Phytoparasitica (2026) 54:38
https://doi.org/10.1007/s12600-026-01368-w

RESEARCH

Impacts of thermal stress on survival, life-history traits, and cellular immunity of the lesser wax moth, *Achroia grisella* F. (Lepidoptera: Pyralidae): implications under climate change

Erinç Çelik Biçer · Aylin Er · Olga Sak

Received: 11 January 2026 / Accepted: 3 March 2026 / Published online: 24 March 2026

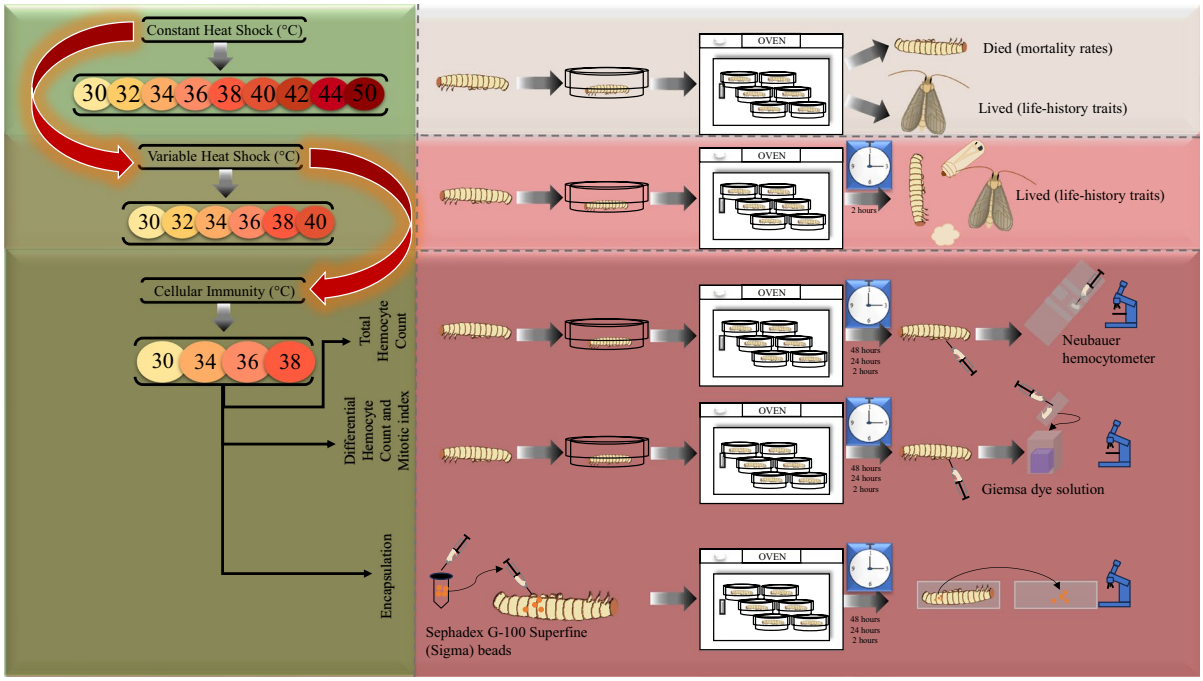
Abstract One of the most prominent features of climate change, rising temperature, disrupts the biological traits and immune systems of pest insects, threatening population stability, agricultural productivity, and the health of stored honeycombs. This study investigates the effects of constant and variable heat stress on mortality, life-history traits, and cellular immunity in the lesser wax moth *Achroia grisella* (Lepidoptera: Pyralidae). Under constant heat shock, adult emergence reached 100% at 30–32 °C, but dropped to 36.67% at 34 °C. The estimated $LTemp_{50}$ and $LTemp_{99}$ were 36.615 °C and 43.016 °C, respectively. Constant heat stress prolonged larval spinning and pupal times, shortened adult longevity, and had no significant effect on the number of eggs produced. Variable heat stress altered all life-history traits, delaying emergence, shortening longevity, and causing fluctuations in egg production, with the highest number at 34 °C and the lowest at 40 °C. In time-dependent immunity assays, compared to 30 °C, heat stress at 34 °C increased total hemocyte counts and strong encapsulation responses while suppressing weak encapsulation and mitotic activity. At 38 °C, the combination of thermal stress and injection-induced injury proved lethal after 24 h, highlighting the limits of immune tolerance in *A. grisella* larvae. These findings highlight the vulnerability of *A. grisella* larvae to rising and fluctuating temperatures, which significantly affect their developmental timing, reproductive capacity, and immune competence in a dose- and time-dependent manner.

E. Çelik Biçer (✉) · A. Er · O. Sak
Department of Biology, Faculty of Science-Literature, Balıkesir University, Balıkesir 10145, Turkey
e-mail: erinc.celik@baun.edu.tr

A. Er
e-mail: asahin@balikesir.edu.tr

O. Sak
e-mail: altun@balikesir.edu.tr

Graphical Abstract



Highlights

- High temperatures alter insect life-history traits and immune responses.
- Development and cellular immunity respond differently to short- and long-term heat exposure.
- *Achroia grisella* shows high sensitivity to both constant and variable thermal stress, and is likely to be affected by increasing temperatures and extreme fluctuations associated with climate change.

Keywords Cellular immunity · Constant and variable heat shock · High temperature · Life-history traits · Mortality

Introduction

Lepidoptera, one of the well-recognized insect orders, includes species that inhabit a wide range of thermal environments. The fact that they remain among the most populous insect groups today has been associated with their ability to withstand significant thermal fluctuations, including both lethal and sublethal extreme temperatures in their ecological habitats (Easterling et al., 2000; Ma et al., 2021). However, with climate change driving a global rise in average temperatures and increasing the frequency and severity of heat extremes, thermal stress is considered a potential challenge for insects (NOAA, 2024). Recent studies suggest that short-term extreme heat events may have a greater impact on insect survival and physiology than gradual warming trends (Ma et al., 2021). Insects can suffer physiological damage during daily peak temperatures, such as between 12:30 and 14:30 (Zhang et al., 2019); however, they can recover their normal functions once temperatures return to optimal levels (Ma et al., 2021). These stressors may affect essential biological processes such as metabolism, respiration, and reproduction.

Rising temperatures associated with climate change may disrupt insect homeostasis, creating physiological stress (Neven, 2000). At the cellular level, processes such as water loss, protein denaturation,

and oxidative stress can lead to functional impairments (Burggren, 2012; Cui et al., 2011; Mironidis & Savopoulou-Soultani, 2010). These damages may contribute to imbalances in energy production, potentially constraining development, growth, and reproductive capacity (Cui et al., 2008; Denlinger & Yocum, 2019). Physiologically induced disruptions are often reflected behaviorally through immobility (dormancy or quiescence) and reduced feeding activity (Burggren, 2012). Consequently, as individuals allocate most of their energy toward maintaining vital functions, less energy may remain for long-term investments such as growth and reproduction. As a result, insects may appear viable after heat exposure but fail to complete development under normal conditions, or exhibit reproductive abnormalities even if they do (Colinet et al., 2015; Cui et al., 2008). Many of these alterations have been linked to endocrine disruptions (Neven, 2000).

Given the close relationship between stress and the immune system, sudden changes in ambient temperature may trigger an immune response in insects (Eggert et al., 2015; Wojda, 2017). Temperature changes can cause deviations in hemocyte counts and types, as well as alterations in cell morphology (Alshabka et al., 2020; Catalán et al., 2012; Moret & Schmid-Hempel, 2000; Pandey et al., 2010). For instance, in *Bombyx mori* (Lepidoptera: Bombycidae), short-term exposure to high temperatures increases hemocyte numbers (Nittono, 1960), whereas prolonged exposure results in their decline (Kiuchi et al., 2008). Other studies have shown that granulocytes and plasmatocytes divide more rapidly under elevated temperatures but lose their structural integrity at higher thermal levels (Amaral et al., 2010; Ghasemi et al., 2013). Thermal stress may also influence the encapsulation response (Dubovskiy et al., 2016). Moreover, accumulated oxidative stress has been associated with irregular phenoloxidase activity and variable antioxidant enzyme responses (Ali et al., 2017; Farahani et al., 2020; Jena et al., 2013; Laughton et al., 2017; Shamakhi et al., 2019). Altogether, these findings suggest that heat stress induces high plasticity in immune responses. Nevertheless, studies that evaluate immune parameters together with biological traits remain limited, representing a significant knowledge gap.

The immense diversity among insect species presents a significant challenge to fully understanding their physiological responses to climate change. Investigating how pest species respond biologically and immunologically to thermal stress is therefore important for characterizing their physiological limits under controlled conditions. In this study, *Achroia grisella* (Lepidoptera: Pyralidae), popularly known as the lesser wax moth, was used as the test species. *A. grisella* is a well-known pest in beekeeping that damages stored honeycombs (Mahgoub et al., 2015) and is also widely used as a host species in parasitoid research (Galindo-Cardona et al., 2019). Despite its importance, no studies have examined how thermal stress simultaneously affects its biological traits and cellular immune parameters. In the present study, we investigated the effects of constant and variable heat shocks of different durations on the biological traits of *A. grisella*, including larval spinning and pupal time, pupal period, adult emergence time, adult weight, longevity, and the number of eggs produced, as well as on cellular immune responses such as total and differential hemocyte counts, mitotic index, and encapsulation. We hypothesized that thermal stress would affect developmental and immune parameters. By evaluating these responses together, this study provides experimental data on the biological and cellular effects of heat exposure in *A. grisella* under laboratory conditions.

Materials and methods

Insects

The lesser wax moth, *A. grisella*, used in this study, was originally collected from several beehives located near Balıkesir, Turkey. Five male and five female adults obtained from the honeycombs were kept in 1-L jars and fed with natural blackened honeycomb to maintain similarity to their natural environment in the hives. Stock cultures of *A. grisella* were maintained at 30 ± 1ºC and 60 ± 5% relative humidity, with a photoperiod of 12:12 h (L:D) in Animal Physiology Laboratory at Balıkesir University, Balıkesir. Prior to experimentation, the population was maintained for multiple generations under standardized laboratory conditions to reduce potential variability associated with field-derived factors.

Treatment and bioassays

Heat exposure

High temperature stress was applied in two ways, as constant and variable heat shocks, to investigate its effects on certain biological traits and the cellular immune system of *A. grisella*. In this study, short-term and long-term heat stress refer to differences in exposure duration. Short-term exposure corresponds to brief heat exposure (2 h, as applied in variable heat shock experiments), whereas long-term exposure corresponds to prolonged heat exposure (as applied in constant heat shock experiments). The experimental design was based on the previous study by Çelik Biçer et al. (2025a). Approximately 30–35 days after hatching (Mahgoub et al., 2015), last instar larvae of *A. grisella* that had not yet entered the pupal stage and weighed 35.7 ± 2.2 mg were randomly selected from stock cultures, without sex differentiation. Each larva was individually placed in a 60×15 mm plastic Petri dish and prepared for heat stress applications.

Constant heat shock experiments began at 30 °C, the optimal developmental temperature for *A. grisella* (Ellis et al., 2013), and continued with 2 °C incremental temperature groups. Preliminary experiments were conducted at 46 and 48 °C; however, these temperatures produced mortality patterns comparable to those observed at 44 °C and were therefore excluded. Although complete mortality occurred at both 44 and 50 °C, 50 °C was selected as the representative lethal temperature due to its more rapid and acute effect on mortality. Therefore, the larvae placed in plastic Petri dishes (60×15 mm) were exposed to nine different temperatures (30, 32, 34, 36, 38, 40, 42, 44, and 50 °C) by placing them into pre-set ovens, where they remained until pupation or death. Prior to the experiments, ovens were adjusted to the target temperatures and allowed to stabilize for 24 h. Temperature accuracy was monitored using both the oven's built-in temperature display and an independent thermometer (TFA 30.5002) placed inside the oven chamber to ensure stable conditions. Mortality assessment procedures varied depending on temperature intensity. At higher temperatures (40, 42, 44, and 50 °C), larvae were observed inside the oven at 30-min intervals without removing them from the experimental conditions. Immobile individuals or those showing visible color changes were immediately removed and recorded as dead. At lower temperatures (30, 32, 34, 36, and 38 °C), where rapid mortality was not observed within minutes or hours, individuals were removed from the oven once daily at a consistent time point (10 min) to evaluate their developmental status and survival. Mortality was confirmed based on the absence of movement and lack of response to gentle mechanical stimulation with a fine brush. This procedure was applied uniformly to all experimental and control groups to minimize handling-related variation. Cumulative mortality (%) was calculated based on the total number of individuals per replicate. The lethal, suboptimum, and optimum temperature thresholds, and the lethal temperature (LTemp), lethal time (LTime), and cumulative mortality (Cl) values were determined for *A. grisella* under constant heat shock. LTemp refers to the minimum temperature that results in complete mortality within a specified period, while LTime indicates the duration needed to achieve a specific level of mortality at a constant temperature. $LTime_{50}$ values were estimated using probit analysis only when mortality reached at least 50%; otherwise, the parameter could not be calculated and was reported as not available (n.a.). Furthermore, the lifespan of an individual larva was determined in hours by tracking the time from exposure to thermal stress to the death (as larva or adult). Finally, we examined in detail how thermal stress affects some biological traits (larval spinning and pupal time, pupal period, and adult emergence time, weight and longevity, and the number of eggs produced) for optimum temperatures with larvae exposed to constant heat shock and reaching the adult stage.

