Final Report

Residual Decline and Efficacy of Commonly Used Insecticides Against Spotted Wing Drosophila in Pennsylvania Wine Grapes
Pennsylvania Wine Marketing and Research Program Board

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Objectives:

Objective 1: Test five commonly used insecticides, which have distinct modes of action, under field conditions to determine the efficacy of residual compound against spotted wing drosophila (SWD) during the grape growing season.

Objective 2: To determine the residual concentrations of five insecticides, used during year two of the proposed research, in grapes by chemical extraction of residual chemicals on field-aged grape clusters.

Objective 3: Compare results with the stated results on the insecticide labels and the New York and Pennsylvania Pest Management Guidelines and inform growers of discrepancies.

Justification and Importance of Proposed Research:

Spotted wing drosophila, *Drosophila suzukii,* (SWD) is an invasive vinegar fly of Asian origin that was recently introduced into the United States. It was first discovered in Pennsylvania's Lake Erie grape growing region in the late fall of 2011. The potential infestation rate of SWD differs from other vinegar flies because the female possess a serrated ovipositor that cuts into healthy fruit to lay eggs. Female SWD can lay eggs into fruit from the time of first coloring through to harvest, so this period is the window of grape susceptibility to SWD. During egg-laying, it is believed that sour rot and fungal disease can also be introduced, further affecting the fruit quality. Spotted wing drosophila overwinter primarily as adult females, and they prefer moderate, cool, wet climates similar to the Lake Erie grape belt. During peak temperatures, a female can lay more than 100 eggs a day. *Drosophila suzukii* is now one of the most serious pests of thin-skinned fruits including blueberry, raspberry, cherry, grape, and strawberry. Upon detection of SWD, it is recommended that the spray intervals be tightened to prevent crop infestation before and during harvest. In high-pressure sites, this has required a 5-7 day spray interval, substantially increasing the cost, health hazards, and workload of the growers. Current insecticides do not have the residual efficiency that the previous broad-spectrum insecticides possessed. Limited information exists regarding field-based residual efficacy of insecticides particularly in the United States (Laskey et al. 2013). Insecticide labels suggest how often to reapply the chemicals, but do not give specific information on how long the sprays are effective. Pesticides' industry and sales personnel are relying on laboratory testing of these insecticides, which is often misleading and sometimes does not convert well to field applications. There are differences between insecticide classes, but there are also differences within insecticide classes and between species. Control of the pest is more complete when the metabolism of the pest is slower, which is generally associated with cooler or moderate temperatures. Similarly, control of some insect species with pyrethroid insecticides decreases as temperature rises. Temperature could then have a significant effect on the efficacy of insecticides when used in the field. Compared with laboratory trials, field-based bioassays more accurately reflect the efficacy of insecticides against insects over the growing season. Consequently, more information is needed to allow those responsible for making pest management decisions to select the best product for the existing environmental conditions.

Objective 1: Test five commonly used insecticides, which have distinct modes of action, under field conditions to determine the efficacy of residual compound against SWD. For year one of this experiment, we used the following 5 insecticides:

Table 1: Insecticides used during year one of the experiment, including the application rate used to spray the grape clusters, and the insecticide class and active ingredient.

Insecticide	Rate Used	Class/Active Ingredient
Avant	6 fl oz./ac	22/ Indoxacarb
Leverage 360	6.4 fl oz/ac	3/4A/ Imidacloprid, B-cyfluthrin
Sniper	6.4 fl oz./ac	3A/ Bifenthrin
Exirel	20 fl oz./ac	28/ Cyantraniliprole
Entrust	2.5 fl oz./ac	5/ Spinosad A & D
Control		No Spray

For year two of this experiment, we used the following 6 insecticides:

Table 2: Insecticides used during year two of the experiment, including the application rate used to spray the grape clusters, and the insecticide class and active ingredient.

Insecticide	Rate Use	Class	Active Ingredient
Scorpion	5.25 fl oz/ac	4A	dinofefuran
Leverage 360	3.2 fl oz/acre	3/4A	Imidacloprid/B-cyfluthrin
Wrangler	1.6 fl oz/ac	4A	Imidacloprid
Sniper	6.4 fl oz/ac	3A	bifenthrin
Tombstone	3.2 fl oz/ac	3A	cyfluthrin
Brigade	6.4 fl oz/ac	3A	Bifenthrin
Control			No Spray

The experimental design for this experiment consisted of small-panel treatments each composed of six panels (18 vines) of grapes, with four rows left between each insecticide treatment. The treatments were timed for sprays to be applied after verasion when grapes are susceptible to SWD infestation. During year one, treatments were applied four times, from August 15th to October 5th, to every replication except the Control (untreated check). Year two treatments were applied three times from August 6th to September 17th. The treatments started at veraison and continued as frequently as possible until close to harvest. All sprays were applied at a pH of 6.0 immediately after mixing. After each application, berries were harvested on Day 1 (same day clusters were sprayed) and Day 3, with continued sampling occurring as long as the insecticides demonstrated greater than a 50% mortality rate in toxicity tests. To insure complete coverage with the spray materials, only grape berries from the outside of the clusters were used for the bioassays. Weather records from the on-site NEWA station were used to determine the weather conditions during the sprays.

Year One Results:

Ten clusters were harvested from each of the treatments for each testing date. Groups of berries were removed from each cluster and placed in five plastic containers for each treatment. (See Figure 1 in Appendix). At the end of forty-eight hours, the total number of alive and dead SWD were counted. Spotted wing Drosophila from the in-house colony were used for this experiment. The in-house colony at LERGR&EC has been in existence for five years. Table 1 in the Appendix lists mean percent survival of larvae for each spray treatment. Means followed by the same letter within columns are not significantly different. Results in red are significantly different than the control. Table 2 shows the high and low temperatures for the days tested and the rainfall amounts. The temperatures of the days between tests were also recorded to ensure no exceptionally high or low temperatures occurred during the non-testing days.

