Sterile Insect Technique (SIT) combined with Mating Disruption for Management of Codling Moth in Pear Orchards

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ABSTRACT

A series of 20 weekly releases of sterile codling moth (CM) were used to examine the potential for use of sterile insect technique (SIT) to augment present pest management tactics, including mating disruption, used to control codling moth in mature California pear orchards. Three orchard blocks were divided into two adjacent plots, with one plot receiving a weekly release of 800 sterile codling moth per acre while the other received all other pest management treatments used in the SIT block. The ratio of sterile to wild moths in traps was variable between weeks and locations, with a median of 7.5 for two locations and 0.67 for a third. Recovery in the traps of all sterile moths released ranged from none to 5.9%. Dispersal was documented by a mean of 22% of the sterile CM were captured in the comparison block. The proportion of wild females mated was greater in the SIT than the comparison plot in all three replicate blocks, although this difference was not statistically significant. These findings confirm that mass-release sterile codling moth performed well in California, demonstrate spatial and temporal variability in performance, and suggest that sterile mass-release CM males bred with wild females in these orchards in the presence of mating disruption.

INTRODUCTION

Sterile insect technique and mating disruption have been used successfully against a variety of moth pests (Simmons et al. 2021). Codling moth (CM) is one of three long-term successful moth SIT programs (Simmons et al. 2021, Thistlewood and Judd 2019). Sterile insect technique is often referred to as "sterile male" technique by the general public, and male only releases are practical and preferred for many dipteran targets. In Lepidoptera, however, both sexes have been sterilized and released in all large-scale cases of SIT for Lepidoptera (Simmons et al. 2021). There is evidence that overflooding with both sexes can, in some instances, be more effective than overflooding with males only (Saour et al. 2022, Ikegawa et al. 2021).

Mating disruption is also widely used against moth pests (Miller and Gut 2015), including codling moth (McGhee et al. 2014). Mating disruption can work by competitive, non-competitive, or hybrid mechanisms (Miller and Gut 2015). Mating disruption against the codling moth works by a competitive mechanism (Miller and Gut 2015, McGhee et al. 2014). Mating disruption against the codling moth has been technically and economically successful, but mating disruption is not as effective for the codling moth as it is for some target pests such as the oriental fruit moth (Miller and Gut 2015). Choices for monitoring codling moth in the presence of mating disruption include a combination of pheromone and pear ester (optionally with acetic acid to increase female capture)(CMDA + AA), or pear ester, nonatriene, linalool oxide, and acetic acid (4K) (Knight et al. 2019, Knight et al. 2022). CMDA + AA is approved for use in organic orchards, whereas 4K is not.

On first consideration, combining mating disruption and SIT as control techniques can meet skepticism, since the former depends primarily on prevention of mating whereas the latter depends on mating between mass-release sterile and wild moths for effectiveness. The counter argument is that the effects may still be added if mating is equally reduced for sterile mass-release and wild moths, and that this effect might be even more likely if both sexes are released. In practice, mating disruption and sterile insect technique (SIT) both have successfully been used together to control codling moth (Horner et al. 2020, Judd and Garner 2005). The present proposal tests vigor and dispersal of codling moth from the Okanagan Kootenay Sterile Insect Release Program (British Columbia, Canada), imported into the U.S. and transported and released by a private contractor (M3 Agriculture, Omak WA). Impact on wild CM populations will also be assessed. Crop damage at harvest was not assessed.

<u>OBJECTIVES</u>

- Determine if overflooding is achieved, the sterile:wild ratio, and if the number of wild moths decrease over the 20 weeks
- Determine dispersal: if sterile moths stay on the release side or disperse evenly across the site.
- Spermatophores — Baseline probability of capturing an unmated vs. a mated Codling moth female in a trap

PROCEDURES

Orchard sites Three orchard sites served as replicate blocks for this experiment. The west (presumably upwind) half of each of these orchards served as a release plot, while the east half of the orchards served as a grower standard (i.e., a plot receiving identical cultural and pest management treatments exception sterile moth release). Each of these orchards was under mating disruption for codling moth.

