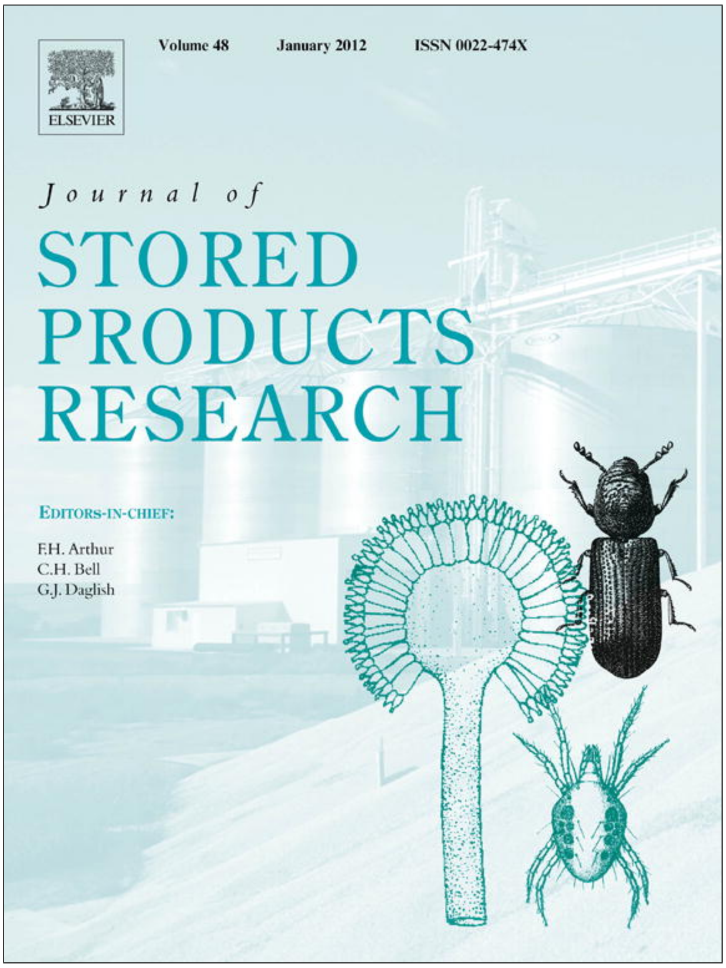

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Journal of Stored Products Research 50 (2012) 15–20

Contents lists available at SciVerse ScienceDirect

Journal of Stored Products Research

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

Comparison *in vitro* and *in vivo* efficiencies of three attractant products against webbing clothes moth *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae)

Ingrid Arnault [a,*], Michael Decoux [a], Emilie Meunier [a], Tom Hebbinckuys [a], Stephane Macrez [b], Jacques Auger [c], Dominique De Reyer [d]

[a] *CETU Innophyt, Université François Rabelais de Tours, UFR sciences et techniques, avenue Monge, 37200 Tours, France*
[b] *Mobilier National, 42 avenue des Gobelins, 75013 Paris, France*
[c] *IRBI UMR CNRS 7261, Université François Rabelais de Tours UFR sciences et techniques, avenue Monge, 37200 Tours, France*
[d] *Laboratoire de Recherche sur les Monuments Historiques, 29, rue de Paris, 77420 Champs-sur-Marne, France*

A R T I C L E I N F O

Article history:
Accepted 19 March 2012

Keywords:
VOCs
Webbing clothes moth
Attractant
Diffusion
Bioassay
Alternatives methods

A B S T R A C T

Moth pests belonging to the Tineidae attack clothing and other animal products, during the processing or manufacturing stage as well as in storage. The insects cause considerable loss or damage to the commodity in terms of quantity, quality and market value. Insect control measures in these products differ depending on the conditions of storage or processing and the relevant cost factor. Fumigation products play an important role in insect pest elimination for the majority of stored animal products. The need of detailed studies on alternatives methods involving the exploitation of insect traps for pest management has been highlighted. In the past years test systems for the evaluation of attractant products have been developed and further refined in our laboratory. In this study, a standardized test system for the evaluation *in vitro* of commercial traps for *Tineola bisselliella*, the webbing clothes moth (WCM), was validated. Three sticky traps baited with female-produced sex pheromone were tested in the laboratory and *in vivo* at the "Mobilier National", a repository of valuable artefacts. In both types of experiment the number of individuals captured was closely linked with the kinetic release of volatile organic compounds (VOCs) from the sticky traps. The test system allowed clear discrimination of the products according their efficiency in a reliable manner but none of the traps tested were effective enough to reduce the damage potential of the pest.

In the light of these results, the characteristics needed for an effective trap in warehouse conditions is discussed.

