

Investigating the overwintering strategy of *Ganaspis kimorum*, a biological control agent of *Drosophila suzukii*

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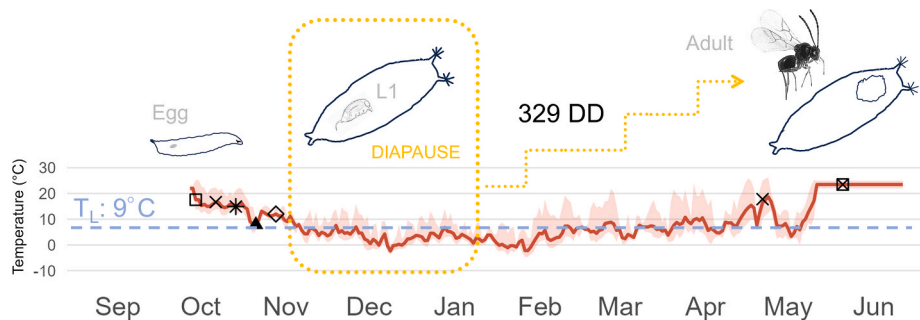
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HIGHLIGHTS

- *Ganaspis kimorum* survived overwintering in the introduction area.
- *G. kimorum* goes through diapause as early larval instar (L1).
- Survival rate varied across sites (0.4–17.6%).
- Extreme low and high temperatures negatively affected overwintering survival.
- A lower thermal threshold of 9 °C and 329 ± 6 post-diapause Degree Days were estimated.

GRAPHICAL ABSTRACT



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ABSTRACT

The larval parasitoid *Ganaspis kimorum* Buffington (Hymenoptera: Figitidae) is a classical biological control agent of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). This study investigated the overwintering strategy of *G. kimorum* under field conditions in Switzerland and Northern Italy. Field experiments conducted from 2021 to 2024 involved exposing *G. kimorum* to naturally variable temperatures in seven sites with different climates. Results indicate that diapause induction may be mediated by temperature, with exposures after late September successfully triggering diapause. Dissections revealed that diapause occurs in the early larval instar (L1) residing within early pupal stages of *D. suzukii*. In four out of seven sites, *G. kimorum* was able to survive winter conditions and emerged as adult. Survival rates varied significantly across sites (0.4–17.6 %). The analysis revealed significant negative associations between insect survival and the count of days with temperatures below 0 °C and above 30 °C. Analysing the variation of Degree Days needed until adult emergence confirmed that *G. kimorum* arrests its development as a first-instar larva and suggests a lower thermal threshold of 9 °C, with 329 ± 6 post-diapause Degree Days needed to develop to the adult stage. The study provides valuable insights into the biology

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of the parasitoid that may be helpful for optimizing field releases and enhancing the effectiveness of *G. kimorum* as a biological control agent of *D. suzukii*.

1. Introduction

As poikilothermic organisms, the development and fitness of insects are strongly influenced by temperature, which shapes their distribution and abundance ((Angilletta, 2009; Nedvěd, 2009; Vieira et al., 2020). For insect predators and parasitoids, understanding the environmental limitations that influence their potential geographic distribution and their overwintering capacity is crucial for their successful application as biological control agents (BCAs) (Hondo et al., 2006; Hughes et al., 2010; Wang et al., 2018b).

Insects often enter dormancy to cope with abiotic stress, such as cold temperatures. Dormancy may involve quiescence, an immediate response to climatic thresholds, or diapause, a hormonally mediated, genetically programmed state characterized by metabolic suppression, developmental arrest, and reproductive inactivity (Denlinger, 2002; Košťál, 2006). Environmental triggers like photoperiod and temperature often influence diapause initiation, but their effects vary among species. For example, diapause in *Anastatus japonicus* Ashmead (Hymenoptera: Eupelmidae) is regulated by photoperiod and temperature (Zhao et al., 2021), whereas photoperiod primarily triggers diapause in *Colpoclypeus florus* (Walker) (Hymenoptera: Eulophidae), and temperature plays a dominant role for *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) (Li et al., 2008; Milonas and Savopoulou-Soultani, 2000).

This study investigates the overwintering capacity and diapause of the larval parasitoid *Ganaspis kimorum* Buffington (Hymenoptera: Figitidae), a biological control agent targeting the invasive spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). Native to East Asia, *D. suzukii* has become a global pest since the early 2000 s, spreading across the Americas, Europe, and Africa (Andreazza et al., 2017; Asplen et al., 2015). Its serrated ovipositor (Atallah et al., 2014), broad host range (Burrack et al., 2013; Kenis et al., 2016), rapid development cycle (Hamby et al., 2016) and high dispersal ability (Tait et al., 2020), enabled this pest to quickly exploit new ecological niches, resulting in significant agricultural losses worldwide (De Ros et al., 2021; Farnsworth et al., 2017; Tait et al., 2021).

Monitoring in the native area and specificity tests (Biondi et al., 2021; Daane et al., 2021; Girod, 2017; Seehausen et al., 2020; Wang et al., 2018a, 2020), strongly suggest that the ecological host range of *G. kimorum* is limited to *D. suzukii* residing in ripening fruit (Stahl et al., 2024), and for this reason was selected as BCA in both Europe and the U. S. (Fellin et al., 2023; Garipey et al., 2024; Lisi et al., 2022). Initial reports suggest that *G. kimorum* can overwinter in introduced regions (Fellin et al., 2023), but exact overwintering strategies and potential diapause remain unknown.

The thermal performance and cold tolerance of *D. suzukii* have been extensively studied. Flies overwinter as adults (Dalton et al., 2011; Enriquez and Colinet, 2017; Shearer et al., 2016; Zerulla et al., 2015), especially when expressing the winter-acclimated phenotype (Colinet and Kustre, 2024; Leach et al., 2019; Sario et al., 2023; Stephens et al., 2015). Adults undergo a reproductive diapause in response to shorter day lengths and cooler temperatures (Grassi et al., 2018). On the other hand, *D. suzukii* larvae are freeze intolerant and die quickly after short-term exposure to relatively mild temperatures (Jakobs et al., 2017; Stockton et al., 2019).

Studies on *D. suzukii* pupal parasitoids such as *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) showed their ability to survive up to three months at constant 12°C under laboratory conditions (Wang et al., 2018b), and under extended natural winter conditions as immature stages (Amiresmaeili et al., 2020; Häner et al., 2022). Research on larval parasitoids focused on *Leptopilina* spp. and *Ganaspis*

lupini (previously *Ganaspis* cf. *brasiliensis* G3 according to Sosa-Calvo et al. (2024)) and found population-specific cold tolerance associated with geographic origin (Gibert et al., 2001). Both species showed a prepupal diapause, with *G. lupini* having a low thermal threshold (T_L) ranging between 5 and 9°C, and *Leptopilina japonica* Novković & Kimura initiating diapause at 15 or 18°C (Hougardy et al., 2019; Murata et al., 2013).

However, most studies on parasitoid diapause and overwintering are conducted under laboratory conditions or rely on temporary field exposures (Amiresmaeili et al., 2020; Häner et al., 2022; Hougardy et al., 2019; Li et al., 2008; Vieira et al., 2020; Wang et al., 2018b) limiting their ecological relevance. Fluctuating temperatures in natural settings can cause physiological and ecological effects that differ from those predicted under constant temperatures (Colinet et al., 2015), and for this reason should be better investigated.

Here, we present findings from two experiments, conducted in winter 2021/2022 and 2023/2024 in Switzerland and Northern Italy, respectively. We exposed *G. kimorum*-parasitized *D. suzukii* larvae to natural conditions at seven sites across different altitudes, monitoring emergence, survival, overwintering stages, and Degree Day (DD) accumulation. DD analysis is a reliable tool for predicting insect development and emergence (Sridhar and Reddy, 2013), and it is particularly valuable in the context of pest control programs (Herms, 2004). By leveraging this approach, our findings provide critical insights into the capacity of *G. kimorum* to establish in release sites (Dietschler et al., 2023; Jenner et al., 2010) and contribute to modelling host-parasitoid interactions.

2. Material and methods

2.1. Insect colonies

Ganaspis kimorum specimens originated from a population collected in 2016 near Tokyo, Japan (Girod et al., 2018a). Parasitoids were reared at CABI in Delémont, Switzerland for experiment 1 and at Edmund Mach Foundation (FEM) in S. Michele all'Adige, Italy for experiment 2. Rearing followed the protocol described by Girod et al. (2018b) and Rossi-Stacconi et al. (2022), under controlled conditions (16:8 light:dark (L:D) photoperiod, $22 \pm 2^\circ\text{C}$ and $70 \pm 10\%$ relative humidity (RH)).

Drosophila suzukii colonies were established from locally collected adults and maintained in climatic chambers with a photoperiod of 16:8 L:D at $23 \pm 2^\circ\text{C}$ and $60 \pm 10\%$ RH.

2.2. Experiment 1

A first experiment was conducted in 2021 in field cages in Switzerland to do preliminary investigations into diapause induction and the overwintering stage of *G. kimorum* under near/natural conditions. This study was part of the large-arena field cage experiment to assess the host-specificity of *G. kimorum* (Seehausen et al., 2022) and

Table 1
Sites description.

Full name	Site	Coordinates	Altitude (m a.s.l.)
Cadenazzo	CAD	46.1616 N; 8.9325 E	225
Delémont	DEL	47.3732 N; 7.3256 E	515
Faedo	FAE	46.1950 N; 11.1670 E	660
Laimburg	LAI	46.3848 N; 11.2865 E	294
Novale di Fié	NOV	46.5312 N; 11.4985 E	655
S. Genesio	GEN	46.5615 N; 11.3244 E	1233
S. Michele A/A	SMA	46.1894 N; 11.1365 E	216

was conducted in two locations, Delémont (DEL) and Cadenazzo (CAD) (Table 1) (Supplementary Fig. S1). In Delémont, a small insect rearing cage (47.5 × 47.5 × 47.5 cm, MegaView Science Co., Taiwan) was installed within a larger polyester netting cage (200 × 200 × 160 cm). From August 20 to September 30, 2021, about twenty freshly emerged *G. kimorum* adults (males and females) were released weekly into the smaller cage. Blueberries infested with one-day old *D. suzukii* larvae were exposed to the parasitoids every week for 3 days. Thus, a total of five parasitoid releases and exposures of blueberries were conducted for the experiment. Following exposure, blueberries were transferred to ventilated plastic containers (10 × 5 cm), closed with a foam lids, and kept in the cages. Because of a shortage of parasitoids, in one case, *D. suzukii* larvae were exposed to parasitoids under laboratory conditions (August 27–30) before being placed in the field cages (E1). A second exposure occurred directly in field between September 10 and 13 (E2). Parasitoid emergence was monitored weekly to determine development time and the onset of winter diapause.

