FINAL PROJECT REPORT
CP-18-104

Project Title: Optimizing sterile insect release of codling moth in Washington

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Total Project Request: **Year 1:** $98,947 **Year 2:** $98,359 **Year 3:** $102,711

Other funding sources: **Awarded**
Amount: $29,724
Agency Name: Western IPM

WTFRC Budget: ***None***

Budget 1
Organization Name: **Contract Administrator:** Shelli Tompkins
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Item	**2018**	**2019**	**2020**
Salaries[1]	58,940	61,298	63,750
Benefits[2]	20,046	20,847	21,681
Wages[3]	6,240	6,500	7,020
Benefits[4]	591	616	665
Equipment	0	0	0
Supplies[5]	5,767	2,200	2,200
Travel[6]	7,363	6,898	7,395
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	98,947	98,359	**102,711**

Footnotes: [1]Salaries for project manager (1 FTE) and technician (0.075 FTE); [2]benefits at 33.5% (project manager) and 41.8% (technician); [3]Wages for time slip: $12.50/hr (yr 2), and $13.50/hr (yr 3) for 13 weeks/summer; [4]benefits at 9.5%; [5]Supplies: computer, printer/software; lab/office supplies, electronics; video camera/accessories, sterile moths and release stations, bands. [6]Travel to plots, motor pool rental, fuel, per diem, travel to industry meetings to present results

Objectives:

1. *Determine the effect of fixed vs variable release rate on efficacy of sterile insect release (SIR).*
   We have completed all three years of releases in the replicated plot study in the Tonasket/Malott area. We collected moth recapture and fruit damage data for all three years.
2. *Compare the non-target effects of broad-spectrum pesticide use versus SIR as a supplement to mating disruption in organic orchards.*
   A broad range of secondary pest and natural enemy samples were taken during the 2018 season. This objective was discontinued in 2019 based on 2018 results.
3. *Examine the synergy between SIR and other tactics using modeling techniques.*
   Models that look at the interaction of multiple mortality/fecundity factors must first be underpinned with field or laboratory estimates of the effects. Initial attempts to investigate the complementarity of mating disruption and SIR using mating tables failed, and an alternative method using molecular markers was unsuccessful.
4. *Investigating sterile moth recapture and behavior.*
   This objective was added in 2019. We investigated recapture of sterile insects near net installations, the effect of high densities of sterile females, and the apparent longevity/viability of sterile moths in the field.

Significant Findings:

- Recapture of sterile moths was low in spring and fall and peaked in midsummer (July and August). The higher (3x) rate was reflected in recaptures in 2018 and 2020, but not in 2019.
- Overflooding ratios ranged from 2 to 20 (2018), 1 to 15 (2019) and 1 to 13 (2020) depending on treatment and time of season.
- Codling moth fruit damage (stings+entries) were significantly lower in the two SIR treatments in 2018, but no significant differences occurred thereafter. Overall, the number of fruit damaged declined each year of the study but did so in all treatments.
- Moths released outside of caged orchards were captured at much lower rates than uncaged orchards, indicating good exclusion by the nets. Moths released over a cage by drone were recaptured at the same rate as moths released by hand inside the cage; in addition, moths sprinkled in the canopy were recaptured at much higher rates than moths sprinkled on the ground (hand release).
- When higher densities of female moths were released, the percent recapture of females was significantly lower than the lower release density treatments.
- Sterile moth longevity/viability in field: In a preliminary study, moth recapture peaked at 3 days post-release with the last detection at 9 days; a larger study found peak recapture 6 days post-release with a final detection at 18 days. The majority of recapture occurred during the first week post-release.

Obj. 1. Determine the effect of fixed vs variable release rate on efficacy of SIR

The plots identified in 2018 were used throughout the project with a few exceptions. In 2019, the trees in one block were removed, and a different block on the same ranch was substituted. In 2020, two check plots were removed (blocks on the same ranch substituted), and two blocks were reduced in size, but remained in the program. Otherwise, the same treatments were applied to the same plots as the previous year (Table 1). Each of the three treatments had four replicate plots, for a total of 12 plots. All orchards are organic apple orchards in the Tonasket/Malott area. Plots range from 4 to 8 acres in size, with range of 0.1 to 1.8 miles between plots to minimize moth spillover. All plots use codling moth mating disruption and received a complete organic control program (petroleum oil, CM virus, and optionally, Entrust) at the grower's discretion.

Table 1. Treatments tested for codling moth SIR

Trt.	Description	Sterile moth rate
1	1x SIR	Std. CM program + std. rate of SIR (800 sterile moths/acre/week)
2	3x SIR	Std. CM program + gradated rate of SIR Base rate increased to 2x (1,600 moths) and 3x (2,400 moths) following wild moth phenology
3	Check	Std. CM program + insecticides; no SIR moths

Moths were transported from the Osoyoos facility and released on Tuesdays using a hexacopter (Hermes V2 UAS) by M3 Consulting of Phoenix, AZ to the eight SIR-treated plots (Trts. 1 and 2). Moths were released weekly for 22 weeks (late April – mid-September) using a release device on the aircraft calibrated to deliver the specified rate evenly over the plot. On a per-acre basis, the 1x (constant) treatment received 22 dishes (17,600 moths) during the season, and the 3x (varying) treatment received 53 dishes (42,400 moths); because of the varying rate, the 3x treatment received only 2.4-fold the number of moths as the 1x treatment.

Moth densities and distribution were sampled using plastic Delta traps baited with the CM-DA+AA lure (codlemone, pear ester, and acetic acid) (Fig. 1A). Traps were deployed at a density of 1/acre in a grid pattern inside the plot boundaries, with four additional perimeter traps ca. 50 ft from the center of each edge. Liners were changed weekly, and the number of moths recorded by sex (Fig. 1B, C), mating status (females only), and origin (sterile, wild). Sterile moths were identified by crushing the abdomen to see the internal red dye used in the larval diet in the Osoyoos rearing facility. Lures were changed every 6 weeks as per manufacturer's recommendations. Trap captures were counted the entire release season plus one week before the initial and one week after the final releases. Trap results were summarized and mapped using GIS software (Fig. 2) and sent to grower-cooperators weekly along with information on the sterile:wild ratio.

