FINAL PROJECT REPORT
WTFRC Project Number: CH-16-102

Project Title: Integrated pest management of spotted wing drosophila in sweet cherry

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Other funding sources

Agency Name: **WSDA SCBG**
Amt. awarded: $237,908 (September 30, 2013 - September 29, 2016)
Notes: Research Intern and time slip is paid from this grant through Sept. 29, 2016; hence the WA request for 2016 is limited to 6 months for these two budget items.
Previous WTFRC SWD project for Beers was used as match for SCBG.

Total Project Funding: $203,420

WTFRC Collaborative Expenses: None

Budget 1 History (Beers):
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email:** arcgrants@wsu.edu/shelli.tompkins@wsu.edu

Item	2016	2017	2018	2019
Salaries[1]	16,042	32,085	33,368	0
Benefits[2]	6,192	12,385	12,880	0
Wages[3]	4,922	8,364	8,699	0
Benefits[4]	118	448	467	0
Equipment	0	0	0	0
Supplies[5]	5,000	1,000	1,000	0
Travel[6]	1,150	1,150	1,150	0
Miscellaneous	0	0	0	0
Plot Fees[7]	2,500	2,500	2,500	0
Total	$35,924	$57,932	$60,064	No Cost Extension

Footnotes: [1]Salaries 0.60 FTE Research Intern, [2]Benefits, Research Intern 38.6%; [3]Wages, student (summer) @$20.51/hr, 20 hrs/week x 12 weeks; Research Assistant $12/hr x 20 hr/week x 13 weeks; [4] Benefits student 2.4%, Research Assistant 10%; [5] Five whole tree cages @$800/cage (2016 only), SWD rearing supplies, traps and lures; [6]Travel to plots, $0.54/mile x 100 miles/year; [7]Plot fees. $1,000/acre x 2.5 acres for Sunrise 4 and Columbia View 14.

Budget 2 History (Shearer):

Item	2016	2017	2018	2019
Salaries[1]	0	0	0	0
Benefits[2]	0	0	0	0
Wages	31,320	0	0	0
Benefits	10,187	0	0	0
Equipment	0	0	0	0
Supplies[3]	3,411	0	0	0
Travel[4]	1,582	0	0	0
Plot Fees	3,000	0	0	0
Miscellaneous	0	0	0	0
Total	$49,500	0	0	0

Footnotes:

[1]Two Temp BSRT1, 6 mo ea, $15/hr, 3% raise each year

[2]Benefits: $850/mo

[3]Traps, lures, sampling equipment, insect rearing

[4]In state travel to research sites, $0.575/mile

Objectives:

1. *Test chemical control products to determine their ability to prevent infestation.* There is an ongoing need to determine efficacy of insecticides on SWD. Establishing the length of residual control, both for mortality and prevention of ovipositions, will aid in optimal timing and sequencing of products. New products must be screened to expand the selection of modes of action available for resistance management.
2. *Test chemical control products to determine ability to kill early stages of SWD in fruit.* If preventive sprays are not applied early enough, fruit infestation may occur under high pressure situations. Killing eggs or larvae in fruit will prevent the development of a complete generation of SWD that can attack nearby vulnerable crops (e.g., blueberry) or later maturing cultivars of cherry.
3. *Test provisional spray thresholds to determine initial and subsequent spray timing in commercial orchards.* In order for IPM to be implemented, action thresholds are necessary. Developing a sensitive and reliable monitoring tool is an essential first step in this process. Historic trap catch data coupled with records of infestation inform the need for insecticidal controls.

Significant Findings:

- Entrust and Delegate consistently provide long-term (up to 21 days) mortality of SWD
- GF-120 is still the most attractive/lethal attracticide tested, although new formulations may provide higher levels control in the field
- Some organic materials may provide overall control either by topical toxicity or oviposition deterrence, but lab studies need to be followed by field tests including phytotoxicity
- The spinosyns (Delegate and Entrust) have consistently provided the longest residual control of SWD, but the pyrethroids and diamides can play a role in resistance management
- The Scentry lure is the most attractive lure, but also has the most by-catch if used in a jar trap. Using this lure in combination with a species- and sex-specific trap (yellow sticky trap) may improve user-friendliness and ultimate adoption
- High pressure years for SWD can be predicted by the use of traps and help guide decisions on the intensity of control measures

Methods – *Field trial.* A field trial was performed in a research orchard to assess insecticide efficacy against SWD. Five insecticides were compared to an untreated check in a randomized complete block design, using single tree plots and rows as replicates. There were buffer rows and trees between the treated plots. Insecticides were applied a single time 2 weeks before harvest using an airblast sprayer calibrated to deliver 100 gpa. Four hundred fruit/replicate were collected and placed in plastic containers, and held at 72 °F for 10 days. The number of adult SWD that emerged from the fruit were recorded.

