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Effects of Short-Term High Temperatures on Survival and Reproduction of *Trabala vishnou gigantina* Yang (Lepidoptera: Lasiocampidae)

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ABSTRACT

The moth *Trabala vishnou gigantina* Yang is a leaf-eating pest, and there have been severe outbreaks of it in *Hippophae rhamnoides* plantations in North China. To study the effects of short-term high temperatures on its feeding, mating, longevity, and fecundity, *T. vishnou gigantina* were cultivated at 30, 35, and 40°C for 1, 2, and 4 h. The results showed that the different intensities and durations of the high temperatures had significant impacts on the moth's survival and reproduction. With increased temperatures and durations of exposure, the development time of *T. vishnou gigantina* was shortened and then prolonged. At 30 and 35°C, with increased exposure time, the development time was shorter than that at 25°C, whereas it was significantly longer at 40°C. The increase in temperatures and treatment times resulted in gradual decrease in mating rate. With increased temperature, the average fecundity, fecundity rate, and average adult longevity (of both the males and females), gradually decreased. Furthermore, with increased treatment times, at the same temperatures, the average fecundity, fecundity rate, and average adult longevity (of both the males and females), also gradually decreased. In conclusion, *T. vishnou gigantina* is significantly impacted on survival and reproduction by short term high temperatures. This study is expected to provide a scientific basis for predicting the population dynamics in *T. vishnou gigantina*.

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Key words
High temperature stress, *Hippophae rhamnoides*, Reproduction, Survival rate, *Trabala vishnou gigantina.*

INTRODUCTION

Trabala vishnou gigantina Yang is a subspecies of *Trabala vishnou* Lefebure*,* from the family Lasioeampidae of the order Lepidoptera, and it is mainly distributed in south-east Asia, inluding India, Myanmar, Thailand, Malaysia, Sri Lanka and Indonesia, and North part of China, and widely parasitizes *Hippophae rhamnoides*, *Ostryopsis davidiana*, Quercus, chestnut, apple, pomegranate, and walnut (Wen *et al*., 2016; Liu *et al*., 2013; Cheng *et al*., 2002). *T. vishnou gigantina* reproduces once per year. The eggs that survive the winter begin to hatch in mid to late May of the following year, and the mature larvae begin to cocoon and pupate in late July. The adults first appear in late August and disappear at the end of September (Liu *et al*., 2016). Major damage is caused by the larvae as they feed on leaves, resulting in holes or nicks in, or in severe cases, destruction of, the entire leaf, leading to the decline of tree vigor or even death. In 2008, there was an outbreak of *T. vishnou gigantina* in the *H. rhamnoides* plantations in Wuqi county, Shaanxi, China. The area of damage has continued to increase since this outbreak, with the overall degree of damage increasing year by year. This has seriously affected the growth of *H. rhamnoides*, and greatly damaged their ecological value and economic benefits.

Insects are poikilothermic animals that are unable to maintain and regulate their body temperatures, which are affected by external temperatures (Gibbs, 2002). Within an appropriate temperature range, the developmental velocity of insects accelerates with the increase of temperature, but when the temperature exceeds a certain value, their growth and development is hindered, which can lead to death (Rasmont and Iserbyt, 2012; Boina and Subram, 2004; Wright *et al*., 2001). Studies have shown that short-term high temperature treatments affected the survival, growth, and development of insects, thereby affecting their populations (Yukawa *et al*., 2016; Ebrahimi *et al*., 2015). The effects of extreme weather on insects have been deeply studied, such as *Carposina sasakii* (Toyoshima *et al*., 2010), *Coleoptera coccinellidae* (Zhang *et al*., 2015), *Pluella xylostella* (Ebrahimi *et al*., 2015), *Liriomyza huidobrenusis* (Huang *et al*., 2015), *Ophraella communa* (Zhou *et al*., 2011), *Cnaphalocrocis medinals* (Bodlah *et al*., 2017), *Drosophila suzuhii* (Green *et al*., 2019), and *Danaus plexippus* (York and Oberhauser, 2002). These studies showed that short-term high temperatures could lead to summer diapause, impaired fertility, heat shock, shortened adult lifespans, decreased survival rates and even death. *T. vishnou gigantina* outbreaks mainly occur between June and August. During these months, the daily maximum

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temperature in Northern China can exceed 40°C, and these high temperatures may affect the growth, development, survival, and reproduction of the *T. vishnou gigantina*.

