Analysis of Codling Moth Mating Disruption Dispensers in a High-Elevation Northern Utah Apple Orchard

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Mating disruption is an insect pest management technology that uses dispensers to saturate an area with the female sex pheromone of the target insect, preventing males from finding females and thereby delaying or stopping mating. Many Utah apple growers use hand-applied mating disruption dispensers for management of codling moth, a pest that is responsible for the majority of fruit loss in Utah due to larval feeding within the fruit. Some growers in the State have expressed concern for the longevity of the polymer membrane of various hand-applied brands due to greater UV exposure at higher elevations. The majority of commercial orchards in Utah are at 4500 – 6000 feet elevation.

A variety of hand-applied dispensers are registered for codling moth mating disruption. The differences between dispensers lie in the delivery and packaging of the pheromone, called codlemone, rather than in the chemical itself. UV and oxygen exposure of the dispensers accelerates isomerization of codlemone into isomers that are antagonistic to male moths (Brown and McDonough 1986, Millar 1995, El-Sayed et al. 1998), which may inhibit success of mating disruption. Manufacturers have made improvements over time through the use of UV-adsorbers and stabilizers and fillers to the dispenser wall, to reduce the rate of pheromone degradation. Ideally, dispensers should release enough pheromone consistently over the codling moths' 2-3 generational life span (approximately 140-150 days) without pheromone depletion or dispenser degradation.

The goal of this project was to provide an unbiased report of the pheromone content and release rate of commonly used mating disruption dispensers for the tree fruit industry in Utah. The information will help growers to make prudent decisions for their pest management needs. It is important that growers have confidence in using mating disruption because this technology allows growers to reduce insecticide sprays, especially the organophosphate products that are currently being phased out.

To test the dispensers, four different hand-applied brands were hung in a northern Utah apple orchard throughout a period of 140 days and tested at various times to determine the amount of pheromone remaining and amount of pheromone being released from each dispenser. It is important to evaluate both residual pheromone and pheromone volatilization in the dispensers because polymerization of pheromone on the dispenser surface can alter actual release rates into the environment.

Materials and Methods

Dispensers: This study tested the following four hand-applied dispenser brands:

1. Cidetrak CM (Trécé, Inc., Adair, OK) is purported by Trécé to last all season due to technology that prevents pheromone degradation. The product uses a novel packaging of the pheromone in an internal and external shell that is said to prevent oxidation.
2. Isomate-C plus (Pacific BioControl, Vancouver, WA) is a polyethylene tube twist tie dispenser loaded with pheromone. This dispenser is considered the industry standard to which others are compared.
3. Isomate-CTT (Pacific BioControl, Vancouver, WA) is similar to Isomate-C plus, but formed of "twin tubes" (TT) that contain double the volume of pheromone, and so are applied at half the rate of the C plus.

4. Checkmate CM-XL 1000 (Suterra LLC, Bend, OR) is a membrane dispenser applied at the same rate as Isomate-CTT.

Trécé Cidetrak is a newly registered brand of dispenser not currently in use in Utah while the remaining three have been used by Utah growers for many years. The two Isomate dispensers were donated from the distributor, Pacific BioControl, Cidetrak was donated from the manufacturer, Trécé, and Checkmate was donated from a grower. All dispensers were shipped to the P.I.'s lab at USU either sealed or wrapped in aluminum foil, and were placed in the freezer until deployment in the field. Table 1 shows the manufacturer's labeled codlemone amount in each dispenser, application rate, and cost/acre.

Table 1. Pheromone amounts, application rates, and cost/acre of codling moth mating disruption dispensers used in this study.

Dispenser	**(*E,E*)- 8,10-dodecadien-1-ol (codlemone pheromone)***		**Application Rate (minimum)**	**Product cost/acre****
Cidetrak CM	2.0% (120 mg)		400/acre	$192
Isomate-C plus	53.0% (205 mg)		400/acre	$100
Isomate-CTT	53.0% (382 mg)		200/acre	$100
Checkmate CM-XL	17.5% (250 mg)		200/acre	$110

*expressed as percentage a.i., and total volume a.i. in each dispenser.

** cost/acre is an average of the product. Average labor cost/acre to install dispensers at 400/acre are approximately $20-40/acre.

Dispenser placement: Dispensers were hung in a commercial 'Fuji' apple orchard in Payson, UT, which sits at approximately 4800 feet, on May 5, 2009 (the day before codling moth biofix in Payson). Dispensers were hung according to labeled directions, in the top third of the tree canopy. Because we were not testing the products for efficacy in the field (via fruit injury or moth trap catch), dispensers were placed at a high density to make retrieval easier. Each brand was hung together in its own row of the orchard, with approximately 10-20 dispensers per tree. Branches with test dispensers were flagged.