Block and plot characteristics are shown in Table 1. Block 1 was established ca. 100 years ago and additional interplanted trees were added ca. 60 years ago, so current spacing is 18 ft rows by 9 ft tree and 16 ft rows by 8 ft tree spacing. Mating disruption was provided by 2 aerosol dispensers (Suterra CheckMate Puffer CM-OFM Standard, Bend OR USA) per acre, and 200 hand dispensers/ acre in the 3 rows adjacent Highway 160 on the North side (Isomate CTT, Pacific Biocontrol, Vancouver, WA USA) placed the week of 20 March, 2023. Insecticides active against CM included Spinosad (Entrust, Corteva Agriscience, Indianopolis IN USA) applied on 10 June.

Block 2 was established ca. 60 years ago and spacing is 16 ft rows by 8 ft tree spacing. Mating disruption was provided by 2 aerosol dispensers (Suterra CheckMate Puffer CM-OFM Standard, Bend OR USA) per acre, placed on 6 April, 2023. No hand applied dispensers were placed in rows along windward edges in this block. Insecticides active against CM included esfenvalerate (Asana, Valent USA LLC, San Ramon CA USA) applied on 28 April, methoxyfenizide applied on 30 May, and spinetoram (Delegate, Corteva Agriscience) applied on 28 June and 12 July.

Block 3 was established ca. 90 years ago and additional interplanted trees were added ca. 25 years ago, so current spacing is 21ft rows by 10.5 ft tree spacing. Mating disruption was provided by 200 hand-applied dispensers with 2 dispensers/tree applied in 1 row along all edges (Isomate CTT, Pacific Biocontrol, Vancouver WA USA) per acre, placed on 1 April, 2023. The only insecticides active against CM was Spinosad (Entrust, Corteva Agriscience) applied on 9 May and Pyrethrin (PyGanic, MGK, Minneapolis MN USA) on 10 August, 2023. Block 3 was certified organic.

Sterile moth releases Sterile codling moth (800 adults per acre) were released each Tuesday for 20 weeks, from 18 April to 29 August, 2023. Releases were shifted a day later due to holidays for the weeks of 30 May and 4 July. Mixed-sex sterile codling moth were sourced from the Okanagan Kooteny Sterile Insect Release (OKSIR) Program (Kelowna, British Columbia, Canada). Adults were exposed to 150gy using a Cobalt Irradiator and imported into the United States by M3 Agriculture Technologies (Omak WA, USA). Codling moth from OKSIR are routinely fed a diet containing a lipophilic red dye (calco oil red) that is retained internally by adults and that permits distinguishing sterile released adults from wild codling moth. Moths were shipped from the US port of entry via parcel overnight express to Sacramento, where they were picked up and applied to fields the following day. Moths were kept at 2°C using pre-chilled refrigerant gel bags while in shipping, or temperature-controlled coolers while transported in vehicles to the field. Field release was conducted using the Hermes V.2 Unmanned Aircraft Systems (UAS)(Esch et al. 2021, Moses-Gonzales et al. 2021). Way points programmed into the UAS provided for release above each of the 9 monitoring points in the release plot (described in the paragraph).

Adult Monitoring Capture of wild and sterile codling moth was examined using 9 sticky traps per plot, arranged in grids of 3 traps in each of 3 orchard rows (Fig. 1). Orange wing traps (Suterra LLC, Bend OR, USA) were modified using bent wire spacers as described elsewhere (Kuenen et al.