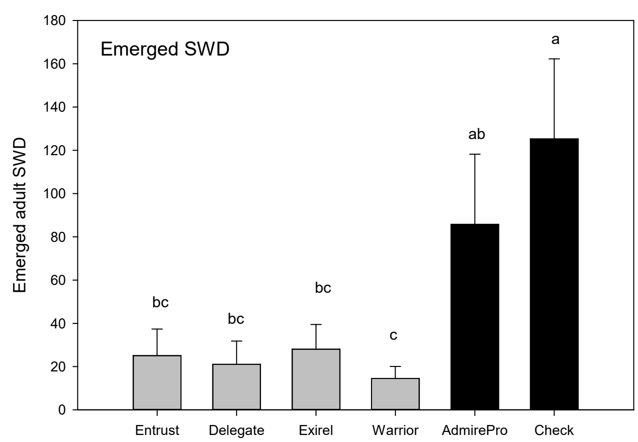

***Fig. 1**. Number of flies emerging from field-sprayed fruit.*

Results – *Field trial.* Fruit were infested to some degree prior to treatment, thus this was a test of both the insecticide's ability to kill pre-imaginal stages in fruit and prevent further infestation. The presence of buffers ensured a high level of pressure in this test. Entrust, Delegate, Exirel, and Warrior all provided similar levels of control. Admire Pro was not significantly different than the check (Fig. 1). This field test confirms observations in previous work that imidacloprid is a weak material against SWD, and the spinosyns, Entrust and Delegate, provide high levels of control. This is the first field trial of a diamide, and it performed better than expected based on field-aged residue bioassays. The Warrior treatment had the lowest number of SWD adults in this trial, confirming the high levels of activity of this pyrethroid.

Methods - *Field-aged residue bioassays.* A series of experiments was performed using similar methods to test the longevity and efficacy of insecticide residues. Insecticides were applied to a 1-acre block of 'Sweetheart'/Mazzard cherries at the WSU Sunrise orchard near Rock Island, WA. The trees were planted in 2007 at a 10 x 14 ft spacing. Replicates consisted of 2-3 trees per treatment, with sufficient fruit on the trees for the bioassay samples. Pesticide treatments were applied with an airblast sprayer calibrated to deliver 100 gpa.

Five replicates of undamaged fruit and leaves for the bioassays were collected 1, 3, 7, 14 and 21 DAT. The fruit and leaves were placed in paper bags and kept cool during transport to the laboratory. SWD used in the bioassay were from a laboratory colony originally collected from a cherry orchard in the fall of 2017. Flies were reared in 30-ml polystyrene vials with commercial *Drosophila* medium. The colony was reared in a controlled temperature room at 22 °C with a photoperiod of 16:8 L:D.

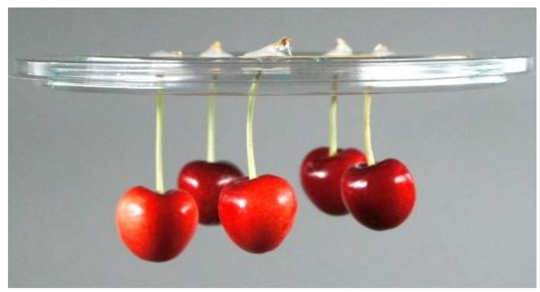

Fig. 1. Sweetheart cherries suspended from lid of bioassay arena.

The bioassay arena consisted of a 32-oz plastic container lined with treated cherry leaves held in place with staples, and a 1-oz plastic portion cup was glued to the bottom of the arena. Five cherries were suspended from the lid of the container by inserting the stem through slits in the top and securing them with hot-melt glue (Fig. 2); allowed females access to all surfaces or the fruit for resting or oviposition. A 10-mm diameter hole was cut in the lid and covered with surgical tape for ventilation. Ten mated female flies were anesthetized with CO_2 and introduced into the arenas. After 16 h, the lid with the suspended cherries was removed and replaced with a standard lid (with honey agar and ventilation). At that time, a second 1-oz cup containing *Drosophila* medium was placed inside the glued cup to provide a food and moisture source in the absence of cherries. After the cherries were removed, the oviposition punctures were counted and recorded. After 48 h, mortality of females was evaluated, after which the females and the original bioassay container with leaves was discarded. The lids with fruit were incubated at 22 °C for 16 d, and the number of emerged adults was recorded at this time. Mortality, oviposition and emergence data were analyzed using a mixed-model ANOVA and the Tukey-Kramer mean separation test.

