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 molts 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 Basin\(^8\) 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...
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. 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.

A life table is an alternative approach to determine the impact of environmental factors on pest survival, development and fecundity, as well as population dynamics. 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.

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. However, to the best of our knowledge, only the embryonic development of European populations has been studied in detail. 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 muslin 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.

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The following population parameters were calculated: (a) the age-specific survival rate (*s*), the probability that a newly laid egg survives to age *x* and stage *j*; (b) the age-specific survival rate (*l*), the probability of survival to age *x*; (c) the age-stage-specific fecundity (*f*), the fecundity of an individual at age *x* and stage *j*; (d) the age-specific fecundity (*m*), the fecundity of the surviving population (number of eggs/individual) at age *x*; (e) the age-specific net maternity (*λ*), 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 oviositional period, the period during which female ovi- position occurs; (h) the total fecundity, the total number of eggs produced per female during her lifetime.

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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 $m_t$ 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’ and ‘survminer’. The adult sex ratio was analysed using a chi-square test.

Lower, optimal and upper temperatures were obtained and the nonlinear models Brière-1, Brière-2 and Lactin-2 were used to compare overall survival curves between the eight temperatures. We used the R packages ‘survival’ and ‘survminer’. The adult sex ratio was analysed using a chi-square test. Lower, optimal and upper temperatures were obtained and the nonlinear models Brière-1, Brière-2 and Lactin-2 were used to compare overall survival curves between the eight temperatures. We used the R packages ‘survival’ and ‘survminer’. 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.

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 stages, we used four mathematical models: a linear model and the nonlinear models Brière-1, Brière-2 and Lactin-2. 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 and Coccinellidae. 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. Initial parameter estimation for the Lactin-2 model was based on Logan. 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.

We recently determined the $H. postica$ oviposition window under Ebro basin conditions. 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 ($S_{ij}$) 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 ($i_{ij}$) is shown in Fig. 2. The overall postembryonic survival curves differed significantly between temperatures ($X^2 = 690$, 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; $X^2 = 8.59$, 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 relationship between development 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...
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 1. Age-stage-specific survival rates ($S_x$) of *H. postica* eggs, first to fourth instar larvae (L1–L4), pupae and adults (male and female) at different rearing temperatures.
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 (X² = 522, df = 15, P < 0.0001). The female survival rate was higher than that of males at 8 °C (X² = 7.8, df = 1, P = 0.05), 12 °C (X² = 6.3, df = 1, P = 0.01), 16 °C (X² = 14.5, df = 1, P < 0.0001) and 20 °C (X² = 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 (fₙ) and age-specific fecundity (mₙ) are shown in Fig. 2, whereas age-specific maternity (lₙ, mₙ) 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).

Figure 2. Age-specific survival rate (lₙ), age-stage-specific fecundity (fₙ) and age-specific fecundity (mₙ) of H. postica at the five temperatures compatible with reproduction.
suggesting that constant temperature dependent developmental rate of the
H. postica population from the Ebro basin. The three nonlinear models predicted an optimal temperature (\( t_{\text{opt}} \)) of between 32 °C (Brière-1) and 35 °C (Lactin-2) for postembryonic and total development. Such high temperatures only occur in the warmest summer period. The Brière-1 prediction was very similar to that reported for an Iranian population.\(^{28}\) On the other hand, the minimum developmental thresholds (\( t_{\text{min}} \)) predicted by the linear and Lactin-2 models were very similar for postembryonic and total development (linear = 6.7 and 6.6, respectively; Lactin-2 = 6.5 and 6.6, respectively), whereas the values predicted by the Brière-1 model were lower (5.0 and 4.3, respectively), suggesting that some development occurs at temperatures slightly below 5 °C. The Brière-2 model estimated a negative minimum temperature threshold, which is not realistic. The \( t_{\text{min}} \) reported for all immature developmental stages in Iranian populations was 8.5 °C\(^{28}\) higher than in our experiments. This supports the presumption that higher latitudes correspond to lower \( t_{\text{min}} \) values.\(^{51}\)

