

## **Efficacy of pheromone-based control system, Exosex<sup>TM</sup> SPTab, against moth pests in European food processing facilities**

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### **Abstract**

The Exosex<sup>TM</sup> SPTab auto-confusion system is a novel pheromone-based method for control of stored product moth pests in both food and tobacco processing and storage facilities. The method uses a female-produced sex pheromone, (Z,E)-9,12-tetradecadienyl acetate, combined with a patented electrostatic powder delivery system known as Entostat<sup>TM</sup> to disrupt mating and interrupt the lifecycle of several important moth pests: *Plodia interpunctella*, *Ephestia kuehniella*, *Ephestia cautella* and *Ephestia elutella*. Male moths are attracted to a compressed tablet of the powder, which contains the sex pheromone. The powder releases pheromone at a slow enough rate to attract males to make contact with the tablet. The powder adheres to the moth cuticle via electrostatic attraction and the moth leaves the tablet coated in female sex pheromone. Flight tunnel studies have shown that this disrupts their ability to locate female moths and they become attractive sources for other males.

Here we will present findings from full scale, long term trials that were conducted under real conditions at commercial food processing facilities across Europe. Populations of target moth species were monitored alongside deployment of the SPTab system and compared with untreated control areas and historical data from the test areas in the years prior to deployment. In all cases populations were reduced compared to the same area in the previous year and compared to untreated control areas under local pest control practices.

The SPTab auto-confusion system could offer the opportunity to actively reduce the use of pesticides and its use as an integrated pest management tool within the food and tobacco processing and storage industry is discussed.

Keywords: Mating disruption, *Plodia interpunctella*, *Ephestia kuehniella*, SPTab, Sex pheromone.

### *1. Introduction*

Concerns over worker safety and residues in our food and the environment have led to a withdrawal of many previously available chemicals to treat stored and processed food environments. Stored and processed food, and tobacco, is subject to attack by a variety of moth pests from the family Pyralidae. Several of these species share a common female sex pheromone component, (Z,E)-9,12-Tetradecadienyl acetate, also known as ZETA (Kuwahara and Casida, 1973).

Sex pheromones have considerable potential in indoor pest management (Phillips, 1997; Cox, 2004), particularly for monitoring moth populations but also for direct control via disruption of mating behaviour. Mating disruption with sex pheromones has achieved widespread use in agricultural settings such as top fruit and vine protection (Cardé and Minks, 1996) but could have great potential for use indoors where airflow is restricted and potential for mated female immigration is less. ZETA has the additional benefit of being a very potent male attractant for the most important stored product moths: *Plodia interpunctella* (Hübner), *Ephestia kuehniella* (Zeller), *Ephestia cautella* (Walker) and *Ephestia elutella* (Hübner) (Kuwahara and Casida, 1973). Thus, there is the potential to control all species with one type of pheromone system. Some experimental assessments of the mating disruption technique by releasing high concentrations of ZETA into the air using pheromone dispensers have shown the potential to reduce moth populations (Hodges et al., 1984; Suss and Trematerra, 1986; Prevett et al., 1989; Fadamiro and Baker, 2002; Ryne et al., 2006).

