Mating Behaviors of *Cydia pomonella* (Lepidoptera: Tortricidae) as Influenced by Sex Pheromone in Electrostatic Powder

J. HUANG,[1,2] L. L. STELINSKI,[3] AND L. J. GUT[1]

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ABSTRACT Entostat is an electrostatically charged wax powder that can adhere strongly to insect cuticle, making it an ideal carrier to deliver pheromone for pheromone-based confusion techniques. We investigated the attractiveness of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) males treated with pheromone-laden Entostat powder to naïve conspecifics as well as mating behaviors of males after such treatment in a laboratory flight tunnel. Male moths exposed to Entostat containing 1% *E*,*E*-8,10-dodecadien-1-ol (codlemone) acquired and retained the largest amount of the powder and became more attractive as point sources to naïve moths compared with those treated with powder containing 5 or 10% codlemone. All Entostat-exposed males remained as attractive as a 0.1-mg codlemone lure for up to 24 h in flight tunnel investigations. Male moth orientation to normally attractive sources of codlemone was completely disrupted directly after treatment with Entostat powder. Males' ability to orient to 0.1-mg lures recovered progressively over a 6-d postexposure interval; however, their responses never reached the levels observed with untreated control moths. Entostat-exposed moths retained detectable amounts of codlemone up to 4 d. Our laboratory flight tunnel results suggest that the mode of action of Entostat powder as an autodissemination control tactic may be due to creating both attractive false point sources after exposure to the powder as well as directly inhibiting contaminated males' capability to orient to pheromone.

KEY WORDS Entostat powder, autoconfusion, flight tunnel, codlemone, mating disruption

Synthetically produced sex pheromones have been successfully used for decades to monitor insect activity patterns and to directly control insect pests through mating disruption or attract-and-kill (Witzgall et al. 2001, Thomson et al. 2001, Gut et al. 2004). Mating disruption is traditionally achieved by releasing relatively large quantities of synthetic pheromone from controlled delivery dispensers into the cropping area to interfere with mate finding behavior of the target pest. Currently, ≈160,000 ha of pome fruits and walnuts is treated with pheromone for control of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) worldwide (Witzgall et al. 2008). Successful implementation of areawide mating disruption has reduced the use of broad-spectrum insecticides against this pest (Calkins 1998, Gut and Brunner 1998). However, the efficacy of mating disruption remains variable and factors such as high pest population densities, small size of disrupted areas, and immigration of females from nondisrupted areas may contribute to failures (Cardé and Minks 1995). Improvement of existing pheromone dispensing technologies and development of new delivery systems to reduce cost and increase effectiveness continues despite several decades of applied pheromone research for *C. pomonella* (Witzgall et al. 2008).

Many commercial controlled release dispensers have been used for the mating disruption of *C. pomonella* (Thompson et al. 2001, Witzgall et al. 2008). Among them, most are hand-applied dispensers including Isomate-C+, rubber tubing, rubber septa, and plastic laminates impregnated with pheromone (Witzgall et al. 2008). Although hand-applied dispensers are effective throughout the season, the cost associated with the hand application has hindered wider adoption of these technologies (Gut et al. 2004). Alternatively, sprayable pheromone formulations that consist of microscopic polymer capsules can be applied using standard agricultural sprayers. However, the cost-saving achieved with ease of application for sprayable formulations is offset by short 2–3 wk residual activity (Stelinski et al. 2007a) and the necessity for multiple applications to achieve season-long control. Another technology that is designed to overcome the cost of hand application are mechanized aerosol-generating Puffers (Shorey and Gerber 1996), but their efficacy against *C. pomonella* has been variable and under certain circumstances unacceptable for commercial apple (*Malus* spp.) production (Stelinski et al. 2007b).

The mechanisms of *C. pomonella* mating disruption have been recently elucidated for several of the technologies described above. False plume following to

[1] Department of Entomology, Michigan State University, East Lansing, MI 48824.
[2] Corresponding author, e-mail: huangju@msu.edu.
[3] Department of Entomology and Nematology, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850.