### 4.3 Adult fitness

The survival and longevity of H. postica adults declined at higher temperatures, as previously reported,\(^{16}\) suggesting that constant high temperatures are detrimental. We observed higher female longevity at 12 and 16 °C. Similarly, cold weather increased the longevity of females, reflecting their reduced activity and/or lower

#### Table 1. Mean (± SE) developmental time for different developmental stages (egg, larval and pupal) of H. postica reared at eight temperatures

| Developmental stage | 8 °C | 12 °C | 16 °C | 20 °C | 24 °C | 28 °C | 32 °C | 36 °C |
|---------------------|------|-------|-------|-------|-------|-------|-------|-------|
| Days                |      |       |       |       |       |       |       |       |
| Egg                 | 90   | 86    | 83    | 86    | 88    | 91    | 99    | 107   |
| L1                  | 94   | 112   | 115   | 137   | 155   | 170   | 193   | 220   |
| L2                  | 104  | 126   | 139   | 170   | 204   | 243   | 313   | 391   |
| L3                  | 118  | 147   | 166   | 197   | 243   | 298   | 398   | 525   |
| L4                  | 138  | 172   | 200   | 243   | 318   | 400   | 534   | 731   |
| Total larval stages | 439  | 563   | 660   | 770   | 965   | 1198  | 1586  | 2108  |
| Pupa                | 43   | 54    | 64    | 74    | 84    | 99    | 123   | 155   |
| Pre-maturation      | 43   | 62    | 78    | 93    | 111   | 134   | 173   | 221   |

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.

### 3.4 Population parameters

Population parameters such as the intrinsic rate of increase \( (r) \), GRR and \( R_0 \) were also affected by temperature \((P < 0.005)\) (Table 4). The \( r \) was zero at 8, 32 and 36 °C due to the absence of reproduction at these temperatures. The highest \( r \) was observed at 24 °C but the highest GRR and \( R_0 \) were observed at 20 °C. The shortest generation time was observed at 24 °C \((P < 0.05)\). These values contrasted with the optimal temperature for development predicted by the nonlinear models.

### 4 DISCUSSION

#### 4.1 Overview

Quantifiable thermal responses in a demographic context are required to understand insect population dynamics, adaptations to climatic conditions and phenological responses in the field. As anticipated, temperature affected the fitness of Spanish H. postica populations, influencing individual parameters such as survival, developmental time and reproduction. In turn, population parameters such as the intrinsic rate of increase and the generation time were also affected. This basic knowledge allows us to predict field activity periods and potential adaptations to the environmental warming that is foreseen in the near future.

#### 4.2 Postembryonic survival and development

The effect of temperature on the postembryonic survival of Spanish H. postica populations is similar to the impact reported on populations from the Asian Middle East and North America.\(^{27,28}\) However, we observed the highest larval survival rate at 16 °C, compared to 24 and 22 °C, respectively, in these earlier studies. We also found that eggs were able to hatch at 8 °C and the survival rate at this temperature was high, whereas no hatching was observed at 9 °C\(^{28}\) and the larval survival rate at 11.5 °C was low. However, eggs have been hatched successfully at 8 °C.\(^{49}\)

The increasing developmental rate from 8 to 32 °C in our experiment was consistent with North America and Asian Middle East populations.\(^{27,28,49,50}\) All four models fitted well with the temperature-dependent developmental rate of the H. postica population from the Ebro basin. The three nonlinear models predicted an optimal temperature \((t_{\text{opt}})\) of between 32 °C (Brière-1) and 35 °C (Lactin-2) for postembryonic and total development. Such high temperatures only occur in the warmest summer period. The Brière-1 prediction was very similar to that reported for an Iranian population.\(^{28}\) On the other hand, the minimum developmental thresholds \((t_{\text{min}})\) predicted by the linear and Lactin-2 models were very similar for postembryonic and total development (linear = 6.7 and 6.6, respectively; Lactin-2 = 6.5 and 6.6, respectively), whereas the values predicted by the Brière-1 model were lower (5.0 and 4.3, respectively), suggesting that some development occurs at temperatures slightly below 5 °C. The Brière-2 model estimated a negative minimum temperature threshold, which is not realistic. The \( t_{\text{min}} \) reported for all immature developmental stages in Iranian populations was 8.5 °C\(^{28}\) higher than in our experiments. This supports the presumption that higher latitudes correspond to lower \( t_{\text{min}} \) values.\(^{51}\)
We also found that female APOP and ovipositional periods decreased significantly as the temperature increased from 12 to 24 °C, as previously reported. 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 but partially contrast with adults from Iran, which were able to lay eggs at 31.5 °C. 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