Instead of relying on pheromone saturation of the air through emission from a fixed number of point sources, a system, Exosex™ SPTab, has been developed that delivers a carrier powder containing ZETA directly to the male moth cuticle. The proprietary carrier powder, Entostat™ (Exosect Ltd, Winchester, UK) is electrostatically chargeable and adheres to insect cuticles via electrostatic attraction (Armsworth et al., 2006; 2008; Nansen et al., 2007a: b). Male moths are attracted to dispensers containing a compressed Entostat tablet, become contaminated with Entostat-containing ZETA (Baxter et al., 2008) and after leaving male moths show reduced responses to calling females and become attractive to conspecific males, spreading the confusion effect (Huggett et al., 2010). The tablets contain less pheromone (10 mg) and have lower release rates than traditional mating disruption systems; for example at recommended application rates, some commonly used dispensers for *Cydia pomonella* result in 7.5-410 g of ai/ha/yr (OECD Environment Directorate, 2001) and SPTab deployment results in 2.4 g of ai/ha/yr, well below the recommended threshold of 375 g ai/ha/yr set by the US EPA for experimental use and waiving residue data. In enclosed indoor environments, lower pheromone rates may be beneficial due to the likely higher expected exposure levels for workers. Traditional mating disruption dispensers work by a combination of factors, including saturation of the air with a fog of pheromone so that males cannot detect the calling female pheromone plumes against the background level in the air (Cardé and Minks, 1996). SPTab has a release rate in the range between a female moth and a typical monitoring lure (Storm, unpublished data) and releases pheromone at a rate to attract males to land on the dispensers. Because the dispensers release pheromone at a lower rate, trap shutdown does not occur and pheromone traps may still be used to monitor populations.

We monitored three separate food processing facilities (one spice processing plant and two flour mills) for one year before and one year after deployment of SPTab using pheromone-based monitoring systems. In addition we collected monitoring data for control areas at each site, which did not receive an application of SPTab and which were separated by at least a closed door or stairwell. Historical data were consulted to ensure control areas had measurable population levels of target moths, similar if possible to the treated area. By obtaining control data, the percent efficacy achieved by SPTab in the treated areas of each location could be calculated using the Henderson-Tilton formula (Henderson and Tilton, 1955). We discuss the merits and limitations of such an approach to evaluate a pheromone control product for storage and processing environments. The potential for the SPTab system for stored product moth control in fully commercial settings are also discussed.

## 2. Materials and methods

### 2.1. Test sites and pest species

Three independent experiments were conducted at commercial food production and processing sites and run for one year. Experiment A) was conducted at a flour mill in the UK between July 2007 and June 2008. An entire area containing the packing lines and ground floor processing area measuring approximately 2000 m<sup>2</sup> was treated with the test product. Experiment B) was conducted in a spice factory in the Netherlands between March 2008 and March 2009. The treated area was approximately 1730 m<sup>2</sup> containing both process and packing lines. Experiment C) was conducted at a flour mill in the UK between January 2009 and January 2010. Two floors containing milling and processing lines and measuring approximately 4200 m<sup>2</sup> in total were treated.

Untreated control areas were monitored at all three sites. At site A the second, third and fourth floor of the processing area, connected to the test area by staircase, were used. At site B the adjoining warehouse area, separated by a plastic-covered doorway, was used as an untreated comparison. At site C three separate untreated areas were monitored. All three areas were separated from the treated area by at least a corridor and a door.

At sites A & C the pest species, typical of low humidity flour milling facilities, was *E. kuehniella*. At the spice factory, site B, the pest species was the more commonly encountered *P. interpunctella*.

### 2.2. SPTab dispensers and application process

SPTab dispensers were deployed as per manufacturer's recommendations. Dispensers were placed at a height of between 1.5 – 2.0 m in an approximate grid pattern with a 5 x 5 m spacing to achieve a calculated density per test area of one dispenser per 25 m<sup>2</sup>. Total dispensers used at sites A, B and C were 80, 69 and 168 respectively. The dispensers were located at even spacing on the walls, on central

structures of the building and where possible on machinery focusing around likely areas of infestation. Pheromone based monitoring trap placement, already established within the test sites, were noted and test dispensers were placed to incorporate them as part of the grid system layout. Once established the pheromone dispensers were maintained at the same location throughout the trial. Tablets were replaced at approximately 60 day intervals to give a total of 6 applications for the one year duration of the trials.

### 2.3. Monitoring and data collection

Standard funnel traps (Killgerm, Ossett, UK) baited with commercial pheromone lures (Spectrum, Ephestia, Killgerm, Ossett, UK) were used to record numbers of adult male moths in both treated and untreated areas for one year before and one year after product deployment. Monitoring points were consistent throughout both the pre and post-treatment periods. Total adult moths were recorded at approximate one monthly intervals throughout. Pest control operator companies servicing each site collaborated to assist with the provision of historical monitoring data, servicing of traps during deployment and replacement of SPTab at 60 d intervals.

