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ORIGINAL PAPER

Development of *Drosophila suzukii* at low temperatures in mountain areas

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Abstract As a fly tracking the availability of fruits along climatic gradients, *Drosophila suzukii* is deemed to be rather flexible in relation to environmental factors, among which temperature is a major player. We sampled potential wild host fruits of *D. suzukii* along two elevational gradients in mountain areas of north-eastern Italy, in order to measure fly performance in relation to temperature. In addition, we used a strong natural temperature gradient in an open-top cave, covering the lower range of temperatures known for *D. suzukii*, to deploy laboratory stock colonies to mimic conditions existing along elevational gradients. At least nine wild host species yielded adults of *D. suzukii* in the mountain area (*Daphne mezereum, Lonicera alpigena, Lonicera caerulea, Lonicera nigra, Lonicera xylosteum, Rubus caesius, Rubus saxatilis, Sambucus nigra,* and *Sambucus racemosa*) when the daily average temperature in the three preceding weeks was at least 11.1 °C. Similar results were obtained with the laboratory colonies reared on an artificial medium in the cave, where oviposition and development from egg to adult occurred at above 11.6 °C. Both values are lower than previously recorded lower thresholds for development at both constant and fluctuating temperatures. These findings indicate that *D. suzukii* performs well at low temperatures, suggesting that population buildup may occur even under these conditions, with likely consequences on crops and wild host reproduction.

Keywords Spotted wing Drosophila · Performance · Fluctuating temperature · Host plant

Key message

- *Drosophila suzukii* is a specialized pest that is tracking availability of small fruits along elevation gradients in both native and introduced areas.
- Oviposition and successful development were observed when daily average temperature was higher than 11.1 °C, a value lower than previously known at both constant and fluctuating temperatures.
- *Drosophila suzukii* has a high potential to colonise mountain habitats where large populations may build up and cause damage to local crops.

Introduction

Temperature is one of the most important abiotic factors affecting the activity, performance, and geographic distribution of insects (Chown and Nicholson 2004; Angilletta 2009; Doucet et al. 2009). In temperate climates, therefore, temperatures seasonally drop below 0 °C, causing overwintering mortality (Williams et al. 2015), determining the life history, and limiting the performance and establishment of an invasive pest species (Doucet et al. 2009). In response, insects have evolved physiological and behavioural strategies to avoid extreme temperatures, such as diapause or migratory flights (Doucet et al. 2009).

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Climate change is dramatically affecting the temperature regime in the world (Field et al. 2014). The projected changes of air temperature for the entire Alpine region are positive in both time horizons and seasons. According to Gobiet et al. (2014), warming will be stronger in winter than in the rest of the year, with an increase of 1.6 °C in air temperature by 2050, with even higher rates of warming at higher elevations. Patterns indicate less precipitation and a decrease of relative humidity in summer, particularly south of the Alps, and potentially more precipitation in winter at the end of the twenty-first century. These increases of temperature, within the vital limits of a species, would allow the spreading of insects northward and upward. Faster development may cause an increase in population density, promoting a further expansion (Battisti 2008). In the Alps, mean air temperature decreases regularly as elevation increases, at a lapse rate of 0.56 °C every 100 m (Theurillat and Guisan 2001); this implies a shift of 200–250 m in elevation.

The spotted wing Drosophila *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), which has recently invaded Europe from Asia (Cini et al. 2014), tracks the availability of wild fruits along latitudinal and elevational gradients, and it is expected to be rather flexible in relation to environmental factors, among which temperature is a major player. In its native range of Japan, *D. suzukii* moves from low to high elevation during summer and then returns to favourable overwintering conditions in the autumn (Mitsui et al. 2010). *D. suzukii* is a relatively cold-intolerant and chill-susceptible species as 50 % of adult flies are killed following a 24-h exposure to temperatures just below 0 °C (Kimura 2004). Adults can also be killed by chronic exposure to 0 °C or by short exposure to chilling temperatures (Jakobs et al. 2015). The migration along elevational gradients has been explained as a mechanism to escape summer heat and to track available host fruits (Kimura 2004; Kinjo et al. 2014). In late summer or autumn, the flies return to low elevation where overwintering conditions are milder (Mitsui et al. 2010). Based on the first studies of Kanzawa (1939), the developmental periods from egg to adult vary between 9 and 25 days at the constant temperatures of 25 and 15 °C, respectively. Recent studies confirmed these results and indicated that development was also possible at temperatures as low as 10 °C on cherry under laboratory conditions (79.4 days), although oviposition was not observed at this temperature (Tochen et al. 2014). Asplen et al. (2015) estimated the lower threshold of development at 6.0 °C and created a temperature-dependent model where the development rate from egg to adult ranged from about 0.02 day^{-1} at 10 °C to 0.1 day^{-1} at 30 °C. All these studies provide laboratory-based indications or model estimation of the lower temperature threshold for development of *D. suzukii*; however, an empirical study under natural conditions is still missing.

As *D. suzukii* has recently invaded the Alps where it has reached pest status (Grassi et al. 2011), we were interested to explore how the insect responds to low temperatures that can be observed in the upper mountains during summer, where adult flies are commonly caught in traps. Thus, we sampled wild potential host fruits of *D. suzukii* along elevational gradients in a mountain area of northern Italy. We also tracked temperature conditions in the weeks before the sampling, in order to verify if data concerning temperature-related development available in the literature match with the exploitation of the hosts, and if there are hosts which cannot be exploited because of limiting temperature. In addition, we used a strong natural temperature gradient, in the lower range known for *D. suzukii*, to study fly performance at low temperatures on an artificial medium, by mimicking the conditions existing along the elevational gradients. We expected to find a temperature threshold for oviposition and development of *D. suzukii* under natural conditions and to assess the insect performance at low temperature. In particular, we wanted to find the lowest temperature allowing development and measure the related offspring yield. The results would help explain the success of the species on natural hosts and to clarify the adaptation potential of the species to host availability in a new invasion area, taking into account the possible effects of climate change.

