

**FINAL PROJECT REPORT**

**Project Title:** Optimization of release strategies for sterile codling moth

**PI:** Dr. Larry Gut (Posthumous)  
**Organization:** Michigan State University  
**Telephone:**  
**Email:**  
**Address:**  
**City/State/Zip:** East Lansing, MI 48824

**PI:** Dr. RT Curtiss  
**Organization:** Washington State University  
**Telephone:** (917) 685-1546  
**Email:** rcurtiss@wsu.edu  
**Address:** 1100 N Western Ave  
**City/State/Zip:** Wenatchee, WA 98801

**Key Person(1):** Nathan Moses-Gonzales  
**Organization:** M3 Consulting Group  
**Telephone:** (480) 239-8942  
**Email:** nmosesgo@m3cg.us  
**Address:** 10009 Mallet Dr.  
**City/State/Zip:** Dayton, OH 45458

**Cooperators:** Dustin Krompetz (M3), Julianna Wilson (MSU)  
**Total Project Request:** Year 1: \$125,000 Year 2: \$125,000 Year 3:

**Organization Name:** Michigan State University **Contract Administrator:** Diane Cox  
**Telephone:** 517-884-4243 **Email address:** contractteam2@osp.msu.edu

**WTFRC Budget:** none

Item	2019	2020	2021
<b>Salary: Project Manager<sup>1</sup></b>	\$25,168	\$25,671	
<b>Benefits: Project Manager<sup>1</sup></b>	\$3,084	\$3,270	
<b>Supplies: Project Manager<sup>1</sup></b>	\$4,248	\$3,559	
<b>Wages: Time Slip Staff<sup>2</sup></b>	\$19,040	\$19,040	
<b>Benefits: Time Slip Staff<sup>2</sup></b>	\$4,809	\$4,809	
<b>Project Vehicle<sup>3</sup></b>	\$4,900	\$4,900	
<b>Fuel<sup>3</sup></b>	\$7,000	\$7,000	
<b>Misc. Field Supplies<sup>4</sup></b>	\$4,751	\$4,751	
<b>Travel<sup>5</sup></b>	\$7,000	\$7,000	
<b>SIR CM, delivery and release<sup>6</sup></b>	\$45,000	\$45,000	
<b>Total</b>	<b>\$125,000</b>	<b>\$125,000</b>	<b>0</b>

**Footnotes:** <sup>1</sup>Project Manager: Rob Curtiss: 50% Salary (\$25,168 +2% increase in year 2) + Fringe (\$3,084 yr 1; \$3,270 yr 2) + supplies (\$4,248 yr 1; \$3,559 yr 2) to be matched 1:1 by FFAR Fellowship. <sup>2</sup>Time Slip Staff: Two technicians at \$14/hr for 40 hr/wk 17 weeks (\$19,040) + 0.0765 fringe rate (\$1,456.56) + Health @419/mo. <sup>3</sup>Project vehicle: One rental vehicle @\$1,225/month for 4 months (Enterprise Car Rental) + Fuel: 240-300 miles/day\*3 days/week\* \$0.54/ mile (MSU 2019 Mileage rate). <sup>4</sup>Misc. Field Supplies: Traps, Liners, lures, etc. <sup>5</sup>Travel: PI and Key person 2 travel to WA field sites 2x/year @\$1,750.00/ trip/ person. <sup>6</sup>Sterile CM Delivery and Release: M3 Consulting Group Sterile Codling Moths + Delivery, and UAS release missions throughout field season and LIDAR missions for 3 orchards.

### ***Original Project Goal and Objectives***

The overall project goal was to provide information to form a best management practices recommendation for the use of sterile codling moths on a farm-scale in Washington State.

- 1 – Determine if orchard factors impact dispersion of SIR CM – **Completed 2019-2020**
  - a) Determine impact of orchard architecture on distribution of released CM,
  - b) Determine impact of topography on distribution, and
  - c) Correlate topography and architecture impacts on distribution of CM.
- 2 – Determine if release factors impact dispersion of SIR CM – **Completed 2020-2021**
  - a) Determine the optimal target release altitude,
  - b) Determine if distributed or point releases are optimal for dispersion, and
  - c) Determine the optimal time of release.

### ***Significant Findings and Benefits to the Industry***

- Significant Finding: Orchard architecture is an important factor in the dispersion and recapture of sterile codling moths
  - Benefits: Simplified release tactics may be employed in orchards with trellised trees
- Significant Finding: Orchard topography is not a significant factor in the dispersion and recapture of sterile codling moths
  - Benefits: Releases of sterile moths do not need to be modified for orchards with steep slopes
- Significant Finding: Release altitude, among those tested, is not a significant factor in recapture or dispersion of sterile codling moths
  - Benefits: Release by Unmanned Aerial Vehicle is effective at any altitude up to 100ft
- Significant Finding: Moths released by hand at a single central location in a 10-acre orchard distribute to the edges of the plot, and fewer leave the area than when they are released in a spread-out pattern
  - Benefits: For blocks up to 10-acres, simplified releases should be employed to ensure that moths that were purchased are not lost
- Significant Finding: The time-of-day moths are released impacts recapture
  - Benefits: Releasing moths before noon appears to give them the best chance at survival and dispersion; avoid releases late in the day to prevent loss of moths

### **Results**

For all experiments, sterile moths, obtained from the OKSIR facility in BC, Canada, were released into commercial apple orchards in Washington State to measure their recapture and dispersion under several test conditions. Releases were replicated up to 18 times per treatment and the average percent recapture was compared among treatments. In addition, the aggregation of the released population was calculated by Morisita's Index of Dispersion ( $I\delta$ ) to determine the degree to which the population dispersed throughout the orchard. \*\*  $I\delta$  is interpreted as follows: 0-1= random dispersion, 1=even dispersion, and >1= aggregated dispersion (higher numbers indicate more aggregation).\*\* When appropriate we also analyzed per trap average recapture distance to determine how far moths dispersed.

#### **Objective 1: Determine if orchard factors impact dispersion of SIR CM**

##### **Obj. 1a: Orchard Architecture: Trellised vs. free standing orchards under mating disruption**

*Recapture:* Significant differences in recapture were found between the three tree training systems ( $F=17.624$ ,  $df=2$ ,  $P<<.001$ ). Tukey's HSD test showed that significantly more moths were

recaptured in both types of trellised orchards than standard planted orchards, but recapture was not different among trellised orchards (Table 1). The mean recapture of moths in standard planted blocks was  $1.6\% \pm 0.3$ , in V-trellised blocks was  $9.2\% \pm 1.5$ , and in Vertical-trellised blocks was  $9.1\% \pm 1.5$ .

