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Spotted wing drosophila in Virginia vineyards: Distribution, varietal susceptibility, monitoring and control

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This report represents work in the final year of funding on this project. The graduate research involved is not yet complete; therefore some questions are not yet resolved. It is anticipated that more complete information will be available upon completion of her PhD program.

Early detection of spotted wing drosophila (SWD) in Virginia Vineyards

Early detection of SWD in Virginia vineyards is critical for determining when the pest has arrived in the vineyard blocks. After the initial detection of SWD it may be possible to monitor the population to see when flies move into the vineyard blocks. This also reiterates the importance of alternative host plants on the first and second generations, respectively, of SWD within the vineyard. By removing alternative host plants the initial SWD populations maybe small, which might keep subsequent population from exploding until later on in the growing season.

Trapping for SWD began on 23 May 2015. Deli style plastic container traps baited with 300mls of a mixture of ACV and Merlot wine (60-40%) were hung in known SWD host plants surrounding grapes at two vineyards. The first vineyard was located in Orange Co. while the second location was in Albemarle County. There were five traps at each vineyard and the alternative host plants where the traps were suspended included: wild cherry, tartarian honeysuckle, blackberry and mulberry. The Albemarle location detected SWD in three of the five traps on 24 June. Two traps hung within cherry trees contained both female and male SWD and a single female was discovered in a trap located near blackberries. The Orange location detected SWD in tartarian honeysuckle in two of the five traps on 29 June. This specific trapping location within alternative host plants allowed for SWD detection two weeks earlier than previous years when trapping was only performed within the vineyard blocks. Both locations trapped male and female flies. Trapping continued for a further two weeks in which trap numbers increased and flies were detected in all traps after that time.

Targeted bait placement for the early detection of SWD in vineyards appears to have been effective in detecting the first incidence of SWD within these two vineyards.

Wine grape susceptibility to SWD oviposition and subsequent larval survivorship

Introduction

The above study showed that SWD are present in the areas surrounding vineyards in June, while subsequent years of trapping has shown that SWD can be expected to enter vineyard blocks in the early weeks in July. This timing of SWD invasion into the vineyard blocks put the grape developmental stage to be around the green pea stage. This is not the observed stage for SWD to oviposit into the grapes; however we have not yet been able to determine the exact point in which the grapes become susceptible to SWD attack. Two experiments were undertaken to answer the question of when exactly do the grapes become susceptible to SWD attack and can the larvae survive in grapes below optimal oviposition conditions.

Material and Methods

The first experiment analyzing grape susceptibility placed six female and six male SWD flies in a twelve oz. clear plastic cup containing five grapes. Flies were left for 48 hours within the cups. Eggs laid on the grape surface as well as those oviposited into the grape were observed and counted. Grapes with eggs oviposited into the flesh were observed for 14 days and emerging adult flies were tallied. Five replicates of this experiment were conducted early in the growing season and eight replicates were conducted after véraison when grapes were at their prime. A second experiment, determining larval survivorship, utilized three grapes per replicate that had been pierced three times with a metal point to create a two to three millimeter hole on the surface of the grape. After the skin had been pierced the three grapes were placed into the aforementioned plastic cups along with four female and three male flies. The flies were kept in the cups for 48 hours. After that time period eggs were counted and recorded. The grapes were observed for 14 days and all emerging adult flies were recorded. Three replicates of this experiment were conducted for the extent of the trial period.

Six grape varieties were analyzed; Viognier, Petit Verdot, Petit Manseng, Vidal, Pinotage and Cabernet Franc. Data collected from the grapes included: skin thickness (cm), penetration force (cnw), and degrees Brix. Twenty replicates were used to collect the data identifying grape susceptibility parameters. Penetration force was collected via a centinewton gauge with a dulled insect pin attached to simulate a female fly ovipositor. Skin thickness data was collected using a digital caliper set to measure millimeters. Degrees Brix were measured using a refractometer. Data represented in tabular form from the last four collection dates ranged from 17 August to 9 September 2015. Data were analyzed using ANOVA, and Tukey-Kramer was used to separate the means.