Fig. 1. Plastic delta trap with CM+DA+AA lures (A); codling moth genitalia, male (B) and female (C); note internal red dye showing through integument of the female.

Results. In all years of the project, sterile moth recapture peaked in mid-summer (mid-July-August) and was lower in the spring and fall (Fig. 3). Studies by Canadian researchers identified the issue of relatively poor performance of the sterile moths at cooler temperatures, possibly related to rearing conditions; however, wild moth flight is also affected by environmental conditions. Overall, the interior traps in the 3x treatment recaptured almost twice as many moths as the 1x treatment in 2020 and 2018. In 2019, the recapture in the 1x and 3x treatments was almost identical. The percentage recapture of sterile moths in 2020 was a little higher for the 1x (0.08%) compared to the 3x (0.05%). The sterile:wild ratios fluctuated during the season, influenced by the response of sterile moths to traps and the generational peaks of the wild population. Ratios were highest during mid-summer and early fall, and somewhat higher in the 3x rate (0.9:9.9, mean 5.32) than in the 1x rate (0.25:12.6, mean 3.74). The proportion of moths captured by the interior vs perimeter traps was consistently higher over time, irrespective of treatment (data not shown), although 30-40% of the total moths recaptured were in the perimeter traps, indicating a moderate amount of off-target drift.

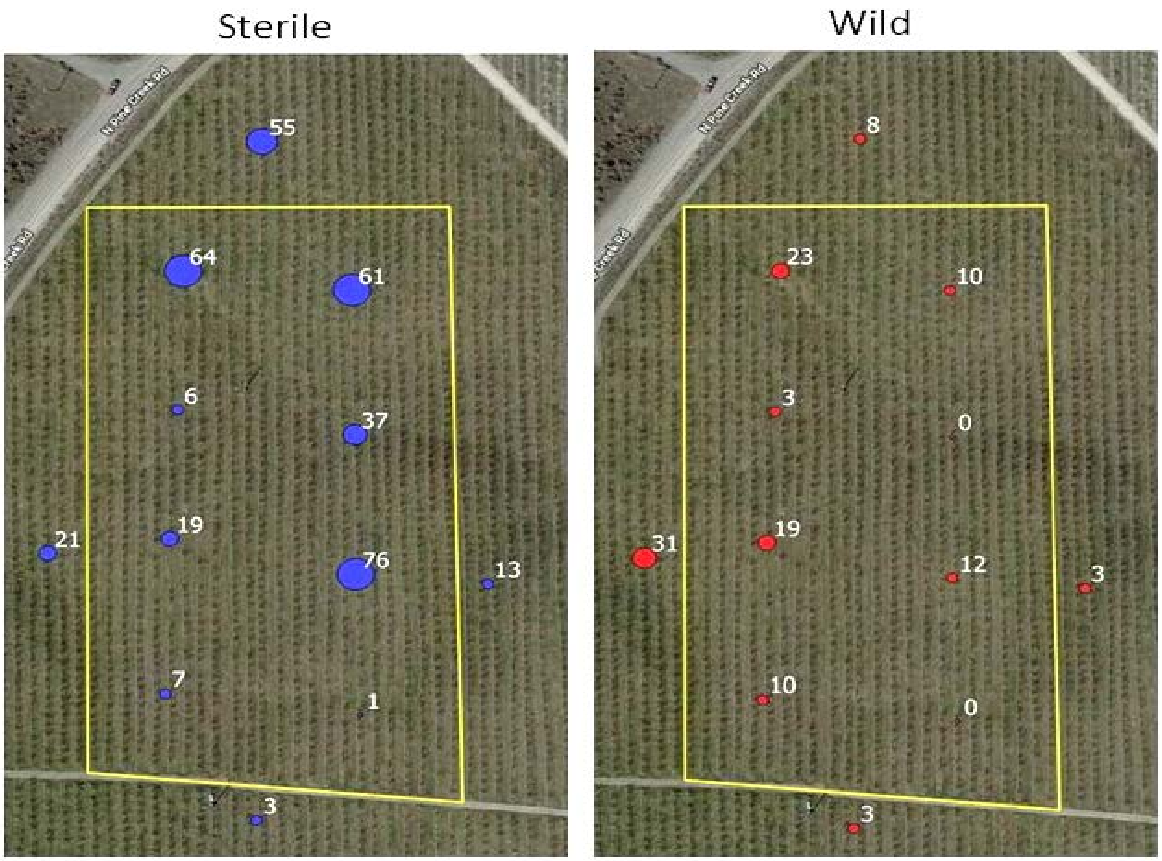

Fig. 2. Bubble maps of weekly trap catches (wild and sterile), 29 May 2018.

Wild moth densities showed a first-generation peak, with the strongest peak in late May (Fig. 3). Wild moth captures declined over the course of the study in all treatments. In 2018, there was a peak of ca 10 moths/trap, but in 2019 and 2020, there were <4 moths/trap/week throughout the season. The second flight in July was fairly distinct in 2018 but was difficult to discern in 2019 and 2020. There were no significant differences between wild moth capture between the treatments.

Plate 1. Visually recording stings and entries, 2018.

The success of the SIR treatments was assessed by intensive preharvest fruit damage samples. Codling moth stings and entries were recorded ca. 7 to 10 days before each block was harvested by visually observing both sides of each row of the blocks and recording the latitude and longitude of each damaged apple observed (Plate 1). This allowed to us to determine and map the spatial pattern of damage in the blocks (Fig. 4). The total number of apples observed was recorded independently to provide information on percent damaged fruit.

