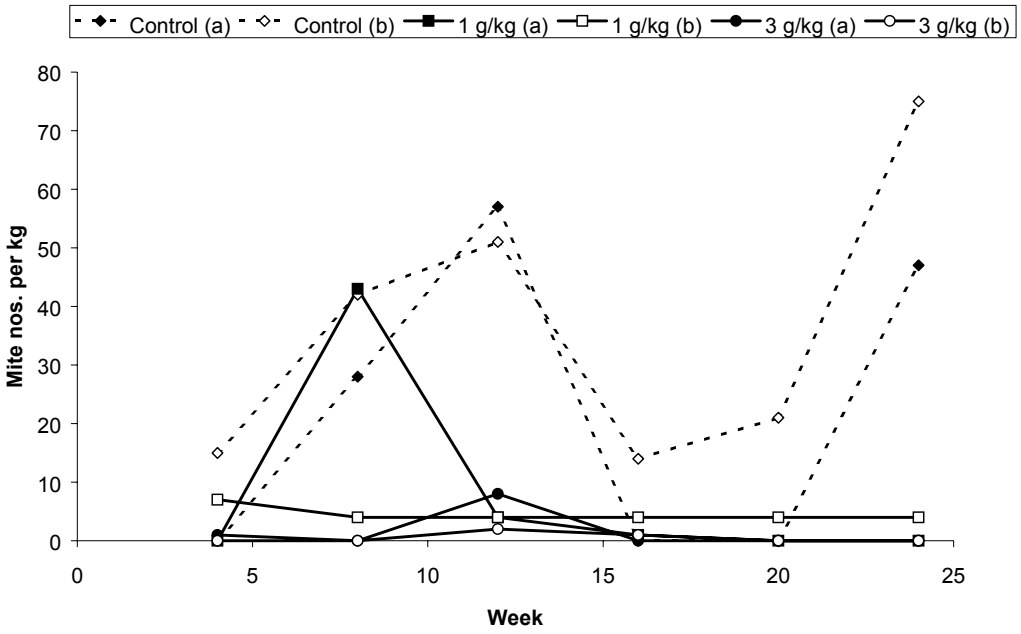

Fig. 4. Surface numbers per kg of *Chyletus eruditus* in 20 t bins of wheat after surface treatment with DE at 0, 1 and 3 g/kg.

C. eruditus tolerates most pesticides applied at the recommended rate and is more tolerant to low humidities than the pest species, so often predominates in the spring, when the ambient RH is relatively low. Although not a pest, this species can be cause for market rejection in the UK. Preliminary laboratory tests with DE against this species has shown it to be tolerant of the doses used here (Fejt, Pers. comm.) but these field results indicate DE to be effective under practical conditions.

Insects

Mean surface numbers of *S. granarius* trapped in the untreated bins peaked at 33 in 9 traps, after cooling had begun (Figure 5.). This was accompanied by a decline at 1 m depth. Since there was no apparent temperature gradient for them to follow, vertical migration (Surtees, 1963) was probably due to either, movement towards the damp upper layer (Howe, 1951), or more likely from disturbance by grain vibration during aeration (Armitage, Day and Lewis, 1983). From week 4, control numbers gradually declined as temperatures rapidly fell below the breeding threshold of 12°C (Eastham and Segrove, 1947). Likewise, numbers in the treated bins also declined and after 17 weeks inhibition was >90% for all doses.

A similar trend was observed for *O. surinamensis*, although surface numbers in the untreated bins peaked at between 10 and 15 weeks, coinciding with a gradual rise in surface temperature, where fluctuations caused by day and night differences became more pronounced. Temperatures were below the developmental threshold of 17°C (Howe, 1956) indicating upwards migration rather than reproduction. It has also been found (Surtees, 1963) that this species shows an increase in activity as temperatures decrease from 25°C to below 20°C. Thus, activity would be expected to increase as aeration produced lower grain temperatures. Again, numbers trapped decreased thereafter, with only a few trapped in one of the control bins at the end of the experiment. Population inhibition followed a similar pattern to *S. granarius* with none present in any of the treated bins by the end of the trial.

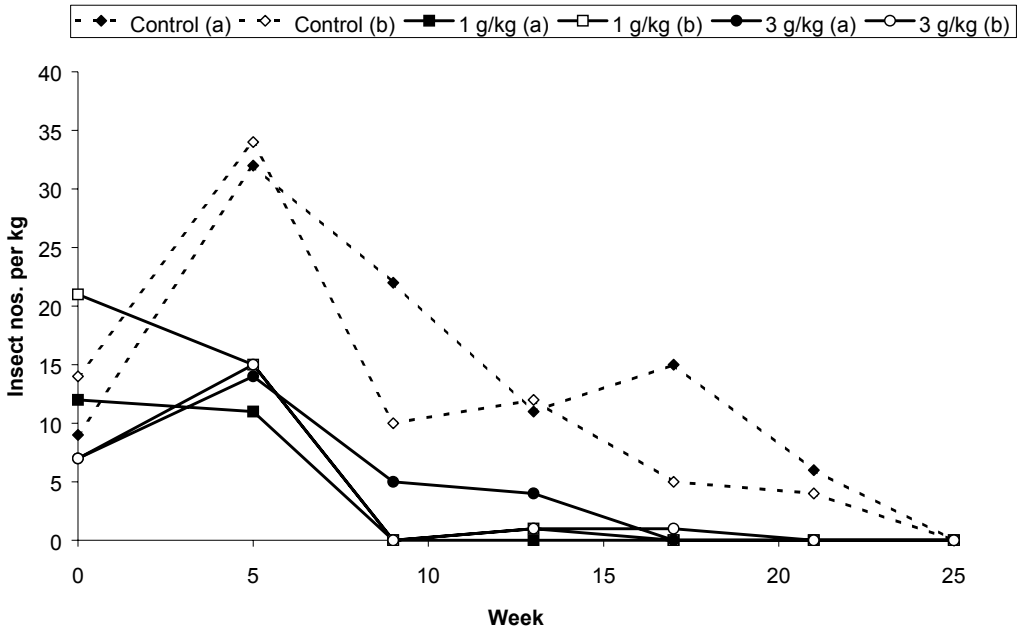

Fig. 5. Surface numbers per kg of *Sitophilus granarius* in 20 t bins of wheat after surface treatment with DE at 0, 1 and 3 g/kg (log scale).

Naturally occurring psocids were also trapped. These were a mixture of *Lepinotus patruelis* and *Lachesilla pedicularia*. After 5 weeks at the surface as many as 494 were recorded in 9 traps, for one of the untreated bins. By the end of the trial, a mean of 16 per untreated bin were still present. No psocids were found in any of the treated bins after 12 weeks. Although these are not primary pests in the UK, increasing worries about allergenic invertebrate contamination (Chambers and Pearson, 1999) could change their pest status in the future.

A recommended dose for top-dressing DE in the UK

The results reported on here, under conditions of high MC and infestation rates designed to challenge the strategy, validate the use of DE's as part of an IPM strategy for stored grain. Previous laboratory work had recommended a dose of 3 g/kg for the UK, three times that used for bulk treatments in dryer countries such as Australia. However, it would be preferable to reduce this dose to minimise the negative effect on bulk density and associated problems with conveying etc. (Jackson and Webley, 1994). These risks are partly minimised because when used as part of an IPM strategy, the dust only needs to be applied to the surface if integrated with cooling. Korunic and Mackay (2000) found that for Hard Red Spring wheat, DE treatment at 7.5 and 5 g/kg, reduced the bulk density by 4.9 and 5.19 kg/hl respectively. Laboratory tests showed that at these doses no more than 10 or 20% of the total grain mass should be treated in order to minimise bulk density reduction on out-loading. However, in practice it has been observed by the authors that the top layer does not mix so uniformly. Nevertheless, in this farm-scale trial, only 10% of the grain mass was treated, so both doses of 1 and 3 g/kg were within these simulated parameters.

In this trial, the higher dose was more efficacious than the lower treatment of 1 g/kg that in turn was more effective than expected. Recent findings that the inter-granular RH can be significantly lowered at the surface during periods of aeration (Dunn, 2001; Cook

unpublished.) may have augmented the effect of the DE. It should also be borne in mind that the surface treatment was calculated on the assumption that the top 0.3m would be treated with this dose. In practice, the raking in probably did not achieve such an even vertical distribution so the dose at the top of this surface layer may have been much higher.

Surface treatment in conjunction with cooling has been successfully used as an IPM strategy in Australia (Nickerson, *et al.*, 1994). Here a lower top-dressing of approximately 0.5 g/kg controlled the more tolerant *Sitophilus* sp. within 8 weeks, compared to 21 weeks at 1 g/kg in this UK trial. However, the former was at conditions of much lower MCs of 9-11% (25-40% ERH) where increased efficacy would be expected.

In summary, at 1 g/kg, insects were completely controlled and only a few *A. siro* (99.9% inhibition) and *C. eruditus* (92%) were present at the end of the trial. As stated, bulk MCs were untypically high allowing a few mites to survive in the bulk that would not normally be present. It is likely that the small number of survivors at the end could have been the result of re-infestation from below. In view of this, the lower dose of 1 g/kg could be commercially viable. Further validation using grain at the recommended lower MC of 14.5% would support this.

Acknowledgements

The authors would like to thank Hedley Technologies Inc. Canada for supplying the 'Protect-it', Alex Buckland for his assistance with the mite assessments and Brenden Llewellin for grain handling.

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Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
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Can diatomaceous earths have an integrated role in small-scale tropical grain storage?

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Abstract: During needs assessment studies in sub-Saharan Africa, farmers repeatedly prioritised the need for improved methods of storage pest control. Reduction of storage losses will help to reduce the vulnerability of small-scale producers by improving household food security and by improving income-generating opportunities. If synthetic chemical pesticides are available in rural areas they are often adulterated, past their expiry dates and expensive, many farmers are fearful of mixing these synthetic chemical pesticides with their food. Inert dusts particularly diatomaceous earths (DE) offer a safer alternative to synthetic chemicals, but information on their efficacy under small-scale farming conditions is limited. Field trials during two storage seasons in Zimbabwe demonstrated that DEs are extremely effective and persistent grain protectants against the major insect pests attacking sorghum, maize and cowpeas in storage. DE treatments outscored the existing grain protection practices during farmer-managed trials. However DE application rates as high as 2 g/kg were needed to reduce bostrichid beetle damage. Further studies in the laboratory found the combination of DEs and very low doses of pyrethroids resulted in high mortality of the bostrichid, *Prostephanus truncatus*. The effect of DEs on natural enemies and their combination with other biological and chemical control options is discussed.

Introduction

Farmers in sub-Saharan Africa lose a sizeable proportion of their grain to insect pests during storage, and consider this loss a serious livelihood constraint (Golob *et al*., 1996; Marsland & Golob, 1996; Donaldson *et al*., 1996). Insect damage threatens not only the household's control over the timing and scale of its grain sales, but also the family's actual food security. This damage affects the nutritional quality, taste, smell and quantity of food available until the next harvest. Farmers have expressed a desire to be able to maintain grain quality by reducing insect damage; they want a method that is not only relatively inexpensive, but is also safe, i.e. one that does not rely on the use of conventional synthetic organophosphate chemicals.

Currently, an acceptable method for protecting grain during storage against insect attack is to apply synthetic organophosphate insecticides. Most developing countries have to import the active ingredient needed to formulate the chemicals, using valuable foreign exchange. Nevertheless, these insecticides are frequently unavailable, adulterated or too expensive for farmers, and their misuse can be a health hazard. Although synthetic pesticides can work well, constraints regarding human and environmental safety and insect resistance, have led to increased international regulation that has reduced the number of 'safe' pesticides available for use. One solution to these problems is to introduce more sustainable methods of pest management through low-cost techniques that are more in tune with the needs of both the population and the environment. Inert dusts, particularly diatomaceous earths (DEs), offer a safer alternative to synthetic organophosphate insecticides for grain protection, but until

recently no information of their efficacy under tropical small-scale farming conditions existed (Golob, 1997). During on-farm trials in three agro-ecological regions of Zimbabwe two commercially available DEs (Protect-it™ and Dryacide®) were shown to be effective in controlling post-harvest insect pests damage in maize, sorghum and cowpeas stored for periods of > 8 months (Stathers *et al*., 2000). The DEs were as effective as the locally recommended insecticide, Actellic Super Dust, and outscored other local grain protection practices during farmers' evaluations (Stathers *et al*., 2000).