The results from year one of this experiment indicated that the tested, commonly used insecticides showed very good efficacy on Day 1 (except Entrust in the August 15th experiment). On Day 3, all insecticides tested showed above a 50% mortality rate in the August 15th experiment. For the other three test dates, only Exirel and Leverage 360 were effective (50% mortality). After Day 3, and in all experiments except the August 15th experiment, only Exirel and Leverage 360 showed efficacy. Leverage 360 was effective for a minimum of 6 days. In the September 12th experiment Leverage 360 was effective beyond October 5th which is twenty-four days past the original spray. We expected the efficacy to increase from the first to the fourth experimental date because of the necessity of performing these experiments with short interval times between the experiments. This did not prove to be the case except possibly with Leverage 360. This also adds credence to the short residual times experienced in these experiments.

<u>Year Two Results</u>:

Year two experiments were conducted according to the exact protocol as year one (See Results year one). Table 4 in the Appendix lists mean percent survival of larvae from the total number for each spray treatment. Results in red are significantly different than the control. Table 5 in the Appendix shows the high and low temperatures for the days tested, and the rainfall amounts. The temperatures of the days between tests were also recorded to ensure no exceptionally high or low temperatures occurred during the non-testing days.

The results from year two of this experiment indicated that all the commonly used insecticides showed efficacy on Day 1 in all three testing periods, apart from Wrangler on September 4th. The August 13th through 22nd experiment, Day 3 (August 15th) Wrangler, Scorpion, Brigade, and Leverage 360 insecticides tested showed above a 50% mortality rate. By Day 5 (August 17th) Leverage 360 and Wrangler showed efficacy above the 50% mortality rate. There were no significant rain events during this testing period, but the heat was above 80˚F on August 14-17th. The September 17th through the 21st experiment, only Wrangler did not perform above the 50% mortality rate on Day 1. None of the insecticides besides Leverage 360 were above the 50% mortality rate by Day 5. There were no major heat or rain events during this period. The third experiment showed efficacy of all the insecticides during Day 1. On Day 3, Leverage 360, Tombstone, and Brigade had efficacy above the 50% mortality rate. By Day 5, only Leverage 360 showed efficacy, which did not last to Day 7. September 21st was the only major rain event and there were no major heat events during this period. We expected the efficacy to increase from the first to the fourth experimental date because of the necessity of performing these experiments with short interval times between the experiments, which did not prove to be the case. This also adds credence to the short residual times experienced in these experiments. Some of the discrepancies in this testing can be attributed to increasing canopy coverage, which is one of the reasons we felt that these experiments would better demonstrate insecticide efficacy under real time conditions.

Objective 2: Determine the residual concentrations of five insecticides, used during year two of the proposed research, in grapes by chemical extraction of residual chemicals on field-aged grape clusters.

This objective would help to lend credence to the conclusion drawn from last year's (2017) research about the residual effects of insecticides on SWD. Therefore, the harvested grapes used in laboratory toxicity tests were chemically extracted, and the residual insecticide concentrations were determined using gas chromatography mass spectrometry (GC-MS).

Insecticide analysis was performed on Day 1 grape samples, as well as the grapes sampled on the last day of the experiment. This allowed for analysis of the change in insecticide concentration over time to determine the degree to which the changes observed in toxicity were due to a decline in efficacy compared to a loss of chemical residue from the grapes.

<u>Chemicals</u>:

Solvents, including acetone (Optima grade) and hexane (Optima grade), were purchased from Fisher Scientific (Thermo Fisher Scientific, Waltham, MA, USA) for extraction of pyrethroid insecticides from Concord grapes. Sodium sulfate (Na_2SO_4) was also purchased from Fisher Scientific to remove any excess water from samples prior to analytical analysis using a gas chromatograph equipped with a mass spectrum detector.

<u>Pyrethroid Extractions from Concord Grapes</u>:

Pyrethroid insecticides present on Concord grape clusters collected immediately post pesticide application, as well as at the end of the toxicity testing period, were extracted using methodologies adapted from Satpathy et al. (2011). Upon collection, grape clusters were stored at -20˚C to prevent degradation of pesticides prior to extraction. To determine the pyrethroid concentrations present on the grape clusters, 100 g of grapes were removed from each cluster and homogenized, with 20% sodium sulfate to remove excess water, using a blender (Whirlwind™, Oster, Sunbeam Products, Inc., Boca Raton, FL, USA). Ten grams of blended grapes were then transferred to microwave extraction vessels with approximately 10 g of sodium sulfate and mixed with 10 mL of acetone: hexane (1:1, vol/vol) to aid in extraction of pyrethroids from grape tissue. The microwave extraction vessels were then capped and sonicated for 10 minutes using a Branson 3200 bath sonicator (Bransonic®, Branson Ultrasonic Corp., Danbury, CT, USA). Following sonication, pyrethroids were extracted from grapes using a Mars 6™ Microwave Digester (CEM Corporation, Matthews, NC, USA) using the following extraction protocol: Ramp 100 watts to 300 watts over two minutes, hold at 300 watts for three minutes, ramp 300 watts to 100 watts, hold at 100 watts for two minutes. After extraction, the acetone: hexane was quantitatively transferred to a 40 mL glass vial, and grape residue was rinsed with 3 mL of hexane, which was subsequently transferred to the 40 mL glass vial containing 2 g of sodium sulfate to remove any residual water. Samples were concentrated under a steady stream of nitrogen using a Turbovap (Biotage, LLC, Charlotte, NC, USA), solvent exchanged with 10 mL of hexane, and concentrated to 1 mL. After concentration, samples were transferred to gas chromatography vials and concentrated to a final volume of 1 mL under a steady stream of nitrogen. Pyrethroid concentrations present in each sample were then analyzed using a gas chromatograph equipped with a mass spectrum detector. Data are reported as ng of pyrethroid per gram of grape (ng/g grape).

<u>Results of Pyrethroid Extractions from Concord Grapes</u>:

Chemical analysis of grape clusters treated with Tombstone (Active Ingredient (AI): Cyfluthrin), Leverage 360 (AI: Cyfluthrin), Brigade (AI: Bifenthrin), and Sniper (AI: Bifenthrin) were conducted for the August 13th, September 4th, and September 17th spray events. Grapes from these experiments were analyzed on Day 1 and the last day that sampling for toxicity testing occurred (Day 8, 12, or 14) (Table 6). In general, the pyrethroid concentrations on the grapes sampled at Day 1 were not different from the pyrethroid concentrations on the grapes sampled on the last day of the experiment (Table 6). The largest decline in pyrethroid concentration was observed for Sniper applied on August 13th. The bifenthrin concentration on August 13th was 399.13 ng/g grape and 131.31 ng/g grape on August 24th (Table 6). This decline in bifenthrin residue could be associated with the increased precipitation during this experimental period compared to the other experimental timeframes (Table 6). Conversely, the cyfluthrin concentration associated with Tombstone applied on September 4th appeared to increase between September 4th and September 11th (Table 6). This is likely an artifact of the sampling design, as the grapes chosen for analysis were randomly selected from the sampled clusters. With the increased canopy cover associated with the grape vines at this period in the growing season, it is likely that the grapes are sprayed unevenly during insecticide application. If more concentrated grapes were sampled from the clusters sampled on September 11th compared to September 4th, it could explain the perceived decline in insecticide residue.

