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Multigenerational Effects of Short-Term High Temperature on the Development and Reproduction of the *Zeugodacus cucurbitae* (Coquillett, 1899)

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Abstract: *Zeugodacus cucurbitae* is an important pest of fruit and vegetable crops in tropical and subtropical regions, and high-temperature stress can have different effects on the development and reproduction of successive generations of *Z. cucurbitae*. To clarify the multigenerational effects of short-time high temperature on the development and reproduction of *Z. cucurbitae*, the newly emerged adults of the contemporary (F_1 generation) and the next generation (F_2 generation) were exposed to short-term high temperatures of 25 °C, 33 °C, 37 °C, 41 °C, and 45 °C for 1 h, and the multigenerational (F_1, F_2, and F_3 generation) effects of these temperatures on the development and reproduction of *Z. cucurbitae* were evaluated. The results showed that (1) when the F_1 was exposed to short-term high temperature, the egg production and lifespan of the F_1 decreased continuously with the increasing temperature, except for the 45 °C treatment for 1 h, which stimulated egg production. Only the 41 °C group had significantly higher egg production and lifespan than the control group in the F_3. (2) In the F_1 and F_2 that were exposed to short-term high temperature, the F_1 and F_3 were consistent with the results of F_1 that were exposed to short-term high temperature. In conclusion, the effects of high-temperature intensity and frequency on multiple generations of *Z. cucurbitae* were different. The results of this study can elucidate the effects of short-term high-temperature stress on the growth, development, and reproduction of *Z. cucurbitae* in different generations, and provide a reference basis for the integrated control of *Z. cucurbitae*.

Keywords: *Zeugodacus cucurbitae*; short-time high temperature; development; reproduction; multigenerational effects

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1. Introduction

Global warming has become one of the main features of today's global climate change, with global temperatures increasing by about 0.68 °C over the past 100 years, and global average temperatures are projected to increase by 1.5–4.5 °C by the end of the century [1,2]. The magnitude and frequency of insects experiencing extreme heat events have greatly increased in the context of global warming [3]. Insects are typical poikilothermal animals with many species, large numbers, and with the potential for rapid reproduction. Insects are very sensitive to environmental changes due to their small sizes, thin body walls, fast heat exchanges with the environment, and their poor ability to self-regulate body temperature. Therefore, the temperature is an important factor affecting their growth and reproduction [4,5]. The findings of studies have shown that when the environmental temperature exceeds the appropriate growth range for insects, they will respond accordingly, and their life activities, such as reproduction, growth, development, and survival, as well as their population dynamics, will undergo a series of changes in response to changes in temperature [6,7]. Different insect species often respond differently to high temperatures. For the *Dysmicoccus neobrevipes* Beardsley, short-term heat treatment does not enhance its heat stress tolerance [8]. The high-temperature treatment and high-temperature stress of the second instars of *Frankliniella occidentalis* significantly reduced the instars' survival and adult fecundity; however, *F. occidentalis* could adapt to high temperatures through rapid thermal domestication [9]. Different generations of the same insect have different responses to high-temperature stress. The survival and immunity of the offspring of *Tribolium castaneum* were significantly reduced when the maternal generation was exposed to 40 °C for 1 h [10]. Gilchrist and Huey found that the higher the temperature experienced by the maternal generation of *Drosophila melanogaster*, the higher the heat tolerance of the offspring within a certain range [11]. Therefore, studying the effects of high temperature on pests and their offspring can elucidate the adaptive responses of pests to high-temperature stress and provide a theoretical basis for integrated pest control.

Zeugodacus cucurbitae (Coquillett, 1899) (Diptera: Tephritidae) is widespread in tropical and subtropical regions, mainly affecting cucurbits, and can parasitize more than 120 species of fruits and vegetables [12]. The female adults pierce their ovipositors into the surface of fruits to lay eggs, which hatch into larvae and feed inside the fruits, causing the rotting of the fruits and causing huge economic losses [13]. In addition to parasitizing fruits, *Z. cucurbitae* can also parasitize flowers and stems, and even root tissue. In Hawaii, pumpkins in pumpkin fields are parasitized before they bear fruit, and female adults lay eggs in unopened female and male flowers, and larvae can develop successfully in the main roots, stems, and petioles, causing great damage to farmland [14]. The fast reproduction rate, high egg production, and wide distribution of the *Z. cucurbitae* make it extremely harmful. *Z. cucurbitae* usually occur in multiple generations a year, so a short-time high temperature can have a profound effect on the growth, development, and reproduction of *Z. cucurbitae* [15]. Studies have found that after a short-term high-temperature treatment for each insect state of *Z. cucurbitae*, the survival, reproduction, and egg hatching rates were significantly inhibited with the increase in treatment temperature. After the adults were treated at 41 °C for 8 h, the adult mortality reached 100%. After the eggs were treated at 42–43 °C for 45 min, the eggs could not hatch [12]. However, after exposure to 45 °C for 1 h, the fecundity and mating amount of adult *Z. cucurbitae* increased significantly, which stimulated the fecundity of *Z. cucurbitae* [16]. Current research on the effects of short-term high temperatures on the survival and reproduction of *Z. cucurbitae* has focused on contemporary times, but under natural conditions, *Z. cucurbitae* experience high temperatures that are often multigenerational. In this study, 33 °C, 37 °C, 41 °C, and 45 °C high temperatures were set to treat the contemporary F_1 generation only and both the F_1 and F_2 generations of *Z. cucurbitae* for 1 h. With 25 °C as the control group, the effects of short-time high temperature on the growth, development, and reproduction of *Z. cucurbitae* for three consecutive generations (F_1, F_2, and F_3 generations) were evaluated to clarify the multigenerational effects of different generations of *Z. cucurbitae* on short-time high-temperature stress.

2. Materials and Methods

2.1. Pest Source for Testing and Pest Management

The insects for this study were collected from balsam pear fields (109°29′ E, 19°30′ N) in Nada Town, Danzhou City, Hainan Province, China, and then bred in the laboratory by preparing the artificial feed. The larval feed formula included 1000 g of pumpkin, 1000 g of cornflour, 200 g of yeast powder, 200 g of sucrose, 6 g of sodium benzoate, 8 mL of concentrated hydrochloric acid, and 1000 mL of water. Adults were fed on a diet made up of sucrose and yeast powder (W:W) in equal proportion. The average indoor temperature was 25 ± 1 °C, with 70 ± 5% relative humidity (RH) and 14 h light: 10 h dark, and a stable laboratory temperature-sensitive population was established. In this study, the newly emerged adults of *Z. cucurbitae* were used for short-term high-temperature treatment.

2.2. Test Reagents and Materials

The reagents used in this study, such as yeast powder, cornflour, sodium benzoate, concentrated hydrochloric acid, and sucrose, were all purchased from Hainan Qingfeng Biotechnology Co., Ltd. (Haikou, China), and the pumpkins were purchased from the surrounding farmers' markets.

2.3. The Setting of Short-Term High-Temperature Treatment

The optimum temperature for *Z. cucurbitae* is 25–30 °C [17]. When the external temperature is greater than the upper limit of the optimum temperature, i.e., >30 °C, this study defines it as the high-temperature range. The F_1 described in this paper is the first generation of *Z. cucurbitae*, i.e., the contemporary *Z. cucurbitae*, the F_2 is the insect source of the egg laid by the F_1 to the adult, and the F_3 is the insect source of the egg laid by the F_2 to the adult. The newly emerged adult was caught in a cage in a specific number to wait for treatment. With the help of the artificial climate chamber, the treatments were set at 25 °C (CK), 33 °C, 37 °C, 41 °C, and 45 °C for 1 h for the F_1 only, and both the F_1 and F_2 of newly emerged adults (Figure 1), and 25 °C-1 h was used as the control for each treatment. Transfer to indoor feeding after treatment was done at 25 ± 1 °C.

