Research Article

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Effects of reservoir dispenser height on efficacy of mating disruption of codling moth (Lepidoptera: Tortricidae) in apple

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Abstract

BACKGROUND: The effect of varying the height of reservoir dispensers for mating disruption of codling moth, *Cydia pomonella* (L.), was investigated. The goal was to improve the effectiveness of *C. pomonella* mating disruption through improved understanding of adult distribution within the tree canopy and the impact of pheromone dispenser placement on disruption. Two dispensers per tree were placed at 2 m and 4 m and/or one dispenser at each height on the tree at a label rate of 1000 units ha^{-1}. Monitoring traps and tethered female moths were deployed in plots at 2 and 4 m heights to assess treatment effects by catches or matings respectively.

RESULTS: Fewest male moths were captured with all dispensers placed at 4 m. Female mating was lowest, and with least variation between females tethered at 2 and 4 m, when dispensers were placed simultaneously at 2 and 4 m (28% mated). Mating was 32% with both dispensers at 4 m, 38% with both dispensers at 2 m and 46% in the no disruption control. Mating was highest across treatments when females were tethered at 2 m and dispensers placed at 4 m (40%), and when females were tethered at 4 m with dispensers placed at 2 m (46%).

CONCLUSION: Traps at 4 m in trees captured more male moths than traps at 2 m, regardless of disruption dispenser positioning. Female mating was lowest when dispensers were placed simultaneously at 2 and 4 m, suggesting that current recommendations for placement of reservoir dispensers in tree crowns may be suboptimal.

Keywords: codling moth; pheromone disruption; dispenser height; competitive attraction

1 INTRODUCTION

Mating disruption of codling moth [*Cydia pomonella* (L.)] using various hand-applied dispensers has become an accepted practice. Approximately 162 000 ha of apple and pear are treated worldwide.[1] Investigations using pheromone-baited sticky traps have indicated that flight of males is concentrated in the top third of the tree canopy. This finding has resulted in the recommendation that dispensers of synthetic pheromone be applied within the top meter of the tree canopy.[2–4] Witzgall *et al*. directly observed male moths flying and 'searching' in branches in the upper half of tree crowns, providing further support for deployment of mating disruption dispensers near the top of the tree canopy.[5]

Epstein *et al*. developed a vacuum sampling technique for direct measurement of the spatial distribution of resting male and female *C. pomonella* within the tree canopy in orchards with and without pheromone mating disruption.[6] In pheromone-disrupted plots, significantly more males were found in the combined lower two-thirds of the tree canopy (0–3.0 m) than in the top third of canopy (3.0–4.5 m). However, female moth distribution was approximately equivalent between the top third and middle third (1.5–3.0 m) of tree canopies (42 and 46% in the top and middle respectively). In plots not treated with pheromone, more male *C. pomonella* were found in the upper two-thirds of trees (1.5–4.5 m) than at lower locations. An equal percentage of females were collected from the top and middle thirds of the tree canopies in non-disrupted plots.[6]

Recent work by Miller *et al*.[7–9] provides strong evidence that competitive attraction is a primary mechanism by which pheromone mating disruption operates for *C. pomonella* with hand-applied reservoir dispensers of pheromone. Disruption should be optimal when dispensers of synthetic pheromone are deployed in areas within the trees where females typically call. Thus, if the distribution of female *C. pomonella* is similar between the upper, middle and lower parts of the tree canopy, then placement of dispensers solely in the top third of the tree canopy may be suboptimal.[6]

The above results, indicating that female *C. pomonella* occur throughout the tree canopy in both disrupted and non-disrupted

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plots and males occur in lower portions of the tree canopy in disrupted plots rather than only near the tree crown, prompted a re-investigation of the optimal placement of *C. pomonella* mating disruption dispensers. This investigation addressed the effects of pheromone mating disruption dispenser height within the tree canopy on mating disruption of *C. pomonella*. Treatment efficacy was evaluated with both male moth catch in attractive pheromone-baited traps (female proxy) and by direct assessment of female mating using tethered virgin female moths. The goal was to improve the effectiveness of *C. pomonella* mating disruption on the basis of improved understanding of the impact of dispenser placement on disruption efficacy.