Isomate-C+ tubes has been directly observed in the field (Stelinski et al. 2004a), and habituation after false plume following has been confirmed in flight tunnel assays (Stelinski et al. 2006). The combination of these two mechanisms as the mode of action of Isomate-C+ was recently confirmed empirically (Miller et al. 2010) and may be the mechanism by which *C. pomonella* is disrupted by other point-source formulations of pheromone described above.

Recently, a novel pheromone dispensing system (auto-confusion system) has been developed based on an electrostatically chargeable powder (Entostat, Exosect Ltd., Winchester, United Kingdom), which exploits the accumulation of electrostatic charges on the insect cuticle during walking and flight (McGonigle and Jackson 2002). Pheromone is formulated within this powder and deployed in the field at bait stations. The premise behind this technology is to lure males into the bait stations containing pheromone-impregnated powder where they become exposed to and contaminated with the powder. Contamination of males with the pheromone-laden powder may inhibit their ability to subsequently find females due to overexposure of the sensory system (sensu Stelinski et al. 2006). Furthermore, contaminated males may act as mobile pheromone dispensers thereby disrupting other unexposed males through the false plume following mechanism (sensu Miller et al. 2006). Potentially very few bait stations per crop area would be required to effectively interfere with mate-finding behavior and thus pheromone and labor costs would be lower than with conventional pheromone-based technologies. Although the mode of action of the Entostat-based pheromone delivery system seems conceptually plausible, the effects of this technology on the behavior of *C. pomonella* have not been hitherto confirmed or quantified. The current investigation assessed the effect of Entostat exposure on 1) behavior of exposed male *C. pomonella* in response to attractive lures in a flight tunnel, 2) the attractiveness of contaminated males to naïve conspecifics over time, and 3) uptake and retention of codlemone on contaminated males.

Materials and Methods

Insects. Codling moths were obtained as late instar larvae in strips of double-sided corrugated card board (2 by 40 cm) from the USDA–ARS Yakima Agricultural Research Laboratory in Wapato, WA. Upon receipt, the strips were transferred into a growth chamber at 24°C, 60% RH, and a photoperiod of 16:8 (L:D) h until they developed into pupae. Pupae were sorted by sex. Male pupae were kept in 30- by 30- by 30-cm cages (Bugdorm-1, Megaview Science Education Services Co., Taichung, Taiwan) and provided with 5% sucrose solution through cotton dental wicks.

Disruption Formulation. The proprietary powder Entostat (Exosect Ltd.) is a surface extract of the leaves of the Brazilian wax palm, *Copernicia cerifera* Martius, with a particle size of 9.2 ± 3.3 μm (Barton et al. 2006). It consists mainly of saturated fatty acids and saturated primary alcohols. The wax binds strongly to insect cuticle due to lipophilicity combined with electrostatic charge (www.exosect.com). Entostat impregnated with 1, 5, or 10% (wt:wt) *E,E*-8,10-dodecadien-1-ol (codlemone, 98% isomeric and chemical purity) were provided by Dow AgroSciences (Indianapolis, IN).

Flight Tunnel Assay Procedures. Male moths (age depended on experimental design) were collected and placed into cylindrical release cages (two males per cage) constructed from aluminum window screening (17 cm in length, 8 cm in diameter) 30 min before the onset of scotophase. These release cages were immediately transferred into a walk-in environmental chamber, where a Plexiglas flight tunnel (1.3 by 0.8 m in cross section and 2.4 m in length) was housed at 21–22°C, for an hour of acclimation before assays. Detailed descriptions of this apparatus and assay procedures were described in Stelinski et al. (2004b). In brief, at the upwind end of the tunnel, a pheromone source was placed 25 cm above the tunnel floor, 1 cm above a horizontally oriented yellow index card (7.6 by 12.6 cm) held by a 9-cm glass rod attached to a steel ring-stand. At the downwind end of the tunnel, two males were released simultaneously from the mesh cage at a height matching the pheromone source. Pheromone sources used were either red rubber septa (The West Company, Lionville, PA) loaded with 0.1 mg of codlemone (henceforth, these dispensers are referred to as 0.1-mg lures) or an Entostat-treated male *C. pomonella* as described below. Moths were allowed 3 min to respond to a pheromone source and then removed by a portable vacuum. Daily wind tunnel assays lasted 2–2.5 h depending on numbers of moths used. The behaviors recorded were wing fanning; nonanemotactic flight from the release cage; anemotactic flight within the pheromone plume toward the source, but without touching the release device (either the yellow index card or 0.1-mg lure, or powdered males); and upwind anemotactic flight followed by landing on the release device (source contact). No detectable behavioral response also were recorded. Treatment order was randomized daily unless otherwise indicated to minimize possible effect of time after the onset of scotophase. After daily use, the interior of the flight tunnel was briefly scrubbed with a paper towel wetted with acetone and immediately rinsed with water so as not to damage the Plexiglas. Release cages, ring stand, and glass rod also were washed with acetone after each use.