**Aggregation:** Significant differences in aggregation were found among the three tree training systems ( $F=6.871$ ,  $df=2$ ,  $P=.0025$ ) (Table 1). Fisher's LSD test showed that released moths aggregated significantly more around the release point in standard planted orchards than in either trellised training system. The mean  $I\delta$  for moths released in standard planted blocks was  $2.57 \pm .44$ , in V-trellised blocks was  $1.59 \pm .09$ , and in Vertical trellised blocks was  $1.39 \pm .05$ . There was no significant difference in degree of aggregation between the trellised blocks.

	Training system	Replicates	ANOVA	Mean $\pm$ SEM
Percent Recapture	STD-32	19	F=17.624	$1.6 \pm 0.3$ a
	V-Trellis	18	df=2,52	$9.2 \pm 1.5$ bc
	Vertical Trellis	18	$P < 0.001$	$9.1 \pm 1.5$ bc
I $\delta$ - Absolute	STD-32	14	F=6.871	$2.57 \pm 0.44$ a
	V-Trellis	17	df=2,45	$1.59 \pm 0.09$ bc
	Vertical Trellis	17	$P=0.0025$	$1.39 \pm 0.05$ bc

Table 1. Average % recapture and  $I\delta$  for sterile codling moths released in three tree training systems.

**Distance:** Moths released in trellised orchards were captured significantly more than in standard planted single, stand-alone tree orchards. Distance from point of release analysis determined that for each distance, 10m ( $F= 18.115$ ,  $df=2$ ,  $P < 0.001$ ), 20m ( $F= 17.176$ ,  $df=2$ ,  $P < 0.001$ ), 30m ( $F=14.434$ ,  $df=2$ ,  $P < 0.001$ ), and 40m ( $F=12.370$ ,  $df=2$ ,  $P < 0.001$ ) from the point of release, there were significant differences among the three tree training systems. Significantly more moths were captured in both trellised systems than in standard planted orchards, and moth recapture in the two trellised orchard types were not different at any of the distances. The estimated maximum population recapture distance of between 55-65 meters from the point of release was estimated as the point at which the three trend lines x-intercept on Figure 1.

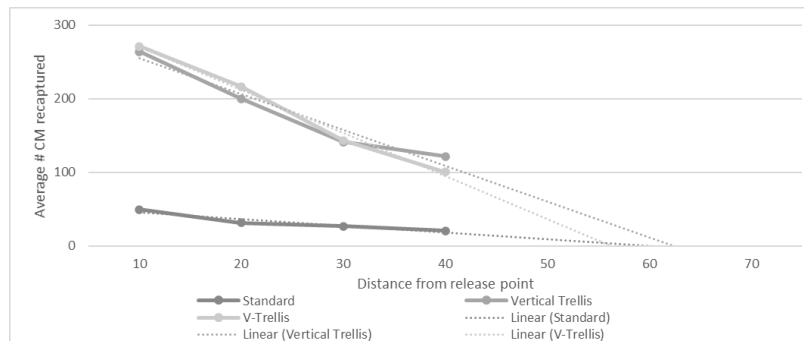


Figure 1. The average number of codling moths captured in apple orchards with three tree architectures at four distances from release.

**Directionality:** There were significant differences found in the direction of sterile moth dispersal in the standard planted orchards ( $F=2.570$ ,  $df=7$ ,  $P=0.0159$ ), but not in the Vertical trellis training system ( $F=0.404$ ,  $df=7$ ,  $P=0.8986$ ), nor the V-trellised system ( $F= 1.387$ ,  $df=7$ ,  $P=0.2157$ ). Moths released in trellised orchards exhibited a slight, but not statistically significant directional preference for movement up and down rows, while those released in standard planted blocks had a minor but significant Westerly preference as revealed by Tukey's HSD. However, the overall impact on dispersion is minimal as moths dispersed to the edges of the blocks in all directions in the three tested tree training systems (Fig. 2).

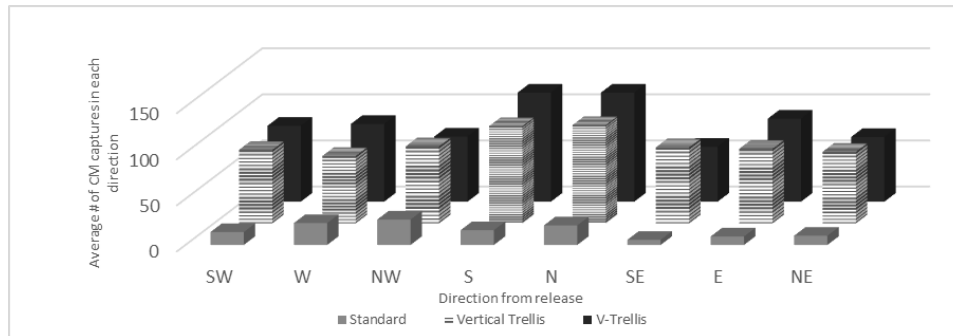


Figure 2. The average number of sterile *C. pomonella* captured at each direction in apple orchards with three different tree training systems. Traps to the North and South are up and down rows while those to the West and East are across rows.

### Obj. 1b: Orchard Topography: Steep slope vs flat planar orchards

**Recapture:** Significantly more sterile moths ( $F=30.991$ ,  $df=1$ ,  $P<0.001$ ) were recaptured in plots on a slope ( $4.8\% \pm 0.9$ ) than without a slope ( $0.9\% \pm 0.3$ ) (Table 2). However, the flat plots were not trellised, and as demonstrated in Obj. 1a, plots of this type typically have lower rates of recapture.

**Aggregation:** There was no significant difference in aggregation in the two treatments based on absolute recapture ( $F=3.128$ ,  $df=1$ ,  $P=0.0878$ ). The  $I\delta$  of absolute number of sterile moths recaptured on orchards with a hill was  $1.53 \pm 0.10$ , and for orchards with less than a  $1^\circ$  slope was  $2.90 \pm 0.90$  (Table 2). There were significant differences in the average trap percent of total capture between the two treatments ( $F=5.366$ ,  $df=1$ ,  $P=0.0281$ ). The aggregation index for traps on the hill was  $1.45 \pm 0.13$  was less than that for traps in a flat orchard was  $3.29 \pm 0.91$ , indicating that moths dispersed in a less aggregated manner.