2 EXPERIMENTAL METHODS

2.1 Experimental design

The experiment was conducted at the Trevor Nichols Research Complex, Fennville, MI (42° 35′ 38.10″ N, 86° 06′ 05.92″ W), within a 16-year-old Red Delicious planting. Trees were planted at a 3 × 6 m tree spacing and were 4–4.5 m in height. A randomized complete block design was used, with four treatments replicated 4 times. Each experimental block consisted of a 1.2 ha orchard divided into four experimental units of 0.2 ha, each separated by a minimum buffer of 20 m. Randomly assigned treatments corresponded to four dispenser height placements: (1) two Isomate C+ dispensers at 4 m within the tree canopy (high); (2) two dispensers at 2 m within the tree canopy (low); (3) simultaneous placement of one dispenser at 2 m and one at 4 m distributed within the tree canopy; (4) no dispensers deployed. All pheromone mating disruption plots were treated with Isomate C+ (Shin-Etsu Chemical Co., Tokyo, Japan) at the full label rate of 1000 dispensers ha^{-1}. Dispensers were uniformly distributed across each treatment and were always spaced by a minimum 1 m within the same tree.

2.2 Moth trapping

Inhibition of moth catch in pheromone traps (female proxy) was the principal means of assessing treatment effects. Four orange-colored traps (LPD Scenturian Guardpost; Suterra, Bend, OR) baited with 1 mg of (*E*,*E*)-8,10-dodecadien-1-ol (codlemone) in rubber septum lures (Suterra, Bend, OR) were placed in each treatment plot. Two traps were placed at 4 m and two at 2 m per plot, and were spaced equidistantly throughout each treatment plot. Traps were affixed to 1.5 m long bamboo poles by placing the wire of the trap through a 2 cm drill hole located 4 cm from one end of the pole, and wrapping the wire around the pole. In all mating disruption plots, traps were positioned at least 1 m away from an Isomate dispenser. Lures were replaced every 2 weeks. Traps were checked twice per week, and flight activity was monitored continuously from 11 May to 24 August 2007. At each collection date, the number of moths captured was recorded for each trap, trap liners were cleaned or replaced and traps at the two heights were rotated within the same trees. Branches were flagged with colored plastic tape at the 2 and 4 m heights to ensure that traps were returned to the same height and compass orientation following rotations.

2.3 Evaluation of mating with virgin females

In addition to measuring orientational disruption to traps, mating disruption was directly assessed using tethered, virgin females. Female *C. pomonella* used for tethering were reared from pupae purchased from Benzon Research Inc., Carlisle, PA.[10] Females were sorted in the pupal stage and placed into 50 mL plastic cages containing 5% sucrose in plastic cups with cotton dental wick protruding from their lids. Adult female *C. pomonella* were deployed within 2 days of emergence for 16 h (17 : 00–09 : 00 h) in all treatment plots on five dates coinciding with peak moth presence during the first and second generations (13 June, 15 June, 26 July, 1 August and 2 August). Female moths were secured to branches of trees with polyester thread (Jo-Ann Stores, Inc., Hudson, OH) tied to the base of the left wing. Moths were given at least 60 cm of thread and were observed for at least 30 s after deployment to ensure that they remained tethered and mobile upon the branch. Four female moths were tethered per treatment replicate (64 total) equidistantly from one another and from plot borders within each 0.2 ha treatment. Two were placed at 2 m and two at 4 m within the tree canopy during each deployment period. Females were always tethered a minimum of 1 m from either a pheromone dispenser or a monitoring trap. Sixty-eight percent of tethered females were recovered over the five deployments. Collected female moths were stored in 70% ethanol alcohol within 8 dram glass vials and dissected in the laboratory within 24 h to determine mating status, based on the presence or absence of a spermataphore in the bursa copulatrix.

