

# Attraction and fecundity of adult codling moth, *Cydia pomonella*, as influenced by methoxyfenozide-treated electrostatic powder

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## Abstract

The attractiveness and responsiveness of adult codling moths, *Cydia pomonella* (L.), exposed to Entostat<sup>TM</sup> powder with or without the ecdysteroid agonist, methoxyfenozide, were investigated in a flight tunnel. Coating males with either Entostat<sup>TM</sup> powder alone or powder plus methoxyfenozide 1 or 24 h prior to flight tunnel assays did not influence the mean percentages of males successfully orienting to a female-equivalent lure relative to unexposed control moths. The fecundity of females paired with males exposed to Entostat<sup>TM</sup> powder plus methoxyfenozide was significantly lower than that for females paired with unexposed males. This reduction in egg output was similar to that observed when methoxyfenozide-treated females were paired with untreated males, indicating that males can successfully pass methoxyfenozide to their partners during copulation. However, Entostat<sup>TM</sup> powder alone carried by male moths did not affect female fecundity after mating. Entostat<sup>TM</sup> powder has the potential to carry pesticides for *C. pomonella* control by autodissemination.

## Introduction

Codling moth, *Cydia pomonella* (L.), remains a principal insect pest of tree fruit orchards throughout the temperate regions of the world (Barnes 1991; Beers et al. 1993). Its larvae bore deep into the fruits of apple, pear and walnut making them unmarketable. The majority of conventional growers still rely on chemical insecticides to control this insect. However, concerns regarding development of insecticide resistance, food safety, environmental contamination, and negative effects on non-target organisms necessitate the development of alternative controls. Loss of registered compounds such as azinphos-methyl limits the insecticidal options for controlling *C. pomonella*. This increases the need for pursuing more selective insecticides as well as reliable delivery systems to: improve the efficiency of insecticide application, minimize impacts on non-target organisms, and reduce insecticide contamination of the environment.

Methoxyfenozide, the newest and most effective member of the diacylhydrazine class, has very high affinity for ecdysteroid receptors in Lepidoptera, where it mimics the insect molting hormone, 20-hydroxyecdysone (Carlson et al. 2001). Thus, it is highly toxic to a range of economically important lepidopteran larvae such as *Spodoptera littoralis* (Boisduval) (Pineda et al. 2007), *Lobesia botrana* (Denis and Schiffermuller) (Irigaray et al. 2005) and *Ostrinia nubilalis* (Hübner) (Trisyono and Chippendale 1997); but it is less harmful to non-target organisms than are conventional insecticides. This compound is essentially non-toxic to adult Lepidoptera by either oral or topical administration (Carlson et al. 2001). However, several studies have reported some effects on fecundity (numbers of eggs laid) and egg hatching following exposure to methoxyfenozide (Sun and Barrett 1999; Sun et al. 2000; Hoelscher and Barrett 2003a,b). For example, exposure to methoxyfenozide-treated surfaces reduced fecundity and

egg hatch of *Argyrotaenia velutinana* (Walker), *Choristoneura rosaceana* (Harris), and *C. pomonella* (Sun and Barrett 1999; Sun et al. 2000) as well as lowered female attractiveness and male responsiveness of these species (Hoelscher and Barrett 2003a,b) as measured in flight tunnels.