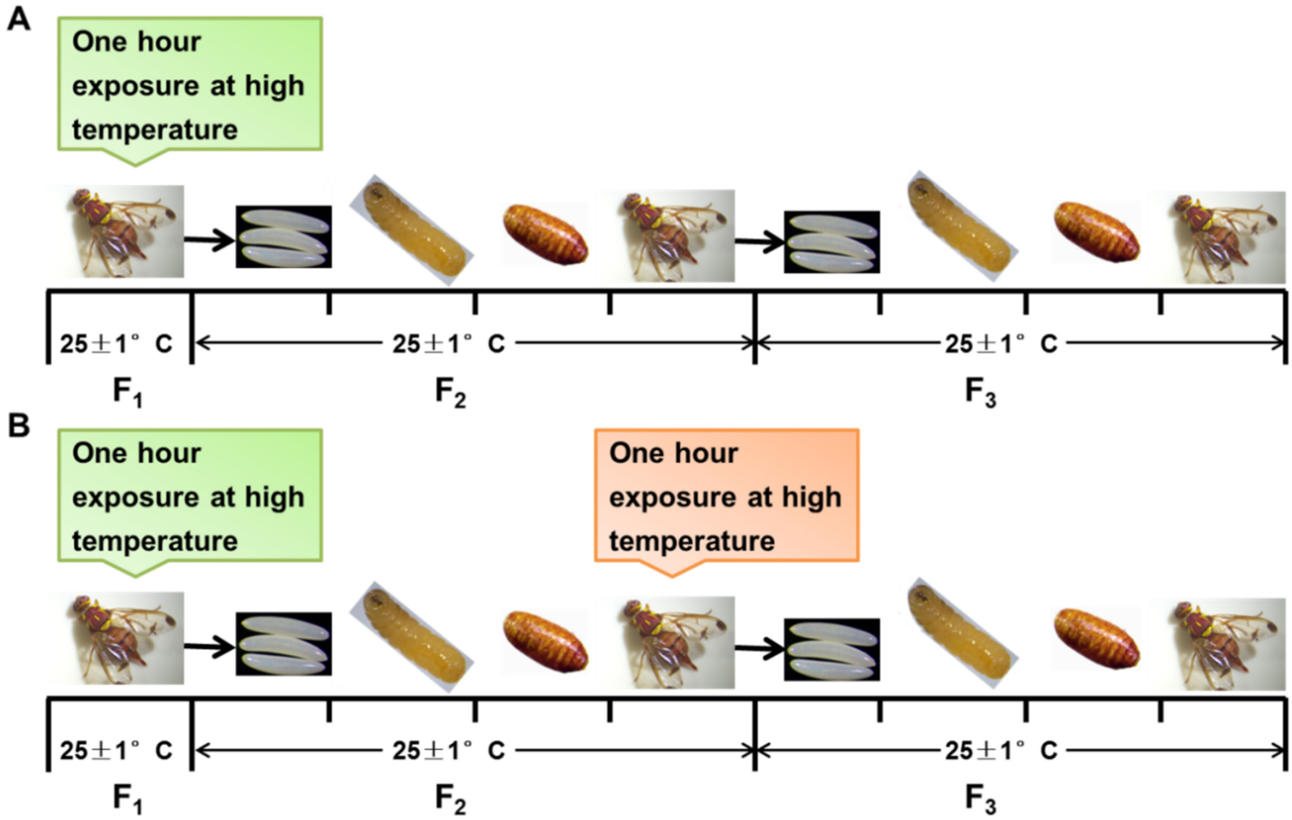

Figure 1. The setting of short-term high-temperature treatment. (**A**) The whole cage with F_1 of *Z. cucurbitae* was exposed to short-term high temperatures. (**B**) The whole cage with F_1 and F_2 of *Z. cucurbitae* was exposed to short-term high temperatures.

2.4. Observations on the Effects of Oviposition Ability and Egg Development

The newly emerged adults of each generation were paired according to 12 females and 12 males per cage, and the cages were equipped with water, artificial feed for adults, and thin slices of pumpkin for egg-laying. After the high-temperature treatment, the total oviposition quantity, daily oviposition quantity, single-female oviposition quantity, early-stage oviposition (d), hatchability of eggs (%), and oviposition days (d) were observed and recorded daily. Each cage of the 12 pairs was considered as 1 replicate, and 6 biological repetitions were set up under each treatment.

2.5. Observations on the Effects of Survival and Development

The survival rate (%) and lifespan (d) of adults in each group and each generation were recorded after the end of short-term high-temperature treatment. The survival rate and lifespan were counted separately for females and males. Each cage of the 12 pairs was considered as 1 replicate, and 6 biological repetitions were set up under each treatment. The larvae of each generation were randomly selected and divided into 120 larvae in each group and 6 replicates in each treatment. Pupae weight (g), pupation rate (%), emergence rate (%), and sex ratio were observed and recorded.

2.6. Statistical Analysis of Data

With the help of Excel (version 2021) (Microsoft, United States of America) and SPSS (version 26.0) (IBM, United States of America), a completely randomized analysis of variance and Tukey's multiple comparisons were performed on the data. The recorded indexes were: single-female oviposition quantity: total oviposition quantity/12; single-female daily oviposition quantity: single-female oviposition quantity/oviposition days; early-stage oviposition (d): time elapsed from the treatment date to the start of oviposition; oviposition days (d): time elapsed from the start of oviposition to the end of oviposition; hatchability of eggs (%): eggs hatched quantity/total oviposition quantity $\times$ 100; survival rate (%): number of surviving adults/12 $\times$ 100; lifespan (d): overall lifespan/12, where survival rate and lifespan were counted separately for males and females; pupation rate (%): number of pupation/120 $\times$ 100; emergence rate (%): number of emergences/total number of pupae $\times$ 100; sex ratio: number of emergence females/number of emergence males.

3. Results

3.1. F_1 Only of Z. cucurbitae Was Exposed to Short-Term High Temperature

3.1.1. Effects of Short-Term High Temperature on Oviposition and Egg Development of *Z. cucurbitae* (F_1)

The effects of short-time high temperature on the oviposition and egg development of *Z. cucurbitae* (F_1) are shown in Figure 2. The egg production of F_1 decreased continuously with the increase in treatment temperatures after being subjected to short-term high temperature, but the total oviposition quantity, daily oviposition quantity, and single-female oviposition quantity increased after 1 h of treatment at 45 °C, which were 5984, 62, and 499 eggs, respectively, and which were significantly higher than those of the control group (5171, 28, and 431 eggs, respectively). The total oviposition quantity, daily oviposition quantity, and single-female oviposition quantity in the 41 °C group were the lowest, at 2168, 19, and 181 eggs, respectively. The early-stage oviposition and oviposition days continued to shorten with increasing temperature, being the shortest in the 45 °C group at 7.4 and 98 d, and the longest in the control group at 10.5 and 184 d, respectively. The increase in treatment temperature caused a decrease in the hatchability of eggs, which was lowest in the 45 °C group at 61.6% and significantly lower than in the control group (91.2%). In summary, except for the 45 °C treatment for 1 h, which could stimulate the egg production of F_1, the short-term high-temperature treatment was generally unfavorable to the egg-laying and egg development of *Z. cucurbitae*.

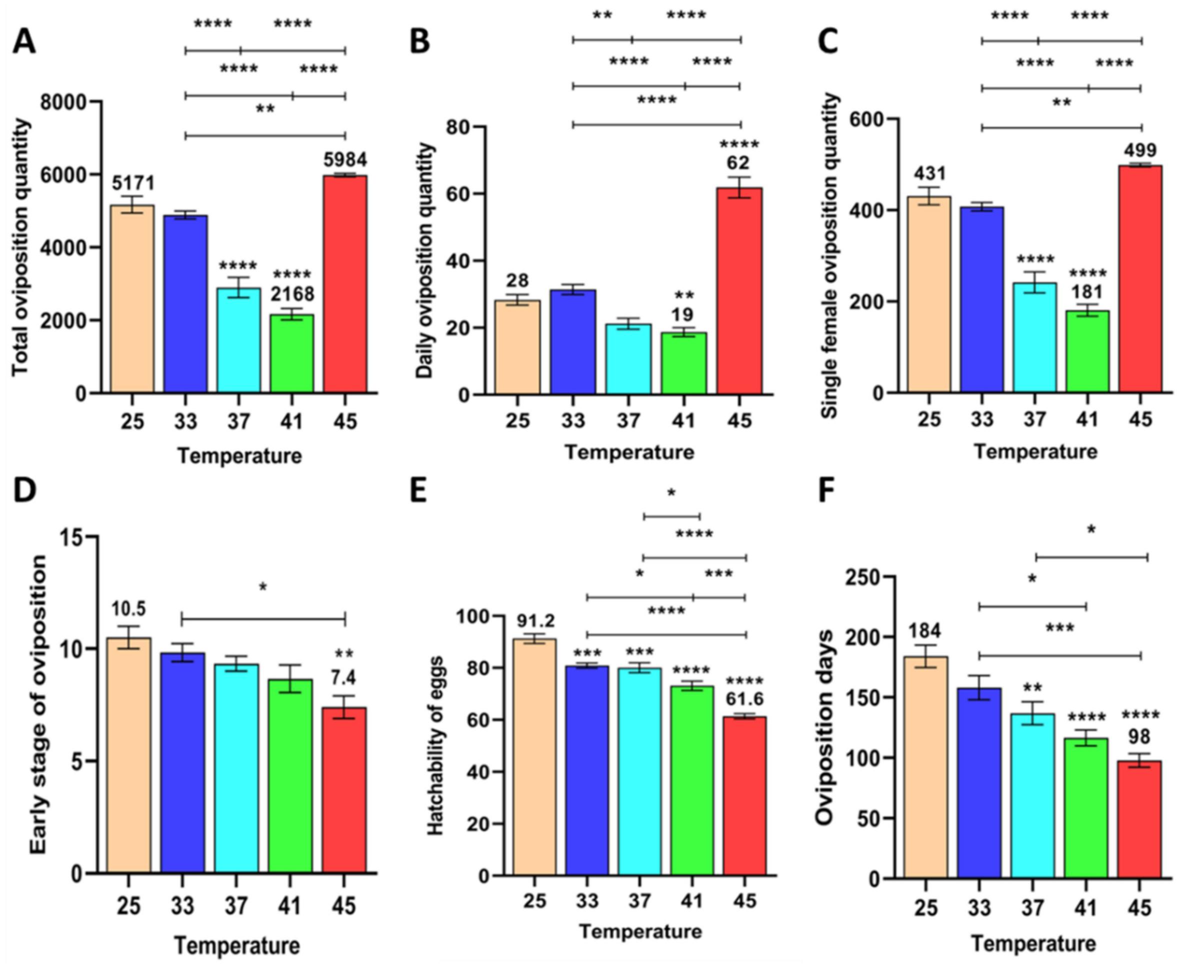

Figure 2. Effects of short-term high temperature on oviposition and egg development of *Z. cucurbitae* (F_1). (**A**) Total oviposition quantity. (**B**) Daily oviposition quantity. (**C**) Single-female oviposition quantity. (**D**) Early-stage oviposition. (**E**) Hatchability of eggs. (**F**) Oviposition days. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.1.2. Effect of Short-Term High Temperature on Oviposition and Egg Development of *Z. cucurbitae* (F_2)

The effects of short-time high temperature on the oviposition and egg development of *Z. cucurbitae* (F_2) are shown in Figure 3. The total oviposition quantity, daily oviposition quantity, and single-female oviposition quantity of the F_2 in the high-temperature-treated group were lower than those of the control group, with the lowest in the 45 °C group (4543, 23, and 378 eggs, respectively) and the highest in the control group (7173, 32, and 597 eggs, respectively). The early-stage oviposition is first prolonged and then shortened with the increase in temperature. The longest time in the 37 °C group was 11.5 days, which was significantly higher than 8.5 days in the control group, and the shortest time in the 45 °C group was 8.2 days. The hatchability of eggs in the high-temperature treatment groups was higher than that in the control group, with the highest being 86% in the 33 °C group, significantly higher than that of the control group (79.5%), and the longest oviposition day was 221 d in the control group and the shortest was 187 d in the 37 °C group. The egg production of the F_2 was higher than that of the F_1, and the hatchability of eggs was above 80%, with no significant differences among the treatment groups.