Attractiveness of Entostat-Treated Males. Male *C. pomonella* (1–2 d old) to be used as release points of Entostat powder were exposed to the formulation containing 1, 5, or 10% (wt:wt) codlemone for 2 min in a petri dish (15 mm in height by 9 cm in diameter) 1–2 h before the onset of scotophase. Each powder-exposed male was subsequently tethered with a thread around a forewing at the upwind end of the wind tunnel to serve as a pheromone point source against a group of 20 untreated (naïve) male moths (2–3 d old). Treated males for each treatment were collected directly after testing for codlemone residual analysis described below. The flight response of another group

of 20 naïve males to a 0.1-mg lure served as a positive control. Yellow index card or naïve male moths or the combination of the two at the upwind end do not attract naïve male moths at the downwind end of the tunnel. Each treatment, including the control, was assayed randomly on each day of testing. This experiment was replicated five times on separate days using different batches of naïve moths and different powder-treated males. Untransformed proportions of moths exhibiting a designated behavior were analyzed as a randomized complete block design by analysis of variance (ANOVA) with day of testing as the blocking factor (SAS Institute 2000). Mean separations were performed using Tukey's honestly significant difference (HSD) tests, with a significance level of $\alpha = 0.05$.

A second experiment was conducted to quantify the duration that Entostat-exposed males are attractive to naïve conspecifics. Based on the results of the first experiment (see Results), Entostat containing 1% pheromone was used to treat male *C. pomonella* as described above. Entostat-treated males were maintained individually in release cages, before assays, within a growth chamber at 24°C, 60% RH, and a photoperiod of 16:8 (L:D) h, with access to sugar solution. At 0 (powder treatment and bioassay were performed on the same day), 1, or 3 d postexposure, five treated males were randomly selected and individually tethered at the upwind end of the flight tunnel to assay for their attractiveness to a group of 15 naïve males. The flight response of another group of 15 naïve males to a 0.1-mg lure served as the corresponding positive control. After flight tunnel assays, the Entostat-treated moths were saved for residual pheromone quantification as described below. The experiment was replicated five times. The proportions of moths performing a designated behavior were analyzed as a 2 by 3 factorial ANOVA, with days after treatment having three levels and treatment having two levels (control versus Entostat-treated) (SAS Institute 2000). Mean separations were performed using Tukey's HSD tests, with a significance level of $\alpha = 0.05$.

Quantification of Codlemone and Entostat Uptake. The objective of this investigation was to quantify the amounts of codlemone that male *C. pomonella* retained after treatment with Entostat containing 1, 5, or 10% codlemone. For each treatment in the experiment described above, all male *C. pomonella* were transferred individually into 1.5-ml microcentrifuge tubes containing 700 μl of acetone immediately after flight tunnel assays. Each microcentrifuge tube was vortexed before acetone solution was transferred into a 1-ml glass vial, which was later concentrated under nitrogen until complete solvent evaporation. Remaining codlemone was extracted by washing the vials three times with 50 μl of acetone. The extractions were combined into a 1.5-ml microcentrifuge tube and centrifuged to discard any solid debris such as scales from moths. The final suspension was transferred into a GC vial (Supelco, Bellefonte, PA) to which 3 μl of methyl myristate (2 μg/μl in acetone, 99% purity; Aldrich, Milwaukee, WI) was added as an internal standard for codlemone quantification. For each sample, 1 μl was injected into a capillary gas chromatograph (Hewlett–Packard HP6890 equipped with a Hewlett–Packard 7863 auto sampler) equipped with an HP-Innowax polyethylene glycol column (30 m by 250 μm i.d., 0.25-μm film thickness), a splitless injector at 250°C, and a flame ionization detector at 300°C. After injection, column temperature was held at 50°C for 5 min, increased at 25°C/min to 155°C and held for 5 min, and then increased at 0.5°C/min to 165°C and held for 3 min. Finally, temperature was increased at 30°C/min to 225 and held for 2 min. Helium was used as a carrier gas at a flow rate of 1.1 ml/min. Data were collected with Hewlett–Packard ChemStation software, and volatile compounds were quantified by comparing their peak areas with that of the internal standard. Based on the amount of codlemone detected by GC, the amount of powder adhering per male *C. pomonella* was calculated by the following equation: amount of powder = amount of codlemone detected/1, 5, or 10% (percentage of codlemone present in powder). Amounts of codlemone and powder acquired by males were subjected to square-root transformation before they were analyzed as a randomized complete block design by ANOVA, with day of testing as the blocking factor (SAS Institute 2000). Mean separations were performed using Tukey's HSD tests, with a significance level of $\alpha = 0.05$.