	Orchard Slope	# Reps	ANOVA	Mean $\pm$ SEM
<b>Percent Recapture</b>	14° steep hill	18	$F=31.763$	$4.8 \pm 0.9$ a
	1° Flat planar	18	$df=1, 34$ $P<<0.001$	$0.8 \pm 0.3$ b
<b>I<math>\delta</math> - Absolute</b>	14° steep hill	18	$F=3.128$	$1.53 \pm 0.10$
	1° Flat planar	12	$df=1, 28$ $P=0.088$	$2.95 \pm 0.97$
<b>I<math>\delta</math> - % of total</b>	14° steep hill	18	$F=5.366$	$1.45 \pm 0.13$ a
	1° Flat planar	12	$df=1, 28$ $P=0.028$	$3.35 \pm 0.99$ b

Table 2. Average % recapture and  $I\delta$  for sterile codling moths released in orchards with two slopes.

**Direction uphill/downhill:** In the two apple orchard blocks with a  $14^\circ$  slope, the traps at the bottom of the slope recaptured a slightly lower but not significantly different number of moths than the traps at the top of the hill ( $F=3.305$ ,  $df=1$ ,  $P=0.078$ ). In the flat blocks, the traps corresponding to the hilltop traps and the hill bottom traps were also not significantly different from each other ( $F=0.652$ ,  $df=1$ ,  $P=0.425$ ). These data indicate that sterile codling moths do not exhibit a significantly elevated uphill movement (Fig. 3).

The traps down slope from the central release point did not capture significantly different numbers of moths than the traps up the slope in the apple orchards on the  $14^\circ$  slope ( $F=1.261$ ,  $df=1$ ,  $P=0.269$ ). The orchards on the flat area also did not capture significantly different numbers of moths in the traps corresponding to uphill and the traps corresponding to downhill ( $F=0.152$ ,  $df=1$ ,  $P=0.700$ ). Further confirmation that *C. pomonella* do not prefer uphill dispersal (Fig. 3).

**Direction across slope:** In the two orchard blocks with a  $14^\circ$  slope, the traps at each extreme side of the block did not recapture different numbers of sterile codling moths ( $F=0.208$ ,  $df=1$ ,

P=0.651). Likewise, the traps on each side of the release point across the slope also did not recapture different numbers of moths ( $F=0.015$ ,  $df=1$ ,  $P=0.905$ ) (Fig. 3).

In the orchard blocks with a flat planar slope, the traps that correspond to those on the extreme sides of hill blocks did not recapture different numbers of moths ( $F=0.250$ ,  $df=1$ ,  $P=0.620$ ), nor did all traps on each side of the release point ( $F=0.100$ ,  $df=1$ ,  $P=0.754$ ) (Fig. 3).

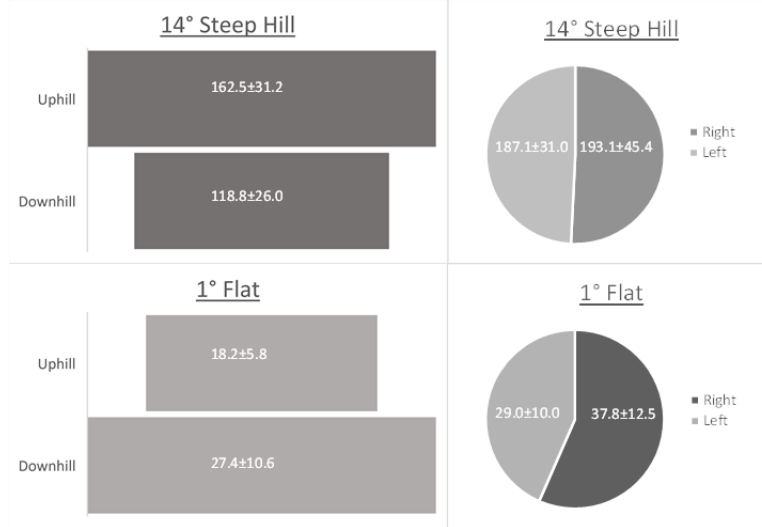


Figure 3. Sterile moth dispersion on steep slopes and flat ground.

**Obj. 1c: Orchard Architecture vs. Topography**

Because Orchard Topography was not found to be a significant factor in the dispersion of sterile codling moths, and orchard architecture was significant, no correlation between the two factors was found.

**Objective 2: Determine if release factors impact dispersion of SIR CM**

**Obj. 2a: Optimal target release altitude**

*Recapture:* There were no significant differences in recapture of moths released by UAS at 30-35m altitude, 20-25m altitude, 10-15m altitude, or by hand at 0-5m altitude ( $F=1.0562$ ,  $df=3$ ,  $P=0.3833$ ). The mean proportion of moths recaptured did not exceed 2.5% in any treatment (Table 3).

*Dispersion:* There were no significant differences found among the four treatments based on absolute aggregation ( $F=0.5726$ ,  $df=3$ ,  $P=0.6377$ ) or aggregation by percent of recapture ( $F=0.815$ ,  $df=3$ ,  $P=0.4964$ ) Overall, all four strategies for releasing sterile *C. pomonella* centrally resulted in 1.1-2.4% recapture and moderate aggregation around the point of release (Table 3).

	Release altitude	# Reps	ANOVA	Mean ( $\pm$ SEM)
<b>Percent Recapture</b>	0-5 m (hand)	8	$F=1.0562$ $df=3, 28$ $P=0.3833$	$2.4 \pm 0.62$
	10-15 m (UAS)	8		$2.1 \pm 0.70$
	20-25 m (UAS)	8		$1.1 \pm 0.39$
	30-35 m (UAS)	8		$1.5 \pm 0.60$
<b>Iδ - Absolute</b>	0-5 m (hand)	8	$F=0.5726$ $df=3, 28$ $P=0.6377$	$2.27 \pm 0.34$
	10-15 m (UAS)	8		$2.14 \pm 0.24$
	20-25 m (UAS)	8		$2.90 \pm 0.48$
	30-35 m (UAS)	8		$2.25 \pm 0.65$
<b>Iδ - % of Total Captured</b>	0-5 m (hand)	8	$F=0.815$ $df=3, 28$ $P=0.4964$	$2.32 \pm 0.36$
	10-15 m (UAS)	8		$2.18 \pm 0.28$
	20-25 m (UAS)	8		$3.20 \pm 0.58$
	30-35 m (UAS)	8		$2.66 \pm 0.67$

Table 3. Average % recapture and Iδ of sterile codling moths after release at four altitudes.

