

An Attempt to Increase Efficacy of Moth Mating Disruption by Co-Releasing Pheromones With Kairomones and to Understand Possible Underlying Mechanisms of This Technique

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ABSTRACT Pheromone-based mating disruption is used worldwide for management of the internal fruit feeding codling moth, *Cydia pomonella* (L.). There has been recent interest in the potential of improving mating disruption of *C. pomonella*, and potentially other insect species in general, by broadcasting combinations of pheromone and attractive host-plant kairomones. Given that such kairomones are attractive by themselves (often to both sexes), and also enhance male moth response to their pheromone, it is possible that the effects of competitive attraction and potentially other mechanisms of disruption might be increased. Herein, we tested the hypothesis that mating disruption of *C. pomonella* could be enhanced by co-deploying pheromone with either of two kairomones: (2*E*, 4*Z*)-2, 4-decadienoate (pear ester), or (*E*)- β -farnesene, as compared with various pheromone blend components alone. When deployed individually, each kairomone caused a low level of synthetic lure trap disruption and (*E*)- β -farnesene also caused disruption of mating as measured by tethering virgin females. However, combined release of either pear ester or (*E*)- β -farnesene with pheromone within the same dispenser or as a co-deployed dispenser treatment, respectively, did not increase the level of mating disruption as compared with deploying pheromone alone. Disruption efficacy did not decline when reducing the amount of (*E,E*)-8,10-dodecadien-1-ol (codlemone) in dispensers by fourfold, when combined with pear ester. *C. pomonella* readily were observed briefly approaching all dispenser types (with and without pheromone) in the field. Exposure of male *C. pomonella* to pear ester alone in a manner mimicking observed field exposures did not reduce the number of males able to contact a female-mimic pheromone lure in flight tunnel assays. Also, reduction of male moth behavioral response to pheromone was similar after exposure to codlemone alone, and codlemone and pear ester after exposures that mimicked those observed in the field and none of the main treatments tested (pheromone versus pheromone and either kairomone) affected male moth antennal response seconds after exposure as measured by electroantennogram assays. Collectively, our data indicate that disruption of *C. pomonella* was not improved by co-releasing pheromone with either kairomone tested from point source devices as compared with pheromone alone at the relatively high loading dosages and associated release rates tested.

KEY WORDS mating disruption, plant volatile, codling moth, pheromone–kairomone blends

The codling moth, *Cydia pomonella* (L.), has been one of the most targeted insect species for management with pheromone-based mating disruption worldwide (Witzgall et al. 2008). Although several technologies are available for dispensing pheromone for disruption of *C. pomonella*, hand-applied dispensers of various types that act as reservoirs for the active ingredients and are deployed at 1,000/units ha have been the most widely used in the previous decade (Witzgall et al. 2008). These dispensers serve as high pheromone release point sources and disrupt mating by diverting

males from females by competitive attraction (Miller et al. 2009) with a supplementary mechanism of habituating male sensitivity to pheromone after close-range exposure to dispensers (Stelinski et al. 2006). There have been recent efforts to improve the effectiveness of such point source dispensers by formulating them to co-release the pheromone of *C. pomonella* with a known kairomone for this species, (2*E*, 4*Z*)-2, 4-decadienoate (pear ester) (Bohnenblust et al. 2011; Knight et al. 2012a, b). The pear ester is not only attractive to male and female *C. pomonella* as a single compound (Light et al. 2001), but it also increases lure potency when combined with (*E,E*)-8,10-dodecadien-1-ol (codlemone) as compared with either compound released individually (Il'ichev 2004, Knight et al. 2005). In general, known host plant volatiles of *C. pomonella* increase behavioral responses of males to

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their sex pheromone (Yang et al. 2004). Therefore, the reasoning behind testing blends of pheromone and pear ester for mating disruption of this species is the possibility of improving mating disruption by enhancing the attractiveness of point sources to males, thus enhancing competitive attraction (Knight et al. 2012b). It is also possible that female behavior may be affected by such dispensers so as to affect mating disruption, given the bisexual attractiveness of pear ester (Light et al. 2001). Although mating disruption of codling moth with pheromone alone often has been successful (Witzgall et al. 2008), there have been cases where efficacy has been variable, despite deployment of the pheromone active ingredients at rates that are far beyond economic feasibility (Stelinski et al. 2006). Also, effective pheromone-based disruption of *C. pomonella* becomes increasingly more difficult to achieve as the population density of moths increases (Epstein et al. 2006). Finally, given the higher cost of mating disruption as compared with conventional insecticides (Agnello et al. 2009), grower expectations for efficacy after investment in this method of pest control are high. Therefore, the prospect of improving the consistency of mating disruption for *C. pomonella* and also potentially reducing cost by admixing less expensive active ingredients with pheromone remains an area of research interest.