Materials and methods

Insect collection

Two mountain elevational gradients where *D. suzukii* was known to occur, at least in the lower part, were selected in Trento (TN) and Verona (VR) districts, respectively, in north-eastern Italy. The gradients consisted of three sites for TN (elevation span 1640–1940 m) and of six sites for VR (elevation span 1100–1820 m) (Tables 1, 2) and were selected in order to mimic the average temperatures tested in the natural gradient experiment, located along the VR gradient (see below). The presence of the fly was assessed with a specific red trap (Drosotrap® Biobest) baited with the food lure Droskidrink (Prantil, 75 % apple cider vinegar, 25 % red vine, 20 g/l sugar) (Grassi et al. 2015; Ioriatti et al. 2015). Along these gradients, fruits of potential host plants were collected whenever available during the summers of 2014 and 2015. For each plant species, fruits were picked as soon as they were ripe, avoiding rotten or damaged fruits. In the laboratory, the precise number and the total weight were measured (Table 1 for 2014 and 2 for

Table 1 Wild fruits collected along elevational gradients in mountain areas, during the summer of 2014, which yielded adults of *Drosophila suzukii* in at least one sample

Species	Family	Site and gradient	Coordinates	Elevation (m)	Dominant landscape	Date	Average daily temperature 3 weeks before collection ± SD	Adult *D. suzukii*	Number of fruits	Fruit fresh weight (g)
Daphne mezereum	***Thymelaeaceae***	**Malga Porcarina (VR)[b]**	**45°40′18″N 11°5′19″E**	**1420**	**Pasture**	**Sep 1st**	**11.1 ± 2.0**	**4**	**91**	**32**
Daphne mezereum	*Thymelaeaceae*	Passo Rolle (TN)[e]	46°17′50″N 11°46′47″E	1940	Pasture	Aug 16th	10.7 ± 1.9	0	57	20
Lonicera alpigena	***Caprifoliaceae***	**Camposilvano (VR)**	**45°37′52″N 11°5′19″E**	**1230**	**Beech forest**	**Aug 18th**	**14.1 ± 2.3**	**60**	**120**	**109**
Lonicera alpigena	***Caprifoliaceae***	**Camposilvano (VR)**	**45°37′52″N 11°5′19″E**	**1230**	**Beech forest**	**Sep 8th**	**13.0 ± 1.4**	**10**	**50**	**45**
Lonicera alpigena	***Caprifoliaceae***	**Malga Porcarina (VR)[b]**	**45°40′18″N 11°5′19″E**	**1420**	**Pasture**	**Aug 18th**	**13.2 ± 2.6**	**15**	**108**	**84**
Lonicera alpigena	*Caprifoliaceae*	Passo Malera (VR)[c]	45°41′31″N 11°6′30″E	1820	Pasture	Sep 1st	10.0 ± 1.8	0	40	23
Lonicera nigra	***Caprifoliaceae***	**Malga Prato di sotto (TN)[d]**	**46°7′20″N 10°52′49″E**	**1660**	**Pasture**	**Aug 19th**	**12.9 ± 2.4**	**17**	**93**	**38**
Lonicera nigra	*Caprifoliaceae*	Passo Malera (VR)[c]	45°41′31″N 11°6′30″E	1820	Pasture	Sep 1st	10.0 ± 1.8	0	10	2
Lonicera xylosteum	*Caprifoliaceae*	Camposilvano (VR)[a]	45°37′52″N 11°5′19″E	1230	Beech forest	Aug 4th	15.5 ± 1.6	0	212	62
Lonicera xylosteum	***Caprifoliaceae***	**Camposilvano (VR)**	**45°37′52″N 11°5′19″E**	**1230**	**Beech forest**	**Aug 18th**	**14.1 ± 2.3**	**38**	**160**	**37**
Sambucus nigra	***Adoxaceae***	**Malga Porcarina (VR)[b]**	**45°40′18″N 11°5′19″E**	**1420**	**Pasture**	**Sep 1st**	**11.1 ± 2.0**	**8**	**250**	**44**
Sambucus racemosa	***Adoxaceae***	**Malga Porcarina (VR)[b]**	**45°40′18″N 11°5′19″E**	**1420**	**Pasture**	**Aug 18th**	**13.2 ± 2.6**	**96**	**600**	**101**
Sambucus racemosa	*Adoxaceae*	Passo Malera (VR)[c]	45°41′31″N 11°6′30″E	1820	Pasture	Sep 1st	10.0 ± 1.8	0	40	3

Plant species are listed alphabetically and according to increasing elevation within each species. Samples that yielded *D. suzukii* are indicated in bold. Plant species that never produced adults are reported in the supplementary material (Table S1). The weather stations are indicated with the site: [a] Pian delle Fugazze 1170 m; [b] Barricata di Grigno 1345 m; [c] Malga Casapinello 1710 m; [d] Viotte, Monte Bondone 1552 m; [e] Malga Prato di sopra, Val d'Ambiez 1880 m. If not specified, an on-site data logger was used

Table 2 Wild fruits collected along elevational gradients in mountain areas, during the summer of 2015, which yielded adults of *Drosophila suzukii* in at least one sample