Excluding these two anomalies within the pyrethroid concentration data collected, the pyrethroid concentrations did not significantly change over the course of the experiments (Table 6). This would suggest that changes in residual toxicity associated with pyrethroid insecticides observed from the toxicity tests is not due to a significant change in the amount of active ingredient present on the grapes. While high temperatures and excessive rainfall would be expected to cause declines in residual effectiveness of the pyrethroids, the hydrophobic nature of pyrethroids likely protects the chemicals from such fate. Pyrethroids are extremely hydrophobic and bind within the waxes and other lipids within the skin of the grapes, preventing loss from rainfall. Similarly, this sequestration likely protects the chemicals from degradation by UV light, preventing the pyrethroid concentrations from significantly declining over the course of the experiment. Therefore, differences in residual toxicity of the insecticide formulations can be attributed to relative differences in susceptibility to the chemicals by the SWD. Leverage 360, the formulation that demonstrated the highest residual toxicity throughout the toxicity tests, is a mixture of both cyfluthrin and imidacloprid, a neonicotinoid. This mixture of active ingredients creates two distinct modes of action to cause toxicity to the exposed pests, such that if the SWD are more resilient in the face of pyrethroid exposure, the combined effects of the neonicotinoid overcome the larvae, resulting in mortality. As such, the results of the current study would suggest that in order to improve residual toxicity of insecticides to SWD, formulations with multiple active ingredients that have different modes of action would best ensure control of SWD with the least investment in continued application of chemicals.

Objective 3: Compare results with the stated results on the insecticide labels and the New York and Pennsylvania Pest Management Guidelines and inform growers of discrepancies.

The results obtained associated with the residual pesticide concentrations and the efficacy of these chemicals to SWD are at odds with one another. The active ingredients within the pesticides appear to be retained within the grapes over the length of the experiment, yet the toxicity associated with these chemicals significantly declines with time. Stating that the guidelines for application outlined on the pesticide labels and within the New York and Pennsylvania Pest Management Guidelines would be inappropriate, as the chemicals are retained within the grapes using recommended applications. Therefore, the decline in efficacy of the pesticides to SWD is likely due to a change in the availability of the chemicals over the length of the test.

Pyrethroids are hydrophobic in nature and are likely sequestered within the waxes of the skin coating the grapes. Fractions of the chemical that are bound to the waxes of the grapes are likely unavailable to the pest insects, decreasing the toxicity of these chemicals within the grapes. As the chemical extraction methods used in the current study are unable to differentiate between bound and free chemical within the grape matrix, we would not necessarily see a decline in the pesticide concentration on the grapes over time. If the pyrethroids are becoming sequestered within the grapes and unavailable to the exposed SWD, however, it would result in the decline in efficacy over time, as noted in the toxicity tests. Therefore, despite proper application methods to deliver active ingredient to the grapes, the pesticides lose efficacy over time due to sequestration of the chemicals within the matrix of the grapes unavailable for exposure to SWD. This is further supported by the sustained efficacy of Leverage 360.

Leverage 360 contains both a pyrethroid (cyfluthrin) and a neonicotinoid (imidacloprid). In comparison to pyrethroids, neonicotinoids are hydrophilic, meaning they are retained within the water rich areas of the grapes. As the SWD deposit their eggs within the grapes and the larvae feed on the grape tissue, they are exposed to the neonicotinoid insecticide, resulting in toxicity. Therefore, even if the effective concentration of cyfluthrin is declining within the grape, the concentration of imidacloprid available to SWD remains high enough to elicit a toxic effect. As such, it would be our recommendation to growers to use Leverage 360, or other pesticide formulations with multiple active ingredients with different modes of toxic action, and proposed application rates documented within the pesticide labels and New York and Pennsylvania Pest Management Guidelines.

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Sparks TC, Shour MH and Wellemeyer EG, 1982. Temperature and toxicity relationships of pyrethroids on three Lepidopterans. *J Econ Entomol* 75:643–646.

Satpathy G, Tyagi YK, and Gupta KR, 2011. A novel optimized and validated method for analysis of multi-residues of pesticides in fruits and vegetables by microwave-assisted extraction (MAE)-dispersive solid-phase extraction (d-SPE)-retention time locked ((RTL)-gas chromatography-mass spectrometry with Deconvolution reporting software (DRS). *Food Chemistry* 127: 1300-1308.

Appendix

Figure 1: Experimental containers used in toxicity tests with spotted wing drosophila.

Table 1: Results of toxicity testing with spotted wing drosophila (SWD) during year one. Mean percent of survival of larvae for each spray treatment is listed. Means followed by the same letter within columns are not significantly different. Results in red are significantly different than the control.

Formulation	15-Aug	17-Aug	19-Aug
Avant	40.0%	43.5%	73.9%
Leverage 360	8.4%	9.1%	35.7%
Exirel	7.5%	33.0%	73.3%
Sniper	5.9%	47.1%	64.1%
Entrust	8.2%	28.8%	78.2%
Control	92.5%	89.9%	90.9%

Formulation	6-Sep	9-Sep	12-Sep
Avant	74.5% b	87.1% a	
Leverage 360	13.7% d	1.7% c	41.1% b
Exirel	35.9% c	15.9% b	82.9% a
Sniper	19.4% d	87.9% a	
Entrust	36.2% c	90% a	
Control	91.6% a	96.3% a	96.8% a

