

A Novel Mating Disruption System Designed for Rapid Deployment of Reservoir Pheromone Dispensers

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Sex pheromone-based mating disruption is among the most effective biopesticides for managing various lepidopteran fruit crop pests, including codling moth (CM). An estimated 544,000 acres worldwide of apple, pear and walnuts, including 190,000 acres in North America, are treated with pheromone to control CM (Witzgall et al. 2008, Miller and

Gut 2015). However, we suspect that less than 15% of the approx. 140,000 acres of apple orchards in the Eastern US use CM disruption. The most widely used CM mating disruption formulations require hand-applying hundreds of devices per acre. Thus, the product and application costs, as well as labor requirements, are high. New pheromone delivery systems are needed to provide Great Lakes growers with economical alternatives to maintain or develop sustainable IPM programs based on the use of mating disruption.

Novel Pheromone Delivery System

Michigan State University researchers have been evaluating a new pheromone delivery system that is easily applied at high point source densities and is potentially less expensive than currently available hand-applied disruption formulations. This new technology, called the Tangler®, is being developed by a Michigan-based company, Deploy Technologies. The Tangler is comprised of a plastic module containing pheromone and a launcher that enables the grower to rapidly apply numerous modules to the upper tree canopy. The module consists of two plastic caps that are attached by a 1-ft length of cotton string (Figure 1). A proprietary pheromone-release system is secured inside one of the caps. During construction, the string is coiled and placed inside the base cap of the module. The pheromone-filled cap joins with the base and seals the module. As a result of the “bola” design, the propelled dispensers readily become tangled in the tree branches (Figure 1). Dispensers are strapped together in a fashion that allows them to be fed into a mechanical launcher operated by compressed gas (Figure 2). They can be assembled into coils of up to 100 to facilitate loading into the launcher.

Application Time

The time required for application of the Tangler, by hand or using the launcher, compared with hand application of Isomate Flex polyethylene twin tubes was directly compared on four Michigan apple farms. Three approaches for applying Tangler dispensers were employed: 1) hand application by a person walking through the orchard tossing modules into the tree canopy, 2) launcher application by a person walking through the orchard launching modules into the tree canopy, or 3) launcher application by two people, one launching modules from the back of a Mitsubishi Mini truck and the other driving the vehicle. Flex dispensers were hand-applied by a person attaching the dispenser to a pole and walking through orchard looping the twin-tubes onto shoots. In all cases, dispensers were

delivered to the upper third of the tree canopy and deployed at a rate of 1-3/tree depending on tree density to achieve a density of 400/acre. Each application method was replicated four times to 1-acre plots. We recorded the total person hours required to apply dispensers to each plot.

Tangler modules took substantially less time to apply than the Flex tubes (Figure 3). The fastest application time of 12 minutes/acre was recorded for the Tangler modules launched from a moving vehicle. Walking through the orchard launching



Figure 1. Tangler dispenser showing the pheromone reservoir secured within the module (upper left) and entwined within the apple canopy (right).



Figure 2. Brett Bunker (right), the inventor of the Tangler, and Dr. Larry Gut (left) prepare to demonstrate the use of the Tangler launcher (Photo credit: Richard Lehnert, Good Fruit Grower).

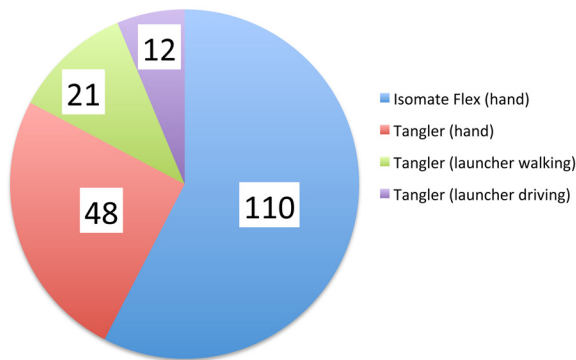


Figure 3. Average time (minutes) required to apply 400 Isomate twin-tube or Tangler dispensers to a 1-acre apple plot using various deployment methods.

the modules took about twice as long, but only required a single laborer. It took over twice as long, or 48 minutes/acre, to walk and toss the modules into the trees. The average time to apply 400 Isomate dispensers was 109 minutes/acre. Thus, automated deployment of the Tangler modules was 2–9X faster than hand application of Flex tubes. All of the modules, regardless of the application method, deployed correctly and tangled within the canopy. The fate of the Tangler modules was observed monthly over the course of the summer. No failures were detected; i.e., all modules remained in the tree for the entire season unless pruned out.