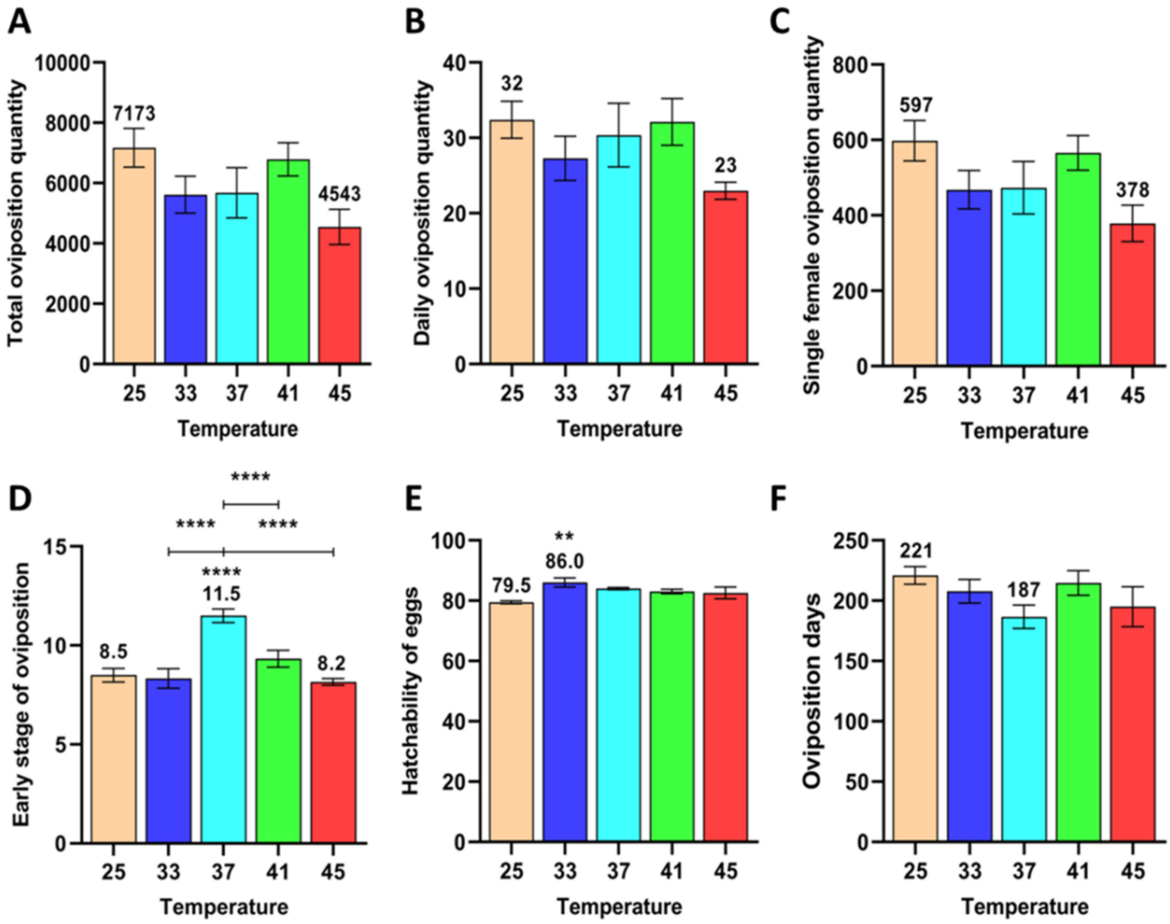

Figure 3. Effects of short-term high temperature on oviposition and egg development of *Z. cucurbitae* (F_2). (**A**) Total oviposition quantity. (**B**) Daily oviposition quantity. (**C**) Single-female oviposition quantity. (**D**) Early-stage oviposition. (**E**) Hatchability of eggs. (**F**) Oviposition days. All values in the figure are represented as mean ± standard error. The "**" and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.01$ and $p \leq 0.0001$, respectively).

3.1.3. Effect of Short-Term High Temperature on Oviposition and Egg Development of *Z. cucurbitae* (F_3)

The effects of short-time high temperature on the oviposition and egg development of *Z. cucurbitae* (F_3) are shown in Figure 4. The total oviposition quantity and single-female oviposition quantity of the F_3 both increased and then decreased with increasing temperature, with the lowest in the 33 °C group (2453 and 204 eggs, respectively) and the highest in the 41 °C group (6307 and 526 eggs, respectively), and the total oviposition quantity and single-female oviposition quantity of both groups were significantly different from those of the control group (4161 and 347 eggs, respectively). The daily oviposition quantity was highest in the 45 °C group with 33 eggs, significantly higher than the control group with 20 eggs, and was lowest in the 33 °C group with 16 eggs. The early-stage oviposition was first shortened and then lengthened with the increasing temperature, with the shortest early-stage oviposition of 7.8 d in the 37 °C group, which was significantly lower than that of the control group (9.8 d), and the longest early-stage oviposition of 10.5 d was found in the 45 °C group. The hatchability of eggs was highest in the 37 °C group with 85.9% and lowest in the control group with 79.6%, and the 33 °C group had the lowest oviposition days—significantly lower than that of the control group (208 d).

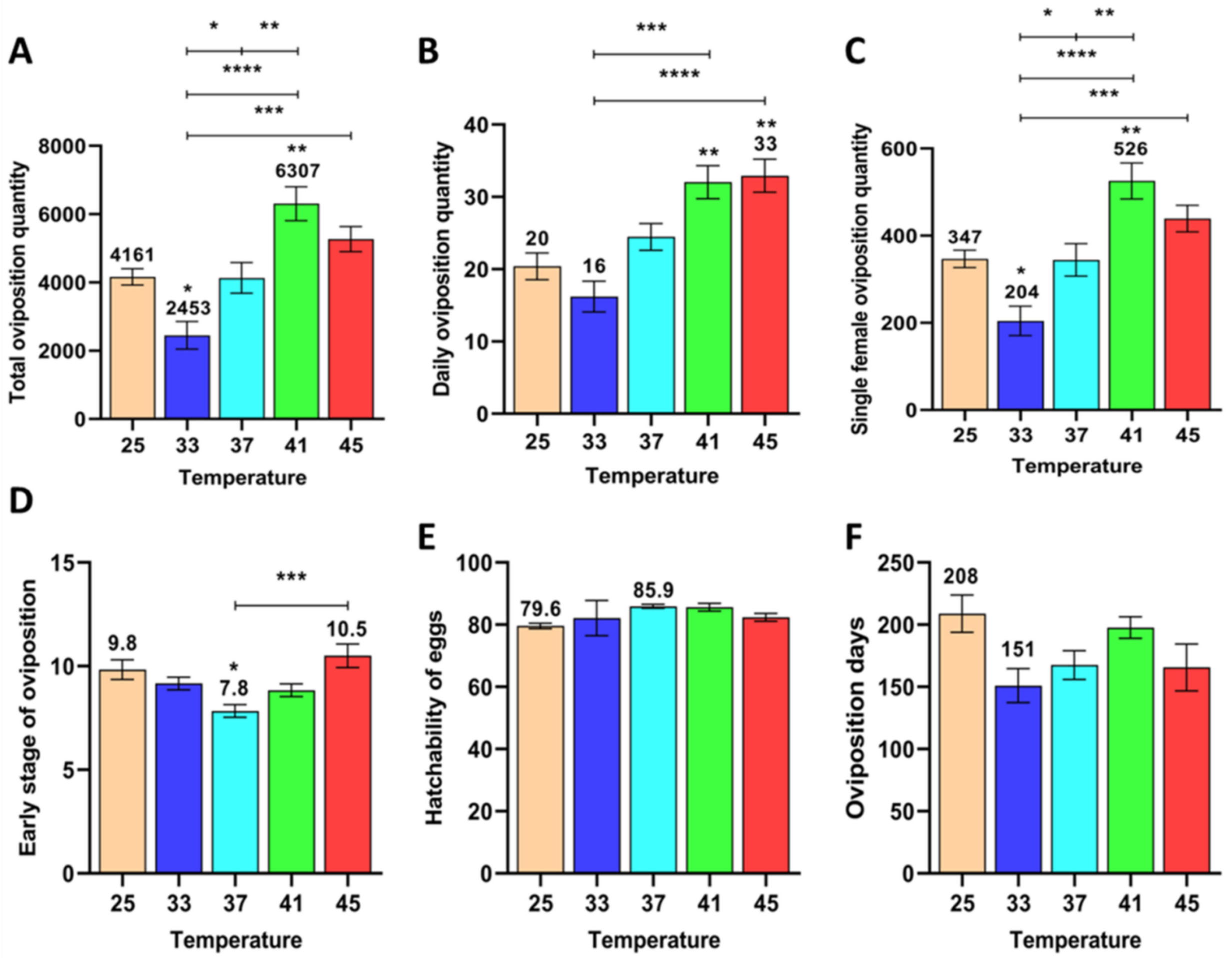

Figure 4. Effects of short-term high temperature on oviposition and egg development of *Z. cucurbitae* (F_3). (**A**) Total oviposition quantity. (**B**) Daily oviposition quantity. (**C**) Single-female oviposition quantity. (**D**) Early-stage oviposition. (**E**) Hatchability of eggs. (**F**) Oviposition days. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.1.4. Effect of Short-Term High Temperature on Survival and Development of *Z. cucurbitae* (F_1)

The effects of short-time high temperature on the survival and development of *Z. cucurbitae* (F_1) are shown in Figure 5. The survival and lifespan of females and males continued to decrease with increasing temperatures after short-term high-temperature treatment, with the highest survival rate of 100.0% for both females and males in the control group and the lowest in the 45 °C treatment group, at 58.3% and 50.0%, respectively, showing a higher survival rate for females compared to males in the same treatment group, indicating that females are more tolerant of high temperatures than males. The lifespan of females was longer than that of males in the same treatment group, with the shortest lifespan of females and males in the 45 °C treatment group being 72 and 59 d, respectively, which was significantly lower than that of the control group (160 and 143 d, respectively).

