

Effects of temperature on the fitness of the alfalfa weevil (*Hypera postica*)

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Abstract

Background: Temperature directly influences the survival, development and reproduction of insects and limits their biological activity. The alfalfa weevil (*Hypera postica* Gyllenhal) is a destructive pest of alfalfa crops in Spain and in most of the alfalfa production countries. The knowledge of how temperature affects the fitness of this pest can be used to predict its activity in current or forecasted environmental conditions and to develop more accurate control strategies.

Results: We evaluated the fitness of alfalfa weevil at eight constant temperatures (8–36 °C) at 4 °C intervals by using life tables and models for the description of temperature-dependent development rates. We found that most of the parameters we studied were temperature dependent. Development was observed at all temperatures. However, postembryonic survival was optimal at 16 °C but very low at 36 °C. Adults did not reproduce at 8, 32 or 36 °C. The highest fecundity and net reproductive rate were observed at 20 °C, but the highest intrinsic rate of increase occurred at 24 °C. We predicted the phenology of the pest based on the heat needed for development, the cumulative degree days from the beginning of October to the end of May, and the day length in the study region.

Conclusion: Our prediction was validated using field data reporting the first occurrence of larval instars and adults, revealing that no more than two generations are possible within an annual cycle. In a hypothetical case where average monthly temperatures increase by 1–2 °C, the number of generations would not change.

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Keywords: two-sex life table; pest control; alfalfa; development; survival; reproduction

1 INTRODUCTION

Hypera postica Gyllenhal (Coleoptera: Curculionidae), commonly known as the alfalfa weevil, is one of the most destructive pests of alfalfa and has spread globally.^{1–4} In Spain, the weevil larvae cause serious damage to the first crop cutting (from March to the end of April) by defoliation, reducing forage quality and yield.⁵ Although the weevil originates from Eurasia,⁶ little is known about its biology, life cycle or ecology in Europe. The European literature where the *H. postica* life cycle has been described follows Hoffmann.⁶ According to this, after a summer aestivation, females lay eggs in clusters inside alfalfa stems. The resulting larvae, hatched at the end of winter and the beginning of spring, feed on leaves and new plant buds, suffer three moults and at the end of the fourth larval development instar pupate between leaflets or under the windrow in white cocoons. Its feeding activity reduces forage yield and the quality of alfalfa. The adults emerge after a few days and continue in the field, but without causing any serious damage. Recent adults mate and females can lay eggs in spring for a time, entering later in a summer aestivation period to be newly active in autumn. The number of generations is variable depending on the climate, and from one to four generations can occur. This life cycle has been also reported in the scarce Spanish literature, where a single generation seems to occur.⁷

However, field observations conducted in the Ebro Basin⁵ suggest that differences in the described life cycle may exist. Therefore, it is necessary to clearly establish the *H. postica* life cycle to properly manage this pest and evaluate the consequences of environmental or crop management changes on its populations.

Insects are ectothermic animals so the temperature is an important abiotic factor that directly influences their survival, development and reproduction, and limits their biological activity.^{8–10} The study of the relationship between temperature and development allows to know how long the different developmental stages and foreseen life cycle in a region will be. Beside this, temperature also affects the reproductive capacity, adult life span and estimate how a population will increase. Therefore, the

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knowledge of how temperature affects the fitness of *H. postica* can be used to predict its activity in current or forecasted environmental conditions and to develop more accurate control strategies.

The relationship between temperature and development time is widely studied and has been described using various models.^{11–17} Such models differ in terms of parameter numbers and basic assumptions about temperature effects near the upper and lower limits. The linear model is the easiest to build, but non-linear models provide a more realistic description of development rate as a function of temperature by estimating the low, high and optimal temperature thresholds for development. Temperature-driven rate models are often used to predict the activity and seasonal population dynamics of pests or their natural enemies, but these models do not consider other factors that also influence insect fitness, such as larval mortality, female fecundity, or adult reproductive time and longevity.¹⁸

A life table^{19–22} is an alternative approach to determine the impact of environmental factors on pest survival, development and fecundity, as well as population dynamics.^{23–26} More precisely, life tables contain information on survival rate, developmental rate and fecundity, but also on stage differentiation. Therefore, insect populations should not be structured only by age but by age-stage.¹⁸

The effect of temperature on the fitness of the alfalfa weevil has been studied using North American and Middle East Asian populations by analysing the temperature-dependent development rate and life tables, and differences between populations of these geographic areas have been reported.^{16,27,28} However, to the best of our knowledge, only the embryonic development of European populations has been studied in detail.²⁹ To address this knowledge gap, we determined the fitness of Spanish alfalfa weevil populations raised at eight constant temperatures, using life tables and models describing the temperature-dependent development rate to obtain essential information that can be used to predict field activity and population dynamics in the current climate and in a perspective of warming due to the climatic change. The data can be used to increase the effectiveness of pest control methods under Mediterranean and European crop conditions.

2 MATERIALS AND METHODS

2.1 Insect rearing

Adult specimens of *H. postica* were collected from a commercial field of alfalfa in Lleida, north-east Spain, in spring 2018. They were reared in 2000-mL glass jars covered with mousseline for proper ventilation, and were maintained at 20 °C and 60–70% relative humidity with an 8-h photoperiod. Fresh alfalfa stems were provided daily for egg laying and feeding. The stems were placed in a glass vial filled with water and sealed with Parafilm to prevent dehydration of the plants and drowning of adult insects. Rearing was refreshed yearly with different location field captured adult individuals to reduce the effects of consanguinity. All *H. postica* individuals used in the experiments were derived from this laboratory population. Experiments were performed during 2019 and 2020.

2.2 Experimental set-up

Separate climate chambers were set to eight different temperatures ranging from 8 to 36 °C at 4 °C intervals, while maintaining the standard parameters of 60–70% relative humidity and an 8-h photoperiod.