DEs exert their effect on insects through physical means. When insects come into contact with the DE particles, waxes are absorbed from the cuticle of the insect resulting in water loss, dehydration, and death (Ebeling, 1971). However, insect species differ in their sensitivity to the various DEs (Fields & Korunic, 2000). During the Zimbabwean field trials, high populations of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) developed in sorghum grain were treated with Dryacide® at doses of 1g/kg or less (Stathers *et al*., 2000). This paper reports on further investigations of the efficacy of DEs against bostrichid beetles including the devastating larger grain borer *Prostephanus truncatus* (Coleoptera: Bostrichidae) which is now endemic in many sub-Saharan African countries where it causes serious storage losses (up to 35% weight loss). Preliminary investigations into the efficacy of DEs combined with low doses of deltamethrin against *P. truncatus* and the survival of *Teretrius nigrescens* (Coleoptera: Histeridae) a known predator of *P. truncatus* in grain treated with DEs are also described.

Methods and materials

Insects

Trial insects *(P. truncatus*, *R. dominica and T. nigrescens)* were cultured under controlled conditions (CTH room at 27±5°C, 60±5% r.h., 12:12 light:dark). *P. truncatus* was reared on maize (*Zea mays*), *R. dominica* on sorghum (*Sorghum vulgare*), and *T. nigrescens* on maize infested with *P. truncatus*. 7-21 day old insects were used in all trials except in the case on *T. nigrescens* where mixed age insects were used.

Trials

Three sets of trials were conducted to assess:

a) The efficacy of different application rates of DEs against the bostrichid beetles *P. truncatus* and *R. dominica* on maize and sorghum respectively. Admixed Dryacide® and Protect-it™ were applied at rates of 0, 0.1, 0.15, 0.25, 0.5, 0.75 and 1%w/w against *P. truncatus,* 40 insects/jar with each treatment replicated three times. Admixed Dryacide and Protect-it were applied at rates of 0, 0.1, 0.125, 0.15, 0.175 and 0.2%w/w against *R. dominica,* 40 insects/jar with each treatment replicated four times.
b) The effect of combining low doses of deltamethrin with DEs against *P. truncatus*. Insects were exposed to admixed deltamethrin doses of 0, 0.025, 0.05 and 0.125 mg/kg alone and in combination with Protect-it™ at 0.05, 0.1, and 0.25 %w/w.
c) The effect of different application rates of DEs on the survival of the predatory *T. nigrescens* in the presence and absence of *P. truncatus*. Protect-it™ was applied at rates of 0.05, 0.1 and 0.25%w/w, 18 *T. nigrescens*/jar with or without 30 *P. truncatus* with each treatment replicated six times.

For all trials, pre-equilibrated commodity (100g, 27±5°C, 60±5% r.h.) was placed in 250 ml glass jars. The DEs Protect-it™ (US origin) and Dryacide (Australian origin) were

obtained from Hedley Technologies Inc.[i] and Dryacide Australia Pty Ltd.[ii] The deltamethrin (2% a.i.) was obtained from Agropharm Ltd.[iii] The required quantity of DE and/or deltamethrin was added to the weighed commodity in each jar, and shaken by hand for exactly one minute. The jars were then sealed with filter paper and molten wax and placed in constant temperature and humidity (CTH) rooms at 27±5°C, 60±5% r.h. The timing of the adult mortality counts differed between trials. The final count of the number of dead and alive insects in the F1 population was conducted at 49 days for all trials and all insects.

Results and discussion

Trial a) Efficacy of different application rates of diatomaceous earths against the bostrichid beetles P. truncatus and R. dominica on maize and sorghum respectively

The data was analysed using GENSTAT an analysis of variance was conducted and orthogonal contrasts were used to compare the treatments. The DE treatments led to increased *P. truncatus* mortality compared to the untreated controls ($p < 0.001$) (Fig. 1.). Protect-it™ treatments caused significantly greater and more rapid adult mortality than Dryacide® treatments ($p<0.001$). The % mortality response to the different application rates of Protect-it™ followed a linear relationship ($p=0.018$), although there was a slight curvature in the relationship.

The DE treatments significantly restricted the appearance of progeny compared to the untreated controls ($p<0.001$), the highest mean number of insects to emerge in the DE treated grain was 88.67 compared to 402.33 in the untreated control (Fig 1.). Adult emergence numbers did not differ between the Dryacide® and Protect-it™ treatments, and for both DEs decreased with increasing concentration following a linear relationship.

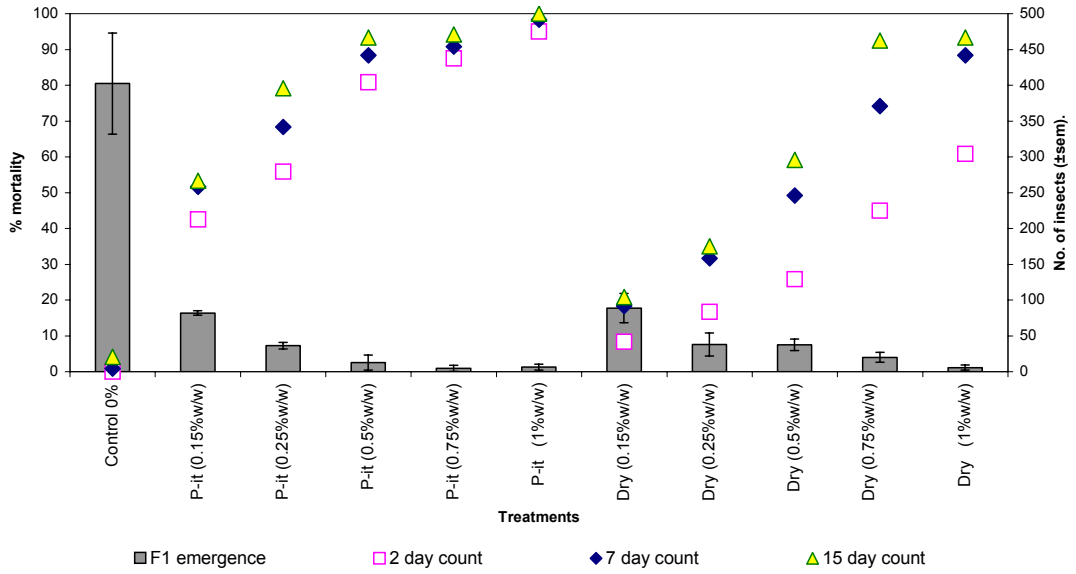

Fig. 1. The effect of a range of Protect-it (US) and Dryacide concentrations on the percentage of mortality of 40 *Prostephanus truncatus* and F1 emergence at 27°C, 60% rh (n=3)

[i] Hedley Technologies Inc., 2600 Skymark Avenue, Suite 101, Building 4, Mississauga, Ontario, L4W 5B2, Canada

[ii] Dryacide Australia Pty Ltd., PO Box 38, Scarborough 6019, Western Australia.

[iii] Agropharm Ltd., Buckingham House, Church Road, Penn, Bucks, HP10 8LN, UK

The lower DE application rates used against *R. dominica* than *P. truncatus*, were based on field trial results in which DE rates of 0.2%w/w were more effective in reducing *R. dominica* damage to sorghum than 0.1%w/w. This current trial found all DE application rates caused less than 20% mortality of *R. dominica* within a 14 day period, with the exception of the Protect-it™ 0.2%w/w treatment (Fig. 2).

Progeny emergence was lower in the Protect-it™ treatments than the Dryacide® ($p<0.001$) and the untreated control ($p<0.001$). Analysis confirmed a linear relationship between Protect-it™ dose and mortality at all three counts and progeny emergence ($p<0.001$). No relationship was found for Dryacide®.

Protect-it™ was found to be more effective than Dryacide® in causing *P. truncatus* adult mortality, however this did not result in any significant difference in the number of progeny emerging from the two DEs. At concentrations of 0.25% w/w, both Dryacide® and Protect-it™ contained *P. truncatus* F1 emergence. Further studies of a range of concentrations around this application rate would provide more detail on optimal application rates against *P. truncatus,* although these are likely to be effected by climatic conditions. None of the DE treatments tried against *R. dominica* caused more than 50% adult mortality or effectively contained F1 emergence, in fact higher mean numbers of progeny emerged from the 0.1 and 0.15% Dryacide® treatments than the untreated control. Similarly when five different DEs (Super Insecolo, Dryacide, Insecto, Insectigone, and Permaguard) were applied at an application rate of 0.1%w/w, the highest mortality of *R. dominica* achieved after 14 days was <10% (Subramanyam, unpubl. data). Higher DE concentrations need to be tested. As feeding, oviposition and larval development occur within the grain, bostrichids are only likely to come into contact with DE on the surface of the grain while searching for a host or mate. This may explain its ability to survive at higher DE concentrations than other stored product pests.

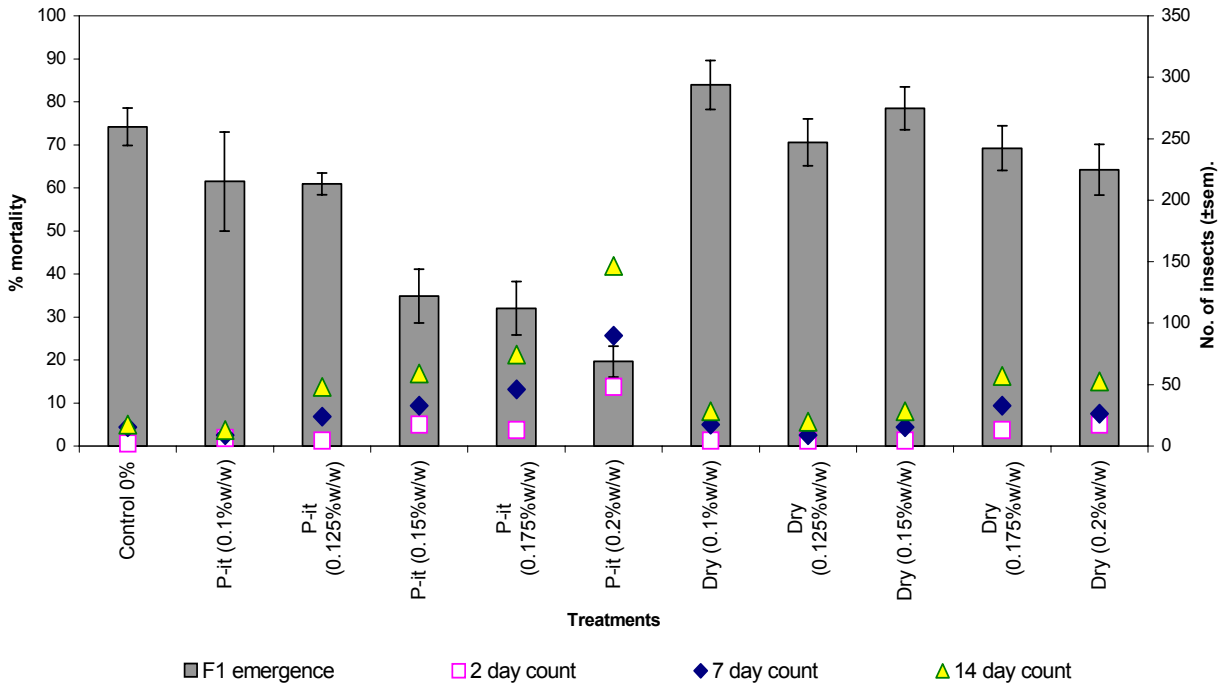

Fig. 2. The effect of a range of Protect-it (US) and Dryacide concentrations on the percentage of mortality of 40 *Rhyzopertha dominica* and F1 emergence at 27°C and 60% rh (n=3)

Trial b) The effect of combining low doses of deltamethrin with DEs against P. truncatus.
Initial studies found deltamethrin application rates as low as 0.25 mg/kg caused rapid high mortality of *P. truncatus* and prevented F1 emergence, no additional effect from combining DEs could be observed (Fig. 3). Further trials found similarly high mortality occurred with deltamethrin rates as low as 0.025 mg/kg. Further studies are underway to investigate the effect of combinations of low doses of Protect-it™ (0.05, 0.1 and 0.25%w/w) with 0.025 mg/kg Deltamethrin against mixed populations of *P. truncatus* and *S. zeamais*. Preliminary observations suggest the quantity of feeding dust produced by the bostrichids may dilute the DEs efficacy against *S. zeamais*. Although combining DEs with insecticides which knock-down insects may result in reduced pick-up of DE particles by insects (Ebeling, 1971; Le Patourel & Singh, 1984), in the current trial deltamethrin doses as low as 0.025 mg/kg caused rapid mortality of *P. truncatus* while *S. zeamais* was unaffected by the deltamethrin. Other researchers have reported slightly higher *T. castaneum* and *S. oryzae* mortality in rice, when DEs were combined with a neem product, in comparison to either treatment alone (Ulrichs & Mewis, 2000).