On December 13, 2021, the containers with the blueberries and potentially parasitized *D. suzukii* were equally divided, placed in two plastic boxes with a mesh window (to allow exposure to natural temperatures), and moved in the ground at the two locations so that the lid was at ground level. Holes in the bottom of the box prevented rain or melting snow from flooding the overwintering individuals. Temperature inside the overwintering boxes was recorded every hour using data loggers (HOBO® Pendant MX, Onset®, USA).

Between February 25 and March 11, 2022, ten *D. suzukii* puparia were removed from the overwintering container in Delémont and dissected under a microscope to determine the overwintering stage of *G. kimorum* according to morphology and size (Wang et al., 2019). The remaining overwintering individuals were transferred on February 25 and March 11 to a growth chamber and reared at 20°C until parasitoid emergence.

2.3. Experiment 2

Following the preliminary findings of Experiment 1, a second experiment was conducted in 2023 to further evaluate the overwintering strategy of *G. kimorum* under natural conditions. This experiment took place in Trentino-Alto Adige in Northern Italy, the first region in Europe where the BCA was released (Fellin et al., 2023). Five sites were selected in forest areas bordering cultivated land at different altitudes: Faedo (FAE), San Genesio (GEN), Laimburg (LAI), Novale di Fié (NOV) and San Michele all' Adige (SMA) (Table 1) (Supplementary Fig. S1). Before taking them into the field, fruit samples were prepared according to the following method: blueberries from the same batch were soaked in a 2% bleach solution for five minutes, rinsed three times, dried, and placed in sterilized 9 cm petri dishes. Blueberries were transferred to *D. suzukii* rearing cages for oviposition (4 h), followed by exposure to *G. kimorum* parasitization for 48 h under laboratory conditions as described above for both species. Blueberries were portioned (150 ± 1 g) in plastic containers (11 × 14 cm), covered with a mesh and transferred to overwintering sites. Control containers were maintained under the same laboratory conditions (16:8 L:D photoperiod, 22 ± 2°C and 70 ± 10% RH).

At each site, samples were kept in large metal cages (5 mm mesh) to prevent external interference (e.g., predation) and monitored for weather parameters using climatic loggers (HOBO® Pendant MX, Onset®, USA). Over nine weeks (September 27 to November 22, 2023), one container per site was exposed weekly, resulting in nine replicates, referred to as exposures (E1–E9). Exposed fruit samples were retrieved between May 1 and 14, 2024, and placed in controlled conditions (16:8 L:D photoperiod, 23.5 ± 1.5°C and 70 ± 10% RH) for 30 days to monitor insect emergence. Every container was monitored weekly to check insects' emergence.

At 63 and 112 days after exposure (DAE), field samplings were conducted to assess insect development stages. *Drosophila suzukii*

juveniles (larvae or puparia) were collected from the fruit samples and taken to the laboratory. The number of sampled juveniles varied depending on availability, ensuring some remained for natural spring emergence. Half of the samples were dissected under a stereomicroscope (LEICA Microsystems, Wetzlar, Germany) to identify developmental stages and vital status. Insects were considered alive if they presented mobility, or if their body shape was intact and coloration clear. Contrary, dead insects did not show mobility and had sign of postmortem discoloration (browning). The categorization of *G. kimorum* development stages were based on the morphology of closely related species (Melk and Govind, 1999). As only parasitoid larval stages were observed throughout the experiment, they were categorized into: first larval instar (L1), second larval instar (L2) and third larval instar (L3). *Drosophila suzukii* juvenile stages were grouped into larvae, early pupae and late pupae based on Chyb & Gompel (2013). Remaining samples were acclimated (12:12 L:D photoperiod, 15 ± 2°C and 70 ± 10% RH) for a week and transferred to rearing conditions (16:8 L:D photoperiod, 22 ± 2°C and 70 ± 10% RH) to assess successful emergence and survival rate (emergence tests).

Overwintering *G. kimorum* adults emerging from the second sampling (112 DAE) were used in parasitization and longevity bioassays conducted at rearing conditions (16:8 L:D photoperiod, 22 ± 2°C and 70 ± 10% RH). Both overwintering and control adult wasps were isolated into Dutscher rearing tubes (2.85 × 9.5 cm) containing a cellulose plug hydrated with 2 mL distilled water and honey applied to the inner lid. Mated females that were 6 ± 1 day old were exposed to 10 ± 0.5 g of blueberries infested with *D. suzukii* larvae (6 blueberries with 5–15 eggs per berry) for 72 h in a cylindrical plastic container (6 × 8 cm). After parasitization, wasps were returned to rearing tubes for assessing their longevity. Mortality was recorded every two days, water and honey were replenished weekly.

2.4. Data analysis

The analysis focused on the first five exposures (E1–E5) because no successful development to the adult stage was observed from E6 onward. From each site, daily parameters of maximum temperature (T_{max}), minimum temperature (T_{min}), and average temperature (T_{ave}) were extracted. As the data did not meet the assumption of normality (as assessed by Shapiro-Wilk test), T_{ave} from different sites were statistically compared with a non-parametric Kruskal-Wallis rank sum test followed by a Dunn's post-hoc pairwise test. Tests were performed in R (4.3.0) (R Core Team, 2020) using the “dunn.test” package (Dinno, 2024). Cumulative time below freezing ($Ct_{<0}$) was calculated as the number of consecutive days where daily T_{ave} was ≤ 0°C. Extreme climatic events were calculated as the count of days where $T_{min} ≤ 0°C$ ($D_{T<0}$) or T_{max} exceeded 30°C ($D_{T>30}$). To evaluate the temperatures conducive to diapause induction, the weekly average temperature for each exposure and site was calculated as the mean of the daily temperatures during the first seven days of exposure.

Degree days (DD) were calculated using the single sine method described by Zalom et al. (1983) according to the following formula:

$$DD = \frac{1}{\pi} \left\{ \left(\frac{T_{max} + T_{min}}{2} - T_L \right) (\theta_2 - \theta_1) + \alpha [\cos(\theta_1) - \cos(\theta_2)] + (T_U - T_L) \left(\frac{\pi}{2} - \theta_2 \right) \right\}$$

$$\theta_1 = \sin^{-1} \left[\left(T_L - \frac{T_{max} + T_{min}}{2} \right) \div \alpha \right]$$

$$\theta_2 = \sin^{-1} \left[\left(T_U - \frac{T_{max} + T_{min}}{2} \right) \div \alpha \right],$$

where T_{max} and T_{min} are daily maximum and minimum temperatures, T_U is the upper thermal threshold, and T_L is the lower thermal threshold. A

T_U of 29.3°C was selected, based on data for the closely related species *Ganaspis lupini* (Hougaard et al., 2019). The lower thermal threshold (T_L) was estimated by doing a sensitivity analysis of the Coefficient of Variation ($CV = \sigma/\mu$, where σ is the standard deviation and μ the mean (Lovie, 2005)) as follows: First, DDs were calculated at T_L values ranging from 0°C to 15°C for two overwintering scenarios: 1) *Ganaspis kimorum* does not have a true diapause but only a quiescence. Therefore, DD accumulation starts at the time of exposure in autumn and ends at adult emergence in the following spring, assuming that the parasitoid continues accumulating DD throughout the entire exposure period. 2) *Ganaspis kimorum* has a true diapause. Thus, an arrestment of development is induced in autumn and terminates after certain time of exposure to cold temperatures, after which DD begin to accumulate. This analysis was conducted with three starting dates of DD accumulation: December 1, January 1 and February 1.

For each scenario, the T_L with the lowest CV was accepted as the developmental threshold in the above-described calculation of DDs (Chmiel and Wilson, 1979), which was conducted using the “degday” package (Lyons, 2022) in software R (R Core Team, 2020). The DD requirements until adult emergence were statistically compared separately for scenario 1) control vs. overwintering parasitoids at T_L of non-diapausing parasitoids, scenario 2) control vs. overwintering parasitoids at T_L of diapausing parasitoids, and a scenario 3), which is as scenario 2 but with an addition of DDs required for the parasitoid to reach the L1 stage. This third analysis was to confirm the diapausing instar that was observed through the above-described dissections. The number of DDs to reach the L1 stage was estimated based on developmental parameters established for *G. lupini* by Wang et al. (2019). At 22°C, *G. lupini*'s L1 larval stage emerges from the egg case at 122 h and transitions to the L2 stage at approximately 142 h (± 6 days). Thus, the resulting number of DDs to reach the L1 stage was estimated to 78. Because the residuals of the ANOVAs did not follow the assumptions of normality (as assessed by Shapiro-Wilk test) and homogeneity of variance (as assessed by Levene's test), the statistical analysis was conducted with a non-parametric Mann-Whitney *U* test in the software R (4.3.0) (R Core Team, 2020).

Parasitoid overwintering survival was calculated for each site and exposure as the total number of adults emerged either after overwintering exposure or from samples collected during winter (emergence tests). The survival rate (SR) was expressed as the absolute emergence corrected by control group, using the formula: $SR (\%) = (A_{OW}/A_C) \times 100$, where A_{OW} is the number of overwintering adults and A_C is the number of adults that emerged under control conditions without overwintering exposure (Häner et al., 2022).