Variable heat shock temperatures (30, 32, 34, 36, 38, and 40 °C) were selected based on survival data obtained under constant heat stress. To simulate realistic climate scenarios, separate larval groups were exposed to a single peak temperatures (32, 34, 36, 38, and 40 °C) for 2 h in the oven, following the approach of Zhang et al. (2019). After heat exposure, Petri dishes were returned to standard rearing conditions (30 °C), where further development was monitored. 42, 44, and 50 °C were excluded due to rapid mortality. As in the constant heat shock experiments, larvae were checked daily at the same time to monitor the effects on some biological traits. All experiments with constant and variable heat shock were performed in three replicates with 10

larvae randomly selected from different populations at different times (30 larvae for each tested group). 30 °C was considered as control temperature while investigating the possible effects of thermal stress on *A. grisella* last instars.

Development and adult traits

The last instars (fifth instar) of *A. grisella* in plastic Petri dishes exposed to thermal stress in the form of constant and variable heat shock were checked at the same time each day. After emergence, female adults were weighed using an analytical balance (Model KERN ABJ-NM/ABS-N, Balingen, Germany) with a precision of 0.0001 mg, individually placed into 80-mL glass cups whose openings were covered with gauze, over which a white paper sheet was placed as an oviposition substrate. Subsequently, all cups were kept in the same room with the stock cultures and checked daily. The longevity of virgin females (individuals isolated from the larval stage onward and never exposed to males) was measured as the time elapsed between adult emergence and death. The number of eggs produced was calculated as the total number of eggs laid by each female throughout her lifespan. Females were allowed to lay eggs on a paper surface placed over the gauze covering the cups. Since females oviposited exclusively on this paper surface, egg collection was performed by replacing the papers daily without direct handling of the insects, and the eggs on the papers were counted under a microscope (Olympus CX21, Japan).

Immune assays

The immune response of *A. grisella* under thermal stress was evaluated at four temperatures (30, 34, 36, and 38 °C), which were determined based on earlier variable heat shock exposures (30–40 °C). The selection was guided by the thermal stress responses of last instars larvae, LTemp values, and temperature-dependent changes in biological traits. Accordingly, the control temperature of 30 °C was included as the initial temperature, from which higher temperatures were subsequently applied. Temperature selection was based on the lifespan (in hours) of individual last instar larvae under heat stress: the first temperature at which mortality occurred without pupation (34 °C), the first temperature at which all individuals died in the larval stage (36 °C), and 38 °C, as despite all individuals perishing as larvae, their lifespan was similar to those at the control temperature. In conclusion, as in the constant and variable heat shock experiments, *A. grisella* last instars of similar weight (35.7 ± 2.2 mg) placed individually into the plastic Petri dishes and exposed to thermal stress for 2, 24 and 48 h at four different temperatures (30, 34, 36, and 38 °C). Each immune parameter (total and differential hemocyte count, mitotic index, and encapsulation response) experiment was repeated four times with a total of 16 larvae (four per replicate).

Total and differential hemocyte counts and mitotic index

Hemolymph samples were taken from the last instars of *A. grisella* after 2, 24, and 48 h to examine the effects of temperature treatments (30, 34, 36, and 38 °C) on total hemocyte count (THC) in circulation, as a measure of cellular immunity. For this purpose, the larvae were punctured with a sterile 19-gaugle needle above their first right hind legs, and 4 µL of hemolymph was collected from each insect using a microcapillary tube (Sigma). The obtained hemolymph sample was transferred to 0.5 mL Eppendorf tubes containing 36 µL of anticoagulant buffer (0.098 M NaOH, 0.186 M NaCl, 0.017 M Na_2EDTA, and 0.041 M citric acid, pH = 4.5), which were kept in an ice-cold environment, and gently mixed with an automatic pipette. In this way, 10 µL was taken from the 1:10 diluted cell suspension with the buffer using an Eppendorf brand automatic pipette (0.5–10 µL) and loaded onto a Neubauer hemocytometer (Improved Neubauer Hemocytometer; Superior, Germany) with a depth of 0.100 mm, and the cells were counted under an Olympus BX51 (Japan) brand microscope.

In order to investigate the effects of thermal stress on differential hemocyte count (DHC), last instars were punctured at the same site as in THC experiment and 5 µL of hemolymph was collected from each insect after 2, 24, and 48 h. The collected hemolymph was immediately spread on sterile slides and left to dry at room temperature to allow the hemocytes to adhere to the glass. After ensuring that the hemocytes were dry, 0.2 mL of methanol (Sigma) was added onto the dried slides, which were placed in a horizontal position, to fix the cells, and the preparations were

left at room temperature for another 10 min. At the end of this period, the excess methanol was drained from the slides and the preparations were left to dry again. The fixed hemocytes were stained by immersing the slides freshly prepared Giemsa dye solution (57 mL PBS + 3 mL Giemsa dye (Merck, Giemsa's Azure Eosine Methylene Blue Solution, pH = 7.4)) for 15 min during each staining procedure. The stained slides were then removed from the dye solution one by one, first rinsed with distilled water and then with PBS, and left to dry in a vertical position for 24 h. After completing all fixation, staining, and drying procedures, DHC was determined by examining the dried preparations under an Olympus BX51 microscope. Hemocyte types were identified based on morphological characteristics described by Er et al. (2010). While determining DHC, the number of hemocytes in the mitotic phase was also assessed. DHC and the mitotic index (MI) data in the preparations made using 5 µL of hemolymph from each last instar were evaluated as percentage.

Encapsulation

The effect of thermal stress on the encapsulation behavior of hemocytes, an immune parameter formed by hemocytes, was investigated in *A. grisella*. Sephadex G-100 Superfine (Sigma) beads (20–50 µm in diameter) were used to assess encapsulation levels. In addition, a 5 µL microsyringe with a replaceable tip and a 32-gauge needle was used during the injection of the bead solution into the last instars. Before injection, beads were stained red in a 1% Congo red solution (Sigma) prepared in PBS (Wu et al., 2014). To ensure proper staining, beads were incubated for 24 h. After staining, the supernatant containing the dye-PBS mixture was removed from the stained bead sediment, and PBS was added to wash the beads (Richards & Parkinson, 2000). Then, 0.5 µL of bead solution was withdrawn. Since the beads in this solution could not be counted, 0.1 µL was taken from the bead-rich bottom layer and 0.4 µL from the upper layer containing PBS before injection into the last instars. After injection, the larvae were immediately placed in pre-heated incubators (30, 34, 36, and 38 °C) and exposed to these temperatures for 2, 24, and 48 h. At the end of these periods, the larvae were removed from the incubators and immediately dissected under an Olympus SZ61 stereomicroscope at 40× magnification. The recovered beads were transferred onto separate slides with a drop of PBS and then covered with a coverslip. These preparations were subsequently examined under an Olympus BX51 microscope to determine the encapsulation levels of hemocytes. The encapsulation response was categorized into three levels based on the hemocyte layers surrounding the beads: absent (no or minimal hemocyte accumulation around the bead), weak (formation of 2–10 hemocyte layers), and strong (formation of 10 or more hemocyte layers) (Fig. 1).

Statistics

Mortality of *A. grisella* last instars under constant heat shock was analyzed using probit analysis to estimate LTemp values with 95% confidence limits (CL). The Kaplan–Meier method was employed to detemine LTime values ($LTime_{50, 99}$) at lethal temperatures. Cumulative mortality (95% Cl) values and the means of biological (larval spinning and pupal time, pupal period, adult emergence time, weight and longevity, and the number of eggs produced) and cellular immune parameters (THC, DHC, MI, and encapsulation) of *A. grisella* were compared using one-way

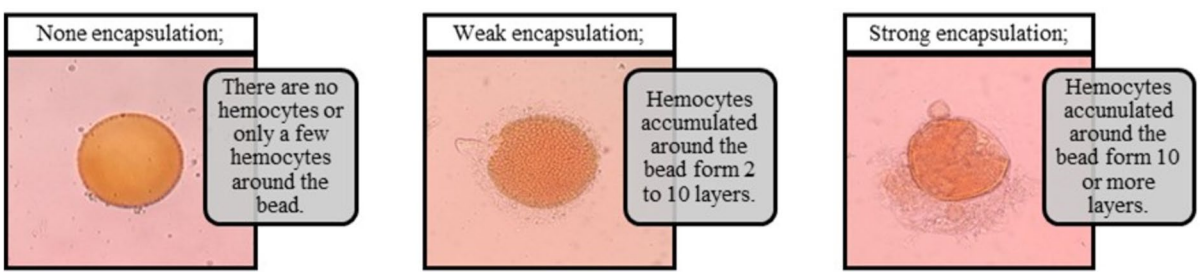


Fig. 1 Encapsulation stages in last instar larvae of *A. grisella*

analysis of variance (ANOVA), as the assumptions for the parametric tests were met. Tukey's post hoc (HSD) test was applied for multiple comparisons where variance homogeneity was satisfied. Also, a multivariate analysis of variance (MANOVA) was conducted to assess temperature effects on multiple continuous life-history traits simultaneously and to account for potential relationships among these variables. Prior to MANOVA, the assumption of equality of covariance matrices was tested using Box's test. Because this assumption was violated, Pillai's Trace was used as the most robust multivariate test statistic. When significant multivariate effects were detected, subsequent univariate analyses were conducted to evaluate temperature effects on each life-history trait individually. Data for THC, DHC, and MI also were subjected to two-way ANOVA to determine the main effects of temperature, time, and their interaction on these values. All statistical analyses were conducted using the SPSS software package (version 18.0 for Windows, SPSS Science, Chicago, IL). Results were considered statistically significant at $P < 0.05$.

Results

Mortality

Three different thermal zones, representing lethal, suboptimum, and optimum temperature ranges, were used to assess the reactions of *A. grisella* exposed to constant heat shock from the last instar larvae (Table 1). Prior to assessing this, we observed that the last instars perished within 1–2 h at 46–48 °C and 0.33–2 h at 50 °C. Therefore, to reduce redundancy and focus on the suboptimum-to-lethal transition range, 50 °C, the first temperature at which death occurred within minutes, was selected as a representative lethal temperature in subsequent experiments. Although complete mortality was also observed at 44 °C, the deaths were more temporally distributed (at 60 and 120 min), in contrast to the rapid mortality observed at 50 °C, where most individuals died within 48 min. Therefore, 44 °C was categorized as suboptimal. At lower high temperatures, survival times were about 8–10 h at 42 °C, 9–10 h at 40 °C, and 6–15 days at 38 °C and 36 °C. The temperatures between 30 and 34 °C were found to be optimal for growth.

Figure 2 shows the mean lifespan in hours for an individual larva subjected to constant heat shock at lethal, suboptimum, and optimal temperatures after three replicates (30 larvae for each tested group) ($F^{8,\ 261} = 354.590, P = 0.000$). All last instars exposed to lethal and suboptimal temperatures (36–50 °C) died before pupation. In contrast, at 34 °C, which is considered an optimal temperature, 33.33% of individuals successfully pupated, whereas 30% died during the pupal stage. The remaining last instars managed to reach the adult stage at 34 °C as well as at the other optimum temperatures of 32 and 30 °C. When the thermal stress-related lifespan (hours) of *A. grisella* last instars were examined, although there was an increase at 32, 34, and 36 °C compared to 30 °C, only 34 and 36 °C were found to be significant. A temperature of 38 °C appears to have a statistically comparable lifespan to 30 °C, but each individual completed its life cycle at 30 °C, whereas all individuals died while still in the larval stage at 38 °C. Moreover, the last instars survived significantly longer at 30, 32, 34, 36, and 38 °C than at 40 °C and above, with mean survival times decreasing sharply from 11.80 ± 0.55 h at 40 °C to 0.74 ± 0.11 h at 50 °C.