The search for plant volatiles attractive to *C. pomonella* has been an active area of research (Witzgall et al. 2008) and has included development of attractive lures for field trapping (Knight et al. 2011), as well as fundamental investigations of neural processing in the peripheral (Ansebo et al. 2005) and central (Trona et al. 2010) nervous system of this species. Although many candidate plant volatiles have been tested for attractiveness to *C. pomonella*, few have resulted in practical tools. For example, various isomers of α -farnesene have proven marginally effective or ineffective as lures in the field (Light and Knight 2005), despite documented attraction in laboratory olfactometer assays (Hern and Dorn 1999). Similarly, hexyl hexanoate and butyl hexanoate proved ineffective under field conditions (Ansebo et al. 2004), despite reports suggesting them to be attractants from laboratory assays (Hern and Dorn 2001, 2004). However, (*E*)- β -farnesene has been reported to attract *C. pomonella* in the field (Ansebo et al. 2004, Coracini et al. 2004). Also, (*E*)- β -farnesene increases response of male *C. pomonella* to its pheromone, when assayed in flight tunnel assays (Yang et al. 2004). Therefore, (*E*)- β -farnesene is a likely choice, in addition to pear ester, as a candidate kairomone for co-release with pheromone to enhance mating disruption of *C. pomonella*. Also, despite recent useful efforts to measure the practical application of dispensers co-releasing the pheromone of *C. pomonella* with pear ester, the possible mechanisms of how such pheromone-kairomone blends may enhance mating disruption have not been investigated previously. Combining field efficacy research with laboratory behavioral and sensory investigations that are governed by direct field observation can improve understanding of how behavior-modify-

ing technologies function (Stelinski et al. 2004, 2006). This, in turn, may reduce the time for bringing a practical tool to market for application in agriculture.

The objective of this study was to test the hypothesis that mating disruption of *C. pomonella* could be improved under high moth population densities by simultaneously releasing its sex pheromone with either one of two known kairomones. Ancillary objectives included determining whether the loading rate of pheromone could be reduced in reservoir dispensers that also co-release a kairomone without reducing mating disruption efficacy and to gain insight into the behavioral mechanisms governing the observed mating disruption. Companion laboratory investigations were conducted to quantify the effect of exposure to pheromones, kairomones, or both from dispensers on moth behavior and antennal sensitivity. These lab-based exposure studies were guided by preceding field observations of authentic moth behavior in the field. In addition, the effect of deploying dispensers releasing plant volatile kairomones alone and in combination with co-deployment of standard pheromone dispensers on mating disruption was tested in the field to determine impacts of individual components.

Materials and Methods

General Methods for Field Study. The field experiment was conducted in the spring and summer of 2006 at the Trevor Nichols Research Complex of Michigan State University located in Fennville, MI. The study was replicated five times within six distinct plots of apples (*Malus domestica* Borkh.) in a randomized complete block design. The blocks were predetermined replicates of our experimental design. These blocks were inserted upon previously planted plots of trees of varying variety within an apple orchard ≈ 64 ha in size. The first block consisted of 31-yr-old 'Delicious' apples. The second block was placed within two plots, with one plot consisting of 14-yr-old 'Empire' apples and the second containing 19-yr-old 'Red Chief' apples. The third block consisted of five-year-old 'Smothee' apples, whereas the fourth and fifth blocks were five-year-old 'Jonamac' apples and 19-yr-old Red Chief apples, respectively. Trees were planted with 3-m spacing within a row and 6 m between row spacing with canopy heights ranging from 2.5 to 4.5 m. The plots received pruning, fungicide, and herbicide according to growers' standards in Michigan, but received no insecticide applications. Each block contained nine randomized treatments, each consisting of 25 trees (0.11 ha) with at least 10 m of buffer between treatments. The first-generation experiment was deployed on 1 May and monitored until 11 July. The second-generation experiment was deployed 12 July and monitored until 28 August.

Insect Cultures. Male and female *C. pomonella* were drawn from 1- and 5-yr-old laboratory colonies established with pupae collected from untreated apple orchards in Michigan and North Carolina, United States, respectively. Moths from North Carolina were supplied by Benzon Research (Carlisle, PA). Moths were

reared on a pinto bean-based diet (*Phaseolus vulgaris* L.) at 24°C, 60% RH, and a photoperiod of 16:8 (L:D) h (Shorey and Hale 1965). Pupae were sorted by sex and adults emerged in 1-liter plastic cages containing 5% sucrose in plastic cups with cotton dental wicks protruding from their lids.

Treatments Measured in Field Experiments. The treatments compared were 1) untreated control plots, or plots treated with 2) Trécé (Cidetrack, Trécé Inc., Adair, OK) polyvinyl chloride (PVC) polymer dispensers loaded with 128.4 mg of (*E,E*)-8,10-dodecadien-1-ol (codlemone); 3) Trécé PVC dispensers loaded with 200.4 mg of (*2E, 4Z*)-2, 4-decadienoate (pear ester); 4) Suterra (Suterra LLC, Bend, OR) experimental membrane dispensers loaded with 200 mg of (*E*)- β -farnesene (first generation only); 5) Trécé PVC dispensers loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively; 6) Trécé PVC dispensers loaded with 34.8 and 200.4 mg of codlemone and pear ester, respectively; 7) Isomate-C Plus dispensers (Shin-Etsu Chemical Co. Ltd., Tokyo, Japan) as an industry standard positive control, which contained 205 mg of 53.0% codlemone, 29.7% dodecanol, 6.0% tetradecanol, and 11.3% inert ingredients per dispenser; 8) Isomate-C Plus dispensers co-deployed with Suterra (*E*)- β -farnesene dispensers; and 9) Isomate-C Plus dispensers co-deployed with Trécé PVC dispensers loaded with 200.4 mg of pear ester (second generation only). All dispensers were deployed at a rate of 1,000/ha. All dispensers were deployed in the upper third of the tree canopy. In cases where two types of dispensers were co-deployed, they were affixed in the tree as a single side-by-side unit. Detailed release rates of Trécé PVC dispensers similar to those measured here have been reported previously (Knight et al. 2012a). Also, release rate of Isomate-C Plus dispensers has been reported previously (Stelinski et al. 2009). Membrane dispensers releasing (*E*)- β -farnesene ($N = 5$) were weighed five and 10 wk after deployment on 1 May to confirm release of volatiles and these weights were compared with otherwise similar blank dispensers.

