DROSOPHILA SUZUKII DEVELOPMENT & ATTRACTION

by

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In the effort to improve the efficacy and sustainability of organic food production, approaches to combat crop pests without the use of pesticides are necessary. The *Drosophila suzukii* pomace fly, known as spotted wing drosophila or SWD, is an invasive pest that causes significant economic damage to important fruit crops. Creating a sustainable integrated pest management program for SWD requires a specific understanding of its development and survival as well as effective approaches for managing quick-growing populations. Part 1 of this study focuses on degree-days required for SWD development and the effects of extreme temperatures on SWD infestation success and survival. Blueberries were infested with lab-reared wild flies to examine the development time from egg to adult, which was found to be significantly faster than previous lab studies under constant conditions. Maximum daily temperatures negatively affected SWD infestation success and may negatively affect SWD survival. Part 2 of this study examined the efficacy of SWD pheromones for short-range attraction for use in mass trapping. Pheromone extracts were used in short-range flight assays and contact assays to test their attractiveness to SWD. Pheromone extracts were not attractive to SWD, so have been concluded to be ineffective as a lure for trapping. These results provide important insights about SWD development and ecology, adding to the collective knowledge of SWD biology to allow the development of a more sustainable approach to controlling SWD in fruit crops worldwide.

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PART I: DEVELOPMENT OF *DROSOPHILA* SUZUKII IN THE FIELD

Background & Introduction

In the effort to improve the sustainability and efficacy of organic food production, approaches to combat crop pests without the use of pesticides are necessary. The *Drosophila suzukii* pomace fly, known as spotted wing drosophila or SWD, is an invasive pest that damages many economically important fruit crops, likely causing millions of dollars of damage. Spotted wing first appeared in cherries in Japan in 1916 and spread through parts of China, Korea, and Russia by the 1930s (Lee et al. 2011). In 2008, SWD was detected in California infesting strawberries and caneberries; by 2009 it had spread to Oregon, Washington, Florida, and British Columbia, and by 2011 it was present in many U.S. states and Mexico (Lee et al. 2011). Since then, it has spread throughout the Americas, most of Europe, and Asia (Asplen et al. 2015).

While other vinegar and pomace flies can only infest rotting fruit, female SWD have a serrated ovipositor—the organ that deposits eggs into fruit hosts—that can saw through the tough outer layer of fruit, allowing them to infest ripening fruit (Atallah et al. 2014). Thus, an infestation of SWD can be an enormous threat to the viability of fruit crops even before they are ready for harvest. SWD exhibits a very wide host range, infesting ripening blackberries, cherries, blueberries, peaches, raspberries, strawberries, table and wine grapes, and damaged or split apples, apricots, persimmons, loquat, greenhouse mandarins, and tomatoes (Lee et al. 2015). Infestations of SWD are a

significant concern across many locations and throughout many different fruit industries.

Effective management of SWD is difficult due to its wide host range and short generation time (Lee et al. 2011). Thus, an infestation of SWD can grow extremely quickly and be difficult to eradicate. Infestations can be very costly to farmers as they require increased expenses for pesticides, additional labor for monitoring and management, and can lead to significant yield losses or rejection of ripe fruit by processing facilities (Goodhue et al. 2011). Currently, management techniques are mostly limited to pesticide sprays on ripening fruit, monitoring for adult activity, and monitoring fruit for larval infestation (Lee et al. 2011). Organic growers have been especially hard-hit by SWD infestations because few organically-labeled pesticides are effective in controlling this pest (Bruck et al. 2011) and infested fruit is not marketable to consumers.

Early studies were done to provide organic and conventional growers with immediate chemical options, but other techniques will be necessary to develop a more sustainable integrated pest management program for SWD; long-term research is examining the use of mass trapping, field sanitation, semiochemicals, biological control, landscape management, and postharvest treatment for controlling SWD (Lee et al. 2011). Biological control research, the management of a pest through the use of natural enemies, has examined the efficacy of larval and pupal parasitoids in native and introduced ranges (Chabert et al. 2012; Kacsoh and Schlenke 2012; Cini et al. 2012; Rossi Stacconi et al. 2013; Gabarra et al. 2015; Stacconi et al. 2015), of predators including Orius sp. (Hemiptera: Anthocoridae), Dalotia coriaria (Coleoptera:

Staphlyinidae), and Labidura riparia (Dermaptera: Labiduridae) (Woltz et al. 2015; Gabarra et al. 2015; Renkema et al. 2015), and of fungal and bacterial entomopathogens (Naranjo-Lazaro et al. 2014; Woltz et al. 2015).

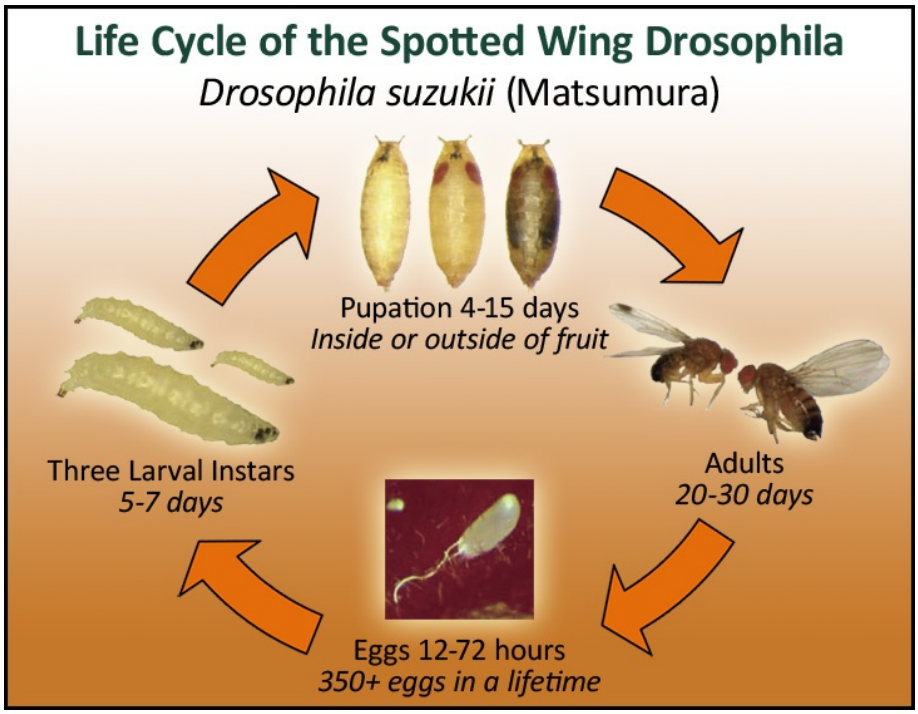

Figure 1: The life cycle of SWD. Courtesy of the Washington State University Extension Service.