1. Introduction

Webbing clothes moth (WCM) *Tineola bisselliella* (Hummel) is a widely distributed pest insect all over the world. The capacity of larvae to digest wool and other hard keratins (Robinson et al., 1993) causes problems in museums, warehouses and households by feeding on textiles such as wool, fur and other products of animal origin. Depreciation caused by keratophagous insects including WCM approximates to one billion dollars annually in the United States alone (Metcalf and Metcalf, 1994). The existing methods of treatment and management do not always control the populations, thus significant economic losses continue. The development of new methods for detection, prevention and management are still awaited (Cox and Pinninger, 2007). *Tineola bisselliella* is highly resistant to physical or chemical stress and the traits of WCM life history have enabled the spread of infestation. The establishment of WCM infestation is closely related to the widespread availability of its food supply and its particular resistance to extreme environmental conditions. WCM resists high and low temperature extremes very well (brief exposures up to 51 °C and tolerant of cold with a supercooling point of −22.6 °C; Chauvin and Vannier, 1997) and is able to develop at 0% relative humidity (r.h.). Indoors, development is continuous and WCM can pass through several generations each year unlike in outdoor habitats (birds' nests). The development of *T. bisselliella* is between 80 and 150 days, depending on the food, temperature and r.h. (Chauvin, 1977). The adults being unable to feed live only about 10 days and are active at night, sheltering during the day. The female scatters up to 250 eggs on suitable substrates and, after 10 days at room temperature, larvae hatch (Griswold, 1944). At the end of the last instar, the

* Corresponding author.
E-mail address: ingrid.arnault@univ-tours.fr (I. Arnault).

0022-474X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jspr.2012.03.004

caterpillar locks itself into its webbing gallery weaving a silk cocoon inside the stiff walls. The silk spun by members of the family Tineidae protects larvae from attack by parasites and predators (Scoble, 1992) and desiccation. The mature larva pupates within the cocoon and the pupa transforms into an adult after 10–15 days.

The interaction between WCM and host material are mediated by semiochemicals. Traynier et al. (1994) showed that the odors from the faeces of conspecific larvae that had eaten diet containing dried yeast increase localized oviposition. Conversely treatment of wool and other products with repellents can reduce WCM damage. Intraspecific interactions are also mediated by volatile organic compounds. The female sex pheromone is composed of two compounds; koiganal I, (2E)-octadecenal and koiganal II, (E, Z)-2,13-octadecadienal; identified by Yamaoka et al. (1985) in Japanese populations of WCM. Takács et al. (2001) have showed that (E, Z)-2, 13-octadecadienol and (E, Z)-2, 13-octadecadienal were also attractive for North American populations of WCM. These two sex pheromones are not species specific and seem to be active in other Lepidoptera (Naka et al., 2007; Mozuraitis and Karalius, 2007). The relative composition of the two compounds in the pheromone mixture affects the level of attractancy to males.

(E, Z)-2, 13-octadecadienal has also been identified as an attractant for *Opogona sacchari* Bojer (Lepidoptera: Tineidae) and is used for monitoring populations of this moth (Jang et al., 2007).

Besides the response to the sex pheromone, aggregation kairomones (9Z)-hexadecanoate and methyl hexadecanoate attract male and virgin female but not mated females of WCM (Takács et al., 2001).

Unless chemical pest management programs are targeted effectively, they could prove noxious to the environment, applicator or treated collection. Furthermore laboratory data on the development of fumigant resistance in insect pests of stored animal products are still needed (Rajendran and Hajira Parveen, 2005). Alternative methods to programmes based on chemicals alone are needed to develop an environmentally sustainable management programme to prevent infestations of WCM becoming established.

The use of extreme temperature as a method of pest control with precaution to prevent damage is very widespread but does not apply for all fabrics and furniture (Stansfield, 1989; Chauvin and Vannier, 1997).

Among biopesticide products, some plants extracts have been reported as repellents or antifeedants against WCM. Extract of neem seeds, containing 1% azadirachtin presented a biopesticidal effect on larvae (Gerard and Ruf, 1995). Extracts of the leaves of the New Zealand native tree *Pseudowintera colorata* showed insecticidal and antifeedant activity (Gerard et al., 1993). Oil of cloves and citronellol in combination with oil of lavender also showed promising repellent effects for 4 weeks (Plarre et al., 1997).