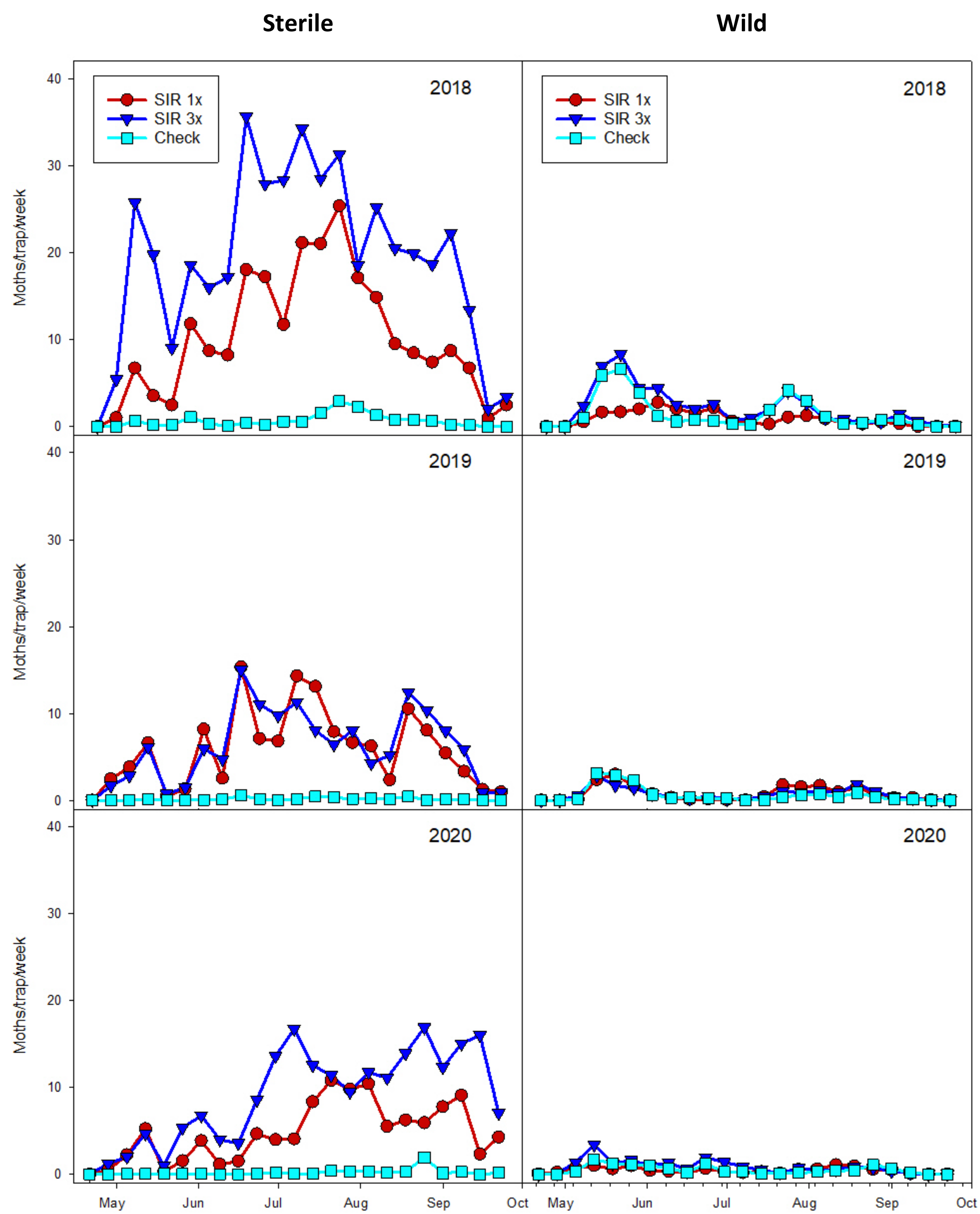

Fig. 3. Sterile and wild moth trap captures in three treatments, 2018-2020.

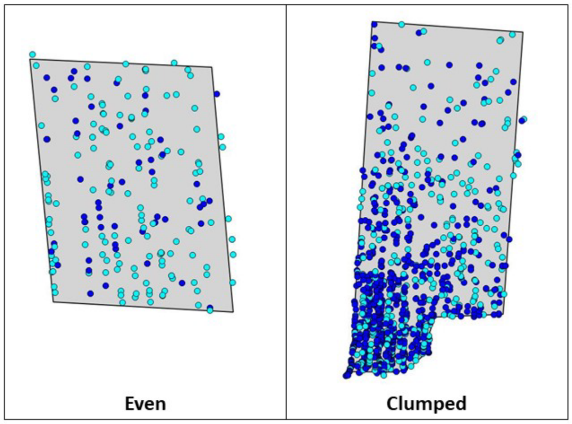

Fig. 4. Even (left) vs clumped (aggregated) (right) distribution of codling moth fruit damage.

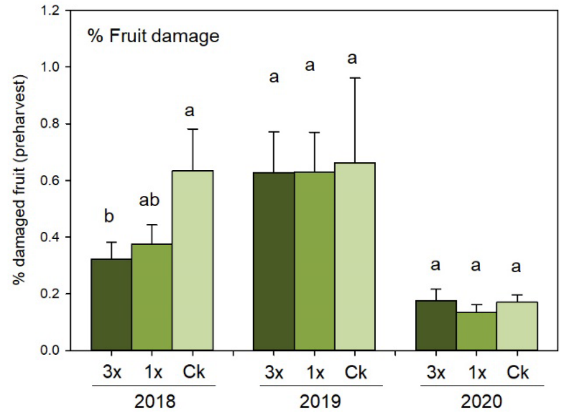

Fig. 5. Percentage codling moth fruit damage, 2018-2020. Replicate 1B was dropped from the analysis in 2020 due to atypical orchard management.

The average fruit damage was <0.66% in all three years of the study for all treatments. Individual blocks were more variable, but never exceeded 1.6% damage. In 2018, codling moth damage was significantly lower in the 3x SIR treatment in comparison to the check, with the 1x treatment intermediate (Fig. 5). There were no significant differences in mean fruit damage thereafter. Fruit damage was about 0.65% in all treatments in 2019, and about 0.16% in 2020.

