

Seasonal Reproductive Biology of *Drosophila suzukii* (Diptera: Drosophilidae) in Temperate Climates

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Abstract

Drosophila suzukii (Matsumura; Diptera: Drosophilidae) is a key pest of sweet cherry and small fruits worldwide. The present studies were designed to describe the reproductive physiology in both sexes, through dissections of their reproductive organs. We extensively dissected female *D. suzukii* throughout the season from 2013 to 2016 and classified the reproductive status flies based on five recognizable ovarian maturation stages: 1) no ovaries; 2) unripe ovaries 3) ripening eggs in ovarioles; 4) mature eggs in ovarioles; and 5) mature eggs in the abdomen. Development was examined as a function of calendar days as well as degree-days (DD). Results obtained from winter collections revealed that females collected from November to March contained a lower percentage of mature eggs than females collected from April to September. These data suggest that environmental conditions during the dormant period induce reproductive diapause. Oogenesis likely increased with an increase in mean monthly temperatures and DD. The first overwintered females with mature eggs were dissected as early as 21 February 2014 in Trento (7 DD). Additionally, we found that a low proportion of males (less than 50%) had sperm in their testes between January and March, yet during the same period females already have sperm stored in their spermathecal. Ivy berries was an alternative but unfavorable non-crop host during the late dormant period, as evidenced by emergence of smaller adults when compared to individuals emerging from cherry fruits. This study showed that *D. suzukii* females have great potential for oviposition early in the season, posing a risk to early season maturing crop hosts.

Key words: ovarian maturation, reproductive status, sperm, spermatheca, *Hedera helix*

Drosophila suzukii (Matsumura; Diptera: Drosophilidae) shows the ability to adapt to changes in climate (Shearer et al. 2016) and flies, which adapt and survive harsh conditions, can damage multiple susceptible crops in subsequent seasons in all major production regions (Asplen et al. 2015). In 2011, the economic losses attributable to *D. suzukii* were estimated at 3 million Euros (De Ros et al. 2015). Observations about its seasonal reproductive biology is essential to inform growers how to best manage *D. suzukii* with particular focus on the important, albeit less studied, winter bottleneck period (Dalton et al. 2011, Stephens et al. 2015, Shearer et al. 2016, Wiman et al. 2016, Enriquez and Colinet 2017).

Effective management of damaging pest populations of *D. suzukii* can benefit producers, extension agents and practitioners, especially if we can identify key periods in the insect's lifecycle to target management (Focks et al. 1995, Almeida et al. 2010, Jones and Wiman 2012, Cianci et al. 2013). Climate-based risk models using the accumulation of heat units or cumulative degree-days (DD) (Baskerville

and Emin 1969, Wilson and Barnett 1983) are often used to determine the timing of management for pests. Such risk models can be used to estimate when the first susceptible crops will be attacked (Zalom et al. 1983). Knowledge of seasonal pest biology can help direct pest management techniques to target a certain time of the season or life stage in order to maximize the impact of such techniques. Previous data have shown that *D. suzukii* has high reproductive levels and a short lifecycle. The population moves through generations rapidly and generations quickly begin to overlap, resulting in significantly restricted management options (Emiljanowicz et al. 2014, Kinjo et al. 2014, Tochen et al. 2014, Wiman et al. 2016). DD phenology models tend to decrease in applicability for pests that show these characteristics. However, DD accumulation may be of particular use in the case of *D. suzukii* during the early portion of the season because generations do not yet overlap and synchrony is visible after the winter diapause period (Dalton et al. 2011, Stephens et al. 2015, Shearer et al. 2016, Wiman et al. 2016, Enriquez and

Colinet 2017). The most useful knowledge from DD accumulation is therefore gained when looking at the period leading to an increase of more favorable conditions when the first susceptible crops become available. Yet, little information is available on whole-season reproductive biology of *D. suzukii* as it relates to climate, and specifically the dormant reproductive biology of *D. suzukii* in temperate regions. Adult winter morph flies, which develop under low temperatures, have the ability to survive cold winter conditions (Shearer et al. 2016, Wallingford and Loeb 2016), but the reproductive status of males and females during these time periods (winter and early spring) is not well understood. Recent studies show that the reproductive status of *D. suzukii* shifts from relatively low ovarian and egg maturity levels to higher levels of maturity as the season progresses from winter to spring and summer (Zerulla et al. 2015, Rossi Stacconi et al. 2016, Ryan et al. 2016, Wiman et al. 2016).

Major factors that affect *D. suzukii* during the winter include abiotic factors of temperature (Dalton et al. 2011, Ryan et al. 2016) and humidity (Gutierrez et al. 2016, Shearer et al. 2016, Tochen et al. 2016b), as well as the presence of essential food resources (Kimura 2004, Mitsui et al. 2006, Mitsui et al. 2007, Lee et al. 2015, Briem et al. 2016, Tochen et al. 2016a). Although DD phenology models may have limited application toward the end of the growing season for a pest such as *D. suzukii*, accumulation of heat units during spring can play an important role in forecasting risk of crop infestation. Temperature-dependent development, survival, and reproductive data are available for all life stages of *D. suzukii* during the summer season under controlled conditions (Emiljanowicz et al. 2014, Tochen et al. 2014, Hardin et al. 2015, Jaramillo et al. 2015). Field observations have also shown that *D. suzukii* goes through reproductive diapause during the winter (Shearer et al. 2016, Wallingford and Loeb 2016). Recent *D. suzukii* modeling has used a combination of mean temperature and calendar-based matrices (Wiman et al. 2014, Asplen et al. 2015). The demographic model produced by Wiman et al. (2016) described the progression of ovarian maturation time from the United States and Northern Italy and indicates a linear temperature-dependent increase in reproductive maturity. This model takes into consideration winter survival, early-season reproductive potential and to a limited extent the availability of hosts (Dalton et al. 2011, Lee et al. 2015, Kaçar et al. 2016). The consistency and ecological consequences of the data over many seasons have not been demonstrated; however, further modeling using a population-based demographic model highlights the contribution of the cold-tolerant *D. suzukii* winter morph toward rapid population growth during springtime in regions of Europe and North America that experience cold winters (Gutierrez et al. 2016).