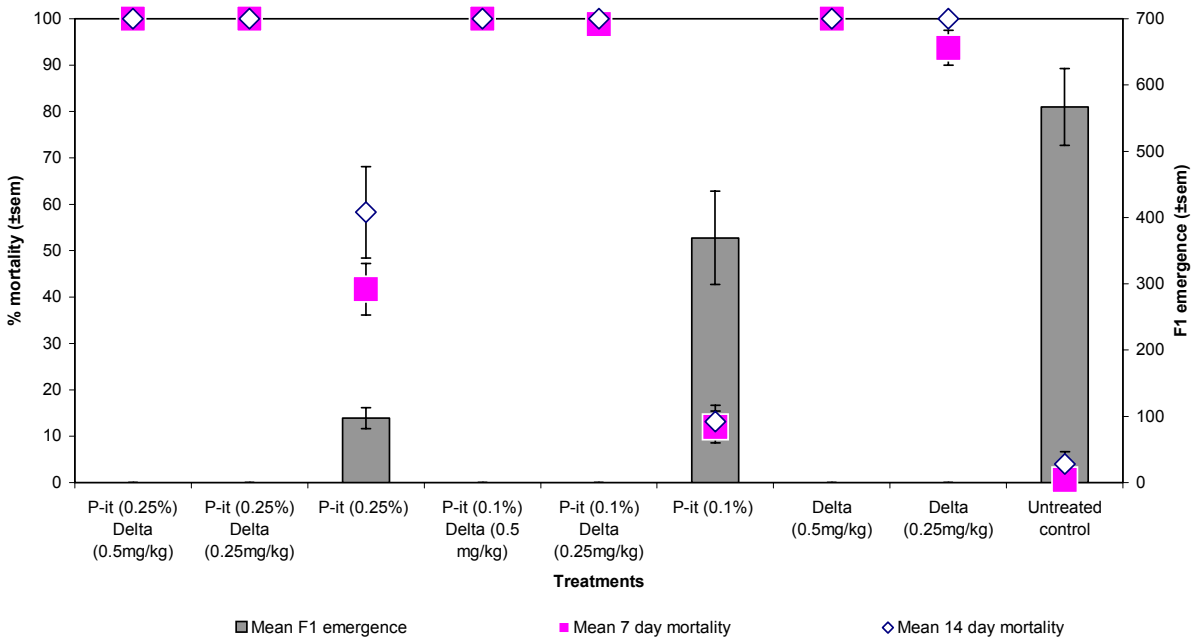

Fig. 3. Effect of mixing low doses of deltamethrin and the diatomaceous earth Protect-it on 35 *Prostephanus truncatus* adults at 27°C and 60% rh (n=5)

Trial c) The effect of different application rates of DEs on the survival of the predator T. nigrescens in the presence and absence of P. truncatus.
T. nigrescens mortality was much higher in all the DE application rates in the absence of *P. truncatus* (Fig 4.). Observations suggest that the large amount of dust produced by *P. truncatus* during feeding, mix with the DEs in effect diluting the DE and reducing the likelihood of *T. nigrescens* coming into physical contact with enough DE to cause dehydration even at the highest application rates of 0.25%w/w. Although *T. nigrescens* host location behaviour is not fully understood, it is usually attracted to grain already infested with *P. truncatus*. These findings suggest that the treatment of grain with DEs would not have a negative affect on *T. nigrescens* survival in the presence of the host. It would be interesting to

assess the survival of *T. nigrescens* with combined low doses of deltamethrin and DEs as studied in Trial b.

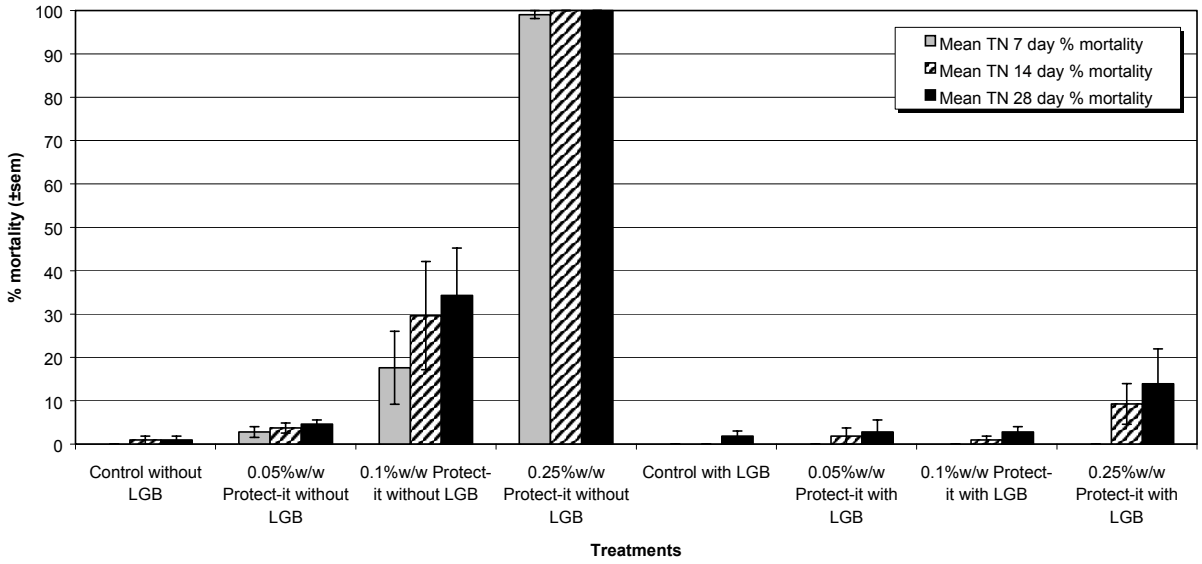

Fig. 4. Effect of different concentrations of admixed diatomaceous earths on the predatory beetle *Teretrius nigrescens* (TN) in the presence and absence of *Prostephanus truncatus* (LGB) at 27°C and 60% rh (n=6)

Few studies have focused on the integration of DEs with predators and/or parasitoids. A recent study found the hymenopteran parasitoid, *Anispoteromalus calandrae* was very sensitive to direct contact with Protect-it™ (Perez-Mendoza *et al*., 1999), confirming field observations in Zimbabwe where the higher populations of *R. dominica* in the low DE treatments compared to the untreated control was thought to be due to both the low efficacy of the DE doses against *R. dominica* coupled with their negative effect on the natural enemy population. *A. calandrae* was observed to avoid DE treated wheat (Perez-Mendoza *et al*., 1999). Parasitoid species differ in their host locating activities, some penetrate grain masses while others remain mainly on the surface, targeting of grain protectants within the grain mass may enhance the combined use of grain protectants and natural enemies (Hodges, pers. comms.).

Acknowledgement

This publication is an output from a research project funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID. Crop Post Harvest Programme.

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Stored product protection with amorphous silica dust (SilicoSec®) in Germany – practical experiences and laboratory trials

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Abstract: SilicoSec is a natural silica powder based on fossilized diatom algae (diatomaceous earth, DE). It contains > 90% amorphous SiO_2 and controls all arthropod pests including weevils, moths, mites etc.. The sharp-edged silica particles destroy the wax layers of the arthropod cuticle and quickly absorb lipids and body fluids, leading to dessication and death. Amorphous silica is completely harmless for all vertebrates and humans. SilicoSec is a registered pesticide for stored grain protection in Germany since 1997.

Cereal grain treated with DE looks different to untreated grain and shows changes in physical behaviour. Flow rate through handling equipment is reduced, bulk volume and the angle of repose are increased. These side effects may limit the use of DE. In order to minimize side effects, it would be necessary to reduce the concentration of SilicoSec within the grain.
In order to check the efficacy of the natural product under field conditions, farmers were asked about their experiences with SilicoSec.

In a laboratory trial it was investigated to what extent either a superficial SilicoSec-covering on grain or a treatment of the upper 30 cm layer of the grain guarantees full protection. Trials were carried out in tubes of 46 cm hight with a diameter of 10 cm. The grain was covered with 0.2, 1, or 2 kg SilicoSec/m² or the upper 30 cm of the grain were treated with 1.5, 3 or 5 kg SilicoSec/t (each n=5). 20 *Sitophilus granarius* were put on each tube and kept in a warm, dry room for 21 days. Grain was sieved in 6 cm layers, active and dead individuals of *S. granarius* were counted out.

65 % of farmers replied to the survey. Four farms treated the empty store, 19 farms the stored product and 3 farms treated both, store and product. The three types of application resulted in successful protection against insect pests in 75, 95 and 100% of the farms. Treatment of the store is useful only in combination with treatment of the stored product or in infested stores which will stay empty for a longer period of time. In order to ensure full protection of the grain SilicoSec should not be applied at less than 1 kg/t.

Results of the laboratory trial showed that a 0.2 kg/m² superficial covering does not prevent *S. granarius* from entering the untreated grain. With 1 kg/m² only a single weevil survived the penetration of the SilicoSec layer, at 2 kg/m² no weevils survived. Increasing the dosage for treatment of the upper 30 cm resulted in a decreased penetration depth of *S. granarius*. Whereas one individual was able to reach the untreated grain through a 1 kg/t treated 30 cm layer, all weevils died within the treated area by the application of 3 or 5 kg/t. Tube trials suggest that >1 kg SilicoSec/m² or ≥3 kg/t should be applied to protect the stored product. Calculating for the protected amount of grain, treating the upper 30 cm layer needs less SilicoSec than a superficial covering.

It can be concluded that SilicoSec ensures a long-term, complete protection of stored products when the whole grain is treated. For superficial treatment and treatment of the upper 30 cm only, more extensive studies are necessary to produce general recommendations.

Keywords: *stored product protection, diatomaceous earth, inert dusts*

Control of the Mediterranean flour moth (*Ephestia kuehniella* [Zeller]) in an automated pig-fattening enterprise

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The European flour moth (*Ephestia kuehniella* (Zeller)) is a common storage pest in mills, mixed fodder plants and food processing plants. A strong infestation of this pest caused serious problems in an automated pig-fattening enterprise, whereby 900 automatic feeders were clogged with larval silk, which was also present in the elevators and fodder bins, thus impeding the automated fodder distribution. The automatic feeders had to be cleaned manually in ever shorter intervals in order to ensure smooth-running operation of the machinery.

In order to reduce infestation pressure, adult moths were controlled by cold atomization with a registered pyrethroid in both the fattening and fodder mixing areas. A monitoring system was installed in these areas and also in the fodder storage area in order to prognosticate infestation rates. Concomitant hygienic measures were also implemented in these areas. A temperature profile in the pig houses was taken using thermohydrographs and showed ideal life conditions for storage pests, with temperatures of 20-25°C and a relative humidity of 80%.

Pheromone funnel traps were set up and controlled weekly in the fodder mixing mill and the pig houses to monitor adult moths. As soon as moth numbers started increasing in spring, the parasitic wasp *Habrobracon hebetor* was released in the pig houses, but were not able to control larvae efficiently. Apparently they could not penetrate the larval silk. An more exact analysis of pest behaviour showed that the moths crawled into the automatic feeders to lay their eggs directly on the fodder mixture. Thus neither they, the eggs nor the larvae could be combated using conventional control measures.

The solution was finally achieved by hanging DDVP strips such as those registered in Germany directly in the automatic feeders, which were additionally closed as well as possible with polystyrene plates that allowed moths to enter but also allowed insecticidal vapor pressure to build up within the feeders. A reduction of infestation to an acceptable level was achieved with this method.

Complete control of Mediterranean flour moth in this enterprise would only be possible with the installation of an entolator at the end of the fodder-mixing process to destroy moth eggs before the fodder is transported to the pig-fattening area.

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IOBC Bulletin Vol. 25 (3) 2002
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Potential of combining silica aerogels with IGRs as protectants of stored rice (paddy) against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae).