The georeferenced fruit damage assessments indicated that some blocks had evenly distributed damage, while some were concentrated in one section of the block (Fig. 4). Information of this type can be very useful to help growers address 'hotspots' in their blocks. Remedial measures could include bands, sprays, sanitation, intensive trapping, or higher rates of SIR moths in the high-damage area.

The spatial fruit damage pattern also allowed us to see a noticeable diminution in damage in a hotspot area in one block over the course of the study (Fig. 6). This occurred even though the moths were distributed evenly in all years of the study; altering moth distribution to address this area may be a future option. There was some correspondence between the trap capture of wild moths (Fig. 7) and the resulting fruit damage (Fig. 4, clumped) in high pressure situations, but in general, the capture of wild moths did not provide an accurate predictor of fruit damage.

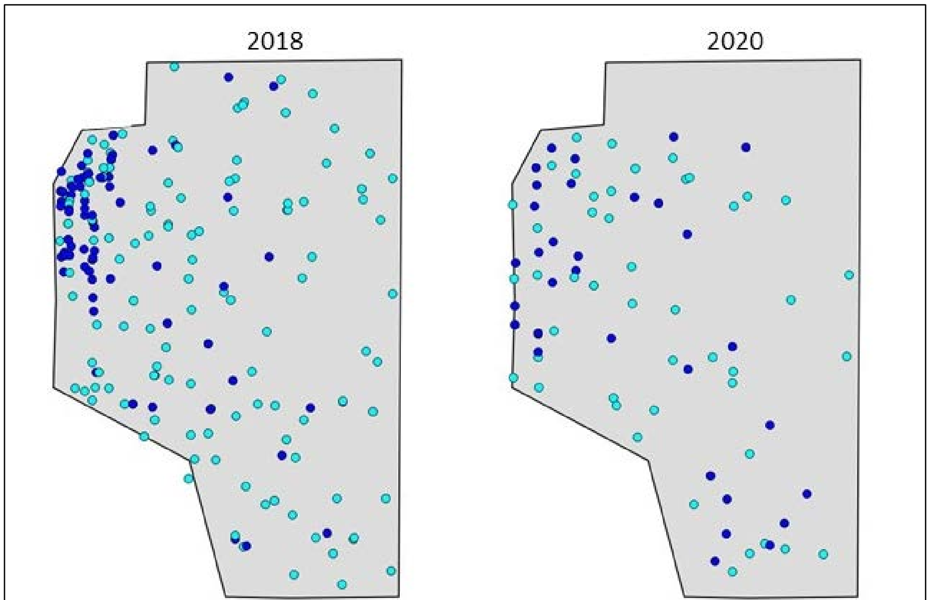

Fig. 6. Amelioration of a codling moth hotspot from 2018 to 2020.

Obj. 3. Examine the synergy between SIR and other tactics using modeling techniques.

In order to construct a predictive model of the interacting effects of multiple control tactics, the magnitude of those effects must first be estimated from laboratory or field data. Development of predictive models dating back to the 1950's have suggested that a critical component to SIR success is the ability of sterile males to compete with wild males for mates. One complicating factor for application to tree fruit pests is the interaction between SIR and mating disruption, which both reduce successful matings, but by very different methods. While pheromone trap captures (the ability to locate a phermone source) are frequently used as a proxy for mating success, there may be great disparities between the total trap capture and the male who mates first with a wild female. Because moths typically only mate once, this order of arrival is critical, and ultimately determines whether mating of wild females leads to fruit damage by larvae. Mating tables are considered the most accurate measure of this rate of larval production, but are laborious to deploy, and the low proportion of mating of tethered females on a given night makes sufficient replication challenging. We propose a more direct measure, that of examining the spermaophore in mated wild females, and determining whether it came from a wild or sterile male. This is based on the assumption that wild and sterile moths have genetic differences that can be detected using molecular methods.

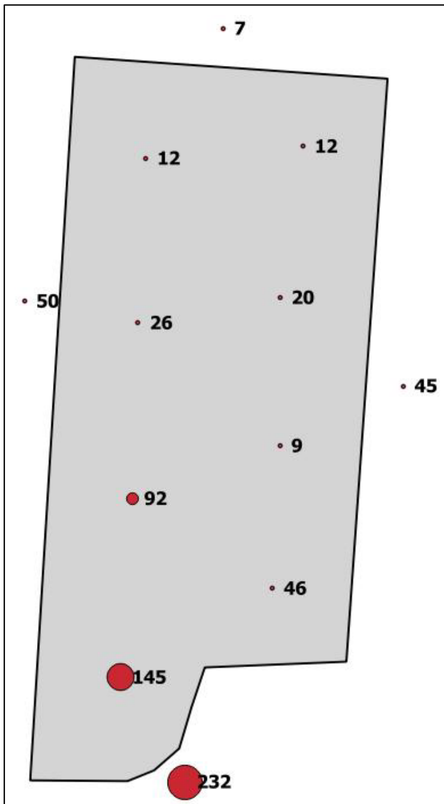

Fig. 7. Aggregated wild moth distribution in pheromone traps (see Fig. 4, right).

Results. In 2019, we sampled the Osyoos colony periodically and preserved moths for future molecular diagnosis to look at variation over time. We also have genetic material from various regions in the state from wild codling moth populations. To date, we have not found a method that will successfully differentiate between a moth from the Osoyoos colony (before or after irradiation) and a wild moth. We continued this effort in 2020, but were still unable to find a viable molecular marker to distinguish between the colony and wild moths.

Obj. 4. Investigating sterile moth recapture and behavior

Moth releases near overhead nets or net enclosures. Net installations, both overhead and full cage, are becoming more common in Washington orchards. The numerous benefits of such installations, coupled with the prevalence of sunburn-sensitive cultivars, make them an attractive option. In addition to horticultural benefits, net installations will have implications (both positive and negative) for pest control. While previous research has been aimed at excluding codling moth, the availability of SIR means we need to explore the question of dispersal, retention, and behavior of codling moth inside or under nets.