Management strategies for *D. suzukii* include chemical (Beers et al. 2011, Bruck et al. 2011, Van Timmeren and Isaacs 2013, Wise et al. 2015), biological (Chabert et al. 2012; Rossi Stacconi et al. 2013, 2015; Cancino et al. 2015; Gabarra et al. 2015; Miller et al. 2015; Nomano et al. 2015; Daane et al. 2016; Mazzetto et al. 2016;

Wang et al. 2016) and cultural (Lee et al. 2016, Tochen et al. 2016b) controls. Other potential control strategies currently being investigated include molecular techniques such as gene modification using CRISPR technologies (Li and Scott 2016, Kalajdzic and Schetelig 2017), RNAi biopesticides (Murphy et al. 2016), and sterile insect technique (SIT, Schetelig and Handler 2013).

Parasitoids known to attack *D. suzukii* have been identified in areas of recent pest invasion (Miller et al. 2015). However, parasitoid success appears generally lower in these regions compared to levels observed in the indigenous range of the pest (Kacsoh and Schlenke 2012, Rossi Stacconi et al. 2015, Daane et al. 2016). In North America and Europe, specialist parasitoid species are absent. These agents may contribute to an overall reduction in local *D. suzukii* populations (Miller et al. 2015, Rossi Stacconi et al. 2015, 2017, Wang et al. 2016, Biondi et al. 2017). It is therefore also important from a biological control perspective to determine the lower temperature threshold for possible egg-laying and larval development in order to optimize different biological control strategies.

The goal of this paper is to provide key insights into how physiological time can be utilized to better understand survival, reproductive status (Avanesyan et al. 2017), and oviposition of *D. suzukii* in changing environments. We specifically ask if 1) there are differences in female ovarian development throughout the year, 2) if females retain sperm from males during different times of the year, 3) if males produce sperm throughout the year, and 4) whether there is early oviposition on ivy (*Hedera helix* L.) and to determine risk of oviposition on early commercial cherry (*Prunus avium* L.). Data analysis here is focused on environmental conditions during the latter portion of winter and early spring coupled with physiological time (DD accumulation) as a determinant factor of female reproductive status.

Materials and Methods

Male and female *D. suzukii* were collected in an area-wide monitoring program in northern Italy during 2013–2016 using traps baited with Droskidrink (Azienda Agricola Prantil, Priò, Trento, Italy). The traps were deployed in six sites (Table 1) and serviced weekly for the duration of the experimental period. *D. suzukii* females used for ovaria dissections were stored 3–4 wk at laboratory conditions (15–25°C), using a 70% alcohol solution as storage medium (Carson and Cappellano 2014). The storage method and time showed minimal impact on the integrity of the reproductive structures (Grassi unpublished). Individuals used for sperm status investigation (both females and males) were similarly kept in physiological saline (0.9% sodium chloride in water solution) and analyzed within 2 d in order to prevent sperm deterioration. Population-level reproductive status was assessed under three criteria: ovarian development in females, presence of sperm in spermatheca and/or in seminal receptacle in females, and presence of sperm in testes in males.

Table 1. *D. suzukii* sampling location, and potential hosts in Trento Province, Italy during 2013–2016

Location	Elevation	GPS coordinates	Hosts	Dissected sex
Trento/Fersina ^a	207 m asl	46° 3'15.26"N	11° 7'24.20"E	Ivy Male, Female
Trento/Orrido	330 m asl	46° 4'31.11"N	11° 9'6.58"E Ivy, Cherry	Female
Serso ^a	600 m asl	46° 4'24.81"N	11°14'47.63"E Cherry	Female
St. Michele ^a	250 m asl	46°11'34.63"N	11° 8'22.90"E Cherry	Female
Romagnano ^a	190 m asl	46° 0'26.49"N	11° 7'3.09"E Cherry	Female
Canale ^a	500 m asl	46° 2'23.08"N	11°13'31.36"E Cherry	Female

^aSites used for climate and reproductive status correlation.

Ovary Dissection

At least 31 female *D. suzukii* ovaries were dissected monthly in order to determine the reproductive status of local populations throughout the year. These assessments were conducted in six locations (Table 1) from January 2013 until December 2016. Individual female *D. suzukii* were classified based on five recognizable ovarian maturation stages (Fig. 1a-d, Boulétreau 1978):

- 1) No ovaries—No ovaries present in the abdomen.
- 2) Unripe ovaries—Ovaries present, but no developing ovarioles (eggs) visible upon dissection (Fig. 1a).
- 3) Ripening eggs in ovarioles—Small developing ovarioles recognizable, but no breathing tubes (spiracles) visible (Fig. 1b).
- 4) Mature eggs in ovarioles—Spiracles visible when dissecting eggs in ovaries, but no free eggs in the oviducts or in the uterus, classified as mature (Fig. 1c).
- 5) Mature eggs outside ovaries (abdominal)—Mature eggs released in the oviducts or in the uterus (Fig. 1d).