Results - *Field-aged residue bioassay Trial 1.* Entrust caused 100% mortality through 14 DAT, and remained high at 21 DAT (Fig. 3). Fruit damage (ovipositions) were significantly lower than the check on 1, 3, and 14 DAT, and numerically lower on other dates. The total numbers of emerged flies (data not shown) was reduced commensurately with mortality and ovipositions, but the numbers of adults successfully emerging from the ovipositions was only occasionally different than the checks (3 and 21 DAT). The two rates of Cormoran behaved similarly, although the lower rate (21 fl oz) was not infrequently better than the higher rate (28 fl oz). Mortality was moderate on

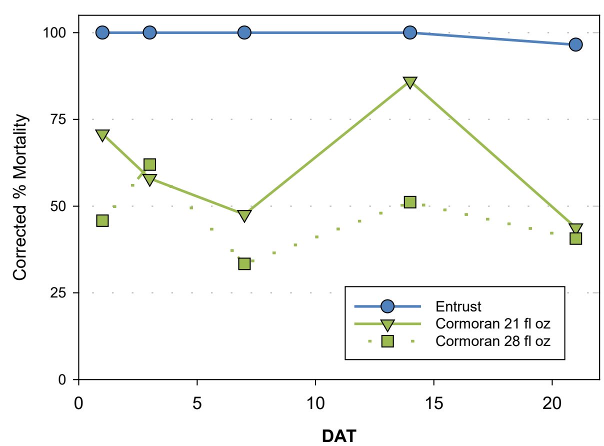

Fig. 2. SWD mortality from 1 to 21 days, Trial 1.

1 DAT, and while significantly higher than the check, it was also significantly lower than Entrust. Mortality in the Cormoran treatments continued higher than the check on 3 and 14 DAT, generally staying between 40 and 90%. Surviving females were able to oviposit normally, and ovipositions resulted in adult development, indicating no effects on egg hatch or larval development.

Results - *Field-aged residue bioassay Trial 2.* Delegate and Entrust caused high levels of mortality (90-100%) through 21 DAT (Fig. 4). Exirel (cyantraniliprole) and Minecto Pro (a mixture of cyantraniliprole and abamectin) caused high levels of mortality initially (3 DAT), which decreased (50-70%) at 7 and 14 DAT, and increased (>80%) again at 21 DAT. V-10433 (a formulation of sabadilla) at 8 fl oz caused ≈70% mortality at 3 DAT, but was low thereafter. This initial high level appears doubtful give that the higher rate (22 fl oz) was low throughout the test. Azera did not cause any mortality of SWD at any point in time. Ovipositions (a measure of fruit damage) were highest throughout the test period in the check V-10433 and Azera treatments, and lower in the Delegate, Entrust, Exirel, and Minecto Pro treatments (Fig. 5).

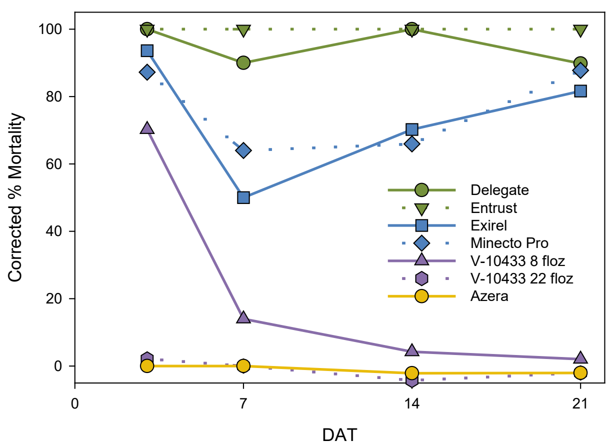

Fig. 4. SWD mortality from 3 to 21 days, Trial 2.