A logistic regression was used with the “glm” function of the stats package in R (4.3.0) (R Core Team, 2020) to analyse the influence of $D_{T<0}$ and $D_{T>30}$ as independent variables on the survival of *G. kimorum* assessed through the dissections conducted during samplings. Survival as the binary dependent variable indicated whether parasitoid larvae examined during dissections ($n = 102$) were alive or dead.

The longevity of post-diapausing *G. kimorum* adults were statistically compared separately for sexes (control vs. male and control vs. females) with a Mann-Whitney *U* test on R software (4.3.0) (R Core Team, 2020), as the data did not meet the assumptions of normality and homogeneity of variances.

Parasitization bioassays measured parasitism using the Apparent Parasitism Rate (APR), defined as the proportion of parasitoid offspring among all emerged insects. APR was calculated for each female using the formula: $APR = n_p/n_t$, where n_p is the number of parasitoid offspring and n_t is the total number of flies and parasitoids emerging (Girod et al., 2018b).

3. Results

Overall average temperatures varied significantly across sites ($\chi^2 = 61.1$, $df = 6$, $P < 0.05$) (Supplementary Fig. S2-S3), with highest temperature of 8.5°C recorded in LAI and lowest temperature of 4.9°C in

GEN (Table 2). The highest T_{max} of 36.6°C occurred in NOV on May 9, 2024, while LAI recorded the second highest (34.2°C) on September 30, 2023. The lowest T_{min} values were observed in GEN (−11.9°C on January 20, 2024) and CAD (−8°C on January 12, 2024) (Table 2). Analysis of extreme climatic events revealed notable site-specific trends (Supplementary Fig. S4). LAI and NOV had the highest number of $D_{T>30}$ where T_{max} exceeded 30°C (21 and 22 days, respectively). Meanwhile, CAD and GEN recorded the highest $D_{T<0}$ values, with 106 and 118 days, respectively (Table 2).

Weekly average temperatures following the first exposure (E1) ranged from $19.9 \pm 0.4^\circ\text{C}$ in LAI to $15.9 \pm 0.5^\circ\text{C}$ in DEL. Apart from DEL, where the second exposure (E2) had a higher average temperature of $18.3 \pm 0.3^\circ\text{C}$, other sites exhibited a steady decline across subsequent exposures, reaching a minimum of $8.4 \pm 0.5^\circ\text{C}$ in GEN (Table 3).

3.1. Experiment 1

From the weekly exposures between August 20 to September 30, 2021, *G. kimorum* emerged before overwintering only from *D. suzukii* that were exposed under laboratory conditions from August 27 to 30. A first male parasitoid was found in the vial on October 25, and two additional males and two females on November 9. In none of the other samples (which were all parasitized in the field cages) did parasitoids emerge before winter. Dissections of *D. suzukii* puparia conducted on February 25 and on March 11, 2022, revealed the presence of second instar larvae (about 1 mm in length and without tail) in the pupal cases. Overwintering survival was confirmed in DEL but not in CAD. Three *G. kimorum* males emerged from samples that were exposed in DEL from September 10–13, 2021. Development from the overwintering stage to the adult parasitoid took in a growth chamber at 20 °C an average of 25 days (23–28 days).

3.2. Experiment 2

During the 63 DAE sampling, 182 juvenile stages of *D. suzukii* were dissected. The highest number of living *G. kimorum* was observed in FAE (22), followed by SMA (12) and LAI (11) (Supplementary Table S1). Among the recorded larval stages of *G. kimorum*, L1 was the most abundant ($n = 58$), followed by L2 ($n = 15$) and L3 ($n = 3$). No later development stages of the parasitoid, such as pupae or adults were detected. Early pupal stages of *D. suzukii* contained the largest number of *G. kimorum* L1 individuals ($n = 32$), whereas L2 stages were more frequently observed in later pupal stages ($n = 12$) (Fig. 1). The rate of living *G. kimorum* L1 within early pupae of *D. suzukii* was 94 %, the highest among the groups analysed (limited to those with > 3 samples) (Fig. 1).

During the 112 DAE sampling, 64 juvenile stages of *D. suzukii* were dissected. The highest number of living *G. kimorum* was observed in FAE ($n = 9$), followed by SMA ($n = 8$) and NOV ($n = 3$) (Supplementary Table S2). Out of the 26 *G. kimorum* that were found, 25 (96 %) were L1, and one was L2; no L3 larvae were recorded. The rate of living L1 *G. kimorum* within early pupal stages of *D. suzukii* was 82 % (Fig. 1).

Emergence data showed a gradual reduction of survival for consecutive exposures for both host and parasitoid (Fig. 2). Survival rates of *D. suzukii* decreased with advancing exposure dates, with adults emerging only up to 67 days after exposure (Supplementary Fig. S5) or from puparia collected at 63 DAE during the first sampling. No emergence occurred from samples collected after 112 DAE or in spring. *Ganaspis kimorum* emerged from samples exposed in three sites out of five and exclusively in spring or from samples taken during the winter (Supplementary Table S3). Relative to non-overwintering laboratory-reared controls, the highest survival rate was recorded in FAE (E1: 17.6 %, E2: 6.6 %) and SMA (E1: 6.3 %, E2: 5.5 %, E3: 3.3 %, E4: 1.2 %, E5: 0.4 %), when compared to the other sites (Fig. 2). In FAE, *G. kimorum* adults emerged from E2 on April 12, May 8, 10, and 12. From the same site, adults emerged from E1 on May 8, 10, and 12. In NOV, one adult

Table 2

Temperature data recorded for the different sites. T_{ave} refers to the average temperature during the entire winter exposure period. Different letters indicate significance according to the post-hoc pairwise Dunn's test ($P < 0.05$). Cumulative days with average temperatures below 0 ($C_{T<0}$). Extreme climatic events: number of days with T_{min} below 0 ($D_{T<0}$), and number of days with T_{max} above 30 °C ($D_{T>30}$).

Site	n	$T_{ave} \pm SEM$ (°C)	T_{max} (°C)	T_{max} (date)	T_{min} (°C)	T_{min} (date)	$C_{T<0}$ (days)	$D_{T<0}$ (days)	$D_{T>30}$ (days)
CAD	196	6.6 ± 0.4c	33.8	Sep. 8	-8	Jan. 12	4	106	10
DEL	196	7.1 ± 0.4bc	33.8	Sep. 8	-6.4	Jan. 27	5	85	10
FAE	217	6.7 ± 0.3c	26.1	Apr. 14	-4.7	Jan. 21	5	36	0
GEN	230	4.9 ± 0.3d	28.6	Oct. 9	-11.9	Jan. 20	11	118	0
LAI	230	8.5 ± 0.4a	34.2	Sep. 30	-6.5	Jan. 22	6	56	21
NOV	230	7.3 ± 0.4bc	36.6	May. 9	-8.1	Jan. 22	8	74	22
SMA	217	8.2 ± 0.4ab	30.8	Apr. 7	-4.4	Jan. 14	4	43	1

Table 3

Weekly average temperature (°C) ± Standard Error Mean (SEM) for all the sites, measured after exposures. Asterisks (*) indicate sites where adults emerged after winter.

Sites	Exposures				
	E1	E2	E3	E4	E5
CAD	15.9 ± 0.5	18.3 ± 0.3	–	–	–
DEL	15.9 ± 0.5	18.3 ± 0.3*	–	–	–
FAE	16.0 ± 0.4*	15.6 ± 0.3*	13.2 ± 1.3	11.1 ± 0.7	10.5 ± 0.5
GEN	15.8 ± 0.6	15.3 ± 0.5	11.0 ± 1.5	9.2 ± 0.6	8.4 ± 0.5
LAI	19.9 ± 0.4	19.5 ± 0.4	15.7 ± 1.4	12.8 ± 0.7	12.0 ± 0.5
NOV	18.4 ± 0.7*	17.7 ± 0.3	14.1 ± 1.4	11.5 ± 0.6	10.7 ± 0.6
SMA	17.6 ± 0.4*	17.3 ± 0.4*	15.2 ± 1.2*	12.8 ± 0.7*	12.3 ± 0.5

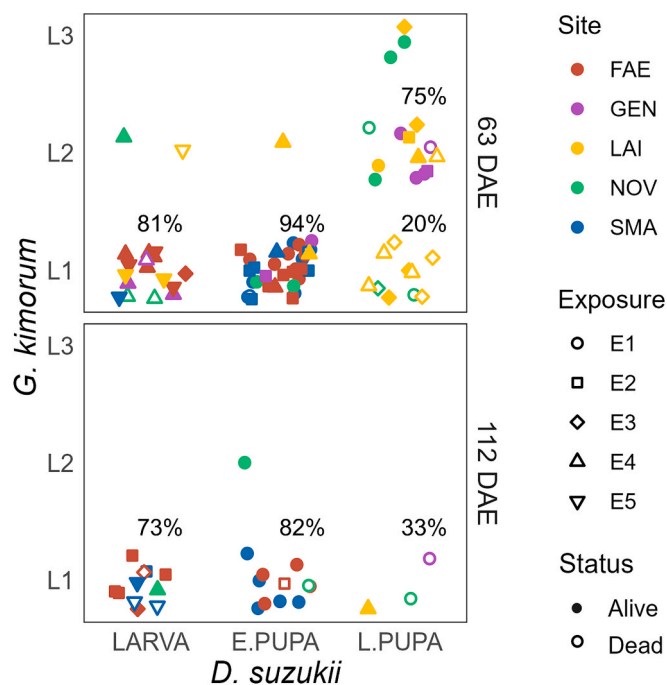


Fig. 1. Correlation between juvenile stages of *Ganaspis kimorum* (L1, L2, L3 larval stages) and *Drosophila suzukii* (larva, early pupa, late pupa) for samples collected at (upper panel) 63 and (lower panel) 112 days after exposure. Numbers on the side of groups (with samples size larger than three) indicate the percentage of alive parasitoids for that specific development stages.

from E1 emerged on May 29. In SMA, adult parasitoids emerged on April 10 (E3), 13 (E1-E2), and 18 (E4) (Supplementary Fig. S2). Survival of parasitoids assessed through dissections was significantly decreased by both the number of days below 0 °C ($D_{T<0}$) ($\chi^2 = 4.3320$; $df = 1,99$; $P = 0.03740$) and above 30 °C ($D_{T>30}$) ($\chi^2 = 3.9166$; $df = 1,99$; $P =$

0.04781).