Determination of Sperm Presence in Females and Males

In order to determine the percentage of individuals containing sperm, a minimum of 18 females and 12 males were dissected during each monthly increment from 2015 to 2017 and 2016 to 2017, respectively using methods similar to Avanesyan et al. (2017). The presence of sperm within the dissected structures was verified by direct observation of the typical striated masses under a dissecting microscope at 400× magnification (OPTIKA SRL, Ponteranica, BG, Italy). For females, both the spermatheca and the seminal receptacle were stained in a 2% aceto-orcein solution and mounted on glass slides with glycerol (Fig. 2a and b). For males, testes were dissected and directly observed in physiological saline (Fig. 3).

Early Oviposition on Ivy and Risk of Oviposition on Early Commercial Cherry

During 14 March to 18 May 2016, ivy fruit were collected at Trento/Orrido (Table 1) in order to determine natural levels of infestation with *D. suzukii*. Each randomly collected fruit sample consisted of a minimum of 100 berries. Fruits were inspected under a dissecting stereo-microscope in the laboratory in order to determine *D. suzukii* egg or larval presence. All infested berries were incubated in sealed polythene bags together with water-soaked paper under laboratory conditions ($20 \pm 5^\circ\text{C}$, $45 \pm 20\%$ RH and 16:8 [L:D] h photoperiod). The bags were inspected twice per week in order to determine adult *D. suzukii* eclosion. Fruit firmness was additionally determined on 25 uninfested berries using a 1.5 mm diameter blunt point digital penetrometer (TR Turoni, Italy).

The average size of *D. suzukii* individuals emerging from ivy berries was compared with the size of individuals reared on commercial cherries (*P. avium*). For both fruit types, a minimum of 16 individuals of each sex was analyzed. The length of the M1 longitudinal wing vein was used as reference parameter and measured using the Leica Application Suite 3.2.1 software (Leica Microsystems Inc, Buffalo Grove, IL).

DD Calculation

The single sine method of DD calculation (Baskerville and Emin 1969) was used with lower, 7.2°C , and upper, 30.0°C , horizontal cutoffs optimized for *D. suzukii* (Tochen et al. 2014) to estimate DD accumulation for this insect in Trento/Orrido during 2016. The early maturing 'Chelan' cherry cv. phenology was correlated to DD accumulation, showing clear trends of cherry fruit development coupled with an increase in DD (D. Gibeaut, unpublished data). Cumulated DD calculations for cherry in Trento/Orrido were used for to estimate the early maturing of fruit of cv. 'Ferrovia' grown in this region during 2016.

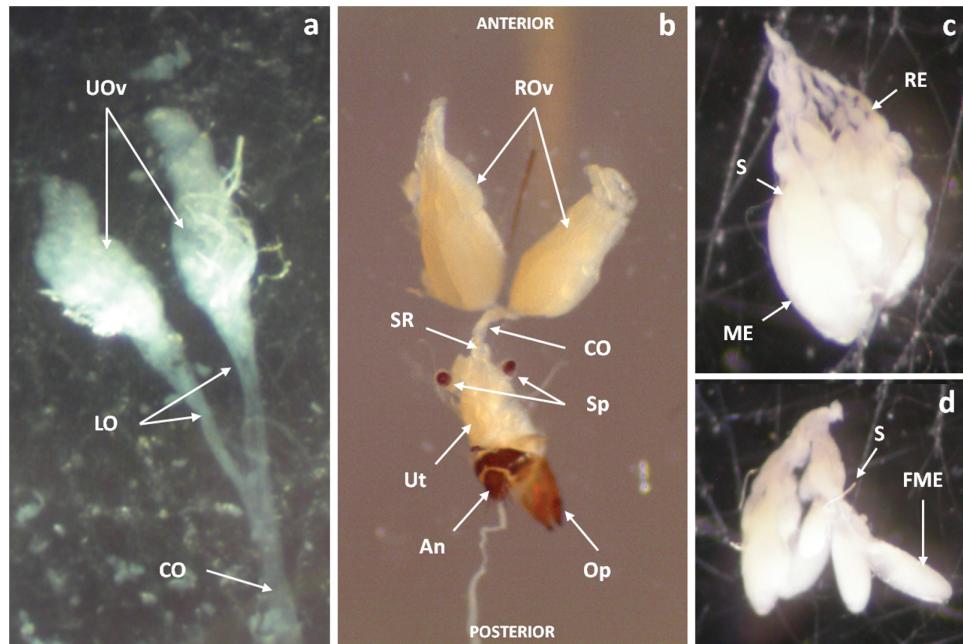


Fig. 1. (a) Unripe ovaries (UOv) of female *Drosophila suzukii*: Ovaries were found, but no developing ovarioles could be seen when dissected. Common (CO) and lateral (LO) oviducts are visible. (b) Ripening ovarioles (ROv): Small developing ovarioles are recognizable, but no breathing tubes (spiracles) were visible on the eggs. (c) Mature ovarioles (MOv): when dissecting the ovaries, both ripening eggs (RE) and mature eggs (ME) bearing spiracles (S) were visible, but none of these eggs were found in the abdomen. (d) Free mature eggs in the abdomen (FME): All of the mature eggs with visible spiracles (S) were found in the abdomen and not in the ovaries. SR: Seminal Receptacle, Sp: Spermathecae, Ut: Uterus, An: Anus, Op: Ovipositor. Photos: A. Grassi.