The effect of temperature on larval development and survival was studied using eggs laid in the previous 24 h, which were placed in transparent PVC rearing cages (diameter = 53 mm, height = 32 mm) in each of the climate chambers. Hatched larvae were individualized in the same rearing cages. Hatching was confirmed when the larva was able to break through the chorion and leave the eggshell. Accordingly, only eggs producing living larvae were considered (no mortality during the egg stage). The larvae were fed regularly on fresh alfalfa. Instar changes were confirmed by the presence of the exuvia. Instar survival and development were recorded daily, along with the pupal stage and adult emergence. The experiment was complete when all individuals had either died or passed through postembryonic development and emerged as adults. For all calculations, events were assumed to occur at the midpoint between two observations. The experiment was carried out in triplicate.

For the analysis of adult reproduction and longevity, individuals were reared as above until postembryonic development was complete. Healthy male and female pairs were then maintained in the same environment under which they were reared. Only adults that emerged on the same day in each group were paired, to rule out the effect of age on survival. Alfalfa was provided on a regular basis for feeding and oviposition. Female fecundity and adult male and female longevity were recorded every 2 days. The experiment was complete when all paired adults had died. The experiment was carried out in triplicate.

2.3 Data analysis

Data were analysed using an age-stage, two-sex life table^{30,31} in TWOSEX-MSChart.³² This takes into account the stage differentiation, the variable developmental rate among individuals, and both sexes. Accordingly, it can properly describe the development, survival and reproduction of all individuals.¹⁸ The egg/larval/pupal data were collected from an individual-reared cohort of 90–160 individuals, whereas the adult data were collected from a group-reared cohort of 105 individuals per temperature. Adult life table data were converted into individual-reared life tables.³³ The bootstrap-random match technique was then used to construct the life table.³⁴ The bootstrap technique with 100 000 resamples was used to estimate the standard errors of the parameters.³⁵ The 0.5th percentile life table was used to calculate the population parameters, whereas the 0.025th and 0.975th percentiles were used to calculate the confidence intervals. The individual parameters were then compared between temperatures using a paired bootstrap test.^{36,37}

The following life table parameters were calculated: (a) the age-stage specific survival rate (s_{xj}), the probability that a newly laid egg survives to age x and stage j ; (b) the age-specific survival rate (l_x), the probability of survival to age x ; (c) the age-stage-specific fecundity (f_{xj}), the fecundity of an individual at age x and stage j ; (d) the age-specific fecundity (m_x), the fecundity of the surviving population (number of eggs/individual) at age x ; (e) the age-specific net maternity ($l_x m_x$) the number of offspring produced by an individual at age x when the survival rate is taken into consideration; (f) the female adult pre-oviposition period (APOP), the time between female emergence and the onset of reproduction; (g) the ovipositional period, the period during which female oviposition occurs; (h) the total fecundity, the total number of eggs produced per female during her lifetime.

The following population parameters were calculated^{25,38}: (i) the finite rate of increase (λ), the population will increase λ -fold per day when it settles down to the stable age-stage distribution as time

approaches infinity; (j) the intrinsic rate of increase (r), the population instantaneous growth rate as time approaches infinity and the population reaches the stable age-stage distribution ($e^r = \lambda$); (k) the gross reproduction rate (GRR), the summation of m_x from age 0 to the last age, ignoring the survival rate; (l) the net reproduction rate (R_0), the total number of offspring that an individual can produce during its lifetime; (m) the mean generation time (T), the length of time taken by a population to increase by R_0 -fold when the population reaches a stable age-stage distribution.

The Kaplan–Meier method was used to estimate postembryonic and adult survival curves at each temperature. Log-rank tests were used to compare overall survival curves between the eight temperatures. We used the R packages ‘survival’³⁹ and ‘survminer’.⁴⁰ The adult sex ratio was analysed using a chi-square test. The effect of temperature, sex and their interaction on adult longevity was assessed by two-way analysis of variance (ANOVA). Data were square root transformed [$\sqrt{x + 0.5}$] to achieve, as far as possible, a normal distribution. When significant differences were detected, Tukey’s honest significant difference (HSD) test was used for multiple comparisons in the ‘multcomp’ R package.⁴¹

We also determined the duration of all developmental stages: egg, larval (different instars and total larval development), pupal, adult (both sexes), postembryonic and whole immature stages, only including individuals that completed development of those stages. We then calculated the corresponding rates of development. To describe the relationship between temperature and developmental rate, we used four mathematical models: a linear model¹¹ and the nonlinear models Brière-1, Brière-2 and Lactin-2.^{13,15} Lower, optimal and upper temperatures were obtained for all immature stages. The three nonlinear models were chosen because they are often used to model coleopteran developmental rates, particularly in species of Curculionidae^{42,43} and Coccinellidae.^{17,44–46} The equations for each of the adjusted models are shown in Table S1. Only nonlinear models allowed the calculation of the optimum temperature (t_{opt}) and the maximum (t_{max}) developmental threshold, whereas all models allowed us to estimate the lower developmental threshold (t_{min}) as the value intercepting the temperature axis. The goodness of fit in the linear and nonlinear models was evaluated by calculating the coefficient of determination (R^2 , a higher value indicating a better fit), the residual sum of squares (RSS, a lower value indicating a better fit), the Akaike information criteria (AIC, a lower value indicating a better fit) and biological criteria. We used the ‘devRate’ R package for data fitting.⁴⁷ Initial parameter estimation for the Lactin-2 model was based on Logan.¹⁴ All statistical analyses referring to model adjustment and comparisons were carried out using R v3.5.2 (R Core Team, 2018). In addition, we calculated the thermal constant (K) of each stage, which can only be estimated using the linear model as the reciprocal of the slope b ($K = 1/b$). This allowed us to predict the occurrence of different development stages in the field. To adjust the linear model, the data value representing 36 °C was omitted because it deviated from the straight line. This was necessary to calculate the parameters K and t_{min} correctly.⁴⁸