Responsiveness of Entostat-Treated Moths to Pheromone Sources Over Time. The objective of this experiment was to quantify the residual longevity of the impact of exposing male *C. pomonella* to codlemone-treated Entostat on their subsequent responsiveness to codlemone over time. Exposure of male *C. pomonella* to Entostat without codlemone does not affect the behavioral response of males to 0.1-mg lures in the flight tunnel (Huang et al. 2009); therefore, this treatment was not included in this experiment. Male *C. pomonella* (1 d old) were individually exposed to Entostat containing 1% codlemone for 2 min 1–2 h before the onset of scotophase. Treated moths were maintained in release cages as groups of two with an access to sugar water in a growth chamber under the temperature and light-cycle conditions described above. At 0, 1, 2, 3, 4, 5, or 6 d after treatment, 10–20 treated moths (depending on moth availability) were randomly selected from the growth chamber, and their subsequent responsiveness to a 0.1-mg lure was tested in the flight tunnel. To account for possible effects of moth age on performance in the flight tunnel, 10–20 naïve moths of equivalent age from another growth chamber were randomly selected and tested daily as corresponding controls. On each day of testing, 10 treated moths were recaptured (selection at random) immediately after testing by using a glass vial (1.5 cm in diameter by 4.5 cm in height) and transferred individually into 1.5-ml microcentrifuge tubes filled with 700 ml of acetone to quantify residual codlemone as described above. The remaining treated and control moths were removed immediately after testing with a portable vacuum. To avoid contamination from treated moths, naïve males were always tested first on each day of testing. The experiment was

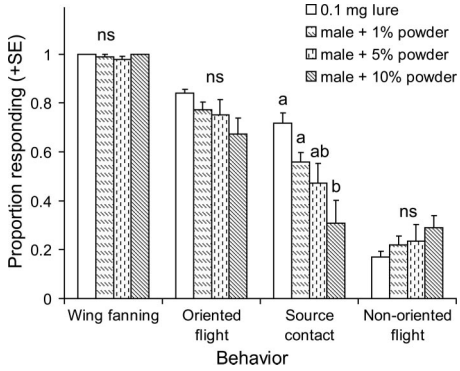

Fig. 1. Attractiveness of *C. pomonella* males exposed to Entostat powder containing 1, 5, or 10% *E,E*-8,10-dodecadien-1-ol (codlemone) to their conspecific naïve males compared with attractiveness of a 0.1-mg codlemone lure. Bars labeled with different letters within columns differ significantly at $\alpha = 0.05$ (Tukey's HSD test). NS indicates no significant difference.

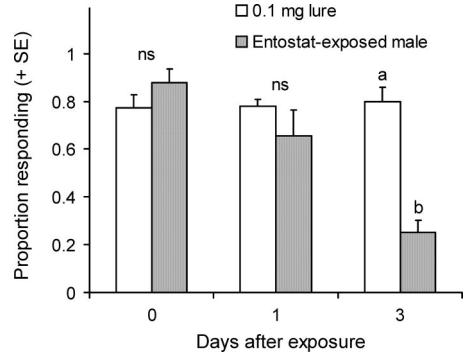

Fig. 2. Proportion of source contact by naïve *C. pomonella* males exposed to Entostat powder containing 1% *E,E*-8,10-dodecadien-1-ol (codlemone) over time. Bars labeled with different letters within columns differ significantly at $\alpha = 0.05$ (Tukey's HSD test). NS indicates no significant difference.

replicated four times on separate days by using different batches of moths.