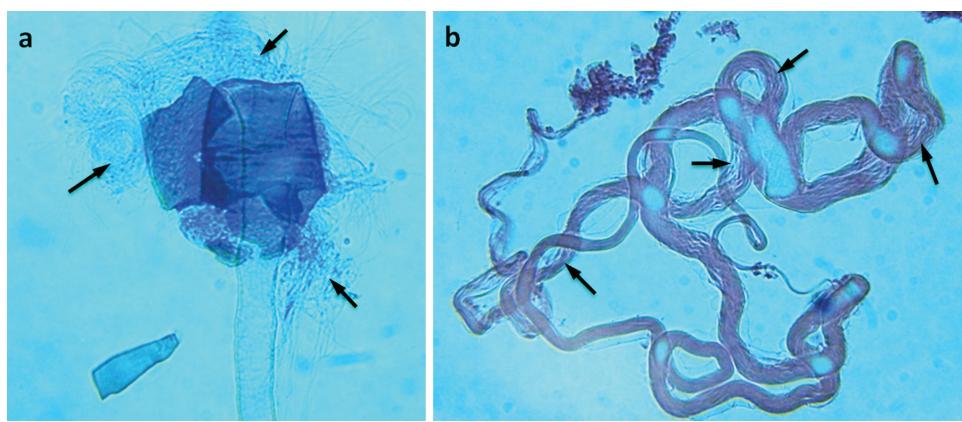


Fig. 2. Spermatozoa (arrows) in dissected spermatheca (a) and seminal receptacle (b) of female *Drosophila suzukii*. Photos: A. Gottardello.



Fig. 3. Dissected testes and striated spermatozoa masses of male *Drosophila suzukii*. The insert shows intact testis. Photos: A. Gottardello.

Statistical Analysis

The reproductive status of the collected individuals from all sites was grouped monthly and the percentage of each maturation stage was represented as a fraction of the whole dissected population for that corresponding month. These data are presented together with the mean daily temperatures for these regions (Fig. 4).

Daily average temperatures from the five highlighted sites in Trentino, Northern Italy were collected from January 2013 to December 2016 (Table 1, Fig. 4). These data allowed the calculation of monthly cumulative DD values with a biofix of 1 January of each year. These reproductive status and cumulative monthly DD data for each region were regressed to estimate the relationship of DD accumulated from 1 January in Trento Province and the five highlighted sites in Northern Italy as a predictor of percent *D. suzukii* ovarian development. The percentage of female *D. suzukii* individuals in each ovarian maturation stage was correlated with the DD accumulation using simple multiple regression.

Differences in the reproductive status of the dissected organs (ovaria, spermathecae and seminal receptacle for females and testes for males) were analyzed using one-way ANOVA followed by Tukey's HSD test to determine differences between months of the year. Levene's test for homogeneity of variances of the mean monthly temperature and reproductive status were conducted in order to highlight differences between years. All analyses were run using the statistics software Statistica 7 (StatSoft. Inc., Tulsa, OK).

Results

Ovaria Dissection

The reproductive status of female *D. suzukii* provided a consistent trend of increasing maturity levels coupled with increasing temperatures and DD (Figs. 4 and 5). Levene's test for homogeneity of variances of the mean monthly temperatures for each year showed that year had no significant impact on temperature. Monthly temperatures were significantly different ($F_{11,31} = 55.376$, $P < 0.001$, differences not shown in Fig. 5a). The statistically lowest temperatures were found from December to February (-2.3 to 1°C). Medium temperatures were found in March, April, and September (5.2 to 8.2°C). Higher temperatures were found in May, June and August (11.1 to 14.1°C). The highest mean temperatures were recorded during July (16.2°C). The linear regression for the region describing the correlation of temperature on egg maturity is $y = 3.0547x + 8.8783$ ($F_{1,10} = 50.93$, $R^2 = 0.84$, $P < 0.038$, Fig. 5b). During the dormant period (from November to February) less than 10% of the analyzed females carried mature eggs (Fig. 5a). This percentage increased to more than 50% during spring (March–May) and decreased to less than 20% during fall (September–November). In all years, mean temperatures above 10°C resulted in more than 50% females carrying mature eggs (Fig. 5). Levels of mature eggs decreased below 50% at lower temperatures. When examining egg dissections over cumulative DD in the five highlighted sites of Trento province, Northern Italy beginning 1 January each year, the percentage