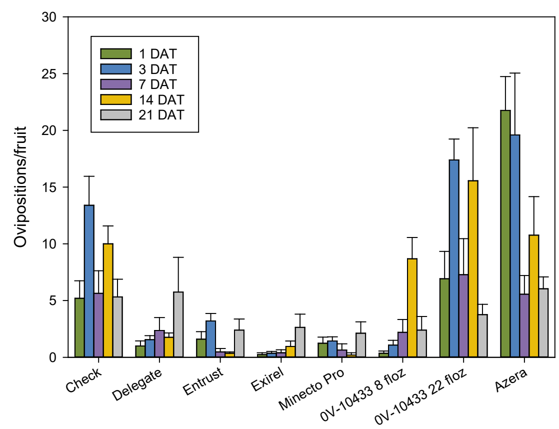

Fig. 5. Ovipositions per fruit, 1 to 21 DAT, Trial 2

Methods - *Laboratory bioassays.* Multiple experiments were conducted to determine if products either deterred female SWD from ovipositing, or killed eggs and larvae in fruit, thus preventing adult emergence. Five of these experiments tested the effects of two IGRs, Rimon and Dimilin, on SWD adults using different routes of exposure (topical, residual, ingestion). In these experiments, untreated leaves and fruit were collected from a research orchard, and treated with the two insecticides in the laboratory. The bioassay arenas were the same as described for the field-aged residue bioassays. For topical exposure, adults were anesthetized and transferred to a 14.7 ml plastic cup. Each group of SWD was sprayed with the specified rate of insecticide in a laboratory sprayer using 2 ml solution at 6.5 psi. Flies were transferred to prepared arenas with untreated leaves and fruit. For residual exposure, previously untreated cherry leaves were sprayed on a metal tray, and then stapled to arenas in groups of 3 leaves. Fruit suspended from arena lids were sprayed in a bucket sprayer with a laboratory mister, turning the lid ¼ turn after each spray in order to cover all fruit surfaces. Fruit and leaves were allowed to dry, and untreated adults transferred to the arena. For the ingestion treatments, the specified rates of the two insecticides were mixed in a 1:1 ratio with corn syrup. Twenty 25-µl droplets were applied to the leaves in the arena, and untreated adults transferred into it. Data collection and analysis of mortality, fruit damage, and adult emergence was the same as for the field-aged residue bioassays (above). In the first experiment, flies were 7 days old when treated; in the second experiment, they were 1 day old; and in the third experiment, they were 7 days old at the time of treatment, but a reduced rate of Rimon was used. In the fourth experiment, Rimon or Dimilin was incorporated into a semi-transparent agar medium where the females oviposited, allowing the eggs and larvae to be dissected out to determine stage of mortality. In the final experiment in this series, the two compounds were sprayed on the surface of the agar medium (versus incorporation).

Results - *Rimon/Dimilin results.* *Bioassay #1***.** Adult female mortality was low regardless of route of exposure, which is typical for IGRs. Oviposition level was quite variable, but lowest in the Rimon/Topical treatment. The most interesting result is the complete shut-down of adult emergence in the Dimilin/Residual treatment, despite the high levels of oviposition and only 16 hours of exposure. The exact mechanism for this shutdown (activity on eggs, larvae, or pupae) cannot be determined from this bioassay, but it represents the potential for an additional tool to suppress SWD populations and prevent fruit cullage. *Bioassay #2*. Both Rimon and Dimilin in the residual and ingestion treatments suppressed oviposition and completely shut down adult emergence when the flies were exposed at a young age. This experiment confirms the results of the first bioassay, which used the same treatments and methods except for the age of the flies at time of exposure (Fig. 6). The impact appears to be greater on flies exposed immediately after eclosion than those that are 7 days old. *Bioassay #3*. Fly mortality, ovipositions/fruit and emerged adults were not significantly different than the check in this test. The Dimilin/residual treatment had the lowest adult emergence, but due to variability, a statistical difference was not detected. This may be an indication that while the higher (Dimilin 16 fl oz/acre, Rimon 40 fl oz/acre) rates were effective, the lower rate of Rimon (24 fl oz) was not; in theory, Dimilin at the same rate should have performed similarly to the first bioassay. The results of the diet incorporation study were striking; egg hatch was greatly suppressed by both materials, and in the Rimon treatment, all of the resulting larvae died. The trend was similar where only the surface of the medium was sprayed, but less pronounced. Egg hatch was reduced by both materials, but only to 60-88%; however, larval mortality was high for both materials (80-90), with a net result of very few surviving larvae. This series of tests indicates that these two IGR may greatly reduce the ability of SWD to successfully reproduce in sweet cherry.

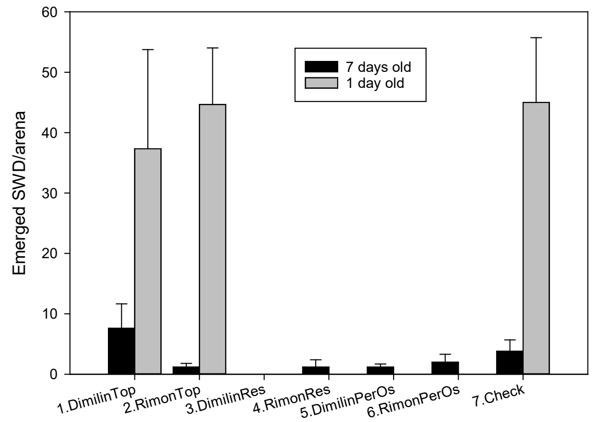

Fig. 6. *1 and 7 day old SWD emerging from fruit treated with Rimon or Dimilin (3 routes of exposure).*

Methods - *Bioassays of organic insecticides.* Additional bioassays were performed to determine if other pesticides (especially those registered for organic production) were effective against any stage of SWD. Contact bioassays were performed on 7-10 day old females in plastic Petri dishes, and sprayed with 2 ml of the solution at 15 psi. Residual control and oviposition deterrence bioassays were performed using the same bioassay arena as in previous experiments with untreated leaves, but with fruit sprayed with the candidate insecticides using a laboratory sprayer. The sprayed fruit were suspended from the lid of the bioassay arena, and 10 female SWD introduced. Mortality and ovipositions were assessed after 24 h, and adult emergence after 15 days. The ability of materials to kill eggs inside the fruit was tested by allowing females to oviposit and then spraying the fruit. A series of bioassays was performed with erythritol, a sugar alcohol known to be toxic to SWD. The first was a contact+residue bioassay (see above), where a food source (drosophila medium) was also contaminated; the second was incorporation in the larval diet (drosophila medium); the third type of bioassay incorporated erythritol into bait droplets, and measure adult mortality.