We recently determined the *H. postica* oviposition window under Ebro basin conditions.²⁹ This period starts in the second week of October and ends at the beginning of February. To predict the occurrence of different development stages in the field, we extended this period from the first week of October to the third week of February. Then, we calculated thermal accumulation (degree days or DD) from the start of oviposition in October to the end of May at weekly intervals, according to the mean temperature method, considering the average weekly temperature of

the last 10 years and the lower temperature threshold (t_{min}). No upper development threshold was taken into account. Data for weekly temperatures were obtained from the Spanish Agency of Meteorology (AEMET) based on reports from four different meteorological stations along the Ebro basin region (Urgell, Segrià, Baja Cinca and Monegros), covering a total distance of 150 km in a straight line. To validate the predicted occurrence of instar larvae, pupae and adults, we contrasted the data with records of insect phenology from >50 alfalfa fields in the Ebro basin during the period 2018–2020. To obtain these records, we sampled commercial alfalfa fields by stem cutting (200 stems) and net-sweeping (five sweeps in 12 sites of the field) from October to May. Samples were brought to the laboratory where they were maintained in a fridge at 5 °C, in the case of the stems, or frozen before sorting, in case of the sweep-net samples. Stems were dissected and the number of eggs and larvae was recorded. Sweep-net samples were observed under stereoscopic microscope and the larval number and development stage, pupae and adults were recorded.

3 RESULTS

3.1 Pre-imaginal survival and development

The alfalfa weevil was able to complete all developmental stages at all temperatures. The age-stage-specific survival rate (S_{xj}) for the different larval instars and pupa are shown in Fig. 1. Overall, the survival rates mainly decreased at the larval stage. The age-specific survival rate (l_x) is shown in Fig. 2. The overall postembryonic survival curves differed significantly between temperatures ($\chi^2 = 690$, $df = 7$, $P < 0.001$). The highest survival rate was recorded at 16 °C and the lowest at 36 °C. The temperature had no significant effect on the sex ratio of the emerging adults (ratio 1:1; $\chi^2 = 8.59$, $df = 7$, $P = 0.25$). The developmental time for eggs, each larval instar, total larval development and pupae at each temperature is shown in Table 1. The duration of development was significantly affected by temperature ($P < 0.05$) at all stages and decreased as the temperature rose from 8 to 32 °C.

The linear model and all three nonlinear models closely fitted the relationships between temperature and the rate of development of the egg, larval, pupal, postembryonic and entire immature stages (Table S2). The minimum thresholds for development (t_{min}) in the linear and Lactin-2 models were very close for postembryonic development (6.68 and 6.50, respectively) and total development (6.64 and 6.60, respectively), whereas the Brière-1 model yielded lower values and the Brière-2 yielded some negative values (Table S2). The optimal temperature (t_{opt}) for rapid development was 32–35 °C according to the nonlinear models (Table S2).

The relationship between temperature and total or postembryonic development rates fitted with the Linear and Lactin-2 models is shown in Fig. 3. The thermal constant K was 122 DD for eggs, 284 DD for larvae and 74 DD for pupae. The heat needed to complete postembryonic development was 358 DD, and the heat needed to develop from egg to adult was 484 DD.

According to the extended oviposition period (and taking into account the heat needed for the completion of different developmental stages), the occurrence of larvae in the field would last from the end of October to the third week of April. Eggs laid in first week of October should become adults by the second week of March, whereas eggs laid in the last week of February should become adults by the end of April (Fig. 4). Field data records revealed that first-instar larvae were first recorded in the second