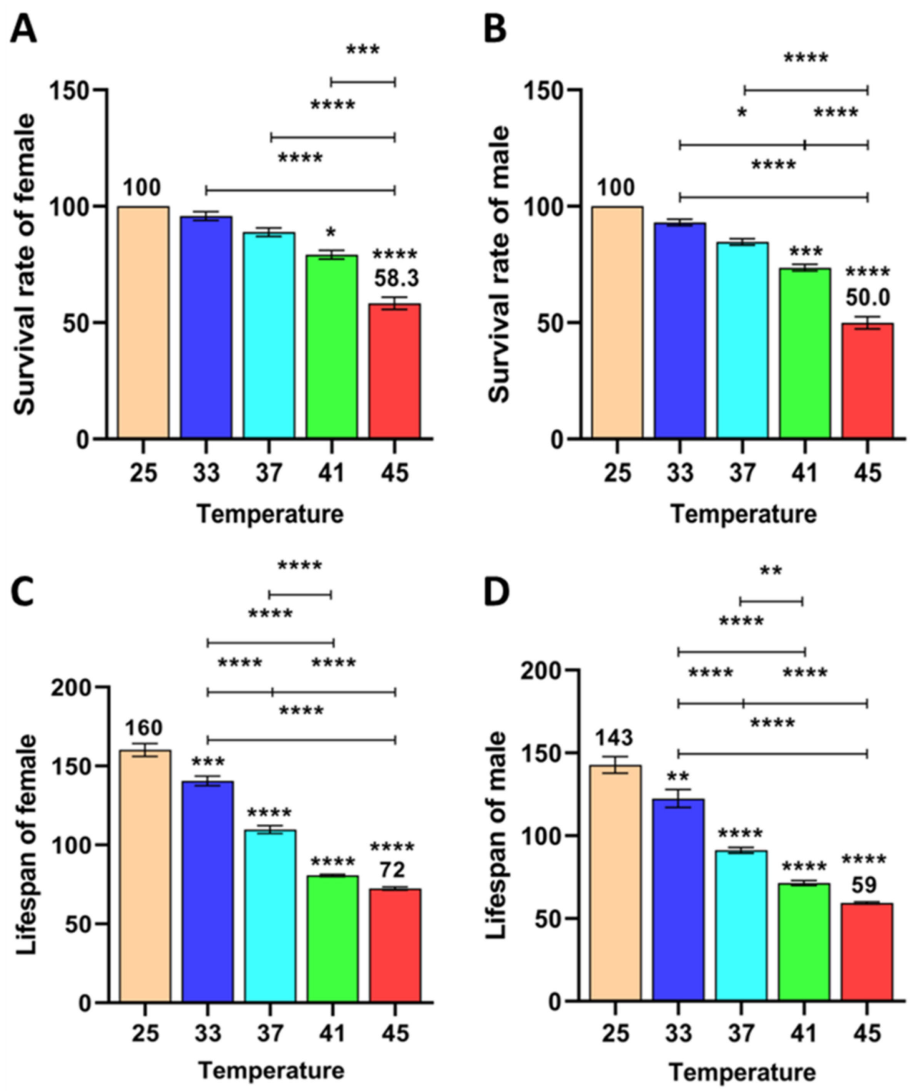

Figure 5. Effects of short-term high temperature on survival and development of *Z. cucurbitae* (F_1). (**A**) Survival rate of female. (**B**) Survival rate of male. (**C**) Lifespan of female. (**D**) Lifespan of male. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.1.5. Effect of Short-Term High Temperature on Survival and Development of *Z. cucurbitae* (F_2)

The effects of short-time high temperature on the survival and development of *Z. cucurbitae* (F_2) are shown in Figure 6. The lifespan of females and males in the 45 °C group was the shortest (79 and 99 d, respectively), while the longest lifespan for females was 144 d for the control group, and the longest lifespan for males was 177 d at 41 °C. The pupae weight, pupation rate, and emergence rate of the F_2 decreased with increasing temperatures, and were the lowest in the 45 °C treatment group, at 0.0075 g, 41.4%, and 51.6%, respectively, which were all significantly lower than the control (0.0170 g, 99.8%, and 96.9%, respectively). The sex ratio increased with increasing temperatures, with a maximum of 3.44:1 at 45 °C, indicating that females were more heat tolerant than males.

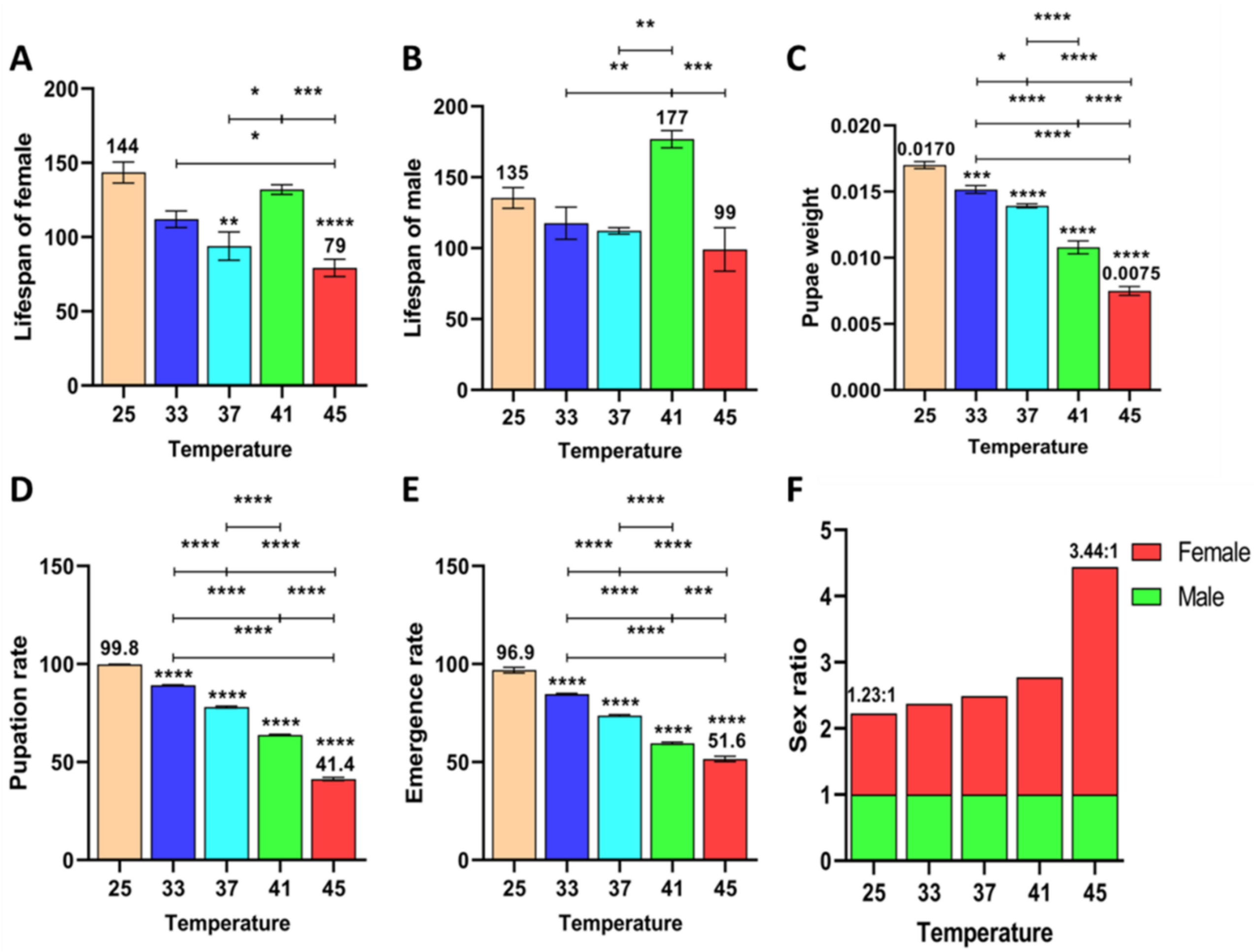

Figure 6. Effects of short-term high temperature on survival and development of *Z. cucurbitae* (F_2). (**A**) Lifespan of female. (**B**) Lifespan of male. (**C**) Pupae weight. (**D**) Pupation rate. (**E**) Emergence rate. (**F**) Sex ratio. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.1.6. Effect of Short-Term High Temperature on Survival and Development of *Z. cucurbitae* (F_3)

The effects of short-time high temperature on the survival and development of *Z. cucurbitae* (F_3) are shown in Figure 7. The lifespan of the females and males was the shortest in the 33 °C group, at 49 and 47 d, respectively, and was the longest in the 41 °C group, at 141 and 149 d, respectively. The female and male lifespans of both groups were significantly different from those of the control group (86 and 88 d, respectively), and the pupae weight, pupation rate, and emergence rate of each treatment group were not significantly different from those of the control group, with the heaviest pupae weight of 0.0162 g found in the 45 °C group and the lightest found in the control group, at 0.0154 g. All treatment groups had high pupation rates, with the highest pupation rate of 100% in the 41 °C group and the lowest of 99.7% in the 33 °C group. The highest emergence rate was 96% in the 33 °C group and the lowest was 76.8% in the 41 °C group, which was significantly lower than the control (90.4%). The sex ratio fluctuated with increasing temperatures, with the highest observed in the 37 °C group at 1.53:1 and the lowest in the 41 °C group at 1.05:1.

