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Efficacy of pheromones for managing of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, in food and feed processing facilities

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DOI 10.5073/jka.2018.463.105

Abstract

In recent years, considerable progress has been made in the monitoring and control of Lepidoptera, by pheromones also used in mass-trapping, attracticide (lure and kill), mating-disruption, auto-confusion methods. In context of IPM "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. In the present paper are reported some promising results offering efficient control of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, populations in food and feed processing facilities based on pheromones and line up a number of remaining questions to be answered to improve the reliability and competitiveness of the methods used. These field researches show potential for successful pheromone-based suppression methods for Mediterranean flour moths in practical applications.

Keywords: Mediterranean flour moth, *Ephestia kuehniella*, pheromones, monitoring, mass-trapping, attracticide method, mating-disruption.

1. Introduction

Pheromones and other semiochemicals have been identified for more than 40 species of stored-product insects over the past four decades. In recent years, considerable progress has been made not only in monitoring but also in direct control of stored-product insects by different techniques (Burkholder, 1990; Chambers, 1990; Phillips, 1997; Trematerra, 2002 and 2012; Phillips *et al.*, 2000; Cox, 2004; Anderbrant *et al.*, 2007; Campos and Phillips, 2010 and 2014; Savoldelli and Trematerra, 2011; Plarre, 2013; Athanassiou *et al.,* 2016; Trematerra *et al*., 2017).

In the present paper are reported the main results obtained in the control of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, populations by means of mass-trapping, attracticide (lure-and-kill), mating-disruption, and auto-confusion methods applied in food and feed processing facilities.

2. Mass-trapping method

As it is know any attempt to suppress the population by mass-trapping would require a sufficient number of trapped males so that nearly all females would be unmated. Theoretical considerations of mass-trapping males take into account the density of males in the population and the potential number of matings that a male is able to secure in its lifetime. If a male can mate with 6-10 females in a lifetime, as is the case of the Indian meal moth, *Plodia interpunctella* (Hübner), 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 (Brower, 1975).

Early attempts of mass-trapping were conducted by using pheromone blends of many target insects species. A major problem was the quantification of the number of traps necessary per unit area to achieve an effective control. Proper experiments of mass-trapping are not easy to conduct due to inadequate controls or poor replication, especially in commercial food/feed storage and processing facilities.

In practice the effectiveness of the mass-trapping technique can be reduced by factors such as: inefficient trap design, saturation of traps especially in situations of high pest density, poor pheromone release or duration, attraction of only one sex, inappropriate positioning of traps and the extensive immigration of new pests from outside the area treated with pheromones (Trematerra and Gentile, 2010).

Food lures used in combination with pheromones may offer a way of enhancing the effectiveness of mass-trapping system for stored product pests trapping males and females of a target species (Chambers, 1990; Toth et al., 2002; Cox, 2004; Trematerra, 2012).

Recent studies have investigated the potential of pheromone based mass-trapping methods to control indoor populations of *E. kuehniella* (Trematerra, 1990; Süss *et al*., 1996; Trematerra and Battaini, 1987; Athanassiou *et al*., 2003; Anderbrant *et al*., 2007; Trematerra and Gentile, 2010). In particular, Trematerra and Battaini (1987) demonstrated that integrated control of *E. kuehniella* could be achieved by mass-trapping. Furthermore, Trematerra (1990) reported results obtained by the practical application of mass-trapping to control an infestation of *E. kuehniella* in a flour-mill.

Subsequently Trematerra and Gentile (2010) presented the 5 years results of applying the mass-trapping method to contain *E. kuehniella* populations infesting a large traditional flour-mill in Central Italy. The study also investigated the effectiveness of mass-trapping, combined with other pest control techniques, at improving the procedures applied to combat *E. kuehniella* infestations using an IPM approach. Over 5 years pheromone funnel-traps baited with 2 mg of (*Z,E*)-9,12-tetradecadienyl acetate (TDA) attracted a total of 54,170 males. The constant presence of the traps caused a marked decrease of *E. kuehniella* populations. The results of the study have shown that the population density of the moth can be effectively reduced and maintained at a low level by means of mass-trapping techniques accompanied by localized insecticide treatments, and careful cleaning of various mill areas and equipment (Figure 1).