Species	Family	Site and gradient	Coordinates	Elevation (m)	Dominant landscape	Date	Average daily temperature 3 weeks before collection ± SD	Adult *D. suzukii*	Number of fruits	Fruit fresh weight (g)
Daphne mezereum	*Thymelaeaceae*	Camposilvano (VR)[a]	45°37′52″N 11°5′19″E	1230	Beech forest	Jul 20th	18.8 ± 1.8	0	140	40
Lonicera alpigena	*Caprifoliaceae*	Camposilvano (VR)[a]	45°37′52″N 11°5′19″E	1230	Beech forest	Jul 20th	18.8 ± 1.8	0	25	9
Lonicera alpigena	*Caprifoliaceae*	Camposilvano (VR)[a]	45°37′52″N 11°5′19″E	1230	Beech forest	Aug 4th	18.0 ± 2.6	0	12	10
Lonicera alpigena	*Caprifoliaceae*	Malga Porcarina (VR)	45°40′18″N 11°5′19″E	1420	Pasture	Aug 4th	15.5 ± 2.0	0	59	31
Lonicera alpigena	***Caprifoliaceae***	**Malga Porcarina (VR)**	**45°40′18″N 11°5′19″E**	**1420**	**Pasture**	**Aug 31st**	**14.9 ± 2.9**	**6**	**70**	**49**
Lonicera alpigena	***Caprifoliaceae***	**Malga Prato di sotto (TN)[d]**	**46°7′20″N 10°52′49″E**	**1600**	**Pasture**	**Aug 15th**	**16.3 ± 2.2**	**22**	**37**	**21**
Lonicera caerulea	***Caprifoliaceae***	**Malga Ben (TN)[e]**	**46°07′39″N 10°53′20″E**	**1880**	**Pasture**	**Aug 15th**	**13.8 ± 2.2**	**4**	**34**	**2**
Lonicera nigra	***Caprifoliaceae***	**Malga Prato di sotto (TN)[d]**	**46°7′20″N 10°52′49″E**	**1600**	**Pasture**	**Aug 15th**	**16.3 ± 2.2**	**4**	**28**	**6**
Lonicera nigra	***Caprifoliaceae***	**Malga Ben (TN)[e]**	**46°07′39″N 10°53′20″E**	**1880**	**Pasture**	**Aug 15th**	**13.8 ± 2.2**	**22**	**42**	**12**
Rubus caesius	***Rosaceae***	**Camposilvano (VR)[a]**	**45°37′52″N 11°5′19″E**	**1230**	**Beech forest**	**Jul 06th**	**14.6 ± 3.2**	**8**	**30**	**15**
Rubus saxatilis	***Rosaceae***	**Malga Prato di sotto (TN)[d]**	**46°7′20″N 10°52′49″E**	**1600**	**Pasture**	**Aug 15th**	**16.3 ± 2.2**	**16**	**150**	**17**
Sambucus racemosa	***Adoxaceae***	**Bosco Chiesanuova (VR)**	**45°38′01″N 11°01′48″E**	**1100**	**Pasture**	**Jul 12th**	**16.3 ± 3.0**	**62**	**400**	**62**
Sambucus racemosa	***Adoxaceae***	**Malga S. Giorgio (VR)[b]**	**45°41′04″N 11°05′02″E**	**1400**	**Pasture**	**Jul 25th**	**17.8 ± 1.8**	**340**	**2000**	**276**
Sambucus racemosa	***Adoxaceae***	**Malga Porcarina (VR)[b]**	**45°40′18″N 11°5′19″E**	**1420**	**Pasture**	**Jul 20th**	**17.5 ± 1.9**	**76**	**2000**	**204**
Sambucus racemosa	***Adoxaceae***	**Malga Porcarina (VR)[b]**	**45°40′18″N 11°5′19″E**	**1420**	**Pasture**	**Jul 27th**	**17.4 ± 1.8**	**37**	**500**	**84**
Sambucus racemosa	***Adoxaceae***	**Passo del Branchetto (VR)[c]**	**45°40′54″N 11°04′10″E**	**1580**	**Pasture**	**Aug 4th**	15.5 ± 2.6	**56**	**1300**	**260**

Plant species are listed alphabetically and according to increasing elevation within each species. Samples that yielded *D. suzukii* are indicated in bold. Plant species that never produced adults are reported in the supplementary material (Table S1). The weather stations are indicated with the site: [a] Pian delle Fugazze 1170 m; [b]Barricata di Grigno 1345 m; [c] Malga Casapinello 1710 m; [d] Viotte, Monte Bondone 1552 m; [e] Malga Prato di sopra, Val d'Ambiez 1880 m. If not specified, an on-site data logger was used

2015). The samples were put in emergence containers (jars with 0.1 mm mesh net) and checked daily. The emerged insects were stored in small tubes with 95 % ethanol. The insects in each vial were counted: dividing *D. suzukii* males and females, other *Drosophila* spp., and parasitoids. For each site along a gradient, a weather station (www.meteotrentino.it) was identified nearby or data-loggers were deployed on site, at 2 m from the ground, to record hourly temperature (HOBO U23 ProV2 with RS1 solar radiation shield, Onset Computer Corporation, Bourne, MA, USA). The average daily temperature 3 weeks before the collection was calculated (Tables 1, 2).

Insect rearing

The *D. suzukii* population used for the experiment was obtained from the laboratory of the author's department of the University of Padua and originated from adults collected from cherry, blueberry, and grape in the Verona district. The flies were reared in plastic vials (Falcon type with 50 ml capacity, diameter 30 mm, length 115 mm) with specific medium for *D. suzukii* rearing (modified by Fondazione Edmund Mach from Bloomington Drosophila Stock Center (Indiana University 2014)). The medium contained raw cornmeal 75 g, dry-yeast 17 g, sucrose 15 g, soybean meal 12 g, agar 5.6 g, propionic acid 5 ml, and water 1000 ml. All components were mixed and heated for 20 min at about 100 °C, with the exception of propionic acid that was added at a temperature lower than 50 °C, just before pouring 15 ml of medium inside the plastic vials. The insect rearing, and the laboratory reference trials, was conducted at the constant temperature of 23.3 °C, 70–80 % relative humidity, and under a photoperiod of 16L:8D in a climatic room. The same type of vials and medium were used to perform the natural gradient experiments.

Cave description

The experiment was conducted in a large, open-top cave well known for its strong temperature gradient (Benetti and Cristoferi 1968), called Covolo di Camposilvano (Velo Veronese, 1220 m, 45°37′34″N, 11°05′34″E) during August and September 2014. The cave had previously been used to study the relationship between temperature and development in the sawfly *Cephalcia arvensis* (Battisti 1994). The choice of the site was motivated by the occurrence of a strong gradient of naturally fluctuating temperatures. Seven sites were chosen, from the bottom of the cave (site 1) to the top (site 7) (Fig. 1, supplementary material Fig. S1). During the study period, sites 1–4 were characterized by differences among sites of 2–3 °C in the average daily temperature, while sites 5, 6, and 7 presented similar average daily temperature but differed in daily temperature variation (supplementary material Fig. S2). All sites had the same natural photoperiod of the season (12–13 h of light). The rearing vials were located close to the ground and protected by a shelter, ensuring the same light intensity but offering rainfall and animal exclusion. The shelter was made of a vertical cylinder of plastic net (diameter 25 cm, square mesh 5 mm × 5 mm) covered by a plastic plate as a roof. Each shelter was sitting on the ground and protected on the sides by stones. The vials were hung on the shelter roof and thus about 10 cm from the ground (supplementary material Fig. S3). The temperature inside each shelter was measured with HOBO data-loggers (previously described) hung close to the vials. Seven data-loggers were used during the experiment in the cave and another one was placed in the laboratory (23.3 ± 1.4 °C). Data were recorded every hour and downloaded twice during the experiment.