The sensitivity analysis suggested a lower thermal threshold (T_L) of 7 °C for the accumulation of DDs without a true diapause, with the lowest coefficient of variation (CV) being 0.129. For the accumulation of DDs by diapausing parasitoids, the analysis suggested a T_L of 9 °C, with the lowest CV being 0.153, regardless of the starting date considered (Fig. 3). For convenience, January 1 was chosen as the starting date for the subsequent calculations of DDs.

The comparison of accumulated DDs between control and non-diapausing overwintering parasitoids at $T_L = 7^\circ\text{C}$ showed that overwintering parasitoids accumulated significantly more DDs compared to the control, with an increase of 55.2 % ($W = 948$, $df = 484$, $P < 0.05$; Fig. 4a). In contrast, the comparison between control and diapausing *G. kimorum* at $T_L = 9^\circ\text{C}$ showed that the overwintering group accumulated significantly fewer DDs compared to control, with an average reduction of 19.6 % ($W = 23662$, $df = 484$, $P < 0.05$; Fig. 4b). The DDs required for the parasitoid to reach the end of the diapausing stage (L1) was calculated as 78 DDs. Adding these DDs to the ones of diapausing parasitoids resulted in no significant difference between overwintering (409 ± 4 DD) and control adults (407 ± 6 DD) ($W = 15183$, $df = 484$, $P > 0.05$; Fig. 4c).

The pairwise comparison showed that overwintering males had a 29 % shorter life span compared to laboratory-reared males ($W = 53$, $df = 15$, $P < 0.05$; Fig. 5). The longevity of overwintering females was not statistically different from laboratory-reared ones ($W = 11$, $df = 9$, $P > 0.05$; Fig. 5). Parasitisation bioassays conducted on overwintering insects showed that two out of five females (40 %) were able to successfully parasitize the host, with an apparent parasitism level of 46 % and 6 %. Only one out of five (20 %) of the control females were able to parasitize the host, with an apparent parasitism level of 38 %.

4. Discussion

Our study confirms that *G. kimorum* is able to overwinter under field conditions in Northern Italy and possibly also in Switzerland. However, the timing of exposures and environmental conditions in the different field sites were critical for diapause induction and survival. Early exposures of parasitized *D. suzukii* conducted in late August in Switzerland led to emergence of adults in late October and early November, indicating that these parasitoids did not encounter the environmental cues required for diapause induction. In contrast, exposures from late September to mid-October resulted in successful overwintering, with emergence of adult parasitoids occurring only after the winter period. This suggests that diapause induction is strongly influenced by the timing of the exposition and associated environmental conditions.

In the second experiment, only the first five of the nine exposures (E1-E5), occurring between late September and late October, yielded insects. This period appears crucial for diapause induction and marks the last activity phase of both host and parasitoid. In Northern Italy, *D. suzukii* females with mature eggs are present until late October (Grassi et al., 2018), and Winkler et al. (2021) estimated that oviposition ceases at a minimum temperature of 13.2 °C, matching conditions in our study

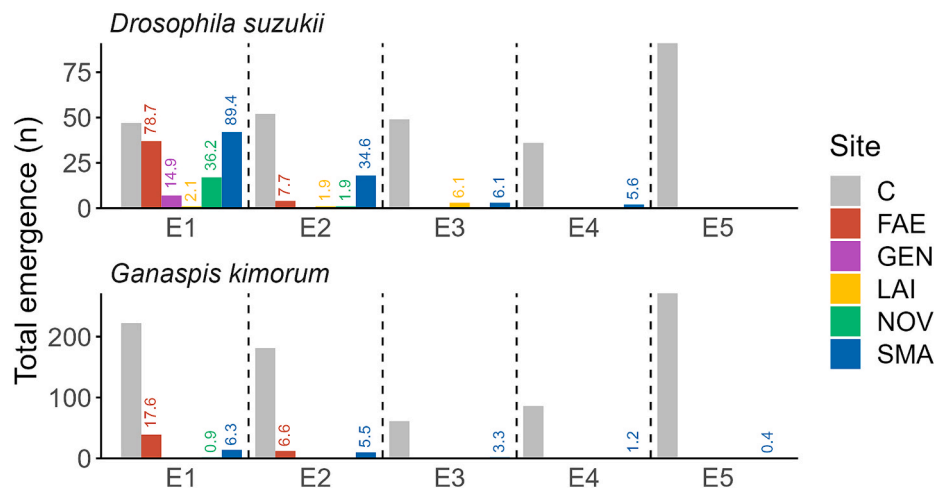


Fig. 2. Overwintering survival in Experiment 2, expressed as total emergences of (upper panel) *Drosophila sukuzii* and (lower panel) *Ganaspis kimorum* for the five exposure dates (E1-E5). Grey bars represent the emergences recorded from the non-overwintering laboratory-reared controls (C), while coloured bars those recorded in the field sites. Numbers on top of the bars indicate percentage of survival relative to the controls.

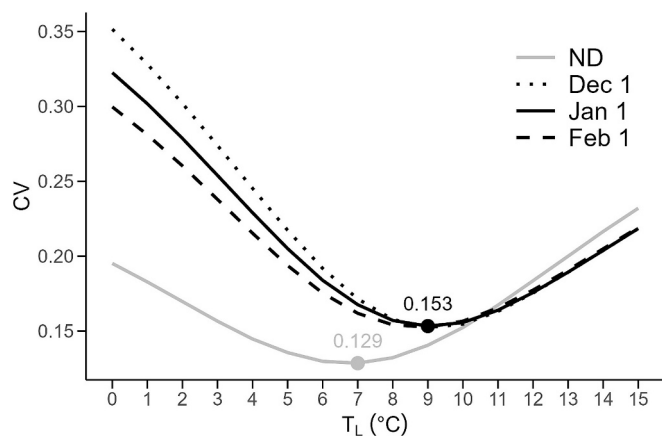


Fig. 3. Sensitivity analysis to find the lower thermal threshold (T_L) of *Ganaspis kimorum*, by using the Coefficient of Variation (CV) for degree day (DD) requirements of the parasitoid to adult emergence. The grey line indicates the change in CV at different T_L for a scenario of non-diapausing parasitoids. The black lines indicate the change for post-diapausing parasitoids, with DD accumulating at three different starting dates. Numbers above the points indicate the lowest CV values, corresponding to the selected T_L .

area during the same period. According to our 2023 monitoring data (M. V.R.S., unpublished), *G. kimorum* was detected in fresh fruit samples collected in the first week of October, indicating activity at least until this date (no further collections were made). Thus, the first five exposures (late September–late October) represent a realistic scenario in which both host and parasitoid coexist and remain ovipositing.

Dissections revealed that the overwintering stage of *G. kimorum* was predominantly the early larval instar (L1), residing inside early host pupae. Our results indicate that *G. kimorum* employs eudiapause, a facultative developmental arrest, likely triggered by environmental factors such as temperature. This dormancy within the endoparasitic phase appears to exploit the lipid-rich resources of the host's early pupal body (Kühnlein, 2012; Liu and Huang, 2013), providing insulation during winter and a nutritional reserve for spring emergence (Sinclair and Marshall, 2018).

Interestingly, differences between our findings and those of Hougardy et al. (2019) might reflect divergence in biology between *G. kimorum* and its closely related species *G. lupini*, which appears to enter diapause at the prepupal stage. This divergence would further

support the recent redescription of *G. kimorum* as a distinct species (Sosa-Calvo et al., 2024), or could be related to the experimental protocol adopted. In our study, later larval instars (L2 and L3) were observed to be present in host larvae and puparia 63 days after exposure to autumn and winter temperatures in the field, particularly in warmer sites and early exposures; however, these stages failed to survive prolonged exposure. After 112 days of exposure, first-instar larvae dominated across all sites, further supporting the hypothesis that diapause occurs in the L1 stage within early stages of host puparia. Also, the presence of L2 stages during samplings conducted in Switzerland in late February and March support our findings, as can be explained by the parasitoids having resumed their development at this time of the year. Since *G. kimorum* diapauses as early larval instar, it needs to arrest host development. This process may be regulated by injected venom proteins, a common mechanism among *Drosophila* parasitoids to suppress the host immune response and manipulate its development (Poirié et al., 2014). This has been observed in both pupal and larval parasitoids. For instance, *T. drosophilae* uses venom to arrest pupal development for nutrient utilization (Pang et al., 2024), while *Leptopilina* spp. use venom to suppress host immune response (Huang et al., 2021). Among the various components of parasitoid venoms, those produced by *Ganaspis* spp. contain metalloprotease enzymes, which are believed to play a role in host development manipulation (Poirié et al., 2014). We hypothesize that *G. kimorum* also manipulates its host through venom, extending the pupal stage to align with its own developmental needs. Supporting this hypothesis, our dissections predominantly revealed *D. sukuzii* in the early pupal stage when parasitized by living *G. kimorum*. This suggests that *G. kimorum* may suppress further host development, maintaining it in this phase, its preferred stage for diapause.

Diapause induction in insects is regulated by token stimuli such as photoperiod and temperature (Gill et al., 2017; Košťál, 2006). Other drosophilid parasitoids like *G. lupini*, *L. japonica*, and *Asobara tabida* (Nees) (Hymenoptera: Braconidae) exhibit temperature-mediated diapause regardless of photoperiod (Hougardy et al., 2019; Kraaijeveld and van Alphen, 1995; Murata et al., 2013). In our field study, photoperiods longer than 14:10 (L:D) in late August failed to induce diapause, while exposures from September onward (13:11 to 11:13 L:D) did not result in premature emergence. This result does not exclude a possible role of photoperiod in diapause induction, but should be further investigated at constant temperatures to uncouple these two factors.