Currently, there are only few reports on the occurrence and damage of *T. vishnou gigantina* outside of China, while the studies in China are mainly focused on its bioecological characteristics, morphological characteristics, behavioral characteristics, spatial distribution, virulence measurements, and natural enemies. Liu *et al*. (2013, 2014) studied its damage levels, behavioral characteristics, morphological characteristics, life history, biological characteristics, and natural enemies. Zhang *et al*. (2012, 2013) studied the spatial distributions of *T. vishnou gigantina* pupae and eggs in *H. rhamnoides* plantations with different aspects and densities, and showed that they clustered in dense forests and were randomly distributed in sparse forests, showing edge effect. Wen *et al*. (2016, 2017) studied the relationship between *T. vishnou gigantina* and its hosts. The results showed that *T. vishnou gigantina* was sensitive to the different volatile substances and inclusions of the host plants, and preferred *Hippophae rhamnoides* and *Ostryopsis davidiana*. However, there are only few reports concerning the effects of high temperature stress on the survival and reproduction of *T. vishnou gigantina*.

The present study aims at determining the effects of short-term high temperatures on the development time, mating rate, food intake, fecundity, longevity, and survival rate of *T. vishnou gigantina*, for better understanding of its tolerance to high temperatures and provide solid theoretical basis for the formulation of appropriate prevention and control strategies.

MATERIALS AND METHODS

Culturing of test insects

The eggs of *T. vishnou gigantina* were collected from *H. rhamnoides* plantations in Xinzhai Township of Wuqi County, Shaanxi Province, China (107°38′57‴ E ~ 108°32′49‴ E, 36°33′33‴ N ~ 37°24′27‴ N), and cultured in a light incubator (MGC-250, Shanghai Yiheng Scientific Instrument Co., Ltd.) at the laboratory, with a photoperiod of 14L:10D, a relative humidity of 80 ± 5%, and a temperature of 25 ± 1°C.

In each day, the fresh *H. rhamnoides* leaves were picked, washed, dried, and put into plastic bottles (15 cm in height and 10 cm in diameter) to cultivate the larvae. Ten larvae were cultivated in each bottle, and the mouths of the bottles were covered with gauze and tied with rubber bands. The leaves were replaced with fresh leaves at 8 am every morning. After the *T. vishnou gigantina* larvae pupated, regular observations were performed twice a day, to inspect and record their emergence status. The adults that emerged on the same day were male to female paired and placed in the same cage, allowing them to mate freely, and finally their fecundity was recorded.

Effects of short-term high temperatures on the development period

The experiment was conducted in an artificial climate box. After the adults laid their eggs, the eggs were soaked in 1 % sodium hypochlorite solution for 10 min, and then rinsed with distilled water. After drying, they were placed on a wet gauze and transferred into plastic bottles. These eggs were treated at 30, 35, and 40°C for 1, 2 and 4 h every day. Each treatment was repeated with 50 eggs for 3 times. They were immediately transferred to normal feeding conditions (same as described in light incubator above) after the high-temperature treatment. *T. vishnou gigantina* cultivated at a constant temperature of 25 ± 1°C as a control (CK) in each treatment. Observations were conducted at 10:00 a.m. and 6:00 p.m. every day until no eggs hatched, and then the average incubation time was calculated. Additional eggs of *T. vishnou gigantina* were treated under the above-mentioned high temperature conditions, without the incubation time calculation, to prepare them for the subsequent experiments.

