

Supplementary material 1: Exploratory experiment, 2017

[Cloutier, C., Guay, J.-F., and Champagne-Cauchon, W. 2022. Postdiapause reproduction of spotted wing drosophila in realistically simulated cold climatic springtime conditions of Québec, Canada. The Canadian Entomologist.]

Methods

Insects

Male and female *D. suzukii* flies were collected in nature in lowbush blueberry fields in the late summer and fall 2016, in the Saguenay–Lac-Saint-Jean region, Québec, Canada. Flies were collected using live traps, and fly age (several months) and mating status were unknown. In spring 2017, the flies were springtime survivors of the overwintering survival experiment reported by Cloutier *et al.* (2021). Postdiapause male and female survivors were monitored at regular intervals during their remaining lifetimes to measure reproductive potential in a springtime regime of abiotic conditions (Fig. 1). In spring 2017, after cold winter treatment, surviving flies were transferred to a growth chamber simulating daily fluctuation of temperature and photoperiod based on 10-year spring–summer data for the Normandin, Saguenay–Lac-Saint-Jean region (48° 50' 30,000 N, 72° 32' 49,000 W). Reproductive potential was measured as lifetime fecundity for postdiapause females and as lifetime fecundity of female partners for postdiapause males (see below).

Available winter survivors ($N = 30$ females, 5 males) had experienced a daily fluctuating 5 °C cold-winter regime with 6 °C amplitude. After six months of dormancy, the flies were transferred to the springtime regime of rising temperature and daylength (Fig. S1). Fecundity monitoring started when the daily maximum temperature exceeded 10 °C for at least three hours each day under a 14:10-hour light:dark photoperiod. Observation units

consisted of small groups of females ($N = 7$) of two to nine individuals each, with a controlled presence of males. The units were maintained in ventilated clear plastic vials with continuous access to fresh blueberries and moist filter paper. Flies were reared as groups, based on the importance of cuticular hydrocarbons as mating pheromones in *D. suzukii* (Revadi *et al.* 2015). Presumably, mating would increase among grouped *D. suzukii*, given reported effects of interindividual contacts on fecundity (e.g., Dekker *et al.* 2015; Ala-Honkola *et al.* 2018). Each group was observed until all individuals died, with a controlled male presence.

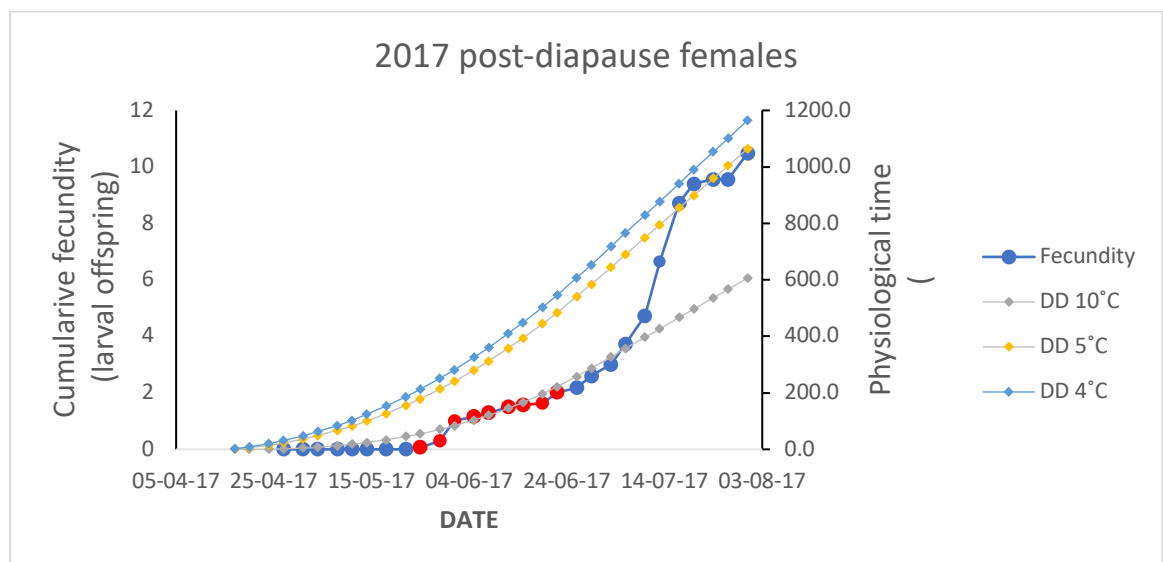


Figure S1. Cumulative fecundity of postdiapause female *Drosophila suzukii* in 2017 in simulated springtime cold–temperate climatic conditions, in relation to physiological time as degree-days above thresholds of 4 °C, 5 °C, and 10 °C. Fecundity values marked with red symbols on fecundity curve represent early reproduction of a post-diapause female coupled with post-diapause males. Oviposition started a month earlier than other females deprived of mating. From 26 June onwards, all other females started to lay eggs after being coupled to young males from the colony.