2.4 Data analyses

Two statistical approaches were used to analyze data. Generalized linear models were employed to assess treatment effects. Moth capture and mating data were normalized using a square root transformation to achieve normality of error distribution. Generalized linear model analyses were performed in R v.2.10.0 (R Development Core Team, 2009, R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org); the mating data were assessed as a binomial distribution. The analysis of variance (ANOVA) command in R was used to test parameter significance of each model using analysis of deviance, and the Tukey multiple comparisons test was used for means separation. Moth catch data were analyzed by a factorial ANOVA, with trap height and dispenser placement as the two factors. Mating data were also evaluated using mean percentages of mated female moths, adjusted by Abbott's formula where mating was considered a survivorship event.[11]

3 RESULTS

Traps placed at 4 m captured more male *C. pomonella* at all dispenser height treatments (df = 1, 11; $F = 12.3$, $P < 0.01$) than traps placed at 2 m (Fig. 1). For traps placed high within the canopy, significantly (df = 3, 11; $F = 9.7$, $P < 0.05$) fewer moths were captured when dispensers were placed at both locations or at 4 m only in comparison with the control (Fig. 1). For traps placed low within the canopy, moth catch was significantly ($P < 0.05$) reduced by each dispenser height placement compared with the control (Fig. 1). The fewest moths were captured when both traps and dispensers were placed at 4 m.

Mating of tethered virgin female *C. pomonella* was significantly lower in all dispenser height treatments compared with the no mating disruption treatment, and was lower when dispensers were placed simultaneously at both 2 and 4 m than at either height alone (df = 222, 219; $z = -3.7$; $P < 0.00025$ for the one dispenser at 2 m and one dispenser at 4 m treatment; df = 222, 219; $z = -3.6$, $P < 0.00042$ for the two dispensers at 4 m treatment; df = 222, 219; $z = -2.9$, $P < 0.00043$ for the two dispensers at

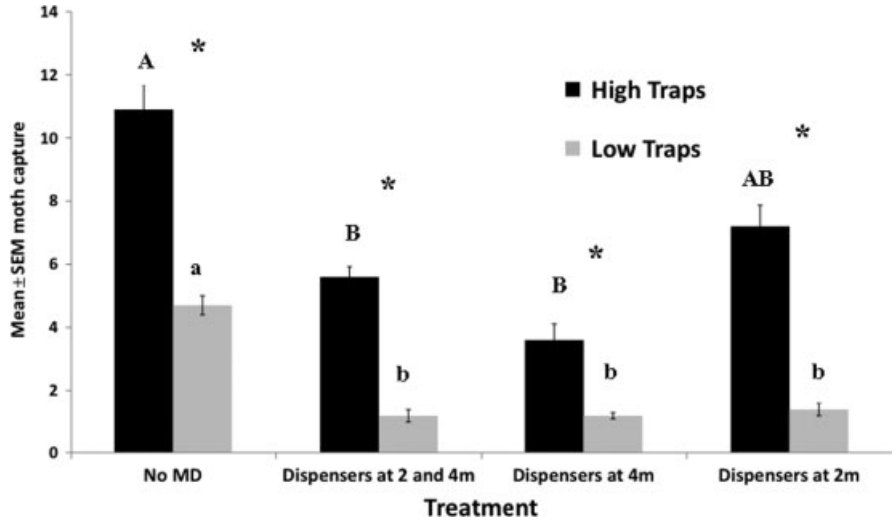

Figure 1. Captures of male moths in plots where pheromone dispenser and trap heights were varied. Means followed by the same upper-case letter between various dispenser height treatments are not significantly different for traps placed high within the canopy at $\alpha < 0.05$. Likewise, means followed by the same lower-case letter between various dispenser height treatments are not significantly different for traps placed low within the canopy at $\alpha < 0.05$. Significant differences between low and high traps are indicated by an asterisk within each dispenser height treatment. No MD = no mating disruption dispensers.

