CONTINUING PROJECT PROPOSAL **YEAR**: 2 of 3
WTFRC Project Number: ST-16-100

Project Title: Spotted wing drosophila management in stone fruit

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Cooperators: stone fruit growers

Total Project Request: **Year 1: $**17,658 **Year 2:** $33,667 **Year 3: $**34,900

Other funding sources: None

Budget 1
Organization: WSU **Contract Administrator:** Joni Cartwright, Carrie Johnston
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Item	2016	2017	2018
Salaries[1]	10,695	22,245	23,135
Benefits[2]	4,128	8,587	8,930
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[3]	1,000	1,000	1,000
Travel[4]	1,835	1,835	1,835
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	17,658	**33,667**	34,900

Footnotes: [1]Salaries 0.40 FTE Research Intern, [2]Benefits, Research Intern 38.6%; [3]SWD rearing supplies, traps and lures, office supplies/electronics; [4]Travel to plots, $0.575/mile x 3,192 miles/year.

Objectives:

1. Determine skin penetration force and flesh firmness levels necessary to allow SWD oviposition
2. Test the use of synthetic lures to predict damage by SWD
3. Determine the number of traps per unit area needed to provide accurate prediction of damage risk.

Significant Findings:

- SWD contaminated with brown rot spores caused infection in ripe and unripe nectarines; the mechanism appears to that of contamination of body parts rather than oviposition wounds
- SWD oviposited in, and emerged from, ripening nectarines at very low levels compared to a known susceptible host, sweet cherry
- Fruit skin penetration and flesh firmness of nectarine decreased as fruit became more mature, but these parameters were unrelated to the ability of SWD to attack the fruit
- On a per-trap basis, the yellow sticky panels at a low trapping density captured more males per trap than those at higher densities, with the liquid traps catching the fewest
- Damage due to SWD was found in harvest samples, but only one male SWD emerged from a damaged fruit out of 12,000 fruit examined.
- While the attack rate by SWD appears to be very low, the association with brown rot requires more investigation

Obj. 1. Determine skin penetration force and flesh firmness levels necessary to allow SWD oviposition

Methods:

Previous research has shown that peaches and nectarines are low risk host crops for SWD. However, this insect can oviposit in them if they are over-mature; thus there must be a point in fruit development when they become susceptible. We will determine where along this continuum this point lies in terms of fruit maturity characteristics.

Fruit maturity was measured for the 2nd year on a sample of 10 fruit/block on 18 dates (31 July to 29 September, 2017). Fruit maturity measurement included weight, flesh firmness, and skin penetration force (Plate 1). On two dates during harvest (first and last pick), an additional 10 fruit were used to bioassay the ability of female SWD from a lab culture to oviposit and successfully develop to the adult stage. Fruit was picked in the morning, transferred directly into individual plastic containers, and exposed to females the same day. Mated female SWD (five per arena, 10 days old) were deprived of an oviposition substrate for 24 h, then exposed to a single nectarine fruit for 16 h. For comparison, a known susceptible host (sweet cherry) was assayed at the same time, using 12-14 cherries to provide an equivalent weight to the single nectarine. A few replicates of Drosophila mediums were also tested to see if these would provide an acceptable surrogate for fruit during the winter months (data not shown) (Plate 2). At the end of the exposure period, females were removed, and the fruit was examined for oviposition punctures with breathing filaments (internal egg deposition) or eggs laid on the surface of the fruit (external oviposition); the latter is an indication of poorer host acceptance.

Plate 1. Fruit texture analyzer used to measure skin penetration force

Based on discussions in the fall of 2016, three additional bioassays were conducted to assess the relationship between SWD and brown rot. The bioassay arenas consisted of a plastic container with a single nectarine in a small cup for support. Fruit were exposed to flies or sprayed with a brown rot spore suspension and incubated as above. Fruit was rated at intervals after treatment as either with or without brown rot, and expressing the results as the proportion of infected fruit. The treatments consisted of permutations of artificially wounded or unwounded fruit, and fruit exposed to SWD with or without previous exposure to brown rot spores. Fruit from the field was surface sterilized, and placed in sealed, ventilated individual containers under a clean hood to avoid accidental introduction of brown rot. The adult flies were exposed by anesthetizing them and placing them on an agar plate with sporulation brown rot (or in the second bioassay, sporulating fruit), so that they were contaminated with spores (Plate 3). Both ripe and unripe fruit were tested. To test the hypothesis that oviposition was necessary for infection, we compared male to female SWD; because males cannot oviposit or wound the fruit, then infection must come from contamination only (the 'dirty feet' hypothesis).