Field Efficacy

Field studies were conducted during the summer in 2011 and 2012 to determine the efficacy of Tangler for CM disruption. The experimental design was a randomized complete block, consisting of three treatments replicated on four apple farms in 2011 and two farms in 2012. All sites were located in the Ridge fruit-growing region near Grand Rapids, MI. Field plots consisted of 15–25-acre apple blocks subdivided into three 5-acre plots with buffers. Treatments were the Tangler applied at a rate of 400 dispensers/acre, Isomate CM Flex (400 dispensers/acre) as a positive control, and a no-pheromone check. If supplemental insecticides and disease management applications were warranted, they were applied to all treatments at a given site.

Captures of male moths in pheromone-baited traps and fruit injury were used to assess treatment effects. Six large Delta traps (Trécé, Inc.) baited with CM L2 lures (Trécé, Inc.) were placed in a grid pattern in the central portion of each plot. Traps were hung in the upper third of the tree canopy by placing them on 8' bamboo poles. New pheromone lures were deployed at the start of each moth generation. Moths captured in traps were counted and removed weekly. Native CM populations were supplemented with releases of sterile codling moths obtained from the Okanagan-Kootenay Sterile Insect Release (SIR) program in Osoyoos, Canada. The addition of SIR moths provided an evaluation using a known and equal pest density across treatments. Groups of 133 (equal numbers of males and females) SIR moths were transferred into 10 oz Solo™ containers and lightly dusted with different color DayGlo pigment powders to distinguish treatments (Figure 4). Moths were transported to field sites in chilled containers. Moths were allowed to warm up before being tossed into the tree canopies at locations no closer



Figure 4. Preparing batches of sterile insects (SIR moths) for release.

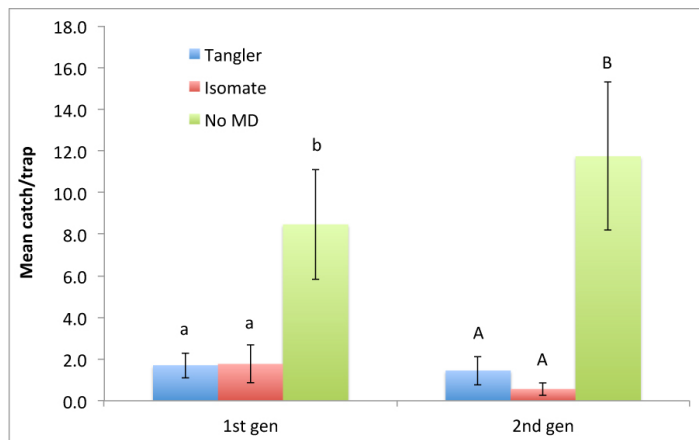


Figure 5. Mean (±SEM) wild codling moth captures in plots treated with Tangler CM, Isomate Flex CM or not treated with pheromone (NoMD), MI 2011–2012. Means within a generation followed by a different letter were significantly different, $P < 0.05$, Tukey's HSD.

than 33 ft from a trap. Releases of equal numbers of SIR moths per treatment block occurred during the weeks of Aug 1, 8, 15 and 22, 2011, and July 2, 16 and 30, 2012. At least 400 moths and no more than 800 moths were released per plot on a given release date. Fruit injury assessments were conducted twice, once after completion of 1st generation CM and again prior to harvest, by visually inspecting 1,200 half-fruits per treatment. Native and sterile moth catch data were square root-transformed, followed by checks for normality by examining skewness and kurtosis, and were then subjected to ANOVA and Tukey's HSD (Systat v13; San Jose, CA) to determine treatment effects.