The proportions of moths exhibiting a designated behavior were analyzed as a 2 by 7 factorial ANOVA, with days after treatment having seven levels and treatment (control versus Entostat-treated) having two levels (SAS Institute 2000). Amounts of codlemone adhering to males were analyzed by ANOVA. Mean separations were performed using Tukey's HSD tests, with a significance level of $\alpha = 0.05$.

Results

Attractiveness of Entostat-Treated Males and Quantification of Formulation Uptake. The behavior of naïve male *C. pomonella* in response to conspecific males that were exposed to Entostat powder and tethered as point sources was similar to that observed in response to the 0.1-mg lure positive control on the same day of testing (wing fanning: $F = 1.29$; df = 3, 12; $P = 0.32$; oriented flight: $F = 2.14$; df = 3, 12; $P = 0.15$; and nonoriented flight: $F = 1.07$; df = 3, 12; $P = 0.4$) (Fig. 1). The proportion of moths contacting 0.1-mg lures was not significantly different from that contacting Entostat-treated males with powder containing 1 or 5% codlemone (Fig. 1). However, fewer males contacted conspecifics treated with the 10% powder than the 0.1-mg lure ($F = 9.46$; df = 3, 12; $P = 0.002$). Male moths treated with Entostat powder containing 1% codlemone remained as attractive as the 0.1-mg lure for up to 24 h after exposure (Fig. 2). Treated moths were significantly less attractive than the 0.1-mg lure at 3 d after exposure; however, >20% of naïve moths still oriented to and contacted treated moths.

The amount of Entostat acquired by *C. pomonella* males decreased as the percentage of codlemone in powder increased ($F = 7.77$; df = 2, 8; $P = 0.01$) (Table 1). The amount of codlemone detected per male *C. pomonella* was the lowest after exposure to powder containing 1% codlemone compared with the higher concentrations ($F = 5.29$; df = 2, 8; $P = 0.03$); however, moths with the lowest amounts of codlemone adhering were the most attractive (Fig. 1). The amount of codlemone required to elicit source contact in this investigation was as low as 1.2 μg.

Responsiveness of Entostat-Treated Moths to Pheromone Sources Over Time. Male *C. pomonella* became quiescent immediately after exposure to Entostat containing 1% codlemone. Source contact and orientation to the 0.1-mg lure was eliminated directly after exposure to Entostat containing 1% codlemone (Fig. 3A and B) (*F* values in Table 2). Also, the proportion of males that exhibited no response to the 0.1-mg lure was significantly greater for Entostat-treated than control males (Fig. 3E), whereas the proportion of males that exhibited wing fanning was significantly lower for Entostat-treated than control males (Fig. 3C). One day after exposure, significantly fewer treated moths engaged in source contact (Fig. 3A), oriented upwind flight (Fig. 3B), and wing fanning (Fig. 3C), whereas significantly more exhibited nonoriented flight and no response (Fig. 3D and E) compared with naïve males. Responsiveness of Entostat-treated males began to increase 2 d after treatment as the proportions of moths that engaged in both wing fanning and those not responding were no longer significantly different from naïve moths (Fig. 3C and E); however, source contact and oriented flight up-

Table 1. Mean amounts of codlemone and Entostat powder per male *C. pomonella* ($N = 5$ per treatment) after exposure to Entostat powder containing 1, 5, or 10% codlemone

Amt (%) of codlemone in the powder	Amt of codlemone detected/male, μg ± SEM	Amt of Entostat powder per male, μg ± SEM
1	1.2 ± 0.2a	116.7 ± 22.1a
5	4.5 ± 0.9b	89.3 ± 17.6ab
10	3.2 ± 0.3b	31.7 ± 2.7b

Means within a column followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey's HSD test).

