


ARTICLE

Postdiapause reproduction of spotted-wing drosophila (Diptera: Drosophilidae) in realistically simulated cold climatic springtime conditions of Québec, Canada

Conrad Cloutier* , Jean-Frédéric Guay, and William Champagne-Cauchon

Département de Biologie, Pavillon Alexandre-Vachon, Université Laval, 1045 avenue de la Médecine, Québec, Québec, G1V 0A6, Canada

*Corresponding author. Email: conrad.cloutier@bio.ulaval.ca

(Received 10 April 2021; accepted 2 November 2021)

Abstract

Knowing the biology and ecology of *Drosophila suzukii* (Diptera: Drosophilidae) is important to predict its potential for permanent establishment in cold climatic regions. We studied the reproductive potential of postdiapause male and female flies collected in lowbush blueberry fields in late season over two years. After being submitted to a six-month cold-winter regime, survivors were exposed to a simulated springtime warming regime to monitor reproductive potential and expected population growth. Overwintered fly survivors of both sexes that were aged up to nine months after field capture reproduced successfully in spring and early summer when mated to nondiapause flies experimentally made available for mating as potential migrant colonisers from lower latitudes. Relative to control nondiapause flies, the lifetime fertility of postdiapause flies was reduced by 84% in females and 70% in males, revealing large fitness costs of reproductive diapause under cold stress. Modelling potential reproduction of overwintered flies in warming conditions using models parametrised for age-dependent fertility rate and diapause status showed that nondiapause migrant flies would be strongly advantaged over locally overwintered postdiapause flies due to precocious reproductive maturity, broader pattern of age-specific fertility, and higher lifetime fertility.

Résumé

La connaissance de la biologie et de l'écologie de *Drosophila suzukii* (Diptera : Drosophilidae) est essentielle pour prédire son établissement éventuel en région tempérée froide. Nous avons étudié pendant deux ans la capacité de reproduction post-diapause de mouches collectées en bleuetières en fin de saison. Après avoir été soumis à un régime hivernal froid de six mois, les survivants exposés à un régime simulé de retour printanier ont été suivis pour déterminer le taux de reproduction et la croissance populationnelle espérée. Des mouches hivernées d'âge réel atteignant jusqu'à neuf mois se sont reproduites avec succès après l'hiver en régime printanier simulé, lorsqu'accouplées avec des partenaires fertiles non-diapausés, considérés comme immigrants potentiels de régions plus au sud. Comparé aux mouches témoins non-diapausées, la fertilité des femelles en post-diapause était réduite de 84 % et celle des mâles de 70 %, révélant un coût potentiel majeur de la diapause en climat tempéré froid en termes de pouvoir reproducteur. Des modèles démographiques dépendant de l'âge et du statut reproducteur relatif à la diapause ont montré que des mouches post-diapausées seraient fortement désavantagées par rapport aux non-diapausées, avec un retard de la maturité reproductive et une diminution de la fertilité dépendante de l'âge et totale à vie.

Subject Editor: Katie Marshall

© The Author(s), 2022. Published by Cambridge University Press on behalf of the Entomological Society of Canada. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Cold winters represent a major barrier to the permanent establishment of invasive species such as *Drosophila suzukii* (Diptera: Drosophilidae) in cold-temperate regions such as Canada (Walsh *et al.* 2011; Zerulla *et al.* 2015; Rossi-Stacconi *et al.* 2016; Shearer *et al.* 2016; Stockon *et al.* 2019). In cold climates, to survive winters until the next season, *D. suzukii* should respond adaptively to cues signalling end of summer by expressing a true reproductive diapause (Danks 2006; Denlinger 2008; Salminen and Hoikkala 2013; Zerulla *et al.* 2015; Rossi-Stacconi *et al.* 2016; Shearer *et al.* 2016; Zhai *et al.* 2016). Then, having responded appropriately, diapausing flies must tolerate cold stress for at least six months at harmful temperatures well below their activity and metabolic thresholds (Rossi-Stacconi *et al.* 2016).

Overwintering in a cold-temperate region also implies that adult longevity should persist long after winter because *D. suzukii*'s oviposition and larval development depend on ripe fruits (*e.g.*, Lee *et al.* 2011; Winkler *et al.* 2020). Postdiapause reproductive maturity would not be recovered until temperatures reach thermal threshold for gametogenesis, estimated to be between 5 °C and 10 °C (Walsh *et al.* 2011; Rota-Stabelli *et al.* 2013; Arnó *et al.* 2016; Gutierrez *et al.* 2016; Hamby *et al.* 2016; Ryan *et al.* 2016; Little *et al.* 2017; Alhmedi *et al.* 2019; Winkler *et al.* 2020). Sufficient physiological time (effective heat units or degree-days above development threshold temperature) must then accumulate until prereproductive maturation, sexual encounter and mating, and ultimately egg laying by fertile females in early maturing fruits (Winkler *et al.* 2020).

In a three-year field study in the Saguenay–Lac-Saint-Jean region, Québec, Canada, Champagne-Cauchon *et al.* (2020) found *D. suzukii* to be a regular, minor pest on lowbush blueberry, where it had been trapped since 2012. In blueberry fields and surrounding areas, *D. suzukii* is widespread and abundant in late summer and fall, but no direct evidence of local overwintering was found. Nevertheless, high densities of dark morph, reproductively diapausing *D. suzukii* flies, occurred in late fall, suggesting the possibility of local overwintering (Champagne-Cauchon *et al.* 2020). Studies to date in the invasive range of *D. suzukii* indicate that cold-acclimated flies are chilling intolerant (Tochen *et al.* 2014; Jakobs *et al.* 2015; Ryan *et al.* 2016; Langille *et al.* 2017; Nikolouli *et al.* 2018; Winkler *et al.* 2020), which would preclude winter survival under natural conditions in Canada and in the northeastern United States of America, except in natural microhabitats protected from freezing or in heated anthropic environments (*e.g.*, Zerulla *et al.* 2015; Stockon *et al.* 2019).

Cloutier *et al.* (2021) studied *D. suzukii*'s winter survival by exposing field collected flies from Québec to realistically simulated winter regimes of cold stress that lasted six months, followed by a return to springtime conditions, while assuming that fall-acclimated flies select microhabitats with low risk of extensive freezing. Despite substantial mortality after 180 days of cold stress, numerous *D. suzukii* flies of both sexes survived. When transferred to springtime rising temperatures and increased daylength based on regional norms, life expectancy distributions indicated that winter survivors (10–30% of flies tested) would still be alive in June and up to July of next year (Cloutier *et al.* 2021). The flies were from late-season, high-density populations (Champagne-Cauchon *et al.* 2020), as previously reported (*e.g.*, Thistlewood *et al.* 2018). Despite the substantial mortality that was seen, it cannot be discounted that many *D. suzukii* might survive local winters to resume reproduction in spring. Even males survived the 180-day cold winter (Cloutier *et al.* 2021), which suggests that both sexes of postdiapause flies could eventually mate after winter to reproduce in the next season. For survivors with no opportunity to mate before diapause, mating after winter would be essential to start a new generation in spring. The need to mate in spring would necessarily apply to males, as well as to females that might have mated before winter but are no longer fertile come spring, as is discussed in Ryan *et al.* (2016) and has been shown in *D. montana* (Ala-Honkola *et al.* 2018).

Reproductive diapause is common among insects that overwinter in cold-temperate climates (Tauber *et al.* 1986; Danks 1987; Denlinger 2008). In multivoltine species such as *D. suzukii*, winter-diapausing flies probably experience fitness costs incurred by overwintering that nondiapausing flies of summer generations do not experience (Tauber *et al.* 1986; Danks 1987; Sadakiyo and Ishihara 2012a, 2012b). Even at a low metabolic rate, the use of stored energy reserves for prolonged diapause likely negatively affects fitness in postdiapause *versus* nondiapause flies (Hahn and Denlinger 2007). Reproductive diapause also implies time and energy after dormancy to recover reproductive maturity, to mate at low population densities caused by winter mortality and among sex-biased conspecifics. For winter-diapausing females, the extra energy expenditures associated with diapause might reduce longevity and fertility compared to nondiapausing females. For males, the additional costs of diapause might be reflected in the reduced fertility of their female partners. Knowing the fitness costs of diapause in *D. suzukii* thus is of considerable interest, given that both sexes can survive cold stress periods equivalent to continental cold-temperate winters (Cloutier *et al.* 2021). Documenting these fitness costs is possible by comparing lifetime fecundity of postdiapause and nondiapause flies in simulated, controlled springtime conditions. Knowing the species' postdiapause reproductive potential would be useful for better predicting *D. suzukii*'s future expansion and potential to establish in temperate regions outside its native range (*e.g.*, Ørsted and Ørsted 2019).

The present study's objective was to experimentally evaluate the early season reproductive dynamics of *D. suzukii* in simulated cold-temperate springtime conditions. We assessed the reproductive fitness of long-lived postdiapause flies that survived a cold winter that is typical of eastern Canada, as reported by Cloutier *et al.* (2021). Following cold-winter stress treatments that lasted six months, surviving male and female flies were monitored for reproductive potential in abiotic conditions that simulated spring and early summer. We expected that postwinter mating might be a critical requirement for postdiapause reproduction, even among females that may have mated the previous fall. We hypothesised that, due to the fitness costs of diapause, fitness would be reduced in postdiapause flies compared to that in nondiapause flies of summer generations. Our objectives were (1) to demonstrate that postdiapause cold-winter survivors of both sexes of *D. suzukii* can reproduce successfully in the next season; (2) to compare the reproductive potential of postdiapause *D. suzukii* to that of nondiapause *D. suzukii* from a laboratory colony (see Supplementary material for details) as a way of evaluating the fitness cost of overwintering in diapausing flies; and (3) to project potential springtime population growth from small numbers of flies, including both potential local winter survivors and nondiapause potential incoming migrant flies from elsewhere.