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Abstract The efficacy of silica aerogels (Gasil 23D and Neosyl TS) used alone and in combination with IGRs (diflubenzuron and fenoxycarb) was evaluated under laboratory conditions against adults of the rice weevil *Sitophilus oryzae* (L.) on stored paddy.

Gasil 23D and Neosyl TS revealed some differences and the LC50s were 0.03% and 0.02% w/w, respectively. The combinations of each of these materials with IGRs also revealed slight differences of effectiveness. However, the joint action of IGRs and Gasil 23D seems to be more synergistic than IGRs with Neosyl TS.

Key words: *Sitophilus oryzae*, activated silicates, insect growth regulators.

Introduction

Rice is one of the most important staple food in Guinea-Bissau. During storage it is often attacked by various storage insects, among them the rice weevil, *Sitophilus oryzae* (L.). The current strategy for controlling pests at the farm level relies upon the use of some traditional measures and contact insecticides which do not provide satisfactory control. Moreover, the awareness of the consequences of environmental pollution, the increasing cost of storage insecticides and the growing problem of insect resistance led pest management specialists to reappraise alternative protectants for stored grains, like the silica aerogels.

Silica aerogels absorb the waxy particles from the insect cuticle surface (Maceljski & Korunic, 1972; Le Patourel *et al*., 1989) and the insect mortality is induced primarily as a result of desiccation. Water loss is a consequence of the destruction of the cuticle (Golob, 1997). The silica aerogels are said to retain activity even at elevated levels of relative humidity (Maceljski & Korunic, 1971). Because the action of these materials is not dependent on metabolic pathways, it has been postulated that insects will not be selected genetically due to the action of these dusts, so that physiological resistance will not occur. Nevertheless, it may be possible for insects to develop a behavioural response to the dust and avoid contact (Ebeling, 1971).

Another advantage over conventional insecticides is the low mammalian toxicity of these materials (e.g. for Insecto the acute oral rat $LD_{50} > 5000$ mg/kg; Subramanyam *et al*., 1994). In the USA, diatomaceous earths are "generally recognised as safe" by the US Food and Drug Administration and are registered for use as food additives (Banks & Fields, 1995). The results of studies related to the efficacy of silica aerogels used alone and in combinations with either contact insecticides or IGRs have been reported against insect species of stored products. For example, several workers have used the commercial aerogel, Dri-Die, and found good control against several insect species including *Sitophilus granarius* (L.)

(Maceijski & Korunic, 1971), *Tribolium confusum* J. du Val and *Oryzaephilus mercator* (Fauvel) (Loschiavo, 1988). McLaughlin (1994) compared the efficacy of several diatomaceous earths, silica aerogels and synthetic silica, all of which are commercially available, though variable in particle sizes. The fumed synthetic silica, Aerosil R974, was the most toxic when applied as a dust to wheat, or as a dust application to aluminium surfaces. The insects tested were *S. granarius* and *S. oryzae*.

Insect growth regulators (IGRs) and inert dusts have proved effective in both laboratory and large-scale trials against a range of storage pests (Dales *et al*., 1994; Golob, 1997; Gudrups *et al*.,1998). A series of precipitated and fumed silicas were screened at NRI and found to be effective in causing mortality of adult *Prostephanus truncatus* (Horn) (Barbosa *et al*., 1994). Its combinations with contact insecticides were also evaluated. Conceição (2000) used combinations of silica aerogels and IGRs against *S. zeamais* Motschulsky. Some additive effects were recorded. The present study was conducted to obtain further information on the control of *S. oryzae* using the silica dusts alone and in combinations with IGRs.

Materials and methods

Insects, activated silicates and insect growth regulators.

Adult insects of unknown age and sex of *S. oryzae* were used for bioassays at laboratory conditions of 28ºC ± 1ºC and 70% ± 5% r.h. The precipitated silica aerogels (Gasil 23D and Neosyl TS 95% pure) and the technical grade IGRs (diflubenzuron and fenoxycarb 97.6% pure) were used to prepare the formulations by impregnating the toxic (IGRs) on the silica dusts. The formulations were analysed by the GTZ (Gesellschaft für Technische Zusammenarbeit) and contained 0.32% diflubenzuron on Gasil 23D and Neosyl TS, respectively. The concentration of fenoxycarb on Gasil 23D and Neosyl TS were 0.33 and 0.27%, respectively. Grain was treated with the different types of dusts by mechanically tumble mixing 100g of paddy with the dust in 300 cm^3 glass cylindrical jars for 10 minutes to ensure good distribution.

Fifty adults were exposed to grain treated with a range of dosages, 0.0125 to 0.20% w/w, for the silica dusts alone. The same range of dosages was used for the formulations with IGRs, which corresponds to 0.375-6 ppm.

Insect adults were deprived from food for 1 hour before being exposed for 5 h to the grain, after which they were removed and kept in separate glass jars without food. Mortality was observed 48 h after exposure to allow any recovery to take place.

Results and discussion

Observed mortalities of adults after treatment of paddy with silica dusts and formulations of IGRs were subjected to probit analysis. The results are shown in Table 1 and Fig. 1.

There are differences in the effectiveness of silica dusts. Neosyl TS (LC_{50} = 0.02% w/w) was more effective than Gasil 23D (LC_{50} = 0.03%w/w). This contrasts with the observations of other workers. Conceição (2000) found that Gasil 23D was more effective than Neosyl TS against *S. zeamais* adults, thought the same 48 h exposure period was used, the LC_{50}'s were 0.037% and 0.111% w/w, respectively.

Not much variability was found for the formulations of silica dusts combined with IGRs. Except for the combination of Neosyl Ts + diflubenzuron (LC_{50} = 0.02% w/w) all the others showed the same level of efficacy. The LC_{50s} were 0.01% w/w, though with some differences on the slope of the regressions. A considerable amount of information was produced by the

use of IGRs alone. Studies involving the joint action of silica dusts and IGRs are not yet well documented. Conceição *et al*. (2000) found the mixture of fenoxycarb and Gasil 23D more effective than other formulations, which is in agreement with the findings of the present study. In fact, the $LC_{99.9}$ for this formulation on *S. oryzae* was 0.09% w/w, which corresponds to 2.7 ppm.

Table 1. Regression analysis of probit mortality of adult *Sitophilus oryzae* exposed 48 h to paddy with different treatments. Data derived from four replicates.

Treatment	Regression	Slope	LC50 (mg/100g)	LC99.9 (mg/100g)	Heterogeneity		
					χ^2	g.l.	P
Gasil 23D	Y=8.7+2.3x	2.3	0.03 (0.02 - 0.03)	0.57 (0.32 – 1.41)	6.17	3	0.1-0.3
Neosyl TS	Y=7.2+1.3x	1.3	0.02 (0.01 - 0.03)	4.64 (1.39 - 49.46)	1.89	3	0.5-0.7
Gasil 23D + dif.	Y=8.4+1.8x	1.8	0.01 (0.01-0.02)	0.65 (0.30 – 2.76)	1.07	3	0,7-0,8
Neosyl TS + dif.	Y=9.1+2.3x	2.3	0.02 (0.01 - 0.02)	0.39 (0.22 – 0.99)	1.28	3	0,7-0,8
Gasil 23D + fen.	Y=10.9+2.7x	2.7	0.01 (0.00 - 0.01)	0.09 (0.05 – 0.79)	2.20	3	0,5-0,7
Neosyl TS + fen.	Y=9.4+2.3x	2.3	0.01 (0.01 - 0.02)	0.27 (0.15 –0.87)	0.96	3	0,8-0,9

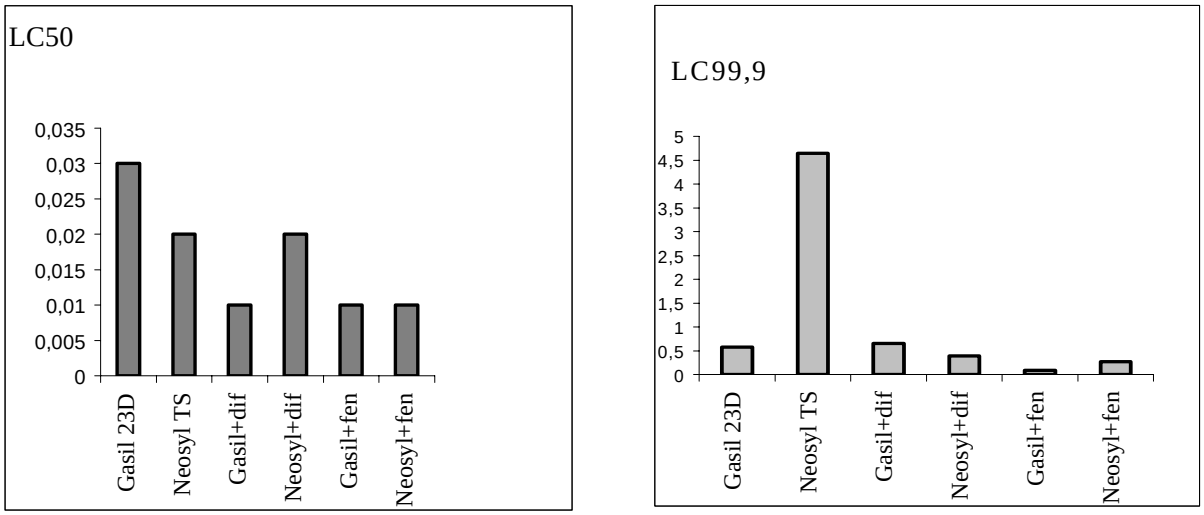

Fig. 1. LC50 and LC99.9 values for *Sitophilus oryzae* on paddy treated with different types of dusts.

Conclusions

The activated silicas might have potential to be used alone or in combination with IGRs as alternative protectants of stored paddy against *S. oryzae*. Gasil 23D and Neosyl TS revealed

some differences and the LC50s were 0.03 and 0.02% w/w, respectively. The combinations of the IGRs with Gasil 23D and fenoxycarb with Neosyl TS revealed the same effectiveness at the LC50 level. At both the levels of LC_{50} and $LC_{99.9}$ the most effective combination was fenoxycarb on Gasil 23D.

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IOBC Bulletin Vol. 25 (3) 2002
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Effect of time of exposure on the effectiveness of silica dusts and mixtures with IGRs against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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Abstract: The effectiveness of two precipitated silica dusts (Gasil 23D and Neosyl TS) used either alone or in combinations with fenoxycarb and diflubenzuron against *Sitophilus zeamais* Motsch. were evaluated under laboratory conditions with different times of exposure.

The data showed differences between the silica dusts. Gasil 23D (LC=0.037%w/w) was more effective than Neosyl TS (LC50=0.113%w/w) when the insects were exposed for 5 hours. Neosyl TS (LC50=0.028%w/w) was more effective than Gasil 23D (LC50=0.037%w/w) at a period of fifteen days of exposure. All the combinations revealed differences in the effectiveness for each time of exposure and the LC50 values decreased significantly (2-3 times) when the insects were exposed during fifteen days. The experiments suggested that the efficacy of some of the treatments increased significantly after a longer exposure of fifteen days.

Key words: *Sitophilus zeamais*, silica dusts, Gasil 23D; Neosyl TS; mixtures

Introduction

In evaluating the effectiveness of silica dusts as grain protectants against insect infestations, several factors are known to influence the response in bioassays. Among them is the exposure period chosen for the target insects on the treated grains.

The work carried out by Mc Laughlin (1994) illustrated the problem of assessing the efficacy of desiccant dusts, like the silica aerogels: they are not directly toxic, are relatively slow acting and typically become increasingly effective with longer exposure times. Various studies on the efficacy of silica dusts have been reported and a range of exposure periods was tested as shown in Table 1.

The objective of this study was to assess the differences on the effectiveness caused by the variation of the exposure period.

Table1. Exposure time used in laboratory experiments by different authors

Author	**Species**	**Dilute dusting powder**	**Duration of exposure**	**Stored grain**
Le Patourel & Singh (1984)	*T. castaneum*	Cab-O-Sil M5 and Aerosil R972	48 h and 168 h	Wheat
Shawir *et al*. (1988)	*S. oryzae*; *T. castaneum* and *T. confusum*	Amorphous silica: -Sipernat 22	72 h	Wheat
Aldryhim (1993)	*R. dominica*	Dryacide	168 h	Wheat
Barbosa *et al*. (1994)	*P. truncatus*	Gasil23D, Aerosil R972, Neosyl TS	5 h	Maize
Haryadi *et al*. (1994)	*S. zeamais*	Zeolite	4; 8 and 12 weeks	Maize

Materials and methods

Insects, precipitated silica and Insect growth regulators

Adult *Sitophilus zeamais* 1 to 3 weeks after eclosion and of unknown sex were used for bioassays at laboratory conditions of 27°C±3°C and 70% ± 10% r.h.. The initial grain moisture content (m.c.) was 10%.