Measurement of Orientational Disruption. Two delta traps (LPD Scenturian Guardpost, Suterra, Bend, OR) were hung in every block ≈ 2 –3 m above ground level in the upper third of the tree canopy. Each trap was baited with a 0.1-mg codlemone red rubber septum lure that was replaced every 2 wk throughout the experiment. Male *C. pomonella* caught in the traps were counted and removed twice weekly.

Measurement of Mating Disruption. Mating disruption was measured by using tethered virgin females. Tethered virgin female *C. pomonella* were attached to at least 60 cm of string by tying a knot around a wing, and then taped onto trees within the treated and control plots. Virgin females were deployed once per week for a 24-h period at a rate of three per plot. They then were collected and dissected to determine mating status by the presence or absence of a spermatophore in the bursa copulatrix. Females were deployed on 15 and 9 nights throughout the first and second

generations, respectively, by targeting nights with no predicted precipitation and low wind.

Direct Observation of Moth Response in the Field. Male moth visits to dispensers during each generation were recorded 2–4 times per week. Treatments to be observed were randomized before each session. Each treatment was observed for 15 min at a time, and 5–7 treatments were observed per night. Data were recorded by speaking observations into a microcassette handheld recorder for future transcription. Data recorded included the following: the number of moths orienting to a dispenser, nearest distance of approach relative to the dispenser(s), and duration of observed visit near the dispenser(s). After dusk, the observations were made by using night vision goggles as described by Stelinski et al. (2004). Observations were conducted on 18 and 13 different nights throughout the first and second generations, respectively, by targeting nights with no predicted precipitation and low wind. We were unable to discriminate approaching moths based on sex in this observational investigation.

Effect of Exposing Males to Dispensers on Subsequent Behavior. Complementary flight tunnel assays were conducted to determine the effect of exposing male *C. pomonella* to chemical release from selected kairomone dispensers with or without codlemone on subsequent behavioral responses to a female-mimic lure. Given logistic limitations, only a representative subset of treatments tested in the field could be measured in these laboratory tests. The assays were designed to mimic the potential exposure (with respect to duration and distance) that males were observed receiving in the field when approaching dispensers (see Results).

Behavioral assays were conducted in a Plexiglas flight tunnel (Stelinski et al. 2005). The flight tunnel was 1.3 by 0.8 m in cross section and 2.4 m long. It was housed in a temperature- and photoperiod-controlled environmental chamber maintained at 23°C, 50–70% RH, and a photoperiod of 16:8 (L:D) h. Light intensity inside the tunnel during photophase was 2–10 lux and was generated by one 95-W fluorescent bulb, model F96T12 (Philips, Amsterdam, the Netherlands) mounted 22 cm above the flight tunnel. Air was pushed through the tunnel by an upwind fan at 0.3 m/s and pheromone emerging from assay chambers was expelled from the tunnel and building through a roof-mounted stack via a pulling fan at the evacuating end of the tunnel.

For preexposures, moths were released within plumes generated by a 1) Trécé PVC dispenser loaded with 128.4 mg of codlemone; 2) Trécé PVC dispenser loaded with 200.4 mg of pear ester; or 3) Trécé PVC dispenser loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively. Exposure to clean air was the negative control. Subsequently, and regardless of the behaviors exhibited during preconditioning, males were assayed 15 min later to a 0.1-mg codlemone lure. The experiment was conducted as a randomized complete block design; 30 replicate groups of two moths were assayed for each treatment. To avoid pheromone contamination between control

and dispenser preexposed moths, these treatments were assayed on different days.

The flight-tunnel assay procedures were a slight modification of those described by Stelinski et al. (2005). Male codling moths (2–3 d old) were collected 0.5 h before the end of a 16-h photophase and placed into cylindrical (8 cm long by 8 cm in diameter) release-cages made from aluminum window screening. Each cage (containing two moths) was placed into the flight tunnel for 0.5 h of acclimation before assays. Subsequently, bioassays ran for a maximum of 1.5 h. At the upwind end of the tunnel, preexposure dispensers were placed 1 cm above a horizontal 7.5- by 12.5-cm yellow card (American Scholar, Bay Shore, NY) 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 down-wind 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 preexposure dispenser, as this was the longest time feral moths remained in close proximity of dispensers in our field observations. However, the majority (>80%) of preexposed males left release cages within 1 min and thus did not receive the full 3 min of exposure. After preexposure, males were assayed to rubber septa loaded with 0.1 mg of codlemone, which elicit maximal responses from unexposed male codling moth in this flight tunnel configuration (Stelinski et al. 2006). During assays after preexposure, males were allowed 3 min to respond. The behaviors recorded were as follows: wing-fanning, nonanemotactic flight from the release cage, anemotactic flight in the pheromone plume without touching the release device, and 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.