Knowledge of the basic biology of SWD development and survival is instrumental to research regarding their management. The life cycle of SWD (Figure 1) begins when a mated female lays eggs into ripe fruit. Upon hatching from the egg, larvae feed on the inside of fruit, causing it to soften, turn brown, and rot (Lin et al. 2014). SWD develop through three larval instars, or stages, while still inside the fruit but may develop into pupae inside or outside of the fruit. Next, they emerge from their pupae shell as adult flies and can mate after approximately 1-4 days (Lee et al. 2011). Understanding development in the field and mechanisms of SWD population increase is

important for conducting research using live SWD, and for timing of pest control measures (Lin et al. 2014).

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Many previous studies establish development times of flies reared in the lab across differing conditions. Hamby et al. (2016) provided a table summarizing recent development time studies. Days to development, also shown as cumulative degree-days in Table 2 as per my calculations, varied based on temperature, relative humidity, and host type (Hamby et al. 2016). A study by Tochen et al. (2014) examined the net reproductive rate and intrinsic rate of population increase of a lab colony of SWD reared across temperatures ranging from 50-86 ˚F (10-30 ˚C). Development time was found to decrease up to an optimal temperature of 83 ˚F (28 ˚C) and survival was lowest outside the 50-86 ˚F (10-30 ˚C) temperature range (Tochen et al. 2014). In a subsequent study, Tochen et al. (2015) examined the effect of relative humidity on SWD development in the lab. With RH ranging from 20-94%, it was found that the shortest development time was in the 82% RH regime; no other significant differences were found among other treatments. RH was also found to have a significant effect on survival: mean survival of females at 20% and 30% RH was less than 3 days, but survival at 71%, 82%, and 94% RH was 20 or more days. Similar results were found in males. The percentage of female flies with mature oocytes, cells in the ovary that will eventually become eggs, and the number of mature oocytes per female increased with RH.

These studies are useful in establishing approximate development times for SWD under different temperature and RH conditions in the lab and for understanding how these factors affect SWD development and survival. These lab studies do not reflect the dynamic conditions of the field environment, where temperature and RH fluctuate daily. Thus, they may not provide accurate development times for SWD in the field. Much of the current research on integrated pest management methods for SWD is conducted in the field under these varying conditions, so a more accurate understanding of development time is instrumental for planning and carrying out these projects. There is a need for a comparison development in the lab to development in the field that accounts for the effect of fluctuating temperature and RH in the outdoor environment.

Questions

My research focuses on degree-days required for SWD development and the effects of extreme temperatures on SWD infestation success and survival. Many plants and invertebrates need a certain amount of heat to develop. The total amount of heat required for an organism to develop is calculated in degree-days (DD), or the accumulated product of time and temperature between the organism's upper and lower developmental thresholds (UC ANR 2014). Understanding SWD in terms of degree-days provides a universal measure, which can be applied to experiments regardless of location and climate. I investigated the following questions: What are the cumulative degree-days (cDD) needed for SWD to develop from egg to adult in the field? What are the observed cDD for development to larvae, pupae, or adult? Do daily maximum and minimum temperatures impact SWD infestation success? Do daily maximum and minimum temperatures impact SWD survival?

Methods

Study site

This study was carried out at the USDA-ARS Experimental Farm (Corvallis, OR) in 'Liberty' blueberries.

Wild D. suzukii collection and rearing

To establish a colony of wild SWD for use in field experiments, I made traps out of Rubbermaid Tupperware sandwich boxes (Figure 2). An 8 x 8 cm square was cut out of the lid and plastic canvas was hot glued over the hole. The openings in the plastic canvas are large enough to allow SWD to enter the trap, but deterred other insects from entering. A hole was poked in each side of the container and wire was attached to hang the traps. Raspberries picked at Oregon State University College of Agricultural Sciences Lewis-Brown Farm (Corvallis, OR) were put inside the traps to attract flies. I hung the traps from wire trellises approximately one meter from the ground at the same farm in ripe blackberry fields where adult SWD were already infesting fruit. To ensure that all flies were approximately the same age, I hung the traps outside for 24 hours, after which I collected them and placed the infested fruit in rearing cages along with a 29.6 mL (1 oz.) cup of *Drosophila* diet made according to Woltz et al. (2015) and a water wick consisting of a soaked sponge placed through a hole cut in the lid of a 60 mL plastic deli cup. I monitored cages for adult SWD emergence and also for emergence of different species of *Drosophila* (which were promptly removed and

destroyed). This process was repeated as needed to keep a viable population of adult lies ready for infestation use.

Figure 2: Sandwich box traps for trapping SWD to establish a wild colony.

Infestation

I enclosed clusters of 15-20 green or blush—slightly pink and blue, but not completely blue—blueberries in mesh bags (Figure 3) with a drawstring closure with a small sponge (approximately 2.5 cm x 2.5 cm x 5 cm in size) to seal the opening. All clusters were bagged at the start of the experiment to prevent damage by birds or natural insect infestation. Within each trial, a set of 14 bagged clusters was established. A trial refers to a group of bagged clusters infested on the same day. A total of 7 trials were established. Each cluster was infested with 8 mated female flies that were collected from the wild colony with an aspirator. Each bag included a soaked sponge for water, a microcentrifuge tube filled with a 20% sucrose solution and plugged with cotton (called a sugar-wick) as a food source, a bent wire to retain bag shape, and was labeled with the

date, trial, and replicate number. Flies were allowed to oviposit in the fruit for 24 hours, after which the flies were killed and the sponge and sugar-wick were removed from the bag.