Results

The first experiment looked at SWD ability to oviposit into intact grapes. There was a statistically significant difference among the grape varieties tested. Testing was blocked by date and was statistically significant. The most susceptible grape variety to SWD oviposition was Viognier, which had flies able to penetrate the skin on 17 August. Viognier also had the most eggs directly oviposited into the grapes with a mean of 4.4 eggs per replicate. This may be due to that variety having the least penetration force (Table 1). Cabernet Franc was the second most vulnerable. Flies were able to oviposit into the flesh of Cabernet Franc a week later on 25 August. Pinotage and Petit Manseng became susceptible to oviposition on 31 August and 9 September, respectfully. Petit Verdot and Vidal were able to escape fly oviposition for the duration of the experiment (Table 2). Viognier also had the most flies emerge from the grapes after oviposition. Survivorship for eggs laid in Viognier and Petit Manseng grapes was around 26% with adult survivorship of 28% for Cabernet Franc.

The second experiment evaluated larval survivorship on grapes that were manually damaged to create a 2-3 millimeter hole. All grape varieties had eggs laid on the grapes that were manually damaged. The grape variety that had the most eggs laid onto the surface of the grapes was Vidal with a mean of 14.5 eggs per replicate. The variety that had the least number of eggs laid was Petit Manseng with only 3.9 eggs laid per replicate. The varieties that had the highest survivorship of eggs to adults were Cabernet Franc and Viognier with survivorship of 37% and 32%, respectfully (Table 3).

Discussion

Viognier appears to be the most susceptible variety to SWD oviposition of the six varieties evaluated. Oviposition by SWD occurred a week ahead of all other varieties. Viognier is an early maturing variety with thin skin and low penetration force. These qualities along with the high sugar content also allow SWD larvae a 32% rate of survival. However, all varieties that were manually damaged had high rates of oviposition and larval survivorship above 15%. This means that all varieties that are mechanically damaged in the field are at risk. The two varieties that escaped direct oviposition were Vidal and Petit Verdot, both of which are later-maturing varieties. Petit Verdot was harvested 3 weeks after Viognier and had a degrees Brix of 16.65 when testing occurred. Furthermore, the thick skin of Vidal might never lend itself to fly oviposition. It is also important to note that early on in the manually damaged experiment when grapes were just past the green pea stage, and had a degree Brix of 5.0, flies laid eggs and adults emerged. Given the opportunity to oviposit on damaged fruit the flies can compete their life cycle in less than optimal fruit conditions.

Table 1. Grape susceptibility factors

Variety	**Mean Penitration Force (Cnw)**	**Mean Skin Thickness (cm)**	**Mean °Brix**
Viognier	8.96 ± 0.24 C	0.0064 ± 0.00044 B	18.35 ± 0.36 C
Vidal	15.18 ± 0.24 A	0.0065 ± 0.00032 B	19.8 ± 0.16 B
Cab Franc	13.97 ± 0.44 B	0.0069 ± 0.00024 B	16.0 ± 0.37 E
Petit Mansang	15.38 ± 0.3 A	0.0079 ± 0.00023 A	17.45 ± 0.69 CD
Petit Verdot	13.16 ± 0.4 B	0.005 ± 0.00031 C	16.65 ± 0.28 DE
Pinotage	13.6 ± 0.22 B	0.0063 ± 0.0003 B	21.95 ± 0.14 A

Table 2. Date of grape susceptibility to fly oviposition and mean number of eggs laid per replicate and adult emergence.