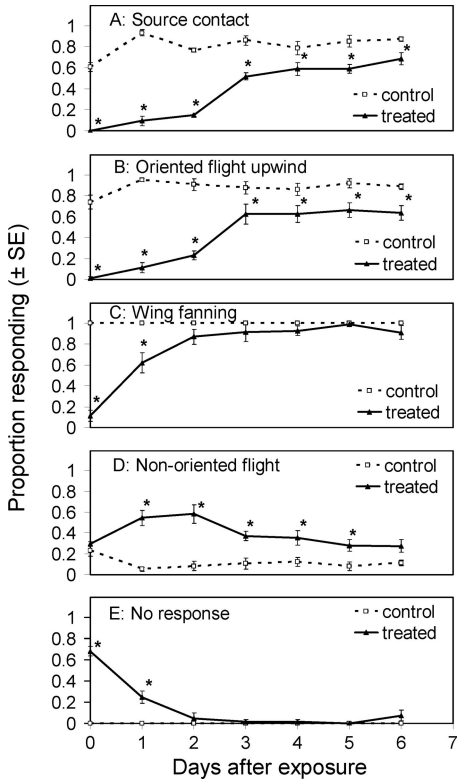

Fig. 3. Behavioral responses of *C. pomonella* males to 0.1-mg codlemone lures at various intervals after exposure to Entostat powder containing 1% *E,E*-8,10-dodecadien-1-ol (codlemone) at various intervals after exposure. Asterisks indicates significant difference within days at $\alpha = 0.05$ (Tukey's HSD test).

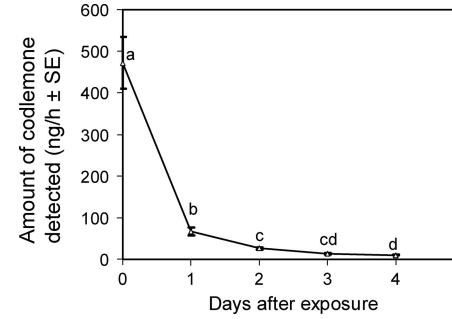

Fig. 4. Amount of *E,E*-8,10-dodecadien-1-ol (codlemone) quantified from *C. pomonella* males exposed to Entostat containing 1% codlemone at various intervals after exposure. Points labeled with different letters differ significantly at $\alpha = 0.05$ (Tukey's HSD test).

wind never reached the levels observed with naïve moths throughout the experiment (Fig. 3A and B).

The amount of codlemone adhering to male *C. pomonella* was greatest directly after exposure to Entostat containing 1% codlemone and dramatically decreased afterwards (Fig. 4). Males retained detectable amounts of codlemone for up to 4 d after exposure to Entostat.

Discussion

The results presented herein are part of an ongoing investigation to develop a novel auto-confusion system for the control of *C. pomonella* (Huang et al. 2009). As expected, *C. pomonella* males were able to pick up and retain the powder after exposure to Entostat containing 1, 5, or 10% codlemone. Surprisingly, the amount of powder retained decreased as the concentration of codlemone increased. Codlemone solidifies at room temperature (20–25°C); therefore, codlemone-treated powder is unlikely to be uniform in size. In fact, we noticed that Entostat powder tended to clump as codlemone loading was increased. This might affect certain physical properties of the powder such as size and charge, which may have affected acquisition of powder by males as codlemone loading was increased. It is also possible that the slightly more coarse powder containing the highest (10%) loading of codlemone might have been groomed off by treated males more effectively or by conspecific naïve moths during source contact. Nevertheless, *C. pomonella* males exposed to the powder containing 1% codlemone were as attractive as 0.1-mg lures to naïve conspecific males for up to 24 h post-exposure. The level of attractiveness decreased markedly by the third day after exposure. Because elevating the amount of codlemone in Entostat beyond 1% decreased powder retention by male as well as their attractiveness after exposure, we suggest not pursuing formulations with loadings of codlemone beyond this dosage.