Electrostatic sprays have found a wide range of uses, including: deploying insecticides onto target-plant surfaces for pest control, surface-coating foods during processing and storage, and pollination (Law 2001). Recently, an insecticide delivery has been attempted using an electrostatically chargeable powder Entostat™ (Exosect Ltd, Winchester, UK) that exploits insects' accumulation of electrostatic charges during walking and flight (McGonigle and Jackson 2002). The powder-based delivery system offers the possibility that males of the target insect can be lured into a pheromone-baited trap where they acquire and retain Entostat™ powder containing an insecticide. Insecticide-contaminated males might then search for a conspecific calling female and transfer insecticide to that female during mating. The lures used with this technology are synthetic sex pheromones, therefore, this powder-based system is highly specific to a particular insect species (Howse and Underwood 2000). The advantages of this type of delivery system over a conventional insecticide application include: reduced insecticide usage, automatic delivery of active ingredients to the targeted pest and reduced environmental contamination due to target specificity (Barton et al. 2006). Although an Entostat™-based insecticide delivery system appears conceptually plausible, the required enabling behaviours of pest insects have thus far not been confirmed and quantified. This study assessed the effects of the Entostat™ insecticide delivery on *C. pomonella* behaviour, fecundity and egg hatch under controlled laboratory conditions.

## Materials and Methods

### Insects

Adult *C. pomonella* were obtained as late instar larvae from the USDA-ARS Yakima Agricultural Research Laboratory in Wapato, Washington. Upon receipt, larvae were transferred into a walk-in growth chamber at 24°C, and 60% RH under a 16 : 8 (L : D) photoperiod until they developed into pupae. Pupae were sorted by sex and kept segregated in a Bugdorm-1 cage (30 × 30 × 30 cm; Megaview Science Education Services Co., Taichung, Taiwan) provided with 5% sucrose in plastic

cups with cotton dental wicks protruding from their lids for adult access.

### Chemicals

The proprietary powder, Entostat™, is a surface extract of the leaves of the Brazilian wax palm, *Copernicia cerifera* (Barton et al. 2006). The particle size of the powder is  $9.2 \pm 3.3 \mu\text{m}$  (Barton et al. 2006). It consists mainly of normal saturated fatty acids and normal saturated primary alcohols. The wax binds strongly to insect cuticle due to lipophilicity combined with electrostatic charge (<http://www.exosect.com>). Entostat™ with and without 5% (w/w) methoxyfenozide were provided by Dow Agro-Sciences (Indianapolis, IN).

### Flight tunnel assays

This experiment determined whether Entostat™ powder with or without methoxyfenozide would affect male *C. pomonella* orientations to optimized codlemone lures in a flight tunnel assay. The flight tunnel apparatus (1.3 × 0.8 m in cross-section and 2.4 m long) was housed in a walk-in environmental chamber maintained at 21–22°C. Detail of the wind tunnel and assay procedures can be found in Stelinski et al. (2004). At the upwind end of the tunnel, a rubber septum lure loaded with 0.1 mg of (*E,E*)-8, 10-dodecadien-1-ol (98% isomeric and chemical purity, Suterra LLC, Bend, OR, USA) was placed 1 cm above a horizontal 7.5 × 12.5 cm yellow card held by a 9-cm glass rod attached to a steel ring-stand. The lure was positioned 25 cm above the tunnel floor. Male *C. pomonella* were released from an 8 cm long × 8 cm diameter cage constructed from aluminium window screening and placed at the downwind end of the tunnel at the same height as the lure.

Moths used were 1–2 days old, and treated 1–2 h before the onset of scotophase. One treatment group of 20 male *C. pomonella* was placed individually into a Petri dish (15 cm high × 9 cm diameter) containing Entostat™ plus 5% methoxyfenozide for 2 min. Another group of 20 male *C. pomonella* exposed to Entostat™ without methoxyfenozide (blank powder) served as the control for the insecticide treatment. Powder-coated moths were transferred to a vial that was tapped gently to remove excessive powder before coated moths were transferred into the release cage. A final group of 20 *C. pomonella*, not exposed to powder, served as a negative control for Entostat™ powder exposure. Each release cage, containing two moths of a particular treatment, was

placed into the tunnel for 0.5 h of acclimation prior to the bioassay. Thereafter, the behavioural responses of treated moths to the 0.1 mg lure were quantified for 3 min. Order of moth treatments was randomized. The behaviours recorded were: wing-fanning only; non-anemotactic flight from the release cage; anemotactic flight in the pheromone plume without touching the release device (either the yellow card or 0.1 mg lure); upwind anemotactic flight followed by landing on the release device. Non-responses were also recorded.