Table 1 The responses of *A. grisella* last instars to thermal stress

Zone	Temp. (°C)	Effect[a]
Lethal	50 ≤	It kills within minutes. (20–120 min)
Suboptimum	44	It kills within hours. (1–2 h)
Suboptimum	42	It stops development. It kills within hours. (8–10 h)
Suboptimum	40	It stops development. It kills within hours. (9–10 h)
Suboptimum	38	It stops development. It kills within days. (6–15 days)
Suboptimum	36	It stops development. It can live as a larva for days. (Sometimes it can stay as a larva for 1 month)
Optimum	34–30	Suitable temperatures for development

[a]Responses to thermal stress can vary depending on the insect species, the developmental stage during heat exposure, and the ambient relative humidity

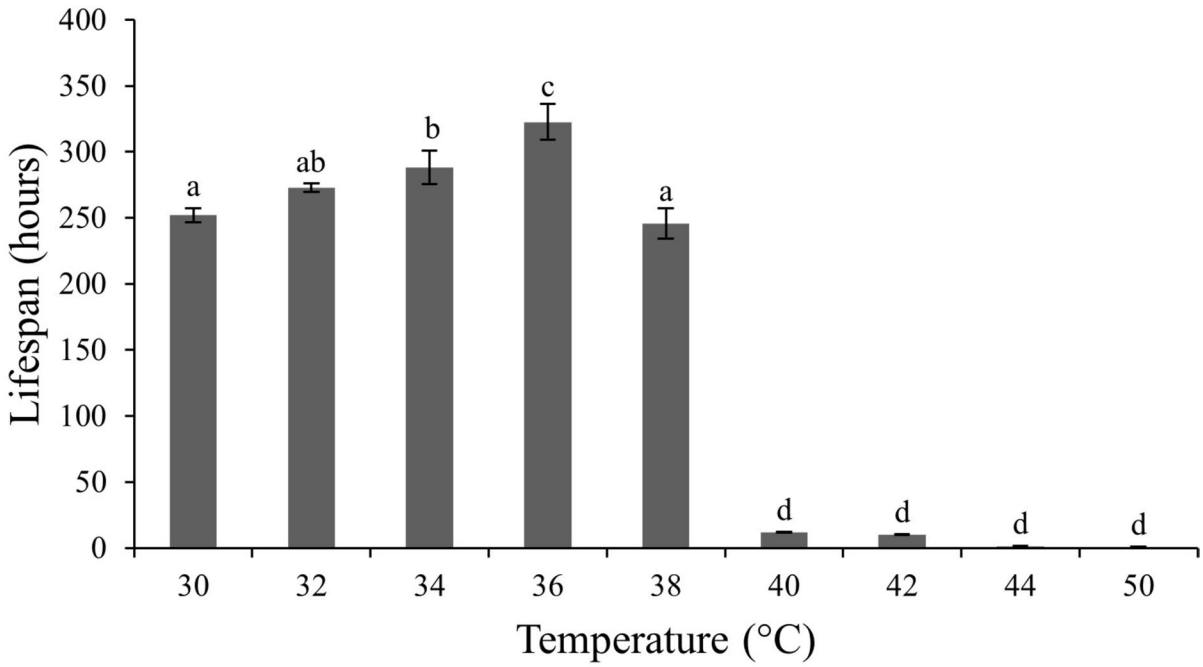


Fig. 2 Heat shock-related changes on the lifespan of *A. grisella*. The same letters are not significantly different (P > 0.05; Tukey's HSD test; d (days)). Data are average of three replicates

The mean adult emergence time at 30 °C (252 h = 10.5 days) was used as a baseline to determine lethal temperature (LTemp), lethal time (LTime), and cumulative mortality in *A. grisella* subjected to constant heat shock during the last instar stage. The $LTemp_{50}$ and $LTemp_{99}$ values obtained from the mortality probit lines are displayed in Table 2 as 36.62 °C (95% CL: 34.29–38.45 °C) and 43.02 °C (95% CL: 40.18–57.59 °C), respectively. For the temperatures (34, 36, 38, 40, 42, 44, and 50 °C) at which larval death was noted under thermal stress, LTime values were calculated using the Kaplan–Meier method (Table 3). Although the $LTemp_{50}$ value for 36 °C could not be computed, it was calculated as 240 h for both 34 and 38 °C. At 36 °C, mortality did not reach 50% during the observation period, making $LTime_{50}$ estimation statistically unfeasible. At temperatures above 38 °C, $LTime_{50}$ was considerably reduced. In addition, the temperature with the longest $LTime_{99}$ occurred at 36 °C (423.2 h), whereas the shortest $LTime_{99}$ occurred at 50 °C (0.74 h) (Table 3). The studies of the thermal stress-related cumulative mortality data of *A. grisella* revealed significant variance in all larval death temperatures (Table 4). The effect of exposure time on cumulative mortality (%) was evaluated for each temperature (Fig. 3). At 34, 36, and 38 °C, the first mortality occurred several days after exposure, whereas

Table 2 Thermal stress dependent LTemp (°C) values of *A. grisella*

	N[a]	X^2 (df)	Slope ± SE	Lethal Temp. (°C) (lower–upper)[b]
Effect of temp. (°C)	240	18.129(5)	33.251 ± 4.439	$LTemp_{50}$ = 36.615 (34.292–38.453) $LTemp_{99}$ = 43.016 (40.182–57.585)

[a]Total number of insects used at the heat shock

[b]Values are displayed with the lower and upper confidence limits. Y(probit) = −51.993 + 33.251 (log dose)

N; Number of individuals

Table 3 Thermal stress dependent LTime values of *A. grisella*

Lethal time estimates (hour)[a]						
Temp. (°C)	N	n	$LTime_{50}$ (95% Cl)[b]		$LTime_{99}$ (95% Cl)	
			Lower–Upper	$\bar{x} \pm SE^*$	Lower–Upper	$\bar{x} \pm SE^*$
34	30	13	n.a.-n.a	240.000 ± n.a	239.554–321.676	280.615 ± 20.95
36	30	7	n.a.-n.a	n.a. ± n.a	386.328–460.072	423.200 ± 18.81
38	30	16	n.a.-n.a	240.000 ± n.a	244.021–304.779	274.400 ± 15.50
40	30	30	10.525–11.475	11.000 ± 0.24	10.711–12.889	11.800 ± 0.56
42	30	30	9.468–10.532	10.000 ± 0.27	9.797–10.603	10.200 ± 0.21
44	30	30	n.a.-n.a	1.000 ± n.a	1.222–1.578	1.400 ± 0.09
50	30	30	0.430–0.570	0.500 ± 0.036	0.527–0.951	0.739 ± 0.11

[a]Time required to kill 50% ($LTime_{50}$) and 99% ($LTime_{99}$) of insects

[b]Cl – confidence limits

$\bar{x} \pm SE^*$; mean value ± standard error of the mean.

n.a.; Value not available, $LTime_{50}$ could not be estimated because mortality did not reach 50%

N; Number of individuals, n; Number of dead larvae

Table 4 Effect of exposure time on cumulative mortality at different temperatures

Temperature (°C)	F	df	P
34	95.571	7, 16	0.000
36	36.177	7, 16	0.000
38	217.796	7, 16	0.000
40	22.307	7, 16	0.000
42	239.393	7, 16	0.000
44	1.000	7, 16	0.466
50	1.000	7, 16	0.466

F; Test statistic, df; Degrees of freedom, P; Probability value. Differences were considered statistically significant at P<0.05

at 40 and 42 °C mortality began within hours. At 44 and 50 °C, mortality occurred within the first hour. Furthermore, it was clearly observed that cumulative mortality rose as temperature exposure time increased.

Impact of constant and variable heat shock on development, longevity, weight, and the number of eggs.

MANOVA was conducted to evaluate the effects of temperature on the combined life-history traits of *A. grisella*. Developmental time, adult weight, and egg production were included as dependent variables for constant heat shock experiments. Because Box's test of equality of covariance matrices was significant ($P = 0.000$), Pillai's Trace was used as the most robust test statistic. The analysis revealed a significant multivariate effect of temperature on the combined life-history variables (Pillai's Trace, $F^{14, 138} = 7.008, P = 0.000$), indicating that constant heat shock significantly influenced overall biological performance. Subsequent univariate analyses were performed to examine temperature effects on each trait individually.

Figures 4, 5, 6, 7, 8, 9 display the larval spinning and pupal time, pupal period, and adult emergence time, weight, longevity, and the number of eggs produced under the effects of constant and variable heat shock on *A. grisella*. The larval spinning time ($F^{2, 74} = 17.797, P = 0.000$) and pupal time ($F^{2, 74} = 23.466, P = 0.000$) of *A. grisella* exposed to constant heat shock from last instars extended significantly at 34 °C when compared to 30 and 32 °C. On the other hand, the application of thermal stress induced fluctuations in pupal period ($F^{2, 74} = 3.733, P = 0.029$) and adult emergence time ($F^{2, 74} = 2.601, P = 0.081$). Among these variations, the decrease at 34 °C compared to 32 °C was statistically significant only for the pupal period (Fig. 4). In addition, the reduction in adult longevity at 34 °C was significant compared with 30 and 32 °C ($F^{2, 74} = 10.613, P = 0.000$). Although there were modest variations in the

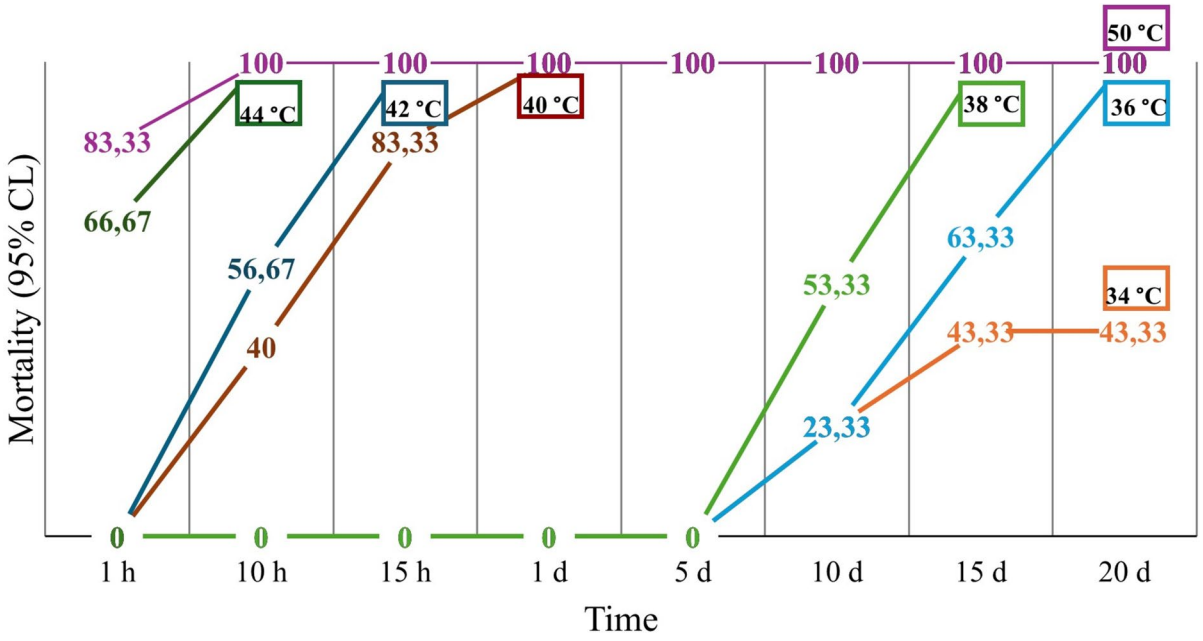


Fig. 3 The constant heat shock-related changes on the cumulative mortality (95% Cl) of *A. grisella*. Mortality rates recorded for each temperature at specified times (d (days), h (hours)). The studies were continued until all insects died

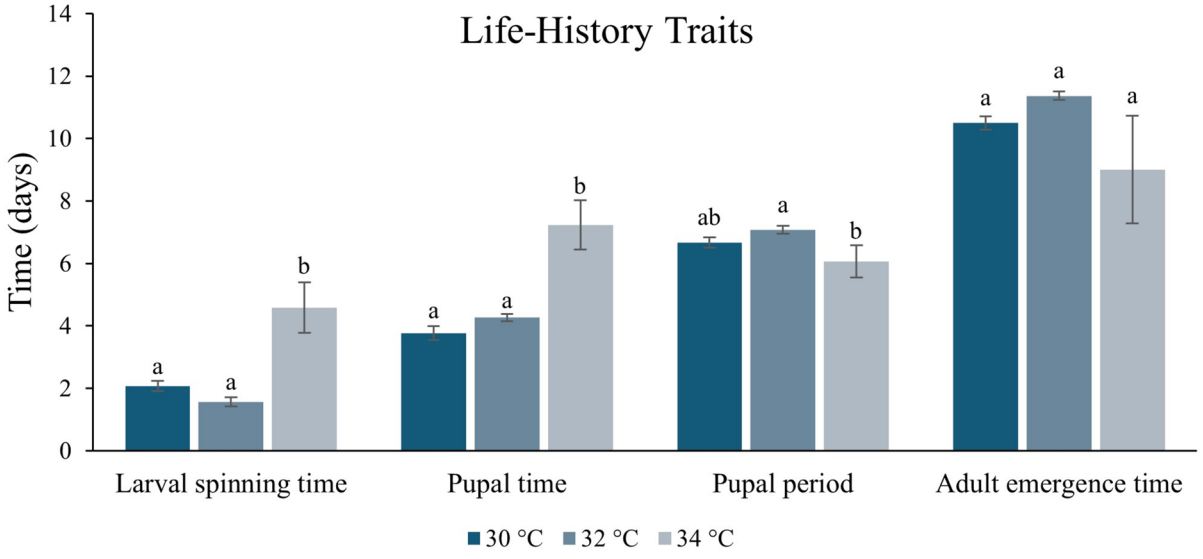


Fig. 4 The constant heat shock-related changes on the larval spinning and pupal time, pupal period, and adult emergence time of *A. grisella*. The same letters are not significantly different (P > 0.05; Tukey's HSD test; d (days)). Data are average of three replicates

weight of females at different temperatures ($F^{2,\ 74} = 1.060, P = 0.352$), the number of eggs produced tended to be higher at 32 °C compared to other temperatures, with the difference approaching statistical significance ($F^{2,\ 74} = 2.532, P = 0.086$). However, these differences in egg production and female weight were not statistically significant (Figs. 5 and 6).