*Distance:* Three of the four release strategies resulted in significant differences in recapture by distance (Fig. 4). There were significant differences in recapture by distance when sterile *C.*

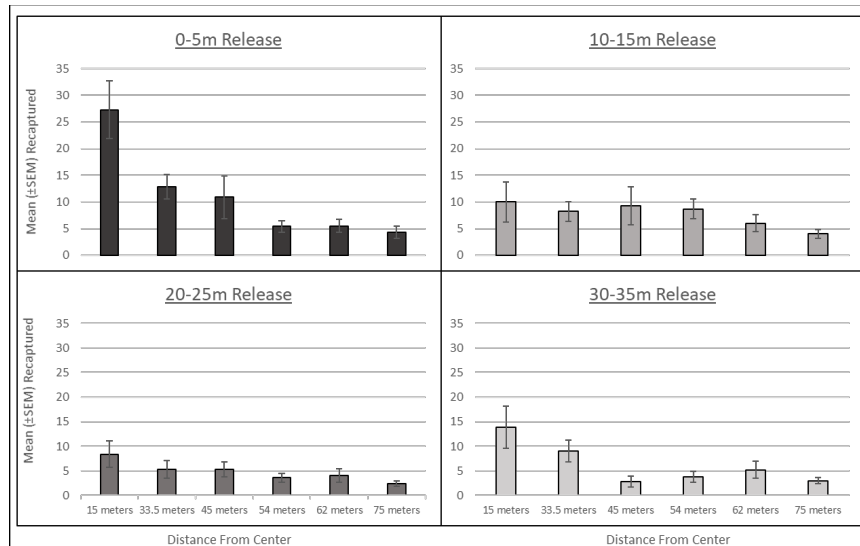


Figure 4. Mean (+/- SEM) per trap sterile moth recapture at 5 distances from release at four altitudes

*pomonella* were centrally released by hand at 0-5m altitude ( $F=9.9738$ ,  $df=5$ ,  $P<<0.0001$ ), by UAS at 10-15m altitude ( $F=4.5111$ ,  $df=5$ ,  $P=0.0007$ ), and by UAS at 30-35m altitude ( $F=4.5364$ ,  $df=5$ ,  $P=0.0007$ ). Hand release resulted in traps at 33.5m recapturing significantly more moths than those at 75m. Following release by UAS at 10-15m altitude, traps closest to the release point (15m) recaptured significantly more sterile *C. pomonella* than traps at 33.5m, 54m, 62m, and 75m. Following release by UAS at 30-35m altitude, more sterile codling moths were recaptured in traps 15m from the center than in traps 45m, 54m, 62m, and 75m away. Although the one-way analysis of variance did not indicate significant differences in recapture by distance following UAS release at 20-25m altitude ( $F=1.8878$ ,  $df=5$ ,  $P=0.0995$ ), there were more moths recaptured in traps closest to the center of the block than in those furthest from the center.

#### **Obj. 2b: Distributed or point releases**

**Recapture:** There were significant differences in moth recapture between by the four treatments ( $F=4.8407$ ,  $df=3$ ,  $P=0.0047$ ) (Table 4). Significantly lower recapture,  $0.8\% \pm 0.3$ , was recorded in hand-nine even points releases than the highest recapture,  $3.6\% \pm 0.7$ , in the UAS evenly spread treatment. There were no significant differences in recapture between the hand-center release ( $1.6\% \pm 0.7$ ) and hand-nine even points release, the hand-center release and UAS Center release ( $2.5\% \pm 0.6$ ), or the hand-center release and the UAS even release.

**Dispersion:** There were significant differences in  $I\delta$  indices calculated using the absolute number recaptured in traps for the four treatments ( $F=2.9316$ ,  $df=3$ ,  $P=0.0431$ ) (Table 4). Fisher's LSD test revealed differences in aggregation between hand-center (mean  $I\delta=2.64 \pm 0.44$ ) and hand-nine even points (mean  $I\delta=1.68 \pm 0.22$ ), and UAS-even (mean  $I\delta=1.43 \pm 0.14$ ) releases, indicating that sterile moths released at the center of the orchard were significantly more aggregated around the central point of release than those released in a spread-out pattern. Moths released by hand-at nine evenly spaced points and UAS-center (mean  $I\delta=1.87 \pm 0.27$ ) were not significantly more or less aggregated from each other or the hand center release or UAS 9 release point treatments.

There were significant differences in dispersion based on  $I\delta$  indices calculated using the proportion of total recaptured moths ( $F=4.021$ ,  $df=3$ ,  $P=0.012$ ) (Table 4). Moths released by hand at the center of blocks were highly aggregated (average  $I\delta=3.25 \pm 0.56$ ), those released by hand at nine even points were more dispersed (average  $I\delta=2.36 \pm 0.44$ ), followed by releases by UAS at the center (average  $I\delta=1.94 \pm 0.28$ ), and the greatest dispersion was for moths released by UAS spread throughout the orchard approximating the hand release at nine evenly spaced points (average  $I\delta=1.40 \pm 0.17$ ). However, Fisher's LSD test revealed that only moths released by hand at the center

were significantly more aggregated on average than those released by both UAS methods. There were no significant differences in dispersion using proportion recapture found between the two hand releases, nor between the two UAS releases.

	<i>Release method</i>	<i>ANOVA</i>	<i>Mean (± SEM)</i>
<b>Percent Recapture</b>	Hand release - Center	F=4.8407 df=3, 52 P=0.0047	1.6 ± 0.7 a,b,c
	Hand release - 9 Points		0.8 ± 0.3 b
	UAS release - Center		2.5 ± 0.6 a,b,c
	UAS release - ~9 Points		3.6 ± 0.7 c
<b>Iδ - Absolute</b>	Hand release - Center	F=2.9316 df=3, 47 P=0.0431	2.64 ± 0.44 a
	Hand release - 9 Points		1.74 ± 0.28 b
	UAS release - Center		1.87 ± 0.27 a,b
	UAS release - ~9 Points		1.43 ± 0.14 b
<b>Iδ - % of Total Captured</b>	Hand release - Center	F=4.0213 df=3, 48 P=0.0124	3.25 ± 0.56 a
	Hand release - 9 Points		2.36 ± 0.44 a,b
	UAS release - Center		1.94 ± 0.28 b
	UAS release - ~9 Points		1.40 ± 0.17 b

Table 4. Average % recapture and Iδ of sterile codling moths after release by hand or UAV at orchard center or manually distributed.