Plate 2. Laboratory bioassay with SWD comparing nectarines, cherries and Drosophila medium

Plate 3. SWD females on agar plate of sporulating brown rot

Results and Discussion:

Oviposition Bioassays. Both skin penetration force and flesh firmness (data not shown) decreased over time as the fruit matured (Fig. 2) in all three orchards. In general, skin hardness was highest on the bottom and lowest on the top, with little difference between the blush and shade sides. Oviposition in nectarine fruits was negligible on both lab bioassay dates (Fig. 3), ranging from 0 to 1.2 ovipositions/fruit. The majority of the ovipositions in nectarine were external (71%) (Plate 4), compared to 4% in the known susceptible host, sweet cherry. Total

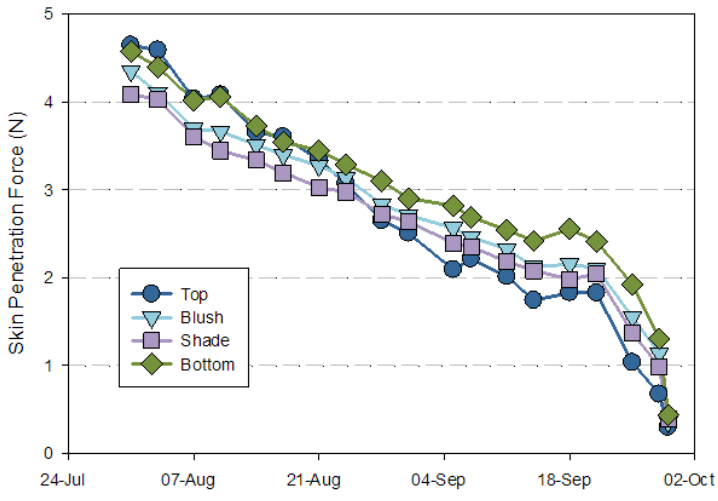


Fig. 2. Skin penetration force at each sampling date, 2017.

ovipositions in cherries were variable, but about 24-fold higher overall than in nectarines (16.1 ovipositions/arena vs. 0.7 ovipositions/arena).

Brown Rot Association. In the first two bioassays (which had similar treatments, but with either ripe or unripe fruit), any fruit exposed to brown rot spores developed brown rot, regardless of wounding (artificial or putative SWD oviposition) or method of contamination (sprayed with a spore suspension, or exposed to SWD contaminated with spores) (Fig. 4, Plate 5). Fruit sprayed with a spore suspension developed brown rot rapidly, and covered the entire fruit surface. As expected, wounded or unwounded fruit sprayed with distilled water did not develop brown rot, nor did fruit exposed to female SWD which were taken directly from the colony, and not exposed to brown rot. The only intermediate case was that of females exposed to either spores on an agar plate or a sporulation fruit, where a proportion of the fruit developed brown rot. *These results indicate that brown rot infection by SWD is possible given the right conditions*.

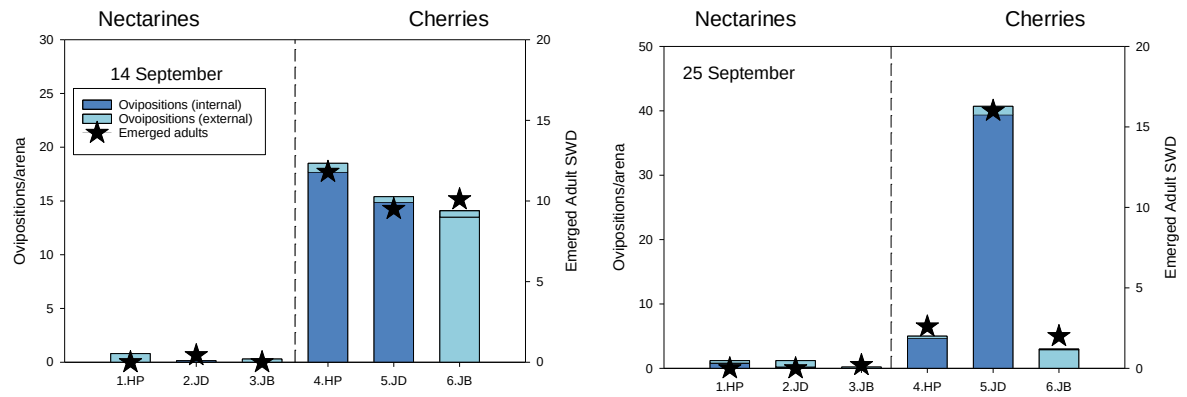


Fig. 3. Ovipositions in nectarines and cherries, 2017

Plate 4. External SWD oviposition on nectarine.