Effect of Exposing Males to Dispensers on Subsequent Antennal Response. Preexposures before electroantennogram (EAG) testing of antennal adaptation were identical to those described for measurements of behavior in the flight tunnel. The EAG system and test protocols were identical to those of Stelinski et al. (2003). EAGs were conducted on male *C. pomonella* 15 min after a preexposure to 1) Trécé PVC dispenser loaded with 128.4 mg of codlemone; 2) Trécé PVC dispenser loaded with 200.4 mg of pear ester; 3) Trécé PVC dispenser loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively; 4) Isomate-C Plus dispensers; or 5) Isomate-C Plus dispensers co-deployed with Sutterra (*E*)- β -farnesene dispensers. EAGs were performed by mounting moths within 60 s of dispenser exposure and were alternated with assays of unexposed moths. Ten replicate moths were assayed per treatment for each preexposure treatment.

Statistical Analyses. For orientational disruption studies, data were transformed to $\ln(x + 1)$ (which normalized the distributions and homogenized variance) and then subjected to analysis of variance (ANOVA). Tethered female mating data were arcsine transformed before ANOVA. In cases of significant

ANOVAs, mean separation was conducted using Tukey's test (SAS Institute 2000). 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 ANOVA, followed by Tukey's test for separation of means. Electroantennogram data were subjected to ANOVA. In all cases, the significance level for mean separation was $\alpha < 0.05$.

Results

Measurement of Orientational Disruption. During the first generation of moth flight, significantly ($F = 28.9$; $df = 7, 32$; $P < 0.05$) more male moths were captured in pheromone traps in untreated control plots than in any of the treatment plots (Fig. 1A). Significantly ($F = 32.0$; $df = 7, 32$; $P < 0.05$) fewer males were captured in plots treated with (*E*)- β -farnesene than in plots treated with pear ester (Fig. 1A). Furthermore, significantly ($F = 19.2$; $df = 7, 32$; $P < 0.05$) fewer males were captured in all of the pheromone treatments compared with the two kairomone treatments; however, there was no significant ($P > 0.05$) difference between the pheromone treatments irrespective of co-deployment with kairomone (Fig. 1A).

During the second generation, there was no significant ($F = 1.1$; $df = 7, 32$; $P > 0.05$) difference between male captures in control plots and plots treated with pear ester (Fig. 1B). Significantly fewer ($F = 47.5$; $df = 7, 32$; $P < 0.05$) males were caught in plots treated with each treatment containing pheromone than in control or pear ester-treated plots. However, there were no significant ($P > 0.05$) differences between male captures in treatments containing pheromone with or without addition of pear ester or (*E*)- β -farnesene (Fig. 1B). Dispensers releasing (*E*)- β -farnesene lost 73 (± 8)% of measurable weight change at 5 wk after deployment and 96 (± 10)% at 10 wk after deployment as compared with similar blank dispensers.

Measurement of Mating Disruption. Mating of tethered females in control plots was above 30% during both generations of moth flight (Fig. 2A and B). Mating of virgin female *C. pomonella* was not significantly ($F = 0.7$; $df = 7, 32$; $P > 0.05$) disrupted in plots treated with pear ester only in either generation as compared with the control (Fig. 2A and B). Significantly ($F = 25.4$; $df = 7, 32$; $P < 0.05$) fewer females were mated in plots treated with (*E*)- β -farnesene during the first generation as compared with the control (Fig. 2A.). Also during the first generation, moth mating was significantly ($F = 12.5$; $df = 7, 32$; $P < 0.05$) reduced in all of the pheromone-containing treatments, with or without co-release of additional kairomones, compared with control plots or kairomone-only plots; however, there were no significant ($P > 0.05$) differences among the pheromone-containing treatments (Fig. 2A). Similarly, during the second generation, mating of female moths was significantly ($F = 11.2$;