CM exclusion. We conducted CM exclusion experiments in 2019 and 2020 in an orchard in Douglas County, WA which had an existing installation of both a full net cage and an overhead net, as well as orchards without nets (check). In 2019, the caged plot was compared to the overhead net and check, but in 2020, only the cage and check were compared. Plots ranged from 10 to 26 acres/plot in 2019, and 10 to 15 acres/plot in 2020. We deployed traps (CM-DA+AA lures in an orange plastic Delta trap) 50 ft inside the perimeter of the blocks. Sterile moths from the OK-SIR facility in Osoyoos, BC, were marked with fluorescent powder and released 50 ft outside of the perimeter of the blocks, with 1 to 6 release points per edge depending on the block size and surroundings (Fig. 8). There were about two-thirds as many traps as release points, plus a trap in the center of the block. Both sterile and wild moths were counted. Treatments were replicated over weeks (n=7) from late July to early September of both years. In 2019, the number of moths available each week was variable; the moths were divided evenly among release cups. In 2020, each release point received a full dish of 800 moths, except for one week, which received 400 moths/release point. The total number of moths released per block per week was the product of moths/release point × release points/block. In 2019, the marked moths were placed in plastic cups which were suspended from a trellis wire about 6 ft from the ground; in 2020, the moths were sprinkled on the tree canopy over a few feet around a flagged release point. The recapture was expressed as the number of released moths recaptured per 1,000 moths released on a whole-block basis.

Results: Overall, the recapture rate of moths was much lower in 2019 than in 2020 (note different y-axes in Fig. 9); this corresponds to lower recapture rates in the SIR pilot project plots in this year. In 2019, the recapture in the overhead net block was slightly lower than the check plot, but not significantly so. In both years, the recapture of moths in the caged block was substantially lower than the check plots (Fig. 10). Interestingly, the same trend occurred each year for wild moths in the cage vs check plots, although different check plots were used, and the underlying wild moth population was unknown.

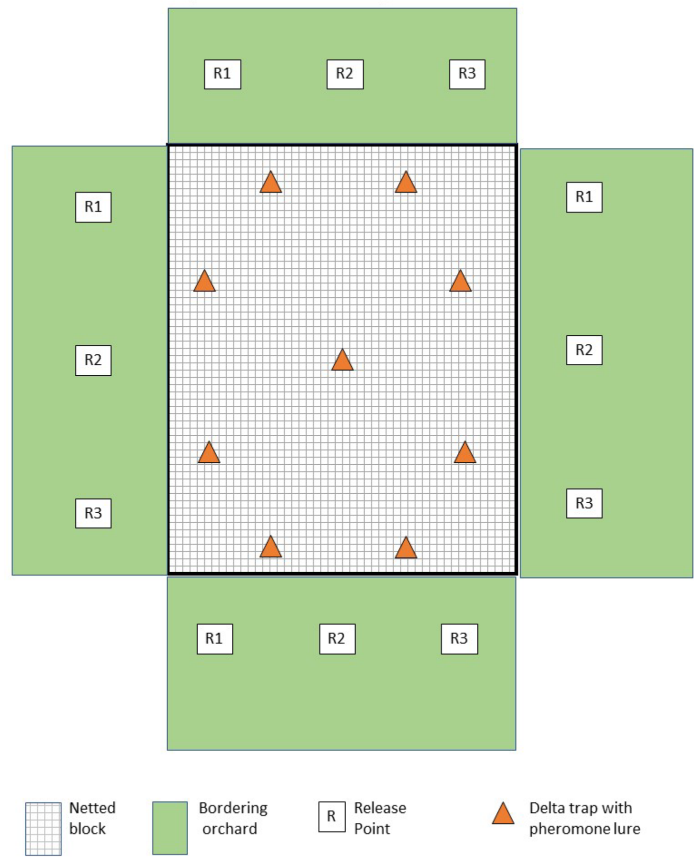

Fig. 8. Treatment schematic for testing codling moth exclusion with nets.

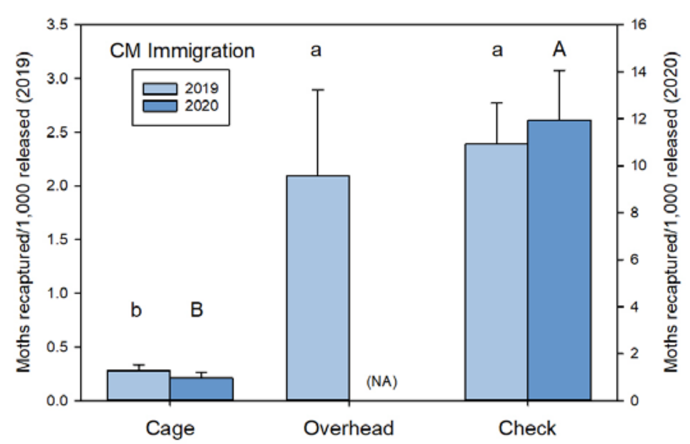

Fig. 9. Exclusion of codling moth from caged and overhead net blocks, 2019-2020.