Figure 3: Bagged blueberry cluster. Includes drawstring and sponge closure and HOBO Data Logger.

Temperature and humidity

I measured temperature and RH with a U-023 HOBO data logger (Onset, Bourne, MA) placed in one bag of each trial. The logger recorded temperature and RH readings every half hour for the duration of the trial.

Dissection

Every day, I examined all bags and recorded the emergence of any adult flies. Within each trial, two bags were collected for dissection every 48 hours beginning 12 days after infestation and ending 24 days after infestation (Table 1). I dissected all berries under a dissecting microscope to record the number of flies present at each developmental stage—larvae, pupae, or adult. I dissected a total of approximately 1666 berries.

Day 0	**Day 12**	**Day 14**	**Day 16**	**Day 18**	**Day 20**	**Day 22**	**Day 24**
Infestation	First dissection	Dissection	Dissection	Dissection	Dissection	Dissection	Last dissection

Table 1: Timeline of infestations.

Data Analysis

Average cDD for Development

The recordings from two representative HOBO data loggers that spanned the duration of the study were chosen, and the daily maximum and minimum temperatures were input into a separate spreadsheet. To determine cDD for daily temperature, I used the University of California Davis Integrated Pest Management Degree-days Calculator <http://ipm.ucdavis.edu/WEATHER/#DEGREEDAYS>. The developmental thresholds used were 44.96 ºF (7.2 º C) (Tochen et al. 2014) as the lower and 86 ºF (30 ºC) (Asplen et al. 2015) as the upper threshold. Daily temperature maximums and minimums were input into the calculator. I chose a double sine method to fit a line to this temperature data, with a steep sine curve from the daily minimum to the daily maximum and a shallow curve from the daily maximum to the next daily minimum. I used a vertical cutoff, which assumes no development above the upper threshold of 86 ºF, because

SWD have been observed to be very sensitive to heat, often dying from above-threshold temperatures. For comparison, the development of adults was also calculated with a horizontal cutoff, which assumes a constant rate of development when temperatures were above 86 ºF.

The experimental results in Hamby et al. (2016), which summarize SWD development times from various other studies, were converted into cDD at the Fahrenheit scale [((Temperature of study – lower threshold) x days to develop) x 5/9].

RH was averaged from HOBO data logger readings from July 27-September 1, 2015.

Cumulative Degree-day Range Per Life Stage

Cumulative degree-days were analyzed from data including live flies to find the range for each life stage.

Infestation Success and Live SWD at Dissection

Using temperatures for the first three days after infestation, I compared average daily minimum and maximum temperatures with two-sided *t*-tests between samples with successful or unsuccessful infestations. Successful infestations were defined as those with any live or dead SWD at the time of dissection, as evidence that at least some eggs were laid and hatched.

Next, I divided the subset of samples with successful infestation into those having live SWD and those with no live SWD at the time of dissection. Using temperatures from the duration of the study, *t*-tests were used to compare daily minimum and maximum temperatures between samples with live or no live SWD. All statistical comparisons were done in JMP® 11.0.0 (SAS 2013).

Percent Live SWD

Percent live SWD for samples containing live flies of any stage was plotted (y-axis) separately with average daily maximum temperature and average daily minimum temperature (x-axis). I linear regression was used to test correlation between average daily minimum or maximum temperature and percent live SWD.

Results

What are the cDD needed for SWD to develop from egg to adult in the field?

It was found that development from egg to adult in the field took nearly half the time as in the lab, and development was much faster using a vertical cutoff than a horizontal cutoff. With a vertical cutoff, the average cDD at adult emergence among 8 individuals was 198.9 ± 5.7 (mean ± SE) with a range from 168-209. With a horizontal cutoff, the average cDD at adult emergence was 287.0 ± 8.0 with a range from 244-299. The average RH for the trial was 63.6 ± 0.56%. In 16 previous development studies, the average cDD for adult emergence was 375.8 ± 11.9 at the Fahrenheit scale, and the average cDD among 4 trials specifically in blueberry hosts was 368.6 ± 11.0. See Table 2 (below) for comparison.

Host	Temp (°C)	Daily DD	Relative Humidity (%)	Days to Development	cDD to Development		Reference
					Celsius	Fahrenheit	
Blueberry	varying	varying	65	ND	110.5	198.85	This study
Blackberry agar	24-27	ND	80	10.2	ND	ND	Bellamy et al. 2013
Blueberry	25	17.8	ND	10.6	188.68	339.624	Jaramillo et al. 2015
Blueberry	20.6	13.4	71	16.3	218.42	393.156	Tochen et al. 2015
Blueberry	22	14.8	60-70	14	207.2	372.96	Tochen et al. 2014
Blueberry	26	18.8	60-70	10.9	204.92	368.86	Tochen et al. 2014
Blueberry agar	24-27	ND	80	10.7	ND	ND	Bellamy et al. 2013
Cherry	22	14.8	60-70	14	207.2	372.96	Tochen et al. 2014
Cherry	26	18.8	60-70	10.8	203.04	365.47	Tochen et al. 2014
Cherry agar	24-27	ND	80	9.7	ND	ND	Bellamy et al. 2013
Grape agar	24-27	ND	80	12.1	ND	ND	Bellamy et al. 2013
Grape	25	17.8	60	16.9	300.82	541.48	Lin et al. 2014a
Media	22	14.8	25	12.8	189.44	340.99	Emiljanowicz et al. 2014
Media	20	12.8	50-65	14.9	190.72	343.30	Hardin et al. 2015
Media	20	12.8	60	16.8	215.04	387.07	Asplen et al. 2015
Media	20	12.8	60	17.1	218.88	393.99	Asplen et al. 2016
Media	25	17.8	ND	11.7	208.26	374.87	Jaramillo et al. 2015
Media	25	17.8	60	11.3	201.14	362.06	Kinjo et al. 2014
Media - molasses	20	12.8	50-65	15.6	199.68	359.42	Hardin et al. 2015
Media - yeast	20	12.8	50-65	15.5	198.4	357.12	Hardin et al. 2015
Peach agar	24-27	ND	80	10.3	ND	ND	Bellamy et al. 2013
Raspberry	20	12.8	50-65	14.7	188.16	338.69	Hardin et al. 2015
Raspberry agar	24-27	ND	80	10.1	ND	ND	Bellamy et al. 2013
Strawberry agar	24-27	ND	80	10.9	ND	ND	Bellamy et al. 2013

Table 2: Degree-days for development of SWD in other experimental studies. Includes 16 studies with varying substrate, temperature, and RH. Grey columns indicate my own data. Adapted from Hamby et al. (2016).