Biological control methods have not been developed because of the difficulties of application in situations where infestations occur. The identification of suitable natural enemies such as parasitic wasps has been little investigated. The larval endoparasitoid *Apanteles carpatus* (Say) (Hymenoptera: Braconidae) is able to complete development in all larval stages and volatile compounds from host larvae habitat play a major role in the location of its host (Takács et al., 1997). The tritrophic interaction between *A. carpatus*, its clothes moth habitats and kairomones of host habitat derived is similar to the well known relationships between parasitoid and herbivore and semiochemicals released from host plants. *Apanteles carpatus* is thus considered as a candidate for biological control (Plarre and Balnuweit, 2003). Furthermore, many strains of the egg parasitoids *Trichogramma* spp. show ready host acceptance *in vitro* conditions (Zimmermann et al., 2003) but no data are available about location of host habitat in practice.

Trapping is known to play an important role in the management of insect pests particularly in museums (Ackery et al., 1999; Trematerra and Fontana, 1996). In museums, sticky ‘blunder’ traps are traditionally used for monitoring insect infestation. Pheromone-baited traps have been found to be particularly effective in the detection and monitoring of *T. bisselliella*, *Anthrenus verbasci* (L.) and *Lasioderma serricorne* (F.). The trapping of *T. bisselliella* using the sex pheromone is available commercially following the patent of Takács et al. (2003). These pheromone-baited sticky traps have been used regularly to successfully monitor *T. bisselliella* populations in museums and historic houses but as they only attract male moths, their efficiency in decreasing the population level is not significant (Trematerra and Fontana, 1996).

Some traps are stated to capture WCM but their real efficiency is unknown. In this study we compared the attractivity of three commercially available sticky traps baited with the female-produced sex pheromone of *T. bisselliella* to reliably assess trap efficiency. Firstly the kinetic release of volatile organic compounds (VOCs) from the traps was monitored by SPME-GC-MS. Secondly,

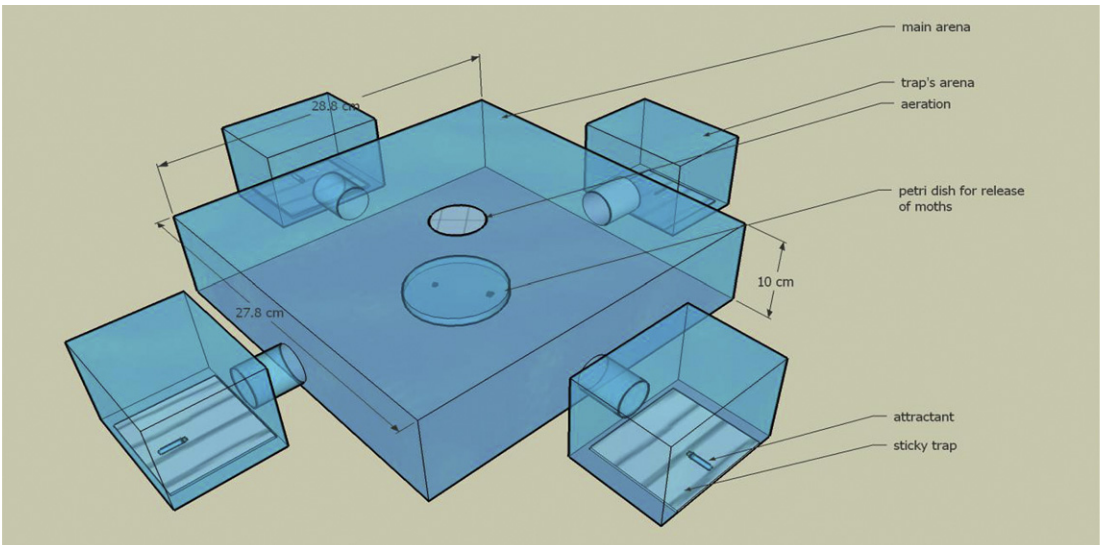

Fig. 1. Olfactometer apparatus.

