2019 Stone Fruit Research Review

Tuesday, November 20, 2018

WSU-Prosser, large conference room

Time	PI	Project Title	Funding period
10:00	Doornink	**Introduction**	
10:15	Beers	Spotted wing drosophila management in stone fruit: ***No-cost extension***	16-18

CONTINUING PROJECT PROPOSAL **YEAR**: 3 of 3 (No-Cost Extension)
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:** Katy Roberts/Kim Rains
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Item	2016	2017	2018	2019
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	**$0**

Footnotes: A no-cost extension is requested for 2019.

Objectives:

1. Determine skin penetration force and flesh firmness levels necessary to allow SWD oviposition (completed years 1 & 2)
2. Test the use of synthetic lures to predict damage by SWD (completed years 1 & 2)
3. Determine the number of traps per unit area needed to provide accurate prediction of damage risk (completed years 1 & 2)
4. Investigate the probability that brown rot could be transferred to a healthy fruit by a brown-rot contaminated SWD under field conditions (new objective for 2018)

Significant Findings:

- No fruit exposed to field-collected SWD developed brown rot
- Only 20% of those fruit exposed to lab-contaminated SWD developed brown rot
- A media selective for brown rot did not allow growth of this pathogen, and thus was not usable as a proxy for fruit

Fig. 1. Male SWD.

This experiment was the third year of experiments carried out on the effect of spotted-wing drosophila (SWD, Fig. 1) on non-cherry stone fruit, specifically nectarines. Previous field and laboratory work done in Washington (2012-2014); and California indicated that non-cherry stone fruit are at low risk from SWD, when harvested 'firm-ripe'. In three years of sampling peaches, apricots, and nectarines, no field infestation occurred during the preharvest or harvest period. Nectarines were the most susceptible crop in laboratory bioassays, when female SWD were caged with fruit. Oviposition occurred at low levels on uninjured fruit, but was generally higher on fruit with some type of injury that broke the skin. Similarly, fruit became more susceptible as it became more mature. Maturity is coincident with a number of changes, but softening of the flesh and reduction in skin penetration force are two of the changes that may affect ovipositional cues and/or success by SWD. These characteristics vary by cultivar, and thus conclusions drawn from one cultivar may not apply to all cultivars. In addition, SWD densities increase in the late summer and fall, and it is probable that later-maturing cultivars are at greater risk of SWD damage, regardless of skin/flesh characteristics.

This experiment was conducted because of a reported heavy infestation of SWD in late-maturing nectarines in 2013 in this growing region. It was investigated by Doug Walsh, and although it appeared SWD emerged from some of the fruit samples, he was unable to draw any firm conclusions regarding whether undamaged fruit had been successfully attacked. The growers also noted that 2013 had been a high pressure year for brown rot, and blamed SWD as promoting the infection. The experiments performed in 2016 were to establish whether SWD could attack this cultivar of nectarines, despite previous research to the contrary. The 2017 experiment continued the bioassays of undamaged fruit, and started preliminary experiments on the relationship of SWD and brown rot.

The 2017 experiments confirmed previous experiments that undamaged fruit were not susceptible to attack by SWD. However, additional experiments established that brown rot could be transferred to a healthy fruit by a brown-rot contaminated SWD. The primary purpose of the 2018 experiments was to establish the probability of such a transfer occurring under field conditions.

Experiment 1 *Methods*:
We carried out a preliminary study using 10 male (Fig. 1) and 10 female SWD taken from a lab colony collected near Mattawa in 2017 that were exposed to a sporulating culture of brown rot (Fig. 2). After approx. one minute we removed the flies from the culture and then confined them for 24 h in 100 × 15 mm Petri dishes, one fly per dish, containing a selective media or in Petri dishes containing standard potato dextrose agar media. After 24 h, we removed the flies from the Petri dishes, evaluating mortality as we did so, and incubated the media for 7 days at 23 ºC (73 ºF) before evaluating for brown rot colony development.

Fig. 2. SWD on a plate of brown rot.

Experiment 1 *Results*:
All of the flies in contact with standard media produced brown rot colonies, while none of the flies on the selective media did so (Table 1). The expected result was that both media types would produce brown rot; clearly, there is some component of the selective media preventing growth. Unfortunately, due to time constraints and harvest schedules, it was not possible to wait for the results of the preliminary study before carrying out the remaining field and laboratory studies also involving the use of the selective media. The inability of the selective media to support the growth of brown compromised the results of all the experiments that used it.