Variety	Date of Oviposition	Mean Number of Eggs Laid	Mean number of Adults Emerging
Viognier	17 August	4.38 A	1.14 A
Vidal	N/A	N/A	N/A
Cab Franc	25 August	2.06 AB	0.58 AB
Petit Manseng	31 August	0.034 B	0.0 B
Petit Verdot	N/A	N/A	N/A
Pinotage	9 September	0.14 B	0.04 B

Table 3. Varietal evaluation of larval survivorship on manually damaged grapes

Variety	Mean Number of Eggs Laid	Mean Number of Adults Emerging	% Survivorship
Viognier	7.83 AB	2.5 A	32
Vidal	14.5 A	2.58 A	18
Cab Franc	6.2 AB	2.33 A	37
Petit Manseng	3.9 B	1.0 A	26
Petit Verdot	7.33 AB	2.08 A	28
Pinotage	11.2 AB	3.0 A	27

Commercially available SWD trap evaluation

Introduction

Ever since SWD was detected on the west coast of the US in 2008 researchers have been trying to develop a trapping system that would be effective at detecting the first generation of flies in the area as well as estimating populations. Trap designs have ranged from simple solo cups with apple cider vinegar (ACV) to elaborate deli cups with fermenting bait cups within the trap. This project evaluated the commercially available trapping systems to determine which system had the best design as well as evaluating the flies captured. Traps were evaluated based on fly numbers caught, ease of assembly and deployment and bait specificity. Trapping was conducted at two vineyards for 5 weeks starting 27 July (just after véraison) through 31 August 2015 (harvest). The first vineyard was located in Orange Co. and the second was located in Albemarle Co. The Orange Co. location was considered a high pressure location while the Charlottesville location was considered low pressure. This experiment will allow growers to select and utilize a trapping system that best suits their SWD pest management needs.

Materials and Methods

Traps to be evaluated were the Trécé Inc. PHEROCON® SWD baiting system, Biobest DROSO TRAP® baited with their DROS'ATTRACT® liquid attractant, and Alpha Scents SWD lure combined with a

8.5” X 11” yellow folding sticky card (Fig. 1). The standard to collect data from these fields has been the ACV and Merlot mixture (60/40) at 300 mls in a clear plastic deli style container and a control of 200 mls antifreeze in a clear deli style container. Five replicates per location were conducted with the traps hung in Cabernet Franc grapes at location one and Viognier grapes at location two. The red grape variety at location two had been recently culled due to disease which left large gaps between vines. Each replicate was randomized within the row. Traps were set two frames in from the edge and were given a two frame buffer between traps. Trapping rows were re-randomized each week with liquid baits changed biweekly and the alpha scents lure change after 3 weeks. Flies were collected, identified and counted back at the Virginia Tech lab in Blacksburg, Virginia. All data were analyzed using an ANOVA, and Tukey-Kramer was used to separate the means.

Results

While only a quarter of the trapping data has been analyzed there seems to be a decisive trend occurring among all the traps. The most attractive trapping systems appear to be the ACV and Merlot (60/40) mixture and the Biobest trapping and baiting system. Both traps capture large amounts of flies, however there are several hundred other species of drosophila captured besides SWD. This significant bycatch takes much time and effort to sort through. None of the traps tested showed any specificity for SWD (Table 4). All traps did capture SWD flies except the blank, but large numbers of SWD were seen in the ACV / Merlot trap as well as the Biobest trap. There was a significant difference between trapping locations and total number of flies caught; however, both locations showed the same fly capture trends among all of the trapping systems.