**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. We also found that female APOP and ovipositional periods decreased significantly as the temperature increased from 12 to 24 °C, as previously reported. 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 but partially contrast with adults from Iran, which were able to lay eggs at 31.5 °C. 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
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.  

### 4.4 Population fitness

Two-sex life table analysis allows the estimation of important population fitness parameters and integrates data concerning preimaginal and adult fitness. 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. In our study, *r* increased in the range 12–28 °C and decreased at higher temperatures. A similar profile was described previously, but there were also noticeable differences between the studies. Our maximum *r* (0.0065) occurred at 24 °C whereas the maximum value in the earlier study was 0.1138 at 29 °C. Furthermore, our *r* values were higher than those previously reported in the low temperature range of 12–14 °C. 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 °C was offset by the net reproductive rate (*R*$_{0}$) and gross reproduction rate (GRR), which reached maximum values at 20 °C. These results suggest that the temperature range associated with the maximum fitness of Spanish *H. postica* populations is 20–24 °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.

### 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. The occurrence of late-stage larvae at the beginning of the reproductive cycle (late March) increases the risk of damage caused by this pest. Previous studies have suggested that *H. postica* produces more than one generation under the conditions in the Ebro basin, thus affecting both the first and second alfalfa intercrops. This is possible given the fast development and high reproductive capacity of this insect at 20–24 °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 (Fig. 4). 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 2. Mean (± SE) adult longevity (in days) of *H. postica* males and females reared at eight different temperatures

| Temperature (°C) | n | Female | | Male | |
|------------------|---|--------|---|---|---|
| 8 °C             | 22 | 80.45 ± 11.23a | 21 | 51.05 ± 7.27a | |
| 12 °C            | 60 | 130.03 ± 3.21a | 63 | 98.98 ± 5.82b | |
| 16 °C            | 54 | 101.63 ± 3.92a | 56 | 71.36 ± 4.42b | |
| 20 °C            | 50 | 71.52 ± 4.10a | 57 | 54.25 ± 3.28a | |
| 24 °C            | 73 | 44.00 ± 1.88a | 58 | 44.62 ± 2.15a | |
| 28 °C            | 41 | 69.76 ± 4.70a | 61 | 76.72 ± 3.84a | |
| 32 °C            | 55 | 59.96 ± 3.68a | 43 | 46.42 ± 3.25a | |
| 36 °C            | 3  | 24.67 ± 2.40a | 6  | 30.00 ± 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.

#### Table 3. Mean (± 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

| Temperature (°C) | 12 | 16 | 20 | 24 | 28 |
|------------------|----|----|----|----|----|
| Female reproduction parameters | n | Days | n | Days | n | Days | n | Days | n | Days |
| APOP             | 55 | 69.24 ± 1.98a | 50 | 50.88 ± 1.17b | 46 | 32.48 ± 1.21c | 56 | 27.67 ± 0.87d | 30 | 51.60 ± 3.95b |
| Ovipositional period | 55 | 25.05 ± 1.82a | 50 | 20.04 ± 1.97a | 46 | 22.13 ± 1.73a | 56 | 13.73 ± 0.86b | 30 | 7.47 ± 1.12c |
| Fecundity (eggs/♀) | 60 | 124.55 ± 14.86b | 54 | 152.35 ± 17.05b | 50 | 277.02 ± 32.25a | 73 | 148.21 ± 15.82b | 41 | 45.22 ± 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.
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 ~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. 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. 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.
Temperature effects on the alfalfa weevil

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