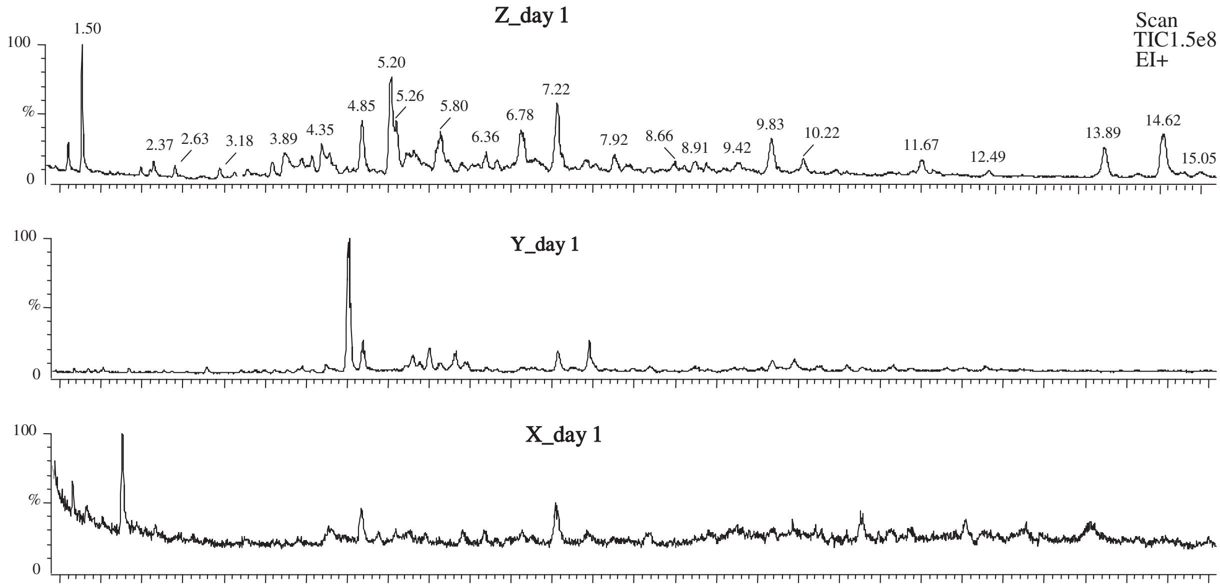

Fig. 2. VOCs profiles of the three products after one day of using.

the behavior of *T. bisselliella* in the presence of the traps was observed under *in vitro* and *in vivo* conditions.

2. Methods and materials

2.1. Commercial products

The three attractant products tested for their comparative efficiency were:

1. Webbing clothes moth "bullet lure", Insect limited Inc 16950 Westfield Park Road, Westfield, IN 46 074, U.S.A
2. CAT-QLURE- WCM Russel IPM®, Unit 68,Third Avenue, Deeside Park, Deeside, Flintshire, CH5 2LA
3. United Kingdom, Finicon® FINICON Sticky Pads

Each sticky trap was supposed to contain the female sex pheromones koiganal I, koiganal II and (E, Z)-2,13-octadecadienol; but the composition of the blend was not really known. They may also have contained semiochemical attractants from larval habitats, food, or male aggregation pheromones (Takács et al., 2003). The three products were named X, Y and Z and subjected to blind tests.

2.2. Chemical analysis

The VOCs released by the products were trapped by SPME (solid phase micro-extraction) and analyzed by GC/MS. GC/MS analyses were performed on a benchtop Perkin–Elmer Turbomass (Shelton, USA) system with a split–splitless injector and a fused-silica capillary column 30 m × 0.32 mm bonded non-polar with a film thickness of 0.25 µm (Biorad, Germany). The carrier gas was helium, injector temperature at 250 °C and detector temperature at 320 °C. The temperature program was 50 °C–250 °C with a heating rate of 5 °C/min. The transfer line and the source temperature were maintained at 250 °C. Total ion chromatograms and mass spectra were recorded in the electron impact ionization mode at 70 eV.

VOCs were extracted head space mode using 50/30 µm DVB/Carboxen/PDMS/StableFlex coated glass fiber (Supelco Co., Bellefonte, USA, Cat-No. 5–7348). Initial conditioning of the fiber is typically required for 1 h at 270 °C. Each product was introduced in a 50 mL flask and VOCs were trapped over a 2-h period, ten times during 3 months.

2.3. In vitro experiments

The mass rearing of WCM was initialized in 2009 with larvae in wool from the "Mobilier National". WCM were reared in plastic boxes with mesh lids at 25 ± 1 °C, 40–60% r.h. and a 12L:12D inversed photoperiod. Larvae were provided with untreated, untanned, dry rabbit pelts with hair. Every day adult webbing clothes moths were collected and their sex determined through the presence of claspers and aedagus (male) or ovipositor (female).

To estimate the efficiency of the three attractants in choice and non-choice conditions, experiments were assessed in a closed arena olfactometer with four arms (Fig. 1). This device was prepared for the laboratory tests and was cleaned with alcohol between every bioassay. Each attractant was randomly placed and 15 individuals (1–4 days old) were released from the Petri dish in the center of the main arena. After 24 h, the number of trapped moths for each attractant was noted. The olfactometer tests were ran in the dark at 20 °C and 70% r.h. All the experiments were conducted at the maximum rate of the VOCs emission between two and 10 days after opening according to the products. The experiment was repeated 3 times.

Statistical analyses were performed using the software XLSTAT Version 2008.6.03.

Table 1
Retention time of the 11 relevant compounds found in the three products; X: detected; –: not detected.

