Supplemental Materials

Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of Drosophila suzukii

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Figure 3

Methods: Monthly *D. suzukii* reproductive status data was summarized from Zerulla et al. (2015) using the month where first maturing eggs were found (April 2013) and the month where the last maturing eggs (November 2012) were found. Shearer et al. (2016) reported bimonthly *D. suzukii* winter morph captures for fall 2012 (14 Aug 2012- 11 Dec 2012) and winter morphs were first trapped in October. The Microsoft Excel solar calculator was downloaded from the National Oceanic and Atmospheric Administration (NOAA) (http://www.esrl.noaa.gov/gmd/grad/solcalc/calcdetails.html, accessed on 5 Oct 2015). Daily sunlight duration data (minutes) was calculated for South Tyrol, Italy (Lat 46.33°, Long 11.30°, Time Zone 1) and Hood River, OR, USA (Lat 45.70°, Long -121.52°, Time Zone -8) from 1 Jan 2012 to 31 Dec 2012. Data was summarized to monthly mean sunlight duration for both sites, and differences between the sites ranged from -5.21 to 5.83 minutes. Therefore, hourly photoperiod is only presented for Hood River, OR. NOAA climate data was downloaded (http://www.ncdc.noaa.gov/cdo-web/datasets#GHCND, accessed on 6 Oct 2015) for the Paganella, Italy weather station (GHCND:IT000160220, Lat 46.15°, Long 11.03°, Elev 2125m) and the Hood River, OR, USA weather station (GHCND:USC00354003, Lat 45.6847°, Long -121.5175°, Elev 152.4m). For Hood River, OR daily temperature summaries (min/max) from 1 Jan 2012 to 31 Dec 2012 were used to generate mean monthly min/max temperatures and all days with missing values were excluded. The 2012 data was chosen because *D. suzukii* winter morph data was reported for Aug-Dec 2012 (Shearer et al. 2016). The Paganella, Italy weather station was chosen for its proximity to South Tyrol. Daily temperature summaries (min/max) from 1 Jan 2013 to 31 Dec 2013 were used to generate mean monthly min/max temperatures and all days with missing values were excluded. The 2013 data was chosen because *D. suzukii* reproductive status was reported for Aug 2012-Sep 2013 (Zerulla et al. 2015).

Lee, unpublished

Methods: Newly emerged SWD females were individually transferred to a 22.9 x 22.9 x 25.4 cm white plastic cage with a clear top and sides, and mesh sleeve kept at 22°C, 16L:8D, and ~70% RH. Each female was paired with a ~3 d to 2 wk old male; a new male was replaced each week. Inside the cage, flies were given a water wick, 20% sucrose water tube, and egg laying substrate (artificial diet, blueberries and wine grapes, see Supplemental Table 1). The substrate was collected and replaced each day, and the number of eggs laid was counted. The diet or fruit were reared out to confirm that SWD were developing. No statistical comparison was made between the different substrates, because each substrate was tested at different times of the year

with a different stock of flies (3 m-old colony, wild flies). Flies were monitored on artificial diet until death; the last female was dead on day 153. Flies were monitored on blueberries and wine grapes for the first four weeks.

Supplemental Table 1. Trial methods.

Trial	Substrate information	Fly information	Dates	n
Artificial diet[1]	9 g in a 1 oz. plastic cup	Laboratory colony initiated 3 months ago	Jan. 30 – June 18, 2010	20
'Elliot' blueberries[2]	5 berries per day	1st generation of wild flies reared on artificial diet	Aug. 7 – Sep. 3, 2010	22
'Pinot Noir' wine grapes[3]	6 grapes per day	1st generation of wild flies reared on artificial diet	Sep. 16 – Oct. 13, 2010	21

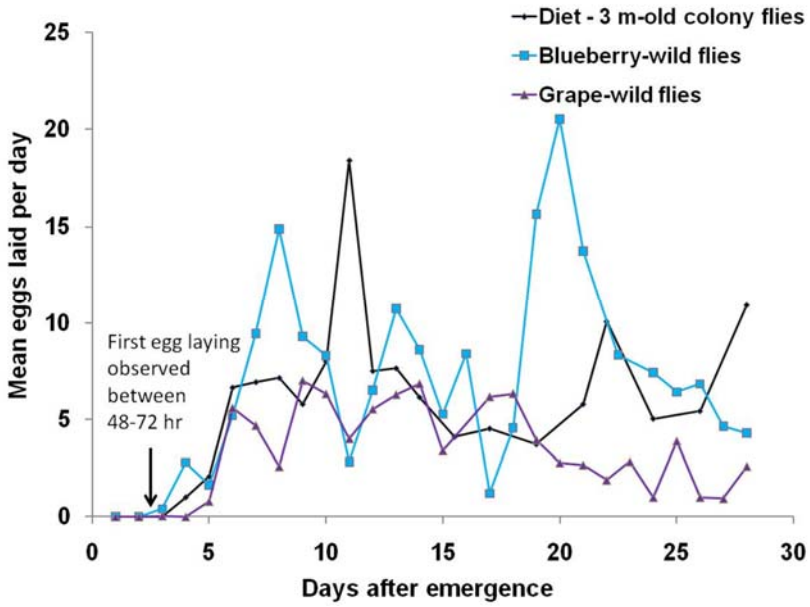
[1] Recipe includes 45g Agar, 125g Cornmeal, 200g Sugar, 70g Nutritional Yeast, 4.7 L dH_2O, 17.7mL Propionic acid, 3.3g Methyl paraben, 33.3mL 95% EtOH, Dry yeast sprinkles
[2] Ripe blueberries collected weekly from research farm in Linn Co., Oregon, Brix° 13.9% ±0.4
[3] Ripening grapes collected weekly from organic vineyard in Polk Co., Oregon, Brix° steadily increased each week from 14.8% ±0.37, 15.3 ±0.28, 17.8 ±0.26 to 22.4 ±0.43