### 2.4. Data analysis

For each site, the effect of the SPTab treatment on the number of moths in monitoring traps in treated areas relative to the control areas was compared using the Henderson-Tilton formula (Henderson and Tilton, 1955); on an annual basis to account for seasonality in moth capture. The mean number of moths caught per trap per year in treated and control areas was entered into the formula to give the % population suppression achieved by the end of one year of treatment ( $\% \text{ moth catch reduction} = (1 - (C1 \times T2) / (C2 \times T1)) \times 100$ ) where C1 = mean trap count total in control area in pre-treatment year, C2 = mean trap count total in control area in post-treatment year, T1 = mean trap count total in treated area in pre-treatment year and T2 = mean trap count total in treated area in post-treatment year). Data presented as mean +/- standard error of the mean.

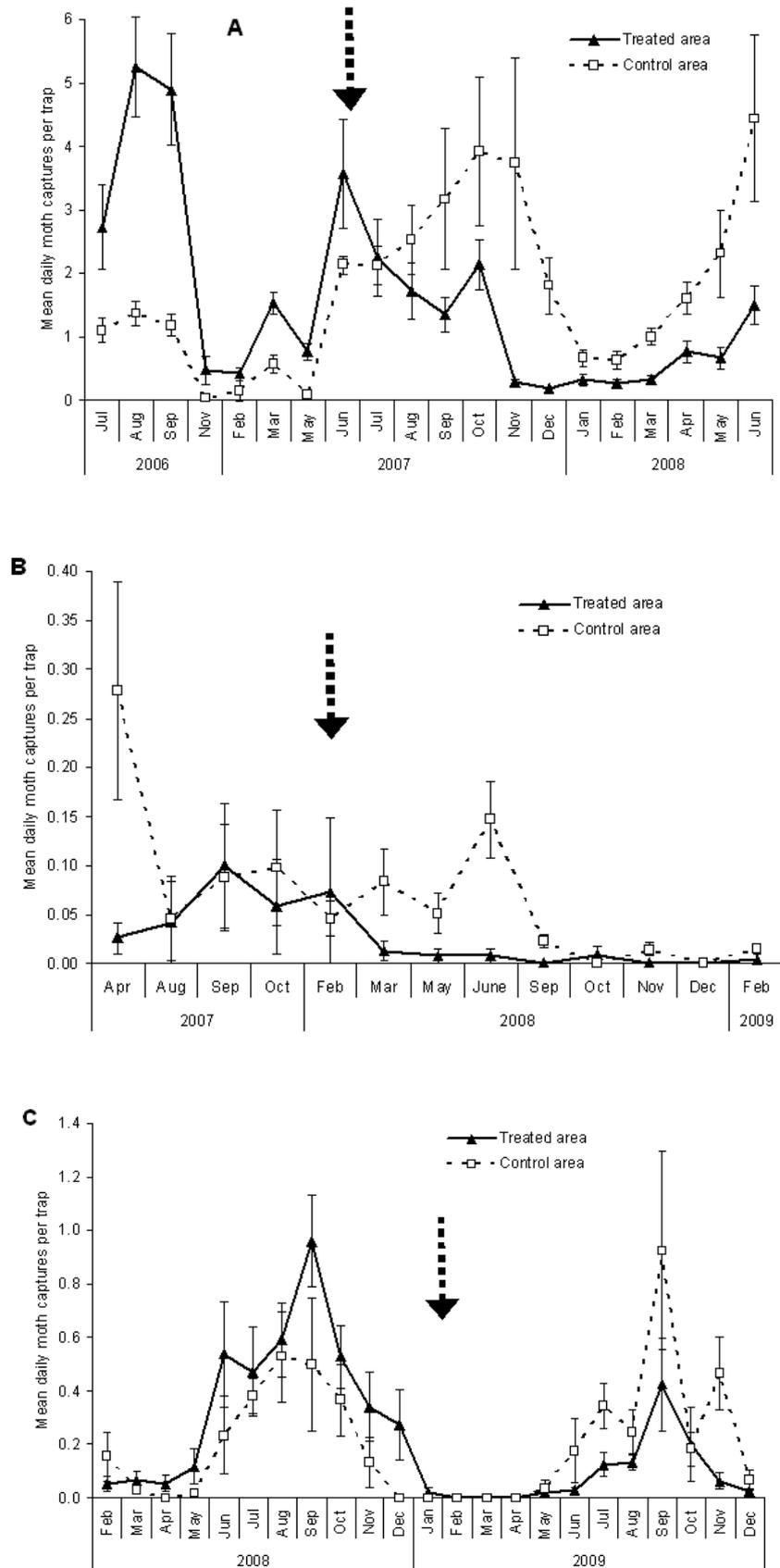
## 3. Results

For each site the seasonal male moth catches over the pre and post treatment periods are represented in Figure 1. Because of the variability in trap servicing, at each service interval the catches per trap were converted to daily trap catches (by dividing the trap catch by the number of days since the last service) and the mean of these, for control and treated areas, at each interval, are represented on the graphs.

At site A, the mean annual moth capture per trap in the treated area decreased from  $835 \pm 94$  in the pre treatment year to  $340 \pm 60$  in the post-treatment year (Figure 1A). Conversely, in the untreated control area, annual moth capture actually increased from  $276 \pm 33$  to  $769 \pm 163$ . The suppression of male *E. kuehniella* numbers in the treated area was calculated as 85% using the Henderson Tilton formula.

At site B, the mean annual moth capture per trap in the treated area decreased from  $18 \pm 16$  in the pre-treatment year to  $2 \pm 2$  in the post-treatment year. In the untreated control area, annual moth capture also decreased, from  $31 \pm 12$  to  $16 \pm 4$  (Figure 1B). The reduction in male *P. interpunctella* moth numbers was greater in the treated area and calculated as 79% using the Henderson Tilton formula.