Retention time	0.64 Acetic acid	1.51 hexanal	2.2	2.6	4.4	4.84 decane	5.1	6.8 undecane	7.2	9.78	14.52
Product X	X	X	_	_	X	X	_	X	X	X	_
Product Y	_	_	_	_	X	X	_	X	X	X	_
Product Z	X	X	X	X	X	X	X	X	X	X	X

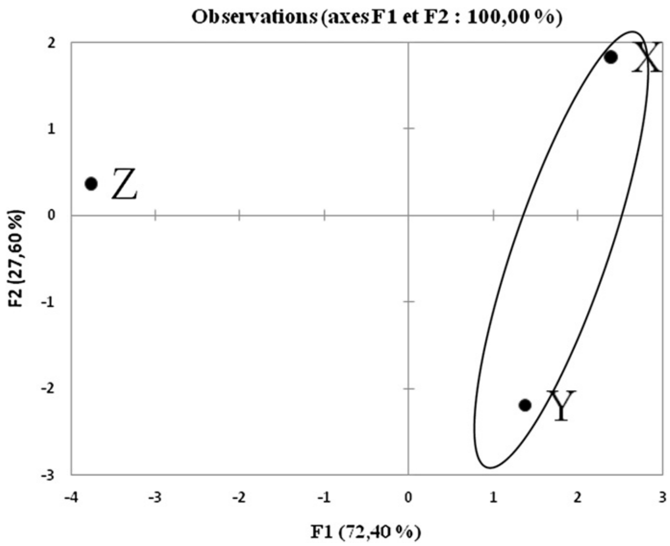

Fig. 3. PCA with the proportion of the 11 VOCs.

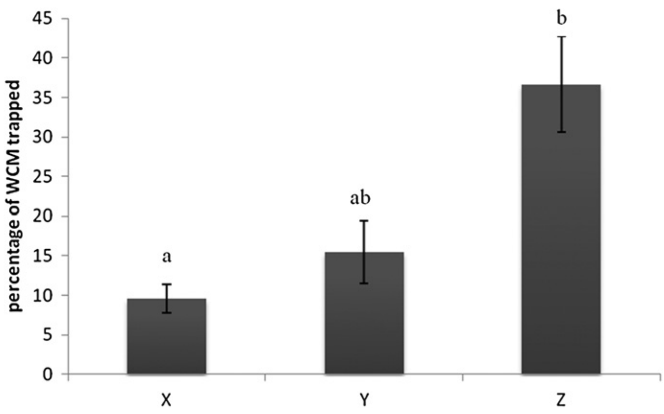

Fig. 5. Average percentage of webbing clothes moth (WCM) trapped for the bioassay *in vitro* (Dunn statistical tests, XLstat©).

2.4. In vivo experiments

The "Mobilier National" is situated in the 13th district of Paris (France) and is a depository for ancient furniture of the "Couronne de France". Their mission is to preserve, repair and maintain more than 80,000 movable objects and textiles (tapestries, carpet).

The three traps were randomly placed on shelves in three areas of the "Mobilier National" housing mainly chairs and carpets. These were rooms of 750 m^2, 5 m high with air-conditioning systems. In these places, the adults of WCM are present all year round but with statistically significant seasonal fluctuations. The rooms were monitored over a 3-year period and regular infestations occurred during summer and spring. The individuals trapped were counted over a 4-month period from May to September 2009.

3. Results

3.1. Chemical analysis

The VOCs profiles obtained with SPME/GC/MS analysis are shown in Fig. 2. Eleven compounds were identified as representative of the VOCs composition (Table 1). Among them, hexanal, decane and undecane were identified with the standard compound. The principal component analysis (PCA) with the proportions of VOCs indicated that Z was different from X and Y (Fig. 3). The results indicated a high attractive potential for Z. We also analyzed the persistence of the VOCs until the threshold of detection of the analytical method was reached. The kinetic of VOC release differed between the products (Fig. 4). In summary, 3 phases of diffusion were distinguished: high release between 0 and 40 days, secondly a phase of low emission between 40 and 60 days and then a last phase of very low emission until 90 days (Fig. 4). The VOCs were not detectable after this last phase. The data indicated that the optimal period for conducting the bioassay *in vitro*: was between 2 and 10 days, according to the product, after opening.

3.2. In vitro experiments

The captures performed by the three traps were quite different from each other (Fig. 5). The highest performance was revealed by the Z trap.

We conducted further experiments with Z *in vitro* to determine the percentage of trapped populations and the corresponding sex ratio. With bioassays containing 55 individuals, 42% the WCM population was trapped and 71% of the males were attracted by Z.