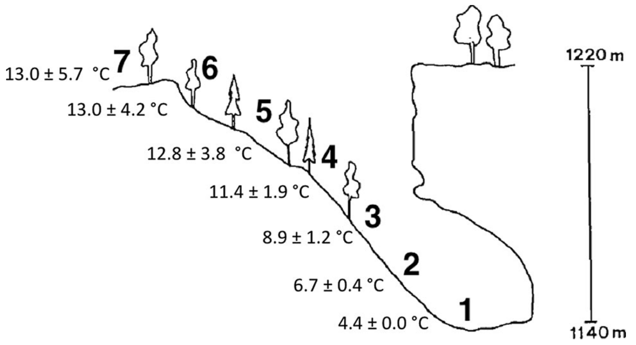


Fig. 1 Cross section of the open-top cave used for the experiment. The *numbers* mark the position of the 7 different sites, with values of the average daily temperature and the average daily temperature variation (maximum–minimum) throughout the period of the experiment (August 4th to September 20th 2014). The numbers on the *right* indicate the elevation in metres above sea level

Natural gradient experiment

Four trials were implemented to test the effect of temperature on both oviposition and development of *D. suzukii* in the cave. Together, these trials aimed to assess a lower temperature threshold for fly development and to test for the capacity of acclimatization within and among generations. Different developmental stages and durations of acclimatization (permanence in the cave sites) were used to quantify possible variations in the response to the lower thermal threshold. In three out of four trials, individuals from constant laboratory conditions (23.3 ± 1.4 °C) were moved to each of the seven sites in the cave. Specifically, larvae were used in Trial 1, while adults were used in Trial 2 for the first 2 days, and in Trial 3 for the remaining 3–14 days of their permanence in the site. The 4th trial was intended to test the effect of temperature on the individuals originating in the cave sites, using the adults developed from the other trials.

The four trials were implemented as follows:

Trial 1 A total of 5 females and 5 males (both 7 days old) from the laboratory rearing culturing (23.3 °C) were placed in individual vials with medium, allowed to mate and oviposit for 5 days in the laboratory before being removed. No mortality was observed during this period. These vials were then checked for the presence of the larvae and brought to the cave on August 6th 2014. Two vials were placed at each of the seven sites, while three vials were kept in the laboratory as a control. The vials were left on site for 32 days and checked biweekly until September 8th, when the insects were either emerged or dead, while the laboratory vials were recorded for 12 days (until August 18th).

Trials 2 and 3 A total of 8 flies (4 female and 4 male) from the laboratory rearing culture were placed in each vial in the laboratory and immediately transferred in a cold box (about 5 °C) to the cave, where three vials were deployed at each site. For these trials, 6-day-old flies were used, in order to have a constant egg-laying rate (Kinjo et al. 2014). For Trial 2, insects were left in the vials for two days, from August 4th to August 6th, then removed and checked biweekly until September 8th (32 days). The same design was used with three additional vials kept in the laboratory for 15 days (until August 21st). For Trial 3, insects that survived the first 48 h in Trial 2 were transferred to new vials, in the number of three vials per site. Insects were left in the vials for 12 days from August 6th to August 18th, when the adults were removed and the vials were checked biweekly until September 8th (21 days). For each trial, the adult mortality was assessed when they were removed from the vials.

Trial 4 New adults emerging from Trials 1, 2, and 3 were removed in two dates (September 1st and 8th) and inserted into new vials with fresh medium. The number of flies and vials used differed among sites. At sites 1–3, no adults were produced, and therefore Trial 4 was not carried out. From sites 4 to 7, a number from 2 to 5 females and from 1 to 5 males for each vial were used, depending on the number of flies emerged at each site/date. In order to assess the effect of the adult age on the offspring yield, when possible adults were removed from the vials after 7 days and transferred into new vials for other 12 days. Due to a sudden temperature drop on September 20th, the vials were taken to the laboratory under a constant temperature of 23.3 °C to follow the development into pupae and adults, with regular checks until October 9th. This trial was also used to verify a possible effect of the time from emergence on the capacity of the flies to generate offspring at low temperatures.

The presence and the number of pupae and adults were assessed in general twice a week. Unfortunately, it was not possible to detect precisely the number of eggs and larvae, although it was possible to detect whether they were present. Dead adults were always left in the vials. The presence of moulds was also annotated at each check and the relevant vials discarded from further analysis. At the end of each experiment (September 8th for Trials 1, 2, and 3, and October 9th for Trial 4), the content of each vial was checked, counting adults (male and female), pupae, and larvae present in the medium. Moreover, the quality of the medium was visually checked, based on its colour/texture, to assess whether development could have been negatively affected.

Data analyses and statistics

All temperature data were referred to as the average daily temperature of each site, calculated using hourly temperature data. The standard deviation (SD) of average daily temperature was also calculated for the period of interest in each site. In addition, degree-days were calculated on an hourly basis using the lower thermal threshold of 5.975 °C as suggested by Asplen et al. (2015). The biweekly checks allowed the calculation of the development rate for pupae and adults. The development rates (r_p for pupae and r_a for adults) were calculated as the reciprocal of days elapsed since the beginning of oviposition to the detection of the first pupa or adult.

$$\begin{aligned}&\text{Development rates } \left(r_p \text{or } r_a\right)\\&\quad = (\text{days from oviposition to first pupa or adult})^{-1}\end{aligned}$$

The counts of all stages of development of insects in the vials at the end of the trials were necessary to assess the yield. The yield was related to the individual female fly per day of oviposition. The number of female flies was considered as the average between the number of females at the beginning of the trial and the number of females alive at the end of the trial, assuming that the mortality rate was constant over time.

We considered the following indices:

- immature yield, as the number of larvae and pupae developed per female per day of oviposition (only for Trial 4),
- pupal yield, as the number of new pupae developed per female per day of oviposition, and
- adult yield, as the number of new adults developed per female per day of oviposition.

The data obtained for each vial were then used for calculating the average and the standard error (SE) at each site.

Statistical analyses were carried out using SPSS version 21.0 for Windows. In consideration of the low number of replicates, the results of Trial 1 were represented with a descriptive statistic only. Conversely, to find the lower temperature threshold for the development for Trials 1, 2, and 3, averages were compared using ANOVA and Dunnett's post hoc test was applied to identify differences from the stock maintained under laboratory conditions (significance level set at $P < 0.05$). Since this test sizably reduces the number of multiple comparisons, it provides a reliable estimation of the difference among means also in the cases of low number of replicates and an unbalanced dataset. To test for the effect of time from the emergence on the capacity to generate offspring at low temperatures (Trial 4), the number of larvae and pupae developed at sites 5–7 was pooled in two separate groups in accordance with the first and the second week of oviposition. Differences between averages of these two groups were tested using the *t* test for paired samples.

