

*Article*



# **The Effect of Temperature and Moisture Content on Population Growth of** *Alphitobius diaperinus* **(Panzer) (Coleoptera: Tenebrionidae)**

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**Abstract:** *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), commonly known as the lesser mealworm, is a significant pest that infests stored grains and other amylaceous commodities. In addition, the species has also been recognized as a viable and environmentally friendly protein source. However, there is still a lack of comprehensive research on the developmental response of this species across various temperature and moisture conditions. This study investigates the impact of temperature and moisture content of the commodity on the population growth of the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). In the first series of bioassays, the progeny production of *A. diaperinus* adults was recorded after 50 days of incubation under a range of temperatures from 25 to 40  $^{\circ}$ C in cracked soft wheat with 11.3% moisture content, while in the second series, the moisture content of the wheat was adjusted to 5, 10, 15, and 20%, at 30 °C. Our results show that temperature largely influences parental adult mortality of *A. diaperinus*. The most suitable temperatures for optimal larval development and adult survival were found to be within the spectrum of 25 to 32 °C. Moreover, we found that the moisture content of the wheat that served as rearing media was also a factor of significance, since a gradual decrease in the larval numbers was observed with an increase in the moisture content. The findings of this study provide data to further enhance the pest control strategies of *A. diaperinus* in poultry farms but also to establish mass rearing standards and facilitate the production of the species for efficient use as food and feed.

**Keywords:** lesser mealworm; abiotic conditions; stored product pests; progeny production; edible insects

### **1. Introduction**

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is a pest of stored grains and related amylaceous commodities. The introduction of insectcontaminated feed into poultry farms led *A. diaperinus* to adapt to the warm and humid conditions that occur in such facilities [\[1\]](#page-7-0), which led to the prevalence of this species as an important pest in commercial poultry production [\[2,](#page-7-1)[3\]](#page-7-2). Nowadays, the pest status of *A. diaperinus* is mostly followed by its association as a vector of serious microbial pathogens, such as *Salmonella* and *Escherichia* [\[4](#page-7-3)[–7\]](#page-7-4). Moreover, *A. diaperinus* larvae and adults are preferred by chicken over their conventional feed, which results in less consumption of poultry feed and therefore less gained weight, factors that can economically affect the overall production [\[8](#page-7-5)[,9\]](#page-7-6).

To this end, the mass production and cultivation of insects as an alternative feed source have been proposed by many as solutions to the food and energy crisis, in order to negate some of the effects of traditional livestock farming that currently requires large amounts of water, nitrogen, and land [\[10](#page-7-7)[,11\]](#page-7-8). Although insects such as *A. diaperinus* have been considered as pests and vectors of diseases, their introduction as an alternative, efficient, and sustainable protein source, especially for livestock feeding is welcomed by the



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European Union (EU). Laws and regulations regarding production, use, and distribution of insect-based protein in fish, poultry, and pig diets have already been established, and are constantly being updated [\[12\]](#page-7-9). Indeed, insect species that are so far allowed to be produced on a large scale for their inclusion in feeds have been proved to provide a sufficient source of nutrients such as fats and proteins, without the need for much space for land, far lower demand for water, and a better feed conversion efficiency than conventional diets [\[13](#page-7-10)[–16\]](#page-7-11). At the same time, insects could be used effectively for food waste management utilizing several agricultural byproducts, lowering the amounts of waste, and consequently facilitating the optimization of food distribution in parts of the world with limited access to resources [\[17–](#page-7-12)[19\]](#page-7-13).

