

## Orientalional Behaviors and EAG Responses of Male Codling Moth After Exposure to Synthetic Sex Pheromone from Various Dispensers

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**Abstract** The effect of brief pheromone exposures on responses of codling moth (*Cydia pomonella* L.) males was tested by flight-tunnel and electroantennogram (EAG) studies. Males were preexposed to pheromone for up to 3 min as they sat in release cages or for shorter times (a few seconds to several min) upon initiating flights or orienting in plumes. Brief exposures to Isomate-C Plus dispensers nearly eliminated moth orientations to 0.1 mg codlemone [(*E,E*)-8,10-dodecadien-1-ol] and 0.1 mg three-component (codlemone/12OH/14OH, 100:20:5 ratio) lures 15 min later. However, there was no associated change in EAG responses between preexposed and control moths. Behavioral responses of Isomate-C Plus-exposed males were normal 24 hr following exposure. The reduced sexual responsiveness observed following exposure to Isomate dispensers appeared to be associated with an elevation of response threshold. Brief preexposure to 0.1 mg codlemone and three-component lures also reduced orientational behavior of males 15 min later, but to a lesser degree than when preexposed to Isomate-C Plus dispensers. Male behavior following preexposure to a 0.1 mg codlemone/pear ester [(*2E,4Z*)-2,4-decadienoate] lure (1:1 ratio) was no different from exposure to codlemone only. Orientational disruption in plots treated with 10 dispensers of Isomate-C Plus per tree was 88.3 and 95.9% for 1.0 and 0.1 mg codlemone lures, respectively. Some males did orient to 0.1 mg codlemone lures so we caution that flight-tunnel experiments on preexposure may overestimate the actual pheromone exposure dosage received by feral moths in treated orchards. Importantly, this work documents that a portion of feral males within a population has the capacity to overcome communicational disruption by high densities of Isomate-C Plus dispensers.

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## Introduction

The codling moth, *Cydia pomonella* (L.), is a worldwide pest of pome fruit (Vickers and Rothschild, 1991). The identification of (*E,E*)-8,10-dodecadien-1-ol (codlemone) as the major pheromonal component (Roelofs et al., 1971) led to attempts to control it by mating disruption (Cardé et al., 1977; Mani et al., 1978; Moffit, 1978). Although a number of studies report successful mating disruption of codling moth and associated crop protection, failures have also been documented, and improvements are called for (Vickers and Rothschild, 1991; Minks, 1997).

A number of laboratory and field experiments have attempted to determine the dosage of pheromone required for effective codling moth mating disruption. A 65% reduction of mating was observed when females were caged with males in 1.2-l containers with 1.0 mg of codlemone (Fluri et al., 1974), whereas by exposing males and females to air moving over one or three 1.0 mg lures of codlemone, there was only a 38% mating reduction (Charmillot et al., 1976). More recently, Judd et al. (2005) established that exposures of male codling moths to ca. 35 µg codlemone/l of air in static air chambers for 10–30 min nearly eliminated response to otherwise attractive lures in a flight tunnel and reduced electroantennogram (EAG) responses. Normal behavioral responses returned after >4 hr and EAG sensitivity after >1 hr. However, caging male codling moths for 30–34 hr in an orchard treated with 1000 Isomate-C dispensers per hectare had no measurable impact on behavioral responses to pheromone in a flight tunnel (Judd et al., 2005). Similar results were obtained by Schmitz et al. (1997) and Rumbo and Vickers (1997) for the European grape moth, *Lobesia botrana* (Denis and Schiffermüller), and the Oriental fruit moth, *Grapholita molesta* (Busck), respectively. Judd et al. (2005) suggested that if male codling moths landed near Isomate-C Plus dispensers for several minutes, they might receive a sufficiently high dose of pheromone to reduce behavioral responses as seen in their preexposure laboratory experiment.

Thirteen minor compounds have also been identified from the sex pheromone gland of female codling moths (Witzgall et al., 2001); dodecanol (12OH) and tetradecanol (14OH) were quantitatively most significant, enhancing behavioral responses of males to codlemone (Einhorn et al., 1984; Arn et al., 1985). Consequently, these two compounds and codlemone are the active ingredients in Isomate-C and -C Plus polyethylene tube dispensers (Pacific Biocontrol Co., Vancouver, WA, USA), which are widely used for mating disruption of codling moth (Thomson et al., 1998).