Methods

Insects

Male and female *D. suzukii* flies were live trapped in lowbush blueberry fields in late summer and fall 2017, in the Saguenay–Lac-Saint-Jean region, Québec, Canada. Postdiapause flies were springtime survivors of the second-year overwintering survival experiment of Cloutier *et al.* (2021). Flies had survived a cold-winter sine-wave fluctuating temperature regime of 3 ± 3 °C. Postdiapause male and female survivors were followed for the remainders of their lives to determine their reproductive potential in a realistic regime of improving abiotic conditions. In April and May 2018, at the end of the six-month cold-winter treatment period, survivors were transferred to a growth chamber that was programmed with daily fluctuating temperatures based on 10-year spring–summer data for Normandin, Saguenay–Lac-Saint-Jean region, Québec, Canada (48° 50' 30 000 N, 72° 32' 49 000 W; Fig. 1).

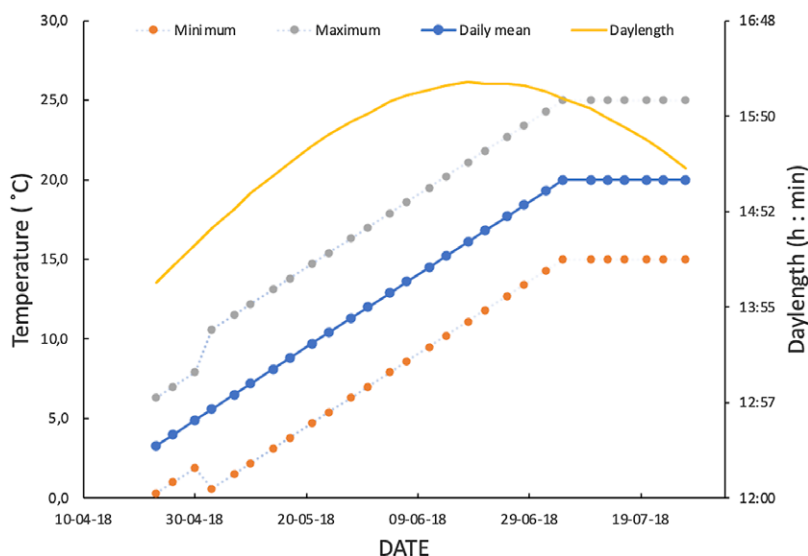


Fig. 1. Spring–early summer regime of daylength and fluctuating temperatures used to simulate temperate climatic conditions in monitoring lifetime fecundity of *Drosophila suzukii*. From first week of July, mean temperature of fluctuating temperature regime was fixed at 20°C to avoid higher values beyond the optimal range of the species.

The reproductive potential of postdiapause females was measured as lifetime fecundity and, for postdiapause males, as lifetime fecundity of female partners (see below). We took into account the results of an exploratory experiment conducted in 2017 (see Supplementary material 1) that showed that postdiapause winter survivors indeed could reproduce in simulated springtime conditions typical of the study region.

Observation units consisted either of isolated females or of couples of *D. suzukii*, for which female lifetime fecundity was measured while controlling for male presence. Dying males in couples were not replaced, but overall, a male was present during 73–85% of females' reproductive lifetime, depending on the mating treatment. Having found previously (Supplementary material 1) that postdiapause mating was critical for postdiapause reproduction, mating treatments were added to examine postdiapause reproductive potential and to estimate the fitness costs of diapause by comparing fertility between diapause and nondiapause flies. Postdiapause females either were matched to a young male from a laboratory colony or remained isolated (no mate) to test the hypothesis that they might still retain viable sperm if they had mated the previous year (2017) before trapping. A colony was initiated in summer 2016 with flies trapped in blueberry fields, and it was maintained on a banana diet using a technique similar to Gonzalez-Cabrera *et al.* (2018), at 20 °C, 65% relative humidity, and a 16:8-hour light:dark photoperiod. The reproductive potential of postdiapause males, which were less numerous than females, was based on the lifetime fecundity of a virgin nondiapause female partner from the colony or of a postdiapause female. The experiment thus involved six mating treatments: (1) virgin nondiapause female from colony coupled with overwintered (postdiapause) male (CFOM); (2) both female and male from colony (nondiapause control treatment; CFCM); (3) virgin colony female without male (CF), testing for possible parthenogenesis; (4) overwintered (postdiapause) female without male (OF), testing for fertility based on viable sperm retention from mating in 2017; (5) postdiapause female coupled to nondiapause male (OFCM); and (6) couples formed by postdiapause females and postdiapause males (OFOM). The results of these treatments would allow us to determine if postdiapause females (OF) could reproduce with and without mating in spring

and if they could mate effectively with either a colony male (CM) or a postdiapause male (OM). Similar reasoning applied in testing the reproductive potential of overwintered (postdiapause) males. Available females and males were randomly allocated to treatments, with the obvious restriction that, because the number of surviving males was lower than females, postdiapause male availability limited the number of replicates for treatments involving overwintered males. For treatments involving nondiapause colony flies (CF, CM), newly emerged (within 24–48 hours) colony flies that had been collected as pupae were used.

For consistency, we use the terminology of Henderson and Southwood (2016, section 11.4) when referring to the life table and reproductive rate of *D. suzukii*. We therefore refer to lifetime fertility of adult flies of known age and mating status – that is, control colony flies – reared in the laboratory or of flies of unknown age and mating status, trapped in nature and experimentally overwintered. Isolated females and couples were followed during their lifetimes in the predetermined regime of simulated springtime conditions (Fig. 1). Consequently, in the case of postdiapause flies, the term, “age-specific”, does not mean the true chronological age of the individual flies: it means their ageing-with-time during the experiment after they had emerged from winter dormancy upon being transferred to the springtime-simulated conditions.

Reproduction was monitored by counting larvae dissected from fruits exposed at three- to four-day intervals during each individual fly’s lifetime. Female fertility therefore represents hatched (larval) offspring born into the next generation – the net reproductive rate (R_0). For each female, R_0 also included the larvae found in blueberries that were dissected to monitor the results of ovipositor marking of exposed fruits by subsamples of females assigned to each treatment. Eggs laid on exposed fruits were not counted, but for up to five consecutive two-day periods, the skin of exposed blueberries was examined under a stereomicroscope to count small slits (ovipositor marks) left by females assigned to each mating treatment. We thus used egg-laying marks as a proxy for the number of eggs laid and compared the number of marks to the number of larvae found when we dissected the fruits after two-day exposure. We did not attempt to determine if eggs remained present in the ovipositor marks because fruits were examined and dissected for larvae within 24 hours of being exposed to flies. To calculate an “egg hatching ratio”, the number of ovipositor marks was compared to number of larvae found upon dissection of each fruit, with the assumption that one mark made on fruit skin corresponds to one egg laid. Low values of this ratio could reveal marks without oviposition – that is, marks made for, for example, feeding or for laying unfertile eggs (Atallah *et al.* 2014). High values could represent more than one egg laid per mark or larval entry into the fruit without an apparent ovipositor stabbing.

Statistical analysis

With enough replicates available, life table data for this experiment were statistically analysed to compare reproductive performance between mating treatments. The normal distribution hypothesis could not be met for many life table variables (see, *e.g.*, Supplementary material 2, Fig. S2-1), based on the Shapiro–Wilk statistic. We therefore used the distribution-free Kruskal–Wallis test, which is a nonparametric version of traditional one-way analysis of variance (Critchlow and Fligner 1991). When the Kruskal–Wallis chi-square statistic was significant ($P \leq 0.05$), we used the `dunnTest` function of package `FSA` of R (Mangiafico 2021; R Studio Team 2021), which implements Dunn’s (1964) method for multiple comparisons, with the Benjamini–Hochberg correction to control the experiment-wise error rate.

To estimate the population growth rates of postdiapause *D. suzukii* under the simulated springtime regime, we first used Henderson and Southwood’s method (2016, Chapter 11, Equation 11.30), based on “intrinsic capacity for increase” (r_c), as generally used by insect ecologists. Capacity for increase was calculated using the equation: $r_c = \log(R_0/T_c)$, where R_0 is

the mean net reproductive rate of the experimental cohorts of flies assigned to each treatment. R_0 was calculated as the sum of all daughter progenies born from females over lifetime, $R_0 = \sum l_x m_x$, where l_x and m_x represent age-specific mortality and fecundity, respectively. T_c is the cohort “generation time”, or pivotal age – that is, the mean age of females at the time of offspring birth, when 0.5 R_0 under experimental conditions (springtime) had been realised, assuming a 1:1 sex ratio among offspring. Age 0 is female chronological age since the beginning of the experiment.

To model and predict springtime population growth based on the reproduction of postdiapause *D. suzukii* flies over lifetime, the cumulative fecundity data (*i.e.*, cumulative larval offspring counts in dissected blueberries) were fitted to a modification of the Gompertz model. This model has been widely used to analyse population growth curves when the growth rate (r) varies in response to population density (Eberhardt *et al.* 2008), as it similarly varied in the present study due to the ageing of females during lifetime. The model formulation used is

$$N(t) = F \exp\{-\exp(-r(t - t_{\max}))\}$$

where F is the asymptotic value of lifetime fecundity, as adult females age with time, r is the maximum rate of population increase during lifetime due to natality, and t_{\max} is the time at the inflexion point of the population growth curve.