Codling moth densities were low to moderate at the six sites, with male captures in pheromone traps averaging 8.5 and 11.7 for the 1st and 2nd generation flights, respectively. Under this level of pest pressure, the Tangler and Isomate mating disruption treatments significantly impeded moth captures (Figure 5). Furthermore, the Tangler formulation provided disruption equivalent to the commercial dispensing system, Isomate CM Flex. In 2011 and 2012, both pheromone treatments consistently inhibited wild male moth orientation to traps relative to the no-pheromone control (Figures 6, 7). Results varied only slightly throughout the season and both dispensing systems lasted for the entire season. Greater than 80% inhibition of wild moth captures in traps was achieved following deployment of either formulation. No fruit injury was recorded in any of the plots.

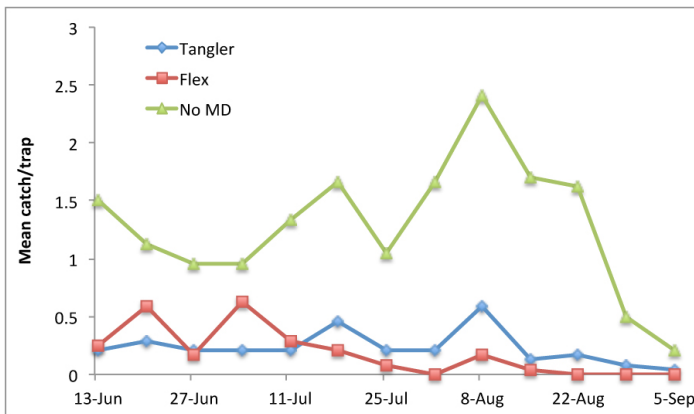


Figure 6. Seasonal captures of wild male codling moths in plots treated with Tangler CM, Isomate Flex CM or not treated with pheromone (NoMD), MI 2011.

Tangler and Flex also performed well under the higher pest pressure provided by the release of sterile moths. Both disruption formulations significantly impeded catch of SIR moths compared with the no-pheromone control (Figure 8). The two formulations had similar effects on male orientation to traps, with catch suppression over 90%.

Dispenser Density Study

The Tangler system provides the opportunity for fruit growers to apply pheromone dispensers at higher densities, due to less time and labor being required for deployment. Thus, a study was conducted in 2014 comparing the efficacy of Tangler CM applied at rates of 300 or 600 dispensers/acre. A no-pheromone treatment was included as a negative control. The experimental design was a randomized complete block, consisting of the three treatments replicated on four apple farms located in the Ridge fruit-growing region near Grand Rapids, MI. Field plots consisted of 15–25-acre apple blocks subdivided into three 5-acre plots with buffers. Fruit injury and captures of males in six pheromone-baited traps per plot were used to assess treatment effects. Native CM populations were supplemented with releases of SIR moths during the weeks of July 7, July 25 and Aug 25. Native and sterile moth catch data were square root-transformed, followed by checks for normality by examining skewness and kurtosis, and were then subjected to ANOVA and Tukey's HSD (Systat v13; San Jose, CA) to determine treatment effects.

Codling moth densities were low to moderate at the four sites, with male captures in pheromone traps averaging 9 and 10.5 for the first and second generation flights, respectively. Under this level of pest pressure, Tangler applied at 300 or 600 dispensers per acre significantly impeded first- and second-generation moth captures (Figure 9). Greater than 80% inhibition of wild moth captures in traps was achieved following deployment of either formulation. Both application rates inhibited wild male moth orientation to traps relative to the no-pheromone control throughout the season (Figure 10). No fruit injury was recorded in any of the plots. Thus, doubling the density of dispensers did not significantly improve disruption efficacy under moderate CM density and inclusion of a few supplemental insecticides in the management program.

The outcome was similar under the higher pest pressure

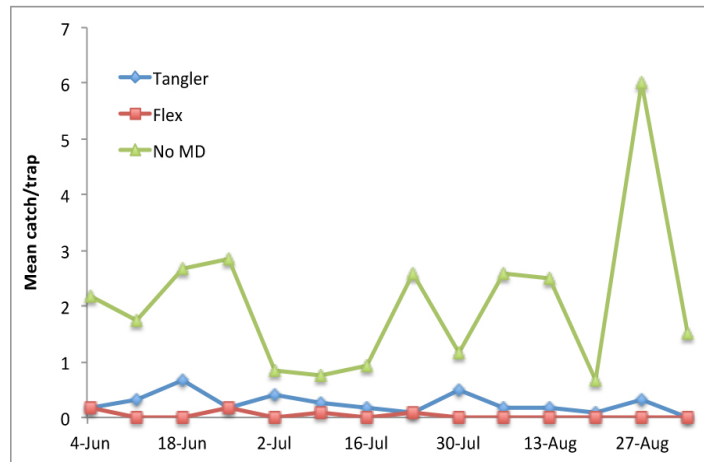


Figure 7. Seasonal captures of wild male codling moths in plots treated with Tangler CM, Isomate Flex CM or not treated with pheromone (NoMD), MI 2012.