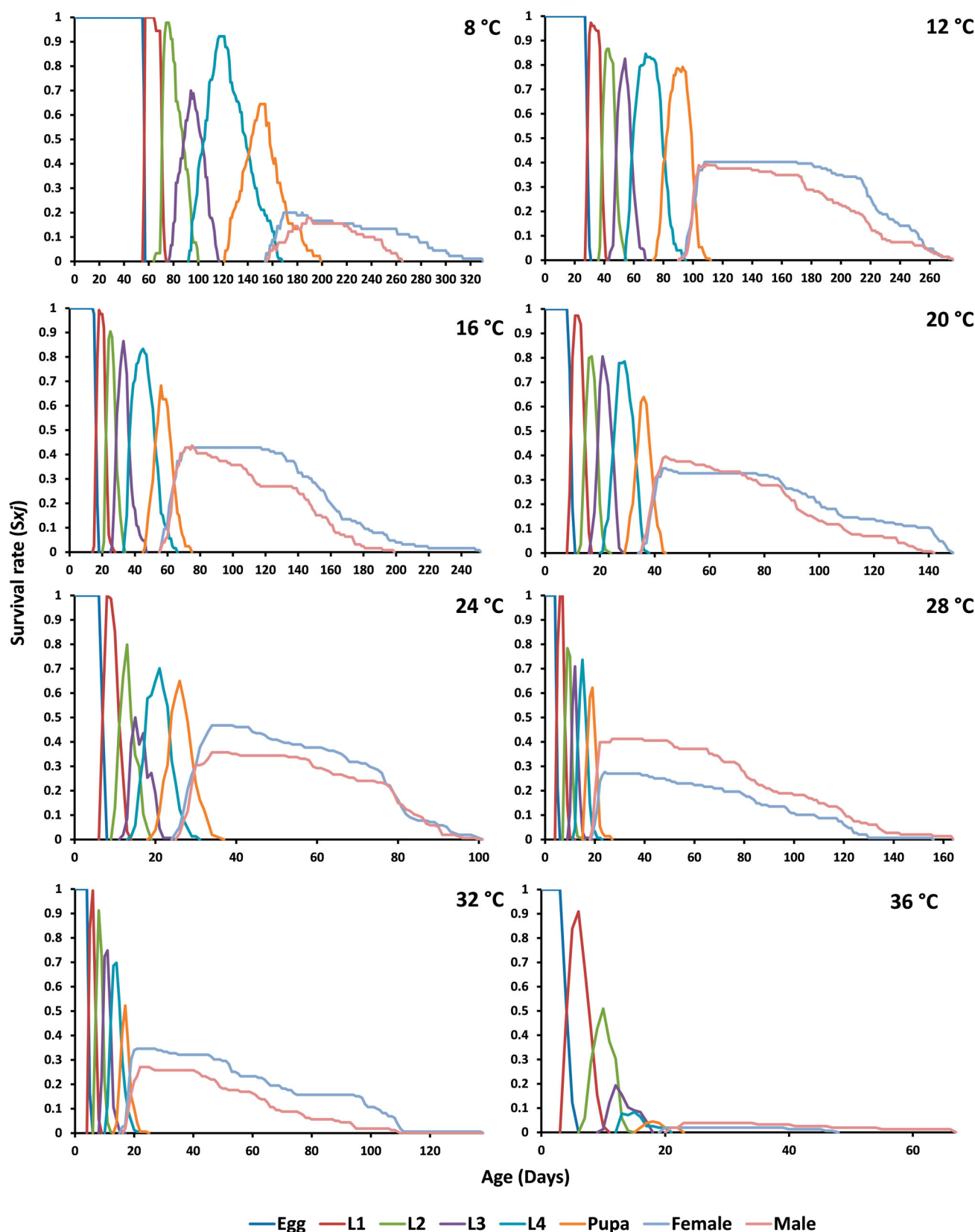


Figure 1. Age-stage-specific survival rates (S_{xj}) of *H. postica* eggs, first to fourth instar larvae (L1–L4), pupae and adults (male and female) at different rearing temperatures.

week of November and the second, third and fourth larval instars in the middle of November, at the end of January and in the middle of March, respectively (Fig. 4). The first adults were recorded in

the field during the fourth week of March (Fig. 4). These records confirm that the oviposition period started in the third week of October and not before. Furthermore, given that the highest

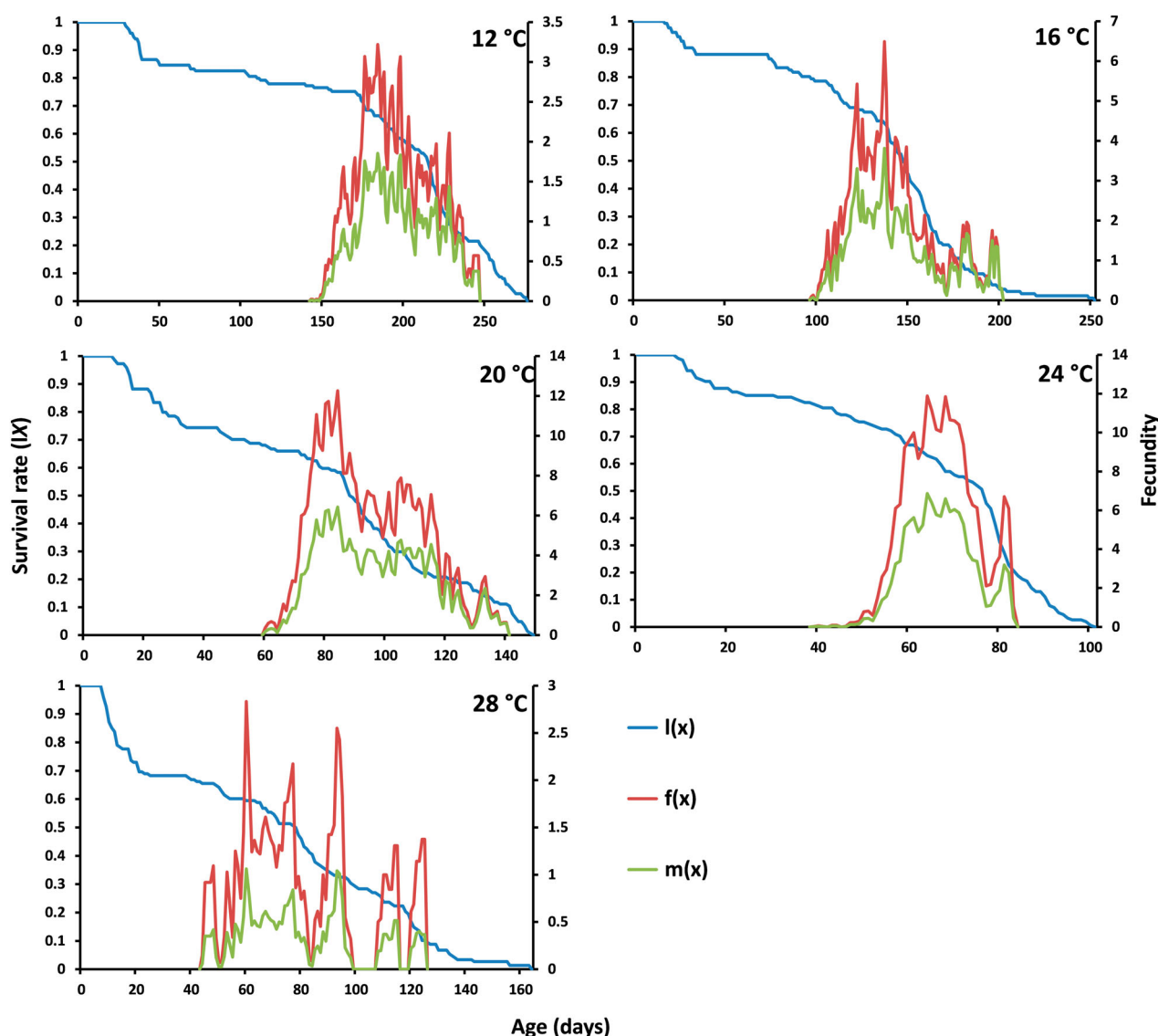


Figure 2. Age-specific survival rate (l_x), age-stage-specific fecundity (f_x) and age-specific fecundity (m_x) of *H. postica* at the five temperatures compatible with reproduction.

proportion of larvae was recorded in the field between the fourth week of March and the third week of April, and that the highest proportion of adults was recorded between the last week of April and the second week of May, our results suggest that the main oviposition period was between the end of January and the second week of February (Fig. S1).

