Behavioural effects of pheromone-based control system, ExosexTM SPTab, on male Indianmeal moth, *Plodia interpunctella*

Huggett, N.J.*#, Storm, C.G., Smith, M.J.
Exosect Ltd, Leylands Business Park, Colden Common, Winchester, Hampshire, SO21 1TH, England
Email: Nicola.huggett@exosect.com

*Corresponding author
Presenting author

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Abstract

We report on laboratory studies to examine behavioural effects of a new pheromone-based control system for stored product moths, Exosex™ SPTab auto-confusion, on virgin male *Plodia interpunctella*. The SPTab comprises a compressed electrostatic powder tablet, containing the female sex pheromone (Z,E)-9,12-Tetradecadienyl acetate; designed to be an attractive source to males and disrupt the mate finding ability of several stored moth pest species.

Our aims were to examine the effects of SPTab contact on male ability to locate females and to be attractive sources to other males. Observations of behavioural effects were made in a moth flight tunnel. Virgin male *P. interpunctella* were treated on SPTabs weathered from 1-8 weeks. After treatment, males were either immediately released downwind of calling females in the upwind section of the flight tunnel, or caged individually for 1 to 48 h before release. The ability of treated males to act as false lures for other males was measured by treating males on different age SPTabs as before, caging them and then placing them upwind of untreated males. In all studies males were scored on making source contact.

Males treated on the SPTab and immediately released downwind of calling females showed a 96.7% reduction in their ability to contact the females. Males held for 48 h before release still showed a significant reduction in source contact compared to untreated controls. Treated males tested after 3 h, were as attractive as calling females with >75% of flights resulting in source contact. Significant reduction in female source contact was still evident when males were treated on SPTab weathered for 4 weeks. Males treated on SPTabs weathered for up to 8 weeks were significantly attractive to untreated males, and equal to calling females.

The results indicate that contact with SPTab significantly reduces 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. The Exosex SPTab has novel methods of causing mating disruption.

Keywords: Mating disruption, *Plodia interpunctella*, Exosex SPTab, Sex pheromone, Flight tunnel.

1. Introduction

Plodia interpunctella (Hübner) (Indianmeal moth), herein referred to as IMM, is a common pest of food manufacturing and stored product areas all over the world. Since treatment of stored products with methyl bromide is to be phased out worldwide by 2015, alternative treatments for stored-product pest protection are being urgently sought (Vassiliou, 2008). Sex pheromones have potential for indoor moth pest management. Adult male IMM, and several other Pyralid species, will positively orientate towards a source of (Z,E)-9,12-tetradecadienyl acetate (ZETA), a compound identified as part of the sex pheromone released by the female (Brady et al., 1971; Kuwahara et al., 1971), by following the plume of sex pheromone upwind to the receptive female. Mass trapping whereby multiple sticky or funnel traps with a pheromone lure are positioned to capture as many moths as possible have been used to reduce moth populations (Cox, 2004). A limitation of this system is that only males of the species are targeted. An alternative moth control technique that has been tested in food processing and storage is the use of sex pheromones to cause mating disruption, which requires the environment to be saturated with pheromone from multiple fixed point sources (Ryne et al., 2006). These point sources can disrupt the response of male moth antennae by causing sensory fatigue or by competing with pheromone plumes produced by calling females (Cardé and Minks, 1995).

Research has shown that electrostatically charged powders can adhere to insect cuticles and be used as carrier particles for active ingredients (Armsworth et al., 2006) such as entomopathogens, insecticides and pheromones. Sex pheromones can be mixed into EntostatTM (Exosect Ltd, Winchester, UK), a proprietary electrostatic wax powder derived from the Brazilian wax palm *Copernica cerifera* Martius (Palmae). Auto-confusionTM pheromone control systems have been developed from this technology. The concept behind auto-confusion is that contact by male moths to the pheromone loaded powder allows the powder to adhere to the moth, moths then leave the point source carrying the powder and confusing pheromone with them. Exosect claim that once they have 'collected' powder, male moths become unable to locate female pheromone plumes and become mobile pheromone point sources, possibly attracting other males, thus reducing the number of fixed point sources needed to distribute the pheromone. EAG tests with male *Lobesia botrana* (Denis and Schiffermüller) treated with pheromone loaded Entostat showed that antennal responses to sex pheromone were reduced in males compared to untreated males, thus they had a reduced response to calling females (Nansen et al., 2007). Baxter et al. (2008) showed that a significant amount of Entostat powder adhered to IMM when artificially treated, and thus could be used as a carrier for pheromone dissemination.