Male presence was delayed, except for two females which continuously had postdiapause male winter survivors present with them in the rearing vial. Considering that only five male survivors were available and that their reproductive potential was unknown,

all males were grouped with the two females at the beginning of the experiment. Temporary restriction of mating opportunity for other female survivors was based on the hypothesis that postdiapause mating might be optional in *D. suzukii* females after winter diapause, assuming pre-diapause mating. Six of the seven female groups were initially deprived of mating for two months until the last week of June 2017. Because no progeny had yet been obtained from unmated postdiapause females, and nearly 40% of the females were dead already, a two- to four-day-old male from a *D. suzukii* laboratory colony was introduced to each vial so that all females had mating opportunity henceforwards. The colony was initiated in summer 2016 with *D. suzukii* flies that had been live trapped in lowbush blueberry fields, and it was maintained on a diet of banana from the supermarket using a technique similar to Gonzalez-Cabrera *et al.* (2018), at 20 °C, 65% relative humidity, and a 16:8-hour light:dark photoperiod. Any progeny produced by female survivors following male introduction would thus suggest either that female winter survivors had not mated in 2016 or that they carried no viable sperm from a previous mating. The two females that had had earlier opportunity for mating were the only females coupled to postdiapause males, and they were permitted to mate freely from the beginning of the simulated springtime season.

All groups of females ($N = 7$) were monitored at intervals of three or four days until death to determine age-specific survival and fertility. Females had continuous access to two fresh blueberry fruits, which were renewed at each observation. Exposed blueberries were incubated at 20 °C, under a 16:8-hour light:dark photoperiod for three weeks. Emerged flies were then counted and sexed, and any remaining pupae were added to calculate individual lifetime fertility (few cases). Age-specific survival and fertility of postdiapause females were thus based on three- to four-day interval data, and overall reproductive performance could be compared between mated and unmated female winter survivors.

Results

Total fecundity of postdiapause female survivors observed as small-group units ($N = 7$) was 32.25 ± 11.78 (standard error) offspring per female. The female units reproduced for 33.17 ± 14.83 (standard error) days, on average, at a mean daily rate of 0.99 ± 0.13 (standard error) offspring per day. The single group consisting exclusively of winter survivors of both sexes (initially two females and five males) had the second-highest reproductive fitness (65 progenies), all produced by the only female that remained alive just before oviposition started. Egg laying by this female started about one month into simulated springtime (observation period 23–26 May; Fig. S1), when the maximum daytime temperature was 17°C (24-hour average = 12.8°C) under a 14:8-hour light:dark photoperiod. One female and three males of this group died before reproduction started, and thus all progenies were born from a single postdiapause female. Her reproductive period lasted 59 days, with a single postdiapause male alive and in attendance until the day before she died. Notably, this female was by far the first to reproduce, starting egg laying about one month before the others.

Introduction of males in late-June to the other female groups ($N = 6$) confirmed that postdiapause females without mating opportunity with a colony male remain infertile. Indeed, egg laying started within two days after male introduction, except in one group (16.5 days delay). Sexing adult flies ($N = 189$) born from postdiapause females ($N = 6$) revealed a moderately male-biased sex ratio (0.414 ± 0.083 standard error). No significant difference occurred between the sex ratios of the offspring of the female mated to postdiapause males (0.484) and of those mated to a colony male (0.444), according to Fisher's exact test ($P > 0.05$). Figure S1 illustrates cumulative fecundity of postdiapause females in relation to physiological time (degree-days), calculated at two potential threshold values (degree-days_{s,c} and degree-days_{10,c}). The postdiapause female allowed to mate freely from the beginning started to oviposit in third week of June, after 165 degree-days_{s,c} (55 degree-days_{10,c}). The

average cumulative fecundity trend in red symbols (Supplementary material, Fig. S2) represents that female's contribution to population growth until about 25 June, but thereafter the trend represents the contribution of at least six females.

Discussion

We conclude that both male and female postdiapause *D. sukii* survivors of six-month cold winter could effectively reproduce in realistic conditions simulating return of warmth and fruit resources for egg laying. Despite being reared gregariously, group size was small and gradually declined. Furthermore, mating was experimentally delayed, eventually allowing to collect fecundity data at individual level. Initially limited by low temperatures, egg laying started about one month after cold winter ended (third week of May 2017), when mean and maximum daily temperatures were 12.8 and 17.6 °C, respectively. In all but one group, delayed reproduction is explainable by delayed mating (Fig. S2) when we realised that postdiapause females might not reproduce at all without mating opportunity. Advanced reproduction in the single group allowed to mate from the beginning of spring with postdiapause males (initial $N = 5$; see above), confirmed that mating opportunity was limiting postdiapause female fertility. In groups with delayed mating ($N = 6$), female oviposition started and peaked soon after young colony males were provided as mates (Supplementary material, Fig. S2). We therefore conclude that either field-collected females in late summer 2016 had not mated or that any sperm acquired before or during capture (in live traps) was no longer viable when spring 2017 began, after six months of reproductive diapause.