Codling moth males have been captured in traps baited with Isomate-C dispensers (Barrett, 1995), and they land within a few centimeters of similar dispensers placed directly in untreated (Witzgall et al., 1999) and pheromone-treated (Stelinski et al., 2005a) orchards. Although attracted moths departed within <60 sec (Stelinski et al., 2005a), as seen with three other tortricids (Stelinski et al., 2004a), it is possible that such pheromone exposure contributes to mating disruption.

The purpose of this study was to quantify the effects of brief (seconds to 3 min) preexposures of male codling moths to various pheromone treatments in a flight

tunnel where moths had the capability of flying from release cages during the preexposure treatment. This context approximated the level of pheromone exposure males could receive during brief and close encounters with pheromone dispensers in the field (Stelinski et al., 2005a). In addition, a field experiment was conducted in which high densities (10/tree  $\approx$  5000/ha) of Isomate-C Plus dispensers were deployed throughout tree canopies in an effort to maximize exposure of feral moths to pheromone. Captures of moths in traps baited with lure loadings assayed in the flight tunnel were used to relate flight-tunnel data to field responses of feral moths.

## Methods and Materials

### Insect Colonies

Codling moth males were drawn from 1- and 5-yr-old laboratory colonies established with pupae from untreated apple orchards in Michigan and North Carolina, USA, respectively. Moths from North Carolina were supplied by Benzon Research (Carlisle, PA, USA). Moths were reared at 24°C and 60% RH on pinto-bean-based diet (Shorey and Hale, 1965) under a 16:8 (L/D) photoperiod. Pupae were sorted by sex, and adults emerged in 1-l plastic cages containing 5% sucrose in plastic cups with cotton dental wicks protruding from their lids.

### Chemicals and Release Devices

Polyethylene tube pheromone dispensers (Isomate-C Plus containing 205 mg of 53.0% codlemone, 29.7% 12OH, 6.0% 14OH, and 11.3% inert ingredients) were used to preexpose moths. The lures were red rubber septa (The West Company, Linville, PA, USA) loaded with 0.1, 1.0, or 10.0 mg of codlemone (Bedoukian Co, Danbury, CT, USA) that was >98% isomerically pure. Three-component red septa lures were loaded with 0.1 mg of codlemone, 12OH, and 14OH in a 100:20:5 ratio (El-Sayed et al., 1999). Codlemone + pear ester lures were loaded with 0.1 mg of each component in a 1:1 ratio into gray halobutyl septa (Trécé Inc, Adair, OK, USA). Pheromone blends were prepared in HPLC-grade hexane and stored at -18°C. All lures were aged in a fume hood for at least 48 hr prior to use in flight-tunnel assays, whereas Isomate dispensers were aged under the same conditions for 2 wk to allow dissipation of excess pheromone that might have built up on their surfaces during shipping and freezer storage. For EAG recordings, stimulus cartridges used to deliver pheromone to insect antennae were prepared according to Stelinski et al. (2003a,b) by using the codlemone described above.

### Flight-Tunnel Assays

Behavioral assays were conducted in a Plexiglas flight tunnel (Stelinski et al., 2004b, 2005b). The flight-tunnel assay procedures were a slight modification of those described by Stelinski et al. (2005b). Male codling moths, 2–3 d-old, were collected 0.5 hr prior to the end of a 16 hr photophase and placed into cylindrical (8 cm long  $\times$  8 cm diam.) release cages made from aluminum window screening. Each cage, containing two moths, was placed into the flight tunnel for 0.5 hr of acclimation prior

to assays. Subsequently, bioassays ran for a maximum of 1.5 hr. At the upwind end of the tunnel, pheromone dispensers (lures or Isomate dispensers) were placed 1 cm above a horizontal  $7.5 \times 12.5$  cm yellow card (American Scholar, Bay Shore, NY, USA) attached to a horizontally clamped, 9-cm glass rod attached to a steel ring stand 25 cm above the tunnel floor. Release cages holding male moths were placed at the downwind end of the tunnel at a height matching that of the pheromone dispenser.

Males were allowed a maximum of 3 min to respond to an inserted pheromone dispenser, as this was the longest time feral moths remained in close proximity of Isomate-C Plus dispensers in treated orchards (Stelinski et al., 2005a). However, the majority (>80%) of preexposed males left release cages within 1 min and, thus, did not receive the full 3 min of exposure. Following preexposure, males were assayed to various lures described below. During assays following preexposure, males were allowed 3 min to respond. The behaviors recorded were wing fanning, non-anemotactic flight from the release cage, anemotactic flight in the pheromone plume without touching the release device, upwind anemotactic flight followed by landing on the platform and touching the release device. Also, the numbers of individuals with no detectable behavioral change were recorded.