Two precipitated silica dusts (Gasil 23D and Neosyl TS) were used. The coformulations were laboratory made by impregnating the IGRs (diflubenzuron and fenoxycarb) on the silica dusts used as carriers. The final concentration of IGRs in the formulations was 0.3% w/w.

Maize grain was treated with the different types of dusts by mechanically tumble mixing 800g of maize with the dust in a 1000 ml cylindrical glass jar for 10 minutes to ensure good distribution. The treated maize was then divided in aliquots of 200g each and distributed in other 350 ml glass jars (4 replicates per dosage).

Fifty adults of *S. zeamais* were exposed to grain treated with a range of dosages (0.0125; 0.025; 0.05; 0.01, 0.2 and 0.4% w/w). Individuals were deprived from food for 1 hour before being exposed for 5 hours or 15 days to the grain, after which they were removed and kept in separate glass jars without food. Mortality was assessed 2 days after exposure to allow any recovery to take place. Observed mortality of adults after the two exposure periods was submitted to probit-analysis (Finney, 1971). The data (LC50s) from all treatments was submitted to analysis of variance and a comparison test using the Least Significant Difference (LSD) method for the LC50s was carried out. A separate analysis of variance was done for silica alone and its combinations with IGRs.

Results and discussion

The results are summarised in Tables 2 and 3.

Table 2. Regression analysis of probit mortality of *Sitophilus zeamais* as a response to maize grain treated with silica dusts used alone.

Silica	Exposure time	Rep.	Regression	LC50 (%w/w)	Slope ± SE	χ^2	p
Gasil 23D	5 h	1	Y= 9.023+2.787x	0.036	2.787± 0.273	19.308	< 0.001
		2	Y=13.139+5.809x	0.040	5.809±0.745	1.700	0.50-0.70
		3	Y=10.641+4.219x	0.046	4.219±0.460	3.868	0.30-0.50
		4	Y= 9.179+2.623x	0.025	2.623±0.313	3.185	0.50-0,70
Neosyl TS	5 h	1	Y= 6.590+1.579x	0.099	1.579±0.179	15.711	0.001-0.01
		2	Y= 7.007+1,934x	0.092	1.934±0.193	7.124	0.10-0.20
		3	Y= 6.528+1.599x	0.110	1.599±0.182	17.811	0.001-0.01
		4	Y= 6.238+1.509x	0.151	1.509±0.183	9.508	0.25-0.05
Gasil 23D	15d	1	Y=12.366+5,293x	0.040	5.293±0.664	4.021	0.30-0.50
		2	Y=14.629+6.537x	0.034	6.537±0.890	2.424	0.50-0.70
		3	Y=12.103+4.882x	0.035	4.882±0.669	2.396	0.50-0.70
		4	Y=11.856+4.781x	0.037	4.781±0.625	4.869	0.30-0.50
NeosylTS	15 d	1	Y= 8.865+2.365x	0.023	2.365±0.290	2.382	0.50-0.70
		2	Y=15.226+6.952x	0.034	6.952±1.063	0.053	> 0.99
		3	Y=12.421+4.750x	0.028	4.75±0.672	0.484	>0.99
		4	*	*	*	*	*

* Too few intermediate responses for probit analysis

Table 3. Regression analysis of probit mortality of *S. zeamais* as a response to maize grain treated with IGRs formulated on actived silica dusts.

Treatment	Exposure	Rep.	Regression	LC50 (%w/w)	Slope± SE	χ2	p
diflubenzuron on Gasil	5h	1	Y=8.440+2.284x	0.031	2.284±0.274	4.191	0.20-0.30
		2	Y=9.175+2.621x	0.026	2.621±0.338	16.505	< 0.001
		3	Y=9.167+2.856x	0.035	2.856±0.348	4.399	0.20-0.30
		4	Y=9.100+2.694x	0.030	2.694±0.307	5.772	0.10-0.20
fenoxycarb on Gasil	5h	1	Y=13.702+5.360x	0.024	5.360±1.205	0.040	> 0.99
		2	Y=13.857+5.595x	0.026	5.595±1.060	0.059	> 0.99
		3	Y=11.840+4.008x	0.020	4.010±0.971	0.284	0.95-0.98
		4	Y=11.775+3.945x	0.019	3.945±0.961	0.322	0.95-0.98
diflubenzuron on Neosyl	5h	1	Y=7.34+1.777x	0.048	1.778±0.221	4.304	0.20-0.30
		2	Y=6.994+1.332x	0.032	1.332±0.212	4.617	0.20-0.30
		3	Y=6.729+1.470x	0.044	1.270±0.204	2.771	0.30-0.50
		4	Y=7.143+1.470x	0.035	1.470±0.215	3.665	0.30
fenoxycarb on Neosyl	5h	1	Y=7.848+2.481x	0.071	2.481±0.268	7.188	0.05-0.10
		2	Y=8.195+2.308x	0.067	2.308±0.270	9.441	0.02-0.05
		3	Y=7.941+2.355x	0,056	2.354±0.286	3.140	0.30-0.50
		4	Y=7.962+2.397x	0.058	2.397±0.284	7.408	0.05-0.10
diflubenzuron on Gasil	15d	1	Y=13.605+4.471x	0.012	4.471±0.930	0.323	0.95-0.98
		2	*	*	*	*	*
		3	*	*	*	*	*
		4	Y=12.870+3.589x	0.06	3.589±1.371	0.081	> 0.99
fenoxycarb on Gasil	15d	1	*	*	*	*	*
		2	*	*	*	*	*
		3	*	*	*	*	*
		4	*	*	*	*	*
diflubenzuron on Neosyl	15d	1	Y=12.771+4.527x	0.019	4.527±0.646	5.506	0.10-0.20
		2	Y=14.805+5.665x	0.019	5.665±0.891	0.929	0.80-0.90
		3	Y=12.127+4.123x	0.019	4.123±0.609	7.818	0.02-0.05
		4	Y=13.808+5.249x	0.021	5.249±0.800	0.063	> 0.99
fenoxycarb on Neosyl	15d	1	Y=11.423+3.545x	0.015	3.545±1.080	0.250	0.95-0.98
		2	*	*	*	*	*
		3	Y=13.583+5.247x	0.023	5.247±1.177	0.044	> 0.99
		4	*	*	*	*	*

* Too few intermediate responses for probit analysis

The variation of the exposure period did not affect the efficacy of Gasil 23D (Table 4). However, the mean LC50 for Neosyl TS was significant decreased with the longest exposure time of 15 days (Figure 1).

Aldryhim (1993) carried out a series of laboratory experiments using an amorphous silica dust, Dryacide, against *Rhyzopertha dominica* (F.), on different classes of wheat, temperature and relative humidity conditions. The exposure periods for the insects were 2 days and 7 days. The LC50 values decreased with increasing time of exposure from 2 to 7 days, for the same classes of wheat, temperature and relative humidity.

This is in agreement with the performance of Neosyl TS from the present study against *Sitophilus zeamais*. The mean LC50 were 0.113% (w/w) and 0.028% (w/w) at 5h and 15d of exposure, respectively.

In contrast, Haryadi *et al*. (1994) found no significant differences at three exposure periods (4; 8 and 12 weeks), using zeolite, a mineral substance containing SiO4 and AlO4, in bioassays against *S. zeamais* on treated maize grains.

Table 4. Mean LC50 (4 replicates) of *Sitophilus zeamais* on maize grain treated with silica dusts alone.

Silica	Exposure	Mean (%w/w)	Significance*
Gasil23D	5h	0.037	b
Gasil 23D	15d	0.037	b
NeosylTS	5h	0.113	a
NeosylTS	15d	0.028	b

*Values followed by the same letter are not significantly different at the 5% level.

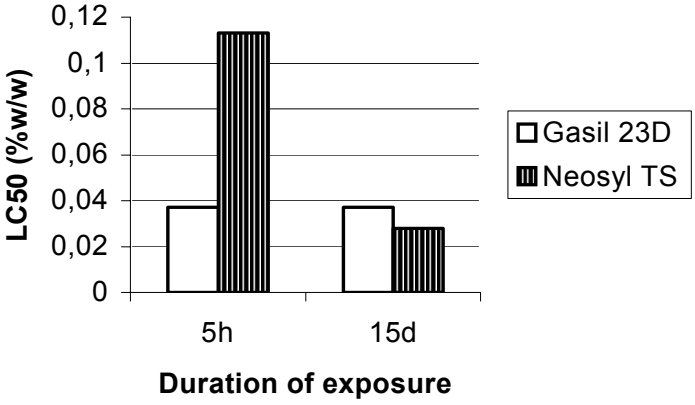

Fig. 1. Efficacy of silica Gasil 23D and Neosyl TS against *Sitophilus zeamais*

The performance of the IGRs formulated on the activated silicates (Table 5) is also illustrated in Figure 2.

Unfortunately, the formulation of fenoxycarb on Gasil 23D was not used for comparisons due to absence of log/probit responses data, for the reason mentioned in Table 3. Nevertheless the data collected for 5 h exposure revealed a mean LC50 of 0.024% (w/w) for this formulation.

The other formulations revealed different performances. The efficacy of those containing Neosyl TS was increased significantly for the longest exposure period. In contrast diflubenzuron on Gasil 23D did not revealed a significant difference at 5h and 15 day of exposure time.

LePatourel & Singh (1984), having worked with two exposure periods (48h and 168 h), also found much lower values for the LC50 for the longest exposure time, at the same concentrations, in bioassays with formulations of pyrethroides on Cab-O-sil (Pyrogenic Silica) against *T. castaneum*.

Table 5. Mean LC50 (4 replicates) of *Sitophilus zeamais* on maize grain treated with IGRs formulated on activated silicates.

Coformulation	Exposure	Mean (%w/w)	Significance*
diflubenzuron on Gasil23D	5h	0.030	bc
diflubenzuron on Gasil 23D	15d	0.036	b
fenoxycarb on NeosylTS	5h	0.063	a
fenoxycarb on NeosylTS	15d	0.019	c
diflubenzuron on NeosylTS	5h	0.040	b
diflubenzuron on NeosylTS	15d	0.020	c

*Values followed by the same letter are not significantly different at the 5% level

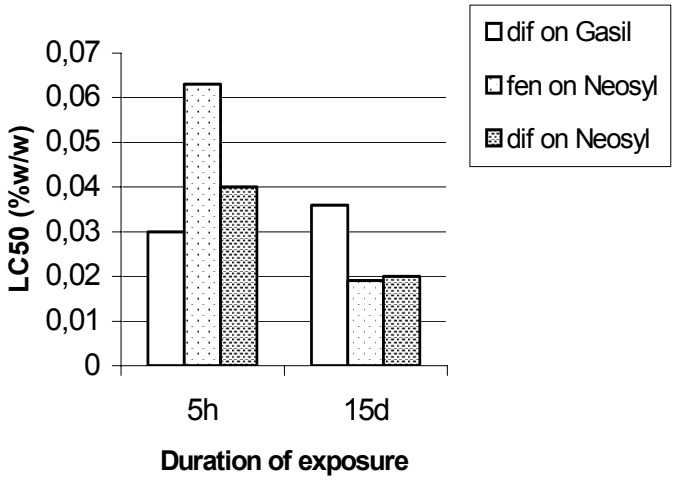

Fig. 2. Efficacy of coformulations against *Sitophilus zeamais*

Conclusions

Under the conditions of the experiments, the time of exposure did affect the effectiveness of the activated silicates, according to the type of silica, among the bioassayed factors.

The efficacy of Neosyl TS, used alone and in combinations with diflubenzuron and fenoxycarb, was increased with the longest exposure time.

However, the efficacy of Gasil 23D, used alone and in combination with diflubenzuron, did not reveal any significant difference for the two exposure times.

Acknowledgements

The authors would like to thank Maria José Monteiro from CEPTA/IICT for her helpful support on statistical analysis.