Dispenser testing: Dispensers were aged in the study orchard for a total period of 140 days. Six dispensers of each brand were collected on 5 testing dates (see Table 2), and sent to Michigan State University to determine pheromone release rate (volatile trapping system) and pheromone content (residual analysis) of each dispenser. In the field, collected dispensers were wrapped tightly in aluminum foil, placed in a labeled Ziploc baggie, and stored in a cooler with ice until they could be placed in a freezer for storage. They were mailed in boxes with cold packs via overnight delivery to the respective labs in Michigan within 3 days of collection. (The "day 0" dispensers were sent directly from the P.I. lab.)

Table 2. Number of dispensers of each brand tested for pheromone release rate (residual analysis) and content (volatile trapping system) on each date.

Date	Days after deployment	Number of dispensers tested	
		residual analysis	VTS
May 5	0	6	6
May 19	14	0	6
August 4	90	6	6
September 1	120	6	6
September 22	140	6	6

Testing protocol, Residual Analysis (modified from: Vince Hebert and Elizabeth Tomaszewska, Michigan State University):
Residual analysis was conducted in the lab of Dr. Jim Miller, Michigan State University, to determine the amount of pheromone remaining within each dispenser. Individual dispensers (cut into 1-1.5 cm pieces) were added to a flask containing 90 mL acetone and 2 mL methyl myristate. After the plugged flask had set for at least 8 hours, the contents were transferred to a volumetric flask with enough acetone to bring the amount up to 100 mL, and the flask inverted several times. One mL of the sample was combined with 2 mL of acetone in a labeled centrifuge tube, and then vortexed and filtered into a labeled gas chromatograph (GC) vial. Residual pheromone was then quantified using a GC.

Testing protocol, Volatile Trapping System:
Testing via the volatile trapping system was conducted by the lab of Dr. Larry Gut, Michigan State University, to determine each dispenser's pheromone release rate. Volatile collection chambers were comprised of 1-liter Teflon transfer containers equipped with two 0.64-cm ports in the lids (Figure 1). During the collection of volatiles the lid was tightly sealed using Teflon taps so that 100% of the air entered and flowed through the chamber (i.e., no leakage).

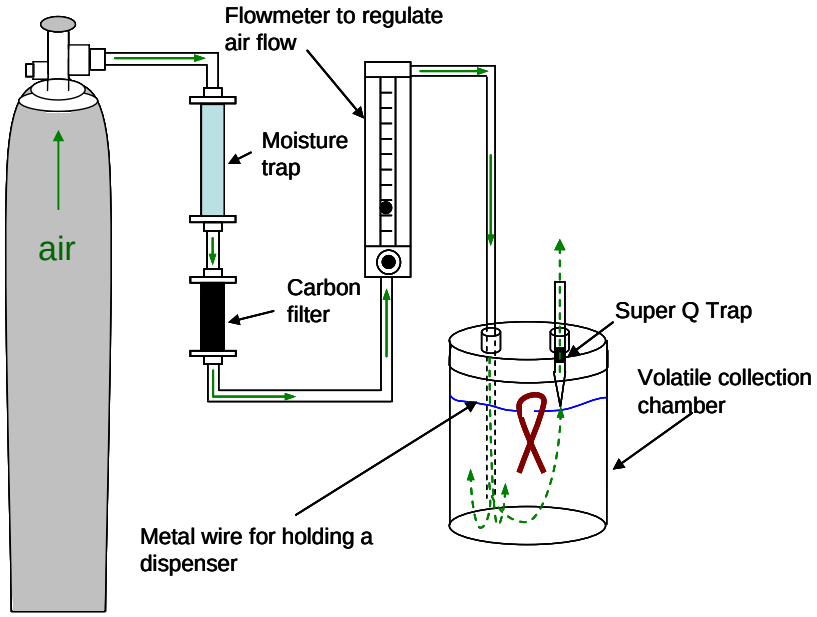


Figure 1. Volatile trapping system configuration

Each dispenser was placed inside a volatile collection chamber, and carbon-purified air entered the Teflon volatile collection chamber at a rate of 1.6L/min. It was passed over the dispenser and then through a trap containing 25 mg of Super Q adsorbent sitting below the exit port. Volatiles were collected for 2 or more hours depending on the amount of pheromone released by a given dispenser. More time was allotted for dispensers releasing very small quantities of pheromone. Between testing, all of the volatile collection devices were washed with acetone three times and baked in an oven over 100º C for at least 2 h.