### Experiment 1

For preexposures, moths were released in plumes generated by a rubber septum loaded with 0.1 mg of codlemone or an Isomate dispenser and then, regardless of the behaviors exhibited during preconditioning, were subsequently assayed 15 min later to a 0.1 mg codlemone lure. Two types of controls were conducted. “Naive” moths had no prior exposure to pheromone or the flight tunnel prior to assay. “Control” moths were preexposed to clean air in the flight tunnel and otherwise handled identically to pheromone-exposed moths.

The experiment was conducted as a randomized complete block design; 47 replicate groups of two moths were assayed for each treatment. To avoid any possibility of pheromone contamination in control and naive moths, pheromone preexposed, air preexposed, and naive moths were assayed on different days. This protocol was used in all other flight-tunnel experiments.

### Experiment 2

Males exposed to either 0.1 mg codlemone lures or Isomate-C Plus dispensers were assayed to 0.1 mg codlemone lures 24 hr after the preexposure treatment. Following preexposure, moths were kept in an environmental chamber under the temperature and light cycle conditions described above for the interval prior to testing. Thirty-seven replicate groups of two moths were assayed per treatment.

### Experiments 3 and 4

In these assays, males were exposed to 0.1 mg three-component lures as preexposure treatments. We chose to assay the effect of preexposure to these three-component lures in addition to the codlemone-only lures given that Isomate-C

Plus dispensers also contain the 14 and 12 alcohols. In addition, these experiments tested the hypothesis that males preexposed to Isomate dispensers would respond differently to three-component vs. codlemone-only lures. Seventy-three and 61 replicate groups of two moths were assayed per treatment for the 15 min and 24 hr postexposure intervals, respectively.

#### Experiment 5

Here we tested the hypothesis that preexposure to Isomate-C Plus dispensers raises the response threshold of male codling moths to subsequent presentations of codlemone. Forty-eight, 34, and 39 replicate pairs of treated and control groups of two moths were assayed to 0.1, 1.0, or 10 mg lures, respectively, 15 min after preexposure to codlemone.

#### Experiment 6

Males were preexposed to a 1:1 mixture of codlemone + pear ester and subsequently assayed to 0.1 mg codlemone lures. Forty-one and 40 replicate groups of two moths were assayed per treatment for the 15-min and 24-hr postexposure intervals, respectively.

#### Experiment 7 (Electroantennograms)

The EAG system and test protocols were identical to those of Stelinski et al. (2003a,b). EAGs were conducted on male codling moths 15 min or 24 hr after a preexposure to a 0.1 mg codlemone lure, a 0.1 mg codlemone/pear ester lure (1:1 ratio), a 10.0 mg codlemone lure, or an Isomate-C Plus dispenser in the flight tunnel. EAGs performed on pheromone-exposed moths were alternated with assays of unexposed, naive moths. Ten moths were assayed per treatment for each preexposure treatment and time interval combination tested.

#### Experiment 8

Oriental disruption of male codling moth by high densities of Isomate-C Plus dispensers was measured in the field. They were hung 10 per tree such that all possible points within the canopy were <1.5 m from a dispenser. The density of dispensers used (5000/ha) was five times the recommended dose to ensure that feral males were exposed to very high levels of pheromone. The paired treatment and control plots of 16 trees (0.07 ha) were at least 15 m apart in a two-by-two design and the five replicates separated by at least 35 m. The treatments were applied at the onset of the second codling moth generation in Michigan, USA, and the experiment ran from 15 July through 30 August 2004. Each plot was monitored with two delta traps (LPD Scenturian Guardpost, Suterra, Bend, OR, USA); one had a 0.1 mg lure and the other a 1.0 mg lure. Traps were hung ca. 2–3 m above ground level in the upper third of the tree canopy in two of the four central trees of each plot. Traps were positioned as far away as possible from Isomate dispensers (at least 60 cm). Moths captured in traps were counted and removed twice weekly.