**Distance:** There were significant differences in recapture by distance from the center of the orchard (F=4.6925, df=5, P=0.0004) for moths released by hand at a single central location (Fig. 5). More moths released at the center of test orchards were recaptured at the closest traps (15m) than at traps 62m, and 75m away (Fig. 5). In addition, moths were significantly more likely to be captured in traps 33.5m from the release than those 75m from the center.

The effect of distance from the center on recapture *within* treatments was variable. Although average recapture was low at all trap distances from the center, there were no significant differences in capture by distance (F=1.5887, df=5, P=0.1634) when moths were released at nine evenly spaced locations. Sterile *C. pomonella* released by UAS at the center of the orchards were significantly more likely (F=3.8202, df=5, P=0.0023) to be captured in traps 15m from the center than those 54m and 75m away (Fig. 5). In addition, they were less likely to be recaptured in traps 75m from the center than in traps 33.5m away from the point of release. There were no significant recapture differences by distance from the center of the block when moths were released by UAS approximating nine evenly spaced points in the orchards (F=0.7507, df=5, P=0.5862); recapture was highest at all distances for this release method among the treatments (Fig. 5).

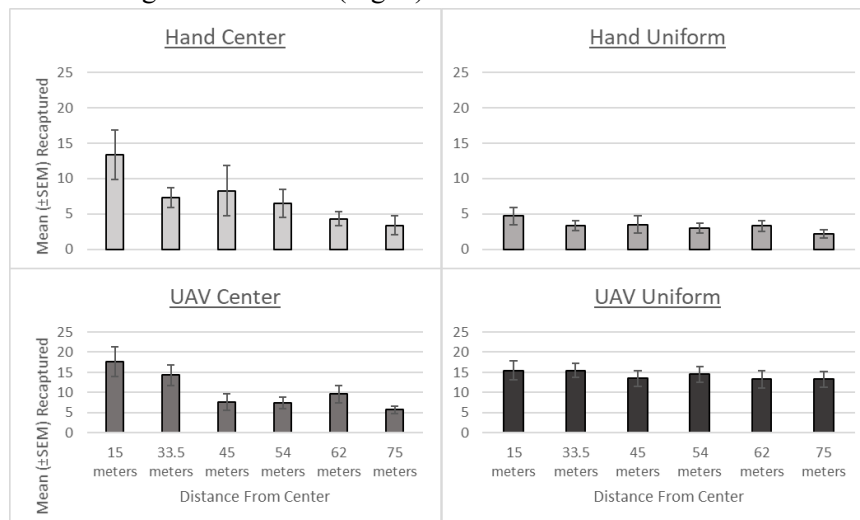


Figure 5. Mean (+/- SEM) per trap sterile moth recapture after release by hand or UAV at center or manually distributed.

The effect of distance from the center on recapture *between* treatments also was variable. There were significantly different treatment impacts on moths recaptured 15m from the center of the orchard ( $F=5.2226$ ,  $df=3$ ,  $P=0.0021$ ). Fewer moths were captured at this distance when they were released by hand at nine locations than by UAS at the center, and UAS approximating the nine locations. There were significant treatment effects on recapture of moths at 33.5m from the center of the orchard ( $F=15.9328$ ,  $df=3$ ,  $P<<0.0001$ ). Fewer sterile *C. pomonella* were recaptured when they were released by hand at nine evenly spaced points than by UAS at the center, and by UAS approximating nine evenly spaced points. There were also significantly fewer recaptured when they were released by hand at the center than UAS at the center, or UAS at nine evenly spaced locations. There were no significant differences in recapture between the two hand released methods at this distance from the center. At a distance of 45m from the center of the orchard, there were significant differences in recapture of moths by treatment ( $F=6.0036$ ,  $df=3$ ,  $P=0.0008$ ); fewer moths were recaptured when released by hand at a single central location and released by hand at nine evenly spaced points than when they were released by UAS approximating nine evenly spaced points. There were significant differences ( $F=14.5591$ ,  $df=3$ ,  $P<<0.0001$ ) in recapture of released moths among the four treatments at a distance of 54m from the center of the orchard. Traps at this distance recaptured significantly more sterile codling moths when they were released by UAV in a pattern approximating nine evenly spaced points than by hand at the center, by UAS at the center, and by hand at nine points. Also, significantly fewer moths released by hand at nine evenly spaced locations were recaptured than those released by UAS at the center of the orchard, and by UAS approximating nine evenly spaced points. Additionally, significantly fewer of the moths released by UAS at the center were recaptured than those by UAS approximating nine evenly spaced points. There were significant differences ( $F=11.5004$ ,  $df=3$ ,  $P<<0.0001$ ) in recapture of moths among release strategies at a distance of 62m from the center of the orchard. Fewer moths released by hand at the center of the orchard were recaptured than moths released by either UAS at the center or UAS approximating the nine evenly spaced points. Likewise, Tukey's HSD test showed that significantly fewer moths released by hand at nine evenly spaced points were recaptured than when moths were released both by UAS at the center and UAS approximating the nine evenly spaced points. At a distance of 75m from the center of the orchard, there were significant differences ( $F=19.7768$ ,  $df=3$ ,  $P<<0.0001$ ) in recapture of moths among the treatments. As indicated by Tukey's HSD test of recapture of sterile *C. pomonella* at this distance from the center of the orchard, more moths released by the UAS approximating nine evenly spaced points were recaptured than those released by hand at the center, hand at nine evenly spaced points, and UAS at the center of the orchard. Also, significantly fewer moths released by hand at the center and by hand at nine evenly spaced locations were recaptured than those released by UAS at the center of the block.

### **Obj. 2c: Release Time**

*Recapture:* There were significant treatment differences found in the recapture of moths released at different times of the day ( $F=4.1328$ ,  $df=5$ ,  $P=0.0039$ ). Releases conducted at 12:00 resulted in numerically higher recapture than all other treatments and Tukey's HSD test indicated significantly more sterile *C. pomonella* were recaptured from releases conducted at 12:00 than releases at 11:00, 15:00, and 19:00 (Table 5).

*Dispersion:* Analysis of absolute recaptures revealed significant differences in  $I\delta$  indices among the treatments ( $F=4.1124$ ,  $df=5$ ,  $P=0.0061$ ). The highest value and thus extent of aggregation was found for releases conducted at 19:00 - dispersion indices at 21:00, 18:00, 11:00, 12:00, and 15:00 were all significantly lower than at 19:00, but were not different from each other (Table 5).

