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Assessing Spotted Wing Drosophila Injury Potential on Grape Production

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Spotted wing drosophila, *Drosophila suzukii,* Matsumura (Diptera: Drosophilae) (SWD) is an invasive vinegar fly of East Asian origin, that was recently introduced into the United States. It was first found in California in 2008 and is now found in all major fruit-growing regions of the country including Pennsylvania. It was first discovered in Pennsylvania's Lake Erie grape growing region in the late fall of 2011. The potential infestation rate of spotted wing *drosophila* differs from other vinegar flies because the female possess a serrated ovipositor that cuts into healthy fruit to lay eggs. Consequently, spotted wing drosophila (SWD) larvae can be found in fruit that is just ripening. During egg-laying, it is believed that sour rot and fungal disease can also be introduced, further affecting the fruit quality. SWD are thought to overwinter primarily as adult females, and they prefer moderate, cool wet climates similar to the Lake Erie grape belt. Adults live approximately two to nine weeks. During this time, one adult female can lay 100 to 600 eggs in fruit. During peak temperatures, a female can lay more than 100 eggs a day. Such a high reproduction rate indicates the SWD's high potential for fruit infestation and their potential for spreading rapidly through a field or a vineyard. Eggs hatch in two hours to three days with the larvae feeding in the fruit for about 3 to 13 days before pupating into adults. Thus, multiple generations occur per year. *Drosophila suzukii* is now one of the most serious pests of thin-skinned fruits including blueberry, raspberry, cherry, grape, and strawberry (Kinjo et. Al. 2013)[1]. Because this pest is similar in appearance to common vinegar flies, the greatest problems have occurred when populations went unnoticed and thus remained untreated until they caused considerable damage to crops.

For the purpose of these experiments two white varieties of grapes; (Vidal and Niagara) and two red varieties of grapes (Chambourcin and Concord) were used. The winters of 2013-2014 and 2014-2015 experienced record breaking low temperatures and many of the wine grape cultivars were lost for the 2014 and 2015 growing seasons. Consequently, the grapes used for this experiment are hardier varieties that were able to withstand the harsh winter.

Objective 1: Assess the risk potential and conduct host emergence and population studies of Spotted Wing *drosophila* on popular juice and wine grape cultivars in the Lake Erie grape growing region.

There are two trap formulas which reportedly are successful in the trapping of SWD. These traps consist of either cider vinegar or a yeast/cornstarch mixture with or without a yellow sticky card. Our previous research, and conversations with other SWD researchers, has indicated that the commercially available traps, such as the Liquid Reuse Fly trap, are less effective than solo cup traps at the beginning of the season. For this reason, we used only the red solo cup traps during the 2014 and 2015 growing season. In previous growing seasons, and at the beginning of the 2014 growing season, we baited traps with yeast/cornstarch, apple cider vinegar, and apple cider vinegar with wine. Trap catches showed only

a very slight variation in SWD numbers. The increase in SWD was not significant enough to justify the use of the yeast/cornstarch bait which is cloudy and spoils rapidly making it more difficult to separate the SWD from the regular fruit flies and this bait requires daily changing. The addition of wine slightly enhanced the traps catches, however, the addition of wine markedly increases the expense of the trap bait. Conclusions from other SWD researchers, with a few exceptions, state that red traps work the best and red is the favored color of SWD. It has been discovered that the more numerous the holes the greater the trap catches, so in 2014 we increased the number of holes contained in each trap to approximately ten on the lid and fifteen on the sides. Although making the traps more difficult to maintain, the addition of sticky cards enhances the trap catches inimitably and is worth using for a more comprehensive picture of SWD's phenology. Our traps were constructed using 18 oz. red plastic cups with holes punched in the lid and the top third of the cup. All of these traps catch numerous species of vinegar flies, so the trap catches were returned to the laboratory for identification. These traps were changed and SWD were enumerated twice weekly.

Twenty traps were set out in diverse locations during the 2014 and 2015 growing seasons. In August of 2014 a new pherocon SWD dual lure became available from Trece, Inc. This lure was designed to be used in combination with apple cider vinegar bait. We set up five traps in side by side comparisons in already existing vineyard locations from August 19th until cessation of trapping on November 14th. The Trece lure decidedly increased trap catches (See Figure 1.). In 2015 Trece improved their lure bait formulation. These improved lures were added to all the SWD traps during the 2015 season. Traps were placed in their locations on May 22, 2014 and May 15, 2015 and trapping cessation occurred on November 14, 2014 and November 20, 2015, when inclement weather conditions made trap collection inhibitive. The first female capture occurred on June 11, 2014 and May 26, 2015; the first male capture occurred on July 29, 2014 and July 14, 2015. The female captures occurred a month earlier in this region than in the 2013 season when the first male and female captures occurred on July 11th. This may be due to improvements in trap design and bait. It is probable from the early onset of trap catches that the SWD is overwintering in this area and could withstand the extreme cold winter temperatures of the 2013-2014 and 2014-2015 winters. Figure 2. illustrates the data from the 2013-15 seasons. SWD were present in all traps regardless of crop or location. Females were caught in traps before males and males were caught in the fall after the females. The numbers greatly increased at the end of the trapping season indicating that the SWD were seeking out food sources. (See Figure 2.).