Results

Development in wild host fruits

Among 25 potential host fruits encountered along the gradients, a total of 9 (Tables 1, 2) resulted in being suitable for the development of *D. suzukii*. The successful development of the fly depended on the site and the date of collection, and was mainly related to temperatures in the period prior to sampling.

In 2014 (Table 1), *Daphne mezereum* yielded adults at 1420 m (11.1 °C) but not at 1940 m (10.7 °C). *Lonicera alpigena* yielded adults at 1230 m (two collection dates) and 1420 m, with temperatures higher than 13 °C, but not at 1820 m (about 10 °C). The number of flies emerged from 1 g of fruit was higher at 14 °C than at 13 °C (0.5 and 0.14–0.20 adults/g, respectively). *Lonicera nigra* yielded adults at 1660 m (12.9 °C) but not at 1820 (10.0 °C). *Lonicera xylosteum* did not yield adults in early August (August 4th) but it did later in the month (August 18th), despite temperatures being higher at the beginning of the month (above 14 °C). *Sambucus nigra* collected at 1420 m in early September (11.1 °C) yielded adults as well as *Sambucus racemosa* collected earlier at the same site (13.2 °C). The latter, however, did not yield adults at 1820 m (10.0 °C).

In 2015 (Table 2), *Daphne mezereum* did not yield adults at low elevation (1230 m, 18.8 °C). Also *Lonicera alpigena* did not yield adults at 1230 m (July 20th), while adults were obtained at the same site from *Rubus caesius*. At 1420 m, adults emerged only from samples collected at the end of August (14.9 °C). At 1600 m, adults emerged during mid-August (16.3 °C). From the sample of *Lonicera caerulea* collected at high elevation (1880 m, 13.8 °C), adults were obtained in abundance (2 adults/g of fruits). *Lonicera nigra* yielded adults at both 1600 m (16.3 °C) and 1880 m (13.8 °C), with higher yield at higher elevation (0.67 vs. 1.83 adults/g of fruits). *Rubus saxatilis* also yielded adults at 1600 m (16.3 °C). *Sambucus racemosa* yielded adults at all collection sites (ranging from 1100 to 1580 m) and dates (July 12th to August 4th). The yield ranged from 0.21 adults/g at lower temperature to 1.23 adults/g at higher temperature.

Natural gradient experiment

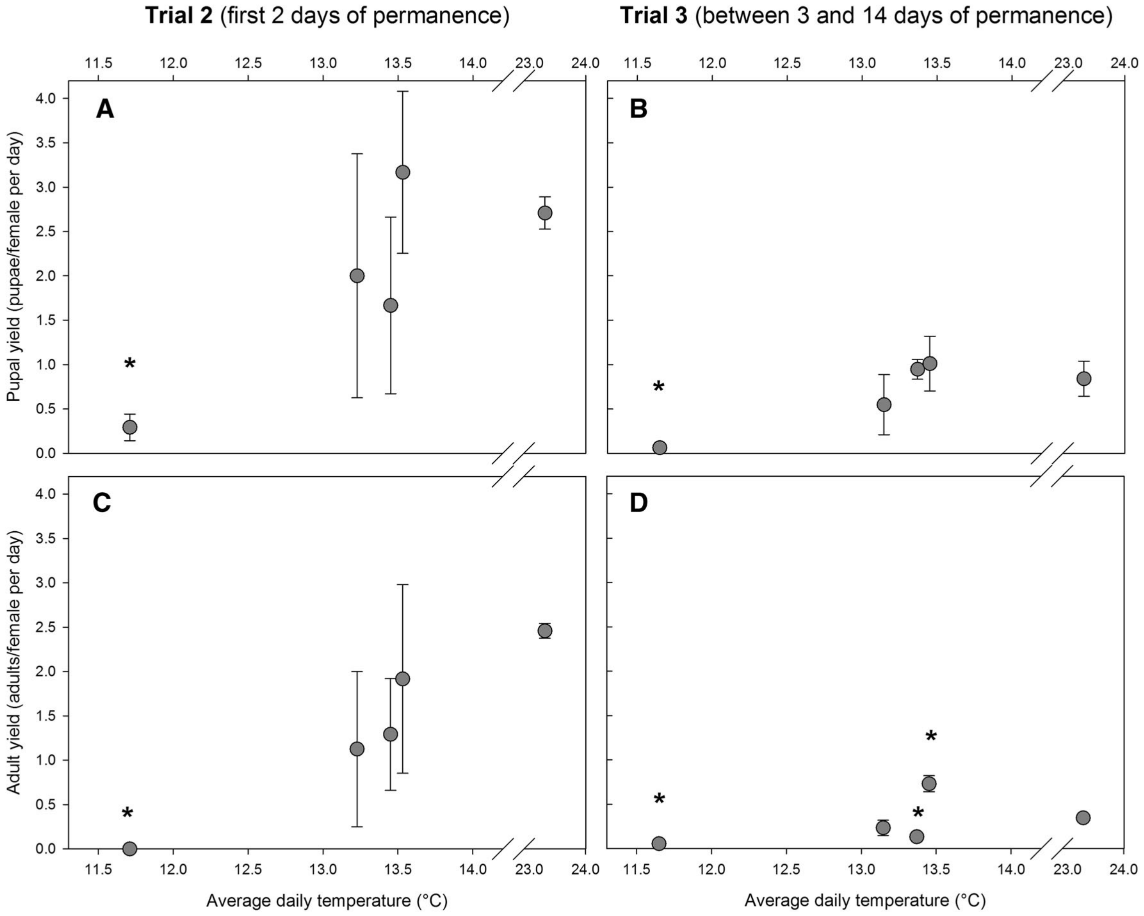
The results of Trial 1 (Table 3) show that the larvae produced in the laboratory developed into pupae at all temperature conditions, even at the lowest site with an average daily temperature of only 4.4 °C. Below 9 °C, however, the yield of pupae was very low, with less than 0.3 pupae/female/day, the remaining larvae were dead. Above 9 °C, the yield increased to values higher than 0.5, although with high variability in individual response (as standard error). With temperatures above 13.3 °C, the yield reached values higher than 1 pupae/female/day, with low variability (1.25 and 1.13 for sites 6 and 7, respectively). In the laboratory (23.3 °C) the yield was 0.8 pupae/female/day. Adults were obtained only at site 4 and above, characterized by temperatures higher than 10.5 °C. The yields were around 0.5 adults/female/day of oviposition, with the exception of site 5 which yielded less (0.2 adults/female/day). In the laboratory (23.3 °C), the adult yield was lower than at the most productive site of the cave, amounting to 0.3 adults/female/day.