2005, Burks et al. 2020). In each orchard traps were >= 32 ft from the edge of the orchard. Traps in Block 1 were approximately 400 feet apart within east-west rows, with trap rows were 230 to 256 ft apart. In block 2 traps were 260 to 300 ft apart within east-west rows and trap rows were 290 ft apart. In the smaller block 3 traps were 100 to 170 ft apart within east-west rows and trap row were 270 to 280 feet apart. Block 3 was wider (more row) in the eastern half than in the western half of the orchard, and there was a 250 ft-wide area on the southeast (non-SIT release area) in which monitoring traps were not placed. Monitoring raps were baited with pear ester (Light and Henrick 2001, Knight et al. 2019) and acetic acid (Landolt et al. 2007) because this combination attracts codling moth in the presence of mating disruption (Knight et al. 2019) and is more effective at capturing females than other attractants currently used (Evan Esch, personal communication). The pear ester and acetic acid bait formulation is not currently produced commercially, but was provided by Trece Inc. (Adair, OK, USA). The lures were changed every 6 weeks. Trap liners were changed weekly and examined in the laboratory to obtain counts of wild and sterile males and females, and to dissect spermatophores in the females to determine mating status.

Adult Monitoring Capture of wild and sterile codling moth was also tracked using I eye-level trap using Megalure 4K lures (Trece', Adair OK) in 2 blocks and CM pheromone DA+AA lures in one block. A trap was positioned in the center of the release area and in the center of the control area of each block. In addition, 22 other locations with these traps were in the block 1 ranch. The results of this trapping are not reported here, except in Fig. 2, showing the proximity of all sterile moth capture adjacent the test block 1. The other 2 of the three test blocks were not adjacent other orchard areas with eye-level traps.

Egg Monitoring Weekly egg searches of fruit clusters using the cut fruit technique were conducted (Zoller and Zoller 2001) 3 cut fruits per trap per orchard = 54 total cut fruit per week per orchard plot, cut 1 week prior to egg searches~~)~~ were maintained as well as random samples of uncut fruit in clusters (306 total uncut fruit per week per orchard plot). Egg monitoring data is discussed in Results, with locations of eggs detected in Table 2. Statistical analysis of egg monitoring data was not performed, as many locations had no eggs.

Data analysis Moths captured in pear ester/day traps were pooled by block, plot, and monitoring interval to examine trap overflooding (defined here as the ratio of dyed to non-dyed moths in traps), percent of sterile moths recovered, and the percent of sterile moths that were recovered in the non-release comparison plot adjacent to the release plot. To examine overflooding, total dyed and non-dyed moths were compared for only the sterile release plots. To examine total recovery, data for dyed moths were compared between the sterile release and the comparison plots. Those data were also used to examine percent dispersal, defined here as the percent of dyed sterile moths that were recovered from the non-release comparison plot. The non-parametric Kruskal-Wallis ANOVA was used to compare overflooding, recovery, and dispersal between the three replicate blocks. A Dunn posterior test with an experiment-wise alpha of 0.05

was used determine significant differences for when the non-parametric ANOVA was significant. The unequal variance Welches t-test was used to compare the proportion of wild CM females between SIT and comparison blocks. Data summary and analysis were performed using R 4.2.1.

RESULTS

Data from only the release plots were used to assess trap overflooding (Fig. 3, Table 3). No dyed or wild moths were recovered on the week of 18 April. During the 19 remaining weeks with overflooding ratio varied between 1 and 62 (mean = 8.9, median = 3.8) (Fig. 3). There was significant variation in the median weekly trap ratio between the three blocks (Kruskal-Wallis, chi-squared = 18.58, df = 2, $P < 0.001$). There was no significant difference in the median overflooding ratio between blocks 1 and 3, but the overflooding ratio in block 2 was significantly lower than either block 1 or 3 (Dunn Test, experiment-wise alpha < 0.05) (Table 3). More wild than sterile moths were found in traps for 2 weeks each for blocks 1 and 3, but for 13 weeks in block 2.

Data from both the release plots and the adjacent dispersal plot were used as a relative index of trap recovery, and to examine dispersal. The percent of sterile CM recovered varied widely between replicate blocks and weeks (Fig. 4). For pooled recapture for all three blocks, the mean percent recovery was 0.43% (Fig. 4). The percent recovery for individual blocks over 19 weeks ranged from 0 to 5.9%. The median capture was similar between blocks 1 and 3 (0.225% and 0.25%, respectively, but significantly lower for block 2 (0.088%) (Kruskal-Wallis with Dunn post-test, chi-squared = 7.3, df = 2; $P = 0.026$).