A second experiment was conducted that was identical to the first, except that males were treated with powder or left untreated 24 h prior to flight tunnel assays and maintained in 1-l plastic containers with access to 5% sucrose in plastic cups with cotton dental wicks protruding from their lids in the walk-in growth chamber where adult *C. pomonella* were kept. The purpose of the 24-h interval between treatment and assay was to allow time for methoxyfenozide absorption into the insect cuticle. We hypothesized that these males may respond differently from those tested immediately after treatment. Both experiments were replicated five times using fresh batches of 20 moths.

We quantified the average weight of Entostat<sup>TM</sup> powder with methoxyfenozide acquired by a male *C. pomonella* to estimate the amount of pesticide active ingredient transferred to each male contacting the powder. Sixty males of *C. pomonella* were individually transferred into BEEM capsules (8 mm ID × 20 mm High; Structure Probe Inc., West Chester, PA) and weighed with a microbalance (Model: Orion Cahn C-35,  $d = 1.0 \mu\text{g}$ ; Thermo Fisher Scientific Inc., Waltham, MA) before and after exposure to the Entostat<sup>TM</sup> powder as described above.

#### Fecundity and egg hatch as influenced by methoxyfenozide

The objective of this study was to determine whether male *C. pomonella* treated with Entostat<sup>TM</sup> powder containing methoxyfenozide transfer it to females during copulation and thus affect female reproductive output. Male (1–2 days old) or female (2–3 days old) moths were exposed to the Entostat<sup>TM</sup> powder containing 5% methoxyfenozide or the blank powder as described above. A treated moth was paired with an untreated conspecific of the opposite sex in a cylindrical cage (13 cm high, 5.5 cm diameter) made from transparency film. The two openings were capped with Petri dish lids (60 × 15 mm). The interior of the cage was fully

covered with wax paper as an ovipositional substrate. A cup with 5% sucrose was provided as food. The following pairings were established: methoxyfenozide powder treated ♂ × untreated ♀, blank powder treated ♂ × untreated ♀, untreated ♂ × methoxyfenozide powder treated ♀, untreated ♂ × untreated ♀. Numbers of eggs laid as well as numbers of eggs hatched were recorded. Each pair was replicated four times for each treatment. The cumulative number of eggs per female laid daily was counted by replacing the wax paper until female death. Numbers of eggs hatching were counted daily under a dissecting microscope. The percentage of females ovipositing was calculated for each treatment. This procedure was repeated four times using different batches of 16 moths of each sex.

#### Data analysis

Data were subjected to analysis of variance (ANOVA) (SAS Institute 2000) after proportions of moths exhibiting designated behaviour, fecundity, and percentages of eggs hatched were transformed  $[\log(x + 1)]$  to normalize distributions and homogenize variance. Mean separations were performed using Tukey's significant difference (HSD) tests.

## Results

#### Flight tunnel assays

Relative to the controls, the behaviours of male *C. pomonella* to 0.1 mg codlemone lures did not differ significantly ( $P > 0.05$ ) at 1 and 24 h following exposure to Entostat<sup>TM</sup> powder with and without methoxyfenozide (table 1). Over 70% of males oriented to and contacted the pheromone source irrespective of treatment. On average,  $326 \pm 23 \mu\text{g}$  of methoxyfenozide-treated powder was carried away per *C. pomonella* male (range 80–1060  $\mu\text{g}$ ). The Entostat<sup>TM</sup> formulation contained 5% of methoxyfenozide by weight, so this translated to an average of 16  $\mu\text{g}$  of active ingredient per male.