The model describes *D. suzukii* population growth resulting from reproduction of flies assigned to the different mating treatments as their reproductive rate changed with time due to ageing in springtime conditions. It is mathematically similar to a two-parameter classical Gompertz model (Eberhardt *et al.* 2008). Asymptotic fecundity (F) is analogous to K in the Gompertz model, and instantaneous fecundity rate (r) is mathematically analogous to the density-dependent per capita growth rate. The third parameter, t_{\max} , is a location parameter that shifts the curve horizontally without changing its shape. It is the time at the inflexion point of the curve for which we have 36.8% of F , the upper asymptote (Tjørve and Tjørve 2017). From this model, we can also estimate the time at which $N(t) = 1$, corresponding to time when fly reproduction started, allowing the estimate of the extent to which the starting time was affected by female diapause and mating status. By rearranging the terms in the model equation, we can estimate time when oviposition started as:

$$t|_{N(t)=1} = t_{\max} - [\log(\log(F))/r]$$

Parameter estimates for fecundity data obtained from different treatments were compared using Cochran’s chi-square test (Cochran 1952).

Results

In spring 2018, *D. suzukii* flies of both sexes were tested for reproductive fitness in a design involving six mating treatments comparing postdiapause and nondiapause flies under simulated springtime conditions. During testing, data on ovipositor marking of fruits by females were collected at two-day intervals for 14 days on average, which allowed us to relate fruit marking to fertility, as confirmed by number of larvae present in marked fruits. Survival data for treatments involving fly couples indicated that males generally died before their female mates. The mean proportion of a female’s lifetime when a live male partner was present varied from 37% (OFOM treatment) to 89% (CFOM), with differences being insignificant ($\chi^2 = 4.0152$, $P = 0.2735$; data not shown).

Table 1. Longevity and reproductive performance (mean \pm standard error) of *Drosophila suzukii* in experiment involving mating treatments. Mated and unmated females were monitored for oviposition on blueberries until they died. Within columns, means sharing a letter are not statistically different based on Kruskal–Wallis analysis of variance.

Mating treatment ¹	N_{obs} ²	N^3	Longevity (d)	N^3	Progenies produced	N^3	Reproductive period (d)
CF	10	10	63.20 \pm 2.74 ab
CFCM	13	13	64.15 \pm 3.94 a	13	58.46 \pm 9.54 a	13	37.23 \pm 4.54 a
CFOM	3	3	51.67 \pm 6.01 ab	3	17.67 \pm 10.84 ab	3	24.67 \pm 3.71 ab
OF	10	10	45.90 \pm 6.12 ab	10	0.10 \pm 0.10 b	10	0.20 \pm 0.20 b
OFCM	11	11	48.27 \pm 5.01 b	11	8.64 \pm 4.95 b	11	11.55 \pm 4.63 b
OFOM	2	2	67.00 \pm 1.00 ab

¹Defined as female (F) of known diapause status (CF, nondiapause from colony; OF, postdiapause – *i.e.*, having survived cold-winter temperatures), left unpaired or crossed with male (M) of similarly known diapause status (CM, OM).

²Total number of females observed for each treatment.

³Sample size for statistical analysis.

Life table schedule

Isolated females without male partners (Table 1; CF treatment, $N = 10$) lived for 63.20 days on average and produced no progenies, as was expected (Table 1). By comparison, mean longevity of the (chronologically much older) postdiapause females without male partners (OF treatment, $N = 10$) was 45.90 days, and none produced progeny (Table 1). In control couples consisting of nondiapause male and female flies from the colony (Table 1; CFCM treatment, $N = 13$), females lived 64.15 days on average. They were fertile for 37.23 days and produced 58.46 offspring (Table 1). By comparison, the mean lifetime of the postdiapause females mated to nondiapause males was 48.27 days; they were fertile during 11.55 days (mean) and produced 9.50 progeny (mean; Table 1). Statistical analysis showed that differences in life statistics between similarly mated postdiapause females (OFOM) and nondiapause (CFCM) females mated to nondiapause males were significant for lifetime ($P = 0.0311$), reproductive period ($P = 0.0017$), and total offspring ($P = 0.0036$). Postdiapause female fertility was thus about six times less than for nondiapause females. Few replicates of treatments involved postdiapause males (OM; Table 1). Postdiapause males mated to postdiapause females (Table 1; OFOM treatment, $N = 2$) survived a modest fraction of her lifetime (0.37 ± 0.10 standard error) but did not reproduce, as their postdiapause partners left no progeny despite extensive mean longevity (67.00 days). The postdiapause males coupled to colony (nondiapause) females (Table 1; CFOM treatment, $N = 3$) survived over most of their female partners' lifetimes (0.89 ± 0.05 standard error), averaging 51.67 days. Postdiapause males were shown to be fertile as their nondiapause female partners produced 17.67 progeny (mean) over a mean reproductive period of 24.67 days. Statistical analysis indicated that the reproductive period and total progenies of nondiapause females mated to postdiapause males did not significantly differ from those of females in the other treatments (Table 1); this is attributable to insufficient statistical power because of the small number of replicates ($N = 3$). Indeed, compared to the fertility of the nondiapause female partners of nondiapause males, postdiapause males appeared about 3.3 times less fertile (Table 1; progeny mean of 17.67 *versus* 58.46).

Monitoring fruit marking

The unmated postdiapause females left no marks on fruits (Table 2). Because of this, this treatment (OF) was excluded from statistical analysis. The total number of marks between other treatments varied markedly but with no significant differences ($P > 0.05$).

Table 2. Ovipositor marking by *Drosophila suzukii* on blueberry fruits in the 2018 experiment (see Supplementary material 2). Postdiapause female (OF) and male (OM) survivors to six months cold-winter treatment are compared to nondiapause females (CF) and males (CM) from a laboratory colony. Within columns, means sharing the same letter are not statistically different based on Kruskal–Wallis analysis of variance.

Mating treatment ¹	N_{obs} ²	N^3	Total marks ⁴	N^3	Number of marks/day ⁴	N^3	Ratio of larvae/marks ⁵
CF	10	10	46.00 ± 7.27 a	10	2.69 ± 0.39 a	.	0.00
CFCM	13	12	46.33 ± 8.98 a	12	3.01 ± 0.44 a	13	2.14 ± 0.53 a
CFOM	3	2	14.00 ± 13.00 a	2	1.00 ± 0.92 a	2	0.76 ± 0.24 ab
OF	10	.	0.00	.	0.00	.	.
OFCM	11	10	17.90 ± 5.82 a	10	1.25 ± 0.38 a	11	0.49 ± 0.22 b
OFOM	2	2	28.50 ± 20.50 a	2	1.58 ± 1.58 a	.	.

¹Defined as female (F) of known diapause status (CF, nondiapause from colony; OF, postdiapause, having survived cold winter), left unpaired or crossed with male (M) of similarly known diapause status (CM, OM).

²Total number of females observed.

³Sample size for statistical analysis (flies in OF treatment marked no fruits).

⁴Daily number of ovipositor marks left on blueberry fruits.

⁵Ratio of number of larvae in dissected fruits to ovipositor marks on fruits.

To account for the monitoring period of fruit marking not being strictly controlled, we calculated daily rate of fruit marking to allow direct comparison of marking frequency between treatments (Table 2). The resulting daily rate of marking ranged from 1.00 marks.d⁻¹ to 3.01 marks.d⁻¹ but had no significant treatments effect (Table 2). Unmated nondiapause (colony) females left abundant marks on fruits, but no offspring emerged from exposed blueberries (Table 2, CF). Nondiapause females coupled to nondiapause males left, on average, the same number of marks on fruit as the unmated nondiapause females did (Table 2, CF *versus* CFCM). The ratio of larvae to marks significantly differed between treatments (Table 2); the ratio was more than four times higher for nondiapause females than for postdiapause females when both were mated to nondiapause males (Table 2, CFCM *versus* OFCM), indicating a significant reduction of egg hatching in postdiapause females. In couples formed of postdiapause flies of both sexes (OFOM), only one of the two females left marks, producing high variance (Table 2). The ratio of larvae to marks for nondiapause females mated to a postdiapause male or to nondiapause male (OFOM *versus* CFCM) did not significantly differ, indicating similar egg-hatching success (Table 2).

Intrinsic population increase rate

Monitoring lifetime fecundity for flies in each treatment involved a maximum of 25 successive observation periods from mid-May to end of July 2018. We defined 25 consecutive age classes of adult flies experiencing similar conditions, for which age-specific mortality (l_x) and age-specific fertility (m_x) were calculated. The intrinsic capacity for increase (r_c) over springtime varied, from 0.0726 d⁻¹ for nondiapause female partners mated to postdiapause males (CFOM) to 0.0771 d⁻¹ for postdiapause females mated to nondiapause males (OFCM; Table 3). By comparison, the (r_c) of nondiapause females mated to nondiapause males (CFCM) in the same conditions was 0.087 d⁻¹; that is, 1.0–1.5% higher than for postdiapause females of nondiapause males and for nondiapause female partners of postdiapause males (CFOM; Table 3).

Modelling population growth potential

The three-parameter Gompertz model provided good fit for the data on female fecundity as affected by age and mating treatments, with R -square values exceeding 98% (Fig. 2). The statistical

Table 3. Expected population growth rates of *Drosophila suzukii* in simulated cold-temperate springtime conditions, as affected by mating treatments in the 2018 experiment (see Supplementary material 2).