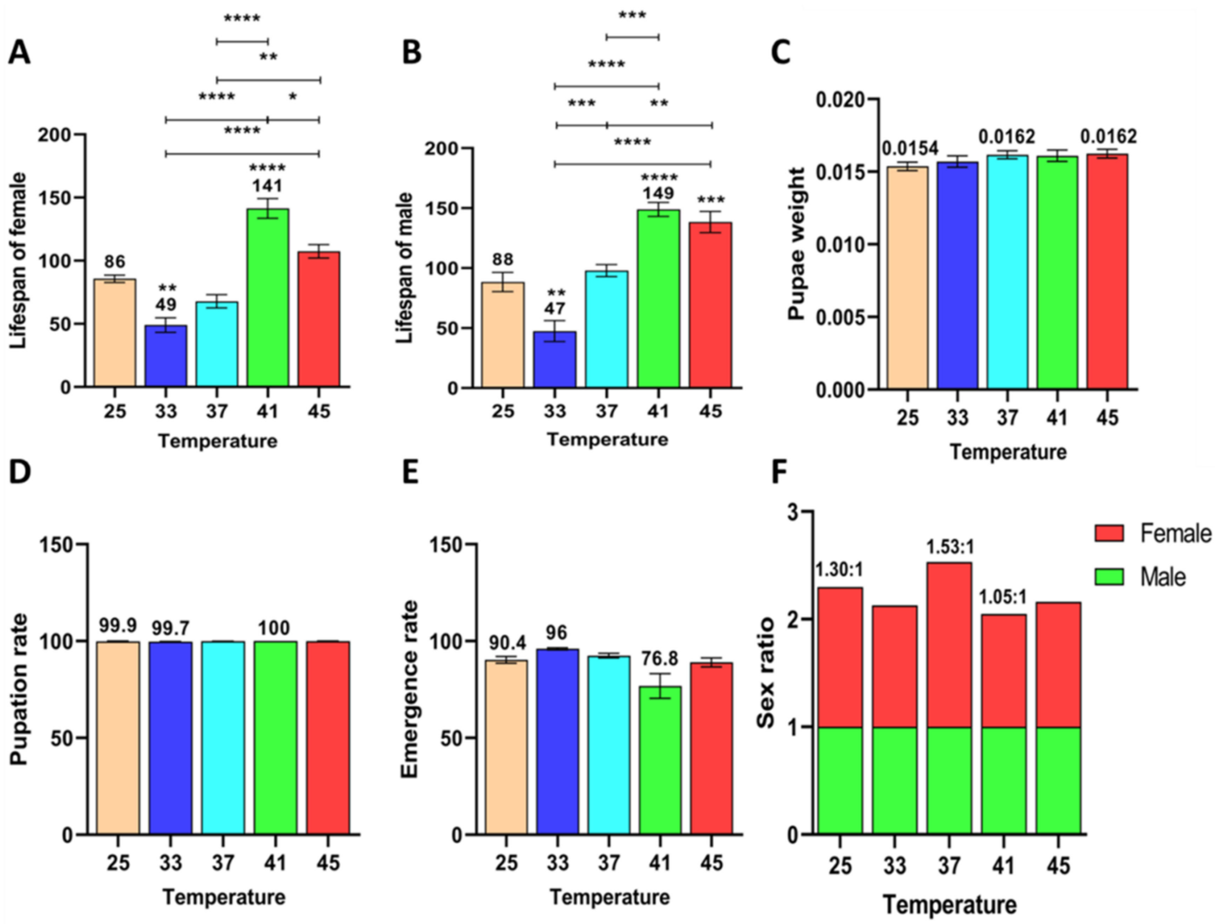

Figure 7. Effects of short-term high temperature on survival and development of *Z. cucurbitae* (F_3). (**A**) Lifespan of female. (**B**) Lifespan of male. (**C**) Pupae weight. (**D**) Pupation rate. (**E**) Emergence rate. (**F**) Sex ratio. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.2. Both the F_1 and F_2 of Z. cucurbitae Were Exposed to Short-Term High Temperature

3.2.1. Effect of Short-Term High Temperature on Oviposition and Egg Development of *Z. cucurbitae* (F_1)

The effects of short-time high temperature on the oviposition and egg development of Z. cucurbitae (F_1) are shown in Figure 8. The egg production of F_1 adults decreased in general with the increase in temperature after being treated with short-time high temperature, but their egg production increased after 1 h of treatment at 45 °C. The total oviposition quantity, daily oviposition quantity, and single-female oviposition quantity were the highest in the 45 °C group, with 7129, 62, and 594 eggs, respectively, which were significantly higher than those in the control group (6289, 35, and 524 eggs, respectively), and the lowest totals were observed in the 41 °C group, with 4587, 31, and 382 eggs, respectively. The early-stage oviposition, hatchability of eggs, and oviposition days decreased with increasing treatment temperatures. The early-stage oviposition and oviposition days were the shortest in the 45 °C. group, at 7.2 days and 115 days respectively, and were the longest in the control group, at 12.5 days and 179 days respectively. The hatching rate of eggs in the 45 °C group was the lowest, at 54.9%, which was significantly lower than that of the control group (92.6%). In conclusion, the short-time high-temperature treatment was not conducive to the reproduction of the F_1, but the treatment at 45 °C for 1 h stimulated its egg production.

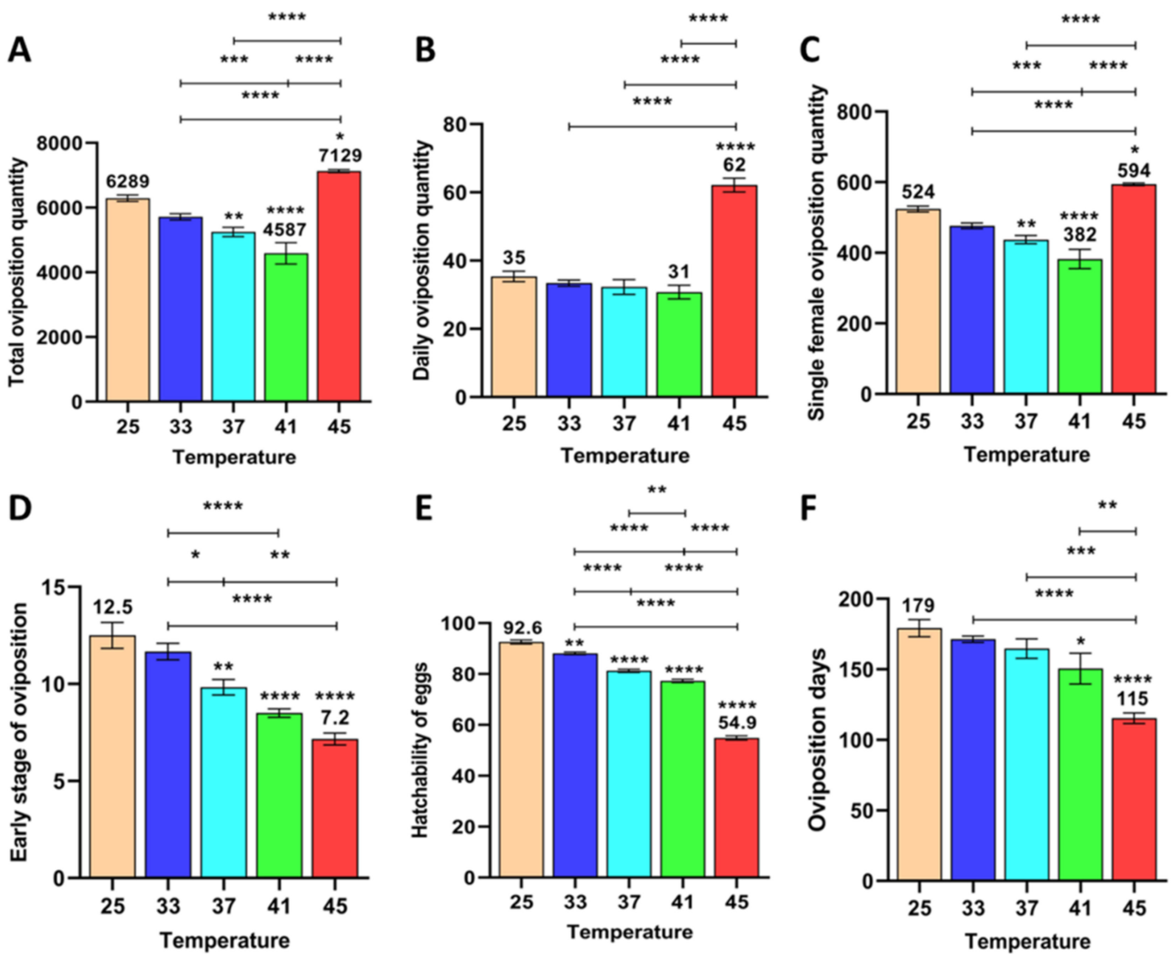

Figure 8. Effects of short-term high temperature on oviposition and egg development of *Z. cucurbitae* (F_1). (**A**) Total oviposition quantity. (**B**) Daily oviposition quantity. (**C**) Single-female oviposition quantity. (**D**) Early-stage oviposition. (**E**) Hatchability of eggs. (**F**) Oviposition days. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.2.2. Effect of Short-Term High Temperature on Oviposition and Egg Development of *Z. cucurbitae* (F_2)

The effects of short-time high temperature on oviposition and egg development of *Z. cucurbitae* (F_2) are shown in Figure 9. The total oviposition quantity, daily oviposition quantity, and single-female oviposition quantity of F_2 adults decreased continuously with the increase in treatment temperature, and were lowest in the 45 °C group, 250, 4, and 27 eggs respectively, which were significantly lower than those of the control group (6323, 41, and 527 eggs respectively). The early-stage oviposition advanced with the increase in treatment temperatures, and the early-stage oviposition was only 7 d in the 45 °C group, which was significantly lower than that of 10.5 d in the control group, and the oviposition days shortened with the increase in treatment temperatures, with the shortest being 48 d in the 45 °C group and the longest being 156 d in the control group. The hatchability of eggs decreased with the increase in temperatures, with the lowest being 37.4% in the 45 °C group, which was significantly lower than the control group (90.1%). In conclusion, F_1 and F_2 of *Z. cucurbitae* were exposed to short-term high temperatures, which adversely affected the egg-laying ability and egg development of F_2.