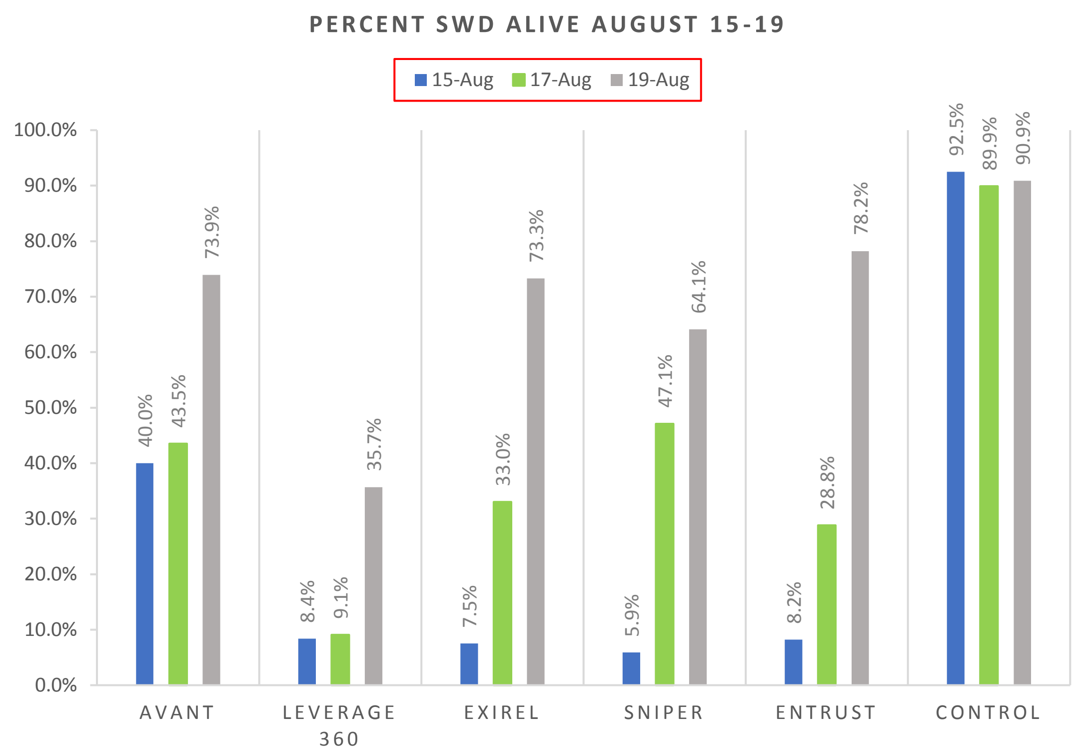
Formulation	22-Aug	24-Aug	27-Aug	29-Aug	1-Sep
Avant	88.2% a	91.9% a			
Leverage 360	5.4% c	13.1% c	27.8% b	17.8% b	74.8% b
Exirel	42.2% b	73.9% b			
Sniper	52.6% b	79.5% ab			
Entrust	62.8% b	92.4% a			
Control	91.4% a	92.7% a	90.9% a	92.7% a	91.7% a

Formulation	12-Sep	15-Sep	18-Sep	22-Sep	27-Sep	5-Oct
Avant	87.7% a	93.8% a				
Leverage 360	22.3% cd	1.4% c	19.1% c	14.7% c	10.7% b	33.4% b
Exirel	56.7% b	21.7% b	82.2% b	75% b		
Sniper	33.9% c	87.3% a				
Entrust	9.5% d	88.4% a				
Control	95.3% a	96.3% a	96.8% a	95.5% a	91.5% a	95% a

Table 2: Temperature and rainfall for the experimental dates. Temperatures in red indicate major rainfall events.

Date	Avg Temp	Max Temp	Min Temp	Rainfall
8/15/2017	72.5	79.6	65.9	0
8/16/2017	70.9	81	60.2	0
8/17/2017	73.9	87.8	63.6	0.78
8/18/2017	75.3	80.6	71.3	0
8/19/2017	71.1	74.8	67.4	0.04
8/22/2017	74.8	81.5	68.3	0.75
8/23/2017	69.1	72.1	66.3	0
8/24/2017	66.1	72.9	59.6	0.01
8/25/2017	63.8	75	54.8	0.02
8/26/2017	63.1	75.1	52.3	0
8/27/2017	66.9	78.6	55.9	0
8/28/2017	68.4	76.8	63.6	0
8/29/2017	67.6	76	62.8	0
8/30/2017	67.9	77.7	59.3	0
8/31/2017	64.6	69.3	59.4	0.28
9/1/2017	57.9	64.7	51.4	0
9/6/2017	59.8	66.4	54.1	0
9/7/2017	57.2	64.4	52	0.33
9/8/2017	56.3	64	52.2	0.54
9/9/2017	56.7	65.2	49.2	0
9/10/2017	57.1	66.8	47.2	0
9/11/2017	60.3	71.2	50.7	0
9/12/2017	62.3	74.7	50.2	0
9/12/2017	62.3	74.7	50.2	0
9/13/2017	68.4	79.7	57.2	0
9/14/2017	65.4	69.7	60.9	0.14
9/15/2017	66.7	76.5	58.8	0
9/16/2017	68.1	78.3	60.4	0
9/17/2017	70.4	80.3	62	0
9/18/2017	71	80.2	64.6	0
9/19/2017	71.3	81.4	65.3	0
9/20/2017	70.3	82	62.3	0
9/21/2017	69.8	80.9	60.9	0
9/22/2017	69.6	80.1	59.5	0
9/23/2017	71.6	86	60	0
9/24/2017	73.8	84.5	63.6	0
9/25/2017	77.1	85.8	70.7	0
9/26/2017	76.4	86.9	69	0
9/27/2017	74	83	66.5	0
9/28/2017	61.7	64.9	59.2	0
9/29/2017	57	66.1	47.9	0.08
9/30/2017	53.6	61.9	45.2	0
10/1/2017	53	65.4	42.5	0
10/2/2017	59.5	73.1	49.4	0
10/3/2017	67.4	80.6	56.3	0
10/4/2017	70.8	77.5	65.5	0.13
10/5/2017	64.4	70.8	57.3	0.4

A.