Many factors affect the success of mating disruption. Moth population density is perhaps one of the most important limiting factors (Cardé and Minks

Table 2. ***F*** **values associated with Fig. 3 for comparing significant differences between control and Entostat-treated male *C. pomonella* at various intervals after exposure**

Days after exposure	Source contact	Oriented flight upwind	Wing fanning	Nonoriented flight	No response
0	78.5**	65.8**	182**	0.56	330.8**
1	149.1**	87.4**	33.5**	34.1**	43.5**
2	81.9**	57.1**	4.1	45.8**	1.78
3	24.5**	7.9*	1.8	9.6**	0.2
4	8.3*	7.0*	1.3	7.4*	0.2
5	14.8**	8.1*	0.1	5.5*	0.1
6	14.8**	8.0*	1.9	3.6	4.1

* significance at $\alpha = 0.05$, ** significance at $\alpha = 0.01$.

1995). Traditional mating disruption technologies depend solely on externally applied synthetic dispensers for pheromone release and sometimes are not effective under high pest populations. In contrast, the auto-confusion system may be effective under high pest population density given that the number of mobile dispensers should increase with increasing pest densities to a certain critical point when mating disruption takes effect. Such contaminated males should act as false plumes distracting conspecific male response from authentic females. Also, the addition of "false females" should intensify competition among authentic ones (Miller et al. 2006). Moreover, secondary transfer of adhesive powders to conspecifics during social interactions has been reported previously (Armsworth et al. 2008). It is possible that secondary transfer may occur in the field with *C. pomonella* when naïve males contact powder-treated conspecifics. However, auto-confusion technology requires that attracted males voluntarily pick up and retain pheromone-laden powder. It is unknown how well the currently described exposure procedure of male *C. pomonella* to Entostat in petri dishes corresponds to uptake of Entostat voluntarily from bait stations in the field. Other insects have been reported to effectively acquire and retain Entostat, including *Ceratitis capitata* (Wiedemann) (Armsworth et al. 2006), *Oryzaephilus surinamensis* (L.) (Nansen et al. 2007a), *Plodia interpunctella* (Hübner) (Baxter et al. 2008), and *Lobesia botrana* (Denis & Schiffermuller) (Nansen et al. 2007b). *P. interpunctella* was able to voluntarily acquire 2.5 μg of Entostat powder deployed in a delta trap (Baxter et al. 2008). Similarly, *C. capitata* picked up ≈1.5 μg of Entostat powder, on average, from field-deployed dispensers (Armsworth et al. 2008).

In addition to serving as false females, Entostat-treated male *C. pomonella* are rendered incapable of effectively following normally attractive codlemone plumes for several days. Immediately after exposure, males exhibited near complete quiescence. This is not surprising because microgram levels of codlemone adhering per *C. pomonella* male are probably well above the airborne codlemone concentration levels known to induce adaptation (Judd et al. 2005) or habituation (Stelinski et al. 2006). Even though behavioral responses of treated males recovered slowly over time, their responses were never fully recovered throughout the 6-d experiment, indicating that sustained exposure resulted in a prolonged desensitization. Unlike briefly preexposing male C. *pomonella* within pheromone plumes (Stelinski et al. 2006), Entostat exposure may have a more permanent effect given that it adheres to males for prolonged periods after they move away from the bait station. It is possible that pheromone loss, which may have been due to grooming or degradation, explains some of the behavioral recovery observed over time. The amount of codlemone retained by exposed *C. pomonella* dropped to 14 and 6% 1 and 2 d after treatment, respectively. Barton et al. (2006) reported that *C. capitata* initially took up 500 μg of Entostat, but only 5% of the powder was retained by 48 h after treatment, which is congruent with our results. Adult *C. pomonella* live for 8 d, on average, under summer conditions in the field (Jones and Wiman 2008). However, life span of *C. pomonella* is significantly longer under laboratory conditions and 14-d-old males were as responsive as 2-d-old males to pheromone sources (J.H. et al., unpublished data). Therefore, male age was unlikely to be a significant contributing factor to the results obtained in this laboratory study.

In summary, treatment of male *C. pomonella* with codlemone-laden Entostat powder created males that were highly attractive to naïve conspecifics and reduced responsiveness of treated males to normally attractive lures for several days. The current results suggest further development of Entostat for pheromone-based control of *C. pomonella* is warranted. Further development should focus on maximizing uptake and retention of codlemone by Entostat-exposed *C. pomonella*, which may be achieved by increasing contact time during visits to bait stations. Further investigation also is warranted to optimize pheromone loading dosage in Entostat.

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