Discussion

Regardless of numbers captured the ease of trap deployment and lure / bait deployment varied among the trapping systems. The ACV / Merlot mixture was the easiest to acquire, set-up and deploy. It was also the least expensive and easiest from which to collect flies. It captured both male and female flies, but had lots of other *Drosophila* to sort through to gather data. The Biobest trapping system and bait also captured lots of flies, but it recommends using their liquid bait in combination with the red trap. The liquid bait smelled similar to our wine bait which may be why it also captured large amounts of flies. It was easy to hang in the vineyard wires, but due to the rounded nature of the trap was sometime tricky to set down when trying to empty the baiting liquid and refill. It also had to be ordered through the mail and may not be cost effective for some growers. Counting flies also became difficult due to a mucus-like film that developed on the surface of the liquid bait. This also hindered fly counts back in the lab. The Trece trapping system had its own container and all that was required was to put the lure into the cup and fill the base with antifreeze. The lures did not smell very pleasant and the holes for flies to get into the trap were set low on the cup, so any sloshing of the trap caused antifreeze and flies to fall out. If the holes were higher on the trap or rectangular towards the top of the trap it would be easier to use and data would not get lost out the sides of the trap. It however did not catch very many flies at all, so we would not recommend this trap. The last lure was from Alpha Scents and was a scented lure in a black plastic sachet. This lure was not pleasant to work with, either, due to the smell of the lure as well as the use of a large sticky card. It was sometimes difficult to find space to hang the trap in the vineyards due to the dense canopy of vines. The sticky card would get caught in the vines and usually only one half of the trap was exposed to flies instead of both sides. It was also difficult to find female flies on the cards due to their ovipositor being difficult to see. Males were easy to see against the yellow, but female flies are more economically important to identify. This lure was not specific for SWD and also had lots of extra flies and other insects attracted to it due to the yellow color. Overall I would recommend the home-made ACV / Merlot trap or the Biobest trapping system to be sure you captured SWD if they were present. If growers would like to look for the first occurrence of SWD early on in the season I would recommend setting the traps along vineyard boarders. If growers want to know when the flies actually are in the vineyard blocks themselves I would set the traps in the grapes. There is still no correlation between trap captures and fly populations know to this date. It is still merely a qualitative tool.

Table 4. Mean number of SWD male and female flies captured in different trapping systems at two separate vineyards in Virginia. Other drosophila captured were also counted and the means per trap represented*.

Vineyard	Trapping System	Mean number of Males	Mean number of Females	Other Drosophila
1	Trece	9.0 C	9.2 B	64.6 B
1	Biobest	51 A	53.2 A	222.2 A
1	ACV /Merlot	24.8 B	53.6 A	171.2 A
1	Alpha Scents	8.8 C	0.2 B	44.6 B C
1	Blank	0.0 C	0.0 B	0 C
2	Trece	8.6 B C	1.8 B	63.4 B C
2	Biobest	23 A	22.2 A	103.4 A
2	ACV /Merlot	19.2 A	22.2 A	228.8 A
2	Alpha Scents	6.0 B C	0.2 B	17.8 C
2	Blank	0.0 C	0.0 B	0.0 C

*Numbers not connected by the same letter are statistically significantly different from one another as separated by Tukey-Kramer at 0.05.