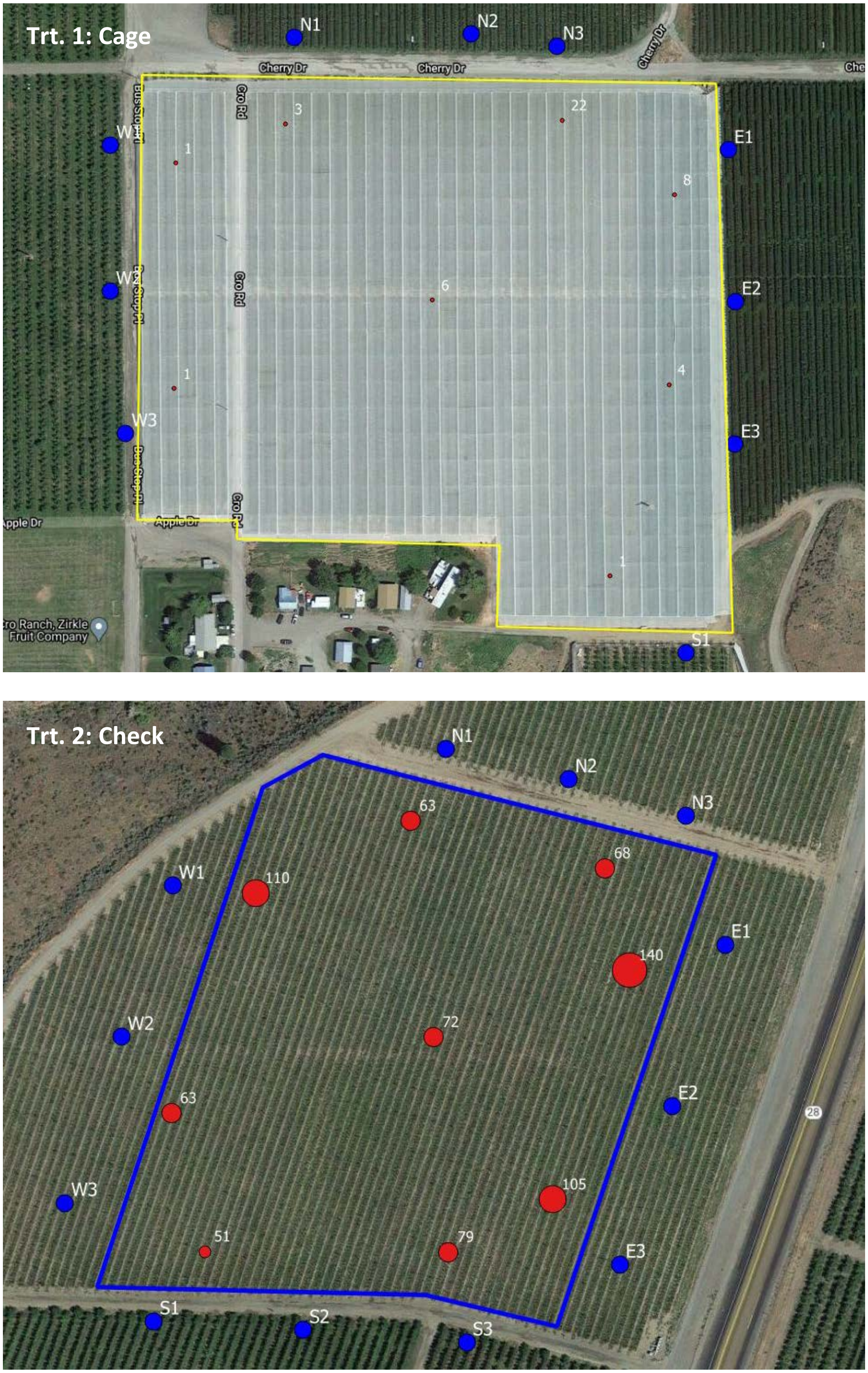

Fig. 10. Recapture of marked sterile moths released outside of a caged (top) or uncaged (bottom) orchard.

CM drone releases over nets. In 2020, we studied the potential for moth release over a caged orchard by drone. The alternative for SIR treatment in caged orchards (or those with overhead nets) is to revert to ground release, either by hand or all-terrain vehicle (ATV). Either method would significantly slow the workflow of releases, and possibly result in poorer moth quality. Previous immigration/emigration experiments indicated that behavior, rather than physical exclusion, plays the greater role in the propensity of moths to traverse a net barrier. Rapid escape from small cages clearly indicated that moths are physically able to cross a 5 x 2 mm mesh of the type commonly used for sunburn protection. However, our 2019 cage experiments (above) also indicated they have little tendency to enter a cage when released outside it at ca. 6 ft. Moths released above a net may exhibit different behavior in order to reach a host tree. If moths can be released by drone above nets, a substantial savings of time would result. The study site was a recently built cage over an 8.3-acre apple orchard. Three of four sides were completely enclosed to ground level, but one side (west) had a partial wall to allow entry/exit of farm equipment. In addition, the top net had apertures at intervals that, while still providing sunburn protection, allowed possible entry points for moths (Fig. 11). We compared drone release above this caged orchard with an uncaged orchard immediately adjacent to the west. In addition, we made releases by hand beneath in both the caged and uncaged orchard, using different colors of fluorescent powder to determine the origin of the moths. Two types of hand releases were compared: spreading the moths on the ground (simulating the Canadian ATV release method) and sprinkling the moths in the tree canopy about 6 to 7 ft above the ground. Moth recapture was determined by a grid of delta traps (1/acre) baited with a CM-DA-AA lure. For all methods, the same rate of moth release was used (800 moths/acre). The drone releases were made by M3 Consulting using their normal flight path for even distribution, and the hand releases were made using 1 release point per acre, thus 1 dish of 800 moths per release point. The treatments were replicated over time for 6 to 10 weeks from mid-July to late August.

Fig. 11. Upper net of cage testing drone versus hand release, 2020 (note gaps).