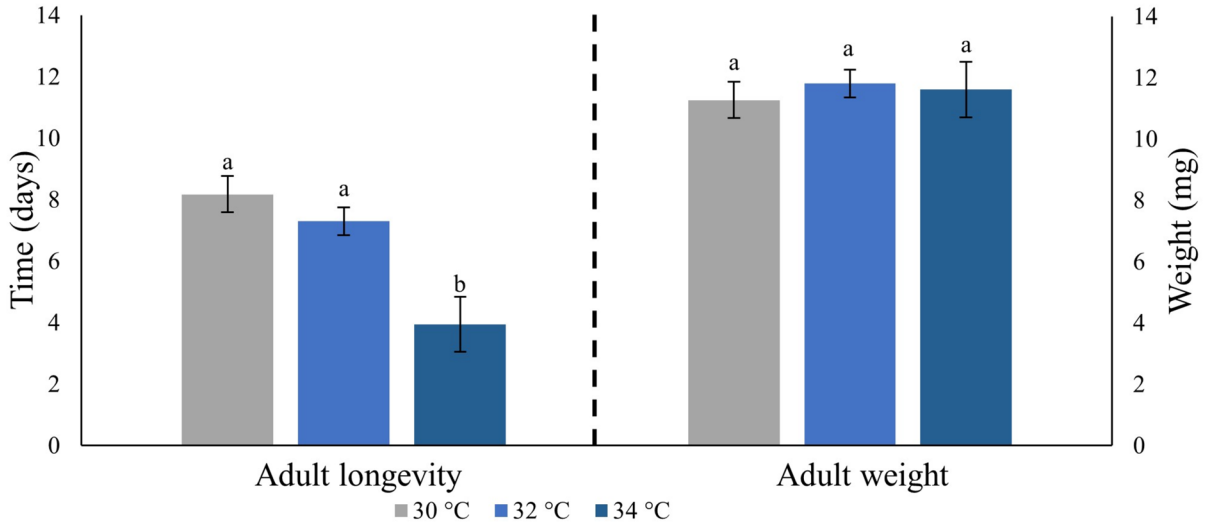


Fig. 5 The constant heat shock-related changes on adult longevity (days) and weight (mg) of *A. grisella*. The same letters are not significantly different (P > 0.05; Tukey's HSD test; d (days)). Data are average of three replicates

MANOVA was performed to assess the effects of temperature treatments on the combined life-history traits of *A. grisella*. For variable heat shock experiments, egg production data were excluded from the MANOVA because the presence of zero values in several treatment groups violated multivariate analysis assumptions and prevented reliable estimation of covariance matrices. Box's test of equality of covariance matrices was significant ($P = 0.000$), suggesting violation of the homogeneity assumption; therefore, Pillai's Trace was considered for interpretation due to its robustness. The MANOVA results indicated a significant multivariate effect of temperature treatments

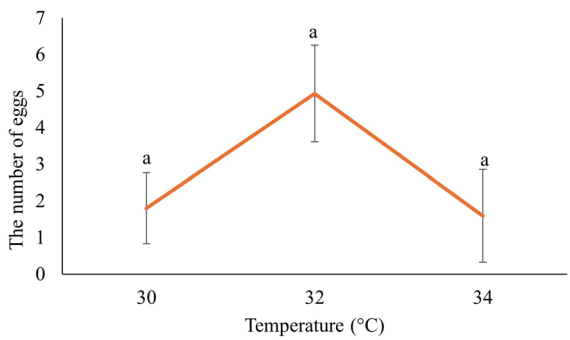


Fig. 6 The constant heat shock-related changes in the number of eggs of *A. grisella*. The same letters are not significantly different (P > 0.05; Tukey's HSD test; d (days)). Data are average of three replicates

(Pillai's Trace, $F^{30,\ 865} = 3.340, P = 0.000$), demonstrating that variable heat shock significantly affected the overall biological responses of the larvae.

The larval spinning and pupal time, pupal period, and adult emergence time of *A. grisella* were significantly altered by variable heat shock treatments, as shown in Fig. 7. As a result of variable heat stress, the spinning time of the larvae increased significantly at 38 and 40 °C compared to 30 and 32 °C. There is also a considerable rise at 34 °C compared to 32 °C ($F^{5,\ 174} = 6.895, P = 0.000$). Furthermore, the pupal period was longest at 38 °C, with a mean duration of 7.87 ± 0.164 days, but the difference was statistically significant only when compared with the 30 °C group ($F^{5,\ 174} = 2.953, P = 0.014$). Pupal time ($F^{5,\ 174} = 9.458, P = 0.000$) and adult emergence time ($F^{5,\ 174} = 9.565, P = 0.000$) were significantly longer at 40 °C than at all other doses (30, 32, 34, 36, and 38 °C). The increase at 38 °C compared to 32 °C was also significant for the adult emergence time. Adult longevity was significantly shorter at 40 °C than at 30, 32, and 36 °C ($F^{5,\ 174} = 3.288, P = 0.007$) (Fig. 8). In addition, the weight of females obtained from variable heat-stressed larvae at 40 °C was considerably lighter than those at lower temperatures except at 30 °C ($F^{5,\ 174} = 4.722, P = 0.000$) (Fig. 8). The number of eggs produced of females reaching the adult stage under thermal stress had the highest value at 34 °C. It was observed that efficiency decreased prominently

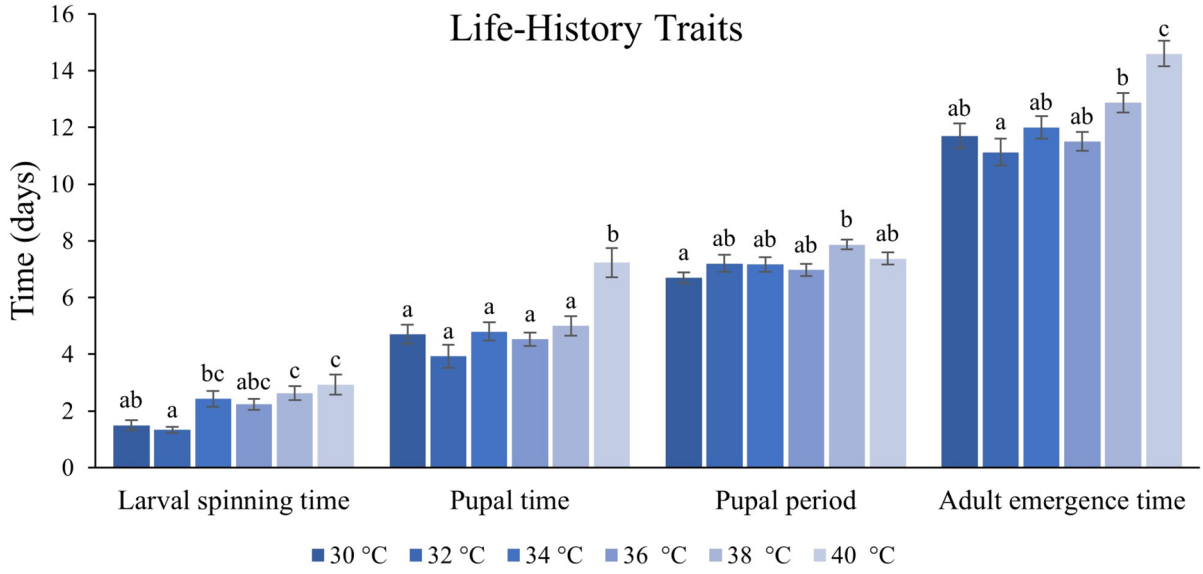


Fig. 7 The variable heat shock-related changes on the larval spinning and pupal time, pupal period, and adult emergence time of *A. grisella*. The same letters are not significantly different (P > 0.05; Tukey's HSD test; d (days)). Data are average of three replicates

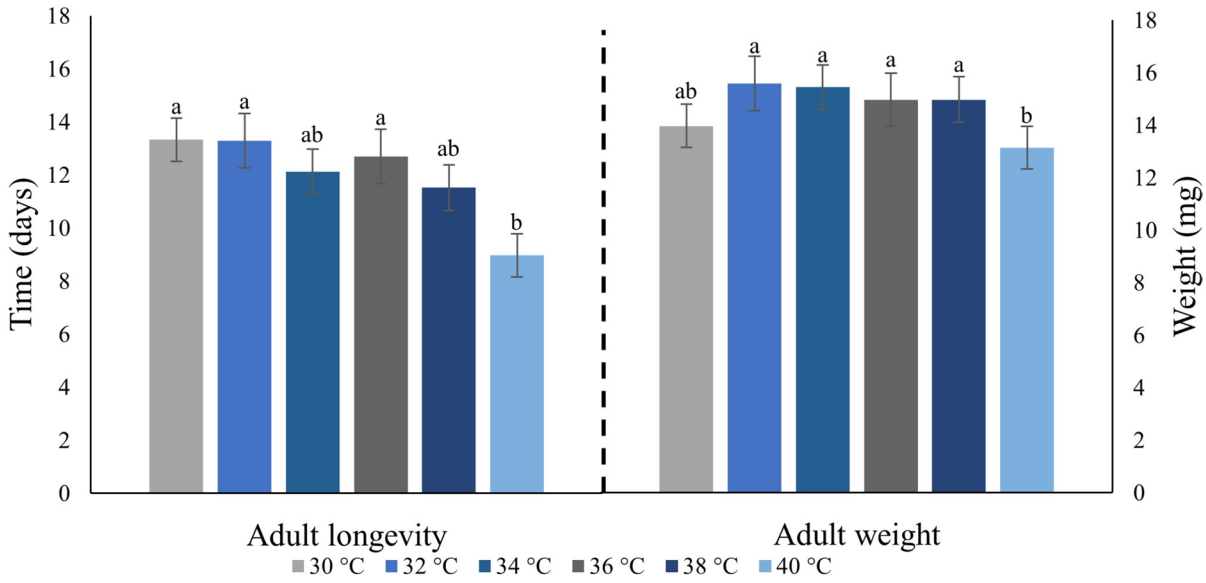


Fig. 8 The variable heat shock-related changes on adult longevity (days) and weight (mg) of *A. grisella*. The same letters are not significantly different (P > 0.05; Tukey's HSD test; d (days)). Data are average of three replicates

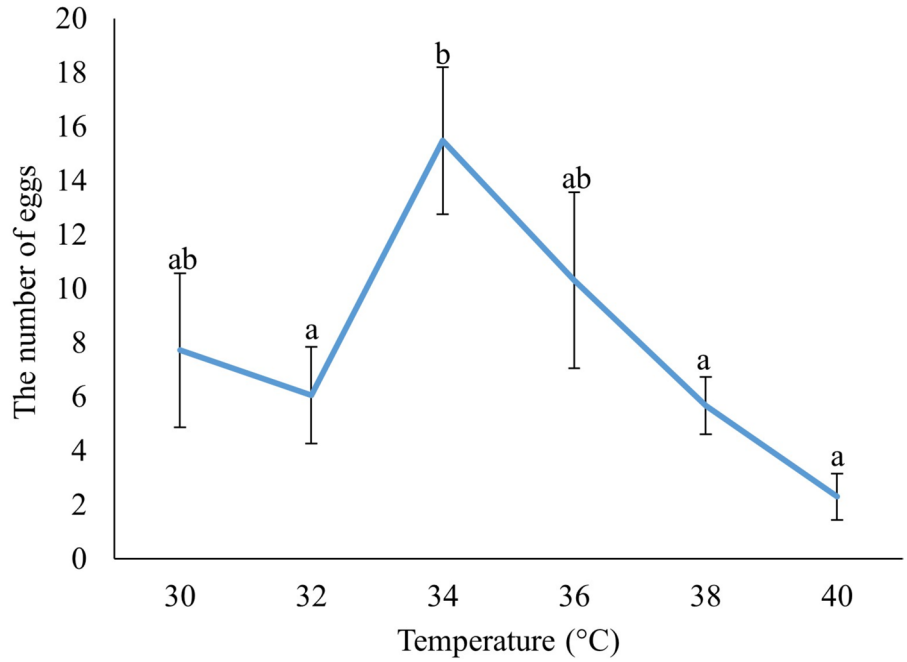


Fig. 9 The variable heat shock-related changes in the number of eggs of *A. grisella*. The same letters are not significantly different (P > 0.05; Tukey's HSD test; d (days)). Data are average of three replicates

at temperatures below and above 34 °C. Statistically, the increase at 34 °C was only significant compared to 32 °C, 38 °C and 40 °C ($F^{5, 121} = 4.608, P = 0.001$) (Fig. 9).

Impact of time-dependent thermal stress on cellular immune responses

Total hemocyte counts

The impact of varying temperature treatments and three distinct time points of THC in *A. grisella* last instars is presented in Table 5. When the changes in THC were evaluated according to the hours of observation, the highest hemocyte count was detected at 24 h for 30 °C, with a significant decrease in cell numbers after 2 and 48 h. In contrast, at 34, 36 and 38 °C, the highest THC was obtained at 2 h, and hemocyte numbers decreased with increasing exposure time. When these decreases in THC were statistically evaluated, they were significant at 34 °C only at 24 h compared to 2 h, at 36 °C after 24 and 48 h compared to 2 h, and at 38 °C after 48 h compared to 2 and 24 h (Table 5). Comparisons of

Table 5 Thermal stress and time-dependent changes in total hemocyte counts ($\times 10^6$ cell/mL) of *A. grisella* larvae

Temperature (°C)	Total hemocyte count ($\times 10^6$ cell/mL) ($\bar{x} \pm$ SE)*			Statistic (ANOVA)		
	Experiment observation hours					
	2	24	48	F	df	P
30	18.38 ± 0.63ax	22.30 ± 1.07aby	12.76 ± 0.79az	32.123	2, 45	0.000
34	25.38 ± 0.75bcx	22.23 ± 0.58aby	23.99 ± 1.21bxy	3.173	2, 45	0.051
36	23.60 ± 0.94bx	18.31 ± 0.68ay	20.46 ± 1.02by	8.958	2, 45	0.001
38	27.89 ± 0.74cx	24.25 ± 1.68bx	12.55 ± 1.59ay	32.649	2, 45	0.000

* Numbers in columns (a-c) and rows (x–y) followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). Data are average of four replicates (Total of 16 individual). SE; Standard error

temperature treatments at each observation time revealed significant fluctuations in THC after 2 h ($F^{3, 60}=27.164$,*P=0.000*), 24 h ($F^{3, 60}=5.224$,*P=0.003*), and 48 h ($F^{3, 60}=23.180$,*P=0.000*). After 2 h of exposure, hemocyte counts increased significantly at all temperatures compared to 30 °C, and the increase at 38 °C compared to 36 °C was also significant. THC was significantly higher only at 38 °C compared to 36 °C at 24 h, whereas at 48 h the increase was significant at 34 and 36 °C compared to 30 and 38 °C (Table 5). The effect of heat stress on THC of last instars was temperature (*P=0.000*) and time (*P=0.000*) dependent, and the relationship between temperature and THC was significantly influenced by the time (*P=0.000*) (Table 6).