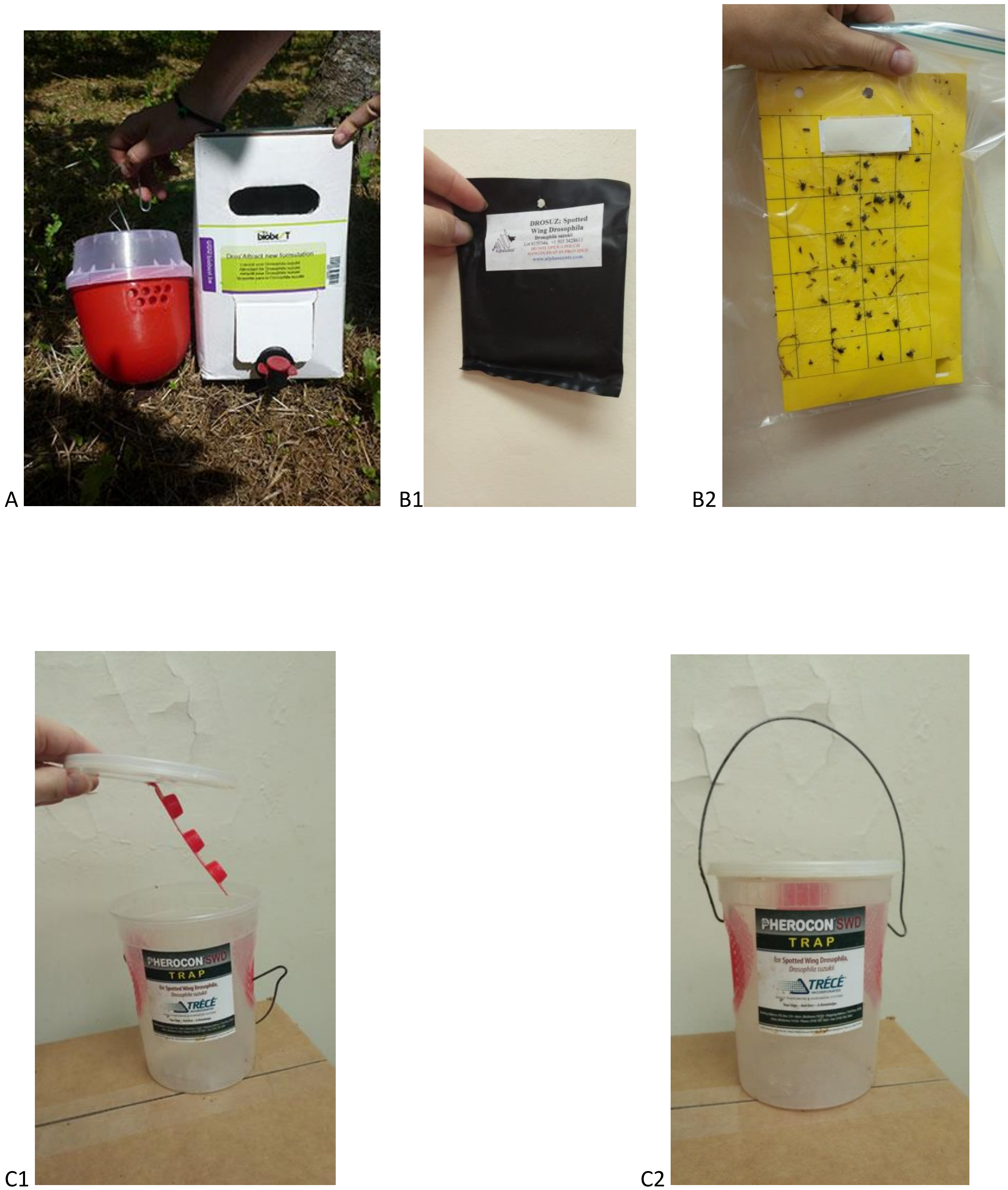

Figure 1. Various trapping systems employed to attract SWD adults. A. Biobest DROSO TRAP® baited with their DROS'ATTRACT® liquid attractant. B1 and B2. Alpha scents SWD lure with yellow sticky card. C1 and C2. Trece Pherocon SWD trap with lure.

Interspecific competition between *Drosophila suzukii* and *Zaprionus indianus* larvae in four varieties of Virginia wine grapes

Introduction

The insect pest community within Virginia vineyards has changed dramatically over the past decade with the introduction of several new invasive species. The latest introductions have been the spotted wing drosophila, *Drosophila suzukii* (SWD), and the African fig fly, *Zaprionus indianus* (AFF). While SWD is a direct pest of wine grapes impacting production by ovipositing into individual grapes, AFF is a secondary pest which may use SWD oviposition wounds as well as cracked grapes as oviposition sites. Both fly species are capable of introducing pathogenic infections such as yeasts into the grape clusters. Depending on the level of larval infestation of the grapes, and the resulting sour rot, the whole cluster may be unsuitable for use in wine production. An observation by a Piedmont wine grower in 2012 estimated 80% of a grape variety was lost due to fly infestation and sour rot. The grower had an infestation of SWD, however the majority of flies in the field and adult flies reared in the lab from infested grape clusters were identified as AFF.

The relationship of fly larvae within a food source has been reported to be competitive, thus leading to the increased mortality, decreased growth and reduced fecundity of the competing individuals based upon density. The degree of interspecific competition was measured by larval and pupal mortality, pupal volume, developmental time and the number of eggs laid by emerging SWD females. The objective of this study was to improve understanding of the interactions of AFF larvae on SWD larvae within the vineyard by using four varieties of Virginia wine grapes.