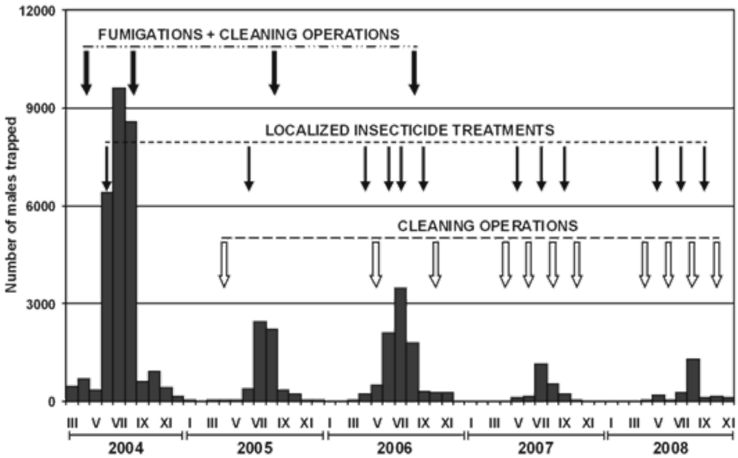


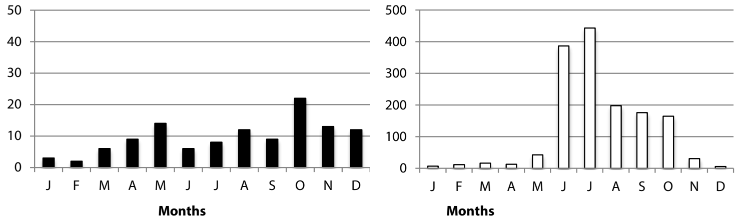
Fig. 1 Mass-trapping, cumulated montly trap catches of *Ephestia kuehniella* males inside a flour-mill.

3. Attracticide (lure-and-kill) method

Attracticide (lure-and kill) method is in some ways 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. There are various promising results on the use of the attracticide concept in flour-mills and confectionary industries in the control of *Ephestia cautella* (Walker) and *E. kuehniella* in Italy (Trematerra and Capizzi, 1991; Trematerra, 1995). Preliminary results on attracticide for *Plodia interpunctella* (Hb.) also have been reported more recently in the United States (Nansen and Phillips, 2002 and 2004; Campos and Phillips, 2010, 2013 and 2014).

In Italian flour-mills, *E. kuehniella* males were successfully lured to laminar dispensers (2 cm x 2 cm), baited with 2 mg of TDA (daily release of 13 µg) and treated with 5 mg of cypermethrin; this caused a marked decrease in moth population. Trematerra and Capizzi (1991) performed behavioural tests involving olfactometer, electroantennogram, and insecticide efficacy evaluations in order to clearly determine the effectiveness of pheromone and toxicant in the attracticide method. In field tests in a practical application was conducted to determine the degree of control of *E. kuehniella*. In the olfactometer tests 80 to 90% of males responded to pheromone plus insecticide dispensers, confirming the low repellency of cypermethrin in sexual behaviour. In this context the possible interaction between optical and pheromone stimuli was also studied.

Later encouranging results were obtained by Trematerra (1995) using attracticide applications in flour-mills placed at 220 to 280 m^3 intervals. This experiment was undertaken in Central Italy in a large mill of 16,000 m^3 that produced flour and semolina. During 2 years of application the attracticide method removed males from *E. kuehniella* populations preventing an increase in the residual population. The prolonged presence of the treated dispensers in the four-mill, particularly during periods when the moths were able to breed, led to a reduction throughout the flour-mill, including areas where no processing occured. After the two years of using the attracticide method, the usual second fumigation of mill proved to be unnecessary. The continuous presence of attracticide dispensers in the mill caused a marked decrease in the *E. kuehniella* population also during the third year.



Figs 2-3 Dynamic population of *Ephestia kuehniella* males in a traditional flour-mill protected by attracticide method (2) and in untreated traditional flour-mill (3).