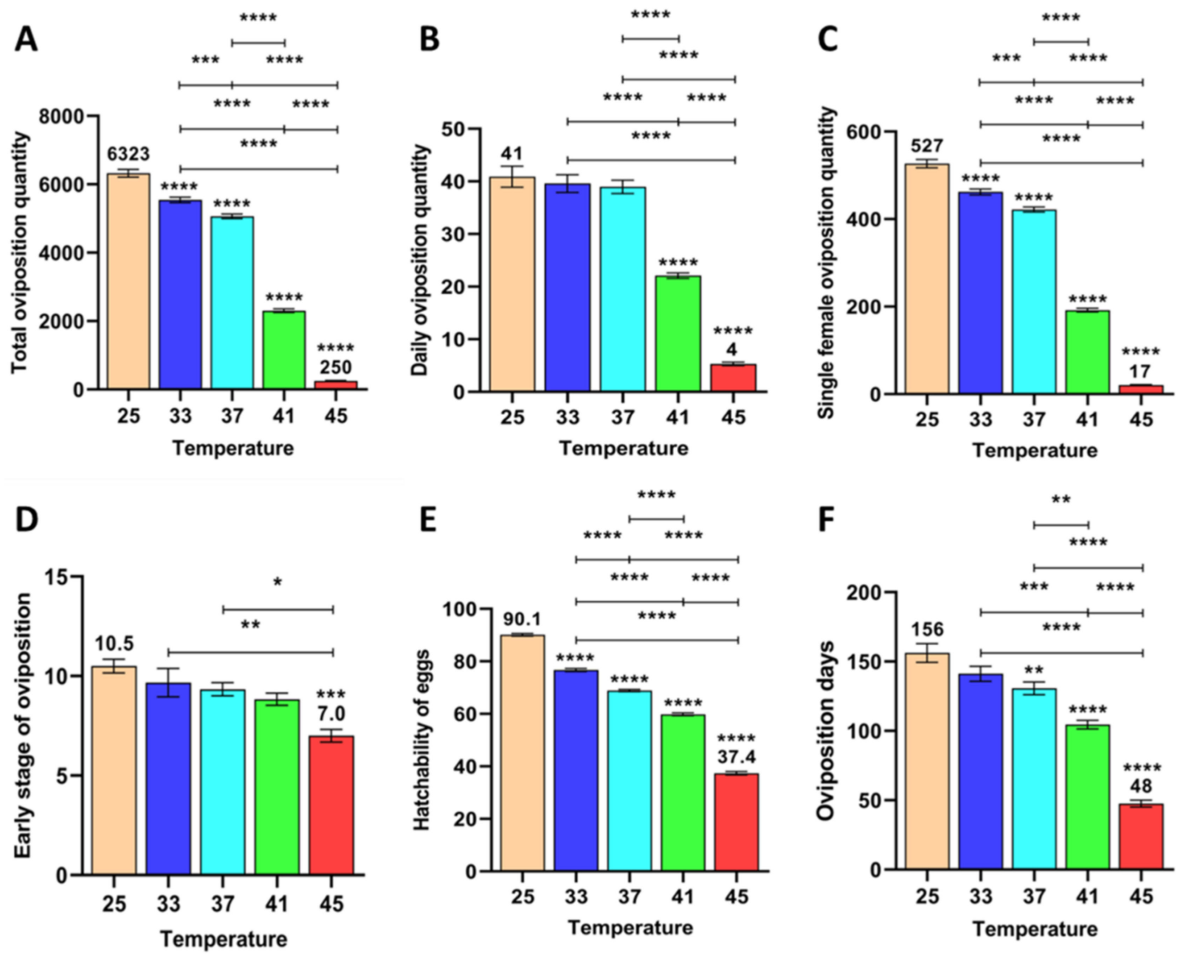

Figure 9. Effects of short-term high temperature on oviposition and egg development of *Z. cucurbitae* (F_2). (**A**) Total oviposition quantity. (**B**) Daily oviposition quantity. (**C**) Single-female oviposition quantity. (**D**) Early-stage oviposition. (**E**) Hatchability of eggs. (**F**) Oviposition days. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.2.3. Effect of Short-Term High Temperature on Oviposition and Egg Development of *Z. cucurbitae* (F_3)

The effects of short-time high temperature on the oviposition and egg development of *Z. cucurbitae* (F_3) are shown in Figure 10. The total oviposition quantity, daily oviposition quantity, and single-female oviposition quantity of the F_3 were the largest in the 41 °C group, with 7867, 48, and 656 eggs, respectively, which were significantly higher than that of the control group (5032, 23, and 419 eggs, respectively), and the smallest totals were found in the 37 °C group, with 1765, 14, and 147 eggs, respectively, which were significantly lower than that of the control group. The early-stage oviposition was the shortest in the 41 °C group at 9.3 d and the longest in the 37 °C group at 15 d. The oviposition days were the shortest in the 37 °C group at 126 d, significantly lower than the 231 d in the control group. The hatchability of eggs was the highest in the 41 °C group at 87.4% and the lowest in the 37 °C group at 80.6%. In the F_3, only the 41 °C group produced significantly more eggs than the control group, but in the F_1 and F_2 groups, the egg production of the 41 °C group was significantly lower than that of the control, indicating that both the F_1 and F_2 were exposed to 41 °C treatment, which inhibited the egg production of the F_1 and F_2, but promoted the egg production of their progeny (F_3).

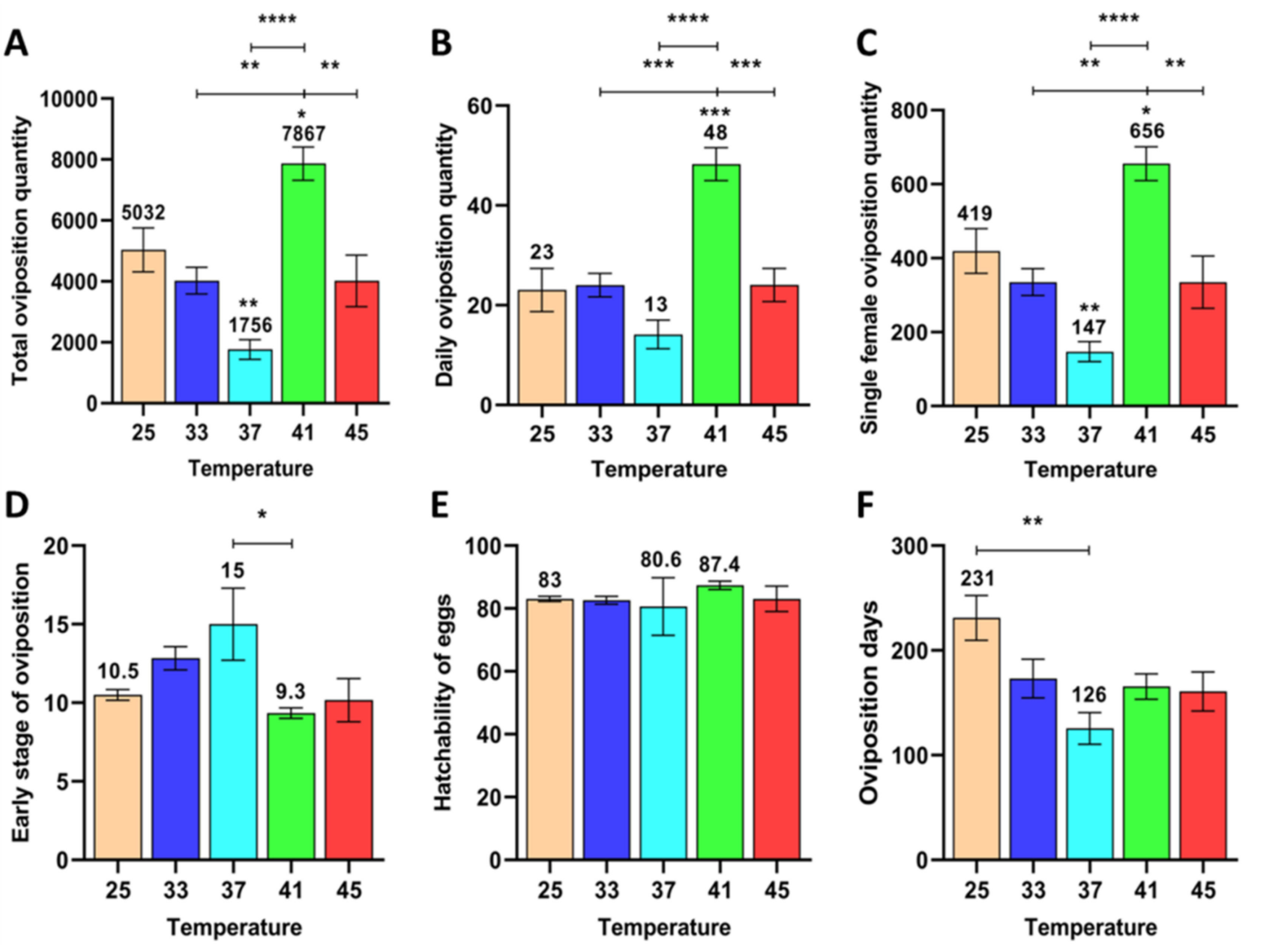

Figure 10. Effects of short-term high temperature on oviposition and egg development of *Z. cucurbitae* (F_3). (**A**) Total oviposition quantity. (**B**) Daily oviposition quantity. (**C**) Single-female oviposition quantity. (**D**) Early-stage oviposition. (**E**) Hatchability of eggs. (**F**) Oviposition days. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.2.4. Effect of Short-Term High Temperature on Survival and Development of *Z. cucurbitae* (F_1)