For this purpose, the EU through the Regulation 2017/893 proceeded with the authorization of certain insect species to be used as insect-based protein in fish feed, and later on, in poultry and pig diets (EU Regulation 2021/1372). Among them, the most widely known species is the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), that has been proved to be an extremely promising nutrient source for certain species of farmed animals, as well as an effective waste management agent [\[20](#page-7-14)[–22\]](#page-8-0). Another species is the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), which has been extensively used to recycle organic waste and then fed to broiler chicken, with promising results [\[17](#page-7-12)[,23](#page-8-1)[,24\]](#page-8-2). Moreover, *T. molitor* meal has also been used in aquaculture, as a substitute of fishmeal, with studies showing promising results, without impacting the growth of fish [\[25–](#page-8-3)[27\]](#page-8-4). Despite the fact that there are additional species that are authorized in the EU as feed sources, the vast majority of the published works has been focused on these two species [\[7](#page-7-4)[,28,](#page-8-5)[29\]](#page-8-6). Indeed, there are disproportionally few data on *A. diaperinus*, despite the fact that this species has been approved by the EU as both food and feed, and thus, could be used with success as feed for different farm animals [\[7\]](#page-7-4).

Insects are poikilotherm organisms, and as such, temperature largely influences their growth and reproduction [\[30\]](#page-8-7). In search of a more sustainable and environmentally safe method compared to traditional chemical practices for effectively controlling stored product insects, temperature manipulation has played a significant role as a physical control approach. For this purpose, heat and cold treatments as management techniques against *A. diaperinus* can be widely applied to disinfest grain storages and food processing facilities [\[31–](#page-8-8)[34\]](#page-8-9). Thus, practitioners in storage and processing facilities must be aware of the pest's thermal requirements, particularly the temperature thresholds beyond which individuals, in all their developmental stages, stop developing. On the other hand, efforts have been made towards the establishment of mass rearing standards in terms of the optimal abiotic rearing conditions, in order to optimize the mass-production in the insect farming industry. For instance, a number of studies have examined the impact of temperature and moisture content on both *H. illucens* [\[35](#page-8-10)[–38\]](#page-8-11) and *T. molitor* [\[39\]](#page-8-12).

In the case of *A. diaperinus*, studies showed that an increase in room temperature to 45 <sup> $\degree$ </sup>C for 24 h resulted in a mortality rate of at least 90% for both adults and larvae [\[34\]](#page-8-9). Other studies have also examined the effect of temperature on the development of different life stages of *A. diaperinus*, as well as the influence of temperature on the metabolic rate of the species, but in most of the cases, the experiments were conducted under a narrow range of temperature values and for a short period of time [\[40–](#page-8-13)[42\]](#page-8-14). Additionally, air humidity and moisture content of the rearing media was not controlled, even though *A. diaperinus* requires a constant moisture source for its development [\[43\]](#page-8-15). On the other hand, the requirement of elevated relative humidity values for optimal growth of *A. diaperinus* presents a potential challenge to control strategies, particularly when aiming to utilize environmentally friendly insecticides [\[44,](#page-8-16)[45\]](#page-8-17). For instance, numerous studies have indicated the significant impact of moisture on the insecticidal effectiveness of inert dusts, such as diatomaceous earth [\[46](#page-8-18)[–48\]](#page-8-19). Therefore, it is evident that the growth and reproductive capabilities of the species are significantly impacted by temperature and moisture. These factors are not only important for controlling the species as a pest, but also for optimizing its mass rearing protocols for food and feed purposes. Based on the above, the aim of the current study was to evaluate, in laboratory conditions, the development and progeny production capacity of *A. diaperinus* under a range of temperature and moisture content values.

### **2. Materials and Methods**

### *2.1. Insects*

Adults of *A. diaperinus* were available at the Laboratory of Entomology and Agricultural Zoology (LEAZ) of the University of Thessaly. The insects were reared in plastic boxes (48 cm length  $\times$  28 cm width  $\times$  10 cm height) with a rectangular screened opening (19 cm  $\times$  27 cm) on the top cover to allow air circulation, at 26  $\pm$  1 °C, with 55% relative humidity (RH) and continuous darkness. Wheat bran (90%) that was obtained from a local mill, and yeast (10%) (Angel Yeast Co., Ltd., Yichang, China) were provided as the rearing commodity, while fresh potato slices were provided in the stock rearings twice a week as a moisture source. Adults (<7 days old) were randomly selected with a fine brush from the rearings and used for the experiments.