## Statistical Analyses

The numbers of moths contacting lures, orienting without source contact, flying out of release cages without orienting, wing fanning without flight, or exhibiting no detectable behavioral change for each treatment were transformed [ $\log(x + 1)$ ] and submitted to analysis of variance (ANOVA), followed by Tukey's test (SAS Institute, 2000) for separation of means. EAG data were subjected to ANOVA (SAS Institute, 2000). Captures of moths in traps in treated and control orchards were compared by using paired *t* tests. The significance level was  $\alpha < 0.05$ . Percent orientational disruption for the field experiment (experiment 8) was calculated as  $1 - (\text{mean moth catch per trap in the Isomate-treated plot} / \text{mean moth catch per trap in the control plot}) \times 100$ . Data in Tables 1, 2, 3, and 4 are presented as proportions of the total number of moths responding in the flight tunnel.

## Results

### Experiment 1

The responses of naive and control moths were virtually indistinguishable for each behavioral category (Table 1). After preexposure to Isomate-C Plus dispensers, significantly more males either remained in their release cage or exhibited nonoriented flight than the control; only 2 out of 74 moths oriented anemotactically to 0.1 mg lures and none contacted the source (Table 1). Significantly fewer males reached the source after preexposure to 0.1 mg lures, but there was no difference in the proportion orientating without source contact (Table 1).

**Table 1** Behaviors of naive, control (air preexposed), and pheromone-exposed codling moths 15 and 24 hr after preexposure to 0.1 mg codlemone lures

	Proportion of males exhibiting				
	No behavioral change	Wing fanning only	Fly out without orientation	Orientation without source contact	Source contact
15 min postexposure					
Naive (no exposure)	0.00b	0.01a	0.13b	0.28a	0.56a
Control (clean air)	0.00b	0.03a	0.11b	0.33a	0.53a
Codlemone lure exposed	0.02b	0.00a	0.46a	0.30a	0.19b
Isomate-C Plus exposed	0.54a	0.06a	0.37a	0.02b	0.00b
24 hr postexposure					
Naive (no exposure)	0.02a	0.00a	0.14a	0.28b	0.56a
Control (clean air)	0.01a	0.20a	0.24a	0.27b	0.46a
Codlemone lure exposed	0.00a	0.00a	0.16a	0.58a	0.26b
Isomate-C Plus exposed	0.00a	0.00a	0.11a	0.36b	0.52a

Numbers in the same column followed by the same letter are not significantly different (ANOVA followed by Tukey's test,  $\alpha < 0.05$ ).

Experiment 2

Twenty-four hours postexposure, there were no significant differences among responses of Isomate-dispenser-exposed, control, or naive moths to 0.1 mg codlemone lures (Table 1). In contrast, significantly more males preexposed to 0.1 mg lures oriented without source contact, although significantly fewer reached the source than in the other treatments (Table 1).

Experiment 3

Significantly fewer males preexposed to Isomate dispensers or three-component lures contacted 0.1 mg three-component lures 15 min postexposure (Table 2). In addition, significantly fewer Isomate-dispenser-exposed males oriented without source contact, whereas more exhibited no behavioral change than control moths (Table 2).

Experiment 4

After 24 hr, the only effect of preexposure was that significantly fewer Isomate-dispenser-exposed males flew out of release cages without orienting compared with the other treatments (Table 2).

Experiment 5

Significantly more Isomate-exposed moths remained in the release cage and exhibited nonoriented flight than control moths; consequently, fewer oriented to and contacted

**Table 2** Behaviors of control (air preexposed) and pheromone-exposed codling moths in response to three-component (Codlemone/12OH/14OH, 100:20:5 Ratio) lures

	Proportion of males exhibiting				
	No behavioral change	Wing fanning only	Fly out without orientation	Orientation without source contact	Source contact
15 min postexposure					
Control (clean air exposed)	0.01b	0.00a	0.24b	0.13a	0.61a
3-Component lure exposed	0.04b	0.01a	0.47a	0.24a	0.24b
Isomate-C Plus exposed	0.47a	0.00a	0.47a	0.04b	0.02c
24 hr postexposure					
Control (clean air exposed)	0.01a	0.05a	0.16a	0.11a	0.66a
3-Component lure exposed	0.04a	0.00a	0.22a	0.16a	0.58a
Isomate-C Plus exposed	0.00a	0.00a	0.04b	0.18a	0.78a

Numbers in the same column followed by the same letter are not significantly different (ANOVA followed by Tukey’s test,  $\alpha < 0.05$ ).