Results - *Bioassays of organic insecticides.* Cinnerate at all rates tested (25-40 fl oz/100 gal) does not appear to have any contact toxicity to SWD, but the contact-only bioassay is a severe test. When tested for oviposition deterrency, the 50 fl oz rate of Cinnerate had fewer ovipositions than the check, but only the standard (1.5% petroleum oil) was significantly lower. Similarly, Cinnerate did not kill eggs after they had been oviposited, but 1.5% petroleum oil reduced the number of adults emerging by over one-half (Fig. 7). In further tests of oviposition deterrence of organic materials, only Ecotrol (0.5%) and methyl benzoate (1%) reduced the numbers of ovipositions in relation to the check; petroleum oils had generally lower numbers of ovipositions, but differences were not statistically significant. Lavender oil residues caused high levels of mortality at 1-5% concentration; the 0.5% level caused a moderate amount of mortality. Conversely, topical applications of sabadilla caused little contact mortality to adult female SWD.

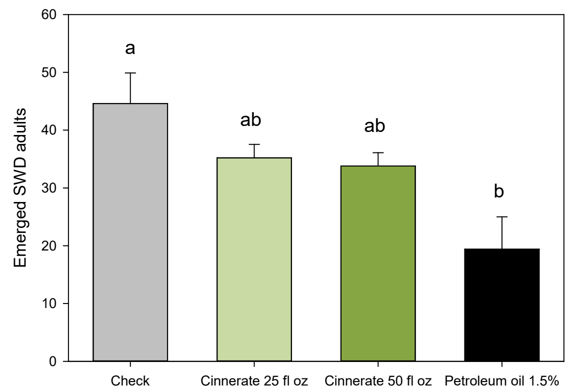

Fig. 7. *:Larvicidal activity of Cinnerate and oil for SWD.*

Erythritol sprayed on the adults (including exposure to residues in the arena and contaminated food) did not cause any mortality. Conversely, when erythritol was incorporated into the larval diet, there were very little survival at the two higher rates of erythritol (Fig. 8. Erythritol incorporated into bait droplets (Fig. 9) caused moderate amount of mortality at 1 DAT, but high levels at 4 DAT; however, there was correspondingly high levels of damage in the check, likely due to starvation. The sucrose standard treatments had the lowest levels of mortality (indicating an adequate nutrient source), with the erythritol+molasses treatments intermediate.

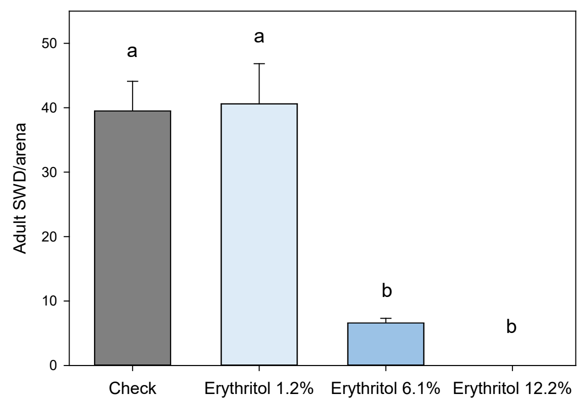

Fig. 8. *Survival of SWD larvae in drosophila medium with various concentrations of erythritol added.*