Materials and methods

Four grape varieties were chosen for this study: Petit Verdot, Cabernet Franc, Petit Manseng and Viognier. The varieties selected included two red and two white varieties. These grapes also had very distinct characteristics from one another such as penetration force needed to pierce the skin, skin thickness, degrees brix as well as having both tight and loose cluster formations. Larval densities on the various wine grapes (SWD:AFF) varied: 2:2 and 1:1 on a single wine grape. Each grape variety was weighed and degrees Brix were recorded. Viognier is the only grape variety that has been fully examined at this time and the mean weight per grape was 1.5 g and mean degrees Brix of 19. Controls for each were 4 and 2 SWD larvae per grape. Ten reps for both the interspecific competition and controls were conducted. L1 larva instead of eggs and were placed onto a single grape. Grapes had been pulled from the cluster to expose the flesh where the pedicle was separated from the grape. This allowed direct access to the grape flesh for the deposited larva to invade. Grapes were then placed into a petri dish and sealed with Parafilm. Dishes were held in a growth chamber at 12:12 L/D at 23° C until pupation.

Competition Evaluation. Larval and pupal mortality were observed and recorded during both studies. Pupae were removed from the grapes and measured (length and width) to determine pupal volume* (Takahashi and Kimura 2005). Pupae were monitored until adult flies emerged.

Developmental time from egg to adult was recorded. The sex of each fly was determined and fitness was evaluated based on the lifetime egg production of any females that emerged.

$$*V = \frac{4}{3}\pi\left(\frac{w}{2}\right)^2\left(\frac{l}{2}\right)$$

Results

Viognier is the only grape variety that has been evaluated thus far since the experiments utilized grapes that were at their prime just before harvest. The three other grape varieties are still being evaluated at this time. The developmental time for SWD at a density of two fly larvae per grape (the lowest density tested) was 11.83 days for females and 11.57 days for males. When SWD and AFF were on a single grape at a density of 1:1 the developmental times increased to 13 days for females and 12.25 days for male flies (Table 5). Pupal volume also decreased when SWD was reared in the presence of AFF at the 1:1 density. There was also significant larval mortality in both the controls and the interaction study, however both pupal mortality and overall mortality were less in the SWD-only grapes. The pupal volume for the four SWD and 2:2 (SWD: AFF) density was not significantly different. However, the developmental time and overall mortality increased for SWD in the presence of AFF. Pupal mortality was also high for SWD when in competition with AFF. When comparing both the four SWD control and the 2:2 (SWD: AFF) densities to the two SWD controls there is a significant difference in both the developmental times and the overall mortality (Table 5).

Discussion

This study shows that SWD in the presence of AFF experiences an increase in mortality at both densities tested in Viognier. This is also seen within the SWD alone controls when the mortality of SWD increased based solely on fly larvae density. This increased mortality within the grape is important to note. It may be that each grape may only be able to support low densities of SWD larvae. If that threshold is reached within a single grape then most of the larvae within the grape should die. An increase of SWD mortality by the presence of AFF or by SWD densities within the grapes themselves may benefit growers by limiting SWD population growth. This was the largest grape variety tested (grams) and if it is not capable of supporting large numbers of SWD; this bodes well for the following varieties left to test in this experiment. The size of the grape may be the greatest factor in limiting the SWD population growth within the vineyard. Another note is if AFF follows every SWD oviposition wound and 80% of what emerges is AFF (seen in lab reared larvae from grapes in 2012), which cannot oviposit into intact grapes, then the SWD populations should decrease. Therefore the amount of damaged clusters should also decrease or remain the same based upon the number of SWD females that emerge.

Table 5. Summary of interspecific density competition study between D. suzukii and Z. indianus on a single Viognier grape.

	Density	Mean Pupal Volume (mm^3)	Mean Developmental Time (days)	Laval Mortality (%)	Pupal Mortality (%)	Total Mortality (%)
Viognier	2 SWD Control	0.5f, 0.39m	11.83f, 11.57m	57.5	17.4	50.0
	4 SWD Control	0.467f, 0.397m	12.26f, 12.18m	57.5	32.4	61.25
	1:1 (SWD: AFF)	0.49f, 0.33m	13.0f, 12.25m	45.0	27.0	60.0
	2:2 (SWD:AFF)	0.51f, 0.39m	13.75f, 13.0m	50.0	42.8	70.0

Control Study Using Differing Modes of Action, and Impacts of Berry Closure Treatment

A chemical control study was performed in a 7-year-oledePinot Noir vineyard block in Amherst Co.