Table 6 ANOVAs of the effects of thermal stress, time, and their interactions on the total hemocyte counts of *A. grisella* larvae

Source	df	MS	F	P	r^2
Temperature	3	299.786	17.592	0.000	
Time	2	677.431	39.754	0.000	0.57
Temperature × Time	6	290.600	17.053	0.000	
Error	180	17.041			

Differential hemocyte counts

The effects of different temperature treatments and three experimental time points on differential hemocyte type ratios in *A. grisella* last instars are shown in Table 7. Morphologically, the hemocytes of *A. grisella* were classified into granulocytes, plasmatocytes, spherulocytes, and other cell types (oenocytoids and prohemocytes) (Fig. 10). When the changes in granulocytes were evaluated according to observation time, there was a significant increase only at 48 h compared to 24 h for 30 and 34 °C. At 36 °C, granulocyte numbers rose considerably after 2 and 48 h compared to 24 h. The granulocyte ratio decreased with increasing exposure time at the highest temperature of 38 °C, but the decrease was only significant at 48 h compared to

Table 7 Thermal stress and time-dependent changes on differential hemocyte counts and mitotic index (% cell/5 µL) of *A. grisella* larvae

Hemocyte type	Temp (°C)	Differential hemocyte count and mitotic index ($\bar{x}\pm$SE)*			Statistic (ANOVA)		
		Experiment observation hours					
		2	24	48	F	df	P
GR	30	18.95 ± 2.30axy	12.64 ± 1.53ax	19.96 ± 2.41aby	3.641	2, 45	0.034
	34	24.65 ± 1.39axy	20.08 ± 1.56bx	26.65 ± 1.72ay	4.682	2, 45	0.014
	36	22.84 ± 1.30ax	16.43 ± 1.46aby	21.70 ± 1.72ax	5.209	2, 45	0.009
	38	24.11 ± 1.65ax	19.01 ± 2.94abxy	13.68 ± 1.86by	6.617	2, 45	0.003
PL	30	75.83 ± 2.44ax	76.03 ± 1.88ax	57.68 ± 2.17ay	21.909	2, 45	0.000
	34	62.90 ± 1.88bx	58.01 ± 1.75bxy	50.13 ± 3.24aby	7.309	2, 45	0.002
	36	65.53 ± 1.83bx	63.08 ± 2.28bx	42.60 ± 2.95by	26.594	2, 45	0.000
	38	61.43 ± 2.17bx	46.41 ± 3.65cy	56.13 ± 2.01ax	7.544	2, 45	0.001
SP	30	2.66 ± 0.29ax	6.57 ± 0.93ay	9.53 ± 1.74ay	13.719	2, 45	0.000
	34	7.79 ± 1.00bx	17.11 ± 2.96bx	16.38 ± 4.22ax	3.147	2, 45	0.053
	36	6.81 ± 0.82bx	16.37 ± 2.53by	31.42 ± 4.10bz	23.436	2, 45	0.000
	38	8.27 ± 1.32bx	30.45 ± 3.64cy	27.56 ± 2.84by	24.090	2, 45	0.000
OCT	30	0.42 ± 0.08ax	0.55 ± 0.04ax	1.84 ± 0.38ay	16.659	2, 45	0.000
	34	0.80 ± 0.14bcx	1.47 ± 0.18by	1.97 ± 0.22ay	14.242	2, 45	0.000
	36	0.52 ± 0.05abx	1.15 ± 0.20aby	1.60 ± 0.29ay	7.153	2, 45	0.002
	38	1.17 ± 0.14cx	1.74 ± 0.48bx	1.26 ± 0.20ax	0.692	2, 45	0.506
MI	30	2.14 ± 0.35ax	4.21 ± 0.38ax	10.99 ± 1.84ay	21.270	2, 45	0.000
	34	3.86 ± 0.29bx	3.33 ± 0.41abx	4.87 ± 0.53bx	2.632	2, 45	0.083
	36	4.30 ± 0.19bx	2.98 ± 0.31abxy	2.70 ± 0.42bcy	6.080	2, 45	0.005
	38	5.02 ± 0.45bx	2.39 ± 0.30by	1.37 ± 0.30cz	29.672	2, 45	0.000

* Numbers in columns (a-c) and rows (x–y) followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). Data are average of four replicates (Total of 16 individual). SE; Standard error. GR; Granulocytes, PL; Plasmatocyte, SP; Spherulocytes, OCT; Other cell types, MI; Mitotic index

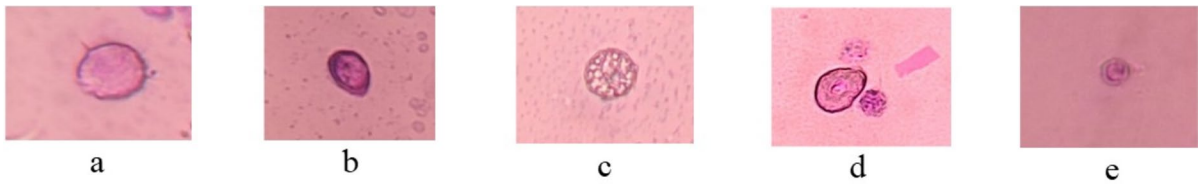


Fig. 10 Hemocyte type of *A. grisella*; **a**) Granulocytes, **b**) Plasmatocytes, **c**) Spherulocytes, **d**) Oenocytoid, and **e**) Prohemocyte

2 h (Table 7). Comparative analysis of granulocyte ratios revealed significant differences among temperature treatments at 24 h ($F^{3, 60}=3.335$,*P* = *0.025*) and 48 h ($F^{3, 60}=8.213$,*P* = *0.000*) except for 2 h ($F^{3, 60}=2.758$,*P* = *0.050*). The highest granulocyte value was at 34 °C at all observation times, while the lowest values were at 30 °C for 2 and 24 h and at 38 °C for 48 h. Temperature-dependent changes were not significant at 2 h, but at 24 h only increases at 34 °C relative to 30 °C and at 48 h only increases at 34 and 36 °C relative to 38 °C were significant (Table 7).

Evaluating plasmatocyte ratios in last instars across different observation times revealed a significant decrease after 48 h compared to 2 and 24 h at 30 and 36 °C. The number of plasmatocytes peaked at 34 °C after 2 h decreased with increasing exposure time, but the decrease was only significant at 48 h compared to 2 h. At 38 °C, the increases at 2 and 48 h compared to 24 h were also significant (Table 7). Comparison of plasmatocyte ratios across different temperature treatments revealed significant fluctuations at 2 h ($F^{3, 60}=10.113$,*P* = *0.000*), 24 h ($F^{3, 60}=24.168$,*P* = *0.000*), and 48 h ($F^{3, 60}=6.713$,*P* = *0.001*). The highest number of plasmatocytes was recorded after 24 h at 30 °C across all observation periods. These higher values at 30 °C were statistically significant for 2 and 24 h compared to all other temperatures (34, 36 and 38 °C). The decrease observed after 24 h at 38 °C compared to 34 and 36 °C was also significant. After 48 h, similar plasmatocyte ratios were obtained at 30 and 38 °C, and the value at 36 °C was significantly lower than these two temperatures (Table 7).

Analysis of spherulocyte counts across different time points revealed that the lowest number among all experimental groups was recorded after 2 h at 30 °C. The ratio of spherulocytes increased with prolonged exposure time at 30 and 36 °C, and this increase was significant at 24 and 48 h compared to 2 h. Furthermore, the increment observed at 48 h compared to 24 h for 36 °C was also significant. Although not statistically important, spherulocyte values of last instars exposed to 34 °C prominently increased at 24 and 48 h compared to 2 h. At the highest experimental temperature of 38 °C, only the increase at 24 and 48 h was important compared to 2 h (Table 7). Significant fluctuations in spherulocyte ratios were detected among temperature treatments at 2 h ($F^{3, 60}=10.311$,*P* = *0.000*), 24 h ($F^{3, 60}=14.384$,*P* = *0.000*), and 48 h ($F^{3, 60}=11.262$,*P* = *0.000*). Spherulocyte ratios increased significantly at all other temperatures compared to 30 °C for 2 and 24 h. The increment observed after 24 h at 38 °C compared to 34 and 36 °C was also significant. After 48 h, only the increases at 36 and 38 °C compared to 30 and 34 °C were significant (Table 7).

Evaluating the ratio of other cell types (OCT) in last instars according to observation time revealed that the OCT value increased considerably at 30 °C only at 48 h compared to 2 and 24 h and at 34 and 36 °C only at 24 and 48 h compared to 2 h. Time-dependent changes were not statistically important at 38 °C (Table 7). Comparisons of temperature treatments at each observation time revealed significant fluctuations in OCT after 2 h ($F^{3, 60}=10.406$,*P* = *0.000*) and 24 h ($F^{3, 60}=5.881$,*P* = *0.001*) except for 48 h ($F^{3, 60}=1.530$,*P* = *0.216*). At 2 and 24 h, only the increases at 34 and 38 °C compared to 30 °C were significant. Furthermore, the increment at 38 °C compared to 36 °C after 2 h was also significant. Temperature-dependent changes were not statistically significant for 48 h (Table 7). The effect of heat stress on DHC (granulocyte, plasmatocyte, spherulocyte, and OCT) of *A. grisella* depended on both temperature (*P* = *0.000*) and time (*P* = *0.000*), and the relationship between temperature and DHC was significantly influenced by the time (granulocyte-*P* = *0.002*,

Table 8 ANOVAs of the effects of thermal stress, time, and their interactions on the differential hemocyte counts of *A. grisella* larvae

Hemocyte type	Source	df	MS	F	P	r^2
Granulocytes	Temp	3	0.070	7.747	0.000	
	Time	2	0.086	9.610	0.000	0.26
	Temp. × Time	6	0.032	3.573	0.002	
	Error	180	0.009			
Plasmatocytes	Temp	3	0.270	25.473	0.000	
	Time	2	0.400	37.797	0.000	0.53
	Temp. × Time	6	0.084	7.948	0.000	
	Error	180	0.011			
Spherulocytes	Temp	3	0.463	27.713	0.000	
	Time	2	0.784	46.890	0.000	0.53
	Temp. × Time	6	0.078	4.686	0.000	
	Error	180	0.017			
Others	Temp	3	0.008	6.227	0.000	
	Time	2	0.028	22.281	0.000	0.32
	Temp. × Time	6	0.004	3.506	0.003	
	Error	180	0.001			
Mitotic Index	Temp	3	0.033	10.448	0.000	
	Time	2	0.009	3.047	0.050	0.48
	Temp. × Time	6	0.065	20.882	0.000	
	Error	180	0.003			

plasmatocyte-$P = 0.000$, spherulocyte-$P = 0.000$, and OCT-$P = 0.003$) (Table 8).

Mitotic index

Analysis of MI in last instars across different time points revealed that MI increased with prolonged exposure time at 30 °C but decreased at 36 and 38 °C. When these changes were statistically evaluated, only the increase at 48 h compared to 2 and 24 h at 30 °C, only the decrease at 48 h compared to 2 h at 36 °C, and the decreases at 24 and 48 h compared to 2 h at 38 °C were significant. Furthermore, the decline observed at 48 h compared to 24 h for 38 °C was also important. However, the exposure time to 34 °C did not considerably affect the MI of hemocytes (Table 7). Comparative analysis of MI ratios revealed significant differences among temperature treatments at 2 h ($F^{3, 60} = 15.714$, $P = 0.000$), 24 h ($F^{3, 60} = 4.404$, $P = 0.007$), and 48 h ($F^{3, 60} = 21.390$, $P = 0.000$). The MI value increased with rising temperature in the 2 h but then showed a decreasing trend after 24 and 48 h. Statistically, the MI value at 30 °C was significantly lower than at all other temperatures after 2 h, but higher after 48 h. Furthermore, the increment observed at 34 °C compared to 38 °C for 48 h was also significant. At the end of 24 h, the only significant decrease was observed at 38 °C compared to 30 °C (Table 7). Two-way ANOVA results showed that the effect of heat stress on MI of *A. grisella* last instars was temperature ($P = 0.000$) and time ($P = 0.050$) dependent, and the relationship between temperature and MI was significantly influenced by the time ($P = 0.000$) (Table 8).