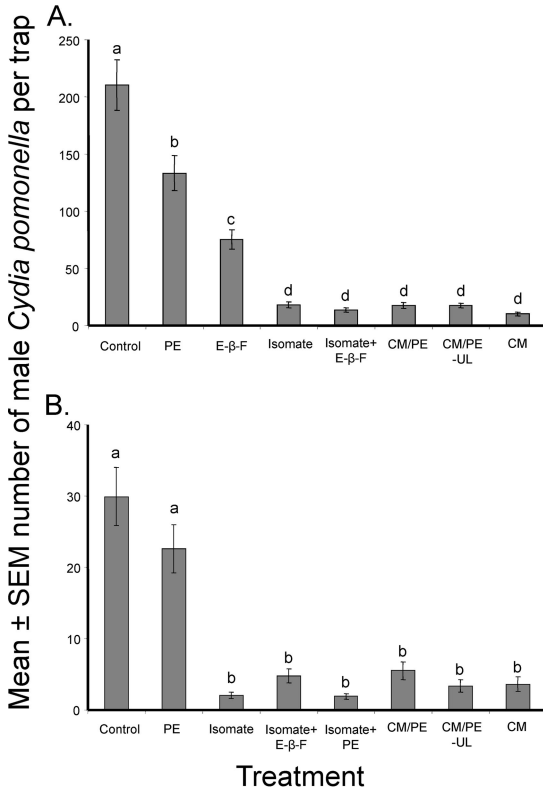


Fig. 1. Mean captures of *C. pomonella* males in lure-baited delta traps in plots containing various pheromone treatments with or without co-release of kairomones during the (A) first (1 May-11 July) and (B) second (12 July-28 August) generations of moth flight. Means followed by the same letter are not significantly different at $\alpha < 0.05$. Abbreviations on the x-axis are as follows: PE: Trécé PVC dispensers loaded with 200.4 mg of (2*E*, 4*Z*)-2, 4-decadienoate (pear ester); *E*-β-F: Sutterra dispensers loaded with 200 mg of (*E*)-β-farnesene (first generation only); Isomate: Isomate-C Plus dispensers, which contained 205 mg of 53.0% (*E,E*)-8,10-dodecadien-1-ol (codlemone), 29.7% dodecanol, 6.0% tetradecanol, and 11.3% inert ingredients per dispenser; CM/PE: Trécé PVC dispensers loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively; CM/PE-UL: Trécé PVC dispensers loaded with 34.8 and 200.4 mg of codlemone and pear ester, respectively; CM: Trécé PVC dispensers loaded with 128.4 mg of codlemone.

$df = 7, 32; P < 0.05$) reduced in all of the pheromone-containing treatments compared with the control or pear ester only treatments (Fig. 2B). There was no moth mating recorded in plots treated with the combination of Isomate C Plus and Trécé pear ester dispensers and in plots treated with Trécé codlemone only dispensers (Fig. 2B).

Moth Observations. During the first moth flight, the majority of moths observed approached Trécé dispensers loaded with pear ester only (approximately two per night) (Fig. 3A). Almost three times as many moths approached pear ester-only dispensers compared with Isomate C Plus dispensers and (*E*)-β-farnesene dispensers (Fig. 3A). During the second

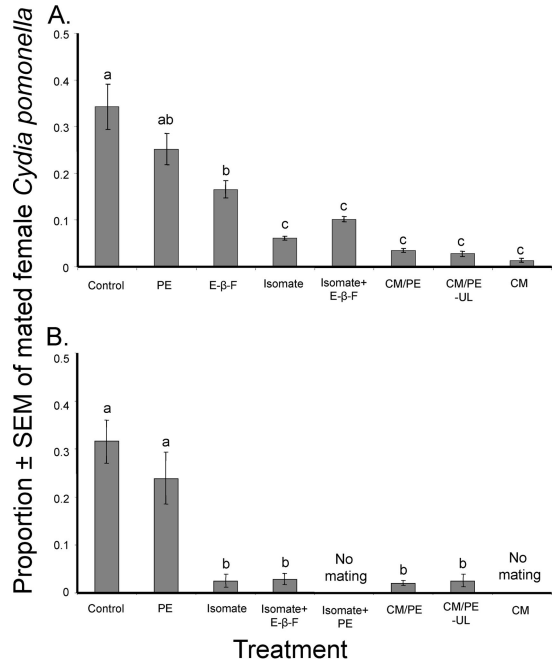


Fig. 2. Mean proportion of virgin female *C. pomonella* mating during 24 h of deployment in plots receiving various pheromone treatments. $n = 45$ and 27 females dissected per treatment in the (A) first and (B) second generations, respectively. Means followed by the same letter are not significantly different at $\alpha < 0.05$. Abbreviations on the x-axis are as follows: PE: Trécé PVC dispensers loaded with 200.4 mg of (2*E*, 4*Z*)-2, 4-decadienoate (pear ester); *E*-β-F: Sutterra dispensers loaded with 200 mg of (*E*)-β-farnesene (first generation only); Isomate: Isomate-C Plus dispensers, which contained 205 mg of 53.0% (*E,E*)-8,10-dodecadien-1-ol (codlemone), 29.7% dodecanol, 6.0% tetradecanol, and 11.3% inert ingredients per dispenser; CM/PE: Trécé PVC dispensers loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively; CM/PE-UL: Trécé PVC dispensers loaded with 34.8 and 200.4 mg of codlemone and pear ester, respectively; CM: Trécé PVC dispensers loaded with 128.4 mg of codlemone.

moth generation, there was no distinct difference between the total numbers of moths observed approaching dispenser treatments, which ranged from approximately one to two moths per dispenser treatment per two nights of observation (Fig. 3B). During both generations, the mean duration of moth visits to dispensers ranged between 4 and 18 s; however, there were no significant ($P < 0.05$) differences in duration of visits between treatments (Fig. 4A). On average, the closest approach distances ranged between 55 and 108 cm from dispensers; but, again there were no significant ($P < 0.05$) differences observed in approach distance between dispenser treatments (Fig. 4B). In all cases, moth sex was not determined.