Exosex SPTab is a pheromone product for the control of IMM and other *Pyralid* species, developed by Exosect Ltd. It is an Entostat powder blend, loaded with 10 mg of ZETA, and compressed into tablets. Exosex SPTab and traditional mating disruption systems likely differ in their modes of activity because the lower concentration of pheromone in SPTab allows the males to make contact with the point source (Huggett, unpublished data). Traditional mating disruption dispensers mask female pheromone plumes by flooding the air with pheromone, and creating false trails to confuse male moths (Cardé and Minks, 1995). If pheromone release rates are too high, contact is unlikely to occur. This work aims to determine how long the SPTab reduces the ability of male IMM to follow a female pheromone plume once they have contacted SPTab, and whether they become attractive sources for other males. It also aims to determine whether weathering of SPTabs reduces their effectiveness; as SPTab is deployed for 8 week intervals. Studies were carried out in a moth behavioural flight tunnel.

2. Materials and methods

For all studies SPTabs were placed into a 31 x 31 x 100 cm stainless steel wind-tunnel, at a constant 25ºC for a maximum of 8 weeks before treating males. IMM were 2 days old for experiments, cultured as per Baxter et al. (2008). All moths were virgins, and separated into gender groups at pupal stage. Gender groups were kept in heated vivariums in separate rooms to prevent pheromone habituation. All flight work was carried out in a custom built wind tunnel similar to a design described in Nansen et al. (2007) with dimensions of 0.75 x 0.75 x 2.5 m, a wind speed of 0.3 m/s and a temperature of 24ºC.

Virgin males for all tests were artificially treated on SPTabs by inverting a glass vial containing individual males onto the SPTab, leaving them for 5 s and re-capturing them in a wire mesh tea strainer release cage (Salt and Pepper Company Ltd, Monmouth, UK). In all studies males were released in the downwind section of the wind tunnel, they were timed and their ability to contact the upwind source was recorded. Each moth was given a maximum 5 min to achieve source contact before it was removed from the wind tunnel. For all studies SPTabs were placed into a 31 x 31 x 100 cm stainless steel wind-tunnel, wind speed 0.5 m/s, temperature a constant 25ºC, for a maximum of 8 weeks before treating males.

2.1. Female source location at different time lags after treatment on 1-week-weathered-SPTab

The purpose of this study was to determine the effect of time since SPTab contact on ability of males to find calling females. Males were treated on an SPTab weathered for one week and either immediately tested (0 h time lapse) or caged individually for 1, 2, 3, 6, 24 or 48 h, and then released in the downwind section of the flight tunnel with two caged calling females in the upwind section. Untreated males were tested as a control source. For each time lapse, and for controls, 30 males were tested.

2.2. Treated male source location at different time lags following treatment on 1-week-weathered SPTab

The purpose of this study was to determine the effect of time since SPTab contact on ability of treated males to attract untreated males. Male IMM were treated and placed in individual mesh cages upwind of untreated males at either 0, 1, 2, 3, 6, 24 or 48 h following treatment on a 1-week-weathered SPTab. Two calling females were tested as a positive control source, and two untreated males were tested as a control

male source. For each time lapse since treatment, 20 untreated male IMM were tested for their response to each source by releasing them individually in the downwind section of the flight tunnel.

2.3. Female source location after treatment on SPTabs weathered 1-4 weeks

The purpose of this study was to determine the effect of weathered SPTab contact on ability of males to find calling females. Males were treated on tablets weathered for either 4, 3, 2 or 1 week, left for 1 h in release cages, released in the down-wind section of the flight tunnel and then their ability to source contact calling females was evaluated. Untreated males were tested as a control source. For each age of tablet and for the controls, 20 males were tested.

2.4. Treated male source location following treatment on SPTabs weathered 1-8 weeks

The purpose of this study was to determine the effect of weathered SPTab contact on ability of treated males to attract untreated males. Male IMM were treated and placed in individual mesh cages upwind of untreated males 1 h following treatment on a 1, 2, 3, 4, 5, 6, 7 and 8-week-weathered SPTab. Two calling females were tested as a positive control source, and two untreated males were tested as a control male source. For each time lapse since treatment, 20 untreated male IMM were tested for their response to each source by releasing them individually in the downwind section of the flight tunnel.

2.5. Statistical analysis

For all experiments the proportion of positive source contact outcomes was compared to the total number of flight observations for each group using the Chi-squared parametric k proportions test; Monte Carlo method. The Marascuilo procedure was also used if the Monte Carlo simulation rejected H0, as the Marascuilo procedure compares all pairs of proportions, which enabled the pairwise differences to be identified. Statistical analysis was performed using XLSTAT (Addinsoft™, New York, NY, USA).