Effects of short-term high temperatures on developmental stages

Ten larvae from the above treatment were randomly selected for continuous observation. The *T. vishnou gigantina* larvae were treated at 30, 35, and 40°C for 1, 2, and 4 h, and the experiment repeated 3 times. The treated larvae were transferred to a light incubator (temperature 25 ± 1°C, relative humidity 80 ± 5 %), and the *H. rhamnoides* leaves were replaced with fresh leaves each day. The larval molting statuses were observed, and the weight of the food taken was measured and recorded at fixed time points, three times a day, until the larvae were mature. Finally, the food intake and development time of the larvae were calculated. In the larvae food intake experiment, a blank control was set to determine the weight change of the *H. rhamnoides* leaves caused by dehydration, to correct for the food intake of the *T. vishnou gigantina* larvae. After the larvae pupated, they were also treated at 30, 35, and 40°C for 1, 2, and 4 h. The treated pupae were transferred to a light incubator, and their development time was recorded. After emergence, they were treated at 30, 35, and 40°C, for 1, 2, and 4 h, respectively, and transferred to a light incubator to record the development time and longevity of the adults after each treatment. *T. vishnou gigantina* at each development stage was also directly placed at 25°C, without being subjected to high temperature treatments, as a control group, for the corresponding development stages.

Table I.- Developmental duration (Mean±SEM) of *T. vishnou gigantina* under short-time high temperature.

Temperature	Exposed for (h)	Egg duration (days)	Duration of larval stage (days)	Pupal duration (days)	Preoviposition period (days)	Generational calendar (days)
CK (25°C)		17.83±0.60 bc	72.16±2.20 c	25.53±1.17 b	1.03±0.05 c	116.55±1.70 d
30°C	1	16.00±0.79 cd	61.50±1.02 d	22.25±1.41 c	0.92±0.03 d	100.67±1.58 e
	2	14.33±0.78 de	57.55±1.17 e	21.33±0.78 cd	0.83±0.04 de	94.04±1.94 f
	4	13.43±1.06 ef	55.98±0.44 e	19.81±0.74 de	0.78±0.02 ef	90.01±1.97 g
35°C	1	11.77±0.46 fg	37.44±1.69 f	18.48±1.07 ef	0.83±0.04 de	74.52±1.11 h
	2	12.77±0.55 gh	40.83±0.84 g	17.07±1.50 fg	0.71±0.02 f	71.37±1.06 i
	4	15.40±0.46 h	43.84±1.09 h	15.53±0.42 g	0.61±0.04 g	69.38±0.94 j
40°C	1	17.93±1.63 bc	73.94±1.69 bc	26.92±0.27 b	1.06±0.08 c	119.85±2.74 c
	2	19.87±2.00 b	76.07±1.22 b	28.97±0.36 a	1.21±0.03 b	126.12±2.28 b
	4	22.27±1.06 a	78.51±1.00 a	30.26±0.37 a	1.51±0.07 a	132.54±0.47 a

CK, Control treatment. Different letters in the same column mean significant difference by Duncan's multiple range test (P<0.05).

Effects of short-term high temperatures on mating and fecundity

Adults that emerged on the same day were immediately placed in an insect cage covered with gauze, with each cage containing one male and one female, and then treated at 30, 35, or 40°C for 1, 2, or 4 h. Each treatment was repeated 10 times. The number of mating pairs and mating times was recorded every 30 min in the dark period (6:00 p.m. to 6:00 a.m. of the next day), and the fecundity and spawning durations of the adults were record continuously until death, and then the adult development time was recorded.

Data analyses

Excel 2010 was used for statistics and SPSS 22.0 software for data analysis. The Student-Newman-Keuls multiple comparison method was applied to compare the significance of the differences in development times, food intake, fecundity, and longevity among the *T. vishnou gigantina* under different short-term high temperature conditions.