PERCENT SWD ALIVE AUGUST 15-19
15-Aug
17-Aug
19-Aug
100.0%
90.0%
80.0%
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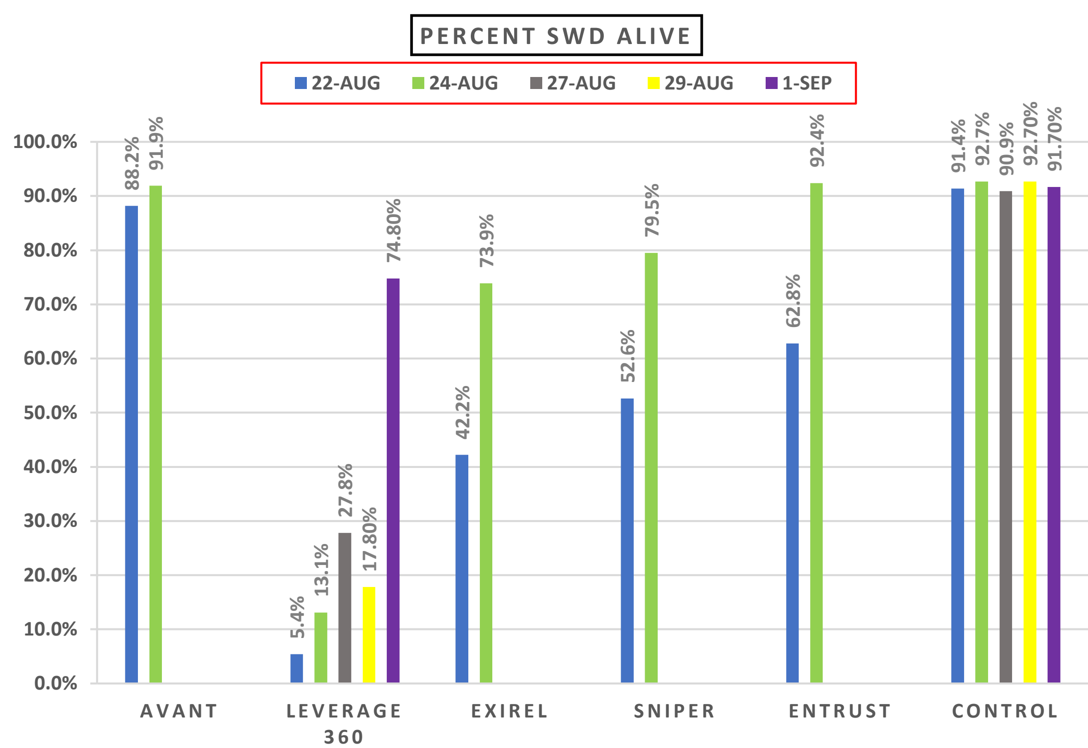
AVANT
LEVERAGE 360
EXIREL
SNIPER
ENTRUST
CONTROL

B.


PERCENT SWD ALIVE
22-AUG
24-AUG
27-AUG
29-AUG
1-SEP
100.0%
90.0%
80.0%
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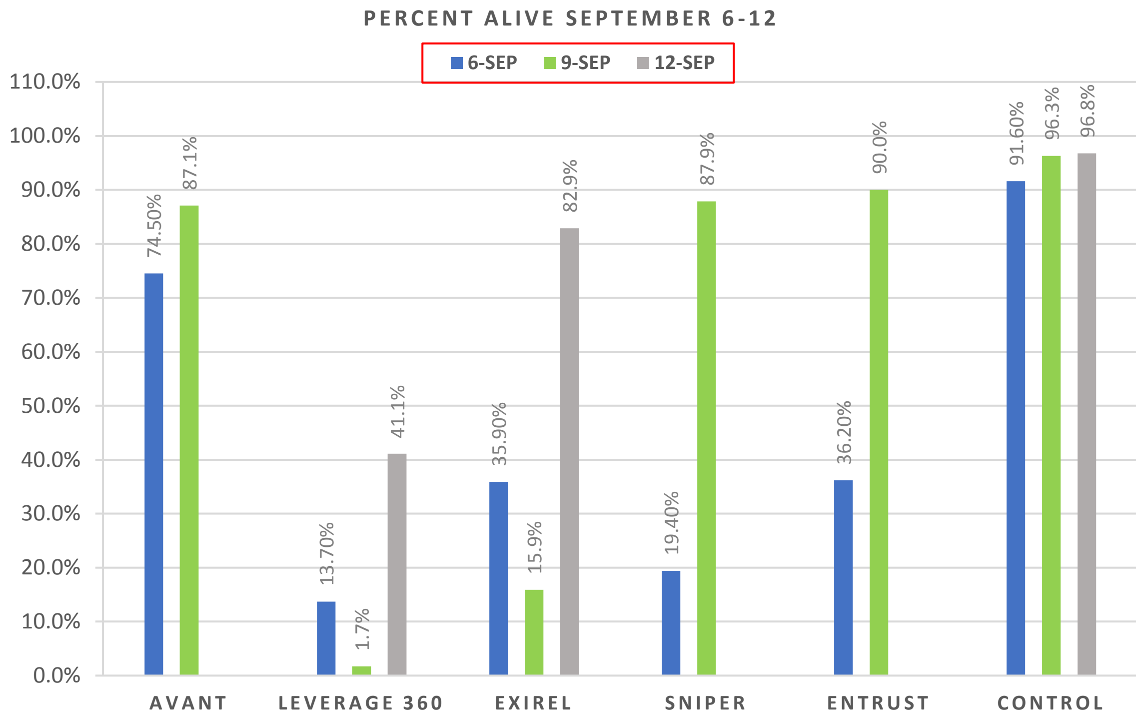