For the three replicate blocks pooled, the mean weekly percentage of dyed moths recovered in the comparison plot (where no release took place) was 22%. Dispersal from the release to the comparison block was more consistent compared to overflooding or overall recovery (Fig. 5). Mean dispersal into the comparison plot was 12% for block 1 compared to 24% for block 2 and 30% for block 3. The difference between block 1 compared to blocks 2 or three were statistically significant, whereas the difference between blocks 2 and 3 were not significant (Kruskal-Wallis with Dunn post-test, chi-squared = 12.1, df = 2; $P = 0.002$).

The total number of wild females captured over the season in the 6 plots ranged from 10 to 65. The proportion of females mated was consistently greater in the SIT treatment plots than in the comparison plots (Fig. 6). However, the overall difference in percent mating between the SIT treatment plots (65 ± 18%, mean ± SE, n = 3) and the comparison plots (32 ± 8%, mean ± SE, n = 3) was not significant ($t = -1.6717$, df = 2.7492, $P = 0.2014$).

Locations of CM eggs detected are in Table 2. Most of the eggs were in block 2, especially in the SW windward area. This particular area lacks the windbreak situation of the block's remainder as well as that of the other two blocks in this study, as described in the Discussion.

DISCUSSION

Generally, the ratio of sterile to wild CM in the monitoring traps suggested that substantial overflooding was achieved with the weekly releases. Since the pink bollworm SIT was closed following the eradication of pink bollworm in the US, the OKSIR SIT program for CM, which provided the sterile CM for the present study, is the most well-established Lepidopteran SIT program in North America (Thistlewood et al. 2019, Simmons et al. 2021). The overall overflooding rate and dispersal is consistent with robust and competitive performance of the sterile mass-release moths in the present study. The variation in overflooding rate and the modest recovery are therefore likely illustrative of expected performance of moths in an SIT program. This type of variation was also noted in a recent study of trapping area for CM using a pheromone-pear ester lure (Horner et al. 2020, Curtiss et al. 2023). In the maximum dispersive distance suggested that traps baited with pheromone and pear ester drew CM males from an area of 10 and 15 acres, respectively, the plume reach (area over which CM actively followed scent trails from attractants) was <15 ft. This is consistent with the modest overall recovery observed in the present study. Moreover, while an average of 20% of CM recovered were captured in traps in the comparison plot, these grids only captured CM that dispersed in one direction. The modest recovery rate reported here is therefore consistent with adequate performance to provide control by SIT.

Variation in results between the orchard sites serving as replicate blocks might be explain in part by differences in the pest management practices used. For example, in block 2 where both overflooding and recovery were much lower, there was greater use of insecticides with contact activity such as esfenvalerate and spinetoram. In block 2 the former was used at the end of April, and the latter was used at the end of June and mid-July. In contrast, Spinosad was used only on 10 June in Block 1 and only on 9 May in Block 3. Another notable feature in which Block 2 differed from the others was the absence of a windbreak on southwest part of the orchard (in the SIT block). Higher damage has been noted just in this area over several years. The other blocks have windbreak trees and buildings on the windward edges (Broc Zoller, personal observation). Damage data are not reported at this time, but generally damage was low (although most in the windward area of block 2 described above) and there was no apparent difference in damage between the SIT and comparison blocks.

Sterile CM have previously been used with mating disruption, both as a means of providing additive crop protection (Judd and Gardiner 2005, Horner et al. 2020) and as a means of testing efficacy and examining mechanisms of mating disruption (Welter et al. 2005, McGhee and Miller 2014, Miller and Gut 2015). Traps baited with pear ester can capture unmated as well as mated CM (Knight 2006), and use of acetic acid with pear ester increases the ratio of females to males captured (Knight 2010). Traps baited with pear ester therefore have the potential to assess the impact of mating disruption treatments on CM females.