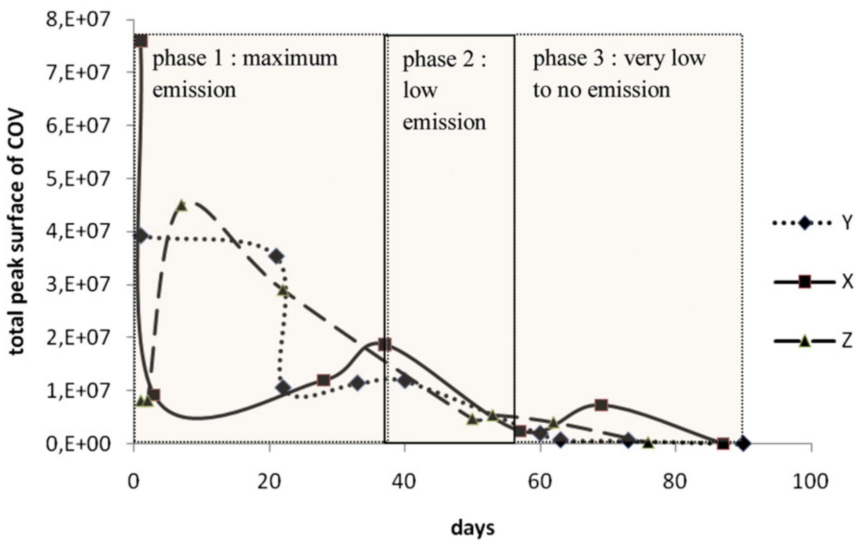

Fig. 4. Release kinetics of VOCs of the three products during three months.

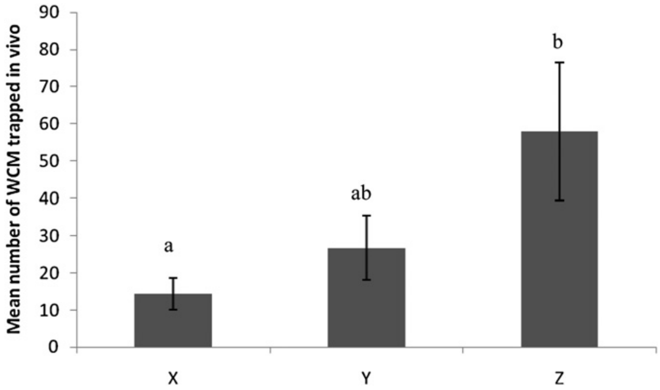

Fig. 6. Mean number of webbing clothes moths (MCM) trapped/per product in the National Reserve during 4 months (Dunn statistical tests, XLstat©).

3.3. Field experiment

The number of individuals trapped by Z was 3–6 times higher than that of X or Y (Fig. 6), which showed similar catch levels. Secondly, the profile of WCM captures followed the pattern of release of VOCs (Fig. 7). The first phase represented the maximum of trapping, the second phase showed less captures and during the third period the trapping of individuals petered out. The chemical analysis and the bioassays *in vitro* and *in vivo* all showed that Z was more attractive than products X and Y.

4. Discussion

The patent related to the chemical composition of a trap for control of WCM was published by Takács et al. (2003). No study has reported the efficiency of the specific semiochemicals present in commercial attractant products in controlling the behavior of WCM. This study brings new information on the efficiency of three traps baited by the synthetic pheromone in different *in vitro* and *in vivo* bioassays.

Field and laboratory tests often give very different results. In our study, the results *in vitro* and *in vivo* conditions matched. Thus the *in vitro* test system composed of a multi-armed closed arena olfactometer has been validated for the study of WCM's behavior in response to attractant products used for WCM control. This apparatus can be further used for any study on *T. bisselliella* behavior under trapping conditions such the sex ratio and the percentage of a population that is likely to be trapped. The study on kinetic release of volatile organic compounds (VOCs) from the sticky traps gave an indication of the duration of efficiency of each.

The results demonstrated statistical differences between the traps in trapping efficiency. All the experiments *in vitro*, *in vivo* and the release of VOCs showed clearly that Z had the highest level of attractancy especially during the first month of using, trapping about 40% of a population, about 70% of the males. VOCs were detectable until 3 months after exposure for all products.

Integrated pest management (IPM) successfully practiced in agricultural systems can be adapted for use in stored products. The integration of physical and biological control methods can be envisaged for WCM. Particularly, real disturbance of the population dynamics of WCM (mating, feeding etc.) would result in a decrease of damage. The population level of the pest insect is the result of balance between two constituents: the potential of reproduction and the potential of survival. The potential of reproduction is inherent in the species, controlled by the expression of the genome of the individual in terms of the fertility, the sex ratio and the voltinism. The potential of survival of a pest is a function of the capacity to adapt to the surrounding environment by modification of its physiology or behavior. The resistance that pests can develop in response of biotic or abiotic stress is also of importance.