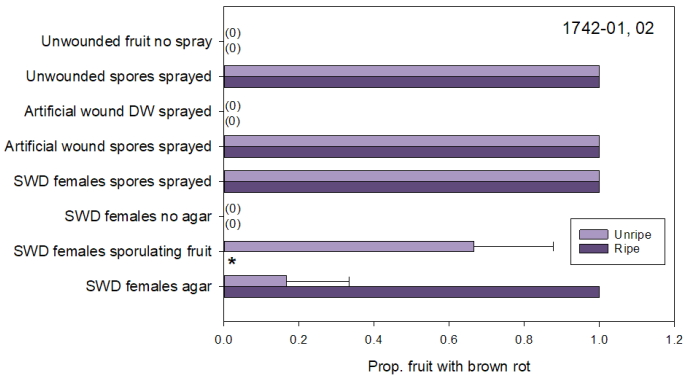
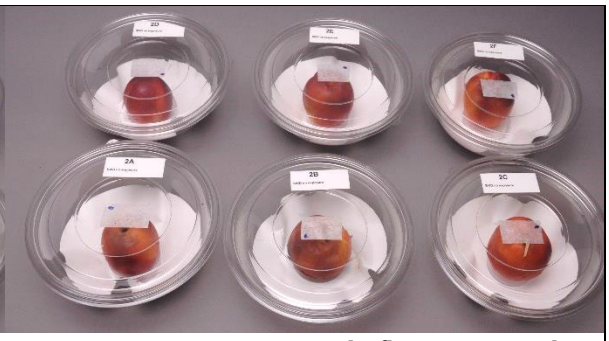


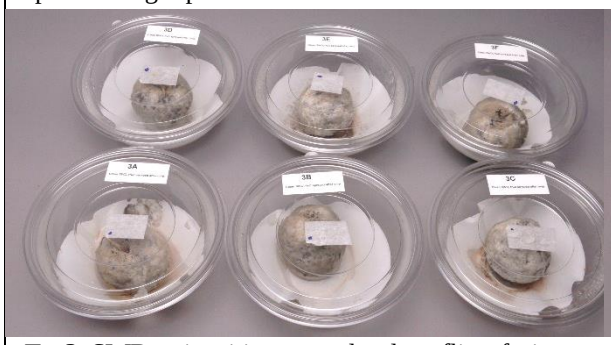
Fig 4. Proportion of fruits with brown rot after exposure to spore suspensions or SWD (* no ripe fruit in the SWD females sporulating fruit treatment)

In the third bioassay, we used very ripe fruit late in the season, comparing male v female SWD to test the dirty feet hypothesis. Both sexes caused contamination of a proportion of the fruit, although less than the positive control (artificial wound + spore suspension), in which all fruit developed brown rot (Fig. 5). However, in this test the negative control also developed brown rot, indication that contamination in the field may have already occurred, and surface sterilization was insufficient to eradicate it.

Trt 1.SWD oviposition wound - flies exposed to spores on agar plate

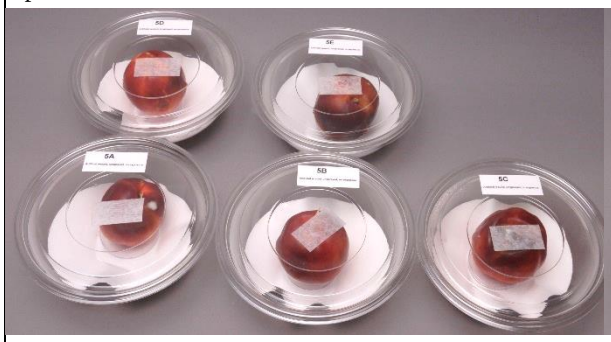


Trt 2. SWD oviposition wound - flies not exposed to brown rot

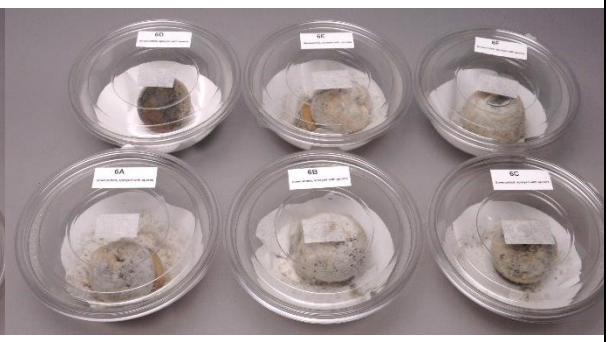


Trt 3. SWD oviposition wound - clean flies, fruit sprayed with spore suspension after oviposition period