Mating treatment ¹	<i>N</i>	Net reproductive rate ² (R_0)	Pivotal age ³ (<i>d</i>)	Capacity for increase (r_c) (d^{-1})
CFCM	13	29.346	39.025	0.0866
OFCM	11	9.250	28.827	0.0771
CFOM	3	17.333	39.300	0.0726

¹Defined as crosses of female (F) and male (M) of known diapause status: CF and CM, nondiapause colony flies; OF and OM, flies having survived cold-winter treatment (postdiapause).

²Expected number of daughters produced during lifetime (cumulative $l_x m_x$).

³Days since the beginning of simulated spring (true chronological age of postdiapause flies unknown).

analysis (Table 4) indicated that maximum instantaneous population growth rate of nondiapause-mated flies (CFCM; $0.0573 d^{-1}$) was significantly lower than that of the postdiapause flies in both the OFCM ($0.1019 d^{-1}$) and CFOM ($0.1435 d^{-1}$) treatments. Paired contrasts (Table 5) showed that all observed r differences were significant, with P -values ranging from 0.001 to 0.0168, with the r estimates for the OFCM and CFOM treatment being the least different. The asymptotic fecundity parameter (F) also varied strongly with treatments: its value for nondiapause flies (CFCM) was 2–3 times higher than for postdiapause flies (OFCM and CFOM; Table 4). Contrasts showed that F parameters for mating pairs involving each sex as the postdiapause individual (OFCM, CFOM) were highly significantly lower ($P = 0.0018$ and $P < 0.001$, respectively; Table 5) than the F parameter for nondiapause male and female mates (CFCM) was. Moreover, the difference between the types of partners for postdiapause males and postdiapause females was not significant ($P = 0.2958$; Table 5). The third parameter, time at inflexion (t_{max}), also varied with treatments (Table 4), indicating that, for both treatments involving nondiapause females (CFCM and CFOM), the maximum rate of egg laying occurred earlier than it did for postdiapause females (OFCM). Paired contrasts showed that t_{max} differences between both treatments involving nondiapause females (CFCM, CFOM) were not significant ($P = 0.1014$; Table 5). In contrast, both differences between treatments involving postdiapause females compared to nondiapause females (OFCM versus CFCM; OFCM versus CFOM) were highly significant ($P < 0.0001$; Table 5). The calculated time when reproduction started ($t_{N(t)=1}$) also varied between treatments (Table 4) and occurred much earlier in the treatment involving nondiapause mates (CFCM) compared to treatments involving a postdiapause mate of either the female or male sex (CFOM, OFCM). Paired contrasts showed that oviposition in treatments involving a postdiapause mate of either sex was significantly delayed compared to the treatment involving both nondiapause mates ($P < 0.0001$; Table 5), with no significant difference between treatments involving a postdiapause male or female ($P = 0.5103$; Table 5).

Ageing effects on female fecundity varied strongly between nondiapause and postdiapause flies (Fig. 3). The broadest, most-productive age-specific fecundity pattern was that of nondiapause mates (CFCM; Fig. 3); however, the highest peak fecundity was attained by nondiapause females mated to postdiapause males (CFOM; Fig. 3).

Springtime population growth and heat accumulation

Results of the above analysis are illustrated in Figure 4 to allow comparisons of initial population growth potential expected at low population density in spring in cold-temperate conditions. Cumulative degree-days in Figure 4 are based on historic climatic records for the region of fly origin, allowing the prediction of potential growth from postdiapause flies that survive winter locally and mate with potential migrant flies colonisers. Offspring production involving postdiapause flies (Fig. 4, trends CFOM and OFCM) started about two months after

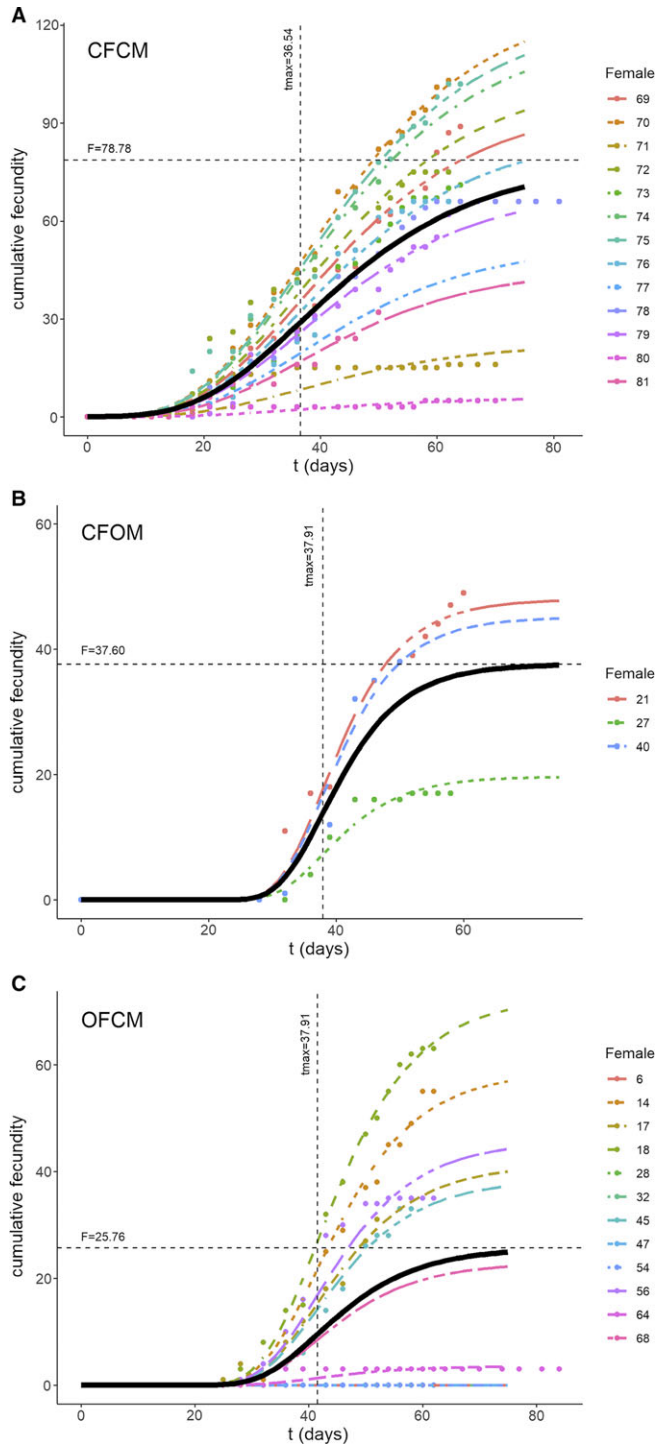


Fig. 2. Three-parameter Gompertz models fitted to data on *Drosophila suzukii* cumulative fecundity over time (t in days) as affected by mating treatments: **A**, fecundity of nondiapausing females mated to nondiapausing males; **B**, postdiapausing females mated to nondiapausing males; and **C**, postdiapausing males mated to nondiapausing females. Black curves illustrate fitted model with parameters specific to each treatment. Horizontal and vertical lines in each graph correspond to estimated parameters asymptotic fecundity (F), and delay before egg laying (t_{max}) following transfer to springtime conditions, respectively.

Table 4. Gompertz model parameters fitted to 2018 data on *Drosophila suzukii* female reproduction to project population growth in the new season and compare time delay before egg laying (intersection with time axis).

Treatment ¹	Parameter ²	Estimate	Standard error	95% confidence limits
CFCM	r	0.0573	0.00247	0.05188–0.06265
CFOM	r	0.1435	0.01664	0.07194–0.2151
OFCM	r	0.1019	0.00509	0.09074–0.1131
CFCM	F	78.7840	10.7742	55.3090–102.26
CFOM	F	37.5999	7.5594	5.0742–70.1255
OFCM	F	25.7579	8.4359	7.1905–44.3253
CFCM	t_{\max}	36.5353	0.6553	35.1074–37.9631
CFOM	t_{\max}	37.9110	0.5254	35.6503–40.1718
OFCM	t_{\max}	41.4708	0.3421	40.7177–42.2238
CFCM	$t_{N(t)=1}$	10.7947	0.9017	9.0274–12.5620
CFOM	$t_{N(t)=1}$	28.9339	1.0248	26.9253–30.9425
OFCM	$t_{N(t)=1}$	29.9127	1.0773	27.8012–32.0241

¹Defined as crosses of female (F) and male (M) flies of known diapause status, either nondiapause from the colony (CF, CM) or having survived cold-winter treatment (postdiapause; OF, OM).

² r , maximum reproductive rate; F , asymptotic cumulative net fecundity; t_{\max} , time to maximum reproductive rate; $t_{N(t)=1}$, time to the start of reproduction (intersection with X-axis).

Table 5. Paired contrasts between model parameters fitted to 2018 data on *Drosophila suzukii* female reproduction as affected by mating treatments.

Parameter	Contrast ¹	t	χ^2	df	P -value
r	CFCM–CFOM	–5.1264	26.2799	1	0.0001
r	CFCM–OFCM	–7.8871	62.2067	1	0.0001
r	CFOM–OFCM	2.3906	5.7150	1	0.0168
F	CFCM–CFOM	3.1291	9.7913	1	0.0018
F	CFCM–OFCM	3.8751	15.0163	1	0.0001
F	CFOM–OFCM	1.0454	1.0929	1	0.2958
t_{\max}	CFCM–CFOM	–1.6579	2.6827	1	0.1014
t_{\max}	CFCM–OFCM	–6.6766	44.5771	1	0.0001
t_{\max}	CFOM–OFCM	–5.6779	32.2384	1	0.0001
$t_{N(t)=1}$	CFCM–CFOM	–13.2888	176.5918	1	0.0001
$t_{N(t)=1}$	CFCM–OFCM	–13.6088	185.2003	1	0.0001
$t_{N(t)=1}$	CFOM–OFCM	–0.6584	0.4334	1	0.5103

¹Contrasted treatments defined as crosses of female (F) and male (M) flies of known diapause status, either undiapause from colony (CF, CM) or having survived cold-winter treatment (*i.e.*, postdiapause; OF, OM).

r , maximum reproductive rate; F , asymptotic cumulative net fecundity; t_{\max} , time to maximum reproductive rate; and $t_{N(t)=1}$, time to the start of reproduction (intersection with X-axis).