## *2.2. Experimental Design*

The experiment was conducted in cylindrical plastic vials (7.5 cm diameter  $\times$  8.8 cm height, Carl Roth GmbH & Co. Kg, Karlsruhe, Germany). In a first series of bioassays, each vial was filled with 20  $\pm$  0.1 g of 100% cracked soft wheat with a moisture content (MC) of 11.3%, as determined with a moisture meter (Multitest, Gode SAS, Le Catelet, France). To obtain homogenous particle size, the wheat was cracked and then sieved with a 2.0 mm and a 0.85 mm sieve. The wheat that passed through the 2.0 mm sieve and was left in the 0.85 mm sieve was used for the experiments. The center of the lid of the vials was cut out, with a diameter of approximately 4 cm, and covered with a thin net, to provide sufficient aeration. Ten *A. diaperinus* adults of mixed sex were placed into each vial. The vials were placed in incubators set at 25 °C, 30 °C, 32 °C, 35 °C, 37 °C, and 40 °C and 55% RH. Different sets of vials were used for each temperature value, while the whole experiment was repeated 9 times. A carrot slice  $(0.4 \pm 0.1 \text{ g})$  was provided three times per week as a moisture source [\[43\]](#page-8-15). Mortality and progeny production was recorded after 50 days of incubation, when the vials were opened, sieved, and alive and dead adults and larvae were counted separately. The alive larvae were classified into two distinct groups based on their instar stage, as outlined in the study conducted by Francisco and Prado [\[49\]](#page-9-0). This classification was achieved by using a 1 mm sieve, whereby larvae that successfully passed through the sieve were determined to be in their 5th larval instar or younger and were referred thereafter as early instar larvae. Conversely, larvae that remained trapped in the sieve were identified as having reached at least their 6th instar, and were referred thereafter as the late instar larvae.

For the second series of bioassays, each vial was filled with  $20 \pm 0.1$  g of 100% cracked soft wheat, with particle size between 2.0 and 0.85 mm, but in this case, the MC of the wheat, which served as a rearing medium, was adjusted to 5%, 10%, 15%, and 20%. To achieve the desired MC values, the wheat samples underwent a two-step procedure. The wheat was initially subjected to a drying process in an oven set at a temperature of 40  $^{\circ}$ C, until the MC of the wheat decreased below 5%. Following this, the wheat was left to reach room temperature for a period of one day. Subsequently, small quantities of distilled water were applied to the wheat samples until the ideal MC values were achieved, as assessed using a moisture meter (Multitest, Gode SAS, Le Catelet, France). Ten *A. diaperinus* adults of mixed sex were placed inside each vial, while a carrot slice  $(0.4 \pm 0.1 \text{ g})$  was introduced in the vials three times per week [\[43\]](#page-8-15), as above. The vials were placed in incubators at 30 ◦C and 55% RH, with different set of vials for each MC value. There was no further evaluation of the MC within the vials during the experimentation. The experiment was based on 6 replicates for each MC value. The number of progenies produced was recorded after 50 days, while progeny production was evaluated as in the case of Bioassay series 1.

# 2.3. Statistical Analysis

All data were checked for normality using the Shapiro–Wilk's test. Adult and larval mortality with the value of temperature as main effect, and adult mortality, larval mortality, and larval progeny (alive early and late instar larvae) with the value of MC as main effect, were analyzed using the Kruskal-Wallis test for non-parametric comparisons. The Dunn's test was followed for post hoc comparisons at 0.05. The larval progeny (alive early and late instar larvae) with value of temperature as main effect was analyzed using a one-way ANOVA for parametric comparison, followed by the Tukey–Kramer HSD test for post hoc comparisons at 0.05. All analyses were performed using the JMP® Software (version 17.0) (SAS Institute Inc., Cary, NC, USA).