Volatiles were eluted from individual traps with 150 µl acetone three times. The elute was either ready for GC analysis or concentrated under a nitrogen stream depending on the amounts of volatiles captured. Methyl myristate was added as an internal standard. For each sample, 1 µl was analyzed by capillary GC (Hewlett-Packard HP6890 equipped with a Hewlett Packard 7863 auto sampler) on an HP-Innowax polyethylene glycol column (30 m × 250 µm i.d., 0.25 µm film thickness) with a splitless injector at 250º C and flame ionization detector at 300º C. Following injection, column temperature was held at 50º C for 5 min, increased at 25º C /min to 155º C and held for 5 min; then increased at 0.5º C/min to 165º C and held for 3 min; finally increased at 30º 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 quantified by comparing their peak areas with that of an internal standard.

Results

Some of the dispenser brands (Isomate and Checkmate) tested in this project also contained minor pheromone alcohols (dodecanol and tetradecanol), however, we are reporting only codlemone ((*E*,*E*)- 8,10-dodecadien-1-ol) amounts because research has shown that the secondary pheromone products do not contribute to a behavioral effect determining attraction and mating disruption in the field (Witzgall et al 2008) or the lab (McDonnough et al 1993).

Results showed that all four dispenser types released codlemone and had codlemone remaining within the membrane after 140 days in the field. Codlemone levels dropped significantly in the first 90 days of deployment from all dispenser types, and then residuals declined slowly from 90 through 140 days in the field (Figure 2). All dispensers initially released a high rate of codlemone which dropped significantly after 2 weeks in the field. Thereafter, release rates declined at a more gradual rate for all dispenser brands through 140 days (Figure 3). The two Isomate brands exhibited the steadiest release rate patterns (Fig. 3). At the end of the study, Isomate-CTT had 32% pheromone remaining in the dispensers while the remaining three brands had from 5-9% remaining.

Figure 2. Average amount of codlemone content (milligrams) within four different brands of mating disruption dispensers tested over time in a Utah orchard, on days 0, 90, 120, and 140.