Encapsulation

The effects of different temperature treatments and three experimental time points on the three levels of bead encapsulation in last instars are presented in Table 9. Since the bead-injected larvae died within one day at the highest temperature of 38 °C, the encapsulation levels at this temperature could only be assessed for 2 h. Comparisons of different temperature treatments following bead injection at each observation time revealed significant variations in the number of unencapsulated

Table 9 Temperature-related changes on encapsulation (%) of Sephadex G-100 beads in *A. grisella* larvae

Temp. (°C)	Number of beads dissected			Extent of encapsulation (% ± SE)* (number of encapsulated beads.)								
				None			Weak			Strong		
	2 h	24 h	48 h	2 h	24 h	48 h	2 h	24 h	48 h	2 h	24 h	48 h
30	396	409	485	45.38 ± 4.93a (168)	11.93 ± 1.30a (49)	16.84 ± 1.17a (81)	45.62 ± 4.28a (189)	25.57 ± 1.37a (105)	49.78 ± 1.61a (242)	8.99 ± 1.28ab (39)	62.50 ± 1.36a (255)	33.39 ± 1.03ab (162)
34	272	256	303	24.13 ± 4.61b (67)	40.22 ± 6.99b (99)	26.26 ± 3.93ab (82)	41.23 ± 4.02a (118)	45.90 ± 5.92a (122)	29.65 ± 2.86b (94)	34.64 ± 4.11c (87)	13.87 ± 2.57b (35)	44.09 ± 4.30a (127)
36	243	162	213	37.11 ± 4.70ab (83)	54.97 ± 6.02b (88)	31.16 ± 4.10b (63)	36.56 ± 3.62a (86)	32.76 ± 6.28a (49)	38.70 ± 3.79b (78)	26.32 ± 5.38bc (74)	12.27 ± 3.45b (25)	30.14 ± 4.50b (72)
38	353	-	-	45.59 ± 5.09a (154)	-	-	50.11 ± 4.46a (182)	-	-	4.30 ± 1.54a (17)	-	-

* Numbers in columns (a-b) and rows (x–y) followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). Data are average of four replicates (Total of 16 individual). SE; Standard error

beads after 2 h ($F^{3,\ 60} = 4.980, P = 0.004$), 24 h ($F^{2,\ 45} = 11.971, P = 0.000$), and 48 h ($F^{2,\ 45} = 3.407, P = 0.042$) (Table 9). The number of unencapsulated beads decreased considerably at the end of the 2 h only at 34 °C compared to 30 °C. Furthermore, the increase observed at 38 °C compared to 34 °C was also important. After 24 h, there were significantly more unencapsulated beads only at 34 and 36 °C than at 30 °C. At the final time point (48 h), the number of unencapsulated beads obtained from the last instars was considerably higher only at 36 °C than at 30 °C (Table 9).

Comparisons of weakly encapsulated beads across different temperature treatments revealed insignificant fluctuations after 2 h ($F^{3,\ 60} = 1.844, P = 0.149$) and 24 h ($F^{2,\ 45} = 2.206, P = 0.122$). However, after 48 h ($F^{2,\ 45} = 12.063, P = 0.000$), significant reductions were only detected at 34 and 36 °C according to 30 °C (Table 9). Comparative analysis of strongly encapsulated bead ratios in last instars revealed that there were significant differences among temperature treatments at each of the following observation times: 2 h ($F^{3,\ 60} = 16.158, P = 0.000$), 24 h ($F^{2,\ 45} = 56.268, P = 0.000$), and 48 h ($F^{2,\ 45} = 4.024, P = 0.025$) (Table 9). By the end of the 2 h, the number of strongly encapsulated beads increased significantly only at 34 °C compared to 30 °C and at 34 and 36 °C compared to 38 °C. After 24 h, strongly encapsulated bead ratios were considerably lower only at 34 and 36 °C compared to 30 °C. After 48 h of thermal stress, the number of strongly encapsulated beads obtained from the last instars was significantly lower only at 36 °C than at 34 °C (Table 9).

Discussion

Survival and life-history traits

The study examined the developmental, biological, and cellular immune responses of last instar *A. grisella* larvae under various high temperature regimes. The effects of short-term thermal changes were examined by exposure to variable heat shock, and long-term thermal changes were examined by constant heat shock. Constant heat shock experiments led to the establishment of lethal, suboptimal, and optimal temperature zones for *A. grisella*. These experiments clarified the thresholds that support or inhibit vital

functions, as well as hinder or halt their development, similar to findings reported for other insect species (Burggren, 2012; Kwadha et al., 2017). *A. grisella* exhibited optimal development between 30–34 °C. Field observations support this, showing peak immature and adult densities at 29–30 °C, with activity declining under cooler conditions (e.g. 19 °C) (Fathy et al., 2017). Under constant heat shock, last-instar larvae survived up to 40 °C, but longevity dropped sharply at higher temperatures. In *Galleria mellonella* (Lepidoptera: Pyralidae), development is optimal at 30–38 °C, but survival drops sharply above 44 °C (Çelik Biçer et al., 2025a). This indicates divergent outcomes for *A. grisella* and *G. mellonella* in both short and long terms (Çelik Biçer et al., 2025a). In the short term, the insect's population may decline as temperatures rise beyond the threshold due to climate change, thereby decreasing its damage to bees and bee products. In the long term, longer larval stage because of elevated temperatures, which is the only feeding period in the life cycle of *A. grisella*, may increase damage within the honeycomb store. However, further behavioral studies are needed to confirm this assumption (Ellis et al., 2013). In both scenarios, the impact on *A. grisella* populations will likely manifest through alterations in the food chain and ecosystem functioning (Çelik Biçer et al., 2025a; Jeffs & Lewis, 2013; Sangle et al., 2015).

Acute lethal temperature (LTemp) and the duration required for mortality (LTime) are the primary parameters studied in insect thermal biology. Thermal stress has a lethal effect on *Bactrocera tau* (Diptera: Tephritidae) (Kells & Goblirsch, 2011), *Cimex lectularius* (Hemiptera: Cimicidae) (Kells & Goblirsch, 2011; Mellanby, 1935; Pereira et al., 2009), and *G. mellonella* (Çelik Biçer et al., 2025a). However, this effect varies depending on the temperature, exposure time, species, and the developmental stage at which the heat is applied. Consistent with the literature (Çelik Biçer et al., 2025a; Cui et al., 2008; Huang et al., 2020; Kells & Goblirsch, 2011; Liu et al., 2021; Mellanby, 1935; Mironidis & Savopoulou-Soultani, 2010; Pereira et al., 2009; Qin et al., 2018), in our study, 50 °C and above were determined lethal for *A. grisella*. Mortality in *A. grisella* and *G. mellonella* requires either longer exposure at comparable temperatures or higher thermal levels than reported for *C. lectularius* (Çelik Biçer et al., 2025a; Kells & Goblirsch, 2011; Mellanby, 1935; Pereira et al., 2009). However, *A. grisella* was more susceptible than *G. mellonella* (Çelik Biçer et al., 2025a), with mortality occurring at lower temperatures or shorter exposures. Under short-term (12 h) thermal stress, different stages of *B. tau* showed similar tolerance ($LTemp_{50}$ = 36.740 °C) to last instars of *A. grisella* ($LTemp_{50,\ 99}$ = 36.615 °C and 43.016 °C). However, our findings for *A. grisella* differed from the $LTemp_{50}$ values of *C. lectularius* ($LTemp_{50,\ 99}$ = 47.5 °C and 54.8 °C) (Kells & Goblirsch, 2011). Thus, lower temperatures (36.615 °C) would suffice to kill 50% of the last instar larvae of *A. grisella* compared to *C. lectularius*. Conversely, *G. mellonella* (Çelik Biçer et al., 2025a) last instars require higher temperatures to reach 50% mortality compared to *C. lectularius* (Kells & Goblirsch, 2011) and *B. tau* (Huang et al., 2020). This may suggest that *A. grisella* exhibits intermediate thermal tolerance characteristics among these species. Additionally, *G. mellonella* ($LTemp_{60}$ = 42.909 °C; $LTemp_{70}$ = 43.194 °C) exhibited a 60–70% mortality rate at the temperature at which 99% of *A. grisella* larvae ($LTemp_{99}$ = 43.016 °C) perished. By focusing on *A. grisella*, a species often overlooked in previous research, this study demonstrates that thermal lethality may vary depending on body mass, metabolic rate, and the developmental stage at which insects are exposed.

Consistent with previous studies on Lepidoptera (Çelik Biçer et al., 2025a; Liu et al., 2021), our data showed that prolonged exposure to lethal temperatures (36–50 °C) progressively reduced *A. grisella* survival. Achieving mortality of *C. lectularius* generally required 48 °C (71.5 min) or > 50 °C (0 min) in all room heat treatments (Kells & Goblirsch, 2011). For *Helicoverpa armigera* (Lepidoptera: Noctuidae) adults, at 40 °C and 46.5 °C, the $LTime_{90}$ was reported as 391.74 min, 12.40 min for males and 375.09 min and 12.46 min for females respectively (Mironidis & Savopoulou-Soultani, 2010). To kill almost all *G. mellonella* larvae ($LTime_{99}$), 516 h were required at 40 °C, 506 h at 42 °C, 20.9 h at 44 °C, and only 1 h at 50 °C (Çelik Biçer et al., 2025a). Evaluating the $LTime_{99}$ values of *A. grisella* at commonly used temperatures (40–50 °C) showed that this species is less heat tolerant than *G. mellonella*, which appears to be the most heat-tolerant wax moth under rising temperatures (Çelik Biçer et al., 2025a).

Nevertheless, both wax moths demonstrate notable thermal tolerance, although not as high as *C. lectularius* (Kells & Goblirsch, 2011). Consistent with the literature (Kells & Goblirsch, 2011; Mellanby, 1935; Pereira et al., 2009), our findings on *A. grisella* confirm that the time required for mortality decreases as temperature increases (Mironidis & Savopoulou-Soultani, 2010). Nevertheless, while their extended survival at moderately high temperatures may offer an ecological advantage under fluctuating thermal conditions, the relative fragility of *A. grisella* may allow the use of shorter heat shocks in biological control.

Exposing *A. grisella* last instar larvae to constant heat shock showed 100% survival at 30–32 °C, but survival dropped to 36.67% at 34 °C and no larvae survived at 36 °C or higher. This threshold suggests that 34 °C is a critical point where physiological stress may disrupt development, potentially through immune or enzymatic pathways (Ali et al., 2017; Ebrahimi et al., 2015; Ghasemi et al., 2013), although this warrants further investigation. Under variable heat shock, the survival rate remained at 100% between 30–40 °C; however, when the temperature exceeded 42 °C, all individuals died. Conversely, *G. mellonella* (Çelik Biçer et al., 2025a) could survive across a much wider temperature range than *A. grisella* when exposed to both constant and variable heat shock. This finding aligns with the literature, suggesting that each insect species has a specific optimum temperature range for development (Burggren, 2012; Mironidis & Savopoulou-Soultani, 2010).

Our findings indicate changes in certain biological traits of developing *A. grisella*, including larval spinning and pupal time, adult emergence time, and adult longevity in both constant (30–34 °C) and variable heat shock (30–40 °C) conditions when exposed to suitable temperatures. Exposing *A. grisella* larvae to variable heat shock increased the pupal period, adult longevity, adult weight, and the number of eggs produced compared to constant heat shock. In both conditions, the longest total lifespan occurred at 30 °C, suggesting this is the most favorable temperature for *A. grisella* (Ellis et al., 2013). At 34 °C, however, larval and pupal mortality increased, reducing adult emergence and shortening adult longevity, consistent with patterns observed in *G. mellonella* (Çelik Biçer et al., 2025a). Similar temperature-method interactions have been reported in other insects. For example, a solitary bee *Osmia bicornis* (Hymenoptera: Megachilidae) (Radmacher & Strohm, 2011) development accelerated under variable heat shock compared to constant heat shock. Furthermore, these bees were not affected by temperature or its application method until they reached the adult stage. Adult mortality of *O. bicornis* increased as the temperature rose, and the adults were less affected by variable heat shock than by constant heat shock (Radmacher & Strohm, 2011). While the rise in *A. grisella* adult mortality with increasing temperature aligns with these findings, effects on the species-specific developmental stage differ. These differences can be attributed to variations in insect species, temperature, application method, and life-stage during temperature application.

The effect of thermal stress on the larval spinning time and adult weight is understudied in the literature. Aligning with previous findings (Çelik Biçer et al., 2025a; Radmacher & Strohm, 2011; Ramachandra et al., 2001), *A. grisella* larval spinning time was shorter under variable heat shock than constant heat shock at all temperatures. However, unlike the previous studies, our results showed no consistent temperature-dependent decrease in larval spinning time. Constant heat shock effects both *G. mellonella* (Çelik Biçer et al., 2025a) and *A. grisella*, whereas variable heat shock affected only *A. grisella*. This suggests that *G. mellonella* may be more resistant to thermal stress than *A. grisella* and other insect species reported in the literature (Radmacher & Strohm, 2011; Ramachandra et al., 2001), considering the mechanisms controlling the larval spinning time. Adult weight of *A. grisella* was unaffected by constant heat shock but increased under variable exposure, consistent with *G. mellonella* (Çelik Biçer et al., 2025a). In contrast, other studies have shown that insects reared at higher temperatures tend to develop smaller body sizes (Davidowitz & Nijhout, 2004; Laughton et al., 2017). Davidowitz and Nijhout (2004) proposed that body size and development duration are regulated by three factors. These factors include the growth rate, the timing of the cessation of juvenile hormone secretion, and the timing of ecdysteroid secretion which facilitates the transition to the pupal stage. The difference between literature and our findings may be due to the larvae in our study being last instars that had nearly completed feeding. The hormonal signals influencing critical weight in these last instar larvae may have already finalized the insect's final weight.