#### **3. Results 3. Results**

Parental adult mortality of *A. diaperinus* was significantly influenced by temperature Parental adult mortality of *A. diaperinus* was significantly influenced by temperature  $(x^2 = 44.7, df = 5, p < 0.01)$ , with mortality rates that increased after 35 °C, while 100% mortality was recorded at 40 °C (Figure [1\)](#page-3-0). Contrarily, mortality of the parental adults were allowed to  $\frac{1}{2}$ remained below 5% between 25 and 32 ◦C spectrum, in which the adults were allowed to oviposit. Temperature also seems to influence larval mortality ( $x^2 = 22.9$ , df = 4,  $p < 0.01$ ), but only under a specific thermal range. Although all larvae were alive at temperatures between 25 and 32 ℃, an increased larval mortality, corresponding to 60%, was observed at 35 and 37 ◦C (Figure [1\)](#page-3-0). No larvae, dead or alive, were observed at 40 ◦C (Figure [1\)](#page-3-0). On at 35 and 37 °C (Figure 1). No larvae, dead or alive, were observed at 40 °C (Figure 1). On the other hand, MC did not seem to affect parental adult mortality ( $x^2 = 2.0$ , df = 3,  $p = 0.55$ ) or larval mortality ( $x^2 = 7.6$ , df = 3,  $p = 0.06$ ), since low mortality rates with no significant differences were recorded among all treatments (Figure [2\)](#page-4-0). In any case, less than 5% larval differences were recorded among all treatments (Figure 2). In any case, less than 5% larval mortality was observed in all MC treatments (Figure [2\)](#page-4-0). mortality was observed in all MC treatments (Figure 2). <sup>2</sup> at a decided a functionally of 21. *auxpertities* was significantly influenced by temperature between  $25$  and  $22$   $\degree$ C, an increased larval mortality, corresponding to  $60\%$ , was observed

<span id="page-3-0"></span>

Figure 1. Mean (%  $\pm$  SE) adult and larval mortality per vial with different temperature values. Means followed by the same uppercase letter are not significantly different among the adult mortality rates, followed by the same uppercase letter are not significantly different among the adult mortality rates, and means followed by the same lowercase letters are not significantly different among the larval mortality rates, where no letter is present no significant differences was recorded, according to the mortality rates, where no letter is present no significant differences was recorded, according to the Dunn's test at  $p < 0.05$ .

Temperature was a key factor for progeny production, in the case of the alive early instar and late instar larvae found in each vial ( $x^2 = 45.0$ , df = 5, *p* < 0.01). More than 80 larvae per vial were found at 25, 30, and 32 ℃, regardless of their instar stage, while at 35 and 37 ◦C, the number of the corresponding progeny reduced drastically, with less than 10 larvae being recorded per vial (Figure [3\)](#page-4-1). Finally, no larvae were observed at 40 ◦C. In search of the optimal temperature for maximum larval production, 30 ◦C yielded the best results in comparison to the corresponding treatments of the other temperatures, with

an average of 101 late instar and 21 early instar larvae per vial. Furthermore, the larval development was observed to be faster at 30 °C in comparison with the respective figures at 25 and 32 ◦C. This was apparent by the ratio of late-to-early instar larvae, which was 5:1 at 30  $\degree$ C, while at 25 and 32  $\degree$ C, the same ratio was approximately 2:1.

<span id="page-4-0"></span>

Where no letter is present no significant differences was recorded, according to the Dunn's test at  $p < 0.05$ . **Figure 2.** Mean (%  $\pm$  SE) adult and larval mortality per vial with different grain moisture contents.  $p < 0.05$ .  $\alpha$  30  $\overline{5}$ 

<span id="page-4-1"></span>

Figure 3. Mean (±SE) number of alive early and late instar larval progenies recorded per vial at each temperature. Means of total larvae followed by the same letter are not significantly different, according to the Dunn's test at  $p < 0.05$ .

Moisture content of the substrate was shown to influence progeny and larval growth for both early and late instar larvae (for total number of larvae:  $F = 7.4$ ,  $p < 0.01$ , df = 3, 20) (Figure [4\)](#page-5-0). The highest progeny was recorded between 5 and 10% MC. The most number of larval progeny (51.2 total larvae per vial) was recorded at 5% MC. However, a higher ratio of early-to-late instar larvae (1:1) was found at 10% MC, versus the 3:1 at 5% MC. This indicates that although more larvae were produced at 5% MC, growth was faster at 10% MC. Both 15 and 20% MC resulted in significantly less total larval production (less than 30 larvae per vial), as compared with the other treatments (Figure [4\)](#page-5-0).