Some specific methods targeting the reproduction and particularly females and eggs of WCM could in theory reduce the population and thus the damage caused. An integrated method for the control of WCM could combine:

- the best available monitor of the population, currently the Z trap
- a very specific semiochemical as an aggregation kairomone simulating larval habitats for the attraction of females (Takács et al., 2001)
- a biological control agent for immature stages: parasitoids such as *Trichogramma piceum* (Dyurich) for eggs (Zimmermann et al., 2003) or *A. carpatus* (Say) for larvae (Plarre et al., 1999).

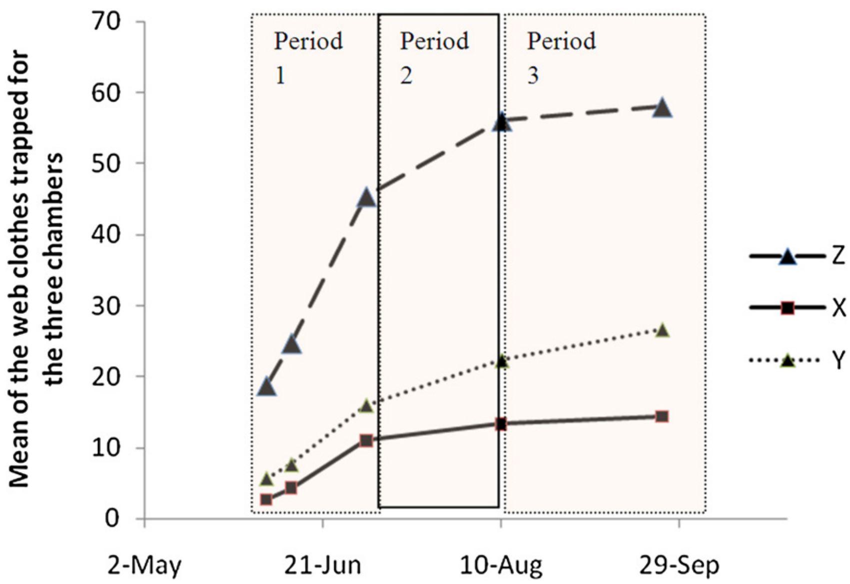

Fig. 7. Number of webbing clothes moths (web clothes) trapped during four months in the Mobilier National (*in vivo*).

This IPM system could be tested for its efficiency with our *in vitro* model together with experiments under *in vivo* conditions over several years to assess whether a significant decrease of WCM populations can be achieved.

Acknowledgments

This study was possible through on-going activities of the Laboratory of Research on Historical Monuments, financially supported by the Government of France represented by the Ministry of Culture.

References

Ackery, P.R., Pinniger, D.B., Chambers, J., 1999. Enhanced pest capture rates using pheromone-baited sticky traps in museum stores. Studies in Conservation 44, 67–71.

Chauvin, G., 1977. Contribution à l'étude des insectes kératophages (Lepidoptera: Tineidae): leurs principales adaptations à la vie en milieu sec. Thesis, Université de Rennes, France, 297 pp.

Chauvin, G., Vannier, G., 1997. Supercooling capacity of *Tineola bisselliella* (Hummel) (Lepdioptera: Tineidae): its implication for disinfestation. Journal of Stored Products Research 33, 283–287.

Cox, P.D., Pinninger, D.B., 2007. Biology, behavior and environmentally sustainable control of *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae). Journal of Stored Products Research 43, 2–32.

Gerard, P.J., Perry, N.B., Ruf, L.D., Foster, L.M., 1993. Antifeedant and insecticidal activity of compounds from *Pseudowintera colorata* (Winteraceae) on the webbing clothes moth, *Tineola bisselliella* (Lepidoptera: Tineidae) and the Australian carpet bettle, *Enthrenocerus australis* (Coleoptera: Dermestidae). Bulletin of Entomological Research 83, 547–552.

Gerard, P.J., Ruf, L.D., 1995. Effect of a neem (*Azadirachta indica* A. Juss, Meliaceae) extract on survival and feeding of larvae of four keratinophagous insects. Journal of Stored Products Research 31, 111–116.

Griswold, G.H., 1944. Studies on the biology of the webbing clothes moth (*Tineola bisselliella* (Hum). Memoirs of the Cornell Agricultural Experimental Station 262, 1–59.