In summary, the present data demonstrate that the sterile CM released for SIT in this study achieved trap overflooding. Variation in performance was similar to that observed in previous studies. Increased mating in wild CM females in the SIT blocks was consistent with mating between sterile mass-release males and wild females.

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Tables and Figures

Table 1. Characteristics of the orchard sites used as the three replicate blocks.

Block	County	Latitude	Longitude	Release area (acres)	Comparison area (acres)	Mating Disruption
1	Sacramento	38.34	-121.55	10	10	Aerosol
2	Lake	39	-122.85	10	10	Aerosol
3	Lake	39.01	-122.85	5.8	5.8	Hand-applied

Figure 1. Schematic diagram of the trap arrangement in adjacent treatment and grower standard plots in each of the three replicate blocks.

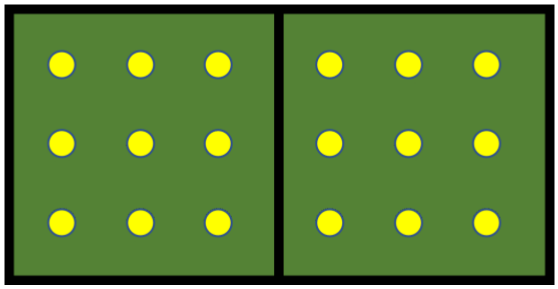

Figure 2. Trap locations where SIT moths were captured in eye-level traps using Megalure 4K lures.

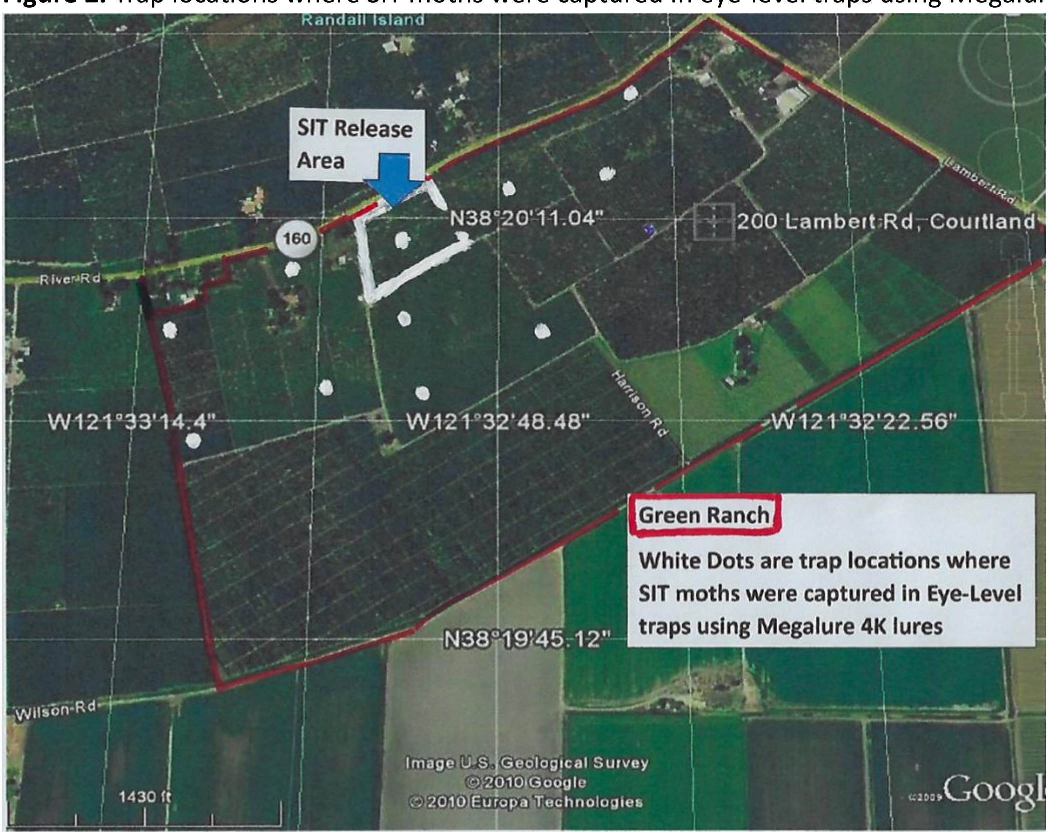

Table 2. Location of detected codling moth eggs using the cut fruit technique.