Traps adjunct to wooded edges from the vineyards catch SWD earlier than the traps in the vineyard and these traps also captured SWD later into the season in greater numbers than the vineyard traps, indicating that the SWD prefer the wooded areas as an overwinter environment. (See Figure 3.) The first peak of SWD activity appeared to occur in early September. Degree Days were recorded for 1st male and 1st female captures. At this time, there appears to be no DD correlation with emergence. This insect is new to the region and to Pennsylvania so more years of data would need to be acquired to make an accurate DD comparison.

	2013	2014	2015
1st Male Caught	July 11	July 29	July 14
Degree Day from Jan 1st Base 50	1175.7	1404.1	1148.7
1st Female Caught	July 11	June 11	May 26
Degree Day from Jan 1st Base 50	1175.7	451.0	356.2

Ten grape clusters from each variety were destructively harvested throughout the grape growing season and placed in a salt water solution to determine if SWD larvae were present. Ten additional clusters were harvested and incubated for ten days to check for emergence of SWD. To test for larva inside the grape berries, we collected five clusters of each variety throughout the season. The individual grape berries were placed in a plastic bag containing a salt solution (4 C water: 1/4 C salt) and gently crushed**.** Employing this method the SWD larvae will float and the fruit will sink to the bottom of the plastic bag. Differentiating SWD from maggots of other species is nearly impossible; the SWD larvae have no obvious head which is not apparent to the naked eye. It is assumed that if the larvae are found in recently ripened and undamaged fruit it is SWD. Ten clusters of the four cultivars used for this experiment were harvested and placed in emergence cages (as pictured below) to see if SWD would emerge. This method is more definitive than the salt solution methods. However, it is not practical for growers to assess infestation using this method, because the SWD infestation will be well advanced by the time the adults emerge in the cages.

During the 2014 season, larva first emerged in the August 29, 2014 sample, but no adults emerged until the October 1, 2014 sample (See Figure 4.). In 2015, larva and adults both emerged from the September 16th sample, and by the last sample (September 30th) larva and adults were found in all four grape cultivars of this experiment (See figure 5). During the 2015 season other grapes cultivars were tested and by September 30th sampling adults were present in all samples except for wild (native) grapes.

Objective 2: To conduct choice and no-choice trials to assess spotted wing drosophila oviposition tendency on Concord, Niagara, and wine grape varieties.

During the 2014 season, No-choice, 2-Choice, and 4 Choice experiments were run and replicated ten times during the growing season. To implement the choice experiment, grape clusters were hung in cages for 24 hours with SWD. The clusters were removed and individually placed in emergence cylinders. (See pictures below) After fourteen days the clusters were removed from the cylinders and the numbers of emerged adults in the cylinders were counted.

It was determined during the 2014 season that SWD do not infest the grapes until after verasion. In the No-choice experiments all of the grape varieties were infested with SWD. Over the course of the 2-choice and 4-choice experiments SWD appeared to show a slight preference for Niagara grapes. In early September 2014 our in-house colony of SWD which is used for bagging experiments were infested with *D. melanogaster*. According to other scientists who raise SWD, this is not uncommon.

During the 2015 we checked our colony weekly and no infestation occurred.

No-choice testing in 2015 revealed that SWD infested all varities tested except the native wild grape. (See Figure 6) The 2-Choice experiment showed significance in the: Concord vs Wild, The Min 1235 vs Tramminette, and the Concord vs. Chambourcin. (See Figure 7) There was no significant differeces between cultivars when 2-Choice tests were compared overall. No significant differences between cultivars were detected in the four choice testing test. SWD did not seem to show a prefernce for color or cultivar.