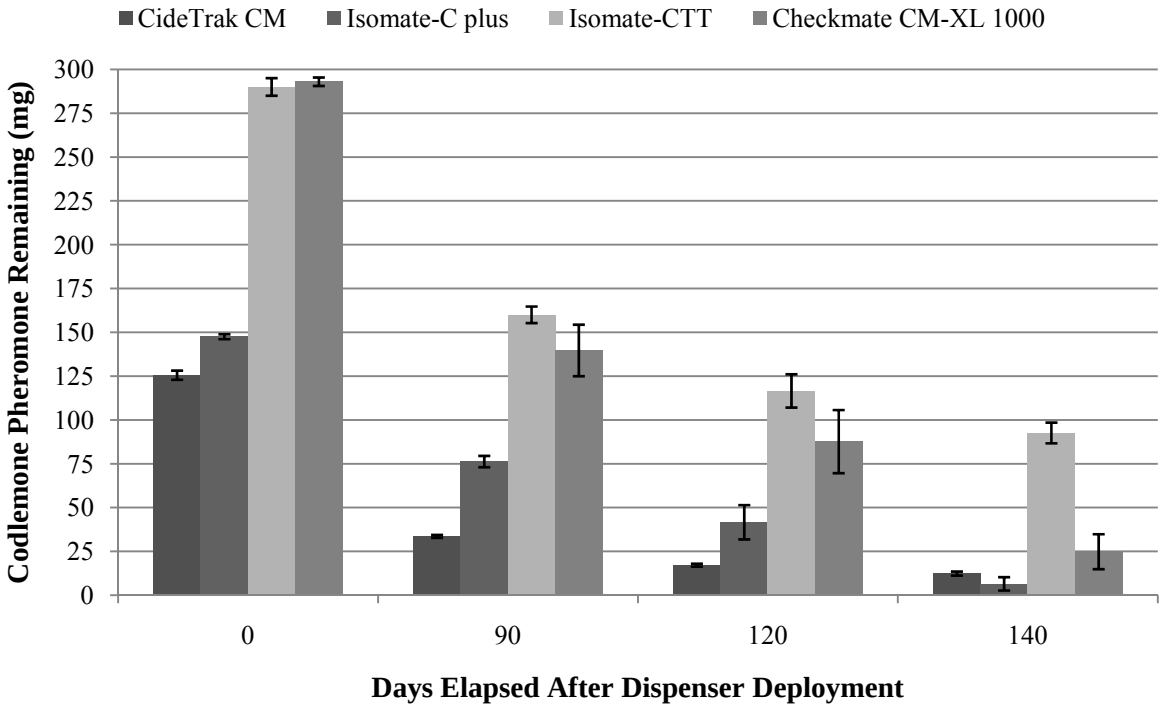
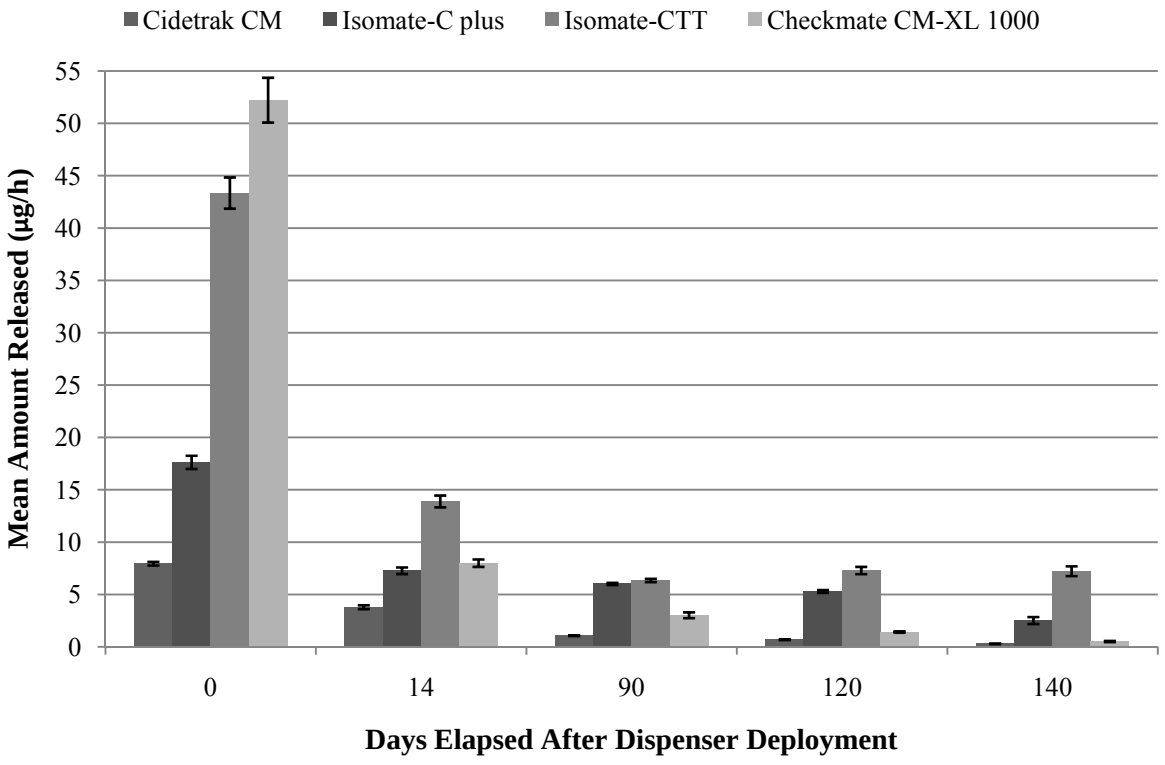


Figure 3. Average amount of codlemone released (micrograms/hour) by four different brands of mating disruption dispensers tested over time in a Utah orchard on days 0, 14, 90, 120, and 140.



Cidetrak: The codlemone release rate for Cidetrak was the lowest of the four brands tested over the entire 140 days. After 14 days, the release rate averaged 3.79 µg/hour, and after 140 days, the release rate averaged 0.28 µg/hr. The amount of codlemone within the dispensers was the lowest of all dispenser types over the first 120 days, and the second lowest at the end of the study (12.37 mg, which was twice as much as in the Isomate-C plus).

Isomate-C plus: The average release rate for this dispenser over the first 14 days of field aging was 7.27 micrograms/hour (µg/hr). This rate remained fairly constant over the next 106 days. It was still releasing pheromone after 140 days in the field, but had dropped to an average rate of 2.51 µg/hr, the second highest of the four (behind Isomate-CTT). The Isomate-C plus dispensers started out with 147 mg codlemone, which gradually decreased to 6.53 mg by the end of the study.