RESULTS

Development duration of T. vishnou gigantina

According to Table I, the short-term high temperatures had significant effects on the development duration of the *T. vishnou gigantina* in each stage. From 25 to 35°C, the development time of each stage was negatively correlated with the temperature, *i.e.,* as the external temperature and treatment time increased, the development time of each stage became shorter. However, when treated at 40°C for 4 h, the development time of each stage was significantly longer than that of the control group.

From 25 to 35°C, the development duration of each stage shortened with the increasing treatment times at the same temperature; at 40°C, with the extension of the treatment times, the development duration of each stage was significantly longer than that of the control group.

The pre-oviposition period of *T. vishnou gigantina* decreased from 1.03 to 0.61 d, when treated at 25-35°C for 4 h, it was 1.06 d when treated at 40°C for 1 h, and 1.51 d when treated at 40°C for 4 h, suggesting that the short-term high temperatures prolonged the pre-oviposition period.

Food intake of T. vishnou gigantina *larvae*

Table II showed the effects of short-term high temperatures on the total food intake of *T. vishnou gigantina*. Since the 1st-instar larvae were fed on eggshells, their food intake was not recorded. At the 2nd instar, the maximum food intake was 0.1932 and 0.1855 g, respectively, when the larvae were treated at 30°C for 4 h and 35°C for 1 h. At the 3rd, 4th, and 7th instars, the food intake was maximized when the larvae were treated at 30°C for 4 h, at 0.4148, 2.6153, and 9.2740 g, respectively. At the 5th and 6th instars, the food intake was maximized at 25°C, as 3.7507 and 5.8353 g, respectively.

Mating rate and mating time of T. vishnou gigantina adults

Short-term high temperatures had a significant effect on the mating rate and mating time of the *T. vishnou gigantina* adults (Table III). The peak period of mating at the different short-term high temperatures appeared from 3:00 a.m. to 4:00 a.m., while the mating rate was highest (68%) when the adults were treated at 30°C for 4 h, and lowest (26%) when treated at 40°C for 4 h. Furthermore, with the increase of temperature and treatment times, the mating rate gradually decreased.

Table II.- Effects of short-term high temperatures on the food intake (Mean±SEM) of *T. vishnou gigantina* larvae.

Temperature	Exposed for (h)	2nd instar (g)	3rd instar (g)	4th instar (g)	5th instar (g)	6th instar (g)	7th instar (g)
CK (25°C)		0.12±0.01 c	0.37±0.02 cd	2.41±0.01 abc	3.75±0.01 a	5.83±0.01 a	8.66±0.13 d
30°C	1	0.13±0.01 c	0.38±0.01 bc	2.44±0.21 ab	3.65±0.10 ab	5.69±0.20 ab	9.07±0.11 b
	2	0.15±0.01 b	0.39±0.01 bc	2.53±0.07 ab	3.69±0.14 ab	5.76±0.21 ab	9.14±0.10 ab
	4	0.19±0.01 a	0.41±0.01 a	2.61±0.04 a	3.65±0.15 ab	5.75±0.20 ab	9.27±0.07 a
35°C	1	0.18±0.01 a	0.40±0.01 ab	2.51±0.01 ab	3.58±0.10 ab	5.64±0.10 ab	8.99±0.03 bc
	2	0.16±0.02 b	0.39±0.01 bc	2.48±0.01 ab	3.52±0.03 abc	5.55±0.10 abc	8.85±0.08 c
	4	0.12±0.01 d	0.37±0.01 cd	2.36±0.05 bc	3.49±0.01 bc	5.45±0.08 bc	8.67±0.08 d
40°C	1	0.11±0.01 d	0.36±0.01 de	2.23±0.09 cd	3.34±0.09 cd	5.32±0.10 c	8.58±0.05 de
	2	0.10±0.01 de	0.34±0.01 e	2.15±0.10 d	3.20±0.05 de	5.26±0.10 cd	8.14±0.11 e
	4	0.09±0.01 e	0.34±0.01 e	2.05±0.10 d	3.14±0.12 e	5.03±0.10 dd	8.78±0.34 f

Different letters in the same column means significant difference by Duncan's multiple range test ($P<0.05$).