The results of Trials 2 and 3 indicate that there was no development of pupae from eggs laid on site when the

Table 3 Temperature (±SD) and the number of pupae and adults developed in Trial 1 per female and per day of the oviposition period

Site	Temperature (°C)	Pupal yield	Adult yield
1	4.4 ± 0.1	0.00–0.28	0.00–0.00
2	6.7 ± 0.2	0.13–0.30	0.00–0.00
3	9.1 ± 0.3	0.47–0.87	0.00–0.00
4	11.6 ± 0.9	0.07–1.02	0.07–0.90
5	13.1 ± 1.7	0.40–0.65	0.20–0.20
6	13.4 ± 1.7	1.20–1.30	0.52–0.62
7	13.4 ± 1.9	1.10–1.17	0.48–0.55
Laboratory	23.3 ± 0.4	0.83 ± 0.12	0.30 ± 0.02

The two values indicate the results of the two vials used at each site, with 10 females in each. The laboratory results are the average (±SE) of three vials with 15 females in each. Hatching and first larval development was in the laboratory. Subsequent larval development and pupae and adult emergence was observed in the cave temperature was below 10 °C. The first pupae appeared at 11.7 ± 1.0 °C in Trial 2 (0.29 pupae/female/day; Fig. 2 panel A) and at 11.6 ± 0.9 °C in Trial 3 with a very low yield (0.06 pupae/female/day; Fig. 2 panel B). Both rate of development and yield increased with temperature, with yields of 2.00, 1.67, and 3.17 pupae/female/day for Trial 2 (Fig. 2 panel A) and 0.55, 0.95, and 1.01 pupae/female/day for Trial 3 (Fig. 2 panel B) for the average temperature of 13.2, 13.4, and 13.5 °C, respectively. Pupal development rate (r_p) increased to 0.071–0.075 days^{-1} at 13.2–13.5 °C for Trial 2 (Fig. 3 panel A) and 0.057–0.075 days^{-1} at 13.2–13.5 °C for Trial 3, respectively, (Fig. 3 panel B). In the laboratory, however, an increase was observed for both yield (2.7 and 0.8 pupae/female/day for Trial 2 and Trial 3, respectively; Fig. 2 panels A and B) and growth rate ($r_p = 0.097$ days^{-1}; Fig. 3 panels A and B). The value for pupal yield reached in the laboratory statistically deviated from the site 4 (11.6 °C) for both trials (Dunnett's test following one-way ANOVA; $F_{7,22} = 5.1$ for Trial 2 and $F_{7,23} = 7.4$ for Trial 3, $P < 0.05$). The laboratory pupal development rate for Trial 2 was significantly higher only in comparison to that observed at the lowest temperature, while for Trial 3 it was always higher, with the exception of the one observed at 13.15 °C (Dunnett's test following one-way ANOVA; $F_{4,12} = 21.4$ for Trial 2 and 10.7 for Trial 3). Adults emerged at temperatures higher than 12 and 11.6 °C for Trials 2 and 3, respectively. In Trial 2, at temperatures around 13 °C the yield in adults increased at values higher than 1 adult/female/day (Fig. 2 panel C) and the development rate reached the values of 0.03–0.04 days^{-1} (Fig. 3 panel C). In Trial 3, the yield in adults slightly increased (0.23, 0.13, and 0.73 for the temperatures of 13.2, 13.4, and 13.5 °C, respectively; Fig. 2 panel D), while the speed of development increased


Trial 2 (first 2 days of permanence)

Trial 3 (between 3 and 14 days of permanence)

Fig. 2 Pupal (**a**, **b**) and adult (**c**, **d**) yields of *Drosophila suzukii* at different temperatures. To test for a potential acclimation, individuals that laid eggs within the first 2 days of permanence (Trial 2, panels **a** and **c**) were compared with those that remained in the cave sites between 3 and 14 days (Trial 3, panels **b** and **d**). Yields under natural conditions (at the *left sides* of the *x*-axis break) were compared with those at constant laboratory condition of 23.3 °C (at the *right sides* of the *x*-axis break). Data below the 11.3 °C were omitted because no oviposition or development was recorded at lower temperatures. Asterisks denote values that are significantly different from the laboratory controls (Dunnett's test following one-way ANOVA; $P < 0.05$). Data are average ± SE. For further explanation see "Materials and methods" section