The temperatures triggering diapause appear to range between 13 °C and 18 °C (see Table 3). In LAI, where average temperatures during the first two exposures exceeded 19 °C, diapause was not induced, and dissections revealed the presence of later larval stages (L2 and L3). These

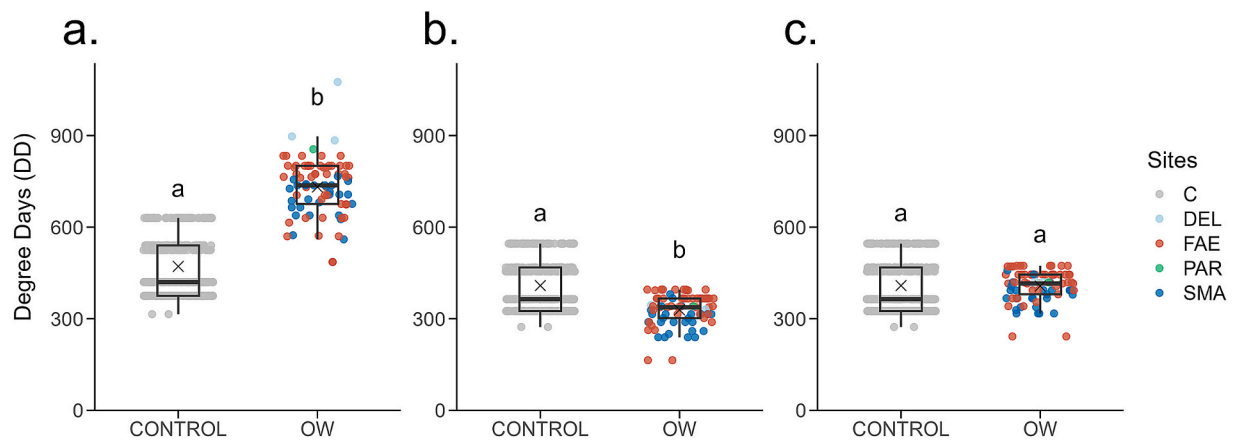


Fig. 4. Degree days needed for development to the adult stage compared between laboratory reared non-diapausing (CONTROL) and overwintering (OW) *Ganaspis kimorum* considering three scenarios: **a** non-diapausing parasitoids with a lower thermal threshold (T_L) of 7 °C and DD accumulation starting at the date of field exposure in autumn, **b** diapausing parasitoids with a T_L of 9 °C and starting DD accumulation on January 1, and **c** diapausing parasitoids as in **b** but with the addition of DDs needed to reach the diapausing stage determined through dissections (first larval stage). Lower case letters above the boxplots indicate significant differences according to the Mann-Whitney U test.

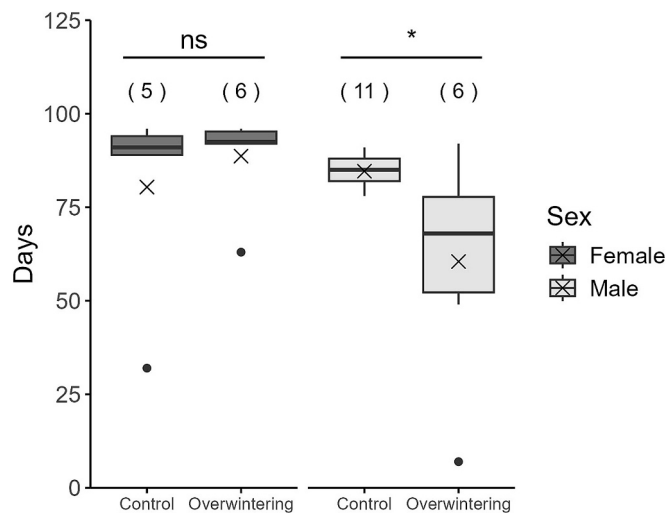


Fig. 5. Longevity of laboratory-reared control and overwintering *Ganaspis kimorum* adults. Boxplots represent the distribution of lifespan for both sexes showing median (line), interquartile range (box) and mean (x). Whiskers extend to the smallest and largest values within $1.5 \times IQR$. Outliers are indicated as individual points. Number in parenthesis are adults tested, asterisk indicate significant difference at $P < 0.05$ and ns that there is no significant difference according to Mann-Whitney U test.

findings align with earlier studies, indicating that Japanese strains of *L. japonica* cease development between 15 °C and 18 °C (Murata et al., 2013). Similarly, *G. lupini* and *L. japonica* have been reported to enter diapause at 11.8 °C and 17.2 °C, but not at temperatures above 19 °C (Hougardy et al., 2019).

Survival of *G. kimorum* was influenced by extreme climatic events, with both low and high-temperature negatively impacting insect's survival. This association was also observed across different sites. Sites with milder winter temperatures, such as SMA, NOV, and FAE, exhibited higher survival rates, whereas cold temperatures in CAD and GEN resulted in no successful overwintering. For example, in GEN, even though insects were exposed at permissive temperatures (15.8 °C and 15.3 °C for E1 and E2 respectively) the cumulative days of average temperatures below 0 °C (11 days) and the days where T_{min} was below 0 °C (118 days) likely exceeded the cold tolerance of *G. kimorum*. By comparison, sites with shorter periods of sub-zero temperatures (e.g.,

SMA and FAE) supported higher survival rates. These findings align with studies conducted on larval parasitoids, such as *Leptopilina* spp., which can only withstand a limited period at 0 °C (Murata et al., 2013), compared to more cold-tolerant pupal parasitoids (Häner et al., 2022). Also, high temperatures and reduced humidity could have compromised overwintering survival (Colinet and Boivin, 2011; Danks, 1987; Gill et al., 2017; Kostál, 2006). In fact, *D. suzukii* immatures cannot survive constant temperatures of 29.3 °C, but can withstand short exposures at temperature above 30.6 °C (Hougardy et al., 2019). The hot days recorded in LAI, where maximum temperatures reached 34.2 °C could have posed a threat for both hosts and parasitoids residing in the fruit samples and could explain the recorded low survival rates. In LAI and CAD, dry conditions likely contributed to the reduced survival of both hosts and parasitoids. Low humidity has been shown to decrease survival rates in other parasitoids (Murata et al., 2013), and desiccation during overwintering may have further exacerbated mortality in these sites.

The survival of *G. kimorum* ranged from 0.4 to 17.6 %, with decreasing probabilities recorded with later exposure dates. The SMA site offers some evidence for this trend, showing survival of 6.3 %, 5.5 %, 3.3 %, 1.2 % and 0.4 % for E1, E2, E3, E4 and E5 respectively. This probability of survival is in accordance with the 4 % recorded for *L. japonica* strain collected in Sapporo (Northern Japan) and exposed to 0 °C for 90 days (Murata et al., 2013). Similarly, larval stages of the pupal parasitoid *T. drosophilae* had a survival of 2 % when exposed to semi-field conditions for 101 days (Amiresmaeli et al., 2020). While the survival calculated in the present study is in accordance with the literature and the recorded climatic data, it may be an overestimation, because it was calculated including the emergence of adults from the samples taken 63 and 112 days after exposure (emergence tests). On the other hand, including solely the emergence occurring in spring would underestimate survival, as a significant number of puparia were removed from the fruit samples to conduct both emergence tests and dissections. Juveniles removed for dissections had the potential to emerge as adults, and their removal possibly compensated the overestimation resulting from the addition of adults from emergence tests. For additional information we suggest to refer to the [supplementary material](#) provided (Table S1, S2 and S3).

The sensitivity analysis to find the lower thermal threshold (T_L) using the Coefficient of Variation and the analysis of the DD accumulation provided insights into the overwintering strategy of *G. kimorum*. Under the non-diapausing scenario ($T_L = 7$ °C), the overwintering group exhibited an unrealistically excessive accumulation of DDs. In contrast, the diapausing scenario ($T_L = 9$ °C) presented a realistic outcome, with

post-overwintering development accounting for 80.5 % of the DDs required for the laboratory-reared control. Incorporating the DDs needed to reach the diapausing stage, the overwintering group achieved 99.6 % of the DDs observed in the laboratory-reared control group, confirming the lower thermal threshold found through the sensitivity analysis, and that *G. kimorum* arrests its development at the late L1 larval stage and therefore undergoes a winter diapause.

Overall, the variance observed in development time (and resulting DD) in the overwintering group was comparable to that of the control group and can be attributed to the plasticity in temperature-dependent development that occurs among individuals of the same species (Colinet and Boivin, 2011). The greater variability noted in FAE, where the first natural emergence and subsequent emergence under laboratory conditions occurred 26 days apart, may be attributed to the effect of fluctuating temperatures within a permissive range. Such fluctuations are known to enhance performance compared to constant temperatures (Colinet et al., 2015) and could have accelerated the timing of the initial emergence.

Cold exposure often imposes significant fitness costs due to chilling effects, starvation, or their combination (Colinet and Boivin, 2011; Ellers and Van Alphen, 2002; Hance et al., 2007). The most notable impacts are typically observed on survival and reproduction. Parasitoids capable of overwintering often experience a reduced lifespan, as fat reserves become a limiting factor. For example, in several *Aphidius* spp., adult survival declines linearly with duration of cold exposure (Colinet et al., 2006; Ismail et al., 2010). In our study, overwintering males of *G. kimorum* exhibited a reduced lifespan, similarly to what was observed in *G. kimorum* larvae in cold storage (Lisi et al., 2024). On the other hand, females were unaffected, successfully parasitizing hosts and producing viable offspring. This sexual difference might be related to sexual allocation. Species like *G. kimorum*, which use haplodiploidy for sex determination (Boulton et al., 2015; Small et al., 2012) can manipulate sex ratio based on host size, favouring females (King, 1993; Seidl and King, 1993). According to this theory, females likely have had access to more nutritional resources hence a longer lifespan compared to males. Another plausible explanation is that females are generally larger in size (Hurlbutt, 1987), possess greater fat reserves, and have the capacity to resorb eggs for additional energy when required (Papaj, 2000; Rosenheim et al., 2000). Consequently, following resource-intensive periods such as winter, females may exhibit a physiological advantage over males. However, this bioassay was conducted on a limited number of parasitoids, as survival rate after overwintering exposures was relatively low. Further studies should be conducted to confirm this theory and support our preliminary results.