Trt 4. Artificial wound - sprayed with spore suspension



Trt 5. Artificial wound - sprayed with distilled water, no exposure to brown rot



Trt 6. Unwounded fruit - sprayed with spore suspension

Trt 7. Unwounded fruit - no exposure to brown rot

Plate 5. Incidence of brown rot after exposure to spore suspensions or contaminated/uncontaminated SWD.

Obj. 2. Test the use of synthetic lures to predict damage by SWD

Methods:

The first synthetic lure was available for testing in 2013, based on the Cha-Landolt blend of acetic acid, ethanol, methionol, and acetoin. Three commercial lures are now available, generally providing higher capture than apple cider vinegar. Several seasons of tests indicate that the Scentry lure consistently captures more SWD, and thus offers the best opportunity for early detection of adult activity in an orchard, and to base a spray threshold on trap capture.

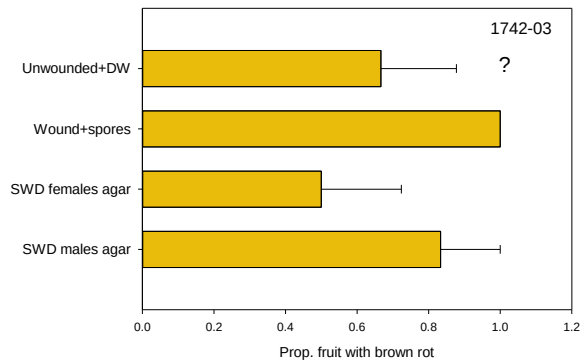


Fig. 5. Proportion of fruits with brown rot after exposure to spore suspensions or SWD

The use of traps for spray thresholds was tested in three nectarine orchards, cv 'Summer Blush' in eastern Washington in August-September of 2017. Traps were deployed in late July and checked twice weekly beginning through the harvest period at the end of September. A 1-acre section of trees was designated as the study area, and six traps were deployed near the center (3 per row with one buffer row between) (Fig. 6). Three of the traps were a liquid-based jar trap (Scentry trap) baited with the Scentry synthetic lure. The drowning fluid was 300 ml of water with a surfactant (liquid dish soap) and a preservative (sodium benzoate) added. The second set of 3 traps was Scentry lures backfolded in AlphaScents yellow sticky traps (Plate 6). The drowning fluid in the liquid traps was collected and replaced at each visit, and the contents counted in the laboratory with the aid of a microscope. The AlphaScents sticky traps were counted *in situ*, scanning only for males, which were removed after counting. The trap positions were rotated between rows at each visit. A provisional threshold of five SWD in any of the six traps per block was the trigger to begin protective pesticide applications, to be continued through harvest at 7-10 day intervals at the grower's discretion.

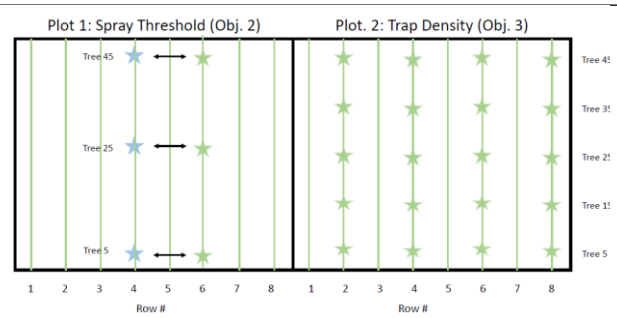


Fig. 6. Plot layout schematic showing trap position (1 acre plots, 'Summer Blush' nectarine).

Scentry Lure | Scentry Trap | AlphaScents Yellow Sticky Card

Plate 6. Lure and traps used in the stone fruit study

The success of the threshold was determined by examining *in situ* 1,000 fruit in each plot. All damaged fruit were collected and returned to the lab to rear out any arthropods found in the fruit.