#### Fecundity and egg hatch as influenced by methoxyfenozide

The blank Entostat<sup>TM</sup> powder did not affect female fecundity when exposed males were paired with unexposed females (fig. 1a). Exposure of male *C. pomonella* to methoxyfenozide-treated powder significantly and dramatically reduced oviposition ( $F = 11$ ; d.f. = 3, 9;  $P = 0.002$ ) by their mates

**Table 1** Behaviours of codling moth males that were untreated, treated with blank powder, or treated with methoxyfenozide powder in response to 0.1 mg codlemone lures

Timing of powder application	Treatment	Mean Percentage of males exhibiting designated behaviours $\pm$ SE				
		No behavioural change	Fly out without orientation	Wing fanning only	Orientation without source contact	Source contact
1 h prior to wind tunnel assays	Untreated	0 $\pm$ 0	13.4 $\pm$ 4.8	1.7 $\pm$ 1.7	4.5 $\pm$ 1.9	80.4 $\pm$ 4.4
	Treated with blank powder	3.8 $\pm$ 2.6	8.1 $\pm$ 2.9	2.4 $\pm$ 2.4	11.6 $\pm$ 1.6	74.1 $\pm$ 4.4
	Treated with methoxyfenozide powder	0 $\pm$ 0	14.2 $\pm$ 3.2	2.2 $\pm$ 1.3	7.5 $\pm$ 3.7	76.1 $\pm$ 4.3
24 h prior to wind tunnel assays	Untreated	1.3 $\pm$ 1.3	15.8 $\pm$ 4.7	0 $\pm$ 0	4.0 $\pm$ 1.7	78.9 $\pm$ 3.4
	Treated with blank powder	0 $\pm$ 0	8.4 $\pm$ 2.2	0 $\pm$ 0	4.5 $\pm$ 3.3	87.1 $\pm$ 3.7
	Treated with methoxyfenozide powder	3.3 $\pm$ 3.3	10.3 $\pm$ 3.2	2.8 $\pm$ 1.8	6.3 $\pm$ 1.8	77.3 $\pm$ 3.6

No significant difference was found.

relative to that of unexposed pairs. This outcome did not differ significantly from that for females exposed to methoxyfenozide-treated powder and then paired with untreated males. Pairing of exposed males with unexposed females reduced female reproductive output by 80%.

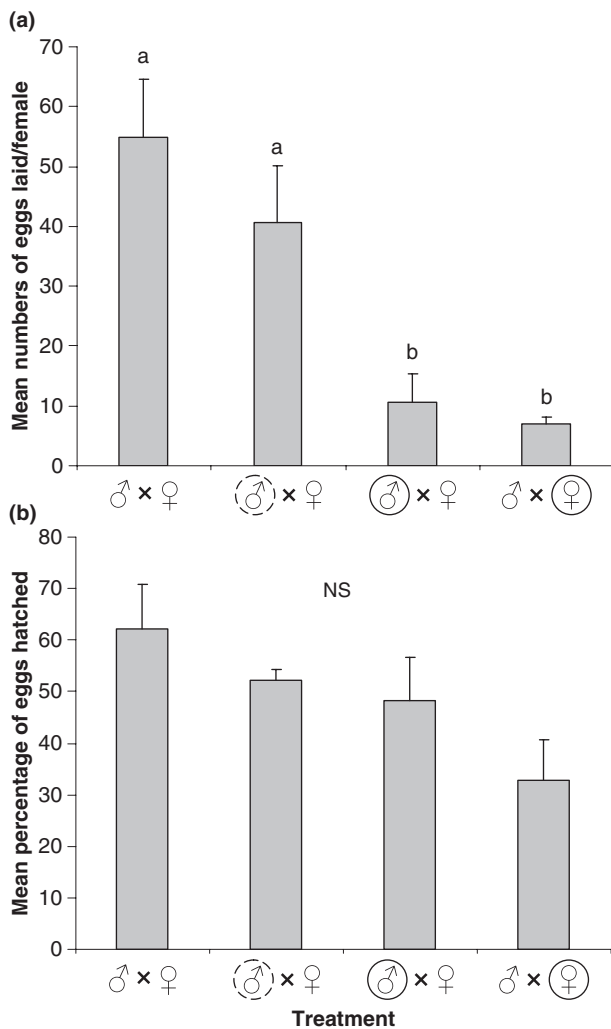
There was no significant effect on percentages of eggs that hatched ( $F = 2.81$ ; d.f. = 3, 9;  $P = 0.1$ ) (fig. 1b) for any of the Entostat<sup>TM</sup> exposure treatments, although the mean percentage of hatch was lowest when female *C. pomonella* were directly exposed to methoxyfenozide-treated Entostat<sup>TM</sup> powder.