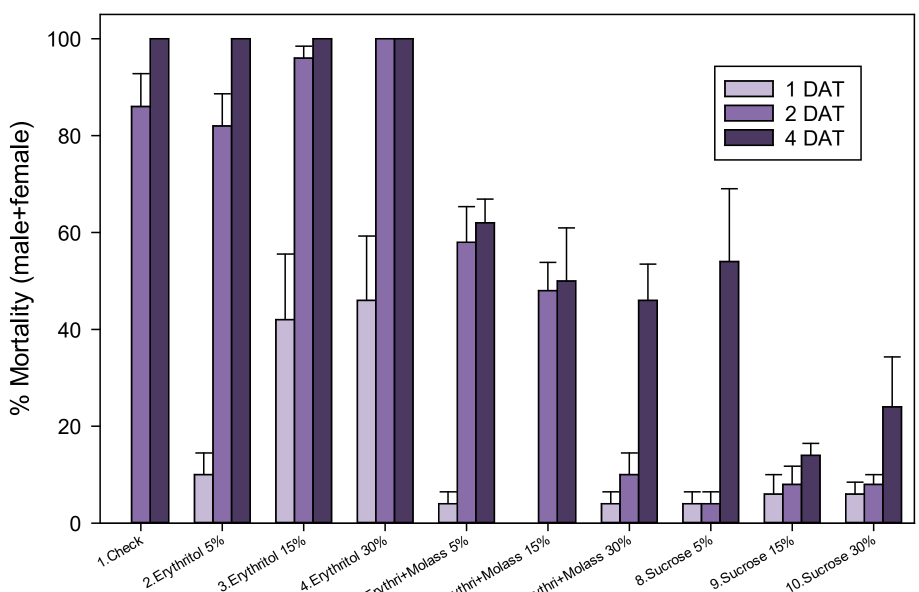

Fig. 9. *Mortality of SWD adults exposed to droplets of erythritol or sucrose.*

Methods - *Attracticides.* One category of insecticidal control, attracticides, was pursued in hopes of reducing the non-target impacts of full canopy sprays. A candidate material made by Scentry consisted of an attractant only, to which different insecticides might be added for resistance management schemes. Initial tests were done in laboratory assays, examining fly mortality when exposed to bait droplets in 1-liter arenas. Further tests were conducted in a field-aged residue bioassay where bait+insecticide droplets applied to leaves in a research cherry orchard, then collected at intervals to determine attractiveness and lethality. In all cases, the toxicant added to the Scentry attractant was spinosad (Entrust), and the comparison material used was GF-120 (which also has spinosad as the toxicant). In addition, control with attracticides was tested in whole-tree field cages, where known numbers of SWD adults could be added to control the amount of insect pressure. This trial compared an airblast application of Entrust to GF-120 bait spray.

Results – *Attracticides*. *Laboratory bioassays.* Neither of the attracticides caused a significant amount of mortality after 6 h, but levels increased at 24 and 48 h. After 24 h, the GF-120 treatment had significantly more mortality than the Scentry attracticide treatment, but they were not different at 48 h (Fig. 10). When field-aged residues were tested, the Scentry attracticide had lower mortality initially than GF-120, and decreased rapidly thereafter (Fig. 11). Overall, the goal of developing an attracticide that is more effective than GF-120 has not been realized. *Field cage trials*. Damage was low overall, and while not significantly different from the check, fruit damage was numerically lower in both the Entrust and GF-120 treatments (Fig. 12).