4. Mating-disruption methods

The mating-disruption technique generally requires use of much larger quantities of pheromones than those employed for mass-trapping and attracticide method. Therefore, the mating-disruption may be practical if the pheromones are inexpensive. In addition, there may be concerns about possible contamination of the stored products in situations where high concentrations of pheromone come in direct contact with the product. Therefore, the application of mating-disruption requires the simultaneous development of a reliable system to monitor if the method is effective for the intended time period. The response of females in the presence of high concentrations of pheromones must be evaluated. The low level of matings and the pheromonal substance present inside the treated area could induce females to leave such areas in favour of outdoor areas and could also stimulate dispersal (Trematerra, 1994; Shani and Clearwater, 2001; Trematerra *et al*., 2013). Also, it has been suggested that insects may evade the effect of mating-disruption by progressive elevation of pheromone production and response threshold or through a change in pheromone composition over consecutive generations to compete with the background pheromone. Studies carried out in field indicate that there is a significant correlation between some of these factors and the spatial distribution of several Lepidoptera in food-processing facilities (Nansen *et al*., 2003; Trematerra and Sciarretta, 2005; Trematerra and Gentile, 2010; Athanassiou *et al*., 2016).

Another disadvantage of mating-disruption is that the method does not prevent mated female immigration from adjacent areas, thus oviposition and subsequent infestation are likely to still occur (Cardè and Minks, 1995; Jones, 1998). Hence, monitoring of female activity and/or oviposition is essential when developing a mating-disruption-based control program (Savoldelli and Trematerra, 2011).

The component pheromone (*Z,E*)-9,12-tetradecadienyl acetate attracts males of several Pyralid moths, thus, this 'multi-species pheromone' has been used successfully for mating-disruption in stored-product facilities. Particularly the use of mating-disruption against pyralid moths, in stored-product facilities has been evaluated, with promising results, in both laboratory and field tests. Several studies from many parts of the world have shown more or less similar results for *E. cautella*, *E. kuehniella*, and *P. interpunctella* (Phillips, 1997; Trematerra, 2002; Plarre, 1998; Ryne *et al*., 2001, 2006 and 2007; Anderbrant *et al*., 2007 and 2009; Sieminska *et al*., 2009; Mueller, 2010; Trematerra *et al*., 2011; Campos and Phillips, 2014).

However, there are consistent methodological problems with evaluating mating-disruption in practice, such as defining what a replicate is and estimation of control based on trap captures (Anderbrant *et al.*, 2009; Sieminska *et al.*, 2009). Ryne *et al.* (2007) compared two adjacent storage rooms, one that was treated with mating-disruption and one that was not, and found using electrophysiological recordings (male antennal response) that there was leakage of pheromone into the untreated room. Mating-disruption-based experiments usually use a single or low number of treatment and control rooms. Each food processing and storage facility is unique that makes finding a 'control facility' which is similar with the treated facility extremely difficult (Sieminska *et al.*, 2009). As a result, there is still inadequate information on mating-disruption effectiveness under different microclimates and in different types of facilities.

Sieminska *et al.* (2009) present results from long-term monitoring of *E. kuehniella* populations in two similar flour mills in Poland. One mill was treated with pheromone for mating-disruption for two years, whereas the other mill was untreated. Thirty pheromone dispensers (one per 100 m^3 factory volume), each releasing about 2 mg TDA per day, were used. The reduction in trap catch during the mating-disruption treatment was about 90% or more, compared with the untreated mill or pre-treatment periods in the mill where mating-disruption was practiced. The reduction was larger during the second year of mating-disruption than during the first year. One of the basic drawbacks of mating-disruption method is that oviposition by mated females that enter areas under treatment from untreated areas can still occur (Jones, 1998; Athanassiou *et al.*, 2003; Campbell and Arbogast, 2004; Trematerra and Gentile, 2010). Consequently, the number of captured males in monitoring pheromone-baited traps may not be a clear indicator of mating-disruption.

To avoid these methodological issues, Trematerra *et al.* (2011) conducted a two-year, large-scale experiment that included eight facilities located in Czech Republic, Greece and Italy. The facilities were flour-mills, food and drug storage rooms, and warehouses storing organic foods, pasta, raisins, or wheat. Dispensers of cellulose pad, each with 50 mg of TDA were placed at a rate of one dispenser per 9 m^2 (or one dispenser per 54 m^3). Based on the results reported in some storage facilities and trap-check dates, the suppression of captures in the mating-disruption-treated areas was <95% in comparison with untreated areas, suggesting that some mating may have occurred.

Generally, there is no clear indication that the moth species made a difference in mating-disruption program effectiveness, so Trematerra *et al.* (2011) proposed that the mating-disruption method had the same efficacy level for *E. cautella*, *E. kuehniella* and *P. interpunctella*. The use of a single pheromone component [(*Z,E*)-9,12-tetradecadienyl acetate] to accomplish simultaneous suppression of more than one pest species is an additional advantage for using mating-disruption in storage facilities (Anderbrant *et al.*, 2009).