3.2 Adult survival and longevity

The temperature significantly affected both female and male survival ($P < 0.0001$). The higher the temperature, the lower the survival rate ($X^2 = 522$, $df = 15$, $P < 0.0001$). The female survival rate was higher than that of males at 8 °C ($X^2 = 7.8$, $df = 1$, $P = 0.05$), 12 °C ($X^2 = 6.3$, $df = 1$, $P = 0.01$), 16 °C ($X^2 = 14.5$, $df = 1$, $P < 0.0001$) and 20 °C ($X^2 = 15.7$, $df = 1$, $P < 0.0001$). Adult survival decreased with advancing age as expected. Survival rates were higher at the beginning of adulthood but decreased more or less abruptly from the middle or last third of the adult lifespan (Fig. 1).

Temperature significantly affected longevity ($F = 48.94$, $df = 7$, $P < 0.0001$). The longevity of both male and female adults

decreased strikingly when the temperature increased from 20 to 36 °C (Table 2). We found a significant interaction between sex and temperature, but only observed significant differences between the longevity of males and females at 12 °C ($F = 5.16$; $P < 0.0001$) and 16 °C ($F = 4.91$; $P < 0.0001$).

3.3 Reproduction

Reproduction was significantly affected by the temperature ($P < 0.005$). No reproduction was observed at 8, 32 or 36 °C (Table 3). The APOP (time required for the onset of egg laying) was also temperature dependent. It significantly decreased as the temperature rose from 12 °C to 24 °C and then significantly increased at 28 °C ($P < 0.005$). Age-stage-specific fecundity (f_x) and age-specific fecundity (m_x) are shown in Fig. 2, whereas age-specific maternity ($l_x m_x$) is shown in Fig. 5. All values differed significantly between temperatures ($P < 0.05$), increasing as the temperature rose from 12 to 20 °C and then decreasing from 24 °C ($P < 0.05$). Females laid the most eggs (277.02 eggs per individual) at 20 °C ($P < 0.005$) (Table 3).

Developmental period

8 °C			12 °C			16 °C			20 °C			24 °C			28 °C			32 °C			36 °C		
Developmental stage	n	Days	n	Days	n	Days	n	Days	n	Days	n	Days	n	Days	n	Days	n	Days	n	Days	n	Days	
Egg	90	56.69 ± 0.05a	149	29.10 ± 0.07b	126	16.76 ± 0.07c	144	9.94 ± 0.05d	154	7.47 ± 0.04e	148	5.22 ± 0.03f	159	5.16 ± 0.03f	155	4.66 ± 0.06ef							
L1	88	14.45 ± 0.16a	129	10.02 ± 0.10b	118	6.19 ± 0.10c	127	5.08 ± 0.09d	142	4.18 ± 0.07e	131	3.37 ± 0.07f	155	2.39 ± 0.04g	94	4.07 ± 0.08cdef							
L2	86	17.12 ± 0.70a	126	9.76 ± 0.22b	113	6.38 ± 0.15c	127	4.61 ± 0.09d	135	3.60 ± 0.09e	122	2.75 ± 0.07f	148	2.47 ± 0.05g	47	3.51 ± 0.16cdef							
L3	83	15.78 ± 0.51a	126	10.84 ± 0.30b	111	8.56 ± 0.23c	115	5.34 ± 0.11d	133	2.98 ± 0.08e	115	2.44 ± 0.06f	129	2.51 ± 0.07fg	23	2.74 ± 0.22efg							
L4	64	34.78 ± 0.74a	123	22.07 ± 0.24b	111	15.56 ± 0.25c	107	8.49 ± 0.12d	131	5.95 ± 0.12e	107	3.79 ± 0.09f	110	3.81 ± 0.10f	13	3.23 ± 0.12ef							
Total larval stages	64	81.11 ± 1.38a	123	52.76 ± 0.39b	111	36.77 ± 0.44c	107	23.59 ± 0.19d	131	16.64 ± 0.19e	107	12.24 ± 0.11f	110	11.23 ± 0.14g	13	13.77 ± 0.53g							
Pupa	43	30.84 ± 1.05a	123	18.41 ± 0.29b	110	9.76 ± 0.23c	107	5.78 ± 0.11d	131	5.28 ± 0.07e	102	3.62 ± 0.10f	98	2.66 ± 0.08g	9	3.00 ± 0.29fg							
Postembryonic	43	111.95 ± 1.34a	123	71.17 ± 0.24b	110	46.53 ± 0.54c	107	29.37 ± 0.23d	131	21.92 ± 0.29e	102	15.86 ± 0.22f	98	13.89 ± 0.19g	9	16.77 ± 0.39f							
Pre-imaginal	43	166.2 ± 1.40a	123	100.26 ± 0.32b	110	63.19 ± 0.39c	107	39.32 ± 0.20d	131	29.43 ± 0.22e	102	21.11 ± 0.14f	98	18.86 ± 0.13g	9	21.33 ± 0.44f							

Different letters in the same row indicate significant differences between temperatures at $P < 0.05$. Standard errors were estimated by 100 000 bootstrap resampling. n = number of individuals.