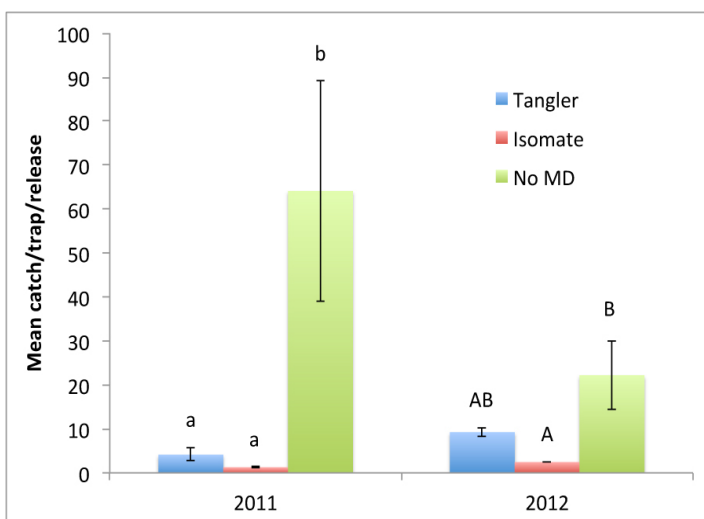


Figure 8. Mean (\pm SEM) released sterile (SIR) codling moth captures in plots treated with Tangler CM, Isomate Flex CM or not treated with pheromone (NoMD), MI 2011-2012. Means within a generation followed by a different letter were significantly different, $P < 0.05$, Tukey's HSD

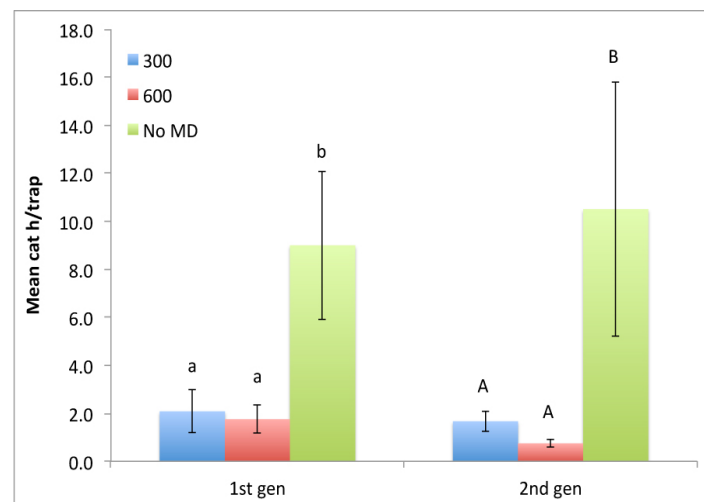


Figure 9. Mean (\pm SEM) released sterile (SIR) codling moth captures in plots treated with Tangler CM at two densities (300/acre or 600/acre) or not treated with pheromone (NoMD), MI 2014. Means within a generation followed by a different letter were significantly different, $P < 0.05$, Tukey's HSD.