In large-scale experiments with mating-disruption dispensers, the 'untreated' areas may not serve accurately as 'controls' because of the potential air permeation from the treated. Also mating-disruption may have a cumulative effect after multiple years of implementation. Historical data from previous years, concerning both adult captures and larval presence for the target facilities, may serve more accurately as 'controls' because it can also reflect seasonal patterns in activity.

Oviposition and/or immature emergence should be monitored, in conjunction with adult activity in pheromone-baited traps, to indicate if successful mating-disruption is occurring. In this regard the pheromone effect on population growth or decrease could be measured by the presence of spermatophores in females (Trematerra and Savoldelli, 2013). Also in this case, one of the most important factors impacting the efficacy of mating-disruption is the population density.

Three years of field trials (from 2007 to 2009) were carried out in Central Italy by Trematerra and Spina (2013) to evaluate MD of the Mediterranean flour moth, dispensers containing the pheromone TDA were placed in two traditional flour mills. Pheromone-baited funnel traps were used to monitor the population fluctuations of moth males throughout the entire experimental period; female oviposition was assessed by placement of petri cups containing wheat germ–semolina flour bait. According to the results, the use of MD dispensers does not interfere completely with the reproduction of *E. kuehniella*. However, looking at the overall data, there was a significant reduction in both adults and larvae in treated mills after the MD application. According to hazard analysis and critical control point procedures (HACCP), treatment should be accompanied by general cleaning of the facilities, including corners and inside machinery, where insects can hide and reproduce.

In integrated pest management programs, the use of MD can lead to a drastic reduction in the need for chemical treatments, with improvement in food quality.

5. Auto-confusion

A particular method of mating disruption is auto-confusion, Baxter *et al.* (2008) and Hugget *et al.* (2010) reported preliminary laboratory studies to examine behavioural effects of auto-confusion on virgin male *P. interpunctella*. The method used TDA, combined with a patented electrostatic powder delivery system to disrupt mating and interrupt the lifecycle of several moth pests. Laboratory flight tunnel studies showed that contact with SP-Tab auto-confusion significantly reduced the ability of male *P. interpunctella* to locate females for up to two days.

These males could increase the confusion effect by becoming competitive attractive point sources for other males (Huggett *et al.*, 2010). Using auto-confusion Pease and Storm (2010) presented preliminary practical trials that were conducted in two flour-mills in UK and in a spice factory in Netherlands. Populations of *E. kuehniella* and *P. interpunctella* were monitored. In all cases populations were reduced compared to the same area in the previous year and compared to untreated control areas in accordance with local pest control practices.

Preliminary results of the SP-Tab auto-confusion system for mating-disruption of *P. interpunctella* in 2008 and 2010 was reported from United States by Campos and Phillips (2014).

Trematerra *et al.* (2013) applied Exosex SPTab dispensers that contained the Entostat powder, at a 5x5 m grid, in three facilities, one feed-mill in Italy and two retail stores in Greece. In the feed-mill, the most abundant pyralid species was *Ephestia kuehniella*. Monitoring through pheromone-baited traps in this facility indicated that the application of the Exosex SPTab dispensers decreased the number of captures 2 months after the initial application. In the case of the facilities in Greece, the most abundant species was *Plodia interpunctella*. In these facilities there was a continuous monitoring of moth populations from January 2008 until February 2011, with pheromone-baited traps and Petri dishes with semolina, which served as oviposition traps. In both facilities, the presence of *E. kuehniella* and of *P. interpunctella* males in the pheromone-baited traps was reduced after the placement of the Exosex SPTab dispensers, in comparison to captures for the same interval from the previous years. At the same time, the number of emerging individuals in the oviposition traps was notably reduced after the Exosex SPTab dispensers placement, in comparison to the previous monitoring interval. Our study documents that the auto-confusion system is an effective and reliable technique that can be used with success against stored-product Pyralidae, to retail stores and feed-mills (Figure 5).

6. Future prospects

As previously reported, there are consistent methodological problems in assessing the efficacy of mass-trapping, attracticide, mating-disruption and auto-confusion methods in practice. Each food storage and processing facility is unique and therefore finding a comparable ‘control facility’ is difficult when pheromone-based control methods are deployed. As a consequence, interpreting effectiveness of pheromone-based control measures in various facilities, at different insect densities, and microclimates becomes difficult. The available data indicate that many factors influence both male and female behaviour.