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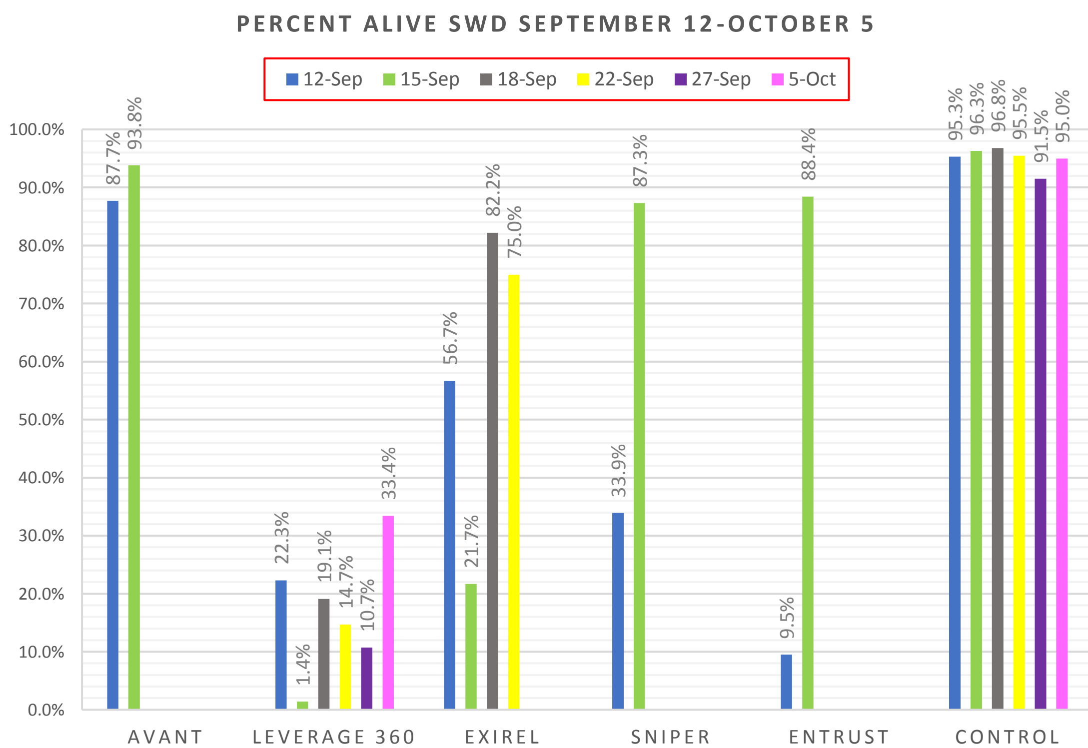


Figure 2: Results of toxicity tests with SWD for insecticide formulations from A) August 15th, B) August 22nd, C) September 6th, D) and September 12th.

Table 3: Percent of spotted wing drosophila alive listed by day for each insecticide used in 2017 experiments.

	Leverage 360				**Exirel**				**Sniper**		
	Day 1	**Day 3**	**Day 6**		**Day 1**	**Day 3**	**Day 6**		**Day 1**	**Day 3**	**Day 6**
15-Aug	**8.4**	**9.1**	**35.7**	**15-Aug**	**7.5**	**33**	**73.3**	**15-Aug**	**5.9**	**47.1**	**64.1**
22-Aug	**5.4**	**13.1**	**27.8**	**22-Aug**	**42.2**	**73.9**		**22-Aug**	**52.6**	**79.5**	
6-Sep	**13.7**	**1.7**	**41.1**	**6-Sep**	**35.9**	**15.9**	**82.9**	**6-Sep**	**19.4**	**87.9**	
12-Sep	**22.3**	**1.4**	**19.1**	**12-Sep**	**56.7**	**21.7**	**82.2**	**12-Sep**	**33.9**	**87.3**	

	Entrust				**Avant**		
	Day 1	**Day 3**	**Day 6**		**Day 1**	**Day 3**	**Day 6**
15-Aug	**8.2**	**28.8**	**78.2**	**15-Aug**	**40**	**43.5**	**73.9**
22-Aug	**62.8**	**92.4**		**22-Aug**	**88.2**	**91.9**	
6-Sep	**36.2**	**90**		**6-Sep**	**74.5**	**87.1**	
12-Sep	**9.5**	**88.4**		**12-Sep**	**87.7**	**93.8**	

Table 4: Results of toxicity testing with spotted wing drosophila (SWD) during year two. Mean percent of survival of larvae for each spray treatment is listed. Means followed by the same letter within columns are not significantly different. Results in red are significantly different than the control.

Formulation	13-Aug	15-Aug	17-Aug	20-Aug	22-Aug
Scorpion	29.2	12.0	49.4	76.3	77.1
Leverage 360	9.4	9.8	6.0	75.0	
Wrangler	71.7	26.2	57.1		
Sniper	37.4	67.0	90.6		
Tombstone	44.5	68.9	55.4	91.6	
Brigade	9.7	26.7	82.5		
Control	93.0	88.8	88.8	96.8	90.8

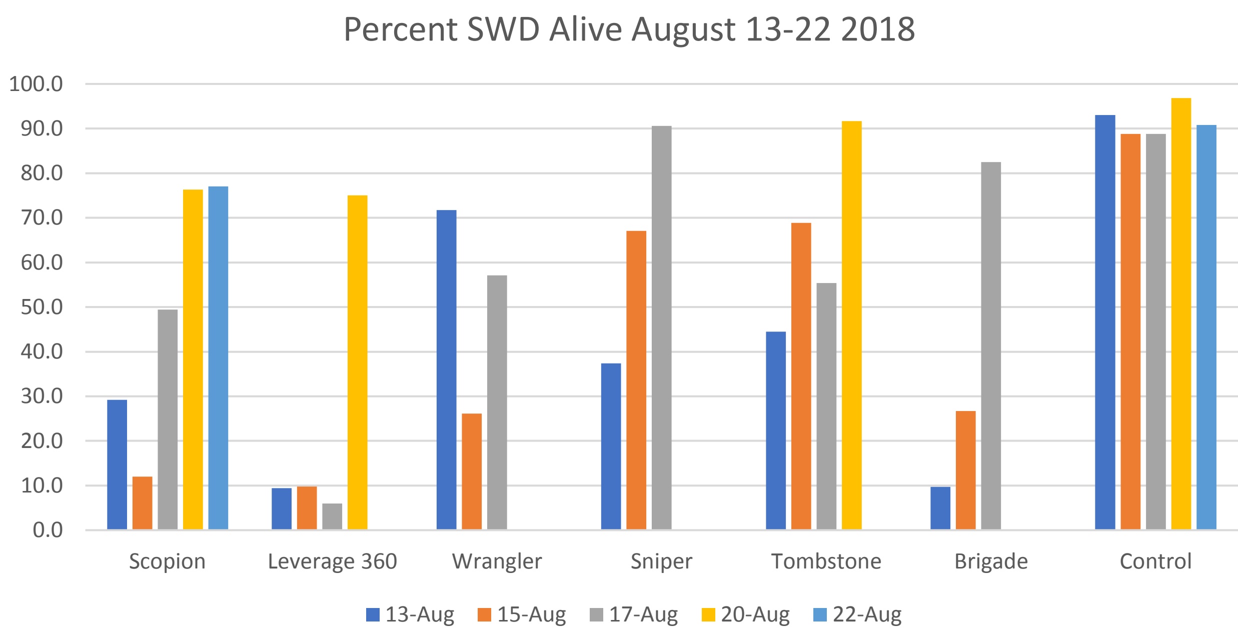
Formulation	4-Sep	6-Sep	8-Sep	10-Sep
Scorpion	7.8	90.3		
Leverage 360	18.7	17.8	15.6	79.8
Wrangler	60.8	90.5		
Sniper	15.9	81.6		
Tombstone	21.6	70.5	86.2	
Brigade	37.7	84.0		
Control	92.2	95.7	91.4	93.3