**Table 3** Behaviors of air preexposed versus Isomate-C plus dispenser-exposed codling moths in response to 0.1, 1.0, OR 10.0 mg codlemone lures 15 min postexposure

	Proportion of males exhibiting				
	No behavioral change	Wing fanning only	Fly out without orientation	Orientation without source contact	Source contact
0.1 mg codlemone lure					
Clean air exposed	0.00b	0.02b	0.19b	0.23a	0.56a
Isomate-C Plus exposed	0.44a	0.12a	0.40a	0.04b	0.00a
1.0 mg codlemone lure					
Clean air exposed	0.26a	0.07a	0.37a	0.27b	0.03a
Isomate-C Plus exposed	0.00b	0.05a	0.30a	0.58a	0.05a
10.0 mg codlemone lure					
Clean air exposed	0.87a	0.01a	0.09b	0.03b	0.00a
Isomate-C Plus exposed	0.21b	0.08a	0.46a	0.29a	0.00a

Numbers in the same column followed by the same letter are not significantly different (ANOVA followed by Tukey's test,  $\alpha < 0.05$ ).

the 0.1 mg lure (Table 3). In contrast, more preexposed males took flight and significantly more oriented to both 1.0 and 10.0 mg lures than controls. However, at both concentrations, there were no differences in the number reaching the source in treated and control treatments (Table 3). For the 10 mg lure, significantly more control males remained in the release cage than the preexposed ones (Table 3).

#### Experiment 6

At 15 min following preexposure to a 0.1 mg codlemone + pear ester lure, significantly more males remained in the release cage and significantly fewer contacted the lure than in the control (Table 4). However, responses were not different from controls 24 hr after preexposure (Table 4).

#### Experiment 7

Mean EAG responses of naive males were indistinguishable, and not statistically different, from those assayed either 15 min or 24 hr after flight-tunnel preexposure

**Table 4** Behaviors of air preexposed versus 0.1 mg codlemone: pear ester lure (1:1 ratio) preexposed codling moths in response to 0.1 mg codlemone lures

	Proportion of males exhibiting				
	No behavioral change	Wing fanning only	Fly out without orientation	Orientation without source contact	Source contact
Clean air	0.06b	0.04a	0.32a	0.06a	0.59a
15 min postexposure	0.22a	0.04a	0.26a	0.19a	0.28b
24 hr postexposure	0.09b	0.06a	0.43a	0.09a	0.51a

Numbers in the same column followed by the same letter are not significantly different (ANOVA followed by Tukey's test,  $\alpha < 0.05$ ).



to a 0.1 mg codlemone, 0.1 mg codlemone + pear ester, or 10 mg codlemone lure or an Isomate-C Plus dispenser (data not shown).

### Experiment 8

Significantly more males were captured in traps baited with either 1.0 mg ( $99.1 \pm 21.8$  vs.  $11.6 \pm 4.2$ ) or 0.1 mg ( $58.2 \pm 13.3$  vs.  $2.4 \pm 1.1$ ) lures in control plots than those treated with 10 Isomate-C Plus dispensers per tree. Orientational disruption was 88.3 and 95.9% for the 1.0 and 0.1 mg lures, respectively.

## Discussion

Brief (seconds to 3 min max) exposures of codling moth males to Isomate-C Plus dispensers in a flight tunnel drastically reduced the proportion of successful orientations to otherwise highly attractive codlemone or three-component (codlemone/14OH/12OH) lures. The effect appeared to be dose dependent; exposure to 0.1 mg lures with codlemone only or with codlemone and the 14 and 12 alcohols did not reduce behavioral responses of males 15 min later to the same degree as did exposure to Isomate dispensers, which release pheromone at ca. 20  $\mu\text{g/hr}$  (Knight, 1995). The effect is likely habituation, given that EAG responses to codlemone were normal in preexposed moths exhibiting reduced behavioral responsiveness. This habituation appears to be a classic threshold elevation (Mafera-Neto and Baker, 1996), as more preexposed moths oriented to elevated dosages of codlemone (1 and 10 mg) than did control moths (Table 3). Conversely, normally attractive dosages of codlemone (0.1 mg) elicited little behavioral response from moths exposed to Isomate dispensers. In field trials, more males are captured in pheromone-treated orchards using higher loadings of codlemone (10.0 mg) compared with lower loadings (1.0 mg), whereas the converse is true in untreated orchards (Charmillot, 1990; Barrett, 1995). Our flight-tunnel data support the hypothesis that pheromone pre-exposure in pheromone-treated orchards raises the behavioral response threshold of codling moth males to subsequent encounters with pheromone plumes (Vickers and Rothschild, 1991). However, the majority of preexposed moths resumed normal behavioral responsiveness within 24 hr of the initial preexposure in the current study. This suggests that a pheromone-induced elevation of response threshold could be reversed in feral males capable of moving to and remaining for some time in pheromone-free air.