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Phosphine combined with low level carbon dioxide for the control of *Tribolium castaneum* (Herbst) at different temperatures [1]

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Abstract Pest control in stored grain and in processing/manufacturing units is going through great difficulties. The need of rapid, low cost and environmentally safe ways of controlling pests requires the generation of new technologies and a better handling of the existing ones. The phasing out of methyl bromide is prompting the search for possible alternatives for effective pest control within a 24 hours period, especially for grain processing units. The reported work assessed the combination of phosphine (PH_3) with carbon dioxide (CO_2) at different temperatures for the control of *Tribolium castaneum* (Herbst). Modified atmospheres with 1g m^{-3} of PH_3 combined with 5% of CO_2 were tested at 20, 25, 30, 35 and 40ºC. Five exposure periods for each temperature were used, according to results obtained in preliminary tests. Experiments were carried out in metallic chambers placed inside a climatic chamber with air temperature and relative humidity control. Mortality with time was assessed for each treatment, and the LT_{50} and LT_{95} values determined for each temperature. Increasing temperatures reduced the time to kill the beetles and at 40ºC, it was possible to control 95% of the insect population with the proposed treatment in 23.2 hours.

Key words: modified atmosphere, storage pest control, stored products, grain processing units.

Introduction

Market changes require constant attention and flexibility. In the food industry, the tendency is towards the consumption of natural food, healthy, of low caloric value, nutritious, and free from pesticide residues and toxic metabolites. Thus modern pest control management programmes inside storage and food processing units must involve fast, low cost and environmentally safe strategies, and this requires the introduction of new technologies and a better handling of the existent ones (Arthur, 1996; Sartori, 2000).

Due to its effectiveness, low cost and easy handling, chemical control has been the most used strategy of pest control, both by fumigation with phosphine and methyl bromide (MB) and the use of residual pesticides. Residual pesticides have been used since the 60's as the main way of controlling storage infestations, especially in countries that store large amounts of grain for internal consumption or export.

The phasing out of MB and the expense of developing and registering new pesticides have contributed to a reduction in available grain protectants. Furthermore, in less than 60 years, insects have become resistant to many insecticides, reducing their effective life (Adler *et al*., 2000). Beyond that, there are several biological, economic, and sociological factors that will continue to contribute to the decline of conventional protectant chemicals (Arthur, 1996).

[1] Part of the Master's Dissertation of the first author

Phosphine usage under inadequate conditions (lack of gas tightness inside storage facilities during its application, insufficient exposure periods and applications in low concentrations) seems to have contributed to insects developing resistance (Champ and Dyte, 1976; Price, 1984; Taylor and Halliday, 1986; Chaudry and Price, 1990; Graver, 1990; Pacheco *et al*., 1990, Sartori *et al*., 1990). Such problems lead to the need for ever-higher concentration of the active product, higher exposure periods and, as a result, an increase in the level of residues on the grain to unacceptable levels (Annis, 1990). Studies carried out by Reichmuth (1990) have also shown that the use of high concentrations of phosphine may cause narcosis in the insects, drastically reducing their respiratory activity and, by that, the toxic effect of the gas.

According to studies carried out by Mueller (1998) an increase in atmospheric carbon dioxide to 5% may increase the respiratory activity of the insects by 300%, and may increase the efficiency of the insecticides used.

A great challenge present today is control pests inside factories/processing units that work almost non-stop, in view of the high cost of shut down to clean/disinfest equipment. Thus the need to seek techniques that allow effective control to be achieved within 24 hours.

The present study sought to assess the viablility of controlling an adult population of *T. castaneum* with a modified atmosphere, consisting of 5% of CO_2 associated with 1.0g m^{-3} of phosphine. Tests were set up at different temperatures to determine lethal exposure times (LT_{50} and LT_{95}) likely to be found for empty premises.

Material and methods

This study was carried out in the Pre-processing of Agriculture Products laboratory at the Federal University of Viçosa (MG – Brazil). Experiments were carried out inside three hermetic metallic chambers of 1 m^3 that were placed inside a climatic chamber with relative humidity and temperature control.

Forty adult *T. castaneum* were placed in each metallic chamber which represented one replicate. An aluminum phosphide tablet of 3g (1g of phosphine) was also placed inside each chamber that was than closed and cylinderised CO_2 gas (100% CO_2) was introduced through a gas-tight register present in the chambers. By another gas-tight register, the concentration of CO_2 was monitored, using a CO_2 analyzer model 425N (NOVA Analytical Systems Inc.®), until 5% was reached.

Five temperatures were used in the study: 20, 25, 30, 35 and 40ºC. The CO_2 concentration was set at 5% and the phosphine concentration used was 1g m^{-3}. Five exposure periods for each temperature were used dependant on preliminary test results.

In order to compare the results, the insects were also treated with atmospheric air (78% N_2, 0,03% CO_2 and 21% O_2) without the presence of phosphine for the longer exposure period at each temperature.

Mortality was measured 12 hours after the ending of each treatment, when the dead insects were counted. Results obtained during the experiment were subject to a probit analysis to determine the LT_{50} and LT_{95} for each temperature. A regression analysis was carried out to determine the equations for the LT_{50} and LT_{95}, as a function of the temperature.

Results and discussion

The main advantage expected from the use of carbon dioxide and phosphine in low concentrations but at elevated temperature was the reduction in the exposure period necessary

for the control of insects. In Figure 1 the observed mortality database is shown. The values obtained by the probit analysis are shown in Table 1.

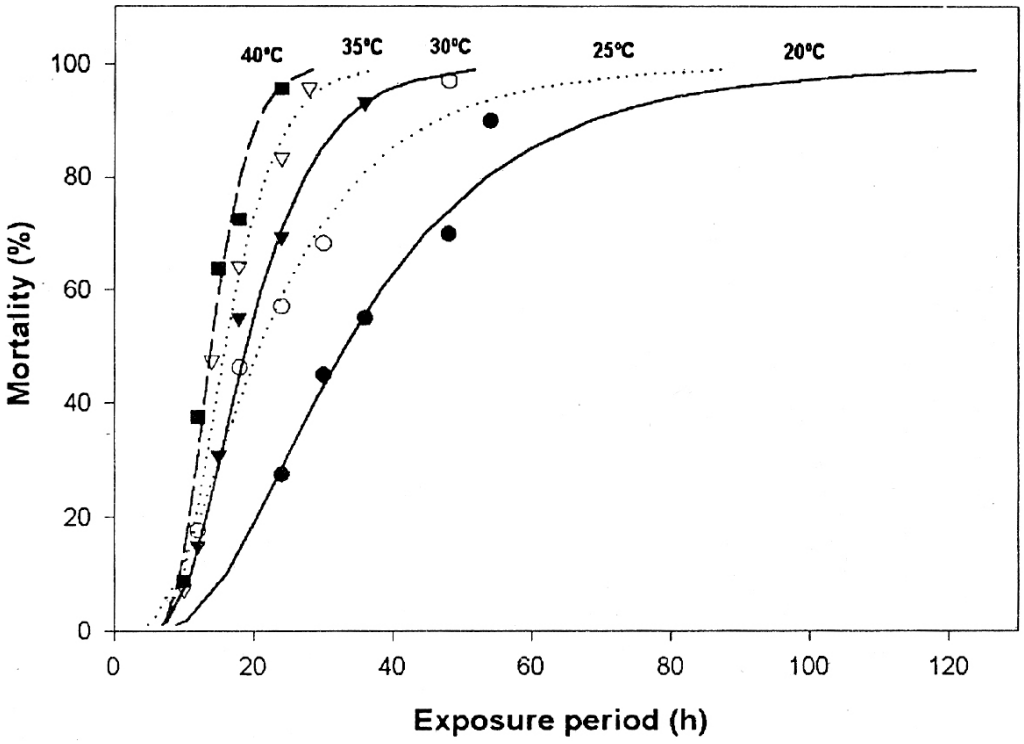

Fig. 1. Mortality (%) of *Tribolium castaneum* treated with 5% of CO_2 and 1 g m^{-3} of phosphine as a function of the exposure period (h) at different temperatures ($R^2 > 0.93$ and $P < 0.0001$).

Table 1. Probit analysis database (*95% Confidence Limits)

T (°C)	**N**	**Slope ± deviation**	**LT_{50} (CL 95%*)**	**LT_{95} (CL 95%*)**	χ^2	**Prob. > χ^2**
20	600	1,50 ± 0,12	33,42 (31,02 – 35,72)	84,36 (69,83 – 115,66)	3,70	0,30
25	593	1,28 ± 0,07	21,00 (19,43 – 22,66)	58,08 (48,35 – 76,10)	3,70	0,30
30	636	1,66 ± 0,07	19,06 (18,01 – 20,17)	38,64 (34,64 – 44,64)	1,63	0,65
35	520	1,85 ± 0,17	16,07 (14,90 – 17,06)	29,16 (26,87 – 32,66)	4,97	0,17
40	517	2,17 ± 0,17	14,06 (13,40 – 14,73)	23,16 (21,34 – 25,87)	5,59	0,13

As anticipated, Figure 1 shows that with an increase in treatment temperature there is a reduction in the required exposure period to control *T. castaneum* adults. However, the target

of control within 24 hours was only reached for the higher temperature used in the treatments - 40ºC.

Figure 2 shows the values of LT_{50} and LT_{95} for *T. castaneum* adults as a function of the different temperatures used. Regression analysis was carried out using the probit analysis database and equations for both LT were determined.

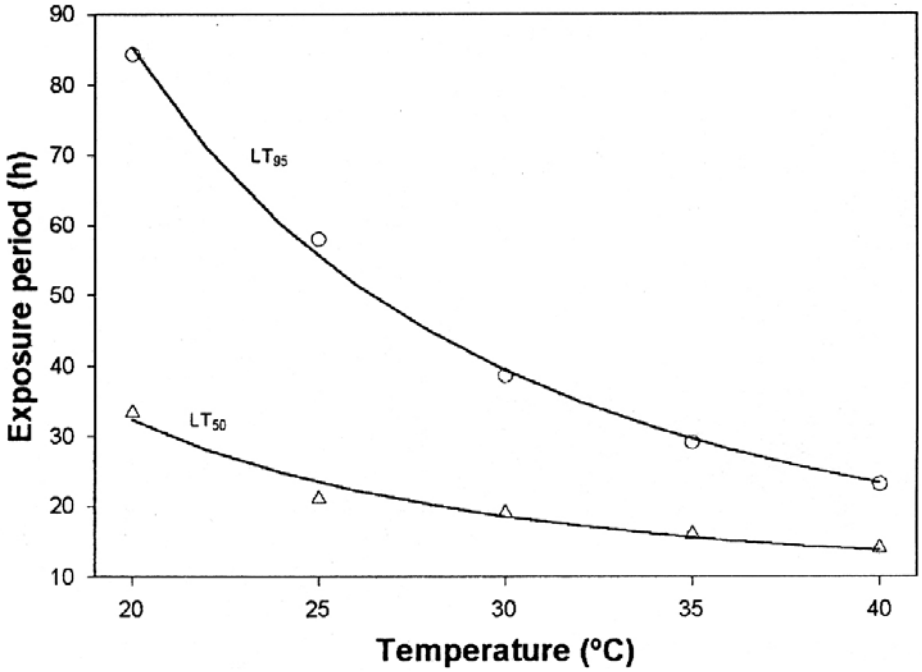

Fig. 2. LT_{50} and LT_{95} of *Tribolium castaneum* adults treated with 1.0 g m^{-3} of phosphine associated with 5% of CO_2 in different temperatures (TL_{50}: $y = 7.49 + 9.94\ 10^3/x^2$; DF = 3; F = 88.82; P = 2.53 10^{-3}; $R^2 = 0.97$; TL_{95}: $y = 2.64 + 3.31\ 10^4/x^2$; DF = 3; F = 914.10; P = 8.00 10^{-5}; $R^2 = 0.99$)

The LT_{95} value obtained in this study for the 30ºC temperature (38.6 h) was lower than the one found by Martinazzo *et al*. (2000) for *Rhyzopertha dominica* adults in wheat grains. In that study, effective control using 1g m^{-3} of PH_3 and 100% of CO_2 at 29ºC required an exposure period of 120h. Coelho *et al*. studying fumigations with 0.75 g m^{-3} of PH_3, combined with 100% of CO_2, at 28ºC to control *T. castaneum* adults in wheat grains, reached an LT_{99} of 95 h. In the same study, with a normal atmosphere of 1.0g m^{-3} of PH_3, the LT_{99} was of 144 h. The results obtained by these authors with a 100% CO_2 atmosphere may prove the theory of narcosis demonstrated by insects when submitted to very stressful environments.