Formulation	17-Sep	19-Sep	21-Sep
Scorpion	19.2	78.9	
Leverage 360	8.4	14.5	19.7
Wrangler	32.0	59.9	
Sniper	39.0	86.9	
Tombstone	36.3	27.4	83.1
Brigade	9.1	18.1	77.0
Control	90.3	91.7	93.1

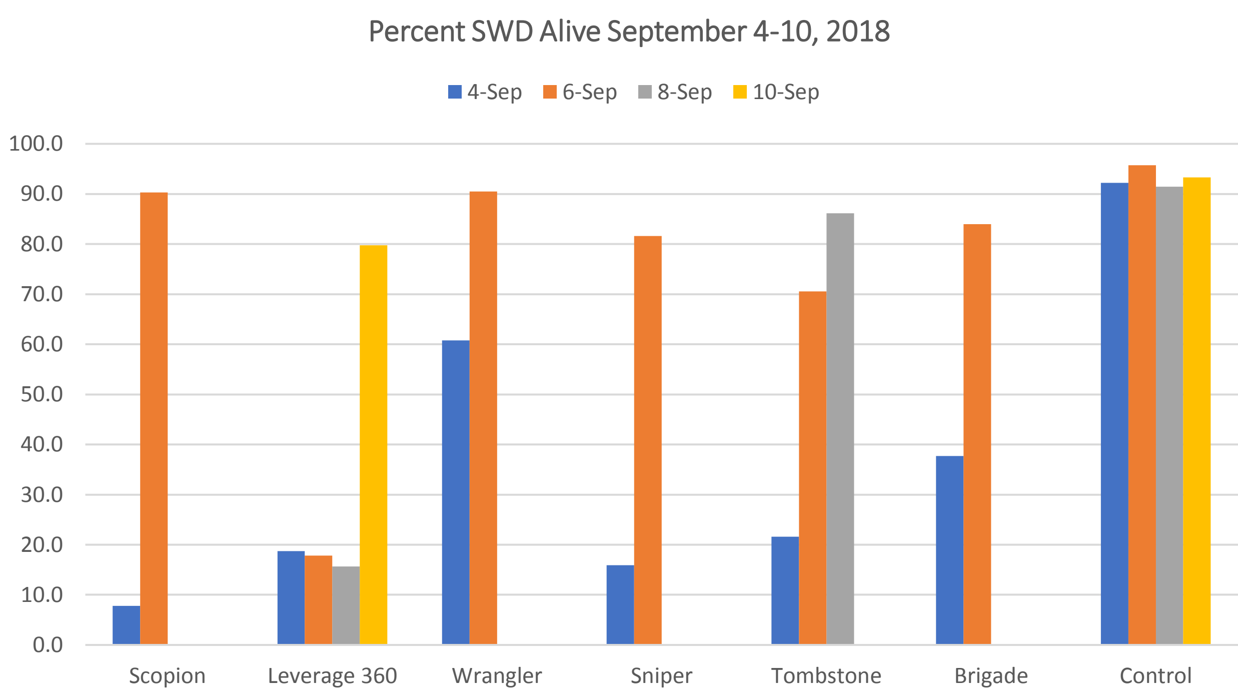
Table 5: Temperature and rainfall for the experimental dates. Temperatures in red indicate major rainfall events.

Date	Avg. Temp.	Max Temp.	Min Temp.	Total Percip.
8/13/2018	71.6	82	61.4	0
8/14/2018	72.3	79.6	65.3	0.56
8/15/2018	75	81.1	68.9	0
8/16/2018	76.6	83.1	70.8	0
8/17/2018	74.7	80.1	70.9	0
8/18/2018	*71.4*	*76.9*	*66.1*	*0.8*
8/19/2018	68.8	76.7	59.6	0
8/20/2018	72.3	79.1	64.7	0.02
8/21/2018	72.7	80.3	69.3	0.73
8/22/2018	68.8	70.7	65.4	0.16
8/23/2018	67.3	74.6	58.9	0
9/4/2018	77.1	85.2	68.5	0
9/5/2018	81.2	88.6	75.4	0
9/6/2018	73.9	78.2	66.2	0
9/7/2018	70.7	79	64.9	0
9/8/2018	62.9	65.8	55.2	0
9/9/2018	54.4	57.2	52.7	0.4
9/10/2018	60.8	68.1	53.2	1.79
9/17/2018	71.5	76.7	66.1	0
9/18/2018	69.5	75.7	62.8	0
9/19/2018	67.8	74.2	60.4	0
9/20/2018	68.8	76	57.9	0
9/21/2018	77.5	87.9	67.5	0.46
9/22/2018	58.4	66	52.1	0
9/23/2018	58.5	68	48.4	0
9/24/2018	62.6	69.8	54.8	0.19
9/25/2018	67.3	73.3	60.3	0.51
9/26/2018	66.9	72.9	61.1	0.5

A.


Percent SWD Alive August 13-22 2018
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Scopion
Leverage 360
Wrangler
Sniper
Tombstone
Brigade
Control
13-Aug
15-Aug
17-Aug
20-Aug
22-Aug

B.


Percent SWD Alive September 4-10, 2018
4-Sep
6-Sep
8-Sep
10-Sep
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Scopion
Leverage 360
Wrangler
Sniper
Tombstone
Brigade
Control

C.