Results, in total number of emerged SWD, in 4-Choice tests:

Niagara	**38**
Vidal	**9**
Concord	**39**
Chambourcin	**25**

Niagara	**28**
Vidal	**11**
Concord	**44**
Chambourcin	**22**

Norton	**28**
NY 81	**32**
Traminette	**39**
Min 1235	**54**

Traminette	**0**
NY 81	**8**
Norton	**35**
Min 1235	**0**

Trammettte	**9**
Vidal	**0**
Norton	**15**
NY 81	**0**

Norton	**16**
Min 1235	**5**
NY 81	**31**
Tramm	**9**

Norton	**78**
Min 1235	**21**
NY 81	**6**
Traminette	**42**

Objective 3: Bagging experiments to determine when the grape varieties are most susceptible to potential fruit injury by SWD.

Nylon bags were placed around 4 clusters of each variety containing approximately 0, 10, and 25 SWD per bag. These were left in the vineyards for 10 days and then the grape clusters were harvested and incubated in emergence cages, to watch for SWD emergence. Ten SWD appeared to be too low a number to guarantee infestation. Infestation did occur in all four grape cultivars during the 2014 season. The results were not conclusive during the latter portion of the season due to the contamination of our in-house SWD colony. Throughout the 2015 approximately 50 SWD were bagged with the four cultivars of grapes. On August 26, 2015 one cluster of Concord and one cluster of Niagara were infested with SWD. By September 9th, at least one cluster from each variety was infested with SWD. September 27th

every cluster in each cultivar tested except for the controls were infested with SWD. October 7th each cluster in all of the samples, barring the controls, was infested with SWD.

Conclusions from this research:

- SWD were present in all traps regardless of crop or location.
- SWD were only a problem on grapes after verasion.
- Females were caught in traps before males and males were caught in the fall after the females.
- In no-choice tests SWD attacked all grapes tested.
- In two-choice test SWD did not show a strong preference for cultivar of grape, color of grape, or brixs.
- SWD does attack injured grapes before non-injured.
- In four-choice tests SWD appeared to attack Vidal slightly less than the other grapes
- SWD is becomes a greater problem the later the grapes are harvested, due to late season rots.
- SWD does not appear to attack native wild grape unless it has no other food source

Impact Statement: The eastern vineyards are the largest producers of wine and juice grapes in North America. Most of these vineyards are located on the shores of the Great Lakes. The vineyards in Erie County are the most concentrated acreage of the Lake Erie grape belt, making SWD infestation between vineyards very likely. Given the propensity for this insect to spread and its potential to infect fruit, it is important to continue this research on the monitoring and management of SWD to minimize the risk of larval developing in fruit and affecting fruit marketability. The above research enhances the ability to forecast the extent and effect of SWD in the Lake Erie grape growing region. This forecasting leads to improvements in our capability to optimally time pest management decisions which should reduce both the direct cost of pesticide treatments and the indirect cost to non-target and beneficial species. This research also informs growers of the most effective ways to trap and scout for these Invasives in their vineyards.

Publications: The research from this grant is conveyed in real time to the Lake Erie Grape team to present at their growers meetings during the season. It is also incorporated into the weekly crop updates, , and the Penn State SWD updates. General information on the invasive species present in our area is presented at the growers' meetings. This information is published in the LERGP winter meeting Proceedings and the PA Wine Symposium Proceedings as well as in the International Cool Climate Wine Conference Proceedings**.**

[1]**.**Kinjo, H., Y. Kunimi, B. Takuya, and M. Nakai. 2013. Oviposition efficacy of Drosophila suzukii (Diptera: Drosophilae) on Different Cultivars of Blueberry. J. Econ. Entomol. 106 (4): 1767-1771.