Table 1. Infection with brown rot following exposure to contaminated SWD

Trt	Treatment	n	Mean colonies/dish
1	Selective media	10	0.0
2	Standard media	10	1.0

Experiment 2 *Methods*:
The purpose of this experiment was to determine what proportion of the flies collected from the orchard were contaminated with brown rot spores, and therefore potentially capable of transmitting them to fruit. We used selective media as a proxy for the fruit in this experiment.

We collected SWD and other drosophila using Trappit Dome traps baited with SWD lures (Fig. 3). The traps were provisioned with 15 ml cups containing drosophila rearing media to provide a food and water source. The traps also contained a 15 ml cup containing selective media destined for Exp. 4. We deployed 10 traps in each of the three Mesa trial orchards (30 total traps) taking part in this study. We also deployed 10 traps at each site that contained cups of selective media only (no lures), and had the entry hole to the trap cover with a porous tape to prevent the ingress of insects but still allow air flow. These traps were also used for Exp. 4.

Fig. 3. Trappit dome in shaded tree canopy.

The traps remained in the orchards for a period of 24 h, we then anesthetized the insects collected using CO_2 and brought them back to the lab (Fig. 4). In the lab, we confined individual insects from the collections (keeping the catch separated by orchard) in Petri dishes containing selective media. After 24 h confinement we removed the insects from the dishes, evaluating mortality as we did so, and incubated the media for 7 days at 23 ºC (73 ºF) before evaluating for brown rot colony development.

Fig. 4. Gas cylinder in back of car, anesthetizing the flies with CO_2

Experiment 2 *Results*:
None of the treatments produced brown rot in this experiment, although Trt. 3 was designed as a positive control that should have produced colonies (Table 2). Without the expected results from the positive control, no confidence may be placed in the results from the remaining treatments.

Table 2. Infectivity of field-collected flies on brown-rot selective media

Orchard	Trt	Fly Source	Media type	n reps	% brown rot development
Orchard 1	1	Field SWD	Selective	10	0
	2	Field other	Selective	10	0
	3	Lab contaminated*	Selective	10	0
	4	Lab uncontaminated	Selective	10	0
	5	No flies	Selective	10	0
Orchard 2	1	Field SWD	Selective	10	0
	2	Field other	Selective	10	0
	3	Lab contaminated*	Selective	10	0
	4	Lab uncontaminated	Selective	10	0
	5	No flies	Selective	10	0
Orchard 3	1	Field SWD	Selective	10	0
	2	Field other	Selective	10	0
	3	Lab contaminated*	Selective	10	0
	4	Lab uncontaminated	Selective	10	0
	5	No flies	Selective	10	0

*Positive control; lab flies were all females.

Experiment 3 *Methods*:
For this experiment, we used field-collected flies captured using Trappit Domes, and nectarines, cv. 'Summer Blush', collected from Orchard 1 on the day that peak harvest was happening. We collected 20 fruit for quality assessment and an additional 30 that were surface-sterilized in the lab by immersing for 5 minutes in a 1% solution of bleach before air-drying. We placed individual fruit in 32 oz salad bowl arenas (Fig. 5) and introduced one fly per arena. After 24 h confinement we

removed the insects from the arenas, evaluating mortality as we did so, and incubated the fruit for 7 days at 23 ºC (73 ºF) before evaluating for brown rot colony development.