3. Results

3.1. Female source location at different time lags after treatment on 1-week-weathered-SPTab

In the untreated control group, 85% of males made contact with calling females. Immediately following treatment (time lapse 0 h), and 3 h following treatment, source contact with females was reduced to 33% and 0%, respectively (Fig. 1). Source contact was still lower than controls at 3.3% and 46.7% at 24 h and 48 h following treatment, respectively. Treatment of males on SPTab had a significant effect on source contact (Chi-square = 91.216, DF = 7, P < 0.0001) (Fig. 1). Males treated on SPTab weathered for one week and left for 0, 1, 2, 3, 6 and 24 h before flight made significantly fewer source contacts with calling female IMM compared to the untreated control male moths. There was no significant difference between the untreated males and males tested 48 h after treatment. There was no significant difference in proportion making source contact between moths released 0 and 1 h after dosing. Releasing males 2, 3 and 6 h following treatment resulted in significantly fewer source contacts than control moths and moths released 0, 1, and 48 h after treatment. There were no significant differences in proportion making source contact between moths released 24 h following dosing and those released 0, 1, 2, 3 and 6 h after dosing.

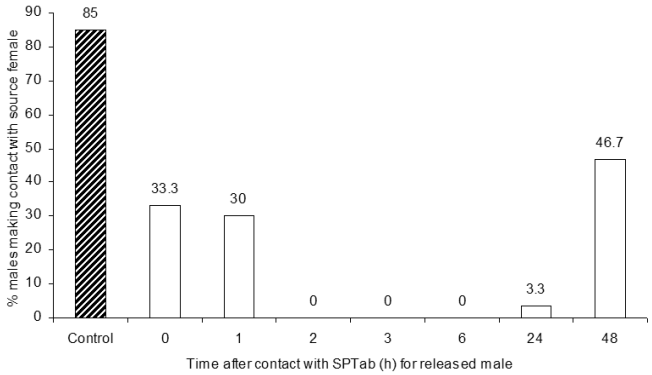

Figure 1 Percentage of virgin *Plodia interpunctella* males making female source contact at different time lags after treatment on 1-week-weathered-SPTab.

3.2. Treated male source location at different time lags following treatment on 1-week-weathered SPTab

In the female control source group 80% of males made contact. No males made contact with untreated control males (Fig. 2). Between 0 and 6 h following treatment the proportion of males making source contact with a treated male was up to 85%. No source contact occurred 24 and 48 h following treatment of the male source. Treatment of source males on SPTab had a significant effect on source contact (Chi-square = 101.157, DF = 8, P < 0.0001). Percent contacts with source males left 0, 1, 2 and 3 h after treatment were not significantly different to the percent contacting calling females. Contacts were significantly fewer than the female control group, but the same as control males, if the treated male source was placed upwind 6, 24 and 48 h following treatment on SPTab.

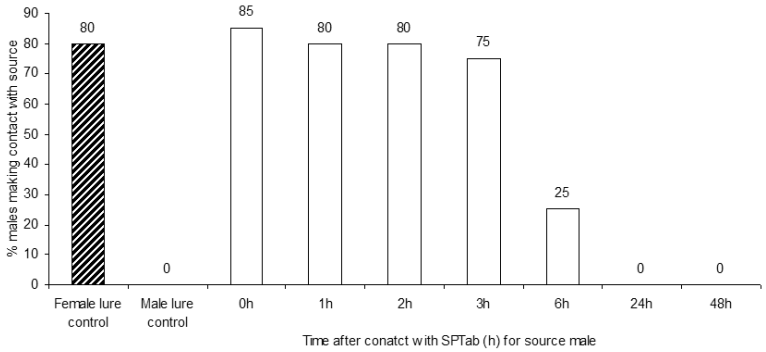

Figure 2 Percentage of untreated virgin male *Plodia interpunctella* making source contact with a male treated on a 1-week-weathered SPTab.

3.3. Female source location after treatment on SPTabs weathered 1-4 weeks.

A maximum of 20% of males treated on SPTabs weathered for either 1, 2, 3 or 4 wks made contact with the female pheromone source compared to 80% in the untreated male control group (Fig. 3). Treatment of males on SPTabs had a significant effect on source contact (Chi-square = 35.020, DF = 4, P < 0.0001). Source contact by released male moths was significantly decreased following treatment on 1, 2, 3 and 4 week weathered SPTabs.

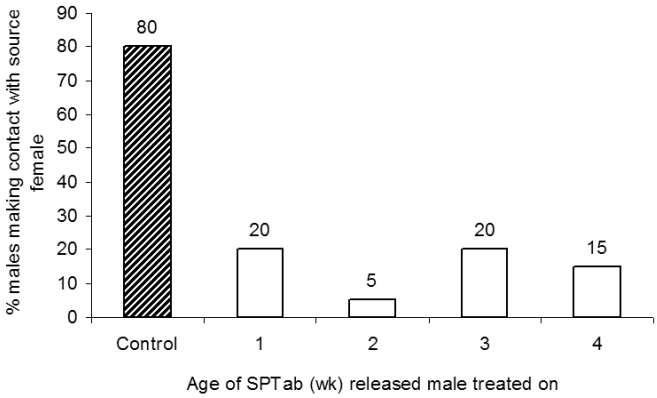

Figure 3 Percentage of *Plodia interpunctella* males making female source contact after treatment on SPTabs weathered 1-4 weeks.