Exposure to methoxyfenozide-treated powder also affected numbers of females laying eggs. All untreated control females paired with untreated or blank powder-exposed males laid eggs. However, only 74% and 78% of females exposed to methoxyfenozide-treated powder or left untreated but paired with methoxyfenozide powder-exposed males laid eggs respectively; although the difference was not significant ( $F = 2.15$ , d.f. = 3, 9;  $P = 0.2$ ).

## Discussion

As recorded in a flight tunnel, exposure to Entostat<sup>TM</sup> powder with or without 5% methoxyfenozide did not affect sex pheromone-stimulated behaviours of male *C. pomonella*. In contrast, Hoelscher and Barrett (2003a) reported that male *C. pomonella* exposed to methoxyfenozide exhibited less sexual excitement and upwind oriented flight to calling females than did water- or surfactant-treated control males. This

might be in part due to the different delivery system for the methoxyfenozide as well as different concentrations of the active ingredient used in the two studies. The effect of methoxyfenozide on fecundity and egg hatch has been shown to be dose-related (Pineda et al. 2007); the numbers of eggs laid by *S. littoralis* and the percentage of eggs hatching decreased progressively as the concentration of methoxyfenozide increased. Hoelscher and Barrett (2003a) dissolved up to 180 ppm of methoxyfenozide in water and immersed all surfaces of an exposure cage in this solution, but actual amounts of methoxyfenozide transferred to an individual *C. pomonella* by direct contact was not quantified. In this study, Entostat<sup>TM</sup> powder was used as the delivery system and moths briefly exposed to powder containing 5% methoxyfenozide, acquired 16  $\mu$ g of methoxyfenozide/moth on average. This quantity may be lower than was achieved by Hoelscher and Barrett (2003a) and might be insufficient to affect male responses to pheromone. Given that methoxyfenozide is essentially non-toxic to adult Lepidoptera by either oral or topical administration (Carlson et al. 2001), perhaps the lack of effects on male behaviour is not surprising. This result suggests that transfer of the methoxyfenozide active ingredient from *C. pomonella* males to females should be possible if this technology was deployed in the field as a control tactic. Male behaviours were unaffected, suggesting that they should be able to find and copulate with feral females after being loaded with methoxyfenozide-containing powder from an auto-dissemination device.



**Fig. 1** Effects of methoxyfenozide on female *Cydia pomonella* fecundity (a) and egg hatch (b). Four pairings are established: ♂ (untreated) × ♀ (untreated), blank powder treated ⊙ × ♀ (untreated), methoxyfenozide powder treated ⊕ × ♀ (untreated), ♂ (untreated) × methoxyfenozide powder treated ⊙. Mean values with different letters are significantly different at  $\alpha = 0.05$ , as determined by ANOVA followed by HSD.

Small amount of methoxyfenozide (16  $\mu\text{g}$  methoxyfenozide/moth in this study) was sufficient to reduce female *C. pomonella* fecundity up to 80% following exposure. Importantly, this significant reduction in mean fecundity also occurred when untreated females mated with methoxyfenozide powder-exposed males, indicating that sufficient methoxyfenozide is passed from male to female during copulation to affect the female's reproductive potential. These results corroborate those of Sun and Barrett (1999) who showed that male *C. pomonella* exposed to methoxyfenozide-treated surfaces

reduced fecundity of untreated mates by transfer of methoxyfenozide during copulation.