				Table 3. Sterile Insect Release Trap Labels Maps									
				With nearby Egg Numbers on Cut Fruit and (dates observed)									
1	**B Block (Joe Green Ranch, Hiway 160 at 200 Lambert Rd, Courtland, CA 95615)**												
			Treated Area (10 acres, W side)						**Un-Treated Area (10 acres, E side)**				
		....	North Border...					....	North Border...				
		101		104		107		110		113		116	
		102		105		108	1 (6/22)	111		114		117	
		103		106		109		112		115		118	
		....	South Border..					....	South Border..				
2	**Hedge (access: go 0.33 miles south of 3250 Finley East Rd, Kelseyville, CA 95451)**												
			Treated Area (10 acres, W side)						**Un-Treated Area (10 acres, E side)**				
		....	North Border...					....	North Border...				
	1 (7/3)	201		204		207		210		213		216	
		202	1 (5/29) 2 (6/19) 1 (7/10)	205		208		211		214	1 (7/17)	217	
	1 (7/17) 1 (7/24) 1 (7/31)	203	1 (6/19)	206		209		212		215		218	
		....	South Border..					....	South Border..				
	Notes: SW corner of the Hedge block is the upwind corner during frquent SW coastal wind drift in the afternoons.												
	There are 2 abandoned pear trees on neighbor's property line just north of Trap 201.												
3	**Rickabaugh (3430 Finley East Rd, Kelseyville, CA 95451)**												
			Treated Area (5.8 acres, W side)						**Un-Treated Area (8.2 acres, E side)**				
		....	North Border...					....	North Border...				
		301		304		307		310		313		316	
		302		305		308		311		314		317	
		303		306		309		312		315		318	
		....	South Border..										
								....	South Border...				

Figure 3. Variation in weekly ratio of sterile release to wild codling moths captured in pear ester/acetic acid traps in three replicate blocks (release plots only).

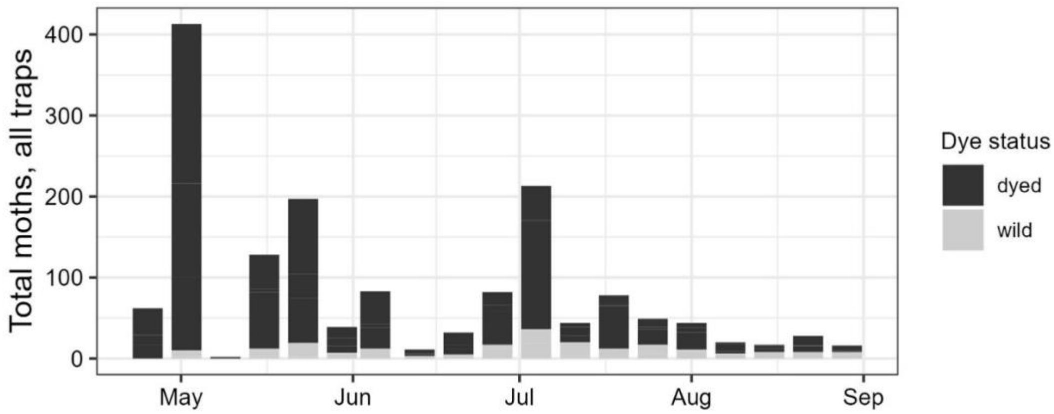

Table 3. Variation in trap overflooding ratios (sterile:wild) (n = 20) between release plots in the three replicate blocks.