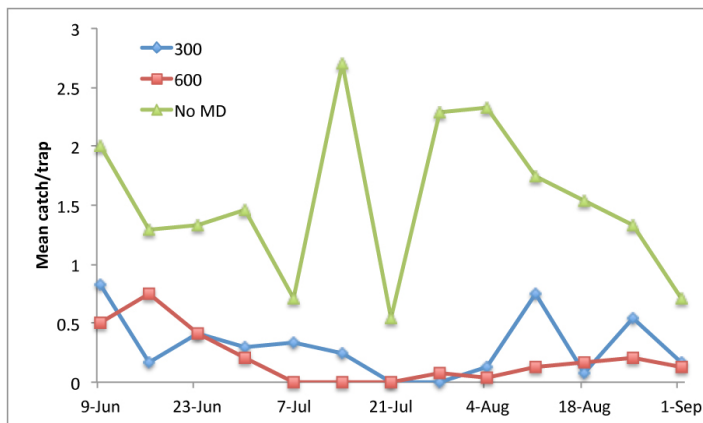


Figure 10. Seasonal captures of wild male codling moths in plots treated with Tangler CM at two densities (300/acre or 600/acre) or not treated with pheromone (NoMD), MI 2014.

provided by the release of sterile moths. Tangler applied at 300 or 600 dispensers per acre significantly impeded catch of SIR moths compared with the no-pheromone control (Figure 11). The two formulations had similar effects on male orientation to traps, averaging 86% catch suppression. We suspect that moth densities substantially higher than 40–60 males recaptured in the control plots might be required to record a measurable increase in disruption efficacy at standard compared with higher application rates.

Pheromone Release Rates

The Tangler pheromone reservoir was designed to provide a sustained release of active ingredient through the entire flight of the target pest. For codling moth, the reservoir was loaded with the main pheromone component, codlemone, as well as additives to protect the pheromone from degrading. To assess pheromone release through the season, field-aged dispensers were analyzed for pheromone emission using a volatile capture system (Tomaszewska et al. 2005, Il'ichev et al. 2012). The system is comprised of 1-liter Teflon transfer containers equipped with two 0.64-cm ports in the lids. Air is purified by passage through a moisture trap and a charcoal column. Air enters the Teflon volatile collection chamber at a rate of 1.6L/min. It passes over a dispenser and then through a trap containing 25 mg of Super Q adsorbent sitting below the exit port. Volatiles are collected on the adsorbent for 2 hours, eluted with acetone, concentrated under a nitrogen stream, and the amounts quantified by gas chromatography.

In 2011, release rates were measured for Tangler and Flex dispensers at day zero and dispensers were collected from the field 14 and 84 days after deployment. In-season codlemone release rates from the two dispensing systems were very similar. Day 14 release rates for Flex and Tangler dispensers averaged 2.8 $\mu\text{g}/\text{hour}$. Day 84 release rates for Flex and Tangler dispensers averaged 1.6 and 1.4 $\mu\text{g}/\text{hour}$, respectively. The two dispensing systems did vary greatly in their day zero release rates. The Tangler released 1.4 $\mu\text{g}/\text{hour}$, a rate very similar to the in-season rates. In contrast, Isomate Flex displayed a “burst” effect, initially releasing codlemone at a very high rate of 34.6 $\mu\text{g}/\text{hour}$.

In 2012 and 2014, four Tangler dispensers were aged in an apple orchard at Collins Farm on the Michigan State University

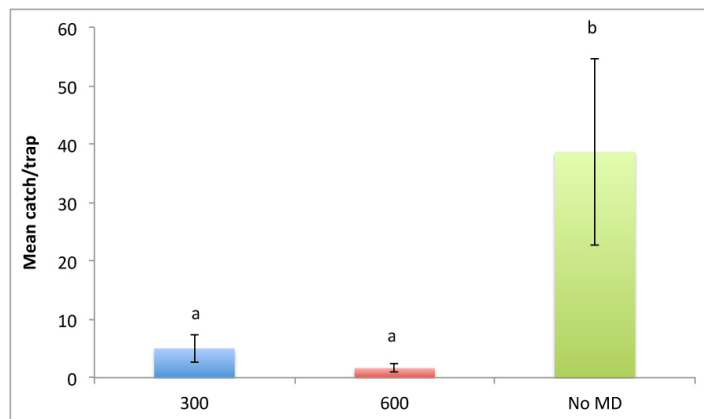


Figure 11. Mean (\pm SEM) released sterile (SIR) codling moth captures in plots treated with Tangler CM at two densities (300/acre or 600/acre) or not treated with pheromone (NoMD), MI 2014. Means within a generation followed by a different letter were significantly different, $P < 0.05$, Tukey's HSD.