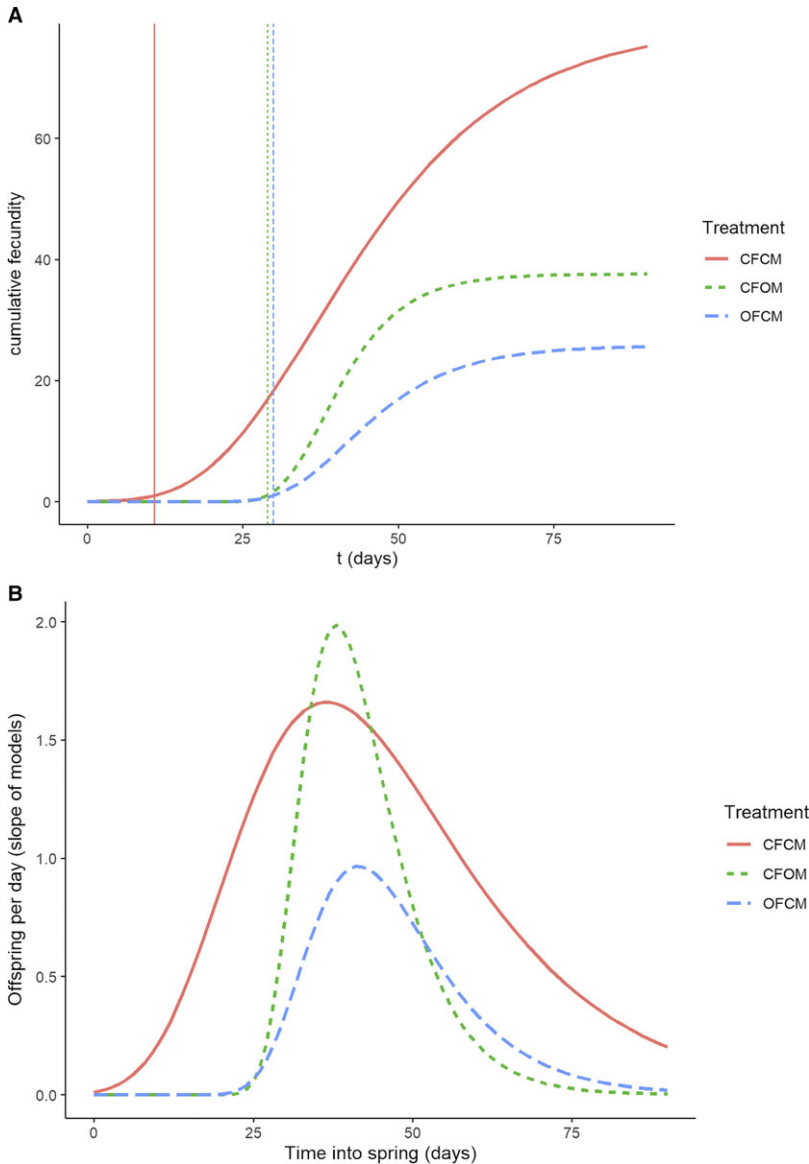


Fig. 3. Age-specific daily fecundity patterns of *Drosophila sukukii* in cold-temperate springtime conditions, as revealed by the instantaneous slope of Gompertz model fitted to larval production data for postdiapause and nondiapause flies from the 2018 experiment (see Supplementary material 2).

winter in early June, first with the females near 1 June, followed by nondiapause females mated to postdiapause males near 8 June. Cumulative physiological time (degree-days) since cold winter ended would then be 118 and 178 degree-days above the 5 °C low-temperature threshold, respectively. Per capita offspring contributed by postdiapause females reached a plateau of 18–19 offspring around 6 July, when heat units would cumulate 640 degree-days_{5 °C} (Fig. 4). For nondiapause females sired by postdiapause males, a total of 10–11 offspring would be attained at the same time near 6 July, with 640 degree-days_{5 °C}. Similar analysis for mating treatments involving nondiapause potential migrant flies (Fig. 4) shows that their per capita

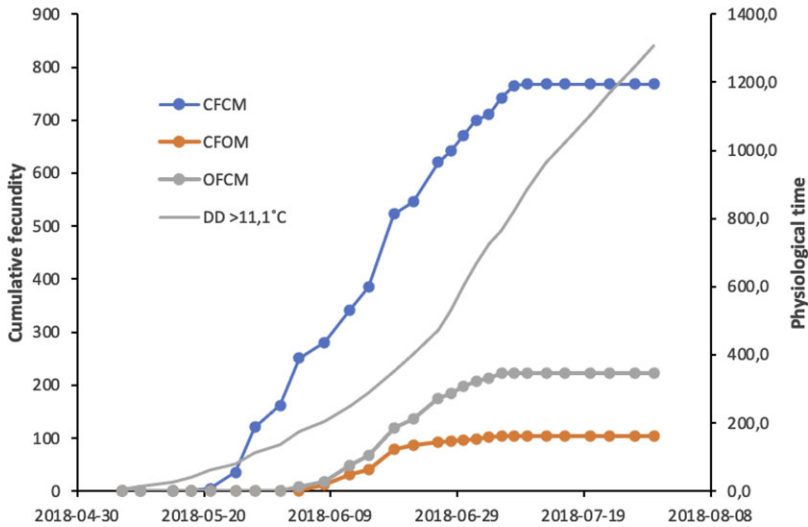


Fig. 4. Cumulative fecundity of postdiapause males (CFOM treatment) and females (OFCM treatment) of *Drosophila suzukii* compared to nondiapause fly mates (CFCM), in relation to cumulative physiological time in degree-days (DD) above the temperature threshold, 11.1 °C. Postdiapause flies of each sex were mated with a nondiapause partner from a colony of same geographic origin.

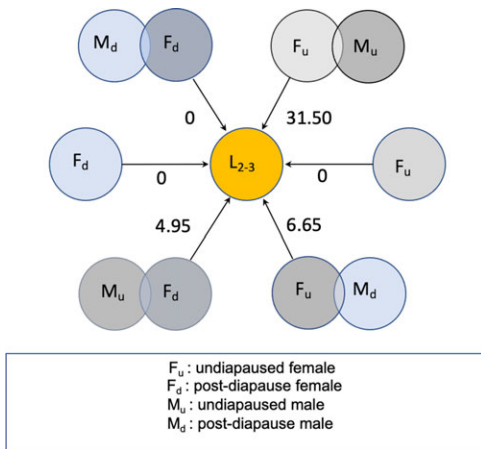


Fig. 5. Schematic representation of net reproductive rate (R_0) as number of daughter larvae (L_{2-3}), expected from postdiapause and nondiapause *Drosophila suzukii* in new season in cold-temperate conditions. Overlapping circles represent mating male and female flies according to diapause status. Unpaired circles represent unmated females, some of which might be fertile with sperm retained from previous mating.

offspring contribution to a new generation could reach 60 offspring around 8 July, with 715 degree-days_{5°C} of cumulated heat units.

Figure 5 schematically summarises results of the 2018 experiment, expressed as per capita lifetime net contribution to population growth as female larval offspring in a new season, assuming a 1:1 sex ratio among the offspring of the flies initiating population growth. Postdiapause females could contribute about five daughters per capita if mated by a fertile nondiapause migrant male. Otherwise, postdiapause females mated by a postdiapause male could contribute a maximum of one daughter offspring, with little evidence they would remain fertile from mating in the previous season. On the other hand, fertile nondiapause females mating with a postdiapause male could contribute eight to nine daughters per capita. By comparison, young nondiapause migrant female flies mated to nondiapause males and arriving early to commence reproduction at about the same time could contribute as many as

30 daughters per capita (Fig. 5), a considerably larger number than that predicted for postdiapause females or for the nondiapause female partners of postdiapause males.

Discussion

The mating behaviour and reproductive biology of *D. suzukii* in relation to winter diapause have been studied previously (e.g., Rossi-Stacconi *et al.* 2016; Grassi *et al.* 2018; Stockon *et al.* 2019), but more work is needed to evaluate the species' potential establishment in cold-temperate regions such as Canada, where the adult survival of diapausing flies challenged by cold stress would need to be much longer. Based on our work in Québec (Cloutier *et al.* 2021), *D. suzukii* flies from local populations can survive a 6-month cold winter despite substantial mortality, with female-biased survival as previously observed (e.g., Stockon *et al.* 2019). The present study extends knowledge of the potential of *D. suzukii* to survive the harsh season and permanently establish in cold climates.