Time of release had a significant effect on dispersion ( $F=5.7990$ ,  $df=5$ ,  $P=0.0005$ ) when  $I\delta$  indices were calculated based on the proportion of a replicate's total capture (Table 5). The aggregation indices for moths released at 11:00 ( $I\delta=2.60$ ), 12:00 ( $I\delta=1.81$ ), 15:00 ( $I\delta=2.65$ ), 18:00 ( $I\delta=3.17$ ), and 21:00 ( $I\delta=3.24$ ) were not significantly different from each other, but all were significantly less aggregated than releases conducted at 19:00 ( $I\delta=8.33$ ).



	<i>Treatment</i>	<i># Reps</i>	<i>ANOVA</i>	<i>Mean (± SEM)</i>
<b>Percent Recapture</b>	1100 release	7	F=4.1328 df=5, 41 P=0.0039	1.4 ± 0.9 a
	1200 release	10		3.7 ± 0.8 b
	1500 release	10		1.2 ± 0.6 a
	1800 release	4		1.2 ± 0.7 a,b
	1900 release	6		0.4 ± 0.2 a
	2100 release	10		1.7 ± 0.3 a,b
<b>Iδ - Absolute</b>	1100 release	4	F=4.1124 df=5, 29 P=0.0061	1.96 ± 0.38 a
	1200 release	10		1.90 ± 0.19 a
	1500 release	5		1.80 ± 0.21 a
	1800 release	4		2.91 ± 0.40 a
	1900 release	3		8.01 ± 3.56 b
	2100 release	9		3.13 ± 0.88 a
<b>Iδ - % of Total Captured</b>	1100 release	5	F=5.7990 df=5, 36 P=0.0005	2.60 ± 0.61 a
	1200 release	10		1.81 ± 0.19 a
	1500 release	9		2.65 ± 0.41 a
	1800 release	4		3.17 ± 0.47 a
	1900 release	4		8.33 ± 2.55 b
	2100 release	10		3.42 ± 0.80 a

Table 5. Mean (± SEM) % recapture and Iδ for sterile *C. pomonella* released at six different times of the day. Means with the same letters are not significantly different (Tukey's  $\alpha = 0.05$ ).

*Distance:* Five of the six release times resulted in significant differences in recapture by distance. When sterile codling moths were released at 12:00 they were more likely to be captured in traps close to the center of the orchard than traps farther from the center (F=16.8678, df=5, P<<0.0001). Tukey's HSD indicated that traps 15m from the center recaptured significantly more sterile *C. pomonella* than those 33.5m, 45m, 54m, 62m, and 75m away. Traps 33.5m from the center of the orchard recaptured significantly more sterile *C. pomonella* than those 75m away from the center, and traps 45m from the orchard center recaptured more than those 62m, and 75m away when moths were released at 12:00. Recapture at all distances when moths were released 1500 day was low, but there were significant differences in the distance from the center where moths were recaptured (F=2.4175, df=5, P=0.0374). Traps 15m from the center of the orchard were significantly more likely to recapture sterile moths than traps 75m away from the point of release. Captures were low when sterile moths were released at 19:00, but there were significant effects globally on recapture based on distance of the trap from the center of the orchard (F=2.6104, df=5, P=0.0283), and Tukey's HSD revealed that moths were more likely to be recaptured in traps 15m from the release point than moths 54m, 62m, and 75m away. Significantly more *C. pomonella* released at 21:00 were recaptured in traps close to the center of the block (F=12.7680, df=5, P<<0.0001) than in traps farther away, and Tukey's HSD test showed that traps within 15m of the center of the orchard recaptured significantly more sterile moths than those 33.5m, 45m, 54m, 62m, and 75m, and traps 33.5m from the center captured more than those 54m and 75m. Although ANOVA indicated that for the 18:00 time of release, the distance from the central release point moths are recaptured was significant globally (F=2.5206, df=5, P=0.0366), overall captures were low, and the Tukey's test did not reveal significant differences among the trap distances. There was not a significant effect on recapture at the six trapping distances when sterile moths were released at 11:00 (F=0.9806, df=5, P=0.4339), but numerically there were more captured in traps close to the center.

All six trap distances resulted in significant differences in recapture by time of release and generated the following results: 15m from the center (F=9.4846, df=5, P<<0.0001), 33.5m from the center of the orchard (F=10.4666, df=5, P<<0.0001), 45m from the center of the orchard (F=7.1408,

df=5,  $P < 0.0001$ ), 54m from the center of the orchard ( $F=11.6886$ , df=5,  $P < 0.0001$ ), 62m from the center of the orchard ( $F=8.1937$ , df=5,  $P < 0.0001$ ) and 75m from the center of the orchard ( $F=5.5968$ , df=5,  $P < 0.0001$ ). Traps placed 15m from the center recaptured more moths when they were released at 12:00 than at 11:00, 15:00, 18:00, 19:00, and 21:00. Also, significantly more moths were recaptured when they were released at 21:00 than 19:00. Traps placed 35m from the center recaptured more moths when they were released at 12:00 than at 15:00, 18:00, 19:00, and 21:00. In addition, moths released at 11:00 and 21:00 were recaptured significantly more than those released at 19:00. Traps placed 45m from the center recaptured more moths when they were released at 12:00 than at 11:00, 15:00, 19:00, and 21:00. Traps 54m from the central release point recaptured significantly different numbers of moths depending on the time of the day they were released, and more of the moths released at 12:00 were recaptured than moths that were released at 11:00, 15:00, 18:00, 19:00, and 21:00. Also, the 19:00 release had significantly lower recapture than the 11:00 release and the 21:00 release. Traps placed 65m from the center recaptured more moths when they were released at 12:00 than at 15:00, 18:00, 19:00, and 21:00. In addition, significantly fewer moths released at 19:00 were recaptured than those released at 21:00. Traps placed 75m from the center recaptured more moths when they were released at 12:00 than at 15:00, 18:00, 19:00, and 21:00.

## Discussion

### Orchard Factors

Our results from these experiments indicate that tree training system and topography impact the dispersal of sterile codling moths, and there are implications for understanding the behaviors of wild moths in these orchard systems. While many studies have focused on the maximum distance that a few CM will travel after release, few have explored the dispersion of a population of moths within the orchard close to the point of release and none have compared dispersion in different orchard training systems or on slopes. Moths released at the center of blocks with trellis disperse more readily than those released into blocks with large, widely spaced single-planted trees. We demonstrated that moths do not prefer dispersing up and down rows versus across them in all tree training systems tested, indicating that the direction of dispersion is not impacted by rows. Hills do not impact dispersion by causing more moths to move upslope than down.