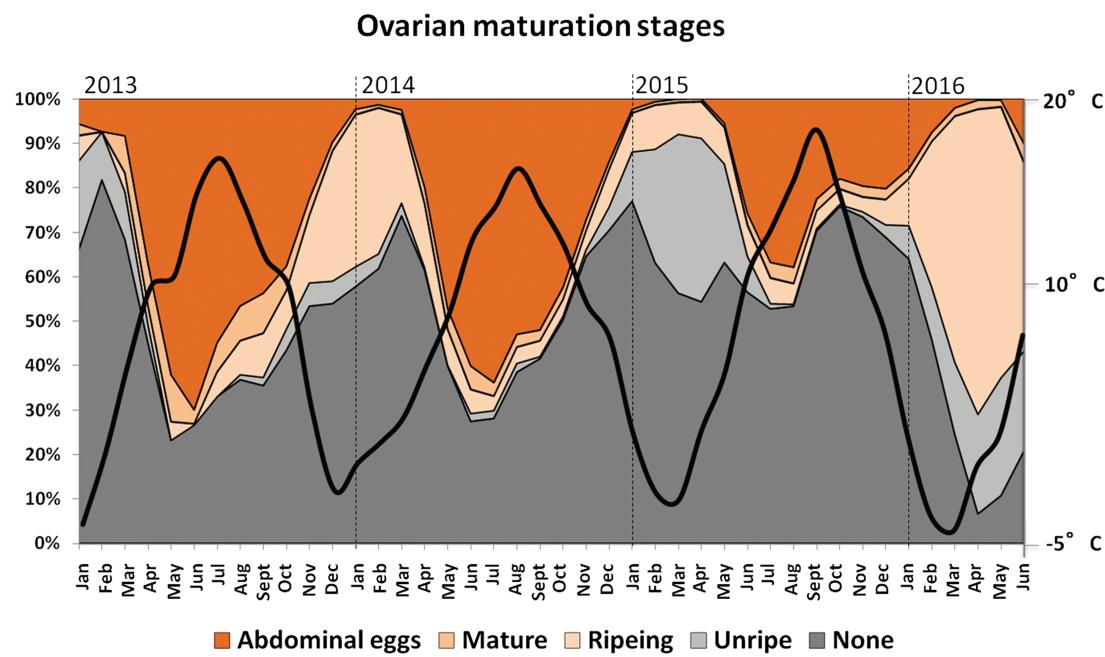


Fig. 4. Seasonal reproductive status of dissected female *Drosophila suzukii* and mean daily temperature in °C from 2013 to 2016 in Trento Province, Northern Italy.

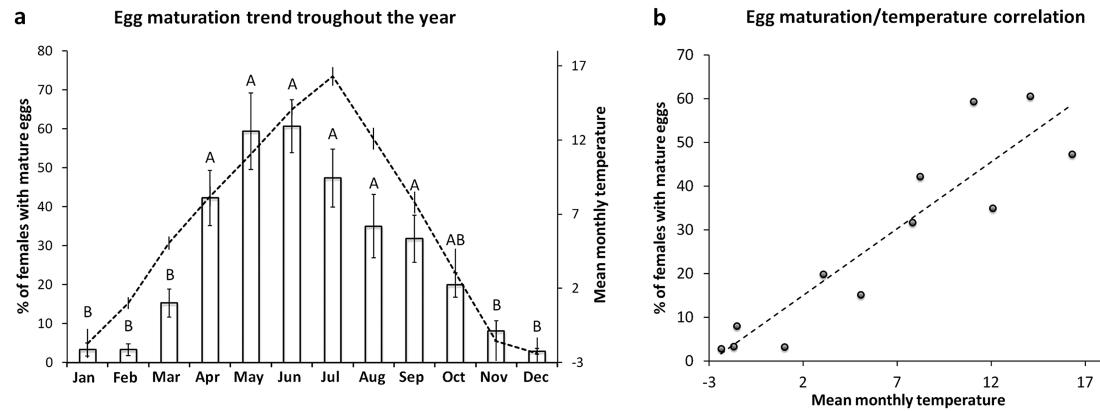


Fig. 5. (a) Monthly average percent (2013–2016) *Drosophila suzukii* females with mature eggs in Trento Province, Northern Italy. For each column, different letters indicate significant differences ($P < 0.05$) after ANOVA followed by Tukey's HSD test. (b) Correlation between the average monthly temperature in Trento Province during 2013–2016 and the percentage of *D. suzukii* females with mature eggs.

of *D. suzukii* with mature eggs showed significant linear relationships for all of the regions (Fig. 6, Table 3). The first flies containing mature eggs all occurred at levels just above 0 DD. Overall, the earliest increase of mature eggs was found at Serso on 21 February 2014 (3 DD), and at St. Michele as late as 15 April 2016 (206 DD). For Trento province overall, the proportion of overwintered females with mature eggs numerically start an increase at 21 February 2014 (24 DD).

Determination of Sperm Presence in Females and Males

The monthly percentage of female *D. suzukii* with sperm within spermatheca and seminal vesicle dissections were significantly impacted by the month of dissection ($F_{9,75} = 6.2$, $P < 0.001$, Fig. 7a). The lowest levels of females with sperm were found from December to March. The numerically lowest levels of sperm were found in February. Increasing levels of sperm were found during April. The statistically highest levels of sperm were found from May to October and decreasing levels of sperm were found during November into December.

The monthly percentage of male *D. suzukii* with sperm within testes was significantly impacted by the month of dissection ($F_{8,24} = 4.6$, $P = 0.002$, Fig. 7b). The lowest proportion of males with sperm was found in March. The numerically lowest levels of sperm were found during January. Slightly higher levels of sperm were found from April to December. Overall, the statistically highest levels of sperm were found from April to December.