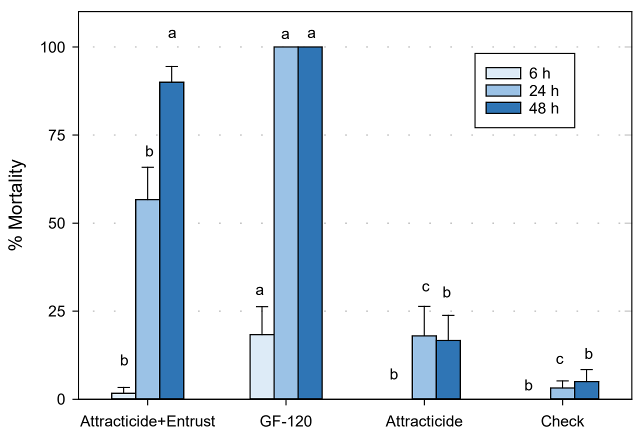

Fig. 10. *Percent mortality of female SWD following exposure to attracticides.*

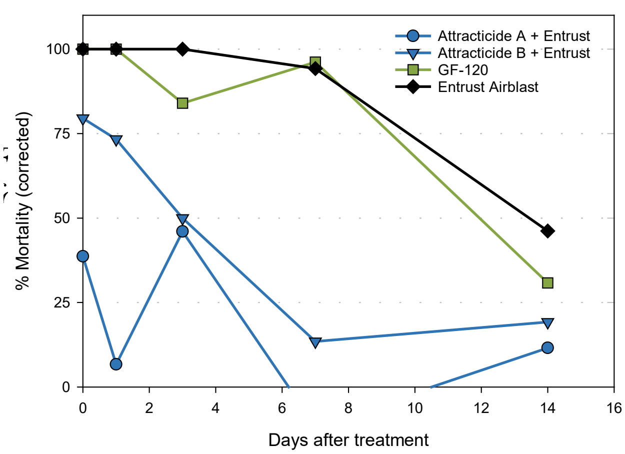

Fig. 11. *SWD mortality from 1 to 14 days, following exposure to attracticide.*

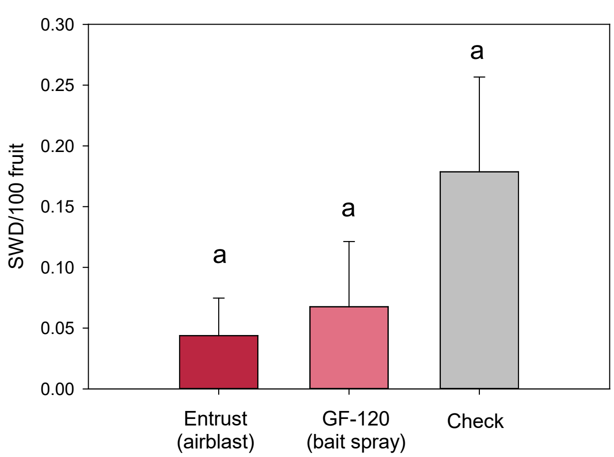

Fig. 12. *Fruit infestation by SWD following exposure to Entrust or GF-120.*

Monitoring. The efficacy of traps and lures were tested over a 4-year period, and provide insight into the most sensitive monitoring tool for SWD. Early trap and bait systems were adequate to detect range expansion, but monitoring for action thresholds will require a trap that is sensitive and (if possible) user friendly. The Scentry lure has consistently provided the highest trap captures of SWD, whether during low density periods (cherry maturation period) or higher densities (fall post-harvest period). This lure also has the highest levels of by-catch, which is problematic with drosophilids, which are small and similar in appearance. Only the spots on the wings of the males distinguishes SWD from non-pest species, but the females (the damaging stage) are difficult to differentiate. The liquid-based traps overall provide the highest level of catch, but the poorest level of user-friendliness. Specifically, the in-house fabricated PBJ trap (Fig. 13) consistently captures the most SWD; the Scentry trap (of the commercial traps) has also performed well. Sticky traps offer an easy-to-use alternative to the liquid traps, but their primary limitation is that while males are relatively easy to identify, the females are more problematic in field counts. Of the sticky traps, the yellow card has a slight edge over other colors. An additional unresolved problem is the preponderance of females in the population in the early season, when spray decisions for cherries are usually made. Whether male capture on these traps is sufficiently sensitive to be used as an action threshold remains to be determined.

Fig. 13. *Clockwise from left: PBJ trap, yellow sticky card, and male SWD on a sticky trap with wing spots clearly visible.*

Regional trends. SWD traps were deployed in orchards in North Central Washington from 2012 to 2019 in the same locations each year to track year-to-year variation in trap capture (Fig. 14). Starting in 2013, we coordinated sample collection and rearing with the WSDA cherry packinghouse inspection program to rear larval *Drosophila* species and determine if they were SWD (Fig. 15). The addition of PCR diagnostic tools in 2015 and 2016 allowed us to identify most of the specimens that died during rearing, and failed to produce an identifiable adult. The packinghouse sample information provides valuable insights into the variation in insect pressure from year to year, and the relevance of cherry season SWD trapping to regional reports of damage. While there many factors (including weather interfering with insecticide applications) that influence the efficacy of control measures, it is clear that high trap numbers in the summer of 2015 corresponded with high numbers of SWD finds in the packinghouse. The packinghouse finds were similar from 2016 to 2018, with an uptick in 2019; this was the second worst year for SWD infestations since 2015. In general, high pressure can be predicted as early as May in order to intensify control measures; however, the lure shortage in 2019 meant no early season data were available to predict the higher risk of damage in 2019.