Cold tolerance and dormancy in *D. suzukii* have often been studied using laboratory strains (Stephens *et al.* 2015; Ryan *et al.* 2016; Shearer *et al.* 2016; Toxopeus *et al.* 2016; Wallingford and Loeb 2016). The *D. suzukii* individuals used in the present study were collected from Québec blueberry fields in late summer and fall and were possibly partially adapted to cold-climatic conditions. They had survived six months of cold stress near 0 °C in simulated winter, and their overall survival extended well into a realistic simulated springtime regime, allowing regeneration of reproductive function after diapause (Cloutier *et al.* 2021). Finding mates in spring would be the most critical factor at the low fly densities that are typical in spring, due to the Allee principle (Jacobs 2009), with decreasing available time and energy (Kramer *et al.* 2009; Yamanaka and Liebhold 2009; Xu and Ma 2020). This would apply mostly to male *D. suzukii*, for which the odds of winter mortality are nearly twice those of females (Cloutier *et al.* 2021), and as indicated by the results of our 2017 experiment (Supplementary material 1).

Focusing on individuals and controlled mating, the 2018 experiment confirmed previous results, showing that field-collected and artificially overwintered postdiapause *D. suzukii* flies can reproduce in a realistic cold springtime regime many months after being captured in the field. However, except for a single female, postdiapause flies of both sexes were fertile only if experimentally mated with nondiapause partners from the laboratory colony (Table 3). Lifetime fertility of postdiapause females mated with nondiapause males (OFCM) was almost half that realised by postdiapause males *via* their nondiapause female mates. To summarise, our results showed that any contribution to population growth of postdiapause female and male flies would depend on the opportunity for mating with fertile partners, here provided as nondiapause flies from the colony. Ryan *et al.* (2016) suggested that mated *D. suzukii* might store sperm over winter to be used when conditions become favourable to laying eggs in the next growing season. We found evidence of sperm retention for at least six months in postdiapause *D. suzukii* females; however, this was observed in only one survivor tested (total $N = 10$; Table 1, OF treatment). Either most *D. suzukii* females were already in reproductive diapause when they emerged as adult flies in the field in late 2017 and were still virgin in spring 2018 or any sperm acquired from mating in the fall of 2017 was no longer available in spring 2018 to fertilise eggs matured in the spring.

Fitness costs of diapause

We refer here to fitness based on the adaptedness concept of Barker (2009, table 1) – that is, the degree to which postdiapause *D. suzukii* flies could contribute to the next generation, relative to the nondiapause adult phenotype of the species. Measured fertility losses imply substantial fitness costs of diapause (Sadakiyo and Ishihara 2012b), inherent to

D. suzukii's persistence in cold-temperate regions. We found no evidence that postdiapause males could reproduce by mating with postdiapause females, which differs from our 2017 results (Supplementary material 1). A slightly different methodology (possible mate choice, multiple mating) might explain the few available postdiapause couples that were fertile in 2017.

In both sexes, fertility losses revealing fitness costs of diapause likely resulted from advanced ageing due to extensive survival under cold stress (Tatar and Yin 2001). Average chronological age during postwinter reproduction in spring 2018 was about seven to eight months older than the age of nondiapause flies used to scale fertility loss (Table 1, CFCM). Fitness trade-offs with prolonged survival are involved in genetically based clinal variation of reproductive diapause expression in *D. melanogaster* in the eastern United States of America (Schmidt *et al.* 2005). Schmidt and Conde (2006) reported that diapause genotypes of *D. melanogaster* had greater lifetime, lower age-specific mortality, and delayed reproduction in adult life when compared to nondiapause genotypes from the same latitudes. A similar trade-off between fitness and prolonged survival under cold stress could explain the reduced lifetime fertility of the longlived, fieldborn *D. suzukii* tested in the present study. What remains to be explained is why the negative effects of diapause on female fertility considerably exceed those on male fertility. A plausible explanation is that male fertility is mediated by their female mates (Margus and Lindström 2020): postdiapause *D. suzukii*'s male fertility was likely mitigated by the potentially high fertility of their nondiapause partners from the colony. It should also be clear that our estimates of fitness costs of diapause in *D. suzukii* are partial and neglect intergenerational effects on offspring quality (Hercus and Hoffmann 2000; Priest *et al.* 2008).

Springtime population growth

Population growth-rate estimates of postdiapause *D. suzukii* in simulated spring conditions suggest that nondiapause immigrant flies of either sex could reproduce faster while mating among themselves rather than with any postdiapause heterosexual fly locally available. Analogous to r_m (Henderson and Southwood 2016), r_c can measure initial growth potential after diapause in the absence of stage structure in winter survivor populations (Amarasake and Coutinho 2013). Ability to recover from low densities after winter most likely depends on natality, assuming adult fly fertility and the presence of suitable fruits to lay eggs. Our r_c estimate for nondiapause *D. suzukii* (circa 0.09 per day) is lower than those presented in other studies on summer-morph flies (circa 0.18, Emiljanowicz *et al.* 2014, Ryan *et al.* 2016; 0.22, Tochen *et al.* 2014), due mainly to the lower temperatures in our simulated springtime regime (Kindlmann *et al.* 2001) and due in part to larval food quality (Tochen *et al.* 2014). Using our r_c estimates to assess fitness costs of diapause indicates a reduction of 11% in postdiapause males, compared to a reduction of 18% in postdiapause females, which agrees with the above-described assessment based on lifetime offspring.

The three-parameter Gompertz population growth model showed how *D. suzukii*'s springtime population growth would be affected by a decreasing growth rate parameter (r), as females aged, according to the female's diapause status or that of the mating partner. Ageing effects on fecundity varied strongly between nondiapause and postdiapause flies (Fig. 3). As expected, the age-specific fecundity pattern involving nondiapause mates (Fig. 3; CFCM) contributed most to spring population growth. However, this was because female fecundity was sustained over a much longer period of time not because of superior peak fecundity. Higher peak fecundity for nondiapause females mated to postdiapause males (CFOM) can be explained by constrained sperm availability from their postdiapause male mates whose age-specific fertility pattern was reduced.

Based on results, *D. suzukii* winter survivors would resume activity in mid to late April if the dynamics are driven mostly by physiological time (degree-days), regardless of other limitations. Wiman *et al.* (2016) estimated that postdiapause *D. suzukii* requires 210 degree-days_{4°C} from 1 January to resume reproductive potential, but this ranges widely.

In 2017 (see Supplementary material 1), reproduction started at approximately 165 degree-days_{5°C} after cold winter ended among postdiapause females mated immediately upon transfer to simulated spring. In 2018 (see Supplementary material 2), oviposition started at 118 degree-days_{5°C} among postdiapause females (OFCM) and at 178 degree-days_{5°C} among nondiapause partners of postdiapause males (CFOM). The between-years difference could be due to field sampling (different years) and differing cold-winter regimes. Females in nondiapause control couples started laying eggs after only 35 degree-days_{5°C}, earlier than Wiman *et al.*'s (2016) estimates. This can be explained by nondiapause females having already developed mature eggs in optimal rearing conditions when they were transitioned to the simulated springtime regime. Larval diet might also have played a role, as banana is superior to blueberry for rearing *D. suzukii* (Little *et al.* 2017; Gonzalez-Cabrera *et al.* 2018). In nature, to recover reproductive maturity, postdiapause flies would normally feed on noncrop protein sources, such as yeasts and other microorganisms in decaying fruits and vegetation (Hamby *et al.* 2016; Fountain *et al.* 2018).

The heat requirements of postdiapause *D. suzukii* winter survivors suggest the flies experience phenological delays in their potential contribution to population growth come spring that potential nondiapause migrant flies colonising the region in early spring may not experience. This is especially true for postdiapause males, which seem unable to regenerate fertility as quickly as females do after diapause. Early reproduction is important in determining insect population growth (see, *e.g.*, Price *et al.* 2011, Chapter 10). Heat-unit accumulation (physiological time) is far from being the sole determinant for insect population growth (Damos and Savopoulou-Soultani 2012) but can be useful to project *D. suzukii*'s early season dynamics while considering mating opportunity and possible immigration. Indeed, springtime populations in a cold-temperate region would consist essentially of small numbers of adult flies that are in search of food, mates, and host fruits for female oviposition (Panel *et al.* 2018).

Fruit-marking behaviour

Results from the present experiment include additional observations on the reproductive biology of *D. suzukii*. We did not attempt to determine the numbers of eggs laid, but we counted ovipositor marks left by females on fruit skins, which are signs associated with egg laying. We limited fruit exposure time to two days to help ensure that the fruit skins remained intact up until the time of counting marks and hatched larvae in dissected fruits (Walsh *et al.* 2011). The unmated nondiapause virgin females that were used as controls left many ovipositor marks on fruits (CF treatment; Table 3), suggesting multiple attempts to lay eggs despite not being inseminated or courted by males. We do not know that all marks represented distinct egg-laying events because there was no evidence of larvae in the dissected fruits; however, we cannot discount that many infertile eggs may have been laid by virgins. The nondiapause virgin females were tested to examine possible facultative parthenogenesis. Tycho parthenogenesis characterises many *Drosophila* species, a lineage that hypothetically evolved in many times as an alternative reproductive strategy that allows host-niche and geographic range expansion (Chang *et al.* 2014). We found no evidence that *D. suzukii* females deprived of mating can reproduce parthenogenetically. Despite that, their marking frequency equalled that of comparable mated females, suggesting that the virgin flies laid infertile eggs. Ryan *et al.* (2016) suggested that sperm-limited females can lay unfertilised eggs, possibly explaining the abundant marks left by nondiapause virgins in the present study. The fruit-marking frequency by females of control couples (CFCM) was similar but led to a high ratio of larvae to marks, thus confirming high oviposition success in blueberries (Atallah *et al.* 2014). In contrast, postdiapause females deprived of mating (OF; $N = 10$) left no marks on fruits, indicating suppressed oviposition compared to nondiapause (CFCM) and postdiapause (OFCM, OFOM) mated females (Table 3). Mated postdiapause and nondiapause

females marked fruits with equal frequency (Table 3). Fruit marking by mated postdiapause females and its absence by unmated ones indicate that marking was likely dependent on male contact – possibly mediated by male olfactory signals (Revadi *et al.* 2015) – as is also reported in *D. montana* in relation to reproductive diapause (Ala-Honkola *et al.* 2018). A final point concerning mated postdiapause female fertility is the low ratio of hatched larvae to oviposition marks (Table 3), which suggests a reduced egg hatchability compared to eggs laid by nondiapause females. This is supported by the reduced offspring production (Table 1), hypothetically as a result of ageing during winter dormancy despite reduced physical and metabolic activity.