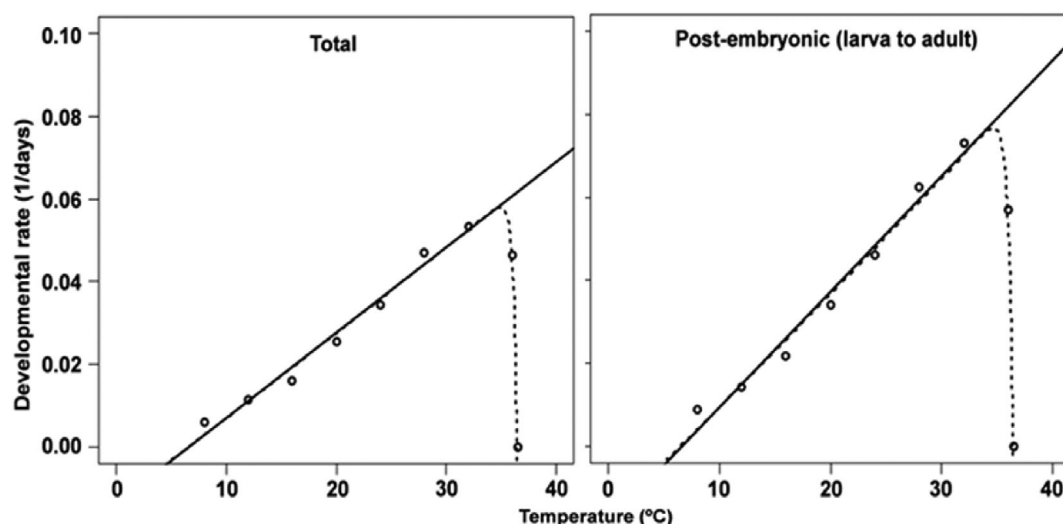


Figure 3. The relationship between temperature and the developmental rate (total and postembryonic) of *H. postica* described by the linear model (solid line) and the Lactin-2 nonlinear model (dashed line).

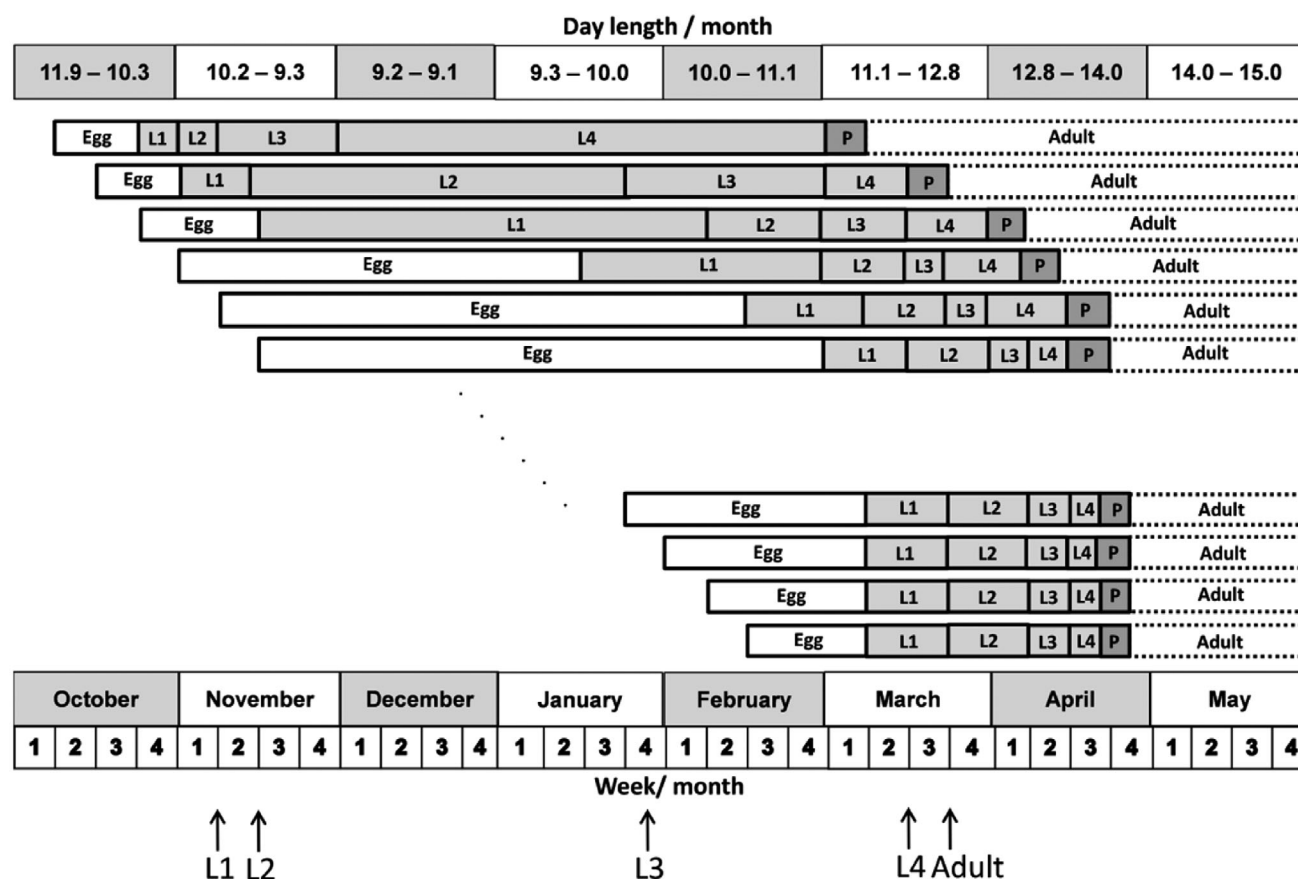


Figure 4. Predicted occurrence of larval instars, pupae and adults according to the degree-days (DD) needed to complete development with an oviposition window from October to February, at weekly intervals (Levi-Mourao *et al.*, 2021). Vertical arrows indicate the first record in the field for each of the larval instars and adults. Dots indicate the period when no reproduction occurred.