What are the observed cDD for development to larvae, pupae, or adult?

Larvae and pupae were both present over a long range of the trial, while adults were only present throughout a short range. Larvae were present from 85-234 cDD (Figure 3), pupae from 85-211 cDD, and adults from 168-216 cDD. This may indicate a varying development time for larvae and pupae. Additionally, as shown in Figure 4, the larval stage dominates from 84-105 cDD, pupal stage from 113-143 cDD and 146-170 cDD, and the adult stage dominates from 208-235 cDD. This information is useful for planning experiments which require us to predict when larvae, pupae, or adults may emerge from infested fruit; having these ranges in terms of cDD allows for the prediction of life stages at any specific location, regardless of weather conditions by simply counting cDD since infestation.

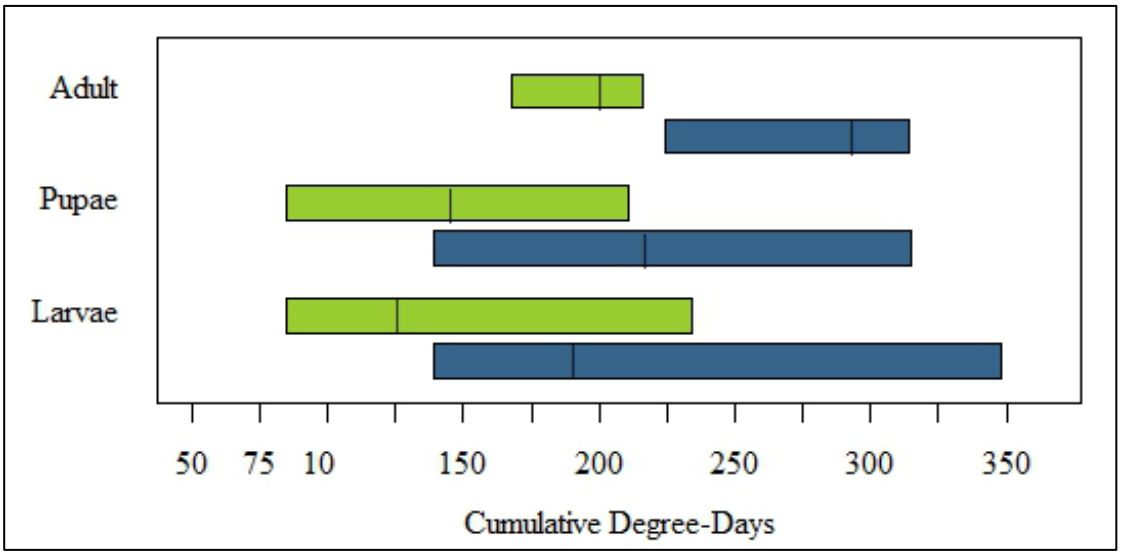

Figure 4: Ranges of cDD for observed larval, pupal, and adult life stages for SWD. Green indicates cDD for the vertical cutoff and blue indicates cDD for a horizontal cutoff.

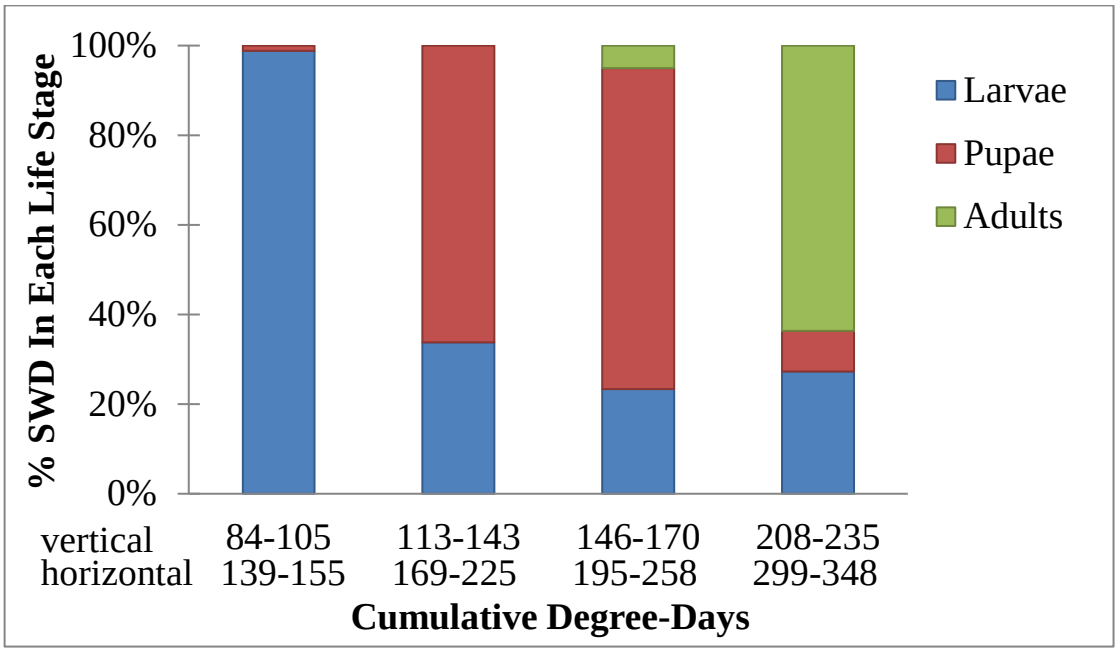

Figure 5: Proportion of each life stage throughout for different cDD ranges.