Conclusions

Knowledge of *D. suzukii*'s reproductive diapause is important to predict potential establishment in cold-temperate climates (reviewed in Nikolouli *et al.* 2018). We showed that most *D. suzukii* that survived a six-month cold winter at temperatures close to 0°C effectively reproduced after winter in simulated springtime conditions. We found negative effects of reproductive diapause on fitness in a sex-biased manner, despite both sexes surviving prolonged overwintering conditions. We also showed that cold stress is not the sole critical factor limiting postdiapause reproduction. Mating in spring with fertile heterosexual partners was critical for females as well as for males, as shown when we provided nondiapause fertile mates tested as potential migrants. Female winter survivors would only exceptionally be able to reproduce without copulating in spring, with little evidence that female flies active in the late prewinter season would continue to be fertile in the next spring as a result of prediapause mating. A population growth model with a variable growth rate parameter (r) determined mostly by ageing of first-generation flies in spring showed that early migrant flies would be strongly advantaged over locally overwintered postdiapause flies, with earlier reproductive maturity, a broader pattern of age-specific fecundity, and higher lifetime fecundity.

Local overwintering with high mortality, in combination with immigration from more favourable southern regions, could facilitate the spotted-wing drosophila's local establishment in Québec and elsewhere in continental Canada.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.4039/tce.2022.3>.

Acknowledgements. The authors thank the Natural Sciences and Engineering Research Council of Canada Cooperative Research and Development programme and Discovery programme, the Syndicat des Producteurs de Bleuets du Québec, the Fonds de recherche du Québec-Nature et technologies, and the Fonds de la recherche agroalimentaire axé sur l'agriculture nordique du Saguenay–Lac-Saint-Jean for research grants to C.C. Special thanks are due to Dr. Valérie Fournier, Département de phytologie, Université Laval, Québec, Québec for useful comments on experimental work and to Gaétan Daigle, Service de consultation statistique, Département de mathématiques et statistiques, Université Laval. Finally, the authors are grateful to the blueberry producers from Saguenay–Lac-Saint-Jean who provided access to their lowbush blueberry fields.

Authors contributions. C.C. conceived the experiments and methodology with J.-F.G., and W.C.C. W.C.C., and J.-F.G. realised the experiments and recorded observations. C.C. statistically analysed the data, with help from J.-F.G. and a professional statistician. All authors contributed to the paper and authorised final publication.

References

- Ala-Honkola, O., Kauranen, H., Tukmaeva, V., Boetzi, F.A., Hoikkala, A., and Schmitt, T. 2018. Diapause affects cuticular hydrocarbon composition and mating behavior of both sexes in *Drosophila montana*. *Insect Science*, **27**: 304–316.
- Alhmedi, A., Clymans, R., Van Kerckvoorde, V., Bylemans, D., and Beliën, T. 2019. Preference and performance of *Drosophila suzukii* on *Prunus* species: a potential eco-friendly pest management tool. *Crop Protection*, **122**: 35–41.
- Amarasake, P. and Coutinho, P.N. 2013. The intrinsic growth rate as a predictor of population viability under climate warming. *Journal of Animal Ecology*, **82**: 1240–1253. <https://doi.org/10.1111/1365-2656.12112>.
- Arnó, J., Solà, M., Riudavets, J., and Gabarra, R. 2016. Population dynamics, non-crop hosts, and fruit susceptibility of *Drosophila suzukii* in northeast Spain. *Journal of Pest Science*, **69**: 713–723.
- Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., and Kopp, A. 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proceedings of the Royal Society B: Biological Sciences*, **281**: 1–9.
- Barker, J.S.F. 2009. Defining fitness in natural and domesticated populations. *In* *Adaptation and fitness in animal populations*. Edited by J. van der Werf, H.U. Graser, R. Frankham, and C. Gondro. Springer, Dordrecht, The Netherlands.
- Champagne-Cauchon, W., Guay, J.-F., Fournier, V., and Cloutier, C. 2020. Phenology and spatial distribution of spotted-wing *Drosophila* in lowbush blueberry in Saguenay–Lac-Saint-Jean, Québec, Canada. *The Canadian Entomologist*, **152**: 432–449. <https://doi.org/10.4039/tce.2020.30>.
- Chang, C.-C., Ting, C.-T., Chang, C.-H., Fang, S., and Chang, H.-Y. 2014. The persistence of facultative parthenogenesis in *Drosophila albomicans*. *PLOS One*, **9**: e113275. <https://doi.org/10.1371/journal.pone.0113275>.
- Cloutier, C., Guay, J.-F., Champagne-Cauchon, W., and Fournier, V. 2021. Overwintering survival of *Drosophila suzukii* in temperature regimes emulating partly protected winter conditions in a cold temperate climate of Québec, Canada. *The Canadian Entomologist*, **153**: 259–278. <https://doi.org/10.4039/tce.2021.6>.
- Cochran, W.G. 1952. The χ^2 test of goodness of fit. *Annals of Mathematical Statistics*, **23**: 315–345.
- Critchlow, D.E. and Fligner, M.A. 1991. On distribution-free multiple comparisons in the one-way analysis of variance. *Communications in Statistics: Theory and Methods*, **20**: 127–139.
- Damos, P. and Savopoulou-Soultani, M. 2012. Temperature-driven models for insect development and vital thermal requirements. *Psyche*, **2012**: 123405. <https://doi.org/10.1155/2012/123405>.
- Danks, H.V. 1987. *Insect dormancy: an ecological perspective*. Biological Survey of Canada (Terrestrial Arthropods), Ottawa, Ontario, Canada.
- Danks, H.V. 2006. Insect adaptations to cold and changing environments. *The Canadian Entomologist*, **138**: 1–23.
- Dekker, T., Revadi, S., Mansourian, S., Ramasamy, S., Lebreton, S., Becher, P.G., *et al.* 2015. Loss of drosophila pheromone reverses its role in sexual communication in *Drosophila suzukii*. *Proceedings of the Royal Society, Biological Sciences*, **282**: 20143018. <https://doi.org/10.1098/rspb.2014.3018>.
- Denlinger, D.L. 2008. Why study diapause? *Entomological Research*, **38**: 1–9.
- Dunn, O.J. 1964. Multiple comparisons using rank sums. *Technometrics*, **6**: 241–252.
- Eberhardt, L.L., Breiwick, J.M., and Demaster, J.P. 2008. Analyzing population growth curves. *Oikos*, **117**: 1240–1246.

- Emiljanowicz, L.M., Ryan, G.D., Langille, A., and Newman, J. 2014. Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet. *Journal of Economic Entomology*, **107**: 1392–1398.
- Fountain, M.T., Bennett, J., Cobo-Medina, M., Conde Ruiz, R., Deakin, G., Delgado, A., *et al.* 2018. Alimentary microbes of winter-form *Drosophila suzukii*. *Insect Molecular Biology*, **27**: 383–392. <https://doi.org/10.1111/imb.12377>.
- Gonzalez-Cabrera, J., Garcia-Cancino, M.D., Moreno-Carrillo, G., Sanchez-Gonzalez, J.A., and Arredondo-Bernal, H.C. 2018. Fresh banana as an alternative host for mass rearing *Drosophila suzukii*. *Bulletin of Insectology*, **71**: 65–70.
- Grassi, A., Gottardello, A., Dalton, D.T., Tait, G., Rendon, D., Ioriatti, C., *et al.* 2018. Seasonal reproductive biology of *Drosophila suzukii* (Diptera: Drosophilidae) in temperate climates. *Environmental Entomology*, **47**: 166–174.
- Gutierrez, A.P., Ponti, L., and Dalton, D.T. 2016. Analysis of the invasiveness of spotted wing drosophila (*Drosophila suzukii*) in North America, Europe, and the Mediterranean Basin. *Biological Invasions*, **18**: 3647–3663.
- Hahn, D.A. and Denlinger, D.L. 2007. Meeting the energetic demands of insect diapause: nutrient storage and utilization. *Journal of Insect Physiology*, **53**: 760–773.
- Hamby, K.A., Bellamy, D.E., Chiu, J.C., Lee, J.C., Walton, V.M., Wiman, N.G., *et al.* 2016. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. *Journal of Pest Science*, **89**: 605–619.
- Henderson, P.A. and Southwood, T.R.E. 2016. *Ecological methods*. Fourth edition. John Wiley & Sons, Chichester, United Kingdom.
- Hercus, M.J. and Hoffmann A.A. 2000. Maternal and grandmaternal age influence offspring fitness in *Drosophila*. *Proceedings of the Royal Society, B Biological Science*, **267**: 2105–2110.
- Jacobs, J. 2009. Cooperation, optimal density and low density thresholds: yet another modification of the logistic model. *Oecologia*, **64**: 389–395.
- Jakobs, R., Garipey, T.D., and Sinclair, B.J. 2015. Adult plasticity of cold tolerance in a continental-temperate population of *Drosophila suzukii*. *Journal of Insect Physiology*, **79**: 1–9.
- Kindlmann, P., Dixon, A.F.G., and Dostáľková, I. 2001. Role of ageing and temperature in shaping reaction norms and fecundity functions in insects. *Journal of Evolutionary Biology*, **14**: 835–840.
- Kramer, A.M., Dennis, B., Liebold, A.M., and Drake, J.M. 2009. The evidence for Allee effects. *Population Ecology*, **51**: 341–354.
- Langille, A.B., Arteca, E.M., and Newman, J.A. 2017. The impacts of climate change on the abundance and distribution of the spotted wing drosophila (*Drosophila suzukii*) in the United States and Canada. *PeerJ*, **5**: e3192. <https://doi.org/10.7717/peerj.3192>.
- Lee, J.C., Bruck, D.J., Curry, H., Edwards, D., Haviland, D.R., Van Steenwyk, R.A., and Yorgey, B.M. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Management Science*, **67**: 1358–1367.
- Little, C.M., Chapman, T.W., Moreau, D.L., and Hillier, N.K. 2017. Susceptibility of selected boreal fruits and berries to the invasive pest *Drosophila suzukii* (Diptera: Drosophilidae). *Pest Management Science*, **73**: 160–166.
- Mangiafico, S.S. 2021. Kruskal–Wallis test [online]. Available at https://rcompanion.org/handbook/F_08.html [accessed 21 January 2021].
- Margus, A. and Lindström, L. 2020. Prolonged diapause has sex-specific fertility and fitness costs. *Evolutionary Ecology*, **34**: 41–57.
- Nikolouli, K., Colinet, H., Renault, D., Enriquez, T., Mouto, L., Gibert, P., *et al.* 2018. Sterile insect technique and Wolbachia symbiosis as potential tools for the control of the invasive species *Drosophila suzukii*. *Journal of Pest Science*, **91**: 489–503.