Jang, E.B., Siderhurst, M.S., Hollingsworth, R.G., Showalter, D.N., Troyer, E.J., 2007. Sex attractant for the banana moth, *Opogona sacchari* Bojet (Lepidoptera: Tineidae): provisional identification and field evaluation. Pest Management Science 66, 454–460.

Metcalf, R.L., Metcalf, R.A., 1994. Attractants, repellents, and genetic control in pest management. In: Metcalf, R.L., Luckman, W.H. (Eds.), Introduction to Insect Pest Management, third ed. John Wiley, New York, pp. 355–356.

Mozuraitis, R., Karalius, V., 2007. Identification of minor sex pheromone components of the poplar clearwing moth *Paranthrene tabaniformis* (Lepidoptera, Sesiidae). Journal of Biosciences 62, 138–142.

Naka, H., Inomata, S.-I., Matsuoka, K., Yamamoto, M., Sugie, H., Tsuchida, K., Arita, Y., Ando, T., 2007. Sex pheromone of two Melititini species, *Macroscelesia japona* and *M. longipes*: identification and field attraction. Journal of Chemical Ecology 33, 591–601.

Plarre, R., Pöschko, M., Prozell, S., Franck, A., Wohlgemuth, R., Phillips, J.K., 1997. Effects of oil of cloves and citronellol, two commercially available repellents, against the webbing clothes moth *Tineola bisselliella* Hum. (Lepidoptera: Tineidae). Anzeiger fur Schädlingskunde, Pflanzenshutz, Umweltschutz 70, 45–50.

Plarre, R., Liber, K., Burkholder, W., Philipps, G., 1999. Host and host instar preference of *Apanteles carpatus* (Say) (Hymenoptera: Braconidae) a possible parasitoid for biological control of clothes moths (Lepidoptera: Tineidae). Journal of Stored Products Research 35, 197–213.

Plarre, R., Balnuweit, O., 2003. Biology of *Apanteles carpatus*, a parasitoid of tineid moths (Lepidoptera: Tineidae). In: Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M., Highley, E. (Eds.), Proceedings of the 8th International Working Conference on Stored Product Protection, 22–26 July 2002, York, UK. CAB International, Wallingford, UK, pp. 169–172.

Rajendran, S., Hajira Parveen, K.M., 2005. Insect infestation in stored animal products. Journal of Stored Products Research 41, 1–30.

Robinson, C.P., Ciccotosto, S., Gaal, A.M., Sparrow, L.G., 1993. Purification of cysteine lyase from larvae of the webbing clothes moth, *Tineola bisselliella*. Insect Biochemistry and Molecular Biology 23, 491–493.

Scoble, M.J., 1992. The Lepidoptera. Form, Function and Diversity. Oxford University Press, UK.

Stansfield, G., 1989. Physical methods for pest control. Journal of Biological Curation 1, 1–4.

Takács, S., Gries, G., Gries, R.M., 1997. Semiochemical-mediated location of host habitat by *Apanteles carpatus* (Say) (Hymenoptera: Braconidae) a parasitoid of clothes moth larvae. Journal of Chemical Ecology 2, 459–472.

Takács, S., Gries, G., Gries, R., 2001. Communication ecology of webbing clothes moth: 4. Identification of male- and female-produced pheromone. Chemoecology 11, 153–159.

Takács, S., Gries, G., Gries, R.M., 2003. Semiochemicals and Sonic Signals for Monitoring and Control of Clothes moths. US Patent Number 6574913.

Traynier, R.M.M., Schumacher, R.K., Lau, D.M., 1994. Oviposition site selection by *Tineola bisselliella*, *Tinea* spp (Lepidopetra: Tineidae) and *Anthrenus flavipes* (Coleoptera: Dermestidae). Journal of Stored Products Research 30, 321–329.

Trematerra, P., Fontana, F., 1996. Monitoring of webbing clothes moth, *Tineola bisselliella* (Hummel), by sex pheromone. Anzeiger fur Schädlingskunde, Pflanzenshutz, Umweltschutz 69, 119–121.

Yamaoka, R., Shiraishi, Y., Ueno, T., Kuwahara, Y., Fukami, H., 1985. Structure elucidation of Koiganal I and II, the sex pheromones of the webbing clothes moth, using capillary GC/MS. Mass Spectroscopy 33, 189–195.

Zimmermann, O., Schöller, M., Prozell, S., 2003. Investigations on the biological control of *Tineola bisselliella* with *Trichogramma* species (Hymenoptera: Trichogrammatidae). In: Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M., Highley, E. (Eds.), Proceedings of the 8th International Working Conference on Stored Product Protection, 22–26 July 2002, York, UK. CAB International, Wallingford, UK, pp. 319–321.