Early Oviposition on Ivy and Risk of Oviposition on Early Commercial Cherry

The ivy berries collected at Trento/Orrido from 14 March to 18 May 2016 showed increasing infestation with *D. suzukii* starting at 68 DD and increased to peak infestations at 521 DD (Fig. 7c, Table 2). Fruit firmness during this period was never less than 53.3cN. Cumulative DD was a significant predictor of higher ivy fruit infestation over time. The simple regression describing this correlation (Fig. 7c) is $y = 0.1002x - 12.845$ ($F_{1,5} = 15.87$, $P < 0.01$, $R^2 = 0.76$). Measurements of wing length from ivy berries and cherry fruit showed significant differences

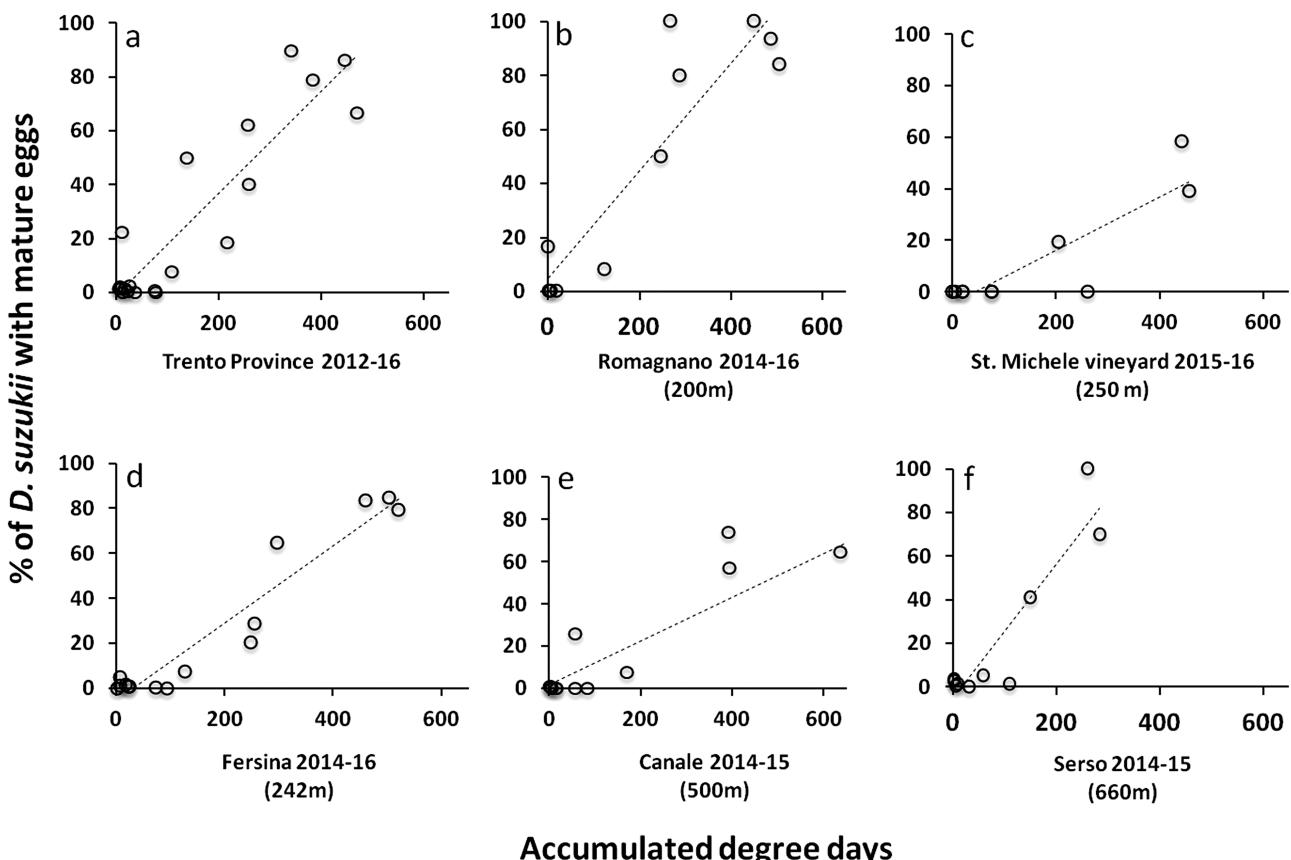


Fig. 6. Percentage of *Drosophila suzukii* with mature eggs over accumulated degree-days from January 1 (Lower and upper thresholds for SWD is set at 7.2 and 30°C, respectively) in Trento Province, Northern Italy (a), and in five production sites (b–f) at different altitudes during 2013–2016.

based on host fruit and insect gender ($F_{1,77} = 79.03$, $P < 0.001$, Fig. 7d). Emerged adults from ivy berries were smaller than those emerging from cherry fruit, and males were smaller than females. Cherry and ivy phenology in relation to *D. suzukii* physiological time (DD) showed that female *D. suzukii* are able to oviposit in ivy berries well before straw-yellow cherry fruit were expected to be susceptible to *D. suzukii* attack (Table 2).

Discussion

The data presented from four seasons of dissections provide a clear picture of *D. suzukii* reproductive biology throughout the year. The data from dissections suggest that female *D. suzukii* have significantly decreased levels of mature eggs during the dormant period (November to March), a factor that possibly contributes to *D. suzukii* reproductive diapause as indicated by lacking or little reproductive activity (Shearer et al. 2016, Wallingford and Loeb 2016). Previous studies (Shearer et al. 2016, Wallingford and Loeb 2016) described the impact of changing seasonal climates on morphology and physiology of *D. suzukii*. Here, we do not report this phenomenon, but unreported data collected during this study confirmed similar trends. The correlated temperature data strongly suggest that unfavorably low temperatures are a likely cause for this phenomenon. It is therefore likely that there is significantly decreased or absent oogenesis during this period. The percentage of females containing mature eggs conversely increased significantly with increasing mean monthly temperatures. This trend is consistent, even when taking into consideration slight variations of temperatures during the same months of different seasons. As shown in Fig. 4, there were slight differences in the percentage of females with mature eggs between years, namely, there were fewer females

with abdominal eggs during the summer of 2015 compared to 2013 and 2014. These differences may be due to subtle changes in both late autumn and winter climatic conditions and variation in the onset of colder winter conditions. It is possible that these differences affect the demographic makeup and size of *D. suzukii* populations at the beginning of the spring period during the following season. This topic is outside of the scope of this paper, but warrants additional investigation.