Table III.- Mating rate and mating time of *T. vishnou gigantina* adults under short-term high temperature.

Temperature	Exposed for (h)	Mating time (a.m.)					Total mating rate
		00:00～01:00	01:00～02:00	02:00～03:00	03:00～04:00	04:00～05:00	
CK (25°C)		5%	12%	21%	44%	18%	100%
30°C	1	4%	10%	23%	47%	16%	100%
	2	2%	10%	21%	59%	8%	100%
	4	2%	9%	15%	68%	6%	100%
35°C	1	3%	11%	18%	55%	13%	100%
	2	4%	8%	26%	42%	14%	94%
	4	5%	15%	21%	38%	13%	92%
40°C	1	3%	10%	30%	35%	12%	90%
	2	4%	11%	27%	29%	8%	79%
	4	4%	13%	25%	26%	7%	75%

Table IV.- Effects of short-term high temperature on fecundity (Mean±SEM %) and longevity (Mean±SEM days) of *T. vishnou gigantina.*

Temperature	Exposed for (h)	No. of eggs layed	Fecundity rate (%)	Average longevity (days)		
				Male	Female	All adults
CK (25°C)		282.25±16.0a	100.00	8.63±0.8a	11.60±0.9a	10.12±0.8a
30°C	1	267.35±9.1a	100.00	8.2±0.5a	9.17±0.4b	8.68±0.5b
	2	233.47±20.6b	100.00	7.03±0.4b	7.93±0.4c	7.48±0.4c
	4	193.35±18.6c	100.00	6.17±0.6bc	7.36±0.9c	6.77±0.8cd
35°C	1	165.12±7.8d	88.09	6.97±0.5b	7.67±0.4c	7.32±0.5cd
	2	136.08±7.0e	75.24	5.57±0.7c	6.63±0.9c	6.10±0.8d
	4	127.52±10.5e	61.58	3.93±0.5d	5.13±0.6d	4.53±0.5e
40°C	1	120.43±4.5e	72.41	4.17±0.4d	4.77±0.9d	4.47±0.6e
	2	87.85±6.6f	47.19	2.9±0.6e	3.87±0.6de	3.38±0.6ef
	4	67.25±12.7f	36.65	1.8±0.3e	3.10±0.4e	2.45±0.4f

Different letters in the same column means significant difference by Duncan's multiple range test ($P<0.05$).

Table V.- Effects of short-time high temperature on the survival rate (Mean±SEM, %) of *T. vishnou gigantina* adults.

Time (h)	CK (25°C)	30°C	35°C	40°C
1	100.00±0.00 a	99.07±0.14 ab	97.82±0.69 ab	93.31±0.78 c
2	100.00±0.00 a	98.19±0.75 ab	94.78±1.29 c	82.48±1.70 e
4	100.00±0.00 a	97.19±0.47 b	91.36±1.03 d	74.50±2.14 f

Different letters in the same column means significant difference by Duncan's multiple range test (P<0.05).

Fecundity and longevity of T. vishnou gigantina

Short-term high temperatures had significant effects on the fecundity, fecundity rate, and longevity of *T. vishnou gigantina* (Table IV). With the increase in temperature, the average fecundity, fecundity rate, and the average longevity of the male and female adults all decreased significantly. Under the same temperatures, the average fecundity, fecundity rate, and the average longevity of the male and female adults also decreased significantly, with the extension of the treatment times.