Results:

Observed fecundities ranged from 85 to 161 over four weeks, and was 419 over the lifetime of flies given artificial diet (Table 2, main article). These results are within the range of observations made in Japan on cherries (161.1 ±23.9, and 382.2 ±33.2) and grapes (105.8 ±15.8) where flies were monitored over a lifetime. While no statistical comparison was made, the fecundity of flies appeared different among the three substrates. Highest fecundity was observed on artificial diet, then blueberry, and lowest on 'Pinot noir'.

Notably only 70% of females from the 3-month-old colony were fertile laying eggs in the artificial diet trial. In this trial, flies from the colony had likely undergone selection, and flies appeared either very fecund or sterile. One female was observed to lay 161 eggs on one day. For this reason, fecundity trials with the other two substrates were conducted with the first generation of flies reared on artificial diet with wild parents.

In these trials, the first females were observed to start laying eggs between 48 and 72 hr, and average age ranged from 3.7 to 7.8 d-old. These flies appeared to lay egg later than flies observed in Japan, where the average onset of egg laying occurred at 47 hr. Egg laying rates were variable from day-to-day (Supplemental Figure 1). A distinct peak in egg laying did not appear consistently.



Supplemental Figure 1. Observed egg laying rates among oviposition substrates.

Margosan, D.[1] previously unpublished scanning electron microscope SEM figures (Figure 2).

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Methods:

Adult mated female *D. suzukii* (age ~ 3 d) were isolated from the USDA-ARS San Joaquin Valley Agricultural Sciences Center in Parlier, CA and placed in 150-mm x 15-mm covered Petri dishes plated with agar media (Bellamy et al., 2013) and a small amount of bakers yeast sprinkled on the surface to stimulate oviposition. When oviposition was observed, the plates were moved to a 4°C refrigerator until all Drosophila movement had ceased. With the use of a dissecting microscope, observed ovipositing flies were removed with forceps and placed in a fixative composed of 4% (v/v) glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.2, at 24°C. The flies were fixed overnight, and then washed in three changes of 0.1M sodium phosphate buffer, 2 hr per change. The flies were post-fixed overnight in 1% (v/v) osmium tetroxide in 0.1M sodium phosphate buffer, pH 7.2, at 24°C, and then washed in three changes of reverse osmosis water, 2 hr per change. The flies were dried in an ethanol series of 10%, 25%, 50%, 75%, 100%, 100%, 100%, three hours per change, and then critically point dried with an Autosamdri-815B (Tousimis, Rockville, MD). The dried flies were mounted on insect pins that were previously attached to a 25-mm diameter Hitachi sem specimen stub. The mounted flies were sputter-coated with gold (SPI Sputter Coater, Structure Probes, Inc., West Chester, PA) then examined and micrographs obtained with a S3500-N scanning electron microscope (Hitachi High Technologies America, Pleasanton, CA).

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Methods:
D. suzukii oviposition was observed every hour on *Drosophila* rearing media for a 24 hour period within a growth incubator. Temperature and light intensity was programmed in order to represent typical early summer conditions. The photoperiod of 12:12 L:D was used. Lights turned on at 7AM(ZT0) and light intensity gradually increased to peak at 4PM(ZT9), after which light intensity declined and turned off at 7PM (ZT12). The daily temperature range was 9.0°C to 21.4°C with the peak temperature of 21.4°C at 6PM (ZT11). The minimum temperature of 9.0°C occurred at 5AM (ZT22). Hourly oviposition per female was recorded and a PearsonVII (Student's t) distribution was fit to generate Figure 1c.

Results:
Minimum egg laying levels started at 11AM (ZT4) (0.05/eggs/female/hour) and increased to a maximum (11/eggs/female/hour) at 5PM (ZT10). Egg laying ceased at 2AM (ZT19) (0.05/eggs/female/hour). Pearson VII distribution fit: $F(1,22)=522$, $p < 0.0001$, adj. $R2= 0.96$ with df=23, location = 18, scale = 1.55.