Do daily maximum and minimum temperatures impact SWD infestation success?

For these analyses, temperatures for the first three days of each trial were used, as they may affect the survival of the mother and thus whether she has the opportunity to lay eggs, as well as the viability of hatching eggs. An infestation was considered successful if there were any live or dead SWD present at dissection. Between groups of successful or unsuccessful infestations, a significant difference between maximum or minimum temperatures will indicate an effect of temperature on infestation. The average maximum temperature of unsuccessful infestations was 99.1 ºF, which is approximately 10 degrees higher and significantly different than the average maximum temperature for successful infestations (Table 3). Minimum temperatures were marginally significantly different, suggesting a marginal effect on infestation success.

	Maximum Temperature (ºF)	Minimum Temperature (ºF)
Successful infestation (n=44)	89.0 ± 0.31	56.8 ± 0.35
Unsuccessful infestation (n=54)	99.1 ± 0.56	57.9 ± 0.24
P-value	0.0068	<0.001
t Ratio	-2.73	-15.9
DF	236	248

Table 3: Differences in maximum and minimum temperatures (mean ± SE) for trials with successful or unsuccessful infestation.

Do daily maximum and minimum temperatures impact SWD survival?

Temperatures from the duration of the trial were analyzed to compare infestations with live or no live SWD. Maximum temperatures between samples with live SWD or no live SWD were marginally different, indicating that daily maximum temperatures may affect SWD survival (Table 4). However, results are not definitive. Despite a low P-value, there is a small effect size, as the mean maximum temperatures for live SWD / no live SWD are extremely close. No correlation was found by linear regression. However, due to extreme temperatures, I did observe reduced SWD survival in my trials, but observation alone is not sufficient to prove or disprove this effect. It remains unclear whether it is biologically significant. Minimum temperatures were not significantly different between samples with live SWD or no live SWD, indicating that minimum temperature does not impact SWD survival (Table 4).

	Maximum Temperature (ºF)	Minimum Temperature (ºF)
Live SWD (n=23)	92.7 ± 0.21	55.1 ± 0.17
No Live SWD (n=19)	92.2 ± 0.15	54.9 ± 0.14
P-value	0.061	0.366
t Ratio	1.93	0.914
DF	40	42

Table 4: Differences in maximum and minimum temperatures (mean ± SE) for trials with live or no live SWD.

Implications

Development in the field was considerably faster than previous lab studies; but, with a sample size of 8, further replication is needed to confirm this quick development time, which was approximately 177 cDD or 89 cDD faster than in published laboratory studies, with a vertical cutoff or horizontal cutoff, respectively. Reasons for quicker development in the field remain unknown, but SWD larvae under stress in the lab have been observed to pupate faster (J. Lee pers. observations). It is possible that the high temperatures experienced during this study represented stressful conditions for the SWD larvae, triggering more rapid development. Additionally, many previous studies used a variety of host substrates, which differentially affect development, possibly impacting the difference found between lab and field development times. Regardless, quicker development in the field suggests that lab studies cannot be extrapolated to completely understand populations of SWD in the wild.

Results from this study showed a varying development time for larvae and pupae, as both were present over a relatively large range of cDD—84-234 cDD for larvae and 85-211 cDD for pupae. This variation suggests that additional factors besides heat affect development time in SWD. For example, the number of larvae developing

within each berry would have affected the resources available and rate of fruit decay, which may have affected development rates unevenly throughout samples.

Maximum temperatures negatively affected infestation success and may affect SWD survival. These results reaffirm results from Tochen et al. (2014), who found more rapid development with increasing temperature and decreased survival above 83 ^{o}F.

The results of this study, though somewhat preliminary, suggest that SWD may be quicker to develop in the field than previously determined in laboratory studies. This information is useful for population modeling and planning SWD field research. Prediction models provide us with expected growth rates of unmanaged SWD populations, and show prevalence of life stages throughout the year to target management practices (Wiman et al. 2014). Knowing that temperature is a major factor in SWD development, these predictive models may need to be adjusted to include temperature parameters specific to development and survival.

In terms of planning research, the USDA-ARS Horticulture Research Unit has ongoing research studying the mechanisms of SWD pupation behavior in the field. These experiments involve estimating the pupation time of SWD within bagged infested clusters. Having a more accurate understanding of the development time in will be helpful in the planning and executing of this project and many other similar field experiments. This study suggests that infested fruit may need to be checked for larvae or pupae in a shorter timeframe than previously expected.

Future Improvements

If completed again or if a similar project is conducted, possible improvements are as follows. Twenty-four hours after infestation, when female flies are killed, it would be useful to put a clean mesh bag over the berry cluster to make counting emerged flies easier. The date of first adult emergence was recorded, but it would be helpful to also record the number of emerged flies. This would provide more observations on development time from egg to adult. Upon dissection, it was difficult to determine whether pupae present in the bag or berries were alive or dead; it would be helpful to rear all pupae in diet cups until emergence to determine their survival. Additionally, beginning dissection on day 8 instead of 12 would likely give a more accurate view of larval development, as some had already pupated before day 12 (pupae were commonly present already by day 12). Furthermore, to avoid maximum temperature-related casualties, it may be beneficial to begin the experiment earlier in the field season, when weather is cooler, in earlier-ripening fruit.

PART II: *DROSOPHILA SUZUKII* SEX PHEROMONE ATTRACTION ASSAY

Background & Introduction

Mass trapping, the use of high trap density (a large number of traps deployed over a given area), is used for detecting and controlling SWD infestations in crops and for experiments. Currently, attractants for these traps are based on fermentation volatiles present in vinegar, wine, and other fermentation products (Burrack et al. 2015, Cha et al. 2015). These attractants do not perform as well when nearby fruit hosts provide competing volatiles (Kanzawa 1939). Efforts are underway to incorporate fruit host volatiles into attractant formulas to improve their effectiveness (Abraham et al. 2015). However, research regarding the efficacy of other types of attractants is necessary to improve mass trapping. Improvements to lures may improve detection and control efforts if they significantly increase trap efficacy, or ability to trap more flies (Landolt et al. 2012).