During the late dormant period in all seasons, there was an increase in the proportion of females with mature eggs coupled with increasing temperatures and DD, suggesting that oogenesis resumes as early as 59 DD. Overall, the lowest mean percentage of females with mature eggs was found in the month of February (3.3%).

Dissected females on average never had lower than 32.14% individuals containing sperm (found in February), indicating that at least part of the population at this time of the year should be able to produce fertilized eggs. February is likely the month with the lowest reproductive potential for female *D. suzukii* populations when looking at both percentage of mature eggs and presence of sperm in the population as a whole in this region. This period also showed comparatively similar low sperm counts in male *D. suzukii* populations (January–March). These data support other earlier studies that the most important bottleneck period for *D. suzukii* pest populations in temperate climates is from January to March (Dalton et al. 2011, Stephens et al. 2015, Shearer et al. 2016, Wiman et al. 2016). Moreover, the low availability of males with sperm in females during this period indicates that females are potentially storing sperm from autumn mating. This stored sperm can be used by the females to fertilize mature eggs as soon as they break diapause without the need to mate with mature males in early spring. Mating behavior of *D. suzukii* at low temperatures need further study (Enriquez and Colinet 2017).

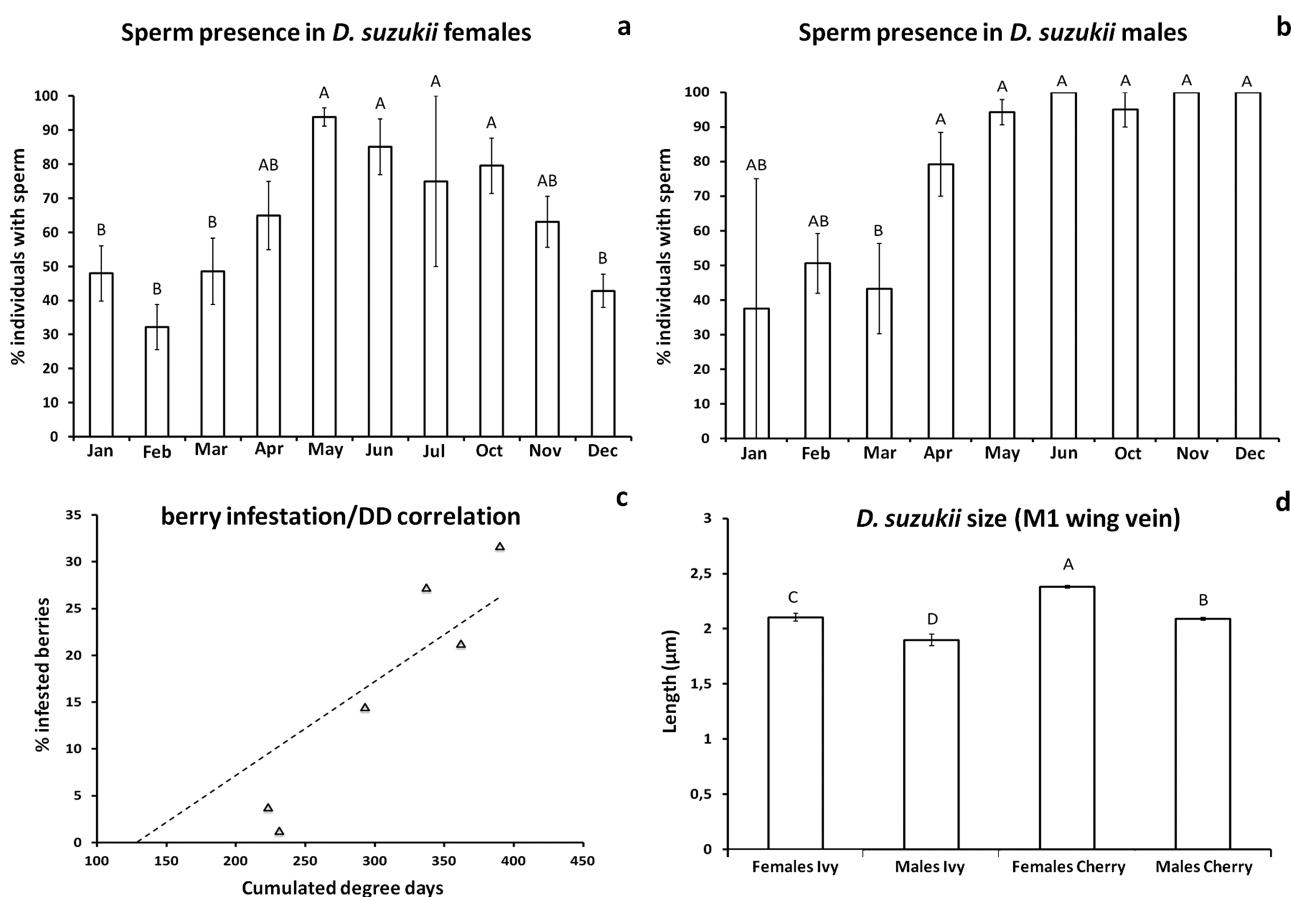


Fig. 7. (a) Monthly percentage of *Drosophila suzukii* females with sperm within spermatheca and/or seminal receptacle in Trento Province, Northern Italy, during 2015–16. (b) Monthly percentage of *D. suzukii* males with sperm within testis in Trento Province, Northern Italy, during 2016–2017. (c) Ivy berry infection rate over degree-days for *D. suzukii* in Trento, Italy during 2016. (d) *D. suzukii* M1 wing vein measurements from Cherry and Ivy-reared individuals. For each column, different letters indicate significant differences ($P < 0.05$) after ANOVA followed by Tukey's HSD test.