Statistic	**Block1**	**Block2**	**Block3**
Minimum	0	0	0
25th Percentile	3.4	0.26	3.1
Median	7.4	0.67	7.5
75th Percentile	22.2	1.9	14
Maximum	45	16.6	197

Figure 4. Variation in weekly recovery of sterile codling moth in all traps compared to the number of moths released, by replicate block. The mean recapture for all weeks and all blocks was 0.43% (horizontal barred line)

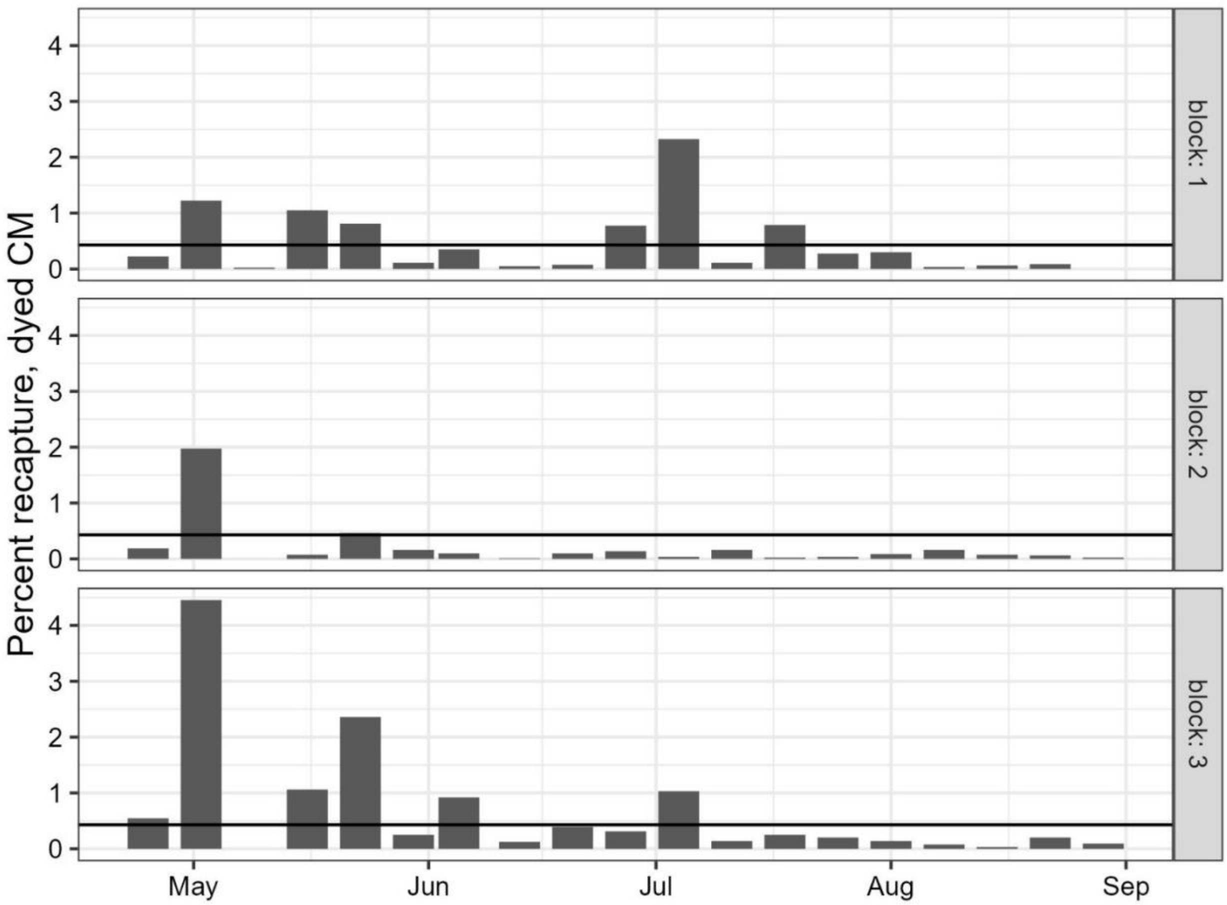

Figure 5. Variation in weekly dispersal, as measured by the percent of sterile CM recovered in the comparison plots.