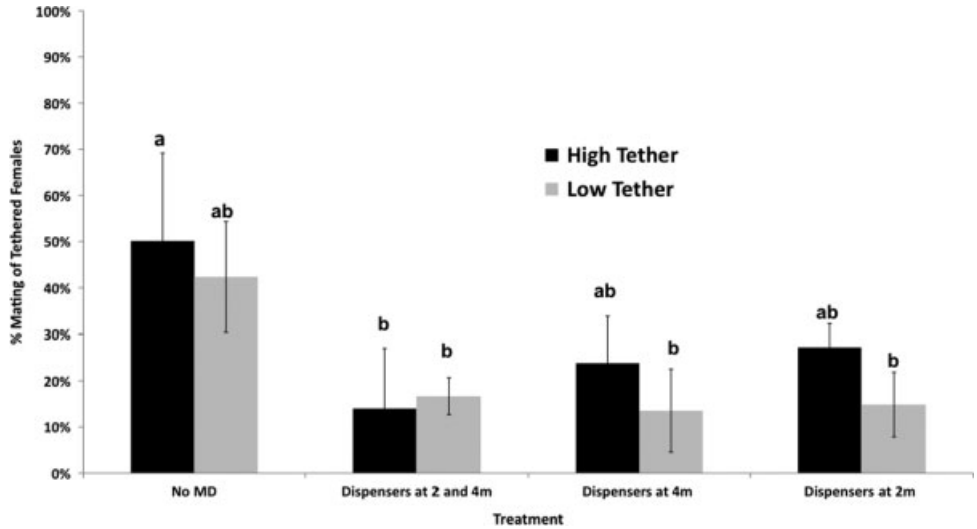

Figure 2. Percent mating of tethered females at 2 m and 4 m in plots where dispenser height was varied. Means represented without Abbott's formula correction (No MD = No mating disruption dispensers).

2 m treatment; df = 222, 219; $z = -2.7$, $P < 0.7893$ for the no dispensers treatment) (Fig. 2). Mean percent mating of tethered female moths corrected with Abbott's formula was 28% in the plots with dispensers at both 2 and 4 m, followed by 32% in plots with two dispensers at 4 m, 38% in plots with two dispensers at 2 m and 46% in control plots. Mating of virgin female moths was highest when females were tethered at 2 m (40%) in plots with dispensers placed at 4 m and when they were tethered at 4 m (46%) in plots with dispensers placed at 2 m (Table 1, Fig. 2).

4 DISCUSSION AND CONCLUSIONS

Optimization of pheromone-based mating disruption and trapping requires an understanding of target insect distribution within crop canopies. Pheromone-baited traps are a common method for surveying male moth presence in crops, as well as evaluating efficacy of pheromone mating disruption applications.[12] Several studies using pheromone-baited traps proved that captures of *C. pomonella* males increase with increasing height of pheromone trap placement within trees, which is congruent with the present results.[2,13,14] However, an interaction between vertical and horizontal placement of pheromone traps within the tree canopy has also been observed for *C. pomonella* male trapping under mating disruption.[13] Specifically, when pheromone traps were placed within and near the tip of the tree canopy, more male *C. pomonella* were captured than when traps were placed at the bottom edge of the canopy; however, traps at both high and low positions captured equivalent numbers of males when placed within the tree canopy.[13] In addition to pheromone trapping, passive sampling techniques that capture flying adults have suggested that *C. pomonella* activity is greatest in the top 1 m of tree canopies, both under mating disruption and in non-disrupted

Table 1. Percent of tethered virgin female *C. pomonella* that mated when deployed on five dates at 2 m and 4 m heights in tree canopies in plots with varying dispenser height treatments

Pheromone treatment	Female height (m)	Mean % mated	Abbott's corrected (%)
No MD	4	50	
No MD	2	42	
2 m and 4 m	4	14	28
2 m and 4 m	2	17	28
2 at 4 m	4	24	25
2 at 4 m	2	14	40
2 at 2 m	4	27	46
2 at 2 m	2	15	30

orchards.[2–4,15] Weissling and Knight found that male response to tethered females was greatest at 4 m within tree canopies in pheromone-disrupted orchards when dispensers were placed at either 2 or 4 m.[14] However, the same investigation reported that there was no difference in the proportion of mated tethered virgin females at 2 and 4 m.[14] Collectively, the above findings have resulted in the recommendation that pheromone dispensers should be placed near the crown of the tree canopy for optimal mating disruption of *C. pomonella*.