At site C, the mean annual moth capture per trap in the treated area decreased from  $120 \pm 31$  in the year prior to treatment to  $30 \pm 12$  in the year of treatment (Fig. 1C). Conversely, in the untreated control area, annual moth capture was very similar in both years:  $72 \pm 21$  in the pre-treatment year and  $74 \pm 16$  in the post-treatment year. The suppression of male *E. kuehniella* numbers in the treated area was calculated as 76% using the Henderson Tilton formula.



**Figure 1** Mean number (+ SE) of *Epesthia kuehniella* (A and C) or *Plodia interpunctella* (B) per trap in each monthly monitoring period for control and treated areas over a one year pre-treatment period and a one year post-treatment period. A = site A, B = site B and C = site C. Closed bars = standard errors. Black dashed arrow indicates time of Exosex SPTab deployment.

#### 4. Discussion

The decrease in moth captures in all three trials potentially shows that population reductions have been achieved. It is likely that the reduction in moth capture was caused by a combination of population reduction and competition with SPTab point sources leading to reduced monitoring trap efficacy. With the current experimental design it is not possible to determine the proportion contribution of each factor to the trap capture reductions. Additional methods of sampling the population should be introduced in future trials to quantify the effects.

The three trials have also shown the ease and safety with which the SPTab system, which utilises comparatively small quantities of pheromone, can be used in enclosed environments where both human health and quality of food produce is of great importance. The feedback we received from the Pest Control Operators from each site was that the SPTab system was both quick and easy to deploy with minimal training and did not require removal of people and produce or interruption to normal operating practices whilst being used.