The mechanism by which methoxyfenozide affects female insects' reproduction directly or indirectly through exposure with treated males still remains unknown. It has been suggested that ecdysone agonists may arrest ovarian growth or block sperm transfer to females (Carpenter and Chandler 1994; Smagghe et al. 1996). Because oogenesis and vitellogenesis in *C. pomonella* already started at the late pupal or pharate adult stage (Webb et al. 1999), the first batch of oocytes as well as vitellin content within these eggs was likely not affected by methoxyfenozide and tebufenozide (Sun et al. 2003a). Vitellin uptake, however, may be reduced in subsequent sets of oocytes, therefore inhibiting formation of new oocytes (Smagghe and Degheel 1994). Alternatively, methoxyfenozide might block choriogenesis (Sun et al. 2003b).

Egg hatch was less affected by methoxyfenozide exposure than fecundity in this study. However, Sun and Barrett (1999) reported that exposure of female *C. pomonella* to methoxyfenozide-treated surfaces significantly reduced both fecundity and egg hatch. Differences in exposure concentrations of methoxyfenozide or in the exposure treatment between these two studies may explain these contrasting results. However, ecdysone agonists affected fecundity but not egg hatch in other insect species as well. Tebufenozide reduced fecundity without affecting egg hatch in *Spodoptera exigua* (Smagghe and Degheel 1994). Although tebufenozide reduced oviposition in a dose-dependent manner, all eggs laid by tebufenozide-treated *S. exigua* were viable (Smagghe and Degheel 1994). Similarly, female oriental fruit moth, *Grapholita molesta* (Busck), exposed to methoxyfenozide laid significantly fewer eggs, but egg hatch was not affected by methoxyfenozide exposure (Reinke and Barrett 2007). Also, egg hatch in tufted apple bud moth, *Platynota idaeusalis* Walker, was unaffected by either methoxyfenozide or tebufenozide (Myers and Hull 2003).

The results presented herein provide evidence that the novel Entostat<sup>TM</sup> insecticide delivery system may have potential as a semiochemical-based control tactic for *C. pomonella*. Entostat<sup>TM</sup> powder with or without methoxyfenozide did not affect mating ability. Male *C. pomonella* acquire large amounts of Entostat<sup>TM</sup> powder during exposure, amounts similar to those reported for the Mediterranean fruit flies, *Ceratitidis capitata* (Wiedemann) (225  $\pm$  25  $\mu\text{g}$ ) (Barton et al. 2006). Entostat<sup>TM</sup> powder forms a fairly uniform layer over all insect body surfaces, including

antennal sensilla and genitalia as shown with *C. capitata* (Armsworth et al. 2006). Our results suggest that males encountering methoxyfenozide-treated Entostat™ powder following orientation to an auto-dissemination device should be able to subsequently orient to authentic calling females. If these males acquire at least 16 µg methoxyfenozide during such an encounter, they should transfer a sufficient quantity of this insecticide to females during subsequent copulations to reduce female fecundity notably.

The success of auto-dissemination using Entostat™ powder will require optimizing all its essential components: attraction, powder uptake and retention, pest release after inoculation, and horizontal transfer among conspecifics (Howse et al. 2007; Baxter et al. 2009). Like *Plodia interpunctella* (Hübner) (Baxter et al. 2009), *C. pomonella* avoided walking directly on the Entostat™-powdered tray in our preliminary study. Recently, Baxter et al. (2009) showed that *P. interpunctella* fell into a pit-fall trap with powder caused moths to acquire more powder than from a tray design where moths were required to walk on the powder. Flight tunnel and field studies examining various means of maximizing contact between Entostat™ powder and *C. pomonella* are ongoing.

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