Early studies exploring intra-orchard self-dispersion of released sterile codling moths (i.e., Howell and Clift, 1974, Mani and Wildbolz, 1977) did not explore the impact of planting system on dispersal, nor did they ascertain more accurate information on within-orchard movement, the impacts of topography, or other orchard conditions on the whole population of released moths. We have assessed the impact of tree training systems on released sterile codling moth recapture, aggregation, and dispersion and found that greater dispersion and recapture of moths is found in orchards with trellis compared to standard planted orchards. This is the first study to test the idea that rows may act as barriers to self-dispersal of released sterile codling moths – they do not.

Previous studies have shown that landscape-level elevational differences are an important factor in the capture of wild *C. pomonella* (Vernon et al. 2006). However, our results refine the notion that elevation may impact the capture of moths by scaling down the test area to single plots and comparing on-farm recapture at different elevations on a hill. We found that released moths are *not* more likely to disperse uphill than downhill or in either direction across the slope, and in flat plots there is also no preference for movement in a directional manner.

It is clear from these experiments that moths released into orchards with trellised trees are recaptured at higher rates than those released into orchards with large old stand-alone trees. This may be explained by the complexity of the systems and canopy density: trellised canopies are less dense and less three dimensionally complex. In trellised systems, not only is there more space for moths to move, but there is also less canopy to interact with as they move from one point to the next. In addition, the odor plume emitted from lures in traps may be more apparent to moths and more defined in orchards with trellis due to the reduced complexity and three-dimensional structure of the canopy.

Our results show that 1) sterile codling moths disperse from the point of release and some moths reached the furthest traps in all directions from the central release point, and 2) that aggregation around the point of release is typically moderate. These data suggest that although some moths may travel far and leave the block, the majority remain in the target area and are available to provide farm-scale control of existing wild populations when they are released at the center of the orchard. Self-dispersal of released sterile codling moths, regardless of orchard conditions, is vital to successfully integrating them into existing management systems. The current study has demonstrated that over short distances the layout and architecture of the orchard impacts how CM disperse throughout the landscape when orchards are treated with pheromone mating disruption. We estimated shorter maximum dispersive distances, less than 245 ft (75 meters), for the population of released moths under mating disruption than studies conducted in the absence of mating disruption, well over 650 ft (200 meters) (i.e., Tremmaterra, 2004; Basoalto et al., 2010).

From a practical standpoint, the movement of sterile codling moths into and out of orchards is of great importance to growers as they strive to make a SIT program economically viable. Farmers do not want to learn that the insects they released have all flown away, nor do they want to suffer the disappointment of knowing that the insects did not fly to the areas of the farm where they were needed most. Our results support employing different release tactics depending on orchard training system. Although moths dispersed to the edges of plot in all directions, it is evident that moths do not disperse as readily in orchards with old stand-alone tree plantings. In these types of plantings, it may be prudent to not release moths at the center of the block for every release: either employ several distributed release points for each weekly release or rotate release points throughout the season based on a schedule or damage sampling. It is equally clear that more moths disperse to the edges of trellised blocks than in stand-alone tree blocks, and release at the center is optimal to retain the maximum number of moths within the target block. It may be possible to utilize fewer sterile moths in orchards with trellis to affect the same level of control as in blocks with large stand-alone trees. Orchards with trees partly on steep hills, or wholly situated on steep slopes should have sterile moths released at the center of the slope because there is not a significant preference for uphill dispersion.

In summary, *C. pomonella* move up and down rows just as readily as they move across them in several planting systems. Rows do not act as a barrier for *C. pomonella*. Rather, released sterile codling moths disperse much more readily in trellised orchards, suggesting that this planting system is highly conducive to the sterile insect technique. Farmers with trellised orchards will find that a single point release at the center of a 10-acre block is sufficient to treat the whole block with sterile codling moths. Orchardists with apple blocks on hills need to understand the ways that sterile codling moths disperse in an orchard with uphill sections and modify releases accordingly.

### Release Factors

There are practical and economic considerations when comparing the use of UAS versus manual releases of sterile *C. pomonella*. A practical advantage of the UAS method over hand release is that pesticide reentry intervals do not impact the ability to release moths in a timely manner. On the other hand, weather conditions and proximity to “no-fly” areas may impede the use of UAS. Wind or rain may delay the application of sterile moths because the aircraft used to release the moths are expensive and precautions to avoid crashes are necessary and may impact the cost of the program.

Regardless of the various methods of release employed to deliver sterile insects, typically the goal is to distribute them quickly and uniformly throughout the target orchards without compromising quality and survival. Knowing that sterile *C. pomonella* are capable of flights of up to 250m, at the outset of this study we hypothesized that on a farm-scale, uniform distribution of moths may not be necessary.

To determine the best means of farm-scale sterile *C. pomonella* release we compared recapture and dispersion of moths following their deployment by hand or by UAS and from a central location vs multiple uniformly spaced sites. Our results show that *C. pomonella* adults released by hand at evenly spaced locations in orchard blocks were recaptured significantly less than when

released by UAS at 35m altitude on a flight path approximating the same locations. Although percent recapture was found to be different in our releases at/above nine evenly spaced points, there was no difference in the degree of aggregation/dispersion found by either release method (Hand or UAS). However, when we compared hand releases at the center of the orchard to hand releases at nine evenly spaced points and also UAS release approximating the same points, we found there was a higher degree of SIT moth aggregation about the center of the block when moths were released by hand at the center than when they were released at nine evenly spaced points by hand or UAS, indicating that more sterile *C. pomonella* were retained in the targeted orchard block when they were released at the center. In addition, release altitude was not significant to *C. pomonella* dispersion or recapture. These findings indicate that *C. pomonella* dispersal capabilities and size may be beneficial for UAS release with less impact from winds and forces associated with aerial release.