For the temperature of 25ºC used in this study an LT_{95} of 2.42 days was obtained, similar to results found by Price & Mills (1988). They found that resistant strains of larval *T. castaneum*, fumigated with 1.03 g m^{-3} of PH_3 at 25ºC, were effectively controlled by an exposure period ranging from 2-4 days.

Acknowledgements

To Praxair – White Martins Industrial Gases S.A. for providing technical support and apparatus; to FIEMG/FAPEMIG for the financial aid during the development of the study; to the Federal University of Viçosa/Agriculture Engineering Department for the opportunity of carrying out this work; to Dr. Cornel Adler for motivating and helping on participating in this

conference; IOBC that provided travel costs support; and to the family of Ana Paula that motivated her on her work and helped her to attend the conference.

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SOCIAL ASPECTS OF POST-HARVEST

Integrated Protection of Stored Products
IOBC Bulletin Vol. 25 (3) 2002
259-267

The erosion of local practices of post-harvest management in times of war – A case study from the north of Mozambique[1]

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Abstract: War can affect African rural societies in many more ways than causing death: it can disrupt entire societies that are forced to displace from their lands to other regions or to neighbouring countries; it can isolate them from the markets preventing people from selling their surplus and buying basic needs like medicines and agricultural instruments; it can spread fear and mistrust breaking sustainable livelihoods. In any case both the productive and the reproductive potential of the societies are affected. Although seed supply and support to crop genetic resources management is now considered an important component of relief and rehabilitation aid to rural communities recovering from war, civil strife and natural disasters, support to post-harvest management is still a frequently forgotten intervention. In this paper, based on field study in the Niassa province of Mozambique, we describe traditional post-harvest management practices of the main agricultural products, how they were affected by war and recent external development interventions in rural societies.

Key words: Gender; participatory R&D; ethno-agronomic approach.

Introduction

Mozambique is a southern African country surrounded by Tanzania in the north, Malawi, Zambia, Zimbabwe and South Africa in the west, Swaziland and South Africa in the south and the Indian Ocean all over the east coast.

The research area – Cuamba and Maúa districts – belong to the Niassa province, one of the Northern regions of the country, surrounded by Cabo Delgado, Nampula and Zambezia provinces and by Tanzania and Malawi. Niassa is the biggest province of the country – about 129 000 km^2 – but has a rather low population density, which according to the last census was of 5.9 inhabitants/km^2 (INE, 1997). The region has a tropical rainy climate (classified Aw by Köppen) characterized by a rainy season of five months that lasts from November till March and two transition months (Uatata, 1994). Small rains usually occur in August and are rather useful for dry season crops. The main ethnic group is the Macua, one of the most representative within the country. At the religious level the population can be divided into two main groups: Muslims and Catholics. Muslim religion has a relevant importance in social and economic life mainly as a consequence of the prohibition to drink alcoholic beverages.

Liberation war in Mozambique began in 1964 and lasted until 1974. With independence in 1975, Frelimo established a one-party regime. However, the new government destroyed the foundations for sustainable social and economic development by breaking the alliance that was established during the liberation war between peasants and the nationalists (Hermele,

[1] - This research was financed by Fundação para a Ciência e Tecnologia (FCT) within the project "The disintegration of African agrarian societies and their potential for reconstruction" (PRAXIS/P/SOC/11110/98).

1989). A deep socialisation process of the countryside was initiated with the creation of communal villages, the marginalisation of the magic-religious and political chiefs – accused of collaboration with the colonial regime – and their substitution by the ruling party representatives (*Secretários do Partido* and *Comités Dinamizadores*) as well as criticism and often even prohibition of traditional rituals and ceremonies.

With the nationalisation of the economy, the market was almost paralysed and peasants were no longer able to buy basic needs. Forced resettlement in communal villages also entailed in most cases a threat to peasants' food security (Geffray, 1991; Roesch, 1993). All these factors led to a disenchantment of the peasants whose support to Frelimo was, according to Geffray (1991), much more based on an anti-colonial feeling than on an understanding and adherence to its socialist project.

Renamo – a party created by Rhodesian secret service – initiated the civil war in 1977, which ended in 1992 with the signature of the Peace Agreement. In the Niassa province this war only began in 1983, in the Maúa district. The region of Muacanha located in this district was Renamo's main military base in Niassa.

Research was conducted in two territories (traditional chieftaincies named Elapo in Macua and *chefaturas* in Portuguese) – Mitukue and Konhomali located respectively in Cuamba and Maúa districts. In this paper the analysis is mainly focussed on the data of Konhomali Elapo, due to the fact that its population was more severely affected by civil war. Actually this is a recent chieftaincy created some years after the war by people belonging to both sides of the conflict, – some were displaced people from several parts of the district who took refuge in Maúa city and some were with Renamo in the Muacanha region. Konhomali Elapo has a population of nine hundred and fifty nine inhabitants (last census in 1997) and two hundred and seventy households (*Ethoco*).

Empirical research was conducted between mid June and the end of August 2001 in Konhomali and Mitukue territories. Semi-structured interviews about the livelihood systems were conducted in a sample of 48 households in Konhomali and 106 households in Mitukue. Research techniques also included participant observation and several Participatory Rapid Appraisal (PRA) techniques.

Social organization and peasants livelihood systems

The basic unit of social organization is the extended matrilineal and matrilocal family – the *Ebumba* – organised in several nuclear families (*Ethoco*) of two to three generations. The *Ebumba* is not only a residential unit, by the fact that it links aspects of the redistribution process (labour and consumption) to the socio-political (lineage and clan) and cosmological spheres of society.

Traditionally, at the level of the extended family compound (*Ebumba*), each nuclear family had their own houses, production fields and granaries and each married woman cooked in her own kitchen. However, consumption was done collectively – at meals all the family members gathered to eat together organised by gender and age. This redistribution process suppressed the deprived situation of some of the family members.

Agricultural work used to begin at the fields of the senior family of the *Ebumba* with the help of all the junior families. Regular reconstruction of houses and granaries of the senior family used to be also performed with mutual aid. Traditionally, sons-in-law had a moral obligation to help the parents of their wives.

Mutual aid on agricultural work occurred also between all the junior families of the *Ebumba* during periods of intensive work, specially weeding (*Olima*). Sweet (*N'toboa*) or alcoholic (O*theka*) drinks made of sorghum or millets were prepared for the invitation of these labour groups to some kind of working parties (*N'Toboa n'olima* or *Otheka u'olima*).

Maize is the main agricultural production in this province and most of the producers have a surplus that in some cases lasts for 2 to 3 years given to market constraints. This cereal is the staple food prepared as a stiff porridge (X*ima*). Sorghum and above all dried cassava are also used to prepare *xima*, mainly in times of food scarcity. More often, sorghum and millet are used to make sweet and alcoholic drinks.

Cropping patterns are rather variable, mostly according to peasants decision-making and experimental skills, availability of seeds, the production level desired for certain crops in each year, soil fertility, specific micro agro-ecological conditions, and potential pests like monkeys, wild pigs and elephants. Upland fields are continuously cropped for six to ten or even more years until the complete exhaustion of the soil. This absence of crop rotation practices is a source of pre- and post-harvest infestation by pests and diseases.

Few peasants have access to external inputs. Mechanical tillage is inexistent while completely generalised in some bordering countries. Pre and post-harvest losses are felt as major constraints. Marketing and road infrastructures are further bottlenecks.

Indigenous storage management of the main productions

Different storage facilities and post-harvest procedures are used for the diversity of crops usually produced by the peasants. Compared to Mitukue territory in Konhomali one can observe a much-reduced variety of storage facilities.

Maize is usually harvested when completely dried and ready to be stored. Nevertheless a delayed harvest can increase field infestation and induce a reduction of initial storage characteristics, because husks tips can dry till broken increasing the exposure of the grain to pests (Tadessa & Eticha, 2000).

The traditional maize storage facility is the *N'huta*, a kind of a fence made of stems stacked on the ground upon which bamboo bars are horizontally tied. Maize cobs are hanged in lines with the tips downwards with a slight angle of inclination that prevents rain to penetrate the husk and damage the grain. In order to be hanged, the cobs must be harvested with a piece of the stem. This system is almost completely abandoned due to its labour-intensive harvest and storage organization; nonetheless, peasants who still use it state that it's the most efficient one.

Nowadays, maize is generally stored in a granary named *N'thata*, which is a square or rectangular bamboo granary – with a wooden locked door - laid on a raised bamboo and wood platform about 1.5 m from the floor. This granary has a straw hip roof that is extremely long protecting the walls from direct sunshine - avoiding rapid and extreme temperature changes and the rain. In Mitukue territory this kind of maize granary is always plastered with mud. In the words of one peasant this practice is not used in Konhomali in order to reduce termite attacks.

Some of the peasants do not use smoke to protect their maize granaries, arguing that ventilation is enough, that attack by pests is quite variable from year to year and above all a matter of individual luck. The floor and the walls (but not the roof supports and beams which are usually covered with old cobwebs) of the *N'thata* granary are beaten and cleaned up with a broom to reduce dust and the pests of the previous stored products. However, the outside of the walls and the floor are not cleaned and so re-infestation may occur. Direct observation showed the need to wash the granaries to completely eliminate the possibility of initial re-infestation.

Some mayor producers have more than one *N'thata* where they separately keep the harvests of different years. Also in rare cases, the old harvest is kept in another kind of granary together with sorghum and millet. Nonetheless, the great majority of peasants organise both harvests (the surplus of the old and the new) side by side in different heaps after

cleaning the *N'thata*. In the same granary also sorghum, millet and dried cassava may be stored. In this case there is a great danger that cross-infestation may occur.

Improved maize varieties - generally named '*Calamidades*' (Calamities) because they were introduced during and after the end of the civil war by relief aid - have rather poor storage characteristics[2]. For that reason, peasants usually store only the seeds they need which are kept in a bunch of cobs suspended outside the granary over the fire to benefit from heat and smoke.

After a year of storage, maize is usually damaged by pests and humidity. According to an Export Marketing enterprise technician, in the Maúa district maize usually comes from the fields «singing», *i.e.*, rather infested by pests. Casual observations indicate that the main pest is probably a weevil (*Sitophilus* sp.). Infested maize grains are given to chicken but when the infestation is rather high and the grain is also rotten the cobs are burnt.

Sorghum and millet are stored over the kitchen fire on an elevated platform made of wood and bamboo. In Konhomali *Elapo* – contrary to what happens in Mitukue - this storage structure has the same name of the maize granary (*N'thata*). The kitchens are little houses that function as storerooms also for other products like beans, pigeon pea, sunflower, sesame and rice, which can benefit from the effects of the smoke.

Sorghum is stored unthreshed. Few peasants inlay the panicles of sorghum as a protection measure against rodent attacks. On the contrary, millet (with the exception of African millet) is threshed and winnowed as a measure to reduce field infestation. By that reason it is kept in polypropylene bags on the *N'thata* also benefiting from smoke.

The drying of sorghum and millet prior to storage may be done in the fields on their plants. However, most peasants prefer to complete the drying operation after harvest on rocks or on low wooden and bamboo platforms (*Thathapo*).

According to peasant statements the main insect pest of sorghum and millet should be a Lepidoptere. Losses attributed to rodents are also considered to be rather high.

Paddy upland and swamp rice are mostly kept in a *Muruthu*. When the harvest is not enough to fill it, then other products may be stored there separated in polypropylene bags or wrapped in cloth. The *Muruthu* is a kind of a drum made of the bark of a certain tree (*Murotho*) with a variable volume between about 50 l to almost 200 l. This container is considered rodent proof. Groundnut, sunflower, beans and pigeon pea are also usually stored inside a *Muruthu* or in polypropylene bags.

Beans and pigeon pea are usually dried in platforms (*Thathapo*) prior to shelling and storage. The drying phase may also be done in a kind of rectangular bamboo granary placed in the verandas (*N'thata napithela*). One peasant stated that if beans are dried in the shadow and over a platform, instead of on the ground, storage losses due to insects are much reduced or null.

Peasants consider some grain legumes (*Vigna unguiculata* and *Phaseolus vulgaris*) highly susceptible to insect attacks. Pigeon pea, however, has the worst storage characteristics of all cropped legumes, and by that fact is usually the first to be consumed or sold when there is a big harvest. On the contrary groundnut, bambara groundnut, and two beans- whose Macua-Xirima name is *Tangaré* (possibly a *Canavalia* sp.) and *Tathape* (*Phaseolus lunatus*) - are considered resistant to insect attacks.