Although pheromone-based trapping is a useful female-proxy method for evaluation of pheromone disruption treatments, measurement of female moth mating by deployment of tethered virgin females is a more direct estimation of actual mating disruption. The current results indicate that disruption of virgin female mating is slightly greater when Isomate C+ dispensers are placed simultaneously at both 2 and 4 m than when all dispensers are placed at either 2 m or the recommended 4 m height in mature 4–5 m tall apple trees. Isomate C+ dispensers are an industry standard for mating disruption of *C. pomonella*.[1] In addition to Isomate C+ polyethylene tubes, other similar hand-applied dispensers are available and are generally characterized by a ca 200–400 g loading of *C. pomonella* pheromone per dispenser and a recommended field application rate of 500–1000 ha^{-1}, which is often roughly equivalent to placement of 1–2 dispensers per tree. Such dispensers require one application per season and are used for *C. pomonella* management throughout the world.[1]

Competitive attraction, involving deactivation after a visit, is the mechanism of *C. pomonella* mating disruption with Isomate C+.[9] For mating disruption to be effective under competitive attraction, synthetic dispensers of pheromone must outcompete feral calling females.[7–9] Therefore, pheromone dispenser placement within the tree canopy, including the proximity of dispensers to calling females, should influence the efficacy of mating disruption. Stelinski *et al*. used Scentry fibers to examine the impact of dispenser position on *C. pomonella* disruption, and found that inhibition of moth captures in traps and mating disruption of tethered females increased significantly as the distance from fibers was decreased.[10] Efficacy also should be related to the relative locations of moths and synthetic dispensers within the tree canopy. Direct sampling of resting *C. pomonella* adults indicated that both male and female adults are distributed evenly between low (0–1.5 m), middle (1.5–3.0 m) and upper (3.0–4.5 m) portions of the tree canopy during periods of female calling and male searching activity.[6] Those results are congruent with the current finding that disruption of tethered virgin female mating was most effective when dispensers were positioned simultaneously at both middle and upper canopy heights.

Although hand-applied pheromone dispensers such as Isomate C+ reduce mating of *C. pomonella* when used properly, their adoption is often hindered by the cost of manual labor.[16] Furthermore, given the species-specific nature of pheromone disruption and the continued development of new cost-competitive and broader-spectrum insecticide formulations, adoption of pheromone mating disruption as an alternative to toxins or insect growth regulators is hindered.[1] Therefore, continued optimization of existing formulations and their deployment practices are needed to maintain the viability of this environmentally sustainable pest control practice. The present results suggest a slight but significant increase in efficacy of hand-applied reservoir dispensers when simultaneously placed both at middle and upper tree canopy positions rather than placed only at the currently recommended upper crown position.

However, the extent to which vertical dispenser placement influences the efficacy of a mating disruption treatment likely depends on the physical structure of the treated crop. deLame and Gut found that the effectiveness of a standard hand-applied dispenser for disruption of oriental fruit moth, *Grapholita molesta* (Busck), varied depending on canopy height.[17] Their recommendation was to place dispensers at an easily reachable height in trees <3.5 m tall, in the upper canopy in 3.5–4.5 m tall trees and in both the lower and upper canopy in trees taller than 4.5 m. The *C. pomonella* studies reported herein were only conducted in apple orchards with canopy heights of 4.0–4.5 m. Further investigation of the impact of vertical dispenser placement on *C. pomonella* disruption is warranted in apple tree canopies of other heights, as well as in walnut tree canopies. Note, however, that the current recommendation for placement of hand-applied pheromone dispensers at a recommended rate of ~500–1000 per two-dimensional space or area (ha), exclusively in the upper tree canopy, may be suboptimal.

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