Table 2. *Drosophila suzukii* (SWD) sampling date, physiological age (DD), reproductive maturity, and commercial cherry phenology estimations together with percent infested ivy berries in Trento/Orrido, Italy during 2016

Sampling date	SWD DD	Estimated SWD with mature eggs (%)	Estimated (Chelan) cherry phenology stage	% infested ivy berries (min 100 berries)	Ivy berries firmness (cN)
14 Mar 2016	68	5.47	Bloom	0.0	
11 April 2016	242	33.51	Post bloom	3.8	nr
12 April 2016	250	34.88		1.3	nr
19 April 2016	315	45.48		14.5	53.35
26 April 2016	361	53.01		27.3	58.64
2 May 2016	389	57.28		21.3	55.80
8 May 2016			Hard green		
18 May 2016	521	62.07		31.7	nr
22 May 2016			Straw		
27 May 2016			Ruby		

The bottleneck periods are not only characterized by smaller *D. suzukii* populations and low temperatures, but also by limited availability of alternate hosts. The collected data strongly support the supposition that ivy berries can serve as a non-crop host during the late dormant period during the latter portion of the bottleneck period. Ivy berries are clearly not the most suitable host for *D. suzukii* development as indicated by the significantly smaller wing measurements in comparison to flies reared on *P. avium*. Presence of ivy berries may nevertheless allow for *D. suzukii* populations to persist and reproduce during the late dormant periods at lower numbers. The

findings that ivy is attacked during the late dormant period suggest that even the earliest commercially produced fruit stand the risk of being attacked by *D. suzukii* as they ripen. Cherries are generally among the first commercially produced susceptible crops of the early season. *D. suzukii* emerged from ivy berries as early as 223 DD (11 April 2015), although dissections suggest that females are physiologically able to lay eggs as early as 3 DD. Both cherry phenology models and observations from the region suggest that cherry fruit become available and are susceptible to *D. suzukii* attack much later than ivy (18 May, 390 DD for *D. suzukii* on cherry fruit), as this

Table 3. Percent *Drosophila suzukii* with mature eggs using egg dissections over accumulated degree-days from 1 January (Lower and upper thresholds for SWD is set at 7.2 and 30°C, respectively) in Trento Province, Northern Italy (Fig. 6a), and five highlighted production regions within Trento Province (Fig. 6b–f) at different altitudes during 2013–2016

Location	Descriptive function	Statistical parameters
Trento Province	$y = 0.1887x - 0.9061$	$F_{1,18} = 88.060$, $P < 0.001, R^2 = 0.83$
Romagnano	$y = 0.1988x + 5.1634$	$F_{1,9} = 42.567$, $P < 0.001, R^2 = 0.83$
St.Michele	$y = 0.1037x - 4.6318$	$F_{1,8} = 27.422$, $P < 0.001, R^2 = 0.77$
Trento/Fersina	$y = 0.1717x - 5.4028$	$F_{1,13} = 154.43$, $P < 0.001, R^2 = 0.92$
Canale	$y = 0.1039x + 1.6808$	$F_{1,11} = 38.351$, $P < 0.001, R^2 = 0.78$
Serso	$y = 0.3076x - 5.3229$	$F_{1,8} = 45.364$, $P < 0.001, R^2 = 0.85$

is when fruit become softer and have higher sugar levels (Lee et al. 2011, Burrack et al. 2013, Lee et al. 2016).

Assessment of female and male *D. suzukii* reproductive biology improves key knowledge about the abilities of early-season populations to utilize non-commercial hosts. This information will aid in the development of new strategies to control the pest. Overall, the alternate host data on ivy illustrate that *D. suzukii* is able to persist at smaller population levels and are very adaptable to low temperatures, together with several additional studies (Dalton et al. 2011, Stephens et al. 2015, Shearer et al. 2016, Wiman et al. 2016, Enriquez and Colinet 2017). It is clear that *D. suzukii* can utilize a wide range of host fruits and can maintain low population levels, even in very harsh conditions where there is limited fruit coupled with suboptimal temperatures. Aside from fruit, it has been demonstrated that *D. suzukii* can utilize non-fruit nectar and saps, as is found in pollen and nectar of cherries and blueberries, as well as extrafloral nectaries (Tochen et al. 2016a). *D. suzukii* can clearly adapt to persist under relatively extreme bottleneck periods, allowing them to oviposit on very early ripening commercial crops. The impact of *D. suzukii* females containing lower fractions of sperm and mature egg loads need additional study. The information from this study clearly points towards the late dormant period as a time when both *D. suzukii* population levels and reproductive potential are low. It is very probable that control strategies including bait sprays, SIT, and biological control can be targeted during this period. These techniques, applied in the early spring and targeting early-season surviving fertile females, are expected to have a significant suppressant population impact. The observations from this study should be incorporated into population models (Wiman et al. 2014, 2016; Gutierrez et al. 2016) and may contribute to make such models more precise and reliable at forecasting persistence of winter populations, and timing of population increase.

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