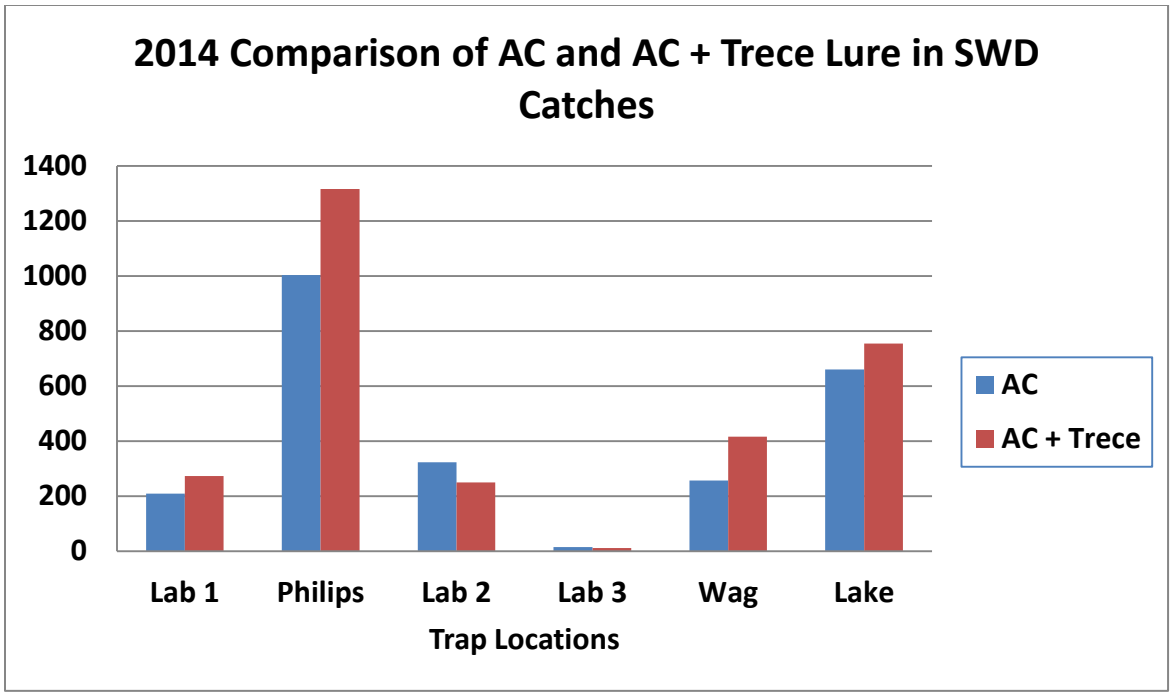

Figure 1. Comparison of traps baited with apple cider vinegar to traps containing apple cider vinegar and the newly developed Trece lure.

SWD Trap Data

	2013	2014	2015
Traps Started	12-Jun	22-May	15-May
1st trap catch male	11-Jul	29-Jul	14-Jul
1st trap catch female	11-Jul	11-Jun	26-May
Spike in trap catches	9-Sep	9-Sep	14-Aug
Verasion Concords	20-Aug	24-Aug	21-Aug
Last female trap catch	N/A	21-Oct	19-Oct
Last trap catch	26-Nov	14-Nov	20-Nov
Traps ended	26-Nov	14-Nov	20-Nov
% of SWD	25	37	24
Total SWD caught	2,887	7,578	13,234
Number of traps	12	19	19

Figure 2. Trap catch data from 2013-2015.

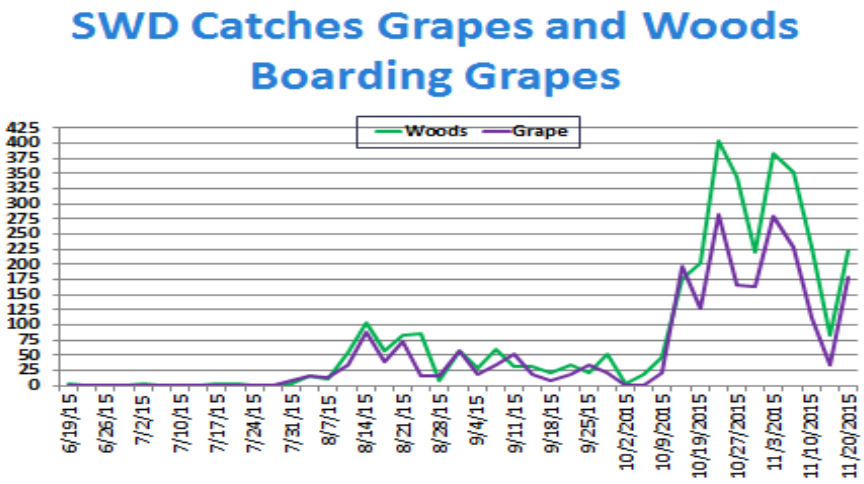

Figure 3. Comparison of a trap placed in the woods boarding grapes and a trap placed in the vineyard edge.

	Concord		Chambourcin		Niagara		Vidal	
	Larva	Adults	Larva	Adults	Larva	Adults	Larva	Adults
7/23/2014	0	0	0	0	0	0	0	0
8/12/2014	0	0	0	0	0	0	0	0
8/29/2014	0	0	0	0	2	0	0	0
9/8/2014	0	0	0	0	1	0	0	0
9/24/2014	0	0	0	0	1	0	0	0
10/1/2014	1	0	0	0	1	2	0	0
10/8/2014	2	1	1	0	3	4	0	0

Figure 4. 2014 Number of Larva and Adults from 10 sampled clusters of each grape variety

Number of Larva and Adults from twenty sampled clusters of each grape variety

	21-Aug		11-Sep		16-Sep		25-Sep		30-Sep	
	Larva	Adults	Larva	Adult	Larva	Adult	Larva	Adult	Larva	Adult
Chancellor	0	0	0	0	0	0	3	25	1	12
Concord	0	0	0	0	1	4	3	12	3	15
Niagara	0	0	0	0	4	8	2	10	4	32
Vidal	0	0	0	0	3	6	5	30	5	29
Min 1235			0		0		11			
Marquette			0				0		3	
LaCrescent			0				0			
Chambourcin			0						1	
NY 81					0		0			
Traminette					0		0		2	
Noiret					0		0			
Wild							0		0	
MN 1189							16			
Norton					0		2			

Figure 5. 2015 Results of field sampling for each cultivar.