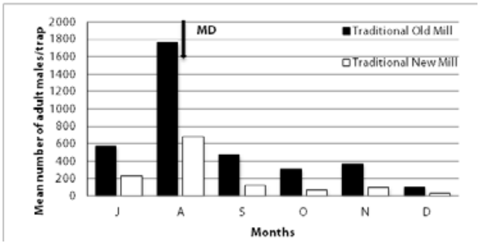


Fig. 4 Mating-disruption: Italy, traditional old and traditional new mill, 2008: mean number of *E. kuehniella* adults/trap caught in the monitoring period.

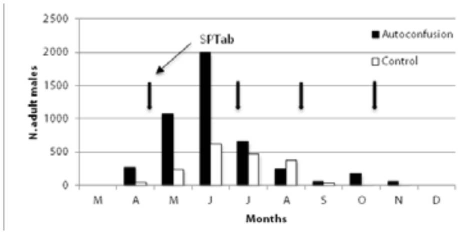


Fig. 5 Dynamic population of *Ephestia kuehniella* males in feed-mill protected with auto-confusion method.

The relative importance of these factors varies among species and among populations of the same species, undoubtedly reflecting the different ecological conditions to which they are normally subjected (Ryne *et al.*, 2007; Trematerra *et al.*, 2011 and 2012; Campos and Phillips, 2014).

In stored-product moth control, the pheromone efficacy was evaluated using the following parameters: male capture in pheromone traps, oviposition and larval emergence from eggs, incidence and frequency of mating as measured by spermatophores in females. The number of captured males or the absence of males in pheromone traps may not be a clear indicator of mating suppression and female oviposition. Oviposition and/or immature emergence should be monitored, in conjunction with adult activity. One of the most important factors, impacting the efficacy of pheromone control is the population density. Historical data from previous years, concerning both adult captures and larval presence in the target facilities, may serve as internal 'control' because such informations shows seasonal patterns of insect activity. The necessity of better controls and adequate replications need to be emphasized. In this regard the pheromone effect on population growth or decrease could be measured by the presence of spermatophores in females' *bursa copulatrix*, which is a good indicator of mating activity (Trematerra and Savoldelli, 2013). The female dissection to count spermatophores, as an estimate of mating reduction, is a more direct method than reduced trap catch or reduced oviposition in diet cups and should probably be used more.

In stored-product protection the increased use of pheromones will help reduce the number of chemical treatments with consequent economic and qualitative advantages. Pheromone-based methods need to be considered as a part of an overall IPM program in food systems (Trematerra, 2013; Trematerra *et al*., 2017). In the future, more efficient formulations of pheromones and other semiochemicals are needed, coupled with research under real-world conditions, for effective management of stored-product insects (Trematerra and Fleurat-Lessard, 2015).

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Influence of low doses of gamma irradiation on cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)

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DOI 10.5073/jka.2018.463.106

Abstract

Phytosanitary irradiation for food commodities has been widely accepted in recent years. Gamma irradiation has been used as a phytosanitary treatment against microbial diseases, insect infestation and food spoilage. The goal of the current study was to determine the lowest possible dose of gamma irradiation that will induce long-term sterility of insects through generations. The effect of four gamma irradiation doses examined were; 20,40, 50 and 70 Gy. Irradiated males were crossed with normal females. For the cowpea beetle *Callosobruchus maculatus*(F.), adult fecundity, hatchability, adult emergence, sterility% was investigated. 100% adult mortality was achieved by 70 Gy dose. Fecundity, hatchability, number of adults emerged, sterility% were significantly reduced when males exposed to 20, 40, and 50 Gy compared to the control. The effect of parental irradiated males exposed to 20 Gy on F2 generation was also studied. Fecundity, hatchability, number of adult emerged, sterility% were significantly reduced in F2 compared to F1 and control progeny. Interestingly, for F1 generation, the effect of gamma rays on adult emergence% exhibit a hermetic effect response although it was not significant. These results demonstrat that pulse irradiation relying on low-doses of gamma radiation induce inherited semi-sterility through generations and is a very promising phytosanitary food technology for post-harvest treatments.

Keywords: inherited sterility, gamma radiation, low-dose effect, sterile male technique, sterile insect technique, hormesis.