Isomate-CTT: One would assume that because the CTT is a "double formulation" model of its counterpart (Isomate-C plus) that codlemone release rates would be twice as much. Evaluation of codlemone release prior to field deployment (day 0) showed that the Isomate-CTT did contain almost twice as much, and was releasing 2.5 times the amount of codlemone as Isomate-C plus. On day 14, the average release rate of Isomate-CTT had dropped to 13.89 µg/hr, still twice that of Isomate-C plus. After 90 days of field aging, the average codlemone release rate of CTT was almost the same as C+, at 6.34 µg/hr. This release rate, however, remained fairly constant through the next 50 days, and after 140 days in the field, the average release rate was the highest of the four tested, at 7.23 µg/hr, almost three times the average amount released by Isomate-C plus. Of the four products tested, Isomate-CTT had the highest amount of codlemone remaining in the dispensers at the end of the study, at 93 mg, 14 times the amount left in the Isomate-C plus, and almost four times the amount left in the Checkmate.

Checkmate: The average codlemone release rate of the Checkmate dispensers before deployment into the field (day 0) was the highest of the four, at 52.22 µg/hour. In 14 days, it had decreased by almost seven-fold to an average of 7.99 µg/hr, and after 140 days, the Checkmate dispensers were releasing an average of only 0.52 µg codlemone/hr. Before deployment in the field, the average amount of codlemone in the Checkmate dispensers was the highest of the four tested, at 293 mg. After 90 days, it had decreased by half, and by 140 days, 24.85 mg remained.

Discussion

Our primary goal was to determine whether the commonly used mating disruption dispensers were still releasing sufficient pheromone after 140 days of field aging. All dispensers were still releasing pheromone and still contained pheromone by the end of the study; however, there were clear differences among the dispenser types. The two Isomate products emitted the greatest amount of pheromone over the course of field aging, and maintained the most consistent release. The Checkmate dispensers were not very efficient in their release of codlemone. They lost the greatest amount of pheromone over the course of the study, but were among the lowest in release rate, suggesting that pheromone was degrading from the dispensers rather than volatilizing into the air. The Cidetrak dispensers had the lowest release rates for all testing dates.

Isomate-CTT and Checkmate are applied at the same rate (200/acre), as are Isomate-C plus and Cidetrak (400/acre). The Isomate-CTT was still releasing the greatest amount of pheromone of all the dispensers

at the end of the study. When compared to Checkmate (which was among the lowest release rates), there was a 14-fold release rate difference between the two. Isomate-C plus released more pheromone than Cidetrak throughout the study, but it should be noted that studies conducted elsewhere that compared Cidetrak with Isomate-C plus found little difference in fruit injury or trap catch between these products in the field (Kahn 2007, Stelinski et al 2006).

It is interesting to note that although the Isomate-CTT is loaded with twice as much pheromone (and applied at half the rate) as Isomate-C plus, the release rates of the two were similar between 90 and 120 days of field aging, which in summer 2009 was during the second half of the second generation moth flight. If higher release rates equate with more successful mating disruption, fields using Isomate-C plus would actually have greater protection during this time period. Alternatively, during protracted growing seasons, the Isomate-CTT might be a good choice because it still contained a significant amount of pheromone by the end of the study. Utah orchards have high codling moth densities due to small, disjointed farms and numerous backyard trees, and growers that choose to use Isomate-CTT might be safer to initially use a higher than labeled rate to reduce local populations.

A quantifiable amount of airborne codlemone required for successful mating disruption has not been determined. Individual codling moth females release approximately 0.005-0.007 µg pheromone/hour during calling (Bäckman 1997), and the two dispensers in this study with the lowest release rates after 140 days were releasing at least 41 times this amount. The level of pheromone necessary for successful disruption would depend upon a variety of variables including initial insect population. As previously mentioned, Utah growers are faced with high pest pressure which may require the high pheromone release rates to successfully suppress mating. The only way to determine whether the two products in this study with the lowest release rates (Cidetrak and Checkmate) are still effective at preventing injury, would be to test them under field conditions for trap shutdown and prevention of fruit injury.

According to the National Weather Service, the summer of 2009 in Utah was cooler than average with a rainy and cool June and dry July and August. Extended periods of hot weather, such as was the case in the summer of 2007, may decrease effective lifetimes of dispensers. As such, we recommend to Utah commercial orchardists using mating disruption, whichever product they chose, to carefully monitor all treated blocks using pheromone traps and injury inspection, particularly toward the end of the season.

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