<span id="page-5-0"></span>

than 30 larvae per vial), as compared with the other treatments (Figure 4).

**Figure 4.** Mean (±SE) number of alive early and late instar larval progenies recorded per vial at each **Figure 4.** Mean (±SE) number of alive early and late instar larval progenies recorded per vial at each moisture content. Means of total larvae followed by the same letter are not significantly different, moisture content. Means of total larvae followed by the same letter are not significantly different, according to the Dunn's test at  $p < 0.05$ .

# **4. Discussion 4. Discussion**

The effect of temperature on insect growth has been studied extensively on a wide The effect of temperature on insect growth has been studied extensively on a wide range of insects by several research groups [\[37](#page-8-20)[,50](#page-9-1)[–52\]](#page-9-2). On the basis of these studies, speciesspecific thermal requirements have been determined for the indication of the thermal activity thresholds and lethal temperatures of several stored product insects, as well as for activity thresholds and lethal temperatures of several stored product insects, as well as for the enhancement of the commercial efficacy of insects as food and feed. [\[53](#page-9-3)[–56\]](#page-9-4). However, literature data on the developmental periods of *A. diaperinus* are not consistent and are literature data on the developmental periods of *A. diaperinus* are not consistent and are usually based on a small number of specimens [7,40,57]. This research has demonstrated usually based on a small number of specimens [\[7](#page-7-4)[,40](#page-8-13)[,57\]](#page-9-5). This research has demonstrated that the development of *A. diaperinus* may be influenced by both temperature and grain that the development of *A. diaperinus* may be influenced by both temperature and grain moisture content. Consequently, these factors should be taken into account in pest control moisture content. Consequently, these factors should be taken into account in pest control strategies as well as when establishing protocols for mass insect rearing. strategies as well as when establishing protocols for mass insect rearing.

*Alphitobius diaperinus* seemed to normally develop within a specific temperature spec-*Alphitobius diaperinus* seemed to normally develop within a specific temperature spectrum of 20 to 38 °C. Temperatures as such are similar in poultry farms, especially on the litter floor where the activity of chickens is more intense [\[40](#page-8-13)[,42\]](#page-8-14). However, to complete a full litter floor where the activity of chickens is more intense [40,42]. However, to complete a life circle under a short period of approximately 30 days, the species requires temperatures me enere under a short period of approximately so days, the species requires temperatures within 30–38 °C [\[7,](#page-7-4)[40\]](#page-8-13). Bjørge et al. [\[42\]](#page-8-14) reported the maximum daily larval growth rate at 31 °C. Our findings stand in accordance with the above, showing that there is indeed at 31 °C. rate at 31 °C. Our findings stand in accordance with the above, showing that there is in-a specific temperature spectrum that enhances the progeny production of *A. diaperinus*. deed a specific temperature spectrum that enhances the progeny production of *A. diaper-*In our case, the optimal temperature range for maximal progeny production was found *inus*. In our case, the optimal temperature range for maximal progeny production was to be 30–32 ◦C, whereas adult and larval survivals dropped dramatically above 35 ◦C. This is particularly important, as this temperature range appears to be crucial for both parental survival and progeny production, and mass rearing protocols should take this into consideration, and  $\frac{1}{2}$  is  $\frac{1}{2}$  into consideration. trum of 20 to 38 ◦C. Temperatures as such are similar in poultry farms, especially on the

Another interesting indicator that was assessed in this study, through larval development, is the fact that at 25 and 32  $^{\circ}$ C, there were approximately two late instar larvae for every early instar larva, while at 30  $^{\circ}$ C, that ratio becomes almost 5:1, suggesting quite evidently that temperature is a key factor not only for progeny production but also for the speed of larval growth. In this context, our data indicate that even an increase of  $5^{\circ}$ C in temperature could have a significant impact on *A. diaperinus* population dynamics. As Bjørge et al. [\[42\]](#page-8-14) has also shown, *A. diaperinus* adults that are reared at 30–31 ◦C exhibit a steep increase in growth rate, and according to our study, they also exhibit the highest survival rate and the highest progeny production capacity, while the produced larvae grow faster than that of any other temperature value. Based on the data available so far, no oviposition or larval development can be achieved below  $17 °C$ , and larval survival below