To the authors' knowledge, this is the first study to examine the overwintering behaviour of *G. kimorum* under natural conditions, providing important insights that support classical biological control programmes. Specifically, the study establishes a lower thermal threshold and identifies climatic conditions that can guide the selection of optimal release sites to ensure successful establishment. Additionally, it provides a starting point to make predictions of parasitoid emergence in spring under field conditions and to model host-parasitoid population dynamics. In Northern Italy, *D. suzukii* terminates its reproductive diapause in March and shows high egg maturation by mid-April (Grassi et al., 2018). Our findings indicate that *G. kimorum* emergence coincides with this period, highlighting the tight coevolution that has shaped the host-parasitoid interactions.

In conclusion, our findings provide important insights into the overwintering biology of *G. kimorum*, confirming its ability to successfully overwinter in introduced area as early larval instars within the early pupal stages of *D. suzukii*. The study underscores the pivotal role of environmental conditions, particularly temperature, in diapause induction and survival. By establishing a lower thermal threshold for development and revealing the parasitoid's diapause strategies, this research lays a strong foundation for the effective use of *G. kimorum* in classical biological control programmes.

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CRedit authorship contribution statement

Lorenzo Fellin: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Marco Valerio Rossi Stacconi:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Gianfranco Anfora:** Writing – review & editing, Supervision, Methodology, Conceptualization. **M. Lukas Seehausen:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Giada Bellotti:** Methodology, Investigation. **Sergio Angeli:** Writing – review & editing, Supervision, Conceptualization. **Silvia Schmidt:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2025.105801>.

References

- Amiresmaeli, N., Romeis, J., Collatz, J., 2020. Cold tolerance of the drosophila pupal parasitoid *Trichopria drosophilae*. *J. Insect Physiol.* 125, 104087. <https://doi.org/10.1016/j.jinsphys.2020.104087>.
- Andreazza, F., Bernardi, D., dos Santos, R.S.S., Garcia, F.R.M., Oliveira, E.E., Botton, M., Nava, D.E., 2017. *Drosophila suzukii* in southern neotropical region: current status and future perspectives. *Neotrop. Entomol.* <https://doi.org/10.1007/s13744-017-0554-7>.
- Angilletta Jr, M.J., 2009. *Thermal adaptation: a theoretical and empirical synthesis*. Oxford University Press, New York, Oxford biology readers.
- Asplen, M.K., Anfora, G., Biondi, A., Choi, D.S., Chu, D., Daane, K.M., Gibert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z.L., Kárpáti, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Véték, G., Vogt, H., Walton, V.M., Yu, Y., Zappalà, L., Desneux, N., 2015. Invasion biology of spotted wing *Drosophila (Drosophila suzukii)*: a global perspective and future priorities. *J. Pest. Sci.* 88, 469–494. <https://doi.org/10.1007/s10340-015-0681-z>.
- Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., Kopp, A., 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related