In the storage of beans and pigeon pea peasants use ashes, capsicum seeds and fruits, seeds of *Tangaré* and quartz stones as protection measures against insects attacks. One producer stated that in her family all the women used also salt to reduce losses caused by

[2] - Compton *et al.* (1993:285) state that the increased production of high-yielding varieties with poor storability is one of the factors responsible for generating higher losses in traditional storage systems in the tropics.

insects. The quantities of all these products were not specified by women and are considered to vary considerably.

When the harvest is rather big, pigeon pea, sunflower and groundnut can be stored in an *Eviri* frequently plastered with mud and located in the veranda or in the dwelling area. If the harvest is small some peasants store the seeds of beans in hermetically closed mud containers (*Muhapo*) usually used to cook and for the preparation of sweet and alcoholic drinks of sorghum, millets and maize bran. The *Eviri* is a kind of bamboo basket with a variable volume of about 200 l to more than 400 l – according to the stored product and the production volume – that is laid in a wooden raised platform. This kind of granary is sometimes plastered with mud and placed in the veranda or in the dwelling area. In this last case it is covered with a straw cone roof.

Another local storage container – the *Epithá* – is a "traditional bag" used to store rice, groundnuts, beans and sunflower but that nowadays is seldom observed. It is made of the bark of a tree, which is beaten with a wooden instrument until reduced to fibre, and the bark is taken out in one piece from the piece of trunk. This material was used as cloth in ancient times and during war in Renamo controlled areas. Once filled with the product the fibre tube is tied up with a string in each end and hanged on two stems to avoid direct contact with the wall and the floor. It is kept in the verandas or in the kitchen.

Cassava is harvested at the end of the dry season, pealed, cut into pieces and then dried on an elevated platform (*Essandjá*) of almost two meters high. There is a total lack of hygienic and preventive measures in the processing of that produce; after pealing, cassava is not washed and during drying it is not kept at home during night or even covered with a mat. The drying process takes a considerable amount of time because cassava reabsorbs air moisture daily, which frequently leads to an infestation by fungi from the beginning of storage. Dried cassava is then stored in an *Eviri* usually plastered with mud.

The extent of losses depends mainly on crop, crop variety, length of storage, climatic conditions, pests, type and sanitation of the storage facility and pre-storage handling of the product. The majority of granaries and containers suffer from not being air tight, insect resistant and rodent proof and main traditional methods aimed to reduce post-harvest losses seem to be rather insufficient. Peasants consider rodents and insects the most important causes of storage losses in all products. Most producers have cats against rodents. Female cats are believed to be more efficient, because male cats are 'travellers'. Smallholders have never mentioned chemical pesticides as a method of pest control used by them.

Each year magic-religious ceremonies are performed to favour good harvests and reduce pre and post-harvest attacks. There is no traditional method to quantify post-harvest losses and the peasants that try to estimate them are considered miser. For the same reason they seldom assess the production volume of the main crops (cereals and dried cassava).

War, social changes and external intervention in rural societies

As Geffray (1991:123-125) states, in the beginning of the war, the settlement pattern was an indicator of political adhesion used by both armies, and peasant houses and granaries were burnt in an attempt to force them to choose a side: the ones that stayed in communal villages were attacked by Renamo and the others that refused to live in villages and stayed in dispersed settlement (in the *mathalani*) were assaulted by Frelimo.

Some decided to create two different scenarios to please (and cheat) both parties: they had a house in the village were they lived during few months of the dry season and another in the *mathalani* where their most important assets like furniture, animals and granaries were kept. That was the case, for instance, of a Frelimo Party Secretary in Mitukue Elapo. During war there was even a rather popular music whose refrain was "with a foot in the village and the other in the *mathalani*".

Research revealed that although the seed system was affected by civil war, few years after it ended peasants were in general able to replace their stocks of traditional seed varieties through buying, gifts by family and friends and exchange of work for seeds[3]. In fact, according to general opinion, people in areas mainly controlled by Renamo – above all in Muacanha – did not have their agricultural production and animal breeding activities affected, although they were completely isolated from market, living without basic needs like salt, soap and cloth.

On the contrary, for the displaced ones that took refuge in the cities, production was affected because they always worked with the fear of being kidnapped, a fact that forced them to reduce production to a minimum. Lack of enough agricultural land around cities and bad storage conditions because of difficulties in collecting materials needed for the construction of granaries and other storage facilities were also constraints to attain food security.

However the most dramatic situation was lived by the ones who stayed in Renamo or Frelimo controlled areas but far from a strong military protection, because they were repeatedly attacked and kidnapped by one or the other of the armies. In that case war completely disrupted production activities, because peasants were frequently fleeing due to military raids. In most cases they had hidden their assets, lived in shelters and had to keep their harvest divided in several storage facilities[4].

Colonial intervention in rural societies, mainly by forced integration in the market economy through the production of cash crops, the socialization process after independence and the long war (anti-colonial and civil) generated a disintegration process of the social fabric of the society. Intra-extended family (*Ebumba*) relations of solidarity and reciprocity were weakened by the growing individualization of the nuclear families.

Since the end of the war, the number of single and divorced women with small children has been increasing, apparently because of the fewer number of men – as men had been killed during the armed conflict – and because of their decline of social responsibility towards women and their own children. Most of the women-headed households have some difficulties in attaining self-sufficiency in food production and in the management of stored products due to the fact that the clearing of new fields and the construction of granaries and some storage containers are tasks for men. Mutual aid, that traditionally occurred between the several *ethokos* of the same *Ebumba*, seldom occur actually and so senior families are facing the same problems.

In general, war may also have had an indirect effect on post-harvest losses when in the rush of emergency aid some seeds were imported without the necessary pest control measures, introducing new pests[5]. The need to introduce seeds of improved varieties when the stock of local germplasm have been eroded by war may also put increased problems in post-war storage management as they usually are much more susceptible to pests.

The migrant behaviour that war developed in rural societies through the need to run from ancestors' territories to more secure places and through the integration in the armies of both parties is now a source of introduction of several agricultural innovations, also in post-harvest management. People were forced to know other regions, discovering better places for living due to better agro-ecological conditions, accessibility to markets, off-farm income

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[3] - The capacity to keep or rapidly replace the stock of germplasm of seeds of local varieties is considered an important indicator of the capacity for reconstruction and reconstitution of African agrarian societies (Temudo & Schiefer, 2001).

[4] - For a comparative analyse with the storage conditions of peasants in Nampula Province during war, see Geffray (1991: 94, 124-7, 131).

[5] - See, for instance, the case of the introduction of a groundnut pest after the end of anti-colonial war in southern Guinea-Bissau (Temudo, 2000).

opportunities and health and education services. This new geographical mobility of Macua people is probably one of the main features of social change.

If war clearly contributed to the breakdown of social organization and to the erosion of social capital, Macua society has also shown a remarkable capacity for reconstruction and reconstitution. Local seed germplasm for the main food productions was recovered almost immediately as stated above, and three to five years after the Peace Agreement peasants began to believe that the war was over, and so came back to their former regions of origin and felt confident to make long-term investments such as building better houses (with mud bricks instead of bamboo plastered with mud) and planting orchards.

In Niassa province, external interventions by the state and by local and international non-governmental organizations (NGOs) seem to have been designed without any knowledge of the social organization and of the farming and livelihood systems of agrarian societies. Recently some baseline studies have been conducted by two NGOs with participatory techniques (ACRIS, 2001; Valente, 2001). However, several key questions have been bypassed by these researches.

After the civil war, external intervention has mainly been oriented towards the support of commercialisation activities through the creation of peasant associations and the credit to small and mid-sized tradesmen on one hand and towards the resettlement of goats through peasant's credit in animals on the other. The associations link directly to the big traders with whom they establish a contract of supplying a certain quantity of a given product at a given price (decided before harvest when the peasant has no means of predicting market demand and possible maximum prices, but the trader has better access to such information). Thatcher (sd) demystifies the impact of associations in improving the living conditions of Mozambican peasants – "Associations and interest groups are just forming and when they do, the motive is often the possibility of benefiting from material support".

Improved cereal seeds, groundnuts, pigeon pea and sunflower have also been distributed by some NGOs without any lasting impact. Again, no efforts have been made to support peasants' initiative of increasing and diversifying fruit production with appropriate planting materials and training for the adoption of improved propagation techniques like grafting and pruning.

Although in the neighbour province of Nampula some international NGOs (like Care and World Vision) are working on integrated protection of stored products, in Niassa no external efforts have been made to generate basic knowledge on local post-harvest management and its improvement. So it seems that the most criticised approach of increasing availability in agricultural foodstuffs by raising production area and by the introduction of improved varieties (in general more susceptible to pests), instead of and intervention in the reduction in pre and post-harvest losses is still in move in development co-operation[6].

For the poor people of Niassa province, food security is much dependent upon the existence of a stock of dried cassava to face the pre-harvest period of food shortage. As we stated before, dried cassava is the most susceptible product to storage pests. Nevertheless, until now interventions directed to improve food security of the most deprived have not considered storage management as a major component of the project design[7].
Empirical research shows, however, an urgent need for a participatory intervention in post-harvest management.

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[6] - Oxfam GB – Niassa Office intended to have an intervention in the reduction of pre-harvest pests. However the difficulties inherent to the control of big pests like elephants, monkeys and wild pigs made it give up (Oxfam, 2000: 7, 17).

[7] - See, Oxfam GB – Niassa program plan on food security and livelihoods for 2000-2003 (Oxfam, 2000) funded by EU.

Conclusions

Local post-harvest management techniques showed to be insufficient and medium to high losses are expected to occur in the main productions for a storage period between six months to a year according to the crop, the variety, the year, the agro-ecological conditions and peasants' storage management practices. Dried cassava, cowpeas, some beans, sorghum and millet are the most affected by pests. Preventive and hygienic measures of pest control are rather weak.

More profound studies should be conducted on the identification of pests and diseases of stored products, levels of losses inflicted by them and the efficacy of traditional protection measures. Given the presence of the large grain borer (*Prostephanus truncatus*) in the neighbouring countries of Tanzania and Malawi (Walker, 1999:2), the risk of its introduction in Niassa is potentially high. Studies should be conducted to confirm or exclude that hypothesis and if that pest is still not present in this province, full measures should be approved to prevent it from happening. However, monitoring with pheromone traps by the Ministry of Agriculture and Fisheries stopped since 1996 due to budgetary constraints (Walker, 1999:9).

There is an urgent need to explore alternative forms of post-harvest management, chiefly through participatory technology development methods. This should be supported by well-targeted, culturally appropriate and realistic measures aimed at improving the management capacity of beneficiaries. Due regard should be paid to environmental, operational, financial and maintenance implications of new technologies.

The use of chemical pesticides is beyond the range of peasants' economic conditions, markets are non reliable and extension services are non-existent or low in performance. General concern for environmental and health problems encourages the study of non-chemical methods of stored product pest control. All these factors lead to the development of integrated pest management (IPM) measures to adapt, modify and complement traditional techniques.

Several plants with insecticidal potentials like *Lantana camara*, *Azadirachta indica*, *Annona reticulata*, *Tephrosia vogelii* and *Cassia sieberana*[8] are locally available and experiments should be conducted to assess their efficacy, feasibility and applicability in local conditions. This should include, as other studies support (GTZ, 1995), the assessment of the possibly toxic residues left in stored products by natural pesticides (above all of *T. vogelii*), and the variation in the concentration levels of active compounds in the composition of plants all through the year and from one region to another and consequently the quantities necessary for attaining efficacy.

Some gender-mainstreamed measures should be conducted considering the specific problems of single, divorced and widowed women and of old men that have no support from their families to built some storage facilities.

External intervention may be characterized by a piecemeal approach to development lacking overall coherence and, in practice, completely bypassing pre- and post-harvest problems.

War had an indirect effect on post-harvest management; nevertheless further studies are needed to fully understand the way in which war affected peasants' livelihood systems.

[8] - The first three are commonly used in traditional methods of storage pest control (GTZ, 1995). The latter two are used locally to fish by poisoning: *Tephrosia vogelii* is well known by its high content in rotenoids and *Cassia sieberana* is used by peasants in southern Guinea-Bissau to reduce losses caused by insects in husked rice (Temudo & Barros, 1998: 147).

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