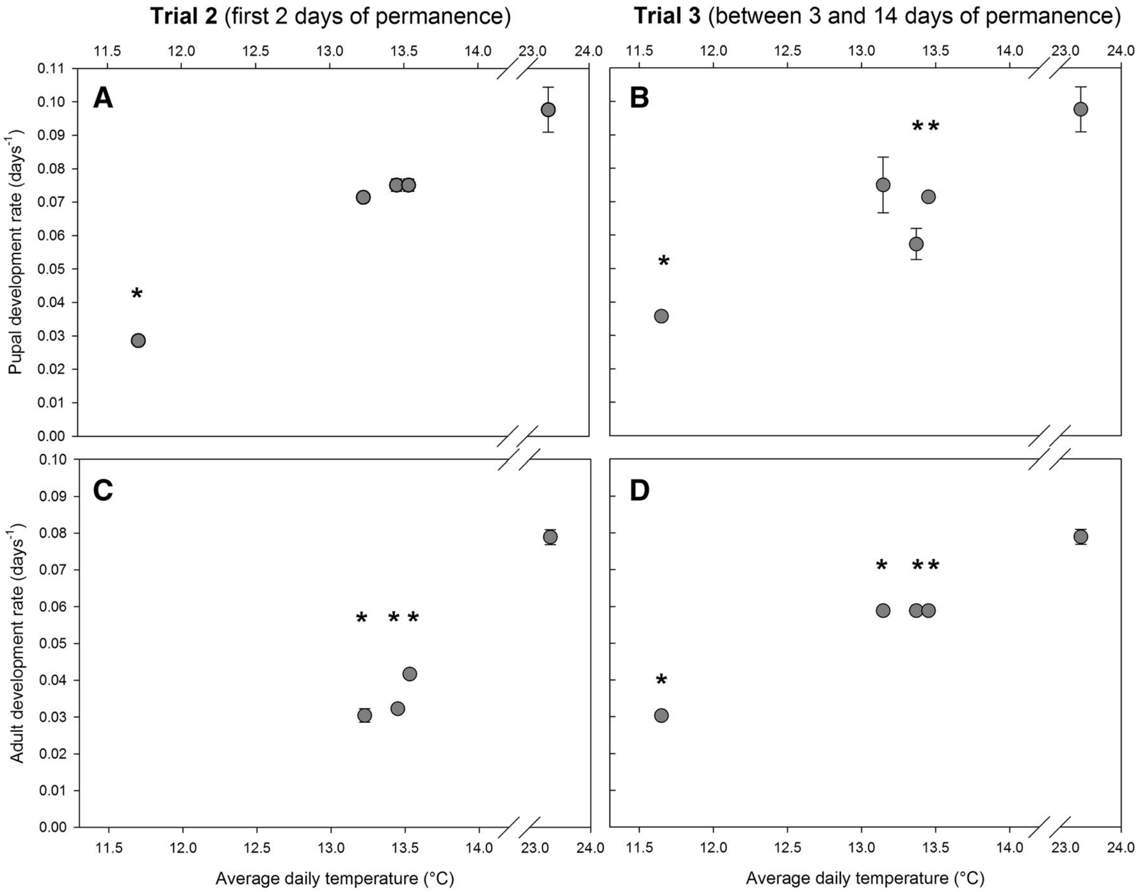


Fig. 3 Development rates from egg to pupa (**a**, **b**) and to adult (**c**, **d**) of *Drosophila suzukii* at different temperatures. To test for a potential acclimation, individuals that laid eggs within the first 2 days of permanence (Trial 2, panels **a** and **c**) were compared with those that remained in the cave site between 3 and 14 days (Trial 3, panels **b** and **d**). Rates under natural conditions (at the *left sides* of the *x*-axis break) were compared with those at constant laboratory condition of 23.3 °C (at the *right sides* of the *x*-axis break). Data below the 11.3 °C were omitted because no oviposition or development was recorded at lower temperatures. Asterisks denote values that are significantly different from the laboratory controls (Dunnett's test following one-way ANOVA; $P < 0.05$). Data are average ± SE. For further explanation see "Materials and methods" section

greatly ($r_a = 0.06$ days^{-1} for the three sites; Fig. 3 panel D). In the laboratory, the development rate was much higher compared to the cave for Trials 2 and 3 ($r_a = 0.079$ days^{-1}; Fig. 3 panel C) with statistical differences with all sites (Dunnett's test following one-way ANOVA; $F_{3,10} = 246.0$ for Trial 2 and $F_{4,12} = 323.0$ for Trial 3). For Trial 2, the yield in the laboratory setting was significantly higher only in site 4 at 11.6 °C (Dunnett's test following one-way ANOVA; $F_{7,22} = 4.1$), while for Trial 3 the yield in the laboratory was similar only to that of site 5 at 13.1 °C (Dunnett's test following one-way ANOVA; $F_{7,23} = 27.6$). The number of degree-days required for the development from egg to adult was between 185 and 237 for Trial 2 and between 125 and 187 for Trial 3, while in the laboratory it was 220.

The results of Trial 4 indicated that at the coldest site where adults were produced from the other trials, they did not generate any progeny (larvae and pupae) both in the first 7 days (average daily temperature 11.6 °C) and in the next 12 days (average daily temperature 10.4 °C). Adults from Trial 1 laid eggs which later developed into new adults both in the first 7 days (average daily temperature 13.0 °C) and in the next 12 days (average daily temperature 11.6 °C) in the sites 5, 6, and 7, with a yield ranging from 0.35 to 0.70 young/female/day. Although in site 4 oviposition did not occur during the first week at temperatures lower than 13 °C, in the upper sites the capacity of developing offspring was maintained after the drop of temperature from 13.2 (adults from Trial 1 at the first week from the emergence) to 11.6 °C (adults from Trial 1 at the second week from the emergence), with no significant differences in the yield (*t* test, $P > 0.05$). Adults from Trials 2 and 3 were tested only at sites 5, 6, and 7 and they were not able to generate progeny, with the exception of 0.02 immature/female/day at site 5 (average temperature of 11.6 °C) and 0.29 immature/female/day at site 7 (second period, average temperature of 11.5 °C).

Discussion

Both experimental results and field surveys conducted in the invasion range of *D. suzukii* in the Alps indicate that the spotted wing Drosophila is a species well adapted to cold conditions and ample fluctuating temperatures typical of mountains, where it presented relatively short development cycles and good reproductive performance. In addition, it invaded progressively the upper elevations during the summer, as shown in its native range in Japan (Mitsui et al. 2010). In Japan, a number of *Drosophila* species specializing on flowers and fruits move from low to higher elevations during summer, some as early as June (*D. unipectinata*) while others (*D. oshimai*, *D. suzukii*, and *D. subpulchrella*) in July. Migration of *D. unipectinata* is considered as a means for avoiding summer heat or exploiting early-summer resources at a higher elevation. On the other hand, *D. oshimai*, *D. suzukii*, and *D. subpulchrella* have the capacity to spend the summer at low elevation, and therefore their migration is assumed to be a means to escape from resource-poor conditions in summer at low elevations or simply to exploit resources at a higher elevation. Other generalist species of *Drosophila*, i.e. those associated with accidentally fallen immature fruits and/or decayed leaves, however, would not perform such extensive movements between low and higher elevations (Mitsui et al. 2010).

The uncertainty associated with the migration behaviour of *D. suzukii* seems to be rather high, as in mountain areas the temperature often drops below 0 °C even during the favourable season, and in general during winter (Williams et al. 2015). Although insects are able to adapt their behaviour, drops in temperature cause first the suspension of reproduction and development, followed by a chilling coma and death (Chown and Nicholson 2004). The escape from adverse conditions through behavioural strategies such as migratory flights (Doucet et al. 2009) is a risky process, which may have important consequences for the population growth as the insects have to find hosts in a stage suitable for oviposition. In turn, it may expose invaded ecosystems as well as crop systems to a sudden herbivore pressure, implying both economic and ecological costs as long as fruit harvest and regeneration potential of wild plants are affected, respectively.