The effects of short-time high temperature on the survival and development of *Z. cucurbitae* (F_1) are shown in Figure 11. The survival rate of the F_1 continued to decrease with increasing temperatures after short-term high-temperature treatment, and the survival rate of both females and males in the 45 °C group was the lowest, at 50% and 44.4%, respectively, which was significantly lower than that of the control group (both 100%), and the survival rate of females in the same high-temperature treatment was higher than that of males, indicating that the females were more heat-resistant than the males. The lifespan of the F_1 continued to shorten with increasing temperatures, and the lifespan of both females and males in the 45 °C group was the shortest, at 92 and 84 d, respectively, which was significantly lower than that of the control group (179 and 161 d, respectively).

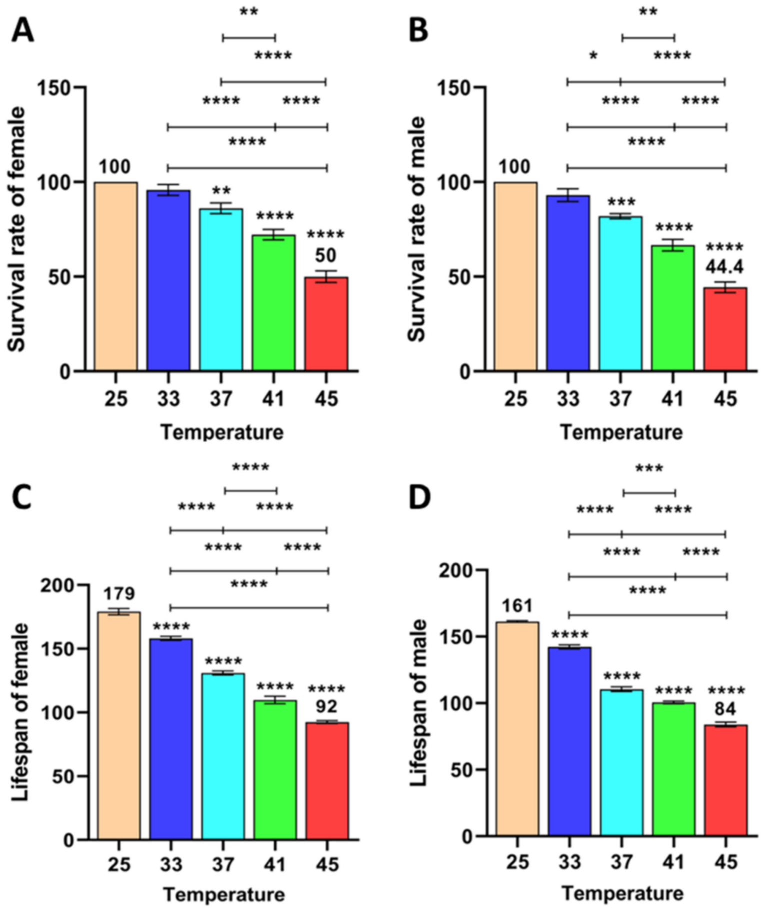

Figure 11. Effects of short-term high temperature on survival and development of *Z. cucurbitae* (F_1). (**A**) Survival rate of female. (**B**) Survival rate of male. (**C**) Lifespan of female. (**D**) Lifespan of male. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.2.5. Effect of Short-Term High Temperature on Survival and Development of *Z. cucurbitae* (F_2)

The effects of short-time high temperature on the survival and development of *Z. cucurbitae* (F_2) are shown in Figure 12. The survival rate of the F_2 decreased with increasing temperatures, and the survival rate of females in the same treatment was higher than that of males, and the survival rates of females and males in the 45 °C group were the lowest, at 37.5% and 33.3%, respectively, which were significantly lower than that of the control group (both 100%), indicating that females were still more heat-resistant than males after two consecutive generations of high-temperature treatment, and their survival rate was overall lower than that of the F_1. Lifespan decreased with increasing temperatures, with the lifespan of females longer than that of males in the same treatment, with the shortest lifespan of 86 and 77 d, respectively, for both females and males in the 45 °C group, respectively, both significantly lower than that of the control group (156 and 143 d, respectively). With increasing temperatures, pupae weights, pupation rates, and emergence rates decreased, with the lowest observed in the 45 °C treatment at 0.0071 g, 38.8%, and 54.3%, respectively, which were significantly lower than that of the control (0.0176 g, 99.4%, and 90.6%, respectively). The sex ratio increased with increasing temperatures, with a maximum of 4.3:1 at 45 °C, indicating that females were more heat tolerant than males.

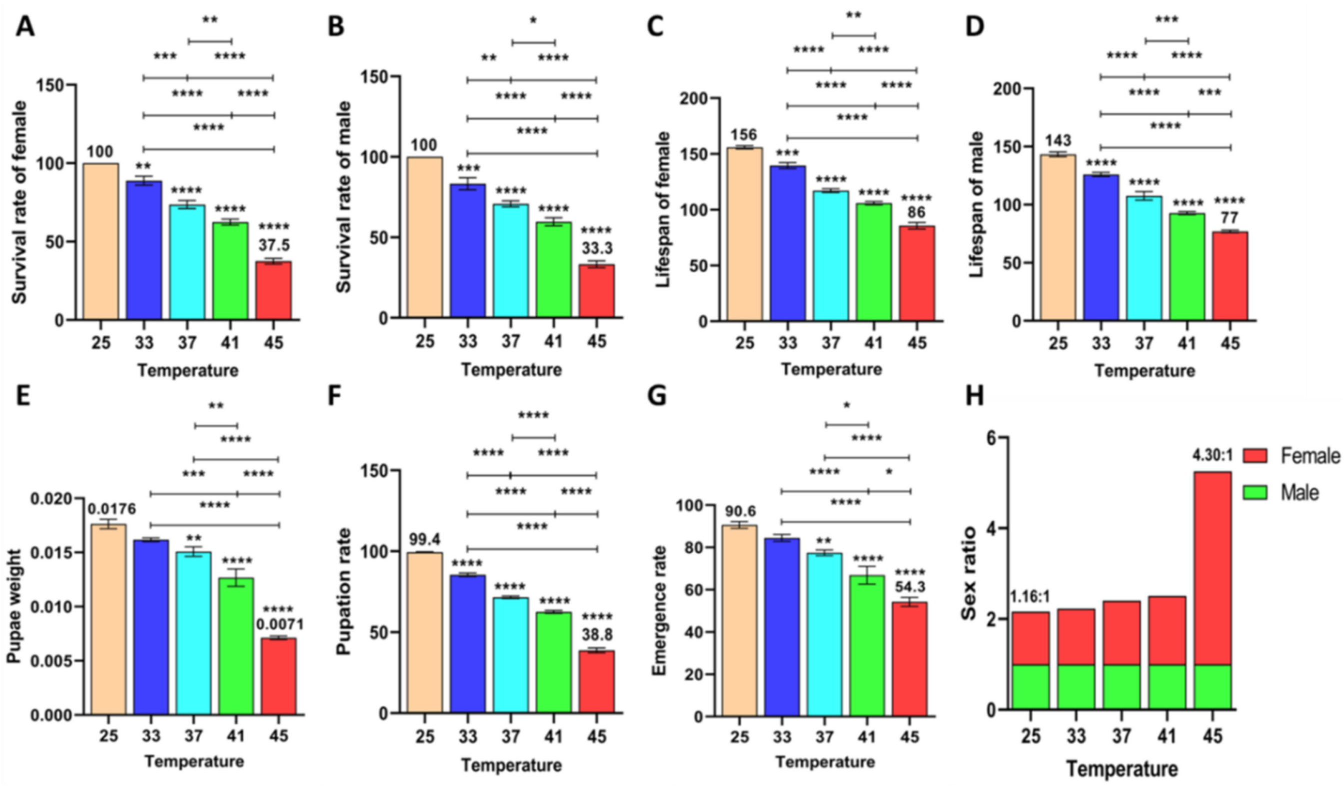

Figure 12. Effects of short-term high temperature on survival and development of *Z. cucurbitae* (F_2). (**A**) Survival rate of female. (**B**) Survival rate of male. (**C**) Lifespan of female. (**D**) Lifespan of male. (**E**) Pupae weight. (**F**) Pupation rate. (**G**) Emergence rate. (**H**) Sex ratio. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

3.2.6. Effect of Short-Term High Temperature on Survival and Development of *Z. cucurbitae* (F_3)

The effects of short-time high temperature on the survival and development of *Z. cucurbitae* (F_3) are shown in Figure 13. The longest lifespan of the F_3 was in the 41 °C group, with 113 and 173 d for females and males, respectively, and the shortest was in the 37 °C group, with 39 and 58 d, respectively. The pupae weights, pupation rates, and emergence rates decreased with increasing temperatures, with the lowest observed in the 45 °C group at 0.0065 g, 19.6%, and 19.1%, respectively, which were significantly lower than the control group (0.0180 g, 97.2%, and 94.3%, respectively). The sex ratio increased with increasing temperatures, with the largest observed in the 41 °C group at 2.08:1. The sex ratio in the 45 °C group was at 1:0, and all emerged adults were female, indicating that both the F_1 and F_2 were subjected to short-time high-temperature treatment, which could reduce the number of emerged F_3 males—or could even result in the males failing to complete emergence.