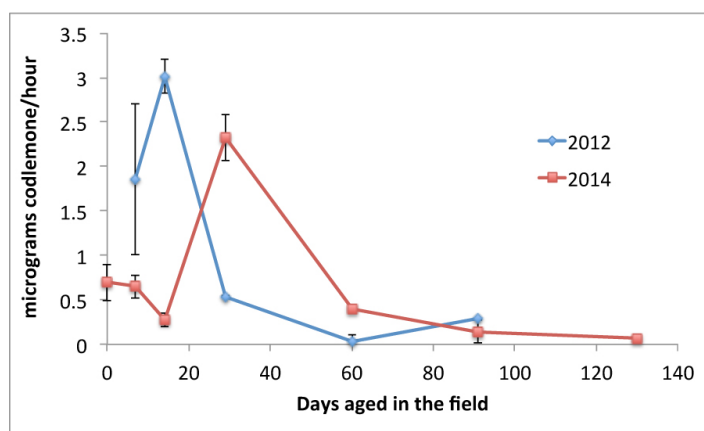


Figure 12. Pheromone emission rates for aged Tangler codling moth mating disruption dispensers using volatile capture analysis.

campus. Dispensers were deployed on July 3 in 2012 and June 19 in 2014. Collections were made at various intervals from day 7 to day 130 post-deployment. Dispensers were held in a fume hood at room temperature for 1 hour and then subjected to volatile capture analysis. Afterwards, the dispensers were transported back to the orchard and hung at their original positions. The rate of release varied considerably, depending on the field age of the dispenser (Figure 12). Moderate release rates of around 0.5 $\mu\text{g}/\text{hour}$ were measured on the majority of sample dates. There was an apparent elevation in release after several weeks of field aging, with the highest rates of 2.3–3.0 $\mu\text{g}/\text{hour}$ recorded for dispensers aged 14–28 days. Pheromone emission below 0.1 $\mu\text{g}/\text{hour}$ was recorded on day 130 in 2013, as well as day 60 in 2012.

A high release at the beginning of the season, with a gradual decline as the dispenser ages is consistent with the general release profiles reported for reservoir-type dispensers (Il'ichev et al 2012, Knight et al 2012). Ideally, we would like to see release rates of at least 1 $\mu\text{g}/\text{hour}$, as measured by our volatile capture system. However, Flex dispensers containing as little as 10% of the standard pheromone load and releasing at rates of 0.3–1 $\mu\text{g}/\text{hour}$ have provided adequate levels of CM disruption (unpublished data). The efficacy of the lower release rates is confirmed by the results of Tangler field studies and the

release rates of aged dispensers. Despite measured release rates on some sample dates below 1 µg/hour, orientational disruption levels of at least 80% were recorded throughout 90 days of CM activity in 2012, 2013, and 2014 field studies.

Prospectus

Field studies indicate that the Tangler system is a viable option for codling moth mating disruption. At densities of 300–400 dispensers/acre, control was equivalent to that provided by the commercial standard, Isomate reservoir dispenser. The novel design of the Tangler allows for rapid deployment high in the apple canopy. It provides growers the opportunity to apply a disruption treatment up to nine times faster than with currently available hand-applied dispensers, thus reducing labor requirements and application costs.

The Tangler CM product was granted US EPA registration this summer after codling moth flight was under way in Michigan and elsewhere in the US. Thus, it was only available for limited use in grower demonstration plots. Commercial product should be available for the 2016 growing season in Michigan and some surrounding states.

Additional Tangler disruption formulations are under development as well. The two-piece modular design is especially well suited for multispecies mating disruption, with reservoirs releasing different pheromones secured to the two halves. In addition to codling moth, MI apple growers must routinely contend with oriental fruit moth (OFM) and obliquebanded leafroller (OBLR). Testing of Tangler formulations designed to disrupt both CM and OFM or CM and OBLR is ongoing.

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2014	\$6,424,581	\$11,130,788	\$3,987,126	\$0.00
2013	\$6,675,387	\$9,694,580	\$3,424,750	\$0.00
2012	\$46,781,438	\$8,463,249	\$2,888,909	\$1,957,004
2011	\$12,141,941	\$7,129,106	\$2,403,012	\$24,295
2010	\$13,992,137	\$7,396,193	\$2,482,527	\$56,395
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