- species. In: Proceedings of the Royal Society 281. Royal Society. Doi: 10.1098/rspb.2013.2840.
- Biondi, A., Wang, X., Daane, K.M., 2021. Host preference of three Asian larval parasitoids to closely related *Drosophila* species: implications for biological control of *Drosophila suzukii*. *J. Pest. Sci.* 94, 273–283. <https://doi.org/10.1007/s10340-020-01272-0>.
- Boulton, R.A., Collins, L.A., Shuker, D.M., 2015. Beyond sex allocation: the role of mating systems in sexual selection in parasitoid wasps. *Biol. Rev.* 90, 599–627. <https://doi.org/10.1111/brv.12126>.
- Burrack, H.J., Fernandez, G.E., Spivey, T., Kraus, D.A., 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive frugivore. *Pest Manag. Sci.* 69, 1173–1180. <https://doi.org/10.1002/ps.3489>.
- Chyb, S., Gompel, N., 2013. Wild-type morphology, in: Chyb, S., Gompel, N. (Eds.), Atlas of *Drosophila* Morphology. Academic Press, San Diego, pp. 1–23. doi: 10.1016/B978-0-12-384688-4.00001-8.
- Chmiel, S.M., Wilson, M.C., 1979. Estimation of the lower and upper developmental threshold temperatures and duration of the nymphal stages of the meadow spittlebug, *Philaenus spumarius*. *Environ. Entomol.* 8, 682–685. <https://doi.org/10.1093/ee/8.4.682>.
- Colinet, H., Boivin, G., 2011. Insect parasitoids cold storage: a comprehensive review of factors of variability and consequences. *Biol. Control.* <https://doi.org/10.1016/j.biocontrol.2011.04.014>.
- Colinet, H., Hance, T., Vernon, P., 2006. Water relations, fat reserves, survival, and longevity of a cold-exposed parasitic wasp *Aphidius colemani* (Hymenoptera: Aphidinae). *Environ. Entomol.* 35, 228–236. [https://doi.org/10.1603/0046-225X\(2006\)035\[0228:WRFRSA\]2.0.CO](https://doi.org/10.1603/0046-225X(2006)035[0228:WRFRSA]2.0.CO).
- Colinet, H., Kustr, A., 2024. The apparent seasonal biphenism in *Drosophila suzukii* stems in reality from continuous reaction norms. *Pest Manag. Sci.* 81, 507–517. <https://doi.org/10.1002/ps.8452>.
- Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in fluctuating thermal environments. *Annu. Rev. Entomol.* 60, 123–140. <https://doi.org/10.1146/annurev-ento-010814-021017>.
- Daane, K.M., Wang, X., Hogg, B.N., Biondi, A., 2021. Potential host ranges of three Asian larval parasitoids of *Drosophila suzukii*. *J. Pest. Sci.* 94, 1171–1182. <https://doi.org/10.1007/s10340-021-01368-1>.
- Dalton, D.T., Walton, V.M., Shearer, P.W., Walsh, D.B., Caprile, J., Isaacs, R., 2011. Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. *Pest Manag. Sci.* 67, 1368–1374. <https://doi.org/10.1002/ps.2280>.
- Danks, H. V., 1987. Insect Dormancy: An Ecological Perspective, 1st ed, Biological Survey of Canada monograph series. Biological Survey of Canada (Terrestrial Arthropods).
- De Ros, G., Grassi, A., Pantezzi, T., 2021. Recent trends in the economic impact of *Drosophila suzukii*. In: *Drosophila suzukii* Management. Springer International Publishing, pp. 11–27. https://doi.org/10.1007/978-3-030-62692-1_2.
- Denlinger, D.L., 2002. Regulation of diapause. *Annu. Rev. Entomol.* 47, 93–122. <https://doi.org/10.1146/annurev.ento.47.091201.145137>.
- Dietschler, N.J., Bittner, T.D., Devine, N.G., Mayfield III, A., Preston, C., Crandall, R.S., Parkman, J., Simek, Z., Thompson, B., Lonsdale, M.E., Veverka, B., Elkinton, J.S., Salom, S.M., Whitmore, M.C., 2023. Overwintering diapause and survival of western *Leucotaraxis argenticollis*, a promising biological control agent for *Adelges tsugae*, in the eastern United States. *Biol. Control* 182, 105233. <https://doi.org/10.1016/j.biocontrol.2023.105233>.
- Dinno, A., 2024. dunn.test: Dunn's test of multiple comparisons using rank sums. CRAN: Contributed Packages. <https://doi.org/10.32614/CRAN.package.dunn.test>.
- Ellers, J., Van Alphen, J.J.M., 2002. A trade-off between diapause duration and fitness in female parasitoids. *Ecol. Entomol.* 27, 279–284. <https://doi.org/10.1046/j.1365-2311.2002.00421.x>.
- Enriquez, T., Colinet, H., 2017. Basal tolerance to heat and cold exposure of the spotted wing drosophila, *Drosophila suzukii*. *PeerJ* 5, e3112.
- Farnsworth, D., Hamby, K.A., Bolda, M., Goodhue, R.E., Williams, J.C., Zalom, F.G., 2017. Economic analysis of revenue losses and control costs associated with the spotted wing drosophila, *Drosophila suzukii* (Matsumura), in the California raspberry industry. *Pest Manag. Sci.* 73, 1083–1090. <https://doi.org/10.1002/ps.4497>.
- Fellin, L., Grassi, A., Puppato, S., Saggi, A., Anfora, G., Ioriatti, C., Rossi-Stacconi, M.V., 2023. First report on classical biological control releases of the larval parasitoid *Ganaspis brasiliensis* against *Drosophila suzukii* in northern Italy. *BioControl* 68, 1–12. <https://doi.org/10.1007/s10526-022-10174-2>.
- Garipey, T.D., Abram, P.K., Adams, C., Beal, D., Beers, E., Beetle, J., Biddinger, D., Brind'Amour, G., Bruin, A., Buffington, M., Burrack, H., Daane, K.M., Demchak, K., Fanning, P., Gillett, A., Hamby, K., Hoelmer, K., Hogg, B., Isaacs, R., Johnson, B., Lee, J.C., Levenson, H.K., Loeb, G., Lovero, A., Milnes, J.M., Park, K.R., Prade, P., Regan, K., Renkema, J.M., Rodriguez-Saona, C., Neupane, S., Jones, C., Sial, A., Smythman, P., Stout, A., Van Timmeren, S., Walton, V.M., Wilson, J.K., Wang, X., 2024. Widespread establishment of adventive populations of *Leptopilina japonica* (Hymenoptera: Figitidae) in North America and development of a multiplex PCR assay to identify key parasitoids of *Drosophila suzukii* (Diptera, Drosophilidae). *Neobiota* 93, 63–90. <https://doi.org/10.3897/neobiota.93.121219>.
- Gibert, P., Moreteau, B., Pétavy, G., Karan, D., David, J.R., 2001. Chill-coma tolerance, a major climatic adaptation among *Drosophila* species. *Evolution* (n y) 55, 1063–1068. <https://doi.org/10.1111/j.0014-3820.2001.tb00623.x>.
- Girod, P., 2017. From Asia to Europe, evaluation of parasitoids for biological control of invasive fruit pest *Drosophila suzukii*. University of Neuchâtel, Switzerland.
- Girod, P., Borowiec, N., Buffington, M., Chen, G., Fang, Y., Kimura, M.T., Peris-Felipo, F. J., Ris, N., Wu, H., Xiao, C., Zhang, J., Aebi, A., Haye, T., Kenis, M., 2018a. The parasitoid complex of *D. suzukii* and other fruit feeding *Drosophila* species in Asia. *Sci. Rep.* 8, 1–8. <https://doi.org/10.1038/s41598-018-29555-8>.
- Girod, P., Lierhmann, O., Urvois, T., Turlings, T.C.U., Kenis, M., Haye, T., 2018b. Host specificity of Asian parasitoids for potential classical biological control of *Drosophila suzukii*. *J. Pest. Sci.* 91, 1241–1250. <https://doi.org/10.1007/s10340-018-1003-z>.
- Grassi, A., Gottardello, A., Dalton, D.T., Tait, G., Rendón, D., Ioriatti, C., Gibeau, D., Rossi-Stacconi, M.V., Walton, V.M., 2018. Seasonal reproductive biology of *Drosophila suzukii* (Diptera: Drosophilidae) in temperate climates. *Environ. Entomol.* 47, 166–174. <https://doi.org/10.1093/ee/nvx195>.
- Hamby, K.A., E. Bellamy, D., Chiu, J.C., Lee, J.C., Walton, V.M., Wiman, N.G., York, R. M., Biondi, A., 2016. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. *J. Pest Sci* 89, 605–619. Doi: 10.1007/s10340-016-0756-5.
- Hance, T., van Baaren, J., Vernon, P., Boivin, G., 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. *Annu. Rev. Entomol.* 52, 107–126. <https://doi.org/10.1146/annurev.ento.52.110405.091333>.
- Häner, N., Amiresmaeili, N., Stähli, N., Romeis, J., Collatz, J., 2022. Overwintering of two pupal parasitoids of *Drosophila* under natural conditions. *J. Therm. Biol* 106. <https://doi.org/10.1016/j.jtherbio.2022.103231>.
- Gill, H.K., Goyal, G., Chahil, G., 2017. Insect diapause: a review. *J. Agric. Sci. Technol. A* 7. <https://doi.org/10.17265/2161-6256/2017.07.002>.
- Hermes, D.A., 2004. Using degree-days and plant phenology to predict pest activity what are degree-days?, in: Krischik, V.A. (Ed.), IPM (Integrated Pest Management) of Midwest Landscapes. Minnesota Agricultural Experiment Station, pp. 49–59.
- Hondo, T., Koike, A., Sugimoto, T., 2006. Comparison of thermal tolerance of seven native species of parasitoids (Hymenoptera: Eulophidae) as biological control agents against *Liriomyza trifolii* (Diptera: Agromyzidae) in Japan. *Appl. Entomol. Zool.* 41, 73–82. <https://doi.org/10.1303/aez.2006.73>.
- Hougaard, E., Hogg, B.N., Wang, X., Daane, K.M., 2019. Comparison of thermal performances of two Asian larval parasitoids of *Drosophila suzukii*. *Biol. Control* 136. <https://doi.org/10.1016/j.biocontrol.2019.104000>.
- Huang, J., Chen, J., Fang, G., Pang, L., Zhou, S., Zhou, Y., Pan, Z., Zhang, Q., Sheng, Y., Lu, Y., Liu, Z., Zhang, Y., Li, G., Shi, M., Chen, X., Zhan, S., 2021. Two novel venom proteins underlie divergent parasitic strategies between a generalist and a specialist parasite. *Nat. Commun.* 12, 234. <https://doi.org/10.1038/s41467-020-20332-8>.
- Hughes, G.E., Alfrod, L., Sterk, G., Bale, J.S., 2010. Thermal activity thresholds of the predatory mirid *Nesidiocoris tenuis*: implications for its efficacy as a biological control agent. *BioControl* 55, 493–501. <https://doi.org/10.1007/s10526-010-9267-0>.
- Hurlbutt, B., 1987. Sexual size dimorphism in parasitoid wasps. *Biol. J. Linn. Soc.* 30, 63–89. <https://doi.org/10.1111/j.1095-8312.1987.tb00290.x>.
- Ismail, M., Vernon, P., Hance, T., van Baaren, J., 2010. Physiological costs of cold exposure on the parasitoid *Aphidius ervi*, without selection pressure and under constant or fluctuating temperatures. *BioControl* 55, 729–740. <https://doi.org/10.1007/s10526-010-9303-0>.
- Jakobs, R., Ahmadi, B., Houben, S., Garipey, T.D., Sinclair, B.J., 2017. Cold tolerance of third-instar *Drosophila suzukii* larvae. *J. Insect Physiol.* 96, 45–52. <https://doi.org/10.1016/j.jinsphys.2016.10.008>.
- Jenner, W.H., Kuhlmann, U., Cappuccino, N., Mason, P.G., 2010. Pre-release analysis of the overwintering capacity of a classical biological control agent supporting prediction of establishment. *BioControl* 55, 351–362. <https://doi.org/10.1007/s10526-009-9264-3>.
- Kenis, M., Tonina, L., Eschen, R., van der Sluis, B., Sancassani, M., Mori, N., Haye, T., Helsen, H., 2016. Non-crop plants used as hosts by *Drosophila suzukii* in Europe. *J. Pest. Sci.* 89, 735–748. <https://doi.org/10.1007/s10340-016-0755-6>.
- King, B.H., 1993. Sex ratio manipulation by parasitoid wasps. In: Wrensch, D.L., Ebbert, M.A. (Eds.), *Evolution and Diversity of Sex Ratio in Insects and Mites*. Chapman & Hall, New York, pp. 418–441.
- Kostál, V., 2006. Eco-physiological phases of insect diapause. *J. Insect Physiol.* <https://doi.org/10.1016/j.jinsphys.2005.09.008>.
- Kraaijeveld, A.R., van Alphen, J.J.M., 1995. Variation in diapause and sex ratio in the parasitoid *Asobara tabida*. *Entomol. Exp. Appl.* 74, 259–265. <https://doi.org/10.1111/j.1570-7458.1995.tb01899.x>.
- Kühnlein, R.P., 2012. Thematic review series: lipid droplet synthesis and metabolism: from yeast to man. Lipid droplet-based storage fat metabolism in *Drosophila*. *J. Lipid Res.* <https://doi.org/10.1194/jlr.R024299>.
- Leach, H., Stone, J., Van Timmeren, S., Isaacs, R., 2019. Stage-specific and seasonal induction of the overwintering morph of spotted wing *Drosophila* (Diptera: Drosophilidae). *J. Insect Sci.* 19. <https://doi.org/10.1093/jisesa/iez067>.
- Li, W., Li, J., Coudron, T.A., Lu, Z., Pan, W., Liu, X., Zhang, Q., 2008. Role of photoperiod and temperature in diapause induction of endoparasitoid wasp *Microplitis mediator* (Hymenoptera: Braconidae). *Entomol. Soc. Am* 101, 613–618.
- Lisi, F., Biondi, A., Cavallaro, C., Zappalà, L., Campo, G., Roversi, P.F., Sabbatini Peverieri, G., Giovannini, L., Tavella, L., Tortorici, F., Bardella, S., Carli, C., Bosio, G., Mori, N., Tonina, L., Zanini, G., Caruso, S., Vaccari, G., Masetti, A., Bittau, B., Bariselli, M., Schmidt, S., Falagiarra, M., Bertignono, L., Bonfanti, R., Giorgini, M., Guerrieri, E., Tropiano, F.G., Verrastro, V., Baser, N., Ibn Amor, A., Endrizzi, S., Tessari, L., Puppato, S., Ioriatti, C., Grassi, A., Anfora, G., Fellin, L., Rossi-Stacconi, M. V., 2022. Current status of *Drosophila suzukii* classical biological control in Italy, in: *Acta Horticulturae. Int Soc Hortic Sci*, pp. 193–200. doi: 10.17660/ActaHortic.2022.1354.25.
- Lisi, F., Biondi, A., Hogg, B.N., Rossi Stacconi, M.V., Wang, X., Daane, K.M., 2024. Cold storage of *Ganaspis kimorum* (Hymenoptera: Figitidae) immature stages for optimized classical biological control of *Drosophila suzukii* (Diptera: Drosophilidae). *J. Econ. Entomol.* 286. <https://doi.org/10.1093/ee/toae286>.
- Liu, Z., Huang, X., 2013. Lipid metabolism in *Drosophila*: development and disease. *Acta Biochim. Biophys. Sin. (Shanghai)*. <https://doi.org/10.1093/abbs/gms105>.