Effect of Exposing Males to Dispensers on Subsequent Behavior. Preexposure of male *C. pomonella* to Trécé dispensers releasing pear ester alone did not significantly ($F = 1.8; df = 3, 116; P > 0.05$) reduce the proportion of moths contacting 0.1-mg codlemone

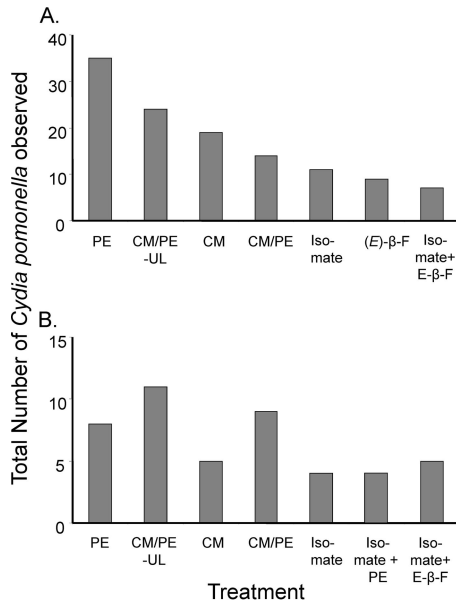


Fig. 3. Numbers of *C. pomonella* observed approaching various dispenser treatments of pheromone with or without additional kairomone release or dispensers in plots receiving various pheromone (\pm kairomone) treatments in the (A) first and (B) second generations, respectively. All moths observed approached within 160 cm of dispensers. Observations were conducted over 18 and 11 nights during the first and second generations, respectively. Abbreviations on the x-axis are as follows: PE: Trécé PVC dispensers loaded with 200.4 mg of (2*E*, 4*Z*)-2, 4-decadienoate (pear ester); *E*-β-F: Sutterra dispensers loaded with 200 mg of (*E*)-β-farnesene (first generation only); Iso-mate: Isomate-C Plus dispensers, which contained 205 mg of 53.0% (*E,E*)-8,10-dodecadien-1-ol (codlemone), 29.7% dodecanol, 6.0% tetradecanol, and 11.3% inert ingredients per dispenser; CM/PE: Trécé PVC dispensers loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively; CM/PE-UL: Trécé PVC dispensers loaded with 34.8 and 200.4 mg of codlemone and pear ester, respectively; CM: Trécé PVC dispensers loaded with 128.4 mg of codlemone. Moth sex was not determined in these in-field observations.

lures 15 min later as compared with exposure to clean air; however, numerically fewer moths made source contact after this preexposure treatment (Fig. 5). Exposure to Trécé dispensers releasing codlemone only and the combination of codlemone and pear ester significantly ($F = 57.5$; $df = 3, 116$; $P < 0.05$) reduced subsequent moth response to 0.1-mg lures compared with the control; however, there was no significant ($P > 0.05$) difference between the two exposure treatments (Fig. 5). Significantly ($F = 63.2$; $df = 3, 116$; $P < 0.05$) more males oriented without source contact after each of the three preexposure treatments compared with air-exposed controls (Fig. 5).

Effect of Exposing Males to Dispensers on Subsequent Antennal Response. Mean EAG responses of unexposed male *C. pomonella* were similar and not statistically different from those of moths assayed within 60 s of flight-tunnel preexposure to Trécé PVC

dispensers releasing codlemone, Trécé PVC dispenser releasing pear ester, Trécé PVC dispensers releasing codlemone and pear ester, Isomate-C Plus dispensers, or Isomate-C Plus dispensers co-deployed with Sutterra (*E*)-β-farnesene dispensers (data not shown).

Discussion

The goal of this investigation was to determine whether mating disruption of *C. pomonella* could be improved by co-deploying known kairomones of this species with its sex pheromone as compared with pheromone-based mating disruption alone. Pear ester and (*E*)-β-farnesene were chosen as candidate kairomones because of their known behavioral activity on *C. pomonella* under field conditions (Light et al. 2001, Ansebo et al. 2004, Coracini et al. 2004, Knight et al. 2005). Although pear ester and (*E*)-β-farnesene were released from different types of dispensers and likely at different rates, both kairomone treatments reduced catch of males in pheromone traps as compared with untreated plots, indicating behavioral activity. The (*E*)-β-farnesene treatment also significantly reduced mating of tethered virgin females; a similar trend was noted in plots treated with dispensers releasing pear ester only. To our knowledge, this may be the first field-based experiment to show disruption of moth catch in traps and that of virgin females by release of kairomones alone without pheromone, suggesting possible future practical application. Despite an indication of behavioral activity from each kairomone dispenser treatment, these results do not suggest practical value was gained by deploying kairomones alone at the release rates tested for disruption of mating.