Nine plant species were suitable for the development of *D. suzukii* in the mountain areas (*Daphne mezereum, Lonicera alpigena, Lonicera caerulea, Lonicera nigra, Lonicera xylosteum, Rubus caesius, Rubus saxatilis, Sambucus nigra,* and *Sambucus racemosa*). With the exception of *Daphne mezereum, Rubus caesius*, and *Rubus saxatilis*, these species were already known as hosts of *D. suzukii*, although not in situations of limiting temperature (Grassi et al. 2011; Lee et al. 2015). The successful development of the fly was obtained only when the average temperature in the 3-week period before the collection was higher than 11.1 °C, as observed in 2014, which was a summer with temperatures much lower than average (ARPAV 2014). On that year, it was also possible to show that performance on the same host (*Lonicera alpigena*) and site increased with temperature, as more flies per unit sample were obtained at 14 °C than at 13 °C. In 2015, temperatures in the 3-week period preceding the collection were higher than those observed in 2014, nevertheless performance on *Sambucus racemosa* also increased from 0.21 adults/g at 15.5 °C to 1.23 adults/g at 17.8 °C. The higher temperatures recorded in 2015 caused an increase in the elevation where successful development of *D. suzukii* was observed, a shift from 1660 to 1880 m. This can be taken as an indicator of how quickly the insect may respond to a temperature shift, likely based on the intense migratory activity, suggesting that the response of *D. suzukii* to climate change (Field et al. 2014) will likely be positive, although strongly dependent on the annual variation of weather (Battisti and Larsson 2015).

The natural gradient experiment in the cave generally supported the field observations using a laboratory population reared on an artificial medium. There were, however, a number of issues that deserve a thorough analysis. First, the larvae carried from the laboratory developed into pupae at all temperature conditions tested. The formation of pupae even at the lower temperature was likely influenced by the advanced stage of the larvae (third instar) taken to the cave. This concerned few individuals at 4.4 °C and progressively more at 9 °C, while above 13.3 °C the number reached values comparable to those obtained in the laboratory (23.3 °C). Adults, however, were obtained only at temperatures above 10.5 °C. The adult yield was similar among temperatures from 10.5 to 13.4 °C, and did not differ from values observed in the laboratory. The ample daily temperature fluctuation observed at the upper sites is likely responsible for the better performance, indicating that *Drosophila* flies are responding to the thermoperiod (Schou et al. 2015) as well as other insect species (the sawfly *Cephalcia arvensis*) tested in the same experimental set-up (Battisti and Cescatti 1994). Adult females taken from the laboratory and allowed to oviposit during the first 48 h at different sites did not produce pupae when the temperature was lower than 10 °C. Pupae, however, appeared at temperatures just below 12 °C and adults above 13 °C. It is likely that adults could have been obtained at lower temperatures, as observed in Trial 3, if the experiment had not stopped because of a sharp drop of temperature. At 10 °C, the duration of development from egg to adult stage has been shown to take more than

70 days, although eggs were laid at 22 °C, since oviposition is not possible at that low temperature (Tochen et al. 2014; Asplen et al. 2015). In addition, it seems very difficult to keep either artificial medium or natural fruits suitable for larval development through that period of time. Anyhow, the development rate and yield increased with temperature up to 13.5 °C for both pupae and adults, when it was comparable to the values observed in the laboratory at 23.3 °C.

The development rate observed for the second oviposition period (starting from the 49th hour of adult female life at different temperatures inside the cave) was significantly higher than that for the first oviposition period. This may be interpreted as an adaptation of the females to the thermal conditions to which they were exposed during the first two days of their life. The observed trade-off with yield was predicted by the phenotypic plasticity theory, where mothers invest larger quantities of nutrients in fewer eggs, which in turn develop faster (Chown and Nicholson 2004). However, yields of pupae and adults obtained from the second oviposition period were significantly lower than those from the first oviposition period. This is probably due to both the competition caused by a higher number of larvae and the aging of the females (8 days old at the beginning of the test, 20 days old at the end). The yield values obtained in the laboratory at 23.3 °C were similar to those obtained in the field (0.35 adults/female/day), perhaps due to the competition-related mortality within the test tubes.

By comparing all the data from the natural gradient experiment, it is possible to derive the minimum threshold of average daily temperature under natural conditions for the egg-to-adult development of *D. suzukii*, which corresponds to 11.6 °C. This value is slightly higher than that obtained by weather stations close to the collection site of wild fruits which yielded adults along the elevational gradient (11.1 °C). Both are lower than the lower threshold (13.4 °C) for intrinsic rate of population increase assessed by Tochen et al. (2014) using nonlinear estimation, indicating that the species is particularly adapted to exploit at best the naturally fluctuating temperatures typical of mountain weather, for both development rate and yield. At the most favourable conditions tested in this study, an egg needed 24 and 17 days to develop into an adult in the first and second oviposition periods, respectively. These development times are similar or shorter than those observed by Kanzawa (1939) and Asplen et al. (2015), at the constant temperature of 15 °C (21–25 days), or by Tochen et al. (2014) at 14 °C (28.8 days), possibly because of the effect of the fluctuating thermal condition. They are, however, in contrast with other data obtained by Kanzawa (1939) under fluctuating low temperatures (10–14 °C, 37 days) but experimental details are not available. The number of degree-days required to complete the development from egg to adult in the laboratory was similar to that observed by other authors under similar conditions, whereas it was considerably lower in the natural gradient experiment likely because of fluctuating temperatures, as hypothesised by Tochen et al. (2014).

This study has shown that *D. suzukii* may quickly adapt to changing conditions, and that the temperature experienced by individuals in a given phase of their life may affect the performance of the subsequent stages. These findings open the way to better understanding the possibility of the fly to colonise mountain habitats, although in a temporary way because of the limiting conditions for overwintering. With the increases in temperature due to climate change, the insect will expand to higher elevations, where it will be able to reproduce depending on the availability of host plants, which in turn will be affected in their reproductive potential. Results also indicate that *D. suzukii* performs well at rather low temperatures, suggesting that population buildup may occur even under those conditions. If high-density populations occur in the mountains at the end of the summer, then the flies may move to low-elevation habitats when the cold arrives, threatening crops in early spring. This pattern is indirectly shown by the high number of flies caught in traps at low elevation during winter (unpublished results). Comparing the elevational distribution of the insect with the temperature-dependent performance over a wider range of temperatures and environmental conditions could contribute to modelling the species performance in the invasion range, as well as to predicting the impact of climate change.

Author contribution statement

LT, NM, and AB conceived and designed the research. LT and AB conducted the experiments. LT, FG, and AB analysed the data. LT, NM, FG, and AB wrote the manuscript. All authors read and approved the manuscript.

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