- Lovie, P., 2005. Coefficient of variation, in: Encyclopedia of Statistics in Behavioral Science. Wiley. Doi: 10.1002/0470013192.bsa107.
- Lyons, A., 2022. degday: compute degree days. CRAN: Contributed Packages. Doi: 10.32614/CRAN.package.degday.
- Melk, J.P., Govind, S., 1999. Development analysis of *Ganaspis xanthopoda*, a larval parasitoid of *Drosophila melanogaster*. J. Exp. Biol. 202, 1885–1896. <https://doi.org/10.1242/jeb.202.14.1885>.
- Milonas, P.G., Savopoulou-Soultani, M., 2000. Diapause induction and termination in the parasitoid *Colpoclypeus florus* (Hymenoptera: Eulophidae): role of photoperiod and temperature. Ann. Entomol. Soc. Am. 93 (3), 512–518. [https://doi.org/10.1603/0013-8746\(2000\)093\[0512:DIATIT\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2000)093[0512:DIATIT]2.0.CO;2).
- Murata, Y., Novković, B., Suwito, A., Kimura, M.T., 2013. Diapause and cold tolerance in Asian species of the parasitoid *Leptopilina* (Hymenoptera: Figitidae). Physiol. Entomol. 38, 211–218. <https://doi.org/10.1111/phen.12024>.
- Nedved, O., 2009. Temperature, effects on development and growth, in: Resh, V.H., Cardé, R.T. (Eds.), Encyclopedia of Insects (Second Edition). Academic Press, San Diego, pp. 990–993. <https://doi.org/10.1016/B978-0-12-374144-8.00261-7>.
- Pang, L., Fang, G., Liu, Z., Dong, Z., Chen, J., Feng, T., Zhang, Q., Sheng, Y., Lu, Y., Wang, Y., Zhang, Y., Li, G., Chen, X., Zhan, S., Huang, J., 2024. Coordinated molecular and ecological adaptations underlie a highly successful parasitoid. Elife 13. <https://doi.org/10.7554/ELIFE.94748>.
- Papaj, D.R., 2000. Ovarian dynamics and host use. Annu. Rev. Entomol. 45, 423–448. <https://doi.org/10.1146/annurev.ento.45.1.423>.
- Poirié, M., Colinet, D., Gatti, J.L., 2014. Insights into function and evolution of parasitoid wasp venoms. Curr. Opin. Insect Sci. 6, 52–60. <https://doi.org/10.1016/j.cois.2014.10.004>.
- R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.
- Rosenheim, J.A., Heimpel, G.E., Mangel, M., 2000. Egg maturation, egg resorption and the costliness of transient egg limitation in insects. Proc. R. Soc. Lond. B Biol. Sci. 267, 1565–1573. <https://doi.org/10.1098/rspb.2000.1179>.
- Rossi-Stacconi, M.V., Wang, X., Stout, A., Fellin, L., Daane, K.M., Biondi, A., Stahl, J.M., Buffington, M.L., Anfora, G., Hoelmer, K.A., 2022. Methods for rearing the parasitoid *Ganaspis brasiliensis*, a promising biological control agent for the invasive *Drosophila suzukii*. J. Vis. Exp. 184 doi: 10.3791/63898 e63898.
- Sario, S., Melo-Ferreira, J., Santos, C., 2023. Winter is (not) coming: is climate change helping *Drosophila suzukii* overwintering? Biology (Basel) 12, 907. <https://doi.org/10.3390/biology12070907>.
- Seehausen, M.L., Ris, N., Driss, L., Racca, A., Girod, P., Warot, S., Borowiec, N., Toševski, I., Kenis, M., 2020. Evidence for a cryptic parasitoid species reveals its suitability as a biological control agent. Sci. Rep. 10, 19096. <https://doi.org/10.1038/s41598-020-76180-5>.
- Seehausen, M.L., Valenti, R., Fontes, J., Meier, M., Marazzi, C., Mazzi, D., Kenis, M., 2022. Large-arena field cage releases of a candidate classical biological control agent for spotted wing drosophila suggest low risk to non-target species. J. Pest. Sci. 95, 1057–1065. <https://doi.org/10.1007/s10340-022-01487-3>.
- Seidl, S.E., King, B., 1993. Sex-ratio manipulation by the parasitoid wasp *Muscidifurax raptor* in response to host size. Evolution (n y) 47, 1876. <https://doi.org/10.2307/2410228>.
- Shearer, P.W., West, J.D., Walton, V.M., Brown, P.H., Svetec, N., Chiu, J.C., 2016. Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. BMC Ecol. 16, 11. <https://doi.org/10.1186/s12898-016-0070-3>.
- Sinclair, B.J., Marshall, K.E., 2018. The many roles of fats in overwintering insects. J. Exp. Biol. 221. <https://doi.org/10.1242/jeb.161836>.
- Small, C., Paddibhatla, I., Rajwani, R., Govind, S., 2012. An introduction to parasitic wasps of drosophila and the antiparasite immune response. J. Vis. Exp. 1. <https://doi.org/10.3791/3347>.
- Sosa-Calvo, J., Forshage, M., Buffington, M.L., 2024. Circumscription of the *Ganaspis brasiliensis* (Ihering, 1905) species complex (Hymenoptera, Figitidae), and the description of two new species parasitizing the spotted wing drosophila, *Drosophila suzukii* Matsumura, 1931 (Diptera, Drosophilidae). J. Hymenopt. Res. 97, 441–470. <https://doi.org/10.3897/jhr.97.118567>.
- Sridhar, V., Reddy, P.V.R., 2013. Use of degree days and plant phenology: a reliable tool for predicting insect pest activity under climate change conditions, in: Singh, H., Rao, N., Shivashankar, K. (Eds.), Climate-Resilient Horticulture: Adaptation and Mitigation Strategies. Springer India, India, pp. 287–294. Doi: 10.1007/978-81-322-0974-4_25.
- Stahl, J.M., Wang, X., Abram, P.K., Biondi, A., Buffington, M.L., Hoelmer, K.A., Kenis, M., Lisi, F., Rossi-Stacconi, M.V., Seehausen, M.L., Vossen, A., Wong, W.H.L., Daane, K.M., 2024. *Ganaspis kimorum* (Hymenoptera: Figitidae), a promising parasitoid for biological control of *Drosophila suzukii* (Diptera: Drosophilidae). J. Integr. Pest. Manag. 15, 44. <https://doi.org/10.1093/jipm/pmae036>.
- Stephens, A.R., Asplen, M.K., Hutchison, W.D., Venette, R.C., 2015. Cold hardiness of winter-acclimated *Drosophila suzukii* (Diptera: Drosophilidae) adults. Environ. Entomol. 44, 1619–1626. <https://doi.org/10.1093/ee/nvv134>.
- Stockton, D., Wallingford, A., Rendon, D., Fanning, P., Green, C.K., Diepenbrock, L., Ballman, E., Walton, V.M., Isaacs, R., Leach, H., Sial, A.A., Drummond, F., Burrack, H., Loeb, G.M., 2019. Interactions between biotic and abiotic factors affect survival in overwintering *Drosophila suzukii* (Diptera: Drosophilidae). Environ. Entomol. 48, 454–464. <https://doi.org/10.1093/ee/nvy192>.
- Tait, G., Cabianca, A., Grassi, A., Pfab, F., Oppedisano, T., Puppato, S., Mazzoni, V., Anfora, G., Walton, V.M., 2020. *Drosophila suzukii* daily dispersal between distinctly different habitats. Entomol. Gen. 40, 25–37. <https://doi.org/10.1127/entomologia/2019/0876>.
- Tait, G., Mermer, S., Stockton, D., Lee, J., Avosani, S., Abrieux, A., Anfora, G., Beers, E., Biondi, A., Burrack, H., Cha, D., Chiu, J.C., Choi, M.Y., Cloonan, K., Crava, C.M., Daane, K.M., Dalton, D.T., Diepenbrock, L., Fanning, P., Ganjisaffar, F., Gómez, M.I., Gut, L., Grassi, A., Hamby, K., Hoelmer, K.A., Ioriatti, C., Isaacs, R., Klick, J., Kraft, L., Loeb, G., Rossi-Stacconi, M.V., Nieri, R., Pfab, F., Puppato, S., Rendon, D., Renkema, J., Rodriguez-Saona, C., Rogers, M., Sassu, F., Schöneberg, T., Scott, M.J., Seagraves, M., Sial, A., Van Timmeren, S., Wallingford, A., Wang, X., Yeh, D.A., Zalom, F.G., Walton, V.M., 2021. *Drosophila suzukii* (Diptera: Drosophilidae): a decade of research towards a sustainable integrated pest management program. J. Econ. Entomol. 114, 1950–1974. <https://doi.org/10.1093/jeet/toab158>.
- Vieira, J.G.A., Krüger, A.P., Scheuneumann, T., Garcez, A.M., Moraes, M.C., Garcia, F.R.M., Nava, D.E., Bernardi, D., 2020. Effect of temperature on the development time and life-time fecundity of *Trichopria anastrephae* parasitizing *Drosophila suzukii*. J. Appl. Entomol. 144, 857–865. <https://doi.org/10.1111/jen.12799>.
- Wang, X., Biondi, A., Daane, K.M., 2020. Functional responses of three candidate asian larval parasitoids evaluated for classical biological control of *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 113, 73–80. <https://doi.org/10.1093/jeet/toz265>.
- Wang, X., Hogg, B.N., Hougardy, E., Nance, A.H., Daane, K.M., 2019. Potential competitive outcomes among three solitary larval endoparasitoids as candidate agents for classical biological control of *Drosophila suzukii*. Biol. Control 130, 18–26. <https://doi.org/10.1016/j.biocontrol.2018.12.003>.
- Wang, X., Nance, A.H., Jones, J.M.L., Hoelmer, K.A., Daane, K.M., 2018a. Aspects of the biology and reproductive strategy of two Asian larval parasitoids evaluated for classical biological control of *Drosophila suzukii*. Biol. Control 121, 58–65. <https://doi.org/10.1016/j.biocontrol.2018.02.010>.
- Wang, X., Serrato, M.A., Son, Y., Walton, V.M., Hogg, B.N., Daane, K.M., 2018b. Thermal performance of two indigenous pupal parasitoids attacking the invasive *Drosophila suzukii* (Diptera: Drosophilidae). Environ. Entomol. 47, 764–772. <https://doi.org/10.1093/ee/nvy053>.
- Winkler, A., Jung, J., Kleinhenz, B., Racca, P., 2021. Estimating temperature effects on *Drosophila suzukii* life cycle parameters. Agric. For. Entomol. 23, 361–377. <https://doi.org/10.1111/afe.12438>.
- Zerulla, F.N., Schmidt, S., Streitberger, M., Zebitz, C.P.W., Zelger, R., 2015. On the overwintering ability of *Drosophila suzukii* in South Tyrol. J. Berry Res. 5, 41–48. <https://doi.org/10.3233/JBR-150089>.
- Zhao, C., Guo, Y., Liu, Z., Xia, Y., Li, Y., Song, Z., Zhang, B., Li, D., 2021. Temperature and photoperiodic response of diapause induction in *Anastatus japonicus*, an egg parasitoid of stink bugs. Insects 12, 872. <https://doi.org/10.3390/insects12100872>.