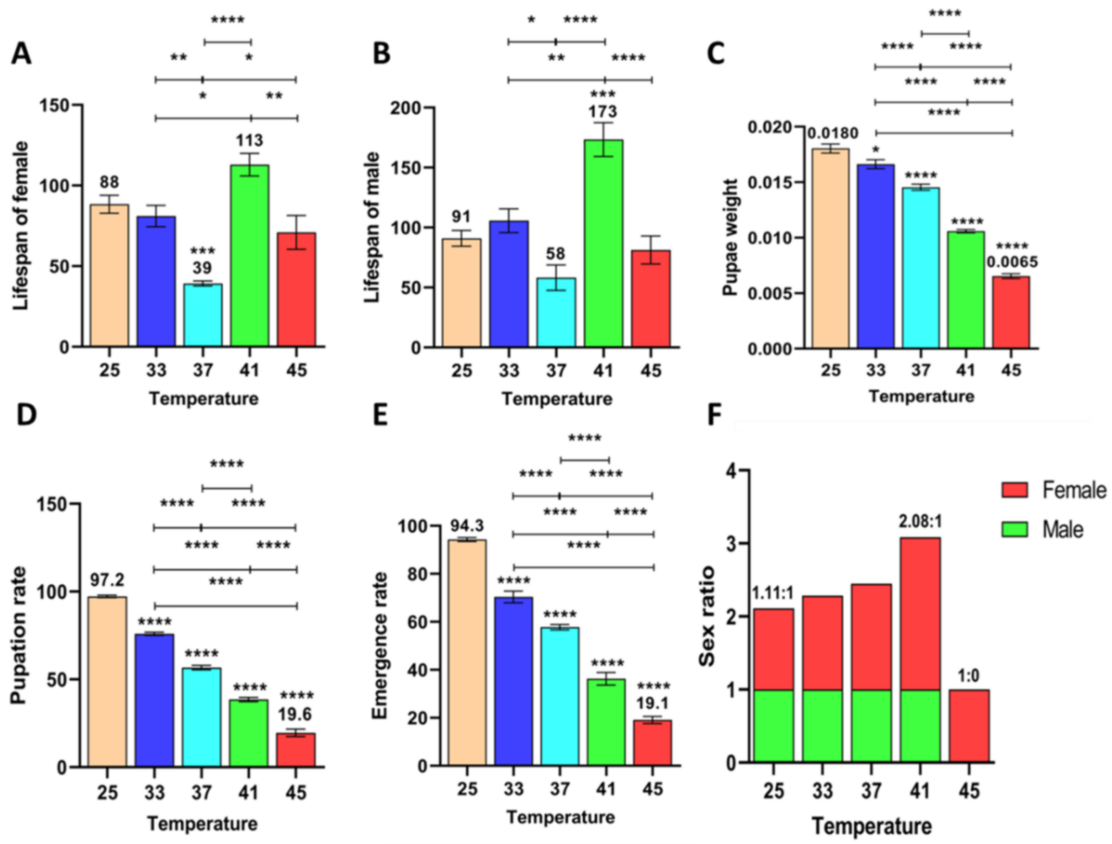

Figure 13. Effects of short-term high temperature on survival and development of *Z. cucurbitae* (F_3). (**A**) Lifespan of female. (**B**) Lifespan of male. (**C**) Pupae weight. (**D**) Pupation rate. (**E**) Emergence rate. (**F**) Sex ratio. All values in the figure are represented as mean ± standard error. The "*", "**", "***", and "****" above the histograms indicate values that differ significantly between treatments ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively).

4. Discussion

In this study, the F_1 of *Z. cucurbitae* produced significantly more eggs and a significantly shorter early-stage oviposition than the control group after 1 h treatment at 45 °C, but the hatchability of the eggs was significantly lower than the control group, indicating that the extreme high-temperature (45 °C-1 h) stimulated the F_1 to lay eggs, but reduced the hatchability of eggs, which is consistent with the results exposing female *Grapholitha molesta* to 38 °C for 4 h, which increased fecundity by 8.9%, but significantly reduced the hatchability of eggs [18]. Insects stimulated by certain environmental conditions such as high temperatures produce a "toxic excitatory effect", a specific temperature-adapted survival strategy, which is also considered a means of survival against heat stress [19]. After 1 h of treatment at 45 °C, the metabolism of *Z. cucurbitae* becomes faster, ovary development is accelerated, and fecundity is enhanced to promote rapid population development [20,21]. Zeng et al. found that the expression of the *Jh-inducible protein* gene was upregulated and that of the *Jh-epoxide hydrolase-2* gene was downregulated in newly emerged adults of *Z. cucurbitae* after treatment at 45 °C for 1 h, which maintained a higher concentration of the juvenile hormone and thus stimulated egg-laying in *Z. cucurbitae* [22]. The effects of high temperature on insects were mainly caused by water loss, alteration of intracellular ion concentration, destruction of the cytoskeleton, disruption of nerve conduction, and structural and functional changes of proteins, DNA, and other biomolecules [23], which further caused the death of insects. In this study, the survival rate and lifespan of the F_1 decreased as the treatment temperature increased, and the survival rate and lifespan of the female were higher than those of males in the same treatment, indicating that the female is more adaptable to short-term high-temperature stress than males, and more heat-resistant than males.

In the F_1 exposed to short-term high temperature, the egg production and hatchability of eggs of the F_2 were not significantly different, and the lifespan of the 45 °C group was significantly lower than that of the control group, indicating that the adverse effects of short-time high temperature on the F_1 continued into the F_2. In the F_1 and F_2 that were exposed to short-term high temperature, the egg production and hatchability of eggs of the F_2 decreased continuously with increasing temperatures, and the egg production of the 45 °C treatment group was much lower than that of the other treatment groups, but its early-stage oviposition was significantly shorter than that of the control group, indicating that two consecutive generations of short-time high-temperature treatment promoted the metabolism of the F_2 and accelerated ovarian development; however, the "toxic excitatory effect" could not be reproduced in the F_2, indicating the instability of the "toxic excitatory effect". The pupae weight, pupation rate, and emergence rate of the F_2 of both treatments decreased continuously with the increase in treatment temperature, indicating that the short-term high-temperature treatment of adults led to a reduction in the hatchability of eggs, pupation rate, and emergence rate of their next generation. The larvae of *Spodoptera littoralis* and *Heliothis virescens* also showed a reduction in pupae weight and pupation rate after high-temperature stress [24]. It has been suggested that small individuals may avoid heat damage by promoting water and nutrient uptake by intestinal symbionts [25] and that insects can regulate and mitigate the effects of heat stress through phenotypic adaptations and rapid evolutionary responses [26]. In this study, the short-term high-temperature treatment of adults reduced the pupae weight of offspring and produced smaller individuals, thus increasing their ability to withstand high temperatures. The sex ratio of the F_2 increased with increasing treatment temperatures in both treatments, which is consistent with the results of Gu et al., further suggesting that the females of *Z. cucurbitae* are more adaptable to short-term high-temperature stress than the males [27].

In both treatments of this study, the oviposition of F_1 was most adversely affected by high temperature in the 41 °C treatment group, but in the F_3, the 41 °C group produced significantly more eggs than the control group, indicating that the adverse effects of short-time high temperature decreased continuously with the increase in generations, and even promoted the development and reproduction of offspring. In the F_1 that was exposed to short-term high temperature, the pupae weight, pupation rate, emergence rate, and sex ratio of F_3 were not significantly different among treatments. In the F_1 and F_2 that were exposed to short-term high temperature, the pupae weight, pupation rate, and emergence rate of F_3 kept decreasing with the increase in temperatures, and the sex ratio of the 45 °C group was 1:0, indicating that after experiencing two consecutive 45 °C treatments for 1 h, the emergence number of F_3 males would be greatly reduced, and some could not complete emergence.

After short-term high-temperature treatment, both the maternal generation and offspring are affected to varying degrees. The environmental conditions (photoperiod, temperature, and nutrition) experienced by the insect maternal generation affect the phenotype of the offspring. This phenomenon is called the "maternal effect", which is a non-genetic effect of the maternal generation on offspring [28]. In this study, except for the 45 °C treatment for 1 h to stimulate egg production, the short-term high temperature was generally detrimental to the growth and reproduction of the *Z. cucurbitae* mother, and the effects on offspring were mainly reflected in the reduction in hatching rate, pupae weight, pupation rate, and emergence rate, and the increase in sex ratio. The egg production and lifespan of the *Z. cucurbitae* maternal generation were much lower than those of the control after 1 h of treatment at 41 °C, while the egg production and lifespan of the offspring were not significantly different from, or even significantly higher than, those of the control. Insect maternal generations feel the changes in the environment, which has an impact on the offspring, increasing the adaptability of the offspring so that they can continue to develop in an adverse environment, so as to ensure the continuation of the population [29].

The short-term high-temperature treatment makes the *Z. cucurbitae* maternal generation encounter an adverse environment, resulting in smaller offspring, and thereby increasing the sex ratio of offspring to ensure the continuation of the population.

In conclusion, this study elucidated the multigenerational effects of short-term high temperature on different generations of *Z. cucurbitae* at the level of apparent biology, but the mechanism at the level of molecular biology is still unclear. For example, after short-term high-temperature treatment, the differential expression of oviposition-related genes such as *Vg* and *VgR* in different generations needs to be further studied, which will promote research on the multigeneration effects of short-term high temperature on *Z. cucurbitae*, and combine the apparent biology and molecular biology to provide a scientific basis for the control of multigenerations of *Z. cucurbitae* in the high-temperature season.

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