Thermal stress and its application method influence both pre-adult stages (pupal time, pupal period, and adult emergence time) and adult stage traits (adult weight, adult longevity, and the number of eggs produced) of *A. grisella*. Exposure of pre-adult stages of *A. grisella* to constant and variable heat shocks revealed different results. Constant heat shock at 34 °C produced particularly strong effects. At 30 °C, pupal time shortened in both wax moths (Çelik Biçer et al., 2025a; Liu et al., 2021), consistent with this being the optimum temperature for last instars. In contrast to these pre-adult stage results, adult longevity in *A. grisella* declined steadily with temperature, in line with previous studies, while egg production showed different responses depending on exposure regime. Under variable heat shock, the highest fecundity was recorded at 34 °C, decreasing at higher and lower temperatures, whereas constant stress had no effect on egg number. The differences can be attributed to species- and stage-specific traits (Ebrahimi et al., 2015; Huang et al., 2020; Liu et al., 2021). Although *G. mellonella* shares many biological characteristics with *A. grisella*, comparative insights from a previous study (Çelik Biçer et al., 2025a) indicate notable differences in thermal responses and developmental dynamics. For example, *A. grisella* prolongs its larval feeding stage at higher temperatures, potentially increasing damage under warming conditions, whereas *G. mellonella* develops faster and may increase its number of generations. Consequently, although *G. mellonella* may increase in population size, the extended larval stage of *A. grisella* could pose a greater risk through cumulative feeding damage.

Cellular immunity

The insect hemogram varies with developmental stage and physiological condition, even within the same species, and is sensitive to environmental factors such as temperature (Ghasemi et al., 2013; Sharma et al., 2008; Wigglesworth, 1973). In our study, exposure duration to elevated temperatures in *A. grisella* led to both increases and decreases in total hemocyte count (THC) and differential hemocyte count (DHC). According to the literature, THC decrease at 30 °C may be attributed to the insect's preparation for the pupal stage (Kiuchi et al., 2008; Nakahara et al., 2003), whereas the decrease at 38 °C may result from dehydration-induced fluid loss due to heat stress (Ghasemi et al., 2013). When examining the hemocyte types of *A. grisella*, plasmatocytes were the most frequently observed cell type at all temperatures, consistent with their central role in immune defense. Elevated temperatures stimulated prohemocyte division and differentiation into immunocytes (Çelik Biçer et al., 2025b; Pandey et al., 2010). Similarly, granulocytes and plasmatocytes can undergo rapid division under stress but may lose membrane integrity at higher thermal levels, leading to rupture and cytoplasmic leakage (Amaral et al., 2010; Ghasemi et al., 2013). Comparable deformities have been reported in other Lepidoptera, such as *Scrobipalpa ocellatella* (Lepidoptera: Gelechiidae), where prolonged heat stress caused severe disruption and lysis of hemocytes (Ajamhassani, 2021; Ajamhassani et al., 2024). Overall, temperature and exposure duration influenced the variations in THC and DHC, as well as hemocyte types responsive to thermal stress (Browne et al., 2014; Çelik Biçer et al., 2025b; Ghasemi et al., 2013; Kiuchi et al., 2008; Laughton et al., 2017; Nakahara et al., 2003; Pourali & Ajam Hassani, 2018). The latter suggest that thermal acclimation may suppress immunity or that THC peaks at the onset of the final larval stage and decreases over time (Ghasemi et al., 2013; Pourali & Ajam Hassani, 2018). Our data similarly showed temperature- and duration-dependent shifts, but the delayed rise in plasmatocytes suggests a time-dependent compensatory response. Differences from previous studies likely stem from methodological variation, including the use of *in vitro* assays or immune pre-stimulation in other species (Laughton et al., 2017; Nakahara et al., 2003; Pourali & Ajam Hassani, 2018).

In Lepidoptera, the regulation of larval hemocytes is closely linked to mitotic cells in the hemolymph (Gardiner & Strand, 1999). Additionally, factors such as the larval instar (Ghasemi et al., 2013) and the state within that instar (Kiuchi et al., 2008) influence mitotic cell numbers. Since our experiments use larvae at the same developmental stage, the observed variations in the mitotic phase of *A. grisella* may result from temperature and exposure duration. At 2 h, rising temperatures increased the mitotic index (MI) alongside hemocyte density, similar to the 2.2-fold increase reported in *E. kuehniella* under heat stress (Ghasemi et al., 2013). This response may indicate accelerated hemocyte division and fluid loss

due to dehydration. Likewise, the MI increase in *A. grisella* at 30 and 34 °C may be a response to thermal stress. Similar patterns have been reported in other Lepidoptera, where thermal stress initially promotes hemocyte proliferation, particularly prohemocytes differentiating into immunocytes (Amaral et al., 2010; Pandey et al., 2010), but prolonged exposure compromises cell integrity, leading to membrane rupture and cytoplasmic leakage (Ajamhassani, 2021; Ajamhassani et al., 2024; Ghasemi et al., 2013). However, prolonged exposure to high temperatures reduced the MI, in agreement with earlier findings in *B. mori* and *G. mellonella* (Çelik Biçer et al., 2025b; Kiuchi et al., 2008). Although the normal MI in hemolymph is around 1% (Jones, 1967a, 1967b; Jones & Liu, 1968), our findings showed that it reached 5.02% in *A. grisella* after just 2 h of heat exposure. This suggests that even short-term thermal stress can significantly affect *A. grisella*.

Temperature may affect encapsulation formation as it influences hemocyte counts. However, existing studies have typically assessed encapsulation in binary terms, encapsulated or not, without evaluating the degree of response (Lynn & Vinson, 1977; Seehausen et al., 2017, 2018). Earlier studies generally reported that encapsulation increases with temperature, and plasmatocyte activation has been proposed as a key driver of this pattern. Similar temperature-linked increases in plasmatocyte numbers were observed in our previous work on *G. mellonella*, where certain combinations of temperature and exposure time enhanced weak or strong encapsulation responses, likely via heightened plasmatocyte activity and peptide release (Çelik Biçer et al., 2025a). In the present study, however, *A. grisella* exhibited a more complex and non-linear pattern. Encapsulation often declined rather than increased at higher temperatures, coinciding with marked reductions in plasmatocyte counts, cells essential for capsule formation. High-temperature mortality further constrained the response: larvae exposed to 38 °C survived only the 2-h treatment, paralleling Lynn and Vinson's (1977) findings where parasitized larvae died under combined thermal and injection stress. A similar interaction between injection injury and heat likely contributed to mortality in our study. Despite this overall suppression, encapsulation strength increased at 34 °C relative to 30 °C at 2 h, accompanied by increases in granulocyte or plasmatocyte numbers. These temperature- and time-dependent shifts highlight that encapsulation in *A. grisella* is not a linear process but reflects dynamic changes in hemocyte composition, particularly the balance and potentially the functional capacity of granulocytes and plasmatocytes. As shown previously for *G. mellonella*, elevated temperature may enhance immune activity under certain conditions. However, in *A. grisella*, high-temperature stress appears to impair encapsulation efficiency, likely due to disruptions in hemocyte structure or spreading ability rather than cell number alone.

Elevated total hemocyte counts under heat stress conditions do not necessarily indicate enhanced immune function. Elevated hemocyte abundance may reflect a generalized physiological stress response associated with mobilization or proliferation of circulating cells rather than enhanced immune efficiency. Therefore, the cellular changes observed in heat-stressed larvae may represent stress-induced physiological adjustment rather than a direct enhancement of immune performance. However, the concurrent alterations in encapsulation responses and mitotic activity suggest that heat stress influences immune-related parameters. These findings indicate that heat stress may alter the relationship between hemocyte abundance and functional immune performance, although the underlying mechanisms require further investigation. Numerous studies have investigated the effects of thermal stress on insect development and immunity, with none focusing on *A. grisella*. In relation to honeybee colonies, *A. grisella* regulates brood nest temperatures (32–36 °C) to support optimal brood development (Petz et al., 2004). Yet, disrupted thermoregulation in weakened colonies may allow comb pests such as *A. grisella* and *G. mellonella* to thrive. Therefore, understanding the host aspect of host-parasitoid interactions will contribute to our interpretation of parasitoid geographical distribution during climate change. The present study findings reveal that thermal stress alters certain developmental and immune parameters in *A. grisella*. Notably, 34 °C, 38–40 °C range, and higher temperatures significantly affected the species' vital activities. These findings demonstrate that short and long-term heat exposures influence the survival, development, and immune responses of *A. grisella* in ways that differ from many previous studies. Different insect species may respond uniquely to similar thermal stress

conditions, influenced by species-specific and size-related biological characteristics. This study contributes to understanding how rising temperatures may affect pest population dynamics under climate change. Therefore, we are currently investigating the effects of thermal stress on antioxidant enzymes in *A. grisella*, as well as immunity and antioxidant enzymes in *G. mellonella*.

Author's contribution Aylin Er proposed the idea and they worked with Olga Sak in the design of the study. Erinç Çelik Biçer performed the experiments. Olga Sak, Aylin Er, and Erinç Çelik Biçer wrote the paper. CRediT authorship contribution statement: Erinç ÇELİK BİÇER: Validation, Investigation, Data Curation, Writing—Original Draft Aylin ER: Conceptualization and Methodology Olga SAK: Writing—Review & Editing and Supervision.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This research was supported by the Balıkesir University Research Foundation [grant numbers 2022, 117].

Data availability Data will be made avaible on request.

Declarations

Consent for publication All authors read and consent to the publication of the manuscript.

Ethics approval This study is a study on Arthropoda. It does not require any Ethical Committee/Institutional Review Board (IRB) approval.

Competing interest The authors declare no competing interests.

References

Ajamhassani, M. (2021). Hemocyte changes of larvae of the beet moth, *Scrobipalpa ocellatella* (Lepidoptera: Gelechiidae) affected by thermal stress. *Journal of the Entomological Society of Iran, 41*(1), 101–103.

Ajamhassani, M., El Aalaoui, M., Valizadeh, B. (2024). Study on hemogram and the effect of thermal stress on hemocytes and development in *Dacus ciliatus* (Diptera: Tephritidae). *Journal of Agricultural Science and Technology,* 1–22.

Ali, A., Rashid, M. A., Huang, Q. Y., Wong, C., & Lei, C. L. (2017). Response of antioxidant enzymes in *Mythimna separata* (Lepidoptera: Noctuidae) exposed to thermal stress. *Bulletin of Entomological Research, 107*(3), 382–390.

Alshabka, Z. E., Naiem, E. A., Dabour, N. A., Seif, A. I., & Meshrif, W. S. (2020). The effects of thermal waves on the biology and immune function of the cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Egyptian Journal of Experimental Biology (Zoology), 16*(1), 85–92.

Amaral, I. M. R., Neto, J. F. M., Pereira, G. B., Franco, M. B., Beletti, M. E., Kerr, W. E., Bonetti, A. M., & Ueira-Vieira, C. (2010). Circulating hemocytes from larvae of *Melipona scutellaris* (Hymenoptera: Apidae, Meliponini): Cell types and their role in phagocytosis. *Micron, 41*, 123–129.

Browne, N., Surlis, C., & Kavanagh, K. (2014). Thermal and physical stresses induce a short-term immune priming effect in *Galleria mellonella* larvae. *Journal of Insect Physiology, 63*, 21–26. https://doi.org/10.1016/j.jinsphys.2014.02.006

Burggren, W. (2012). Temperature. In: Harrison JF, Woods HA, Roberts SP, editors. *Ecological and Environmental Physiology of Insects*. New York (NY): Oxford University Press. p. 64–97. https://doi.org/10.1086/669295

Catalán, T. P., Wozniak, A., Niemeyer, H. M., Kalergis, A. M., & Bozinovic, F. (2012). Interplay between thermal and immune ecology: Effect of environmental temperature on insect immune response and energetic costs after an immune challenge. *Journal of Insect Physiology, 58*(3), 310–317. https://doi.org/10.1016/j.jinsphys.2011.10.001

Colinet, H., Sinclair, B. J., Vernon, P., & Renault, D. (2015). Insects in fluctuating thermal environments. *Annual Review of Entomology, 60*(1), 123–140. https://doi.org/10.1146/annurev-ento-010814-021017

Cui, X., Wan, F., Xie, M., & Liu, T. (2008). Effects of heat shock on survival and reproduction of two whitefly species, *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B. *Journal of Insect Science, 8*(1), Article 24.

Cui, Y., Du, Y., Lu, M., & Qiang, C. (2011). Antioxidant responses of *Chilo suppressalis* (Lepidoptera: Pyralidae) larvae exposed to thermal stress. *Journal of Thermal Biology, 36*(5), 292–297. https://doi.org/10.1016/j.jtherbio.2011.04.003

Çelik Biçer, E., Sak, O., & Er, A. (2025a). Constant and variable heat shock effects on *Galleria mellonella* L. (Lepidoptera: Pyralidae) mortality and biological traits in the

context of climate change. *International Journal of Tropical Insect Science, 45*(2), 759–771. https://doi.org/10.1007/s42690-025-01466-y

Çelik Biçer, E., Sak, O., & Er, A. (2025b). Influence of thermal stress on the cellular immunity of *Galleria mellonella* F. (Lepidoptera: Pyralidae), and the biological traits of *Bracon hebetor* (Hymenoptera: Braconidae) in a host–parasitoid interaction: Implications under climate change. *Journal of Insect Physiology*. https://doi.org/10.1016/j.jinsphys.2025.104907

Davidowitz, G., & Nijhout, H. F. (2004). The physiological basis of reaction norms: The interaction among growth rate, the duration of growth and body size. *Integrative and Comparative Biology, 44*(6), 443–449. https://doi.org/10.1093/icb/44.6.443

Denlinger, D. L., Yocum, G. D. (2019). Temperature sensitivity in insects and application in integrated pest management. In: Hallman GJ, Denlinger DL, editors. *Temperature Sensitivity in Insects and Application in Integrated Pest Management.* New York (NY): CRC Press. p. 1–7. https://doi.org/10.1201/9780429308581

Dubovskiy, I. M., Kryukova, N. A., Glupov, V. V., & Ratcliffe, N. A. (2016). Encapsulation and nodulation in insects. *Invertebrate Survival Journal, 13*(1), 229–246. https://doi.org/10.25431/1824-307X/isj.v13i1.229-246

Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R., & Mearns, L. O. (2000). Climate extremes: Observations, modeling, and impacts. *Science, 289*(5487), 2068–2074. https://doi.org/10.1126/science.289.5487.2068

Ebrahimi, N., Talebi, A. A., & Fathipour, Y. (2015). Effects of short-term heat shock of eggs on the development and fecundity of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Journal Crop Protection, 4*(1), 73–83.