Survival rate of T. vishnou gigantina *adults*

According to Table V, short-term high temperatures had a significant effect on the survival rates of *T. vishnou gigantina* adults. From 30 to 40°C, the survival rates of the adults were significantly lower than those of the control groups. After 1 h treatments at 30, 35, and 40°C, the survival rates decreased by 0.93, 2.18, and 6.69%, respectively. With the increase of treatment time, the survival rates decreased. After 4 h treatments at 30, 35, and 40°C, the survival rates decreased by 2.91, 8.64, and 25.5 %, respectively.

DISCUSSION

As poikilothermic animals, insects are very sensitive to high temperatures, as they not only reduce their survival rates, but also reduce or eliminate their fecundity (Mironidis and Savopoulou, 2010; Kang *et al*., 2009; Wang *et al*., 2004). Previously, there have been many reports on the effects of short-term high temperature on insect survival and reproduction. Insects vary between species in their degree of tolerance to high temperature stress (Zhang *et al*., 2020; Rameswor *et al*., 2017). The results of this study indicated that short-term high-temperature treatments had a significant effect on the larval development of *T. vishnou gigantina*. From 25 to 35°C, the development time of the larvae shortened with the increased temperatures, whereas it was significantly longer at 40°C than that at 25°C. This was consistent with the study results in *Athetis lepigone* (Liang *et al*., 2016) and *Callosobruchus chinensis* (Rameswor *et al*., 2017), which indicated that short-term high temperatures could lead to increased larval development times. Therefore, when the temperature is 25-35°C in the summer, the possibility of *T. vishnou gigantina* damage greatly increases, and timely prevention and control measures should be taken. However, temperatures above 35°C have an inhibitory effect on *T. vishnou gigantina*. It was also observed that the number of deaths ascended with the increase of the treatment time and the temperature, between 25-40°C. Similar findings also exist in the study of *Sitobion avenae* (Chiu *et al*., 2015), *Cydia pomonella* (Neven, 2000) and *Myzus persicae* (Jeffs and Leather, 2014), which indicated that high temperature accelerated the death of insects.

The larval stage of *T. vishnou gigantina* is the most damaging for crops, and is mainly from the 4th to the 7th instar. This study showed that short-term high temperatures had little effect on the larval food intake. The food intake at the 2nd instar was very small, but it started to increase at the 3rd instar, and continuously and gradually increased from the 4th to the 7th instars. Hence, even in high temperatures, the best time for control is before the 3rd instar.

This study found that short-term high-temperature treatments significantly affected the fecundity, fecundity rate, and longevity of the *T. vishnou gigantina*. With the increase of temperature and treatment time, the fecundity, fecundity rate, and longevity all decreased. The fecundity decreased from 282 ± 16 eggs (25°C) to 67 ± 12 eggs (40°C, treated for 4 h); the fecundity rate decreased from 100% to 36%; the average longevity (of males and females) decreased from 10.12 ± 0.8 d to 2.45 ± 0.4 d. As the temperature and the treatment times increased, the survival rate of the *T. vishnou gigantina* adults decreased, which was consistent with the study results of the effects of the short-term high temperatures on the *Helicoverpa armigera* (Liu *et al*., 2004) and *Harmonia axyridis* (Zhang *et al*., 2014) indicating that short-term high temperatures reduced the adult survival rate.

Different insects have different degrees of tolerance to high temperatures. This study was based on the results from experiments in indoor constant temperatures, whereas field temperatures vary rather than staying constant. Given that insects develop faster under varying rather than constant temperatures, actual field conditions would differ to some degree from the study results. Therefore, it is necessary to comprehensively consider factors of all aspects in actual work.

CONCLUSION

This study concluded that the feeding, mating, longevity, and fecundity of *Trabala vishnou gigantina* is obvious impacted by short term high temperatures. This information will be extremely valuable when understanding the mechanism of its population changes in high temperature season, revealing its population dynamics in extreme high temperature and developing control strategies in the future.

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Ethical compliance

There are no researches conducted on animals or humans experiments.

Statement of conflict of interests

The authors declare no conflict of interest.

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