The use of pheromones for trapping has been effective for some other insects, such as widely-used pheromone-based traps for the coddling moth (Witzgall et al. 2008). Semiochemicals, including pheromones, play an important role in search by insects for mates, food, and oviposition sites (Flint and Doane 2016). Sex pheromones of SWD are involved in courtship interaction and short-range attraction (Landolt et al. 2012). *Drosophila* produce non-volatile pheromones that are embedded in their cuticular hydrocarbons—important molecules on the surface of all insects—and sensed through taste sensilla on their legs and proboscis (Dekker et al. 2014). Thus far, no

Drosophila pheromones have been found to be useful in lures or traps, but there have been no movement or flight assays done with SWD pheromones published to-date.

Questions

While the role of pheromones on cuticular hydrocarbons in short-range attraction has been identified for SWD (Landolt et al. 2012), no published study has examined their ability to elicit behavioral responses, such as flying or walking toward the pheromone over a short range. If pheromones can attract SWD in the short range, they may be combined with long range volatile cues to improve trap monitoring. I ask the following research question: Can pheromone extracts from male or female SWD be used as attractants at a short range?

Methods

Separating Virgin Flies for use in Pheromone Extracts

Virgin flies were used to create pheromone extracts because they are more likely to have the most attractive pheromones, as they have not yet mated. To obtain virgin flies for use in extracts and assays, I put newly emerged flies into individual glass vials and examined them under a dissecting microscope. Females were distinguished by a serrated ovipositor and males by the presence of claspers in the genital region. Males and females were then put into separate cages (labeled by sex and date) with water and artificial diet until further use.

Pheromone Extracts

To create pheromone extracts, I aspirated 10 virgin female flies using a handheld aspirator and stored them in plastic vials. I labeled three sets of ten small glass vials (Thermo Scientific 12 mm x 32 mm DP clear glass vials) to use in the extraction process: one set of vials as controls, the second set of vials for soaking the flies in hexane, and the third set of vials for storing the extracts. A Hamilton 250 µL glass syringe was used to put 200 µL hexane into one set of extract vials and one set of control vials, which were then capped. Aspirated flies were anesthetized with CO_2 (using a Genesee Scientific CO_2 dispenser) and placed into each extract vial using metal forceps. Flies soaked in hexane for 10-20 minutes to extract their cuticular hydrocarbons, after which a glass syringe was used to remove the hexane and place it into the third set of extract vials, which were then capped. Control vials only had hexane added to them. I used a stream of N_2 gas from a pressurized canister for approximately two minutes to evaporate the hexane from the extract and control vials. Vials were stored at -4 ˚F (-20 °C) until assays were set up.

Short-Range Flight Assays

I set up individual assays in small plastic Bug Dorm cages (29.2 cm (11.5 in) x 29.2 cm x 29.2 cm in size) with mesh sides and large metal Bioquip cages (60.96 cm (2 ft) x 60.96 cm x 60.96 cm in size) with mesh sides. Each cage included two traps: one containing the pheromone extract and one containing the control (Figure 6). I made the traps for the small cages by hot gluing a 25 mL glass vial into the bottom of 295.7 mL (10 oz.) plastic cup. A drowning moat solution was created by filling the trap with ~91 mL soapy water, made with 4 mL unscented Seventh Generation Natural Dish Liquid to

3.8 L (1 gallon) of water, to just below the top of the glass vial. Traps for the large cages were made in the same way, but I used taller 25 mL glass vials and 946.4 mL (32-ounce) plastic cups, which were filled with ~370 mL soapy water. An extract or control vial was placed in the center of each trap by removing its lid, wrapping the lip in twist tie wire (Bond brand) to prevent it from falling, and placing it in the top of the 25 mL vial. I used cotton to seal the opening between the smaller and larger glass vials to deter flies from entering the 25 mL vial.

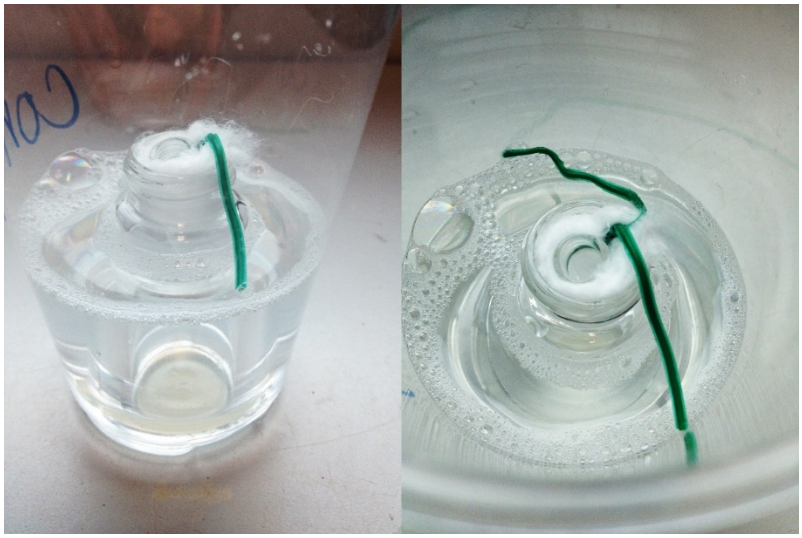

Figure 6: Pheromone traps.

For each assay, a single extract trap and control trap were present in the cage (Figure 7). In small cages, 50 virgin male or virgin female flies approximately 1-7 days old were added, and in large cages, 200 flies mixed sex flies approximately 7-14 days old were added. The cage was left for approximately 24 hours, after which I removed each trap and counted the number of trapped flies in each. Live flies remained in the cage and were re-used for the next trial that used the same response group. Cages were cleaned and traps were removed prior to being re-used.

Figure 7: Set-up of traps in Bug Dorm Cage. Photo by Jana Lee.

For a positive control to confirm that the arena and trap arrangement could elicit a positive response from flies, I set up five assays with apple cider vinegar (ACV) as the extract. ACV is a known attractant for SWD and is often used in monitoring traps. All combinations of extract and response group are shown in Table 5.