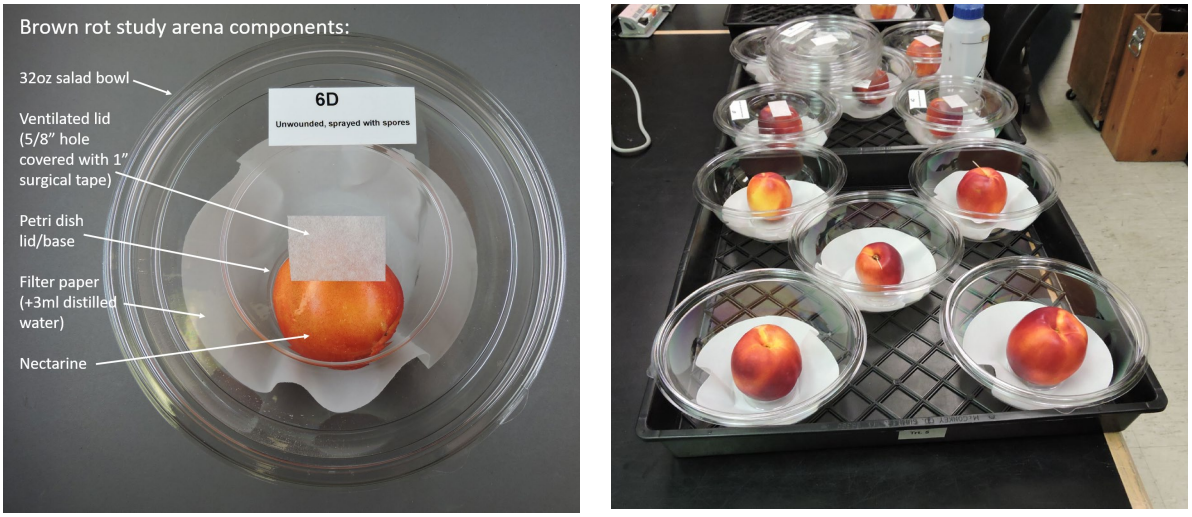

Fig. 5. Nectarines in salad bowl arenas.

Experiment 3 *Results*:
Because no selective media was used in this experiment, this is the sole one whose results may be considered valid. None of the field-collected flies (test treatment) or uncontaminated lab flies (negative control) caused a brown rot infection of nectarines held in the lab (Table 3). However, under the test conditions, only 20% of replicates using deliberately contaminated flies (positive control) caused brown rot (Fig. 6), indicating a (potential) low but measurable rate of transfer. By comparison, in previous experiments 100% of ripe fruit and 20% of unripe fruit was infected by lab-contaminated females. A further evaluation at 16 days (24 Sep) showed a 50% infection rate in Trt. 2 fruit, possibly linked to maturity/senescence. No flies emerged from any treatment, and no brown rot had occurred in Trts. 1 and 2 at 16 days.

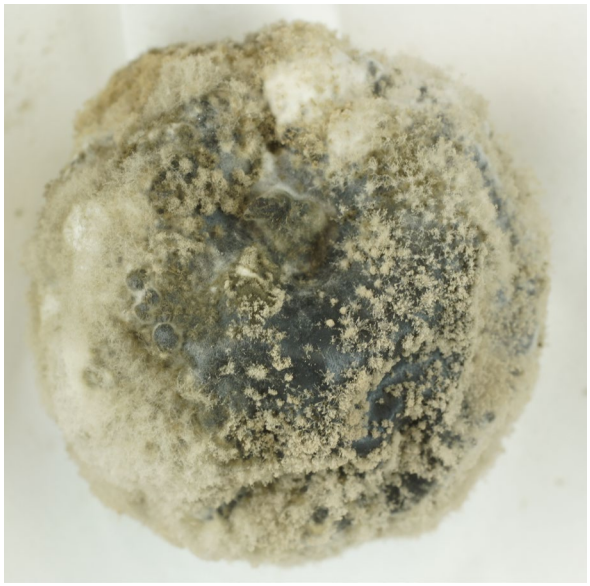

Fig. 6. Brown rot infected nectarine

Table 3. Infection potential of adult SWD on nectarine fruit, 2018

Orchard	Trt. #	Treatment	n reps	% SWD survival	% brown rot development
Orchard 1	1	Field collected flies	10	70	0
	2	Lab flies contaminated	10	70	20
	3	Lab flies uncontaminated	10	90	0
Orchard 2	1	Field collected flies	10	66.67	0
	2	Lab flies contaminated	10	70	20
	3	Lab flies uncontaminated	10	90	0
Orchard 3	1	Field collected flies	10	77.78	0
	2	Lab flies contaminated	10	70	20
	3	Lab flies uncontaminated	10	90	0

Experiment 4 *Methods*:
This experiment was to determine if brown rot spores could be transferred by SWD to selective media in the field and compare them to media exposed to the atmosphere, but from which insects were excluded. We placed cups of selective media inside Trappit domes with Scentry lures and the bottom entry hole left uncovered. A second set of traps contained cups of selective media only (no lures), and insects were excluded by placing screening materials over the bottom entry hole. After 24 h exposure in the orchards we put lids on the cups and brought them back to the lab for incubation for 7 days at 23 ºC (73 ºF) before evaluating for brown rot colony development.

Experiment 4 *Results*:
This experiment was designed to test the percentage of SWD in the field contaminated with brown rot. Selective media was used as a proxy for fruit in order to use SWD traps to capture flies. No brown rot occurred in the treatment of interest (open trap) nor in the negative control (insects excluded), likely due to the inability of the selective media to support the growth of brown rot (Table 4).

Table 4. Potential infectivity of SWD in the field on brown rot selective media, 2018

			% Brown rot infection		
Trt. #	Treatment	n	Orchard 1	Orchard 2	Orchard 3
1	Trappit dome, selective media cup, SWD lure, no mesh	10	0	0	0
2	Trappit dome, selective media cup, with mesh	10	0	0	0