An exception was that 24 hr following initial exposure, fewer moths preexposed to 0.1 mg codlemone contacted identical lures compared to those preexposed to Isomate-C Plus (Table 1). This difference is likely due to the type of pheromone preexposure received, as <2% of moths touched the Isomate source during preexposure, compared with >50% of those preexposed to 0.1 mg codlemone (Table 1). It is possible that codlemone was adsorbed onto the insects' cuticle during contact and, thus, exerted a longer-lasting effect. If true, then dispensers, such as hollow fibers (Moffitt and Westgard, 1984; Knight, 2003), that elicit direct source contacts could impact male responsiveness for longer periods than dispensers that do not.

Preexposure of caged male codling moths to an Isomate dispenser for 24 hr increased their behavioral responsiveness to the pear ester [(2*E*,4*Z*)-2,4-decadienoate] in flight-tunnel assays (Zhihua et al., 2005). In addition, captures of both male and female codling moths in pear-ester-baited traps were higher in pheromone-treated vs. untreated orchards (Zhihua et al., 2005). In the current investigation, brief preexposure of males to a low dose of codlemone + pear ester did not affect their subsequent behavioral responses any more than preexposure to codlemone alone. This does not rule out the possibility of habituation at higher dosages of pear ester.

Codling moth males exhibit a prolonged reduction of EAG responses following 10–30 min exposures to codlemone, lasting ca. 1–1.5 hr (Judd et al., 2005; Stelinski et al., 2005c). Similar “long-lasting” adaptation has been reported for other moths, including polyphemus moth, *Antheraea polyphemus* (Cramer) (Kaissling, 1986), the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Stelinski et al., 2003a), and the Pandemis leafroller, *Pandemis pyrusana* Kearfott (Stelinski et al., 2005c). In cases where the airborne concentration of pheromone has been quantified (Stelinski et al., 2003b; Judd et al., 2005), micrograms per liter of air are required to induce long-lasting adaptation. In field trials, long-lasting antennal adaptation in male obliquebanded leafrollers following 24 hr of exposure to Isomate dispensers was seen if males were held 1–2 cm from dispensers but not if the distance was increased to 2 m. Given that long-lasting adaptation was not recorded following any of the preexposure treatments in the current study, males probably did not receive the  $\approx 355 \mu\text{g min}^{-1} \text{l}^{-1}$  dose required to induce long-lasting adaptation (Judd et al., 2005). Stelinski et al. (2005c) questioned the importance of long-lasting adaptation as a contributing factor to mating disruption, as airborne concentrations of pheromone to induce the phenomenon are much higher than those recorded in pheromone-treated orchards (Koch et al., 1997, 2002).

Since exposure of codling moth males to Isomate-C Plus dispensers strongly reduced subsequent responsiveness to codlemone in the flight tunnel, we postulated that feral males, landing near such dispensers in the field would be effectively eliminated from an olfaction-based search for females on that night, given the short diel window of male and female sexual activity (Castroville and Cardé, 1979). However, some feral males were captured in traps baited with 0.1 mg lures despite having 10 Isomate-C Plus dispensers hung throughout tree canopies. This spotlights a vulnerability of polyethylene tube dispensers for pheromonal control of codling moth under high population densities. Given that delta traps limit the importance of close-range visual cues, it is possible that disruption of feral females may have been even lower.

The data from our field experiment suggest that some feral males may receive a pheromone exposure below that imparted by our flight-tunnel preexposure treatments. Thus, we suggest that disruption outcomes in the laboratory, where male moths have been carefully preexposed to microgram per liter dosages of pheromone for several minutes and subsequently shown to exhibit reduced sexual response, may need to be interpreted with caution. These exposures are likely higher than those received by feral males under disruption protocols in the field. Additionally, feral males may actively modulate their exposure by flying to pheromone-free air or to areas of lower pheromone concentrations. Alternatively, those males captured in traps with 0.1 mg codlemone lures in our field experiment

might represent a tolerant phenotype selected by years of pheromone application at this study site.

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