Co-deployment of either kairomone did not improve the level of *C. pomonella* mating disruption as compared with plots treated with pheromone only. The two kairomones were tested in different types of dispensers because of our limitation in formulating release devices. In the case of pear ester, kairomone and codlemone were released individually or as combined blends from recently developed Trécé PVC dispensers (Bohnenblust et al. 2011, Knight et al. 2012a). The current results are consistent with previous measurements of mating disruption with a combination of pear ester and codlemone (Knight et al. 2012a). One recent study reported significantly lower male catch in virgin female-baited traps in plots treated with Trécé PVC dispensers releasing a combination of codlemone and pear ester as compared with plots treated with Isomate-C Plus dispensers releasing three components of the sex pheromone and no kairomone (Knight et al. 2012b). However, our results are consistent with previous investigations in that Trécé PVC dispensers releasing a combination of codlemone with pear ester did not cause greater disruption of *C. pomonella* as compared with otherwise identical dispensers releasing codlemone alone (Knight et al. 2012a, b). Therefore, there is no evidence to date that co-release of pear ester with codlemone enhances mating disruption of *C. pomonella* as compared with deploying codlemone alone and the

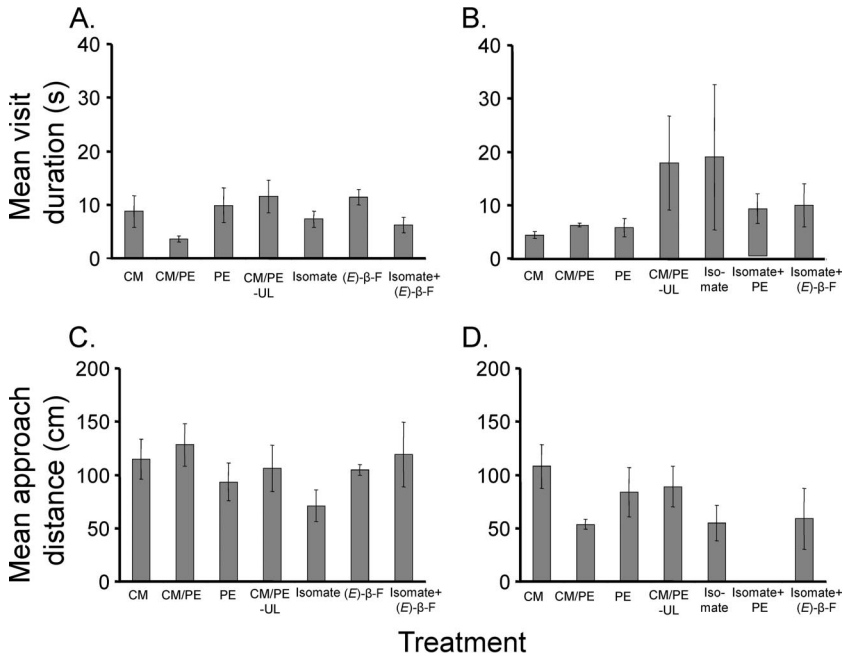


Fig. 4. Mean duration (A) and (B) of observed exposure during orientation and nearest distance of approach to dispenser treatments (C) and (D) by *C. pomonella* in the field during the first and second generations, respectively. Abbreviations on the x-axis are as follows: PE: Trécé PVC dispensers loaded with 200.4 mg of (2*E*, 4*Z*)-2, 4-decadienoate (pear ester); *E*-β-F: Sutterra dispensers loaded with 200 mg of (*E*)-β-farnesene (first generation only); Isomate: Isomate-C Plus dispensers, which contained 205 mg of 53.0% (*E,E*)-8,10-dodecadien-1-ol (codlemone), 29.7% dodecanol, 6.0% tetradecanol, and 11.3% inert ingredients per dispenser; CM/PE: Trécé PVC dispensers loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively; CM/PE-UL: Trécé PVC dispensers loaded with 34.8 and 200.4 mg of codlemone and pear ester, respectively; CM: Trécé PVC dispensers loaded with 128.4 mg of codlemone. Moth sex was not determined in these in-field observations.

superiority of Trécé PVC dispensers, as compared with Isomate-C Plus, reported by Knight et al. (2012b), was similar whether the dispensers released codlemone alone or codlemone with pear ester.

In the case of (*E*)-β-farnesene, we tested the hypothesis that the efficacy of the standard Isomate-C Plus dispenser treatment, which releases three components of the codling moth pheromone blend, could

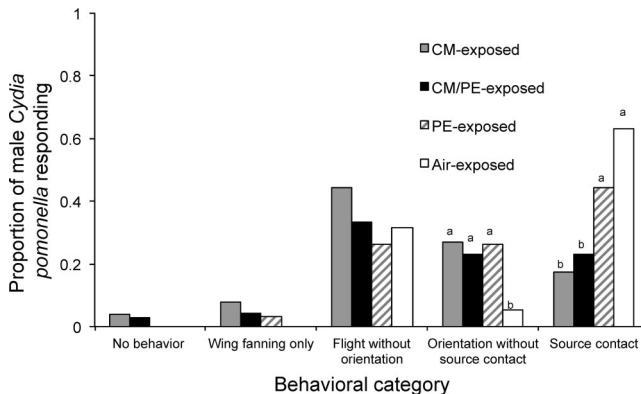


Fig. 5. Behaviors of air preexposed (control) versus dispenser-exposed *C. pomonella* males in response to 0.1-mg codlemone lures 15 min postexposure. Treatments consisted of seconds-long exposures to the following: 1) Trécé PVC dispenser loaded with 128.4 mg of codlemone; 2) Trécé PVC dispenser loaded with 200.4 mg of pear ester; 3) Trécé PVC dispenser loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively), or clean air. Abbreviations in figure legend are as follows: CM: Trécé PVC dispensers loaded with 128.4 mg of codlemone; CM/PE: Trécé PVC dispensers loaded with 139.2 and 200.4 mg of codlemone and pear ester, respectively; PE: Trécé PVC dispensers loaded with 200.4 mg of (2*E*, 4*Z*)-2, 4-decadienoate (pear ester).