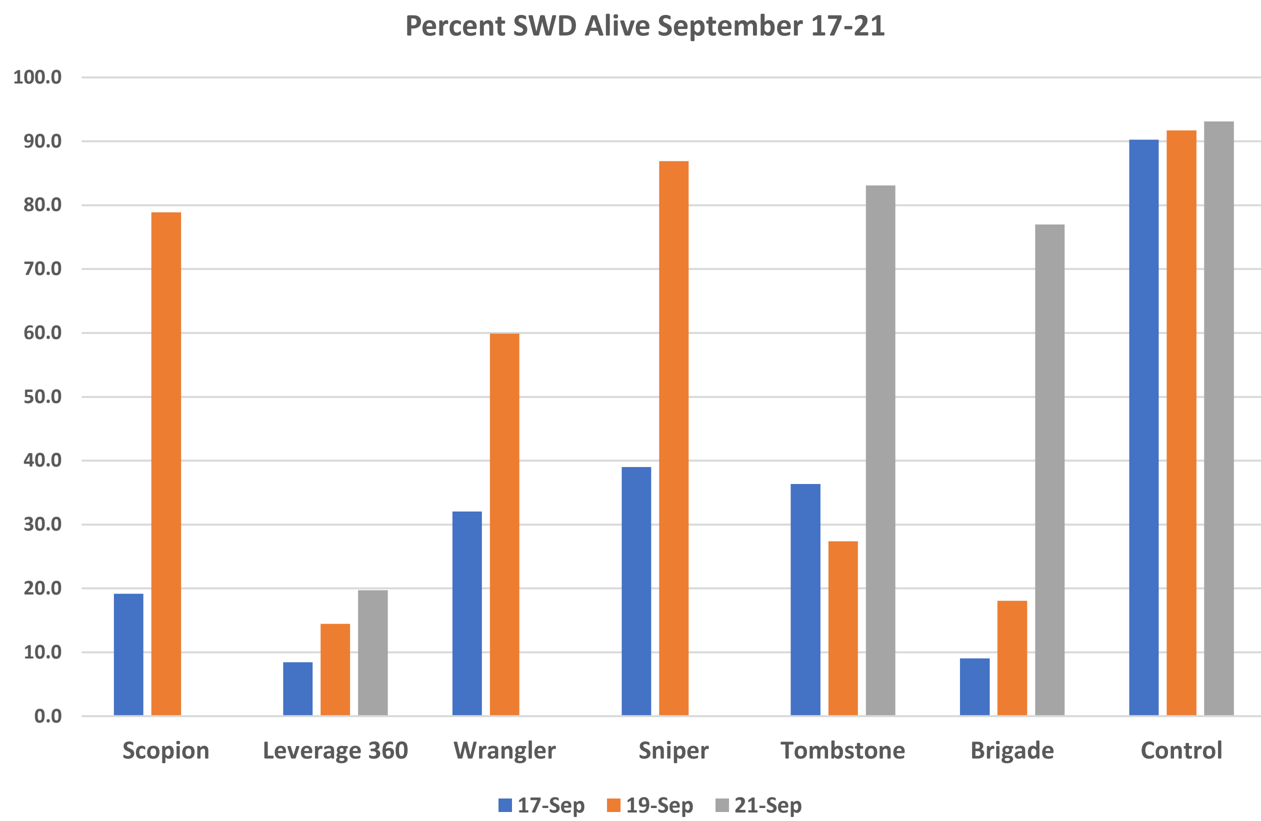


Figure 3: Results of toxicity tests with SWD for insecticide formulations from A) August 13th, B) Septmeber 4th, and C) September 17th.

Table 6: Pyrethroid concentration extracted from Concord grapes at various points in the growing season after formulation application to combat Spotted Wing Drosophila pests. The formulation name, active pyrethroid ingredient in each formulation, date of application, date of grape sampling, and the average pyrethroid concentration in nanograms pyrethroid per gram of grape (wet weight) is reported. Relative percent difference among replicates (n=2) is reported as values in parentheses.

Formulation	Pyrethroid	Application Date	Sample Date	Pyrethroid Concentration (ng/g grape)
Tombstone	Cyfluthrin	8/13/2018	8/13/2018	55.59 (1.69)
Tombstone	Cyfluthrin	8/13/2018	8/24/2018	55.03 (62.35)
Tombstone	Cyfluthrin	9/4/2018	9/4/2018	12.50 (NA)[ab]
Tombstone	Cyfluthrin	9/4/2018	9/11/2018	88.00 (1.14)
Tombstone	Cyfluthrin	9/17/2018	9/17/2018	102.94 (46.92)
Tombstone	Cyfluthrin	9/17/2018	9/30/2018	145.44 (14.96)
Leverage 360	Cyfluthrin	8/13/2018	8/13/2018	44.19 (37.62)
Leverage 360	Cyfluthrin	8/13/2018	8/24/2018	41.62 (38.51)
Leverage 360	Cyfluthrin	9/4/2018	9/4/2018	85.69 (14.25)
Leverage 360	Cyfluthrin	9/4/2018	9/11/2018	85.53 (19.74)
Leverage 360	Cyfluthrin	9/17/2018	9/17/2018	207.81 (0.42)
Leverage 360	Cyfluthrin	9/17/2018	9/30/2018	198.71 (38.79)
Brigade	Bifenthrin	8/13/2018	8/13/2018	147.00 (7.82)
Brigade	Bifenthrin	8/13/2018	8/24/2018	222.91 (63.71)
Brigade	Bifenthrin	9/4/2018	9/4/2018	271.78 (15.30)
Brigade	Bifenthrin	9/4/2018	9/11/2018	235.56 (18.63)
Brigade	Bifenthrin	9/17/2018	9/17/2018	546.94 (21.60)
Brigade	Bifenthrin	9/17/2018	9/30/2018	411.39 (5.66)
Sniper	Bifenthrin	8/13/2018	8/13/2018	399.13 (24.12)
Sniper	Bifenthrin	8/13/2018	8/24/2018	131.31 (13.01)
Sniper	Bifenthrin	9/4/2018	9/4/2018	820.49 (5.49)
Sniper	Bifenthrin	9/4/2018	9/11/2018	783.36 (18.59)
Sniper	Bifenthrin	9/17/2018	9/17/2018	371.20 (20.27)
Sniper	Bifenthrin	9/17/2018	9/30/2018	290.88 (31.71)

[a]Estimated based on reporting limits of analytical analysis, as sample concentration was below lowest calibration standard.
[b]Low confidence reported in analysis of chemical concentrations, as it appears bifenthrin was present instead of cyfluthrin.