Cages were held in a greenhouse at the USDA-ARS Horticulture Research Unit (Corvallis, OR) with high ambient light and an approximate temperature of 71.8 °F. All assays were conducted between December 9 to 23, 2015.

Extract Origin	**Virgin Female**	**Virgin Female**	**Virgin Female**	**Virgin Male**	**Virgin Male**	**ACV**
Response Group	Virgin Male	Virgin Female	Mix	Virgin Female	Mix	Mix
# Trials	9	6	7	6	4	5

Table 5: Combinations of assay set-ups.

Contact Assay

To determine whether SWD pheromone attraction is touch-based, I set up a landing assay. Extracts were only taken from virgin females for this assay. To create the

extracts and controls for this assays, the same procedure was followed until the evaporation step. Once the extract was removed from the fly soak, the hexane from the extract and control vials was poured into glass petri dishes (Pyrex glass dishes, 9.5 cm diameter) and allowed to evaporate for approximately 5 minutes.

To set up the assay, I aspirated 4 vials of 20 virgin males. The same Bug Dorm cages were used and each contained one extract plate and one control plate. One vial of flies was emptied into each, and all cages were monitored approximately every 15 minutes for 4 hours. I recorded fly behavior and the number of flies present on each plate.

Data Analysis

To determine if the response to each extract was significant, the mean proportion of flies trapped in the extract trap within each trial was tested for deviation from 0.5 by a two-sided *t*-test in JMP 11.0.0. The proportion of flies trapped must be significantly greater than 0.5 to indicate attraction. The same method was used to determine the attractiveness of the extract plate in the contact assay.

Results

Short-Range Flight Assays

I found that no extracts, except the ACV positive control, were significant attractants for SWD (Table 6). Although some extracts had a proportion response over 0.5, none were significant except the ACV positive control.

Extract Origin	Response Group	Proportion Response (Mean ± SE)	P-value
ACV	Mix	0.74 ± 0.083	0.0462
Virgin Female	Virgin Male	0.56 ± 0.094	0.5136
Virgin Female	Virgin Female	0.56 ± 0.135	0.6726
Virgin Female	Mix	0.51 ± 0.055	0.8987
Virgin Male	Virgin Female	0.39 ± 0.077	0.2151
Virgin Male	Mix	0.47 ± 0.092	0.7322

Table 6: Proportion of SWD response in various trials.

Contact Assay

The attractiveness of the pheromone extract plate was not significantly different than the attractiveness of the control plate. Over 4 hours and a total of 16-17 observations per cage, the number of flies that landed on each plate was totaled. I found proportion of landings on the extract plate was not significant (mean ± SE= 0.31 ± 0.200, P=0.408 from *t*-test).

Implications

Cuticular hydrocarbon extracts from virgin females or virgin males were not attractive in various short-range flight assays with SWD. Additionally, the presence of virgin female extracts was not attractive to virgin males in the glass contact assay. A possible explanation is that flies are not very attracted to each other's volatiles over short ranges, despite demonstration by Dekker et al. (2014) that SWD are responsive to cuticular hydrocarbons through direct contact on their antennae. A second possibility is that my extract method was ineffective. My methods differed slightly from the methods used by Dekker et al. (2014). Their extracts used only 50 µL hexane, which was evaporated slowly, as opposed to 200 µL evaporated relatively quickly. It is possible

that the flies were soaked in hexane for too long or the hexane was evaporated too quickly to adequately capture the extremely delicate volatiles. This could be confirmed through further testing of the extract method and testing of the extracts for the presence of pheromones. A third possible explanation is that SWD are attracted to cuticular hydrocarbons, but they are ineffective for trapping, as suggested by Landolt et al. (2012). Even though Dekker et al. (2014) found antennal responses, they did not test these hydrocarbons in eliciting responses of SWD over small distances. At present, the Lee lab will not likely further pursue pheromone extracts as a method of bait improvement.

PART III: STUDY CONCLUSIONS

Overall, my results show that infestation success is closely related to temperature at the time of egg-laying, suggesting a need to adjust current predictive models for egg-laying success and SWD survival due to extreme temperature. Also, different climates may strongly affect population dynamics. For example, SWD population dynamics in California's Central Valley are not the same as in Oregon's Willamette Valley. Predictive models must be able to understand location-specific temperature conditions to be the most effective. Additionally, population dynamics in a given location may change with changing climates. Global climate change is predicted to cause periods of unusually warm weather may occur more frequently, likely affecting SWD infestation success, survival, and population dynamics, perhaps altering the usual infestation regime in relation to fruit crops. A quantitative understanding of the relationship between temperature and SWD development will be important to adapting to these changes.

In addition to population modeling, effective trapping is a key part of creating a sustainable integrated pest management program for SWD. Efforts to improve bait and attractants are ongoing, so eliminating the possibility of pheromones as an effective attractant informs future research regarding bait improvement.

Although small in scale, this research provides important insights about SWD development and ecology, adding to the collective knowledge of SWD biology that informs the management of this destructive crop pest and allows us to move toward a more sustainable approach to controlling SWD in fruit crops worldwide.

WORKS CITED

Abraham, J., Zhang, A., Angeli, S., Abubeker, S., et al. (2015). Behavioral and Antennal Responses of *Drosophila suzukii* (Diptera: Drosophilidae) to Volatiles from Fruit Extracts. *Environmental Entomology.* v. 44(2), p. 356-367.

Asplen, MK., Anfora, G., Biondi, A., Choi, D-S., Chu, D., et al. (2015). Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science.* v. 88(3), p. 469-494.

Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., Kopp, A. (2014). The making of a pest: the evolution of a fruit-penetrating ovipositor in the *Drosophila suzukii* and related species. *Proceedings of the Royal Society B.* v. 281.

Bruck, D., Bolda, M., Tanigoshi, L., Klick, J., Kleiber, J., DeFrancesco, et al. (2011). Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. *Pest Management Science.* v. 67, p. 1375–1385.