Eggert, H., de Didns- Buhr, M. F., & Kurtz, J. (2015). A temperature shock can lead to trans-generational immune priming in the red flour beetle, *Tribolium castaneum*. *Ecology and Evolution, 5*(6), 1318–1326. https://doi.org/10.1002/ece3.1443

Ellis, J., Graham, J. R., & Mortensen, A. N. (2013). Standard methods for wax moth research. *Journal of Apicultural Research, 52*(1), 1–17. https://doi.org/10.3896/ibra.1.52.1.10

Er, A., Uçkan, F., Rivers, D. B., Ergin, E., & Sak, O. (2010). Effects of parasitization and envenomation by the endoparasitic wasp *Pimpla turionellae* (Hymenoptera: Ichneumonidae) on hemocyte numbers, morphology, and viability of its host *Galleria mellonella* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America, 103*(2), 273–282.

Farahani, S., Bandani, A. R., Alizadeh, H., Goldansaz, S. H., & Whyard, S. (2020). Differential expression of heat shock proteins and antioxidant enzymes in response to temperature, starvation, and parasitism in the carob moth larvae, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). *PLoS ONE, 15*(1), Article e0228104.

Fathy, D. M., Fathy, H. M., Mansour, H. M., & Ziedan, M. A. R. (2017). Activity of the greater wax moth *Galleria mellonella* L. and the lesser wax moth *Achroia grisella* F. in apiary and storage in Kafr El-Sheikh Province. *Journal of Plant Protection and Pathology, 8*(10), 497–500.

Galindo-Cardona, A., Achar, J. D., González-Brizuela, G., Martín, E., Salvo, S. A., & Monmany-Garzia, A. C. (2019). First report and molecular determination of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae), a parasitoid of the lesser wax moth *Achroia grisella* F. (Lepidoptera: Pyralidae) in Northwest Argentina. *Journal of Apicultural Research, 58*(4), 550–552.

Gardiner, E. M., & Strand, M. R. (1999). Monoclonal antibodies bind distinct classes of hemocytes in the moth *Pseudoplusia includens*. *Journal of Insect Physiology, 45*(2), 113–126. https://doi.org/10.1016/s0022-1910(98)00092-4

Ghasemi, V., Moharramipour, S., & Sendi, J. J. (2013). Circulating hemocytes of Mediterranean flour moth *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae) and their response to thermal stress. *Invertebrate Survival Journal, 10*(1), 128–140.

Huang, Y., Gu, X., Peng, X., Mei, T., Chen, G., & Zhang, X. (2020). Effect of short-term high temperatures on growth, development, and reproduction in the fruit fly *Bactrocera tau* (Diptera: Tephritidae). *Scientific Reports, 10*(1), Article 6418.

Jeffs, C. T., & Lewis, O. T. (2013). Effects of climate warming on host–parasitoid interactions. *Ecological Entomology, 38*(3), 209–218. https://doi.org/10.1111/een.12026

Jena, K., Kar, P. K., Kausar, Z., & Babu, C. S. (2013). Effects of temperature on modulation of oxidative stress and antioxidant defenses in testes of tropical tasar silkworm *Antheraea mylitta*. *Journal of Thermal Biology, 38*(4), 199–204. https://doi.org/10.1016/j.jtherbio.2013.02.008

Jones, J. C., & Liu, D. P. (1968). A quantitative study of mitotic divisions of haemocytes of *Galleria mellonella* larvae. *Journal of Insect Physiology, 14*(8), 1055–1061. https://doi.org/10.1016/0022-1910(68)90043-7

Jones, J. C. (1967a). Changes in the hemocyte picture of *Galleria mellonella* (Linnaeus). *Biological Bulletin, 132*(2), 211–221. https://doi.org/10.2307/1539889

Jones, J. C. (1967b). Normal differential counts of haemocytes in relation to ecdysis and feeding in *Rhodnius*. *Journal of Insect Physiology, 13*(8), 1133–1141. https://doi.org/10.1016/0022-1910(67)90087-X

Kells, S. A., & Goblirsch, M. J. (2011). Temperature and time requirements for controlling bed bugs (*Cimex lectularius*) under commercial heat treatment conditions. *Insects, 2*(3), 412–422. https://doi.org/10.3390/insects2030412

Kiuchi, T., Aoki, F., & Nagata, M. (2008). Effects of high temperature on the hemocyte cell cycle in silkworm larvae. *Journal of Insect Physiology, 54*(2), 454–461. https://doi.org/10.1016/j.jinsphys.2007.11.004

Kwadha, C. A., Ong'amo, G. O., Ndegwa, P. N., Raina, S. K., & Fombong, A. T. (2017). The biology and control of the greater wax moth, *Galleria mellonella*. *Insects, 8*(2), Article 61. https://doi.org/10.3390/insects8020061

Laughton, A. M., O'Connor, C. O., & Knell, R. J. (2017). Responses to a warming world: Integrating life history, immune investment, and pathogen resistance in a model insect species. *Ecology and Evolution, 7*(22), 9699–9710. https://doi.org/10.1002/ece3.3506

Liu, Y., Li, X., Yan, X., Li, G., Luo, C., & He, Y. (2021). Effects of short-term high temperatures on survival and reproduction of *Trabala vishnou gigantina* Yang (Lepidoptera:

Lasiocampidae). *Pakistan Journal of Zoology, 54*(1), 145–151. https://doi.org/10.17582/journal.pjz/20201105081124

Lynn, D. C., & Vinson, S. B. (1977). Effects of temperature, host age, and hormones upon the encapsulation of *Cardiochiles nigriceps* eggs by *Heliothis* spp. *Journal of Invertebrate Pathology, 29*(1), 50–55. https://doi.org/10.1016/0022-2011(77)90171-9

Ma, C. S., Ma, G., & Pincebourde, S. (2021). Survive a warming climate: Insect responses to extreme high temperatures. *Annual Review of Entomology, 66*(1), 163–184. https://doi.org/10.1146/annurev-ento-041520-074454

Mahgoub, M. O., Lau, W. H., & Omar, D. B. (2015). Observations on the biology and larval instars discrimination of wax moth *Achroia grisella* F. (Pyralidae: Lepidoptera). *Journal of Entomology, 12*(1), 1–11. https://doi.org/10.3923/je.2015.1.11

Mellanby, K. (1935). A comparison of the physiology of the two species of bed-bug which attack man. *Parasitology, 27*(1), 111–122. https://doi.org/10.1017/S0031182000014992

Mironidis, G. K., & Savopoulou-Soultani, M. (2010). Effects of heat shock on survival and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) adults. *Journal of Thermal Biology, 35*(2), 59–69. https://doi.org/10.1016/j.jtherbio.2009.11.001

Moret, Y., & Schmid-Hempel, P. (2000). Survival for immunity: The price of immune system activation for bumblebee workers. *Science, 290*(5494), 1166–1168.

Nakahara, Y., Kanamori, Y., Kiuchi, M., & Kamimura, M. (2003). In vitro studies of hematopoiesis in the silkworm: Cell proliferation in and hemocyte discharge from the hematopoietic organ. *Journal of Insect Physiology, 49*(10), 907–916.

Neven, L. G. (2000). Physiological responses of insects to heat. *Postharvest Biology and Technology, 21*(1), 103–111.

Nittono, Y. (1960). Studies on the blood cells in the silkworm, *Bombyx mori* L. *Bulletin of the Sericultural Experiment Station, 16*, 171–266.

NOAA National Centers for Environmental Information. 2024. Monthly Global Climate Report for January 2024. Published online February 2024. Accessed March 29, 2024. https://www.ncei.noaa.gov/access/monitoring/monthly-report/global/202401

Pandey, J. P., Mishra, P. K., Kumar, D., Singh, B. M. K., & Prasad, B. C. (2010). Effect of temperature on hemocytic immune responses of tropical tasar silkworm, *Antheraea mylitta*. *Research in Immunology, 3*, 169–177.

Pereira, R. M., Koehler, P. G., Pfiester, M., & Walker, W. (2009). Lethal effects of heat and use of localized heat treatment for control of bed bug infestations. *Journal of Economic Entomology, 102*(3), 1182–1188. https://doi.org/10.1603/029.102.0342

Petz, M., Stabentheiner, A., & Crailsheim, K. (2004). Respiration of individual honeybee larvae in relation to age and ambient temperature. *Journal of Comparative Physiology B, 174*, 511–518.

Pourali, Z., & Ajam Hassani, M. (2018). The effect of thermal stresses on the immune system of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Journal of the Entomological Society of Iran, 37*(4), 515–525. https://doi.org/10.22117/jesi.2018.116103

Qin, J. Y., Liu, Y. Q., Zhang, L., Cheng, Y. X., Luo, L. Z., & Jiang, X. F. (2018). Effects of temperatures on the development and reproduction of the armyworm, *Mythimna roseilinea*: Analysis using an age-stage, two-sex life table. *Journal of Integrative Agriculture, 17*(7), 1506–1515. https://doi.org/10.1016/s2095-3119(17)61856-2

Radmacher, S., & Strohm, E. (2011). Effects of constant and fluctuating temperatures on the development of the solitary bee *Osmia bicornis* (Hymenoptera: Megachilidae). *Apidologie, 42*, 711–720. https://doi.org/10.1007/s13592-011-0078-9

Ramachandra, Y. L., Bali, G., & Rai, S. P. (2001). Effect of temperature and relative humidity on spinning behaviour of silkworm (*Bombyx mori* L.). *Indian Journal of Experimental Biology, 39*(1), 87–89.

Richards, E. H., & Parkinson, N. M. (2000). Venom from the endoparasitic wasp *Pimpla hypochondriaca* adversely affects the morphology, viability, and immune function of hemocytes from larvae of the tomato moth, *Lacanobia oleracea*. *Journal of Invertebrate Pathology, 76*(1), 33–42.

Sangle, P. M., Satpute, S. B., Khan, F. S., & Rode, N. S. (2015). Impact of climate change on insects. *BioScience Trends, 8*(14), 3579–3582.

Seehausen, M. L., Cusson, M., Régnière, J., Bory, M., Stewart, D., Djoumad, A., et al. (2017). High temperature induces downregulation of polydnavirus gene transcription in lepidopteran host and enhances accumulation of host immunity gene transcripts. *Journal of Insect Physiology, 98*, 126–133. https://doi.org/10.1016/j.jinsphys.2016.12.008

Seehausen, M. L., Naumann, P. H., Béliveau, C., Martel, V., & Cusson, M. (2018). Impact of rearing temperature on encapsulation and the accumulation of transcripts putatively involved in capsule formation in a parasitized lepidopteran host. *Journal of Insect Physiology, 107*, 244–249. https://doi.org/10.1016/j.jinsphys.2018.04.013

Shamakhi, L., Zibaee, A., Karimi-Malati, A., & Hoda, H. (2019). Effect of thermal stress on the immune responses of *Chilo suppressalis* Walker (Lepidoptera: Crambidae) to *Beauveria bassiana*. *Journal of Thermal Biology, 84*, 136–145. https://doi.org/10.1016/j.jtherbio.2019.07.006

Sharma, P. R., Sharma, O. P., & Saxena, B. P. (2008). Effect of sweet flag rhizome oil (*Acorus calamus*) on hemogram and ultrastructure of hemocytes of the tobacco armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Micron, 39*(5), 544–551. https://doi.org/10.1016/j.micron.2007.07.005

Wigglesworth, V. B. (1973). Haemocytes and basement membrane formation in *Rhodnius*. *Journal of Insect Physiology, 19*(4), 831–844. https://doi.org/10.1016/0022-1910(73)90155-8

Wojda, I. (2017). Temperature stress and insect immunity. *Journal of Thermal Biology, 68*, 96–103. https://doi.org/10.1016/j.jtherbio.2016.12.002

Wu, G., Zhao, Z., Liu, C., & Qiu, L. (2014). Priming *Galleria mellonella* (Lepidoptera: Pyralidae) larvae with heat-killed bacterial cells induced an enhanced immune protection against *Photorhabdus luminescens* TT01 and the role of innate immunity in the process. *Journal of Economic Entomology, 107*(2), 559–569. https://doi.org/10.1603/EC13455

Zhang, Y. B., Zhang, G. F., Liu, W. X., & Wan, F. H. (2019). Continuous heat waves change the life history of a host-feeding parasitoid. *BioControl, 135*, 57–65. https://doi.org/10.1016/j.biocontrol.2019.04.013