energy expenditure during reproduction.⁵² We also found that female APOP and ovipositional periods decreased significantly as the temperature increased from 12 to 24 °C, as previously reported.¹⁶ Fecundity was also temperature dependent, and females reared at 8, 32 and 36 °C did not lay eggs. These results largely agree with studies of American populations^{27,53–55} but

partially contrast with adults from Iran, which were able to lay eggs at 31.5 °C.¹⁶ These variations indicate the differences between geographically separated *H. postica* populations. When the age-specific fecundity (m_x) was tempered with the age-specific rate of survival (l_x), the highest reproductive rate was observed at 20 °C, decreasing at 24 and 28 °C. These results

Table 2. Mean (\pm SE) adult longevity (in days) of *H. postica* males and females reared at eight different temperatures

Temperature ($^{\circ}$ C)	<i>n</i>	Longevity (days)			
		Female		Male	
8 $^{\circ}$ C	22	80.45 \pm 11.23a	21	51.05 \pm 7.27a	
12 $^{\circ}$ C	60	130.03 \pm 3.21a	63	98.98 \pm 5.82b	
16 $^{\circ}$ C	54	101.63 \pm 3.92a	56	71.36 \pm 4.42b	
20 $^{\circ}$ C	50	71.52 \pm 4.10a	57	54.25 \pm 3.28a	
24 $^{\circ}$ C	73	44.00 \pm 1.88a	58	44.62 \pm 2.15a	
28 $^{\circ}$ C	41	69.76 \pm 4.70a	61	76.72 \pm 3.84a	
32 $^{\circ}$ C	55	59.96 \pm 3.68a	43	46.42 \pm 3.25a	
36 $^{\circ}$ C	3	24.67 \pm 2.40a	6	30.00 \pm 5.06a	

Standard errors were estimated by 100 000 bootstrap resampling. Different letters in the same row indicate significant differences between sexes at $P < 0.05$. *n* = number of individuals.

suggest that Spanish populations of *H. postica* do not perform optimally at high temperatures. Indeed, most of the activity of this insect in the study area is observed during early spring.²⁹

4.4 Population fitness

Two-sex life table analysis allows the estimation of important population fitness parameters and integrates data concerning pre-imaginal and adult fitness.^{56,57} The intrinsic rate of increase (*r*) integrates the effects of survival and fecundity in a single value determined by the pre-imaginal survival, developmental rate, sex ratio and female fecundity and longevity, all of which are affected by temperature.¹⁸ In our study, *r* increased in the range 12–28 $^{\circ}$ C and decreased at higher temperatures. A similar profile was described previously,²⁸ but there were also noticeable differences between the studies. Our maximum *r* (0.0065) occurred at 24 $^{\circ}$ C whereas the maximum value in the earlier study was 0.1138 at 29 $^{\circ}$ C.¹⁶ Furthermore, our *r* values were higher than those previously reported in the low temperature range of 12–14 $^{\circ}$ C.¹⁶ These results suggest that Spanish *H. postica* populations are less well-adapted to higher temperatures than Iranian populations, again highlighting the differences between geographically separated populations.

The highest *r* at 24 $^{\circ}$ C was offset by the net reproductive rate (*R*₀) and gross reproduction rate (GRR), which reached maximum values at 20 $^{\circ}$ C. These results suggest that the temperature range associated with the maximum fitness of Spanish *H. postica*

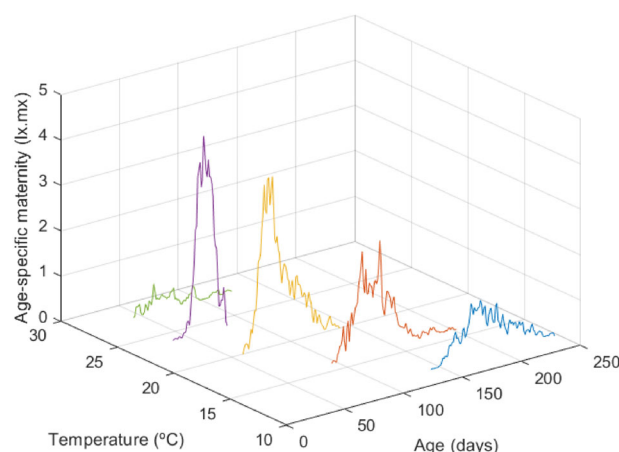


Figure 5. Age-specific temperature dependent maternity ($l_x m_x$) of *H. postica* at the five temperatures compatible with reproduction.

populations is 20–24 $^{\circ}$ C. This is lower than the temperature predicted for the faster developmental rate because the latter parameter refers to development and not the overall effect on the population fitness, which also takes survival and fecundity into account.¹⁸

4.5 Phenology and the damage risk period of *H. postica* in the Ebro basin

We predicted the occurrence of *H. postica* at different developmental stages, taking the estimated values of t_{min} and *K* from the linear model into account, as previously reported.^{42,43,58,59} The occurrence of late-stage larvae at the beginning of the productive cycle (late March) increases the risk of damage caused by this pest.^{5,29,60,61} Previous studies have suggested that *H. postica* produces more than one generation under the conditions in the Ebro basin,^{5,29} thus affecting both the first and second alfalfa intercrops.⁷ This is possible given the fast development and high reproductive capacity of this insect at 20–24 $^{\circ}$ C.