Treatment protocol:

All treatments were applied as foliar sprays by hand using a CO_2 powered backpack sprayer set at 40 psi with a single wand equipped with an 8008VS stainless steel spray tip. Treatments were applied in the fruit zone until run-off. A single replication consisted of two adjacent vines.

Surround treatments were applied on 25 June prior to cluster closure. Pairs of vines were either sprayed with Surround WP or untreated.

Late season treatments were applied on 11 and 18 August, in both the 25 June treatments. Each treatment was applied to two pairs of Surround-treated vines and two pairs of non-treated vines. Plots were sampled prior to treatments on 11 August, and again on 14 August (3 DAT), 18 August prior to the second treatment application (7 DAT), and 25 August (7 days after second treatment). Commercial harvest began on 15 August.

The following treatments were included:

June 25: Surround WP (25 lb/acre) or Untreated; replicated 8 times

August 11 and 18
(replicated 2 times over June 25 Surround and Untreated vines):

1. Spinetoram (Radiant SC*) 12 oz/acre
2. Pyrethroid (Mustang Max) 8 oz/acre

3. Kaolin (Surround WP)   25 lb/acre
4. Untreated;

*Note: Radiant SC contains same active ingredient (spinetoram) as Delegate WG

Sampling protocol:

In a pre-spray sample on 11 August, one cluster of grapes was collected from each pair of vines to be treated. All grapes were then dissected and inspected for *Drosophila* injury and / or presence. Very little damage and no *Drosophila* larvae were detected in the pre-spray sample.

For all the other (post treatment) sample dates, one cluster per plot was harvested and kept in zippered plastic bags at ambient temperature for approximately 48 hours. All grapes were then dissected and inspected for *Drosophila* injury and / or presence. Percent injured berries were calculated. Sugar content was calculated from 10 randomly selected berries using a refractometer.

Percent injured berry data for 18 and 25 August samples are presented in Tables 6-7. Statistical analyses are pending; however, it appears that a berry closure application of kaolin (Surround) reduces subsequent injury at harvest by 30-50% (Table 8). This is noteworthy because while a spray timed at berry closure is much earlier than the time that berries become susceptible to SWD oviposition, this is the last time to get some active residue into the interior of clusters, where oviposition sites often occur.

All three modes of action (kaolin – particle film technology), spinetoram (spinosyn), and zeta-cypermethrin (pyrethroid) appeared to provide control of SWD, and control was usually enhanced by an earlier, berry closure spray of kaolin.

Table 6. Effect of berry closure (25 June) application of kaolin on percent Pinot Noir berries injured by spotted wing drosophila treated on 11 and 18 August with various insecticides, sampled 18 Aug.

18 August Sampling	**Late treatments (11, 18 Aug)**			
Berry Close Trt (25 June)	Spinetoram	Zeta-cypermethrin	kaolin	No late treatment
No Surround	3.9	2.7	7.6	9.0
Surround	2.6	1.3	5.0	6.0

Table 7. Effect of berry closure (25 June) application of kaolin on percent Pinot Noir berries injured by spotted wing drosophila treated on 11 and 18 August with various insecticides, sampled on 25 Aug.

25 August Sampling	**Late treatments (11, 18 Aug)**			
Berry Close Trt (25 June)	Spinetoram	Zeta-cypermethrin	kaolin	No late treatment
No Surround	7.8	5.2	9.9	13.6
Surround	3.6	7.8	4.5	14.0

Table 8. Percent reduction in SWD injury resulting from a berry close (25 June) application of kaolin.

	Sample Date	
Insecticide Applied Aug 11,18	**18 Aug (19.3° Brix)**	**25 Aug (20.5°)**
Spinetoram	32.9	53.7
Zeta-cypermethrin	50.9	(50.7)
Kaolin	34.6	54.7
Control	32.8	(2.5)