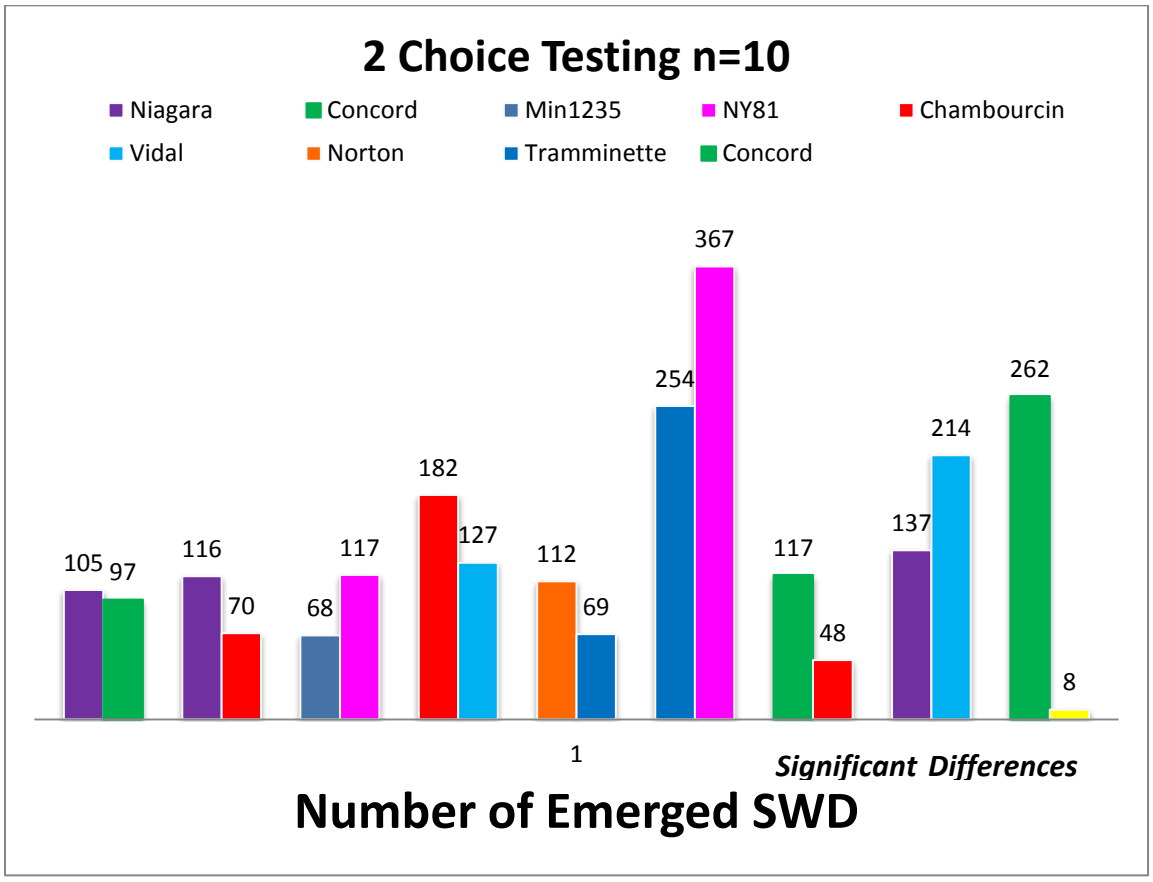

Figure 6. Two Choice testing results. Numbers of emerged SWD above each bar.

Budget Expenditures:

	2014	2015
Wages:		
Summer University Student Help	3633.00	1313.00
Fringe	279.52	104.20
Wage Payroll Employee		2007.00
Fringe		743.00
Travel:	593.99	
Supplies:		
Bags for netting grapes, rearing supplies (SWD food, Plastic buckets for rearing cages,) Bags for samples, salt for emergence test, sticky cards	928.49	1440.17
LERGR&EC Grape Crop Maintenance		978.83
TOTAL:	**5,435.00**	**6,586.20**