20  $\degree$ C could be severely affected [\[40\]](#page-8-13). Our findings add onto this, as the adults were able to survive and reproduce for few days at 35  $\degree$ C, but the eggs or the newly emerged larvae could not survive at this temperature. Rueda and Axtell [\[40\]](#page-8-13) found that temperatures above 38  $\degree$ C inhibited their development, which is also readily apparent in our study, as less than 50% of adults survived at 37 ◦C and above. Progeny and larval development were also minimal, and a fairly high death rate (over 50%) for adult insects was observed. Both adult survival rate and therefore progeny production was completely suppressed at 40 ◦C (0% survival rate and progeny), which was expected, given that this temperature is lethal for other related beetle species as well [\[58](#page-9-6)[,59\]](#page-9-7).

Previous studies conducted on *H. illucens* have shown a correlation between moisture content of the rearing media and the survival and growth of larvae [\[60](#page-9-8)[,61\]](#page-9-9), suggesting that purposeful modification of moisture values may produce better results for insect mass rearing. Lower substrate moisture content yielded lower larval growth rates for *H. illucens*. Studies conducted on another beetle species, the rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), showed that progeny increased between 11.3 and 14.8% moisture content in whole corn kernels, but fell rapidly above 14.8% [\[62\]](#page-9-10). According to Axtell [\[2\]](#page-7-1), the development and survival at all life stages of *A. diaperinus* depend primarily on temperature rather than humidity, since the species can tolerate a broad range of humidity regimes, including relatively dry habitats [\[7\]](#page-7-4). In our study, the relative humidity in which the experiment was conducted was maintained at 55%, since it was difficult to retain higher relative humidity values, as it resulted in substantial fungal growth of the wheat grain, lowering progeny numbers.

We found that the total progeny production of *A. diaperinus* declined as moisture content increased above a certain threshold. This phenomenon is contrary to the general belief that the species requires a constant moisture supply and consequently higher moisture values, indicating that there may be other factors that influenced our experiments. We assume that the presence of carrot slices in each vial may have impacted the equilibrium between grain moisture and relative humidity, as the moisture content of grains is known to be influenced by relative humidity [\[62\]](#page-9-10). The authors acknowledge that this factor may have had an indirect impact on the reproductive output of *A. diaperinus*; however, the specific mechanisms underlying this effect remain unknown. In other words, despite the differences in the percentages of moisture content among the tested grains, it is likely that the occurrence of the carrot increased the relative humidity within the vial, and the speed of this increase could be related with the initial moisture content value. Additional experiments that investigate the fluctuations in relative humidity and moisture content within each vial during the incubation period could yield further insights into the influence of these two abiotic factors on the development of *A. diaperinus*. The objective could be to gather empirical evidence on whether adjusting the moisture levels in the rearing medium has the potential to substantially improve the production of offspring, and consequently, to establish the significance of this parameter as a critical factor to consider in the large-scale rearing of *A. diaperinus*.

### **5. Conclusions**

In retrospect, by optimizing the breeding and developmental temperature, as well as the grain moisture content, significant changes in population dynamics can be expected, as progeny and larval growth rapidly increases. The 25–32 ◦C range is the temperature spectrum in which *A. diaperinus* populations grow faster, as compared with other temperature values, with 30–32 ℃ being the optimum range, at least as far as adult survival and progeny production are concerned. We also found that this species can easily develop on wheat with moisture content value that is even lower than 10 %, a value which is sufficiently lower than the suitable value of other stored product beetle species. The results of this study suggest that even small changes in temperature or substrate moisture content may noticeably affect the progeny and growth of *A. diaperinus*. Given that temperature range was tested here at one single moisture content value and vice versa, we suggest that additional experimental

work is required to define specific combinations of these two factors that may enhance growth and progeny production of this species.

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**Data Availability Statement:** The data will be made available upon request.

**Conflicts of Interest:** The authors declare no conflict of interest.

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