be enhanced by co-deployment with dispensers releasing the kairomone only. For this purpose, we deployed (*E*)- β -farnesene in custom-made membrane dispensers patterned after the Suterra CheckMate technology. As observed with pear ester, there was no evidence that co-deploying (*E*)- β -farnesene with pheromone improved disruption of *C. pomonella* as compared with plots treated with the positive control pheromone only treatment. Similarly, co-deploying Isomate-C Plus dispensers with separate Trécé PVC dispensers releasing pear ester only did not increase the level of mating disruption as compared with deploying Isomate-C Plus dispensers alone. A limitation of our field investigation was the small plot size required to accommodate the number of treatments tested. Plot sizes on the scale described in Knight et al. (2012a, b) or larger may be necessary to adequately resolve the potential additional benefit of deploying kairomones with pheromone as compared with pheromone alone for disruption of this or other species.

The complementary behavioral and EAG experiments yielded results that were either largely consistent with the results of previous investigations or were otherwise predictable. *C. pomonella* were observed to closely, yet briefly, approach all dispenser types as has been observed previously for the various Trécé PVC dispensers by Knight et al. (2012a) and Isomate-C Plus dispensers by Stelinski et al. (2006). As we predicted, *C. pomonella* also approached dispensers releasing (*E*)- β -farnesene only. There was no apparent correlation between the number of observed moth visits to dispenser treatments with effectiveness of mating disruption. For example, the most moths were observed approaching dispensers releasing pear ester only; however, such dispensers did not significantly reduce mating of virgin females. The greater number of moth visits to pear ester only dispensers as compared with the pheromone dispenser treatments may have been because of attraction of both sexes as compared with males only to pheromone-only dispensers and the suboptimally high release rate of pheromone. These results also support the hypothesis that pure competitive attraction as defined in Miller et al. (2009) was not functioning alone to cause disruption, but supplemented by sensory desensitization after moth exposure to pheromone from dispensers as suggested by Stelinski et al. (2006). The results of our flight tunnel experiment corroborate this hypothesis in that brief and close range exposures of male *C. pomonella* to Trécé PVC dispensers releasing pear ester only did not reduce subsequent male moth response to pheromone nearly as greatly as identical exposure to dispensers that released pheromone. However, there was a discernable effect of pear ester only exposure on subsequent orientation of male *C. pomonella* to codlemone, which does not completely exclude the possibility that this kairomone may indeed contribute to sensory desensitization of males to pheromone for practical exploitation. In a related experiment, exposure of males to Trécé PVC dispensers releasing codlemone only had the same effect as exposure of males to dispensers releasing the combination of codlemone and pear es-

ter, suggesting that the blend does not induce a greater level of habituation as compared with the pheromone alone. Overall, the effects on male orientation behavior observed after exposure to the Trécé PVC dispensers releasing codlemone were similar to those observed previously after exposure to Isomate-C Plus dispensers (Stelinski et al. 2006). Finally, brief and close range exposures of male *C. pomonella*, mimicking those observed in the field, to dispenser treatments with or without kairomones, did not affect male antennal responses, as recorded by EAG, within 60 s of preexposure, congruent with similar results after exposure to Isomate-C Plus dispensers (Stelinski et al. 2006).

Collectively, the current data set tends to falsify the hypothesis that disruption of *C. pomonella* is increased by releasing a combination of an attractive plant kairomone with sex pheromone as compared with releasing pheromone alone. However, the limitation of small plot size necessitates further investigation. This was tested for two known attractive kairomones and two types of hand-applied release devices when deployed at 1,000 units/ha. These results are consistent with previous investigations measuring Trécé PVC dispensers releasing codlemone with pear ester as compared with those releasing codlemone alone (Knight et al. 2012a, b). Although disruption may be improved by further increasing the release rate of either kairomone, the practicality of this approach may be questionable, given potential economic and environmental limits of the amount of synthetic kairomone released as compared with release of pheromone alone. It may be possible that addition of kairomone may allow reduction of the pheromone active ingredient per dispenser, although still maintaining overall efficacy of disruption. Unfortunately, the current and previous investigation of a Trécé PVC dispenser treatment with reduced codlemone loading and co-release of pear ester (Knight et al. 2012b) did not have an available low rate, codlemone only positive control treatment; therefore, this is still an open question. It is also possible that co-release of kairomones with pheromone to enhance mating disruption of *C. pomonella* (or other insect species) would benefit from releasing several kairomone components simultaneously with pheromone as compared with individual compounds combined with pheromone.

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