Results and Discussion:

Two fruit damage assessments were made (1,000 fruit/plot) on the dates of the first and last pick (14 and 25 September for HP and JB, or 18 and 28 September, 2017 for JD) in the six study plots. Fruit were examined *in situ*, and damaged fruit were picked and returned to the lab for rear out any insects present. A total of 55 damaged fruit were found in the first pick sample; however, no SWD or other *Drosophila* were found in the incubated fruit. A total of 14 damaged fruit were found on the last pick sample. A single male SWD was reared out form a fruit from JD, plot 2.

Obj. 3. Determine the number of traps per unit area needed to provide accurate prediction of damage risk

Methods:

Little is known about the source of SWD occurring in blocks, specifically whether the major source comes from habitat surrounding the block, or from within the block itself. This makes the number and position of traps used for action thresholds difficult to determine. Observations to date indicate that the older ACV traps have a limited range of attraction, but newer lures are untested.

To address this question, the same blocks used in Obj. 2 were used, locating a second 1-acre plot next to the Obj. 2 plot (Fig. 6). In contrast to the low trap density used in Obj. 2, and the second plot had a high trap density, using only the Scentry lure/yellow AlphaScents sticky trap combination. Traps were laid out in a grid pattern throughout the block, 5 traps in each of 4 rows, or 20 traps per 1-acre plot. Traps were checked twice weekly *in situ*, without changing the lure or trap, and removing males after counting. The same threshold of five SWD (males) in any trap used in Obj. 2 was used, as well as the same method of determining success of the threshold.

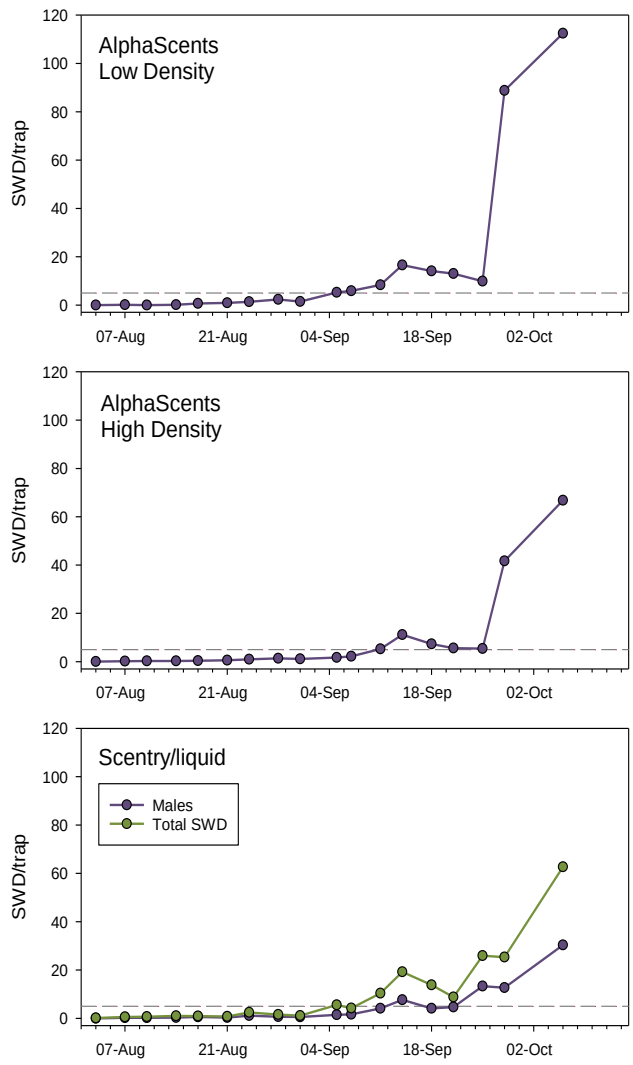


Fig. 7. SWD caught in two trap types and trapping densities, 2017

Results and Discussion:

The low density yellow traps caught the most male SWD (mean=15.6), followed by the high density yellow traps (mean=8.45) and the liquid traps (mean=4.6) (Fig. 7). This is similar to tests in cherry, where sticky traps have been male biased, but the reverse of what occurred in this study in 2016. Also unlike 2016, there were almost twice the number of males caught in the low density as the high density sticky traps. Trap captures of males were below 5/trap until the first half of September, then increased to about 30-112/trap by the end of the study in early October. Both the high and low density yellow sticky traps reached a threshold of 5/trap (average) a week or two before harvest, depending on the orchard.

Given the extremely low attack rate in laboratory bioassays and emergence from damaged fruit in the field, the threshold may still be too conservative if only SWD damage is considered. However, until the association with brown rot is more thoroughly explored, maintaining preventative sprays for both SWD and brown rot seems prudent.