Results. In the caged plot, releases made by drone were not significantly different than releases made by hand (sprinkling moths in the canopy) (Fig. 12); this is a positive indicator that under certain conditions, SIR moths can be distributed by drone with no penalty in moth performance. The two hand-release methods gave strikingly different results; placing the moths in the canopy (Plate 2) resulted in higher recapture than placing them on the ground (Fig. 13). This difference was apparent in both the caged and uncaged plots. Interestingly, the drone release resulted in 4× higher recapture of the caged plot than over the uncaged plot (data not shown); the reasons for this are not immediately apparent, but are still a hopeful indicator that drone releases over nets will be as effective as those made by hand

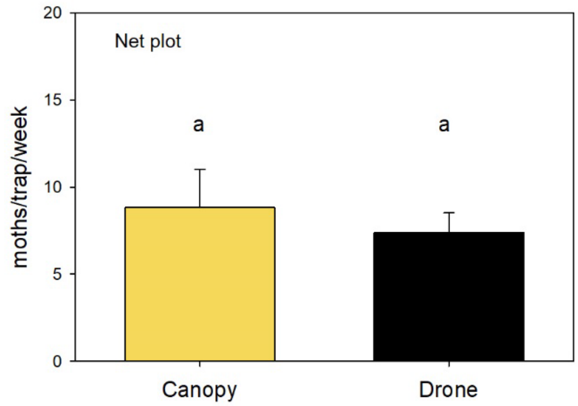

Fig. 12. Recapture of marked sterile moths released by drone or by hand (sprinkled in canopy).

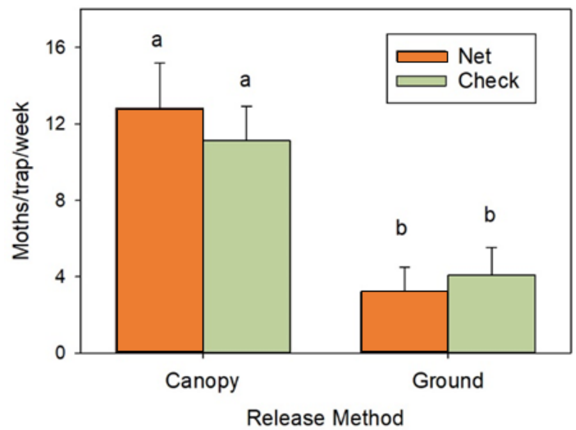

Fig. 13. Recapture of marked sterile moths released by hand in the canopy or on the ground.

Sterile moth accumulation in traps over time
Two experiments were conducted to see how long sterile codling moths remain viable in the field, as evidenced by trap recapture. The first preliminary experiment was in a small 2-acre research pear orchard not under mating disruption at the WSU Tree Fruit Research and Extension Center in Wenatchee, WA. There was a single release of 3,200 moths (4 points x 800 moths/point) on 8 July. Recapture was determined by a grid of 8 traps all within ca. 200 ft of the release point. Traps were check daily until no more moths were recorded.

Plate 2. Marked codling moths in orchard canopy.

The second experiment was conducted in a 21.6-acre organic apple block under mating disruption in a commercial apple orchard near Rock Island, WA. We had 12 traps baited with CM-DA-AA lures. Traps in the release block were placed 200 ft apart in a north-south line (10 traps), with an additional 2 traps 200 ft to the east and west of the release point. These were eight additional traps (400 ft apart) in surrounding blocks to the west (the east side abutted a cliff); four in Fuji organic transition and four in conventional Granny Smith (Fig. 14). There was a single release of moths on 15 July 2020 of 10 dishes (8,000 moths) at the central release point. Traps were equipped with cameras, and a photo of the trap liner and moths was taken ca. 10 am daily. Liners were changed weekly and brought into the lab to match the moths captured to the photos to determine the day of capture. The traps were left in place for three weeks post-release. This experimental design allowed us to look at movement of moths over a greater distance, and well as the recapture curve.

Results. In the preliminary experiment, 93 of the 3,200 moths released in the pear block were recaptured, or 2.9%. Peak recapture occurred on 3 days after release, and 98% of all moths recaptured occurred in 7 days. The last moth was detected 9 days after release (Fig. 15A).

In the second experiment, a total of 113 moths released in the apple block were recaptured, or 1.4% of the total released (avg. 5.65 ± 1.71 moths/trap). Moth recapture was low on day 1 and peaked on day 6. Recapture steadily decreased quickly after day 6 (Fig. 15B). There was a small peak of recapture on day 12. This suggests that sterile moths may have greater longevity than we may have previously thought. There was one moth recaptured on day 18, almost three weeks after release. Overall, the majority of recaptured moths were caught in 8 days, and in the release block; only 2 were found in a neighboring block. The greatest numbers (43%) were found in the two adjacent traps along the north-south transect, and 58% in the four traps in four cardinal directions (all 200 ft from the release point) (Fig. 14). Although treated as a single management unit, the block had two bisecting east-west road that may have impeded movement. Recovery ≥800 ft from the release point was extremely low.