- Ørsted, I.V. and Ørsted, M. 2019. Species distribution models of the spotted wing drosophila (*Drosophila suzukii*, Diptera: Drosophilidae) in its native and invasive range reveal an ecological niche shift. *Journal of Applied Ecology*, **56**: 423–435.
- Panel, A.D.C., Zeeman, L., van der Sluis, B.J., van Elk, P., Pannebakker, B.A., Wertheim, B., and Helsen, H.H.M. 2018. Overwintered *Drosophila suzukii* are the main source for infestations of the first fruit crops of the season. *Insects*, **9**: 145. <https://doi.org/10.3390/insects9040145>.
- Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.L., and Kaplan, I. 2011. *Insect ecology: behavior, populations and communities*. Cambridge University Press, New York, New York, United States of America.
- Priest, N.K., Galloway, L.F., and Roach, D.A. 2008. Mating frequency and inclusive fitness in *Drosophila melanogaster*. *The American Naturalist*, **171**: 10–21. <https://doi.org/10.1086/523944>.
- R Studio Team. 2021. R Studio: integrated development for R [online]. R Studio, Boston, Massachusetts, United States of America. Available from <http://www.rstudio.com/> [accessed January 2021].
- Revadi, S., Lebreton, S., Witzgall, P., Anfora, G., Dekker, T., and Becher, P.G. 2015. Sexual behavior of *Drosophila suzukii*. *Insects*, **6**: 183–196.
- Rossi-Stacconi, M.V., Kaur, R., Mazzoni, V., Ometto, L., Grassi, A., Gottardello, A., *et al.* 2016. Multiple lines of evidence for reproductive winter diapause in the invasive pest *Drosophila suzukii*: useful clues for control strategies. *Journal of Pest Science*, **89**: 689–700.
- Rota-Stabelli, O., Blaxter, M., and Anfora, G. 2013. *Drosophila suzukii*. *Current Biology*, **23**: PR8–R9. <https://doi.org/10.1016/j.cub.2012.11.021>.
- Ryan, G.D., Emiljanowicz, L., Wilkinson, F., Kornya, M., and Newman, J.A. 2016. Thermal tolerances of the spotted-wing drosophila *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, **109**: 746–752.
- Sadakiyo, S. and Ishihara, M. 2012a. Cost of male diapause indirectly affects female reproductive performance. *Entomologia Experimentalis et Applicata*, **143**: 42–46.
- Sadakiyo, S. and Ishihara, M. 2012b. Cost of diapause on life-history traits under restricted resources in multivoltine bruchid *Acanthoscelides pallidipennis* (Coleoptera: Bruchidae). *Annals of the Entomological Society of America*, **105**: 422–426.
- Salminen, T.S. and Hoikkala, A. 2013. Effect of temperature on the duration of sensitive period and on the number of photoperiodic cycles required for the induction of reproductive diapause in *Drosophila montana*. *Journal of Insect Physiology*, **59**: 450–457.
- Schmidt, P.S. and Conde, D.R. 2006. Environmental heterogeneity and maintenance of genetic variation for reproductive diapause in *Drosophila melanogaster*. *Evolution*, **60**: 1602–1611.
- Schmidt, P.S., Paaby, A.B., and Shaneheschel, M. 2005. Genetic variance for diapause expression and associated life histories in *Drosophila melanogaster*. *Evolution*, **59**: 2616–2625.
- Shearer, P.W., West, J.D., Walton, V.M., Brown, P.H., Svetec, N., and Chiu, J.C. 2016. Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. *BMC Ecology*, **16**: 1–18.
- Stephens, A.R., Asplen, M.K., Hutchison, W.D., and Venette, R.C. 2015. Cold hardiness of winter-acclimated *Drosophila suzukii* (Diptera: Drosophilidae) adults. *Environmental Entomology*, **44**: 1619–1626. <https://doi.org/10.1093/ee/nvv134>.
- Stockon, D., Wallingford, A., Rendon, D., Fanning, P., Green, C.K., Diepenbrock, L., *et al.* 2019. Interactions between biotic and abiotic factors affect survival in overwintering *Drosophila suzukii* (Diptera: Drosophilidae). *Environmental Entomology*, **20**: 1–11.
- Tatar, M. and Yin, C.M. 2001. Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms. *Experimental Gerontology*, **36**: 723–738.
- Tauber, M.J., Tauber, C.A., and Masaki, S. 1986. *Seasonal adaptations of insects*. Oxford University Press, Oxford, United Kingdom.

- Thistlewood, H., Gill, P., Beers E.H., Shearer P.W., Walsh, D.B., Rozema, B.M., *et al.* 2018. Spatial analysis of seasonal dynamics and overwintering of *Drosophila suzukii* (Diptera: Drosophilidae) in the Okanagan–Columbia basin, 2010–2014. *Environmental Entomology*, **47**: 221–232.
- Tjørve, K.M.C. and Tjørve, E. 2017. The use of Gompertz models in growth analyses, and new Gompertz-model approach: an addition to the Unified-Richards family. *PLOS One*, **12**: e0178691. <https://doi.org/10.1371/journal.pone.0178691>.
- Tochen, S., Dalton, D.T., Wiman, N., Hamm, C., Shearer, P.W., and Walton, V.M. 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. *Environmental Entomology*, **43**: 501–510.
- Toxopeus, J., Jakobs, R., Ferguson, L.V., Garipey, T.D., and Sinclair, B.J. 2016. Reproductive arrest and stress resistance in winter-acclimated *Drosophila suzukii*. *Journal of Insect Physiology*, **89**: 37–51.
- Wallingford, A.K. and Loeb, G.M. 2016. Developmental acclimation of *Drosophila suzukii* (Diptera: Drosophilidae) and its effect on diapause and winter stress tolerance. *Environmental Entomology*, **45**: 1081–1089.
- Walsh, D.B., Bolda, M.P., Goodhue, R.E., Dreves, A.J., Lee, J., Bruck, D.J., *et al.* 2011. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*, **2**: G1–G7. <https://doi.org/10.1603/IPM10010>.
- Wiman, N.G., Dalton, D.T., Anfora, G., Biondi, A., Chiu, J.C., Daane, K.M., *et al.* 2016. *Drosophila suzukii* population response to environment and management strategies. *Journal of Pest Science*, **89**: 653–665.
- Winkler, A., Jung, J., Kleinhenz, B., and Racca, P. 2020. A review on temperature and humidity effects on *Drosophila suzukii* population dynamics. *Agricultural and Forest Entomology*, **22**: 179–192. <https://doi.org/10.1111/afe.12381>.
- Xu, Q. and Ma, C.S. 2020. Aged virgin adults respond to extreme heat events with phenotypic plasticity in an invasive species, *Drosophila suzukii*. *Journal of Insect Physiology*, **121**: 104016. <https://doi.org/10.1016/j.jinsphys.2020.104016>.
- Yamanaka, T. and Liebhold, A.M. 2009. Mate-location failure, the Allee effect. *Population Ecology*, **51**: 337–340.
- Zerulla, F.N., Schmidt, S., Streitberger, M., Zebitz, C.P.W., and Zelger, R. 2015. On the overwintering ability of *Drosophila suzukii* in South Tyrol. *Journal of Berry Research*, **5**: 41–48.
- Zhai, Y., Lin, Q., Zhang, J., Zhang, F., Zheng, L., and Yu, Y. 2016. Adult reproductive diapause in *Drosophila suzukii* females. *Journal of Pest Science*, **89**: 679–688.

Cite this article: Cloutier, C., Guay, J.-F., and Champagne-Cauchon, W. 2022. Postdiapause reproduction of spotted-wing drosophila (Diptera: Drosophilidae) in realistically simulated cold climatic springtime conditions of Québec, Canada. *The Canadian Entomologist*. <https://doi.org/10.4039/tce.2022.3>.