Moths released at different times of the day behaved generally like those in other trials – when released at the center of the orchard they dispersed throughout the 4.05ha area. Moths released at noon were recaptured at a higher rate than those released at other times of the day while also not having major differences in dispersion. Causes of this may be: 1) moths released late in the evening do not have sufficient time to acclimatize to field conditions before cessation of normal evening activities, thus are subject to 24 hours of potential mortality causes before conditions are appropriate for dispersing, 2) moths released in the middle afternoon are susceptible to shock caused by the rapid transition from chill-coma to >95°F causing either direct mortality or non-lethal damage resulting in low response rates, and 3) moths released at noon do not experience the previous two conditions at their release time and have sufficient time to acclimatize to temperatures and day lengths in the field. Typically, the warmest part of the day at our field sites was from 13:00-17:00. Successful mating usually occurs between 18:00 and 20:00 and longevity decreases with increasing temperatures. Moths released at or after typical evening mating periods do not experience the cues to elicit a mate-finding response. These data show that late afternoon and evening releases should be avoided, and moths should be released by noon the day they are received.

Application costs are a major consideration when implementing any SIT program and are acutely important for commercial applications of the technique by individual farms. For the past few years, several research programs have explored UAS as a means of delivering sterile *C. pomonella* to target areas. A UAS flying at about 30 meters above the orchard can distribute 30,000 sterile moths over a 6ha orchard in less than 10 minutes. However, the cost remains high. Season-long release of moths is currently accomplished in Washington State by a commercial applicator for over \$1100/ha. Wider adoption of this method of release will require new approaches that focus on improved efficiencies. Releasing only during peak generation flight may provide a significant cost savings and still provide substantial *C. pomonella* population suppression. Similarly, releasing fewer than the currently accepted full release density of 2000 mixed sex sterile moths per hectare may be more cost-effective. SIT is density dependent and thus operates much like pheromone-based mating disruption. With mating disruption, the fraction of added control achieved by applying 1000 rather than only 750 dispensers per acre may not be worth the added cost. The same may be the case with SIT, adding more moths to achieve the theoretical overflooding ratio of 40:1 sterile to wild males for eradication may not be worth the additional cost when used in conjunction with other management techniques. Also, deployment of SIT females in combination with synthetic pheromone mating disruption may have an additive impact on confusing wild males and increasing suppression and control.

Our findings reveal that hand-applying sterile *C. pomonella* is a viable option. Hand and aerial release of moths provided similar recapture and dispersal of released moths. Labor costs for hand application versus the costs of paying for a UAS or service to release moths should be compared when deciding which approach is best for a given situation. In the approximately 10-ac experimental blocks, an individual could walk from the truck to the pre-marked center of the orchard and back for a hand release in less than five minutes and walk to the nine evenly spaced locations in 10-15 minutes. In contrast, UAS releases applied moths to 10-ac plots in five minutes from the time the flight began to when it returned for all methods tested.

## Conclusions

The sterile insect technique for codling moth in Washington State is a valuable new management tool that should be carefully employed. Our studies have shown that released moths are vulnerable to a variety of conditions that may impact their effectiveness as a control tactic. The two main factors that we studied, those inherent to the orchard, and those controllable during the release were both found to be significant in the recapture and dispersion of released sterile codling moths. Users of SIT for on-farm *C. pomonella* control should carefully plan where and when to release moths to maximize their effectiveness. Based on the findings reported herein, a single release at the center of a 10-ac orchard either manually or by UAS at any altitude is sufficient to allow moths to disperse independently to the edges of the block while retaining the maximum number of moths within the targeted treatment area. Requiring only a single central release in each 10-ac orchard plot should reduce the cost of application. Additionally, moths should be released prior to noon. If moths are unavailable for release in the morning, applicators should consider holding them in cold storage until the next day. Our findings have shown that trellised orchards are more conducive to the spread of sterile moths than large single plantings, moths do not only move up slope in orchards with hills, hand release is not better or worse than UAS release at any altitude, and moths should be released by noon to ensure they have the best chance at being effective on the farm.

## Literature Cited

- Basoalto, E.; Miranda, M.; Knight, A.L.; Fuentes-contreras, E. 2010. Landscape Analysis of Adult Codling Moth (Lepidoptera: Tortricidae) Distribution and Dispersal Within Typical Agroecosystems Dominated by Apple Production in Central Chile. *Environmental Entomology* 39(5): 1399-1408.
- Trematerra, P.; Gentile, P.; Sciarretta A. 2004. Spatial analysis of pheromone trap catches of codling moth (*Cydia pomonella*) in two heterogeneous agro-ecosystems, using geostatistical techniques. *Phytoparasitica* 32: 325-341.
- Howell, J.F.; Clift, A.E. 1974. The Dispersal of Sterilized Codling Moths Released in the Wenatchee Valley, Washington. *Environmental Entomology* 3(1): 75-81.
- Hu, M.; Whitty, M. 2019. An evaluation of an apple canopy density mapping system for a variable-rate sprayer. *IFAC-Papers OnLine*, 52 (30): 342-348.
- Mani, E. and Wildbolz, T. 1977. The dispersal of male codling moths (*Laspeyresia pomonella* L.) in the Upper Rhine Valley. *Z. ang. Ent.* 83, 161-168.
- Vernon, B.; Thistlewood, H.; Smith, S. 2006. Case Study in Data Analysis: Variables related to codling moth abundance and the efficacy of the Okanagan Sterile Insect Release Program. *The Canadian Journal of Statistics* 34(3): 1-38.

## **Executive Summary**

**Project title:** Optimization of release strategies for sterile codling moth

**Key words:** Codling Moth, Dispersion, Sterile Insect Technique, Integrated Pest Management

**Abstract:** Sterile codling moth release as a control tactic recently became commercially available to Washington apple farmers. A better understanding of how released sterile codling moths behave on-farm in the presence of existing control tactics was necessary before this technology is recommended for adoption on an industry-wide scale. We studied how both factors inherent to modern orchards and factors that may be controlled at release impact dispersion and recapture of released sterile moths. We found that dispersion and recapture are impacted by orchard training systems, release locations, and release time of day, but not impacted by release altitude and orchard slopes. We demonstrate that sterile codling moth release is highly conducive to use in modern trellised orchards. Moths are flexible in the release and orchard conditions they will tolerate; they quickly distribute throughout the orchard when released at a single central location despite facing a variety of conditions. Based on the assumption that high moth recapture and slightly aggregated dispersion equates with effective on-farm control, we recommend use of a single central release location before noon in 10-ac trellised apple orchards. Unmanned aerial systems or hand releases are both effective tactics for deploying moths within orchards.

Final report prepared and submitted to WTFRC by Dr. RT Curtiss on behalf of Dr. Larry Gut who passed away before the conclusion of the project.