Whilst large mean reductions were recorded in all three trials over the course of a full year compared to both control and historical records, there was large variation between individual monitoring traps both spatially and temporally. This was often found to be correlated with known hotspots of infestation, as reported by facility pest control technicians during the trial and related to historical trap counts. Other trials (Pease, unpublished data) have also shown that under high levels of infestation it may also be possible to detect such hotspot areas within commercial facilities with increased clarity following deployment with this system in conjunction with standard pheromone monitoring traps. Where large populations have often been visible in several traps over greater areas prior to deployment, unequal reductions post-deployment of SPTab, by reducing the overall population level, have drawn attention to smaller more defined areas where key harbourage sites have existed. When used as part of an integrated pest management (IPM) system in conjunction with deep cleaning and hygiene this may allow for a more targeted approach. It was noted that the expectations and interpretation of results by both pest controllers and facility personnel would require management where resulting reductions in trap catches were not instant in comparison to expectations with traditional pesticide use. SPTab and other pheromone-based control systems work by long-term reduction of populations, and they do not provide the instant knockdown of mainly adult life-stage seen with current fogging and ULV type treatments.

It has been acknowledged that there are limitations when measuring population reduction success with the use of pheromone baited monitoring traps in trials that test mating disruption products (Ryne et al., 2006). With increased point source release of pheromone after installing such a system there may be interference competition with monitoring traps and hence apparent reduction within the population may not be wholly assigned to the effect of the product on lifecycle disruption. To minimise these effects the trials were conducted over long time periods to see the long-term effects, and the product placement took into account the positioning of monitoring traps within the deployment grids. It has also been observed that point source competition can be largely off-set by increased flight activity of adult male moths following SPTab deployment. Large initial increases in trap catch have been recorded in pheromone monitoring traps in other trials (Pease, unpublished data), perhaps due to the pheromone drawing males out of harbourage sites. This was always followed by significant reductions at two to three months post-deployment, coinciding with a single full life-cycle of the pest and indicating that disruption of mating and not interference with monitoring traps is the more likely observed effect.

It is also acknowledged that designing and conducting full scale field trials of this type has additional limitations. Mating disruption control systems require large areas over long-time periods to determine efficacy. The complexity at such fully commercial sites, in terms of both pest population dynamics and commercial activities makes it difficult to establish a robust comparative control area for the duration of the trial (Ryne et al., 2006. Pease, unpublished data). Likelihood of adult moth migration and re-infestation via contaminated material is also possible and un-quantifiable. This has been minimised by using long-term historical data in addition to untreated control areas within these trials in order to determine population reduction over a full one year period. Sites were also selected according to likelihood of re-infestation. Processing and packing areas within the flour mills and the spice processing plant were tested as resident populations are more likely the result of inherent breeding and not due to continual re-infestation from an external source.

Future trials should attempt to incorporate other methods of population monitoring, such as water traps (Chow et al., 1977) and measurement of oviposition (Nansen et al., 2006), which could be used to offset the problem of pheromone point source competition. However, unlike traditional mating disruption systems that rely on environmental flooding with pheromone, immediate trap shut-down is neither expected nor desired with SPTab. Volatile capture analysis of pheromone release from SPTab has indicated that release rates are slightly below the rate of standard monitoring lures whilst much greater than that reported for calling females (Storm, unpublished data). We hypothesise that this explains why male moths are still able to locate and travel up the pheromone plume from monitoring traps. Valuable interpretation of data using the industry standard pheromone monitoring traps is therefore still possible, which is considered advantageous in commercial working sites where methods such as water trapping may not be practically possible.

In conclusion, the SPTab system has demonstrated good efficacy against *P. interpunctella* and *E. kuehniella*, and offers an alternative to pesticidal treatment of stored product moths, of which few alternatives are available. SPTab could offer an environmentally safer alternative by reducing risk to pest control operators and workers and by reducing residue exposure to the fabric and produce within such facilities. In addition to this is an indication that by not causing trap shut-down this system may also be used in conjunction with current industry standard monitoring traps as part of an efficient and effective IPM approach to stored product pest control.

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