Burrack, H., Asplen, M., Bahder, L., Collins, J., et al. (2015). Multistate Comparison of Attractants for Monitoring *Drosophila suzukii* (Diptera: Drosophilidae) in Blueberries and Caneberries. *Environmental Entomology*. v. 44(3), p. 704–712.

Cha, D., Hesler, S., Park, S., Adams, T., Zack, R., et al. (2015). Simpler is better: fewer non-target insects trapped with a four-component chemical lure vs. a chemically more complex food-type bait for *Drosophila suzukii*. *Entomolgia*. v. 154(3), p. 251-260.

Chabert, S., Allemand, R., Poyet, M., Eslin, P., Gibert, P. (2012). Ability of European parasitoids (Hymenoptera) to control a new invasive Asiatic pest. *Drosophila suzukii. Biological Control,* v. 63, p. 40-47.

Cini, A., Ioriatti, C., Anfora, G. (2012). A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology*, v. 65, p. 149-160.

Dekker, T., Revadi, S., Mansourian, S., Ramasamy, S., Lebreton, S., Becher, PG., Angeli, S., Rota-Stabelli, O., Anfora, G. (2015). Loss of *Drosophila* pheromone reverses its role in sexual communication in *Drosophila suzukii*. *Proceedings of the Royal Society of London B: Biological Sciences.* v. 282.

Flint, H.M, Doane, C.D. 2016. "Understanding Semiochemicals with Emphasis on Insect Sex Pheromones in Integrated Pest Management Programs" *Radcliffe's IPM World Textbook.* http://ipmworld.umn.edu/flint. (accessed date April 8, 2016).

Gabarra, R., Riudavets, J., Rodriguez, G., Pujade-Villar, J., Arno, J. (2015). Prospects for the biological control of *Drosophila suzukii*. *BioControl*, v. 60, p. 331-339.

Goodhue, R., Bolda, M., Farnsworth, D., Williams, J., Zalom, F. (2011). Spotted wing *Drosophila*- infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. *Pest Management Science*, v. 67, p. 1396- 1402.

Hamby KA, E. Bellamy D, Chiu JC, Lee JC, Walton VM, Wiman NG, York RM, Biondi A (2016) Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. Journal of Pest Science, doi 10.1007/s10340-016-0756-5

Kacsoh, B., Schlenke, T. (2012). High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii*, a relative of *D. melanogaster*. *PLoS ONE*. 7(4):e3472

Kanzawa, T. (1939) Studies on *Drosophila suzukii* Mats. Kofu. Yamanashi Agricultural Experimental Station, Yamanashi, Japan. pp. 1–49.

Landolt, P., Adams, T., Rogg, H. (2012). Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumara) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. *Journal of Applied Entomology*. v.136 (1-2), p. 148–154.

Lee, J., Dreves, A., Cave, A., et al. (2015). Infestation of Wild and Ornamental Noncrop Fruits by *Drosophila suzukii* (Diptera: Drosophilidae). *Annals of the Entomological Society of America*. v. 108.

Lee, J., Bruck, D., Dreves, A., Ioriatti, C., Vogt, H., Baufeld, P. (2011). In Focus: Spotted wing drosophila, *Drosophila suzukii*, across perspectives. *Pest Management Science,* v. 67, p. 1349- 1351.

Life Cycle of Spotted Wing Drosophila. Digital image. *Spotted Wing Drosophila*. Washington State University Extension, n.d. Web. 12 Oct. 2015.

Lin, Q., Zhai, Y., Zhang, A., Men, X., Zhang, X., Zalom, F., Zhou, C., Yu, Y. (2014). Comparative Developmental Times and Laboratory Life Tables for *Drosophila suzukii* and *Drosophila melanogaster* (Diptera: Drosophilidae). *Florida Entomological Society,* v.97, no. 4, p. 1434-1442.

Naranjo-Lazaro, J., Mellin-Rosas, M., Gonzalez-Padilla, V., Sanchez-Gonzalez, J., Moreno-Carillo, G., Arrendondo-Bernal, H. (2014). Susceptibility of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) to entomopathogenic fungi. *Southwestern Entomologist*, v. 39, p. 201-203.

Rossi Stacconi, M., Buffington, M., Daane, K., Dalton, D., Grassi, A., Kacar, G., Miller, B., Miller, J., Baser, N., Ioriatti, C., Walton, V., Wiman, N., Wang, X., Anfora, G. (2015). Host stage preference, efficacy and fecundity of parasitoids attacking *Drosophila suzukii* in newly invaded areas. *Biological Control,* v. 84, p. 28-35.

Rossi Stacconi, M., Grassi, A., Dalton, D., Miller, B., Ouantar, M., Loni, A., Ioriatti, C., Walton, V., Anfora, G. (2013). First field records of *Pachycrepoideus vindemiae* as a parasitoid of *Drosophila suzukii* in European and Oregon small fruit production areas. *Entomologia,* v1:e3, p. 11-16.

Tochen, S., Dalton, D., Wiman, N., Hamm, C., Shearer, P., Walton, V. (2014). Temperature- Related Development and Population Parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on Cherry and Blueberry. *Entomological Society of America,* v. 43, no. 2, p. 501-510.

Tochen, S., Woltz, J.M., Dalton, D.T., Lee, J.C., Wiman, N.G., Walton, V.M. (2015). Humidity affects pop of *Drosophila suzukii* (Diptera:Drosophilidae) in blueberry. *Journal of Entomology,* published online 2 July, 2015.

University of California Agriculture and Natural Resources (UC ANR). (2014). About degree-days. http://ipm.ucdavis.edu/WEATHER/ddconcepts.html (accessed Feb. 26, 2016)

Witzgall, Peter, et al. (2008). Codling moth management and chemical ecology. *Annual Review of Entomology,* v. 53, p. 503-522.

Wiman, G., et al. (2014) Integrating Temperature-Dependent Life Table Data into a Matrix Projection Model for *Drosophila suzukii* Population Estimation. *PLoS One.*

Woltz, J.M., Donahue, K.M., Bruck, D.J., Lee, J.C. (2015). Efficacy of commercially available predators, nematodes and fungal entomopathogens for augmentative control of *Drosophila suzukii. Journal of Applied Entomology.*

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