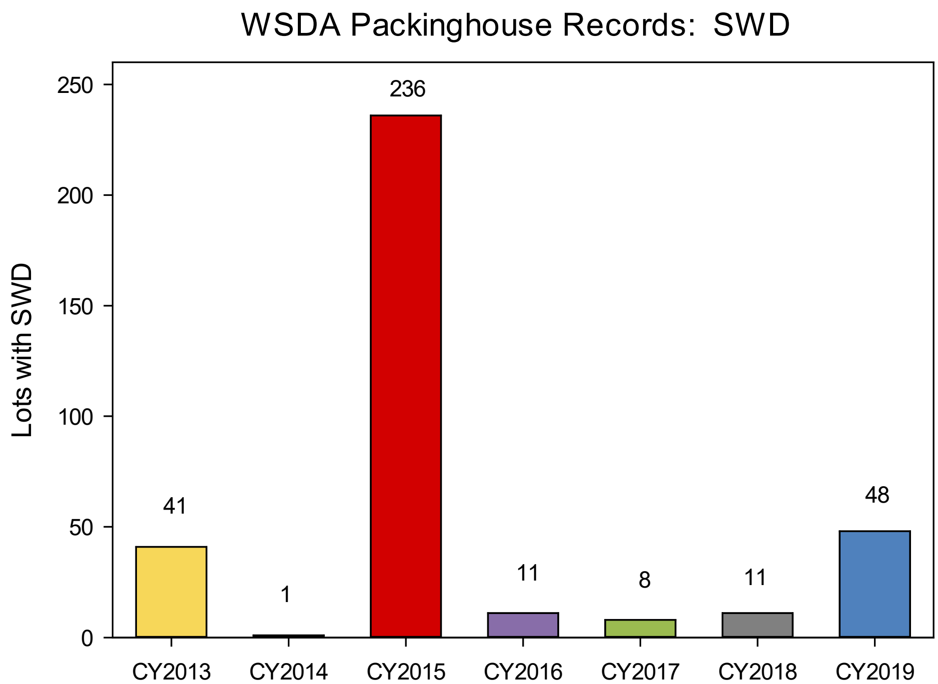

Fig. 14. *WSDA larval SWD detections in packinghouses, 2013-2019.*

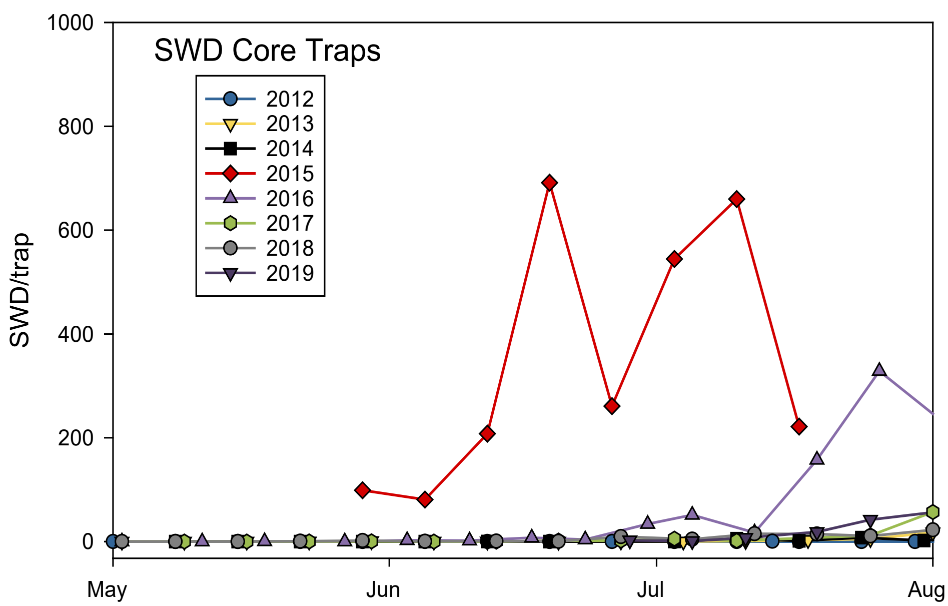

Fig. 15. SWD trap captures during cherry maturation and harvest, 2012-2019.

Executive Summary

Project Title: Integrated pest management of spotted wing drosophila in sweet cherry

Keywords: spotted wing drosophila, *Drosophila suzukii*, attracticide, oviposition deterrent

The first detection of spotted wing drosophila in eastern Washington caused a sudden shift in the insect management programs of sweet cherry growers in the region. While populations and damage levels have varied from year to year, this pest continues to be a challenge to cherry growers. Early research focused on assessment of chemical control options, and the cherry industry has picked 2-3 materials that give consistent results. Unfortunately, several are in the same chemical class, spinosyns, and warning were issued about the possibility of resistance. A previous project set baselines for some of the primary active ingredients, and can be used to track incipient resistance in the future. In the meantime, new techniques and approaches are needed to address the single-tactic approach to control.

On the research side, a continuing challenge is the unpredictability of this pest's appearance in prospective plots, and the reluctance of producers to risk part of a valuable cherry crop. WSU's two research orchards have had a testable population in only one year since 2010, viz., the high-pressure year of 2015. While laboratory or field-lab residue tests have help fill in the details of the characteristics of the candidate insecticides, field-level testing is necessary in order to validate these findings.

This project focused on alternatives to the standard adulticidal insecticides. The impetus for this arose from the high level of fruit infestation in 2015: if eggs or larvae are already present, how can I kill them? While less desirable than preventing infestation, adverse weather conditions or unusually high overwintering survival may result in some level of infestation. Many of the same materials that are used as adulticides also kill larvae developing the fruit. Laboratory tests have demonstrated there are two IGRs (Rimon and Dimilin) which cause little adult mortality, but cause a high level of egg and larval mortality. These may provide rotational materials for the currently used adulticides.

Another approach that was explore was the use oviposition deterrents. In theory, because the compound does not kill the female spotted wing drosophila, there should be less selection of physiological resistance. Many of the compounds screened are essential oil products (lavender, rosemary, cinnamon, garlic) and thus suitable for use in organic production. Erythritol shows promise as a toxicant for use in a bait spray, and should be tested further. The need for rotational materials in organic sweet cherries is acute, give the dominance of spinosad use in these orchards. The need is even more urgent because the same active ingredient is used in both organic and conventional orchards, or effectively industry-wide. Resistance to spinosad has already been documented from California, and may spread to other growing regions.

Finally, we have explored the possibility of a replacement for GF-120 that is engineered especially for spotted wing drosophila versus western cherry fruit fly, the original target. To date the candidate compounds have been less attractive than GF-120 in laboratory test, which only provided about 50% suppression in the field. Further research in collaboration with private companies may yield progress in the future.