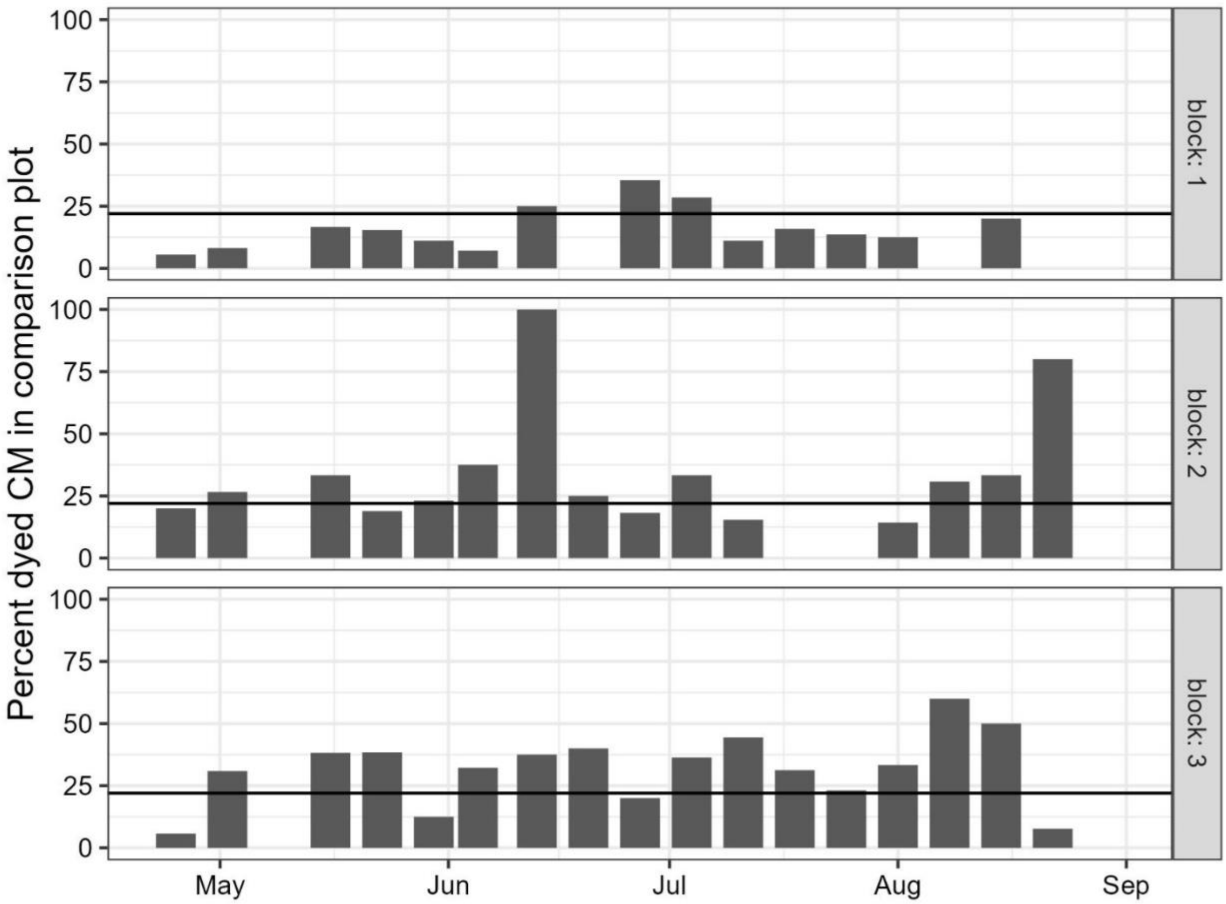

Figure 6. Percent of wild females mated in SIT and comparison plots, by replicate block.