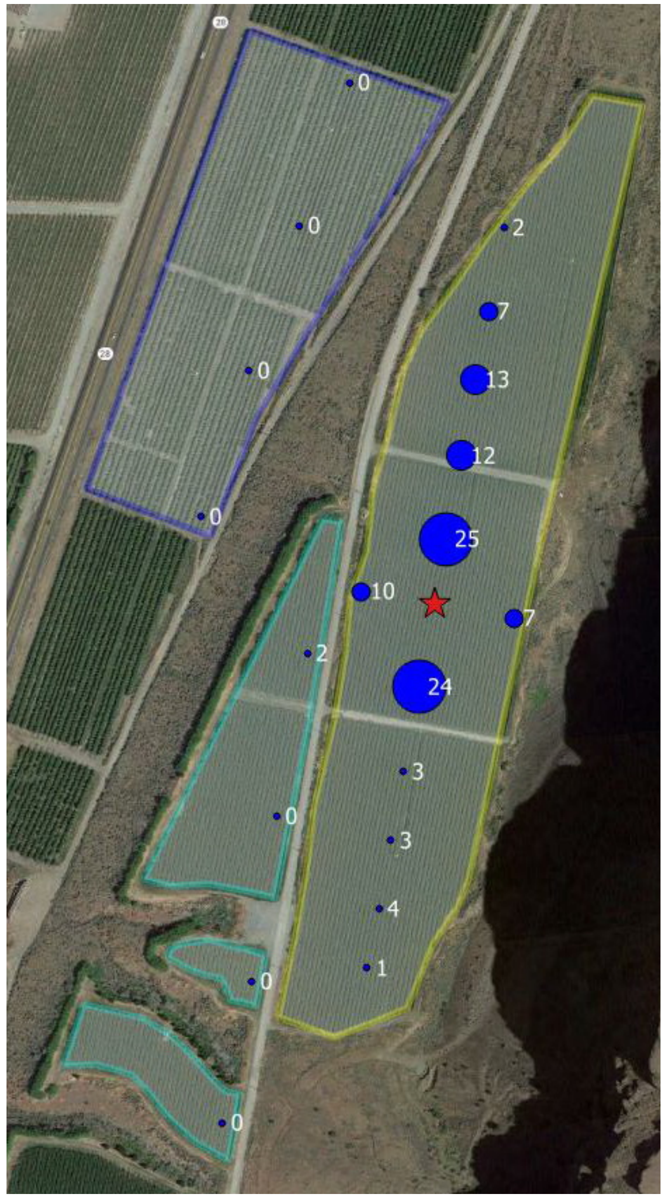

Fig. 14. Recapture pattern of moths in a 21-acre organic apple block, 2020.

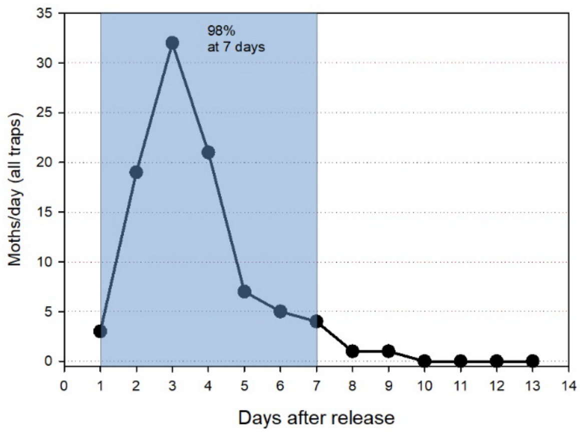

Fig. 15A. Recapture of released sterile moths over time, pear block, 2019.

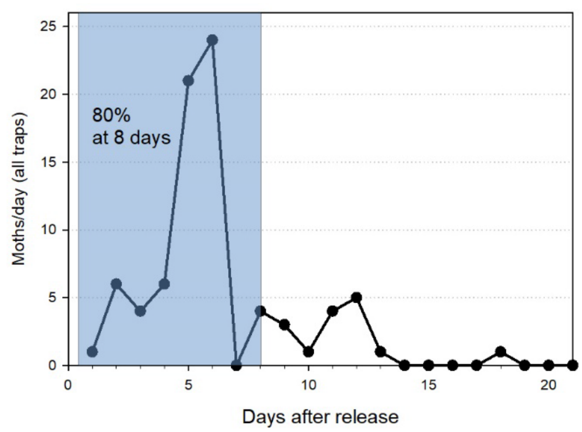

Fig. 15B. Recapture of released sterile moths over time, apple block, 2020.

EXECUTIVE SUMMARY

Project Title: Optimizing sterile insect release of codling moth in Washington

Keywords: sterile insect release, SIR, codling moth

Abstract: The Canadian SIR program in British Columbia has been in place since the early 1990s, and had successfully suppressed this key pest of apples and limited the need for pesticides. Interest in this technique intensified in Washington in recent years due to the availability of excess moths from the Canadian facility in Osoyoos, a few miles from the Washington border. A pilot project was conducted in Washington from 2018 to 2020 in Okanogan County, WA to determine if sterile moth release could be used as an IPM tactic outside of an areawide program, the latter being the norm for most SIR projects. Two rates of sterile moth releases were compared to a check without SIR. Fruit damage in the first year was significantly reduced in the SIR treatments, but no differences occurred in subsequent years. However, fruit damage declined steadily in most of the plots throughout the study. This preliminary study provides a positive indicator that SIR can be helpful in suppressing codling moth when used on limited acreage, although more experience is needed to confirm this.

Summary: Codling moth (CM) has been the key pest of Washington apples since the early 1900s and remains so today. The development of pheromone mating disruption (MD) in the late 1980s helped stabilize pest management programs under constant threat of pesticide resistance and became the foundation of codling moth management for ca. 85 to 90% of the state's apple acreage.

Despite the efficacy and widespread use of MD, codling moth pressure has been building in recent years in Washington. The problem is more acute in organic orchards, where insecticidal inputs lack the high mortality levels and long residual control of conventional materials. Sterile insect release (SIR) provides a potential new tool that can be used in both organic and conventional production, with advantages to resistance management in both regimes. However, SIR is normally used on an areawide basis, with compulsory releases on all affected acreage in a region under a sponsored government program. The paradigm of SIR on a voluntary, open market, and block-by-block basis is generally considered unfeasible due to the high initial input costs of rearing, sterilization, and distribution. The availability of sterile moths from the (existing) Canadian program makes this new approach possible. Unsurprisingly, very little of the foundational research addressed this type of use; thus exploration of the integrated pest management (IPM) approach was needed.

To explore this concept, we released Canadian SIR moths from 2018 to 2020 in Okanogan County, WA. We used three treatments, the standard release rate of sterile moths (1x, or 800 moths/acre/week), a high rate of moths (3x), and a control where no moths were released. The recapture rate of sterile moths throughout all three growing seasons did not always mirror the release rates. There was also much better recapture of SIR moths in the middle (warmer) part of the season, with poor rates in spring and fall. The overflooding ratios (sterile:wild moths), a key concept to SIR, varied correspondingly.

The true test of efficacy of any IPM technique is fruit damage. In the first year, the use of SIR moths significantly reduced damaged when compared to the check. No differences among treatments were found in succeeding years, although damage had declined overall by the third year, irrespective of treatment. Our experience in this pilot project indicates that the underlying insect pressure and management intensity greatly affects the outcome for a given block, suggesting that each block is in effect a case study. The transition from a single-tactic approach to a multi-tactic approach thus is dependent on the efficacy of deployment of available tactics, and their integration into a comprehensive program.