3.4. Treated male source location following treatment on SPTabs weathered 1-8 weeks.

In the female control group, 80% of released males made contact. No males made source contact with the male control group (Fig. 4). Up to 90% of male moths made contact with treated male sources when the sources were treated on SPTabs weathered for 1, 2, 3, 4, 5, 6, 7 and 8 weeks. Treatment of male sources on SPTabs had a significant effect on source contact (Chi-square = 71.604, DF = 9, P < 0.0001). There was no significant difference in source contact between all the treated groups and the positive female control source group, but significantly more contact for all those groups than in the untreated control male source group.

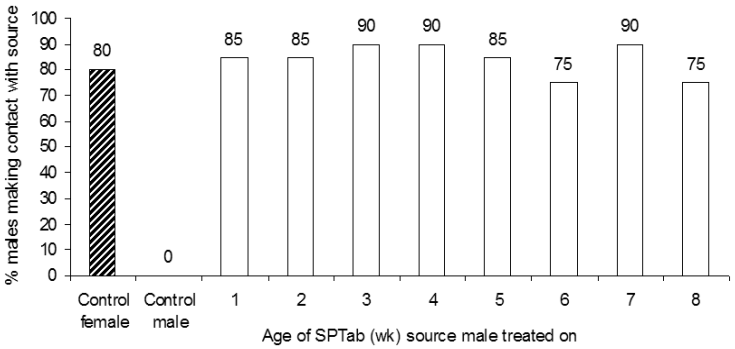

Figure 4 Percentage of untreated virgin male *Plodia interpunctella* making source contact with a male treated on 1 to 8-week-weathered SPTabs.

4. Discussion

This study has shown that very low concentrations of pheromone, if placed into an appropriate carrier, can successfully disrupt the mate-finding behaviour of male IMM once contact with the carrier has been made, a process termed auto-confusion. Contact of male IMM with SPTab reduces the ability of the males to locate females for over 24 hours. After 24 h, the pheromone from the powder may have completely volatilised, or the powder may have been removed by grooming, allowing the males to locate pheromone plumes again, from either calling females or SPTabs. Baxter et al. (2008) showed that 89.8% of Entostat was lost from a male IMM 48 h following artificial treatment in a dosing tube. The delay in males finding females was effective when males had made contact with SPTabs weathered for up to 4 weeks. Males that contacted SPTab were also highly attractive sources of pheromone to other males for over 3 hours following SPTab contact, causing males to follow a 'false' pheromone plume. Attraction to these 'false' sources was equal to two calling females even if the SPTabs had been weathered for up to 8 weeks. Untreated males were not at all attractive to other males; attraction to treated males must have been because of pheromone transfer with the powder, which adhered to the male cuticle after contact with SPTab. The attraction of males to an SPTab treated male also shows that the single major component of the female pheromone, ZETA, can elicit the full suite of flight behaviour, despite Zhu et al. (1999) indicating the minor components were required.

If males follow false male lures, or are unable to locate females, then mating is either delayed or prevented altogether. Even delayed mating reduces the chances of female moths laying fertile eggs (Huang and Subramanyam, 2002) thus a significant effect on the population is possible.

The inability of males to locate females following SPTab contact may have been due to sensory habituation. Stelinski et al. (2005) showed that habituation to pheromone following exposure to pheromone in the air can take up to an hour, and in this study a stronger reduction in source contact was seen in males left for 2 h than if tested immediately. The habituation effect observed when males are exposed to high pheromone concentrations in air (Stelinski et al., 2005) could be extended with the SPTab system due to males actually being in direct contact with the pheromone after visiting the dispenser.

Traditional mating disruption systems release large quantities of pheromone, which can cause sensory habituation in males, masking of female pheromone plumes and false trail following (Cardé and Minks, 1995). The lower amounts of pheromone released from SPTab compared to traditional mating disruption dispensers and monitoring lures (Storm, unpublished data) means that trap shutdown is not observed, thus allowing the continued use of monitoring traps (Pease and Storm, 2010).

This study has shown that the mode of action of SPTab is auto-confusion. SPTab may have similar effects on other Pyralid moths attracted to ZETA. This system could be a safer, greener alternative to pesticide sprays in indoor storage and food processing environments.

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