Another factor that should be taken into account is the effect of photoperiod on the induction of adult diapause and aestivation. Larval stages reared under long-day conditions with a day length >12 h induce imaginal diapause.^{54,62–68} In the Ebro basin, the day length is <12 h from November to the middle of March, but it increases to 12.8 h by the end of March and to 14 h in April (Fig. 4). Larvae developing early in the season (reaching the final instar before April) therefore do not produce adults with reproductive diapause, whereas this is the more likely fate of larvae

Table 3. Mean (\pm SE) pre-oviposition (APOP) and ovipositional periods in days, and fecundity as the total number of eggs produced per *H. postica* female reared at the five temperatures compatible with reproduction

Female reproduction parameters	Temperature ($^{\circ}$ C)									
	12		16		20		24		28	
	<i>n</i>	Days	<i>n</i>	Days	<i>n</i>	Days	<i>n</i>	Days	<i>n</i>	Days
APOP	55	69.24 \pm 1.98a	50	50.88 \pm 1.17b	46	32.48 \pm 1.21c	56	27.67 \pm 0.87d	30	51.60 \pm 3.95b
Ovipositional period	55	25.05 \pm 1.82a	50	20.04 \pm 1.97a	46	22.13 \pm 1.73a	56	13.73 \pm 0.86b	30	7.47 \pm 1.12c
Fecundity (eggs/f)	60	124.55 \pm 14.86b	54	152.35 \pm 17.05b	50	277.02 \pm 32.25a	73	148.21 \pm 15.82b	41	45.22 \pm 10.61c

Different letters in the same row indicate significant differences between temperatures at $P < 0.05$. Standard errors were estimated by 100 000 bootstrap resampling. *n* = number of individuals.

Table 4. *H. postica* population parameters (mean \pm SE)

Temperature (°C)	<i>n</i>	Finite rate of increase λ (days ⁻¹)	Intrinsic rate of increase <i>r</i> (days ⁻¹)	Gross reproduction rate (GRR)	Net reproduction rate <i>R</i> ₀	Mean generation time <i>T</i> (days)
12	149	1.02 \pm 0.00084d	0.020 \pm 0.00083d	89.07 \pm 12.77b	50.14 \pm 7.78b	187.59 \pm 1.96a
16	126	1.03 \pm 0.00121c	0.032 \pm 0.00118c	130.67 \pm 22.08b	65.29 \pm 9.88ab	130.15 \pm 1.67b
20	144	1.05 \pm 0.00199b	0.053 \pm 0.00191b	234.02 \pm 38.13a	96.18 \pm 15.64a	85.80 \pm 1.41c
24	154	1.06 \pm 0.00246a	0.065 \pm 0.00231a	120.21 \pm 14.70b	70.25 \pm 9.55ab	65.02 \pm 0.61d
28	148	1.04 \pm 0.00518c	0.036 \pm 0.005c	28.04 \pm 6.33c	12.52 \pm 3.35c	68.94 \pm 3.55d

Finite rate of increase, intrinsic rate of increase, gross reproduction, net reproduction rate and mean generation time were calculated for the five temperatures that were compatible with reproduction. Different letters in the same column indicate significant differences at *P* < 0.05. Standard errors were estimated by 100 000 bootstrap resampling. *n* = number of individuals.

developing from eggs laid at the end of the autumn–winter period, which reach the final larval instar during April.

The first occurrence of spring adults was predicted by the end of March, as confirmed by field sampling (Levi-Mourao *et al.*, unpublished). At this time, the mean air temperature is \sim 12 °C and mated females are not able to lay eggs for nearly 70 days. Although the temperature increases from the end of April to June, it is unlikely that this pre-oviposition period is <1 month (Table 3). In such cases, a theoretical new generation could arise in 2–3 months (Table 4), but the larvae would develop under conditions that induce reproductive diapause and no further generations would arise in the same year. In the context of a warming climate, a temperature increase of 1–2 °C would radically change the first spring adult occurrence. If the temperature were to increase by 1 °C, the first spring adults would appear in the middle of February, but if it increased by 2 °C, they would appear in the third week of December. Even in these hypothetical situations, the temperature would never reach the minimum threshold that allows reproduction (8 °C) until the middle of February, and females would need nearly 2 months to become reproductive (Table 3). Accordingly, the larvae would always develop under the day-length conditions (end of March and April) that induce diapause in the adults.

The success of these two potential generations would be influenced by crop management practices in the Ebro basin. The first alfalfa cutting is usually performed by the end of April or the beginning of May, removing most of the larvae and pupae present on the foliage. However, some larvae may remain in the alfalfa furrow and can infest new alfalfa buds if the forage is not withdrawn quickly.^{5,69} Advancing this cutting as far as possible without compromising crop development may therefore help to reduce damage and economic loss. Further cuttings would also help to limit the growth of weevil populations. An additional winter cutting is a good control method to reduce the population and the damage caused in the study region during the first intercut.⁶⁹ Based on the annual cycle of the weevil in the region, the best time for cutting is late February.

5 CONCLUSION

We found that the two-sex life table and developmental rate models can help to predict the effect of temperature on the fitness of *H. postica*. Two-sex life tables provide more realistic data on whole-life performance. For example, under Ebro basin conditions, the optimal temperature range for *H. postica* is 20–24 °C. The models (especially the linear model) can determine the minimum development threshold temperature and the heat needed

to accomplish each developmental stage. The information derived from both types of approaches allowed us to predict the annual cycle of the alfalfa weevil in the Ebro basin, which has been validated by field records. This knowledge will facilitate the introduction of more successful control strategies, such as the management of cutting to prevent weevil population growth.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization: Xavier Pons and Alexandre Levi-Mourao. Methodology and validation: Alexandre Levi-Mourao and Xavier Pons. Formal analysis: Alexandre Levi-Mourao, Filipe Madeira and Xavier Pons. Investigation: Alexandre Levi-Mourao and Roberto Meseguer. Resources: Xavier Pons. Writing—original draft preparation: Alexandre Levi-Mourao and Xavier Pons. Writing—review and editing: Alexandre Levi-Mourao, Filipe Madeira, Roberto Meseguer and Xavier Pons. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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