

Effect of photoperiod and temperature on the reproductive responses of *Protaetia brevitarsis*

Seonghyun Kim*, Hae-Chul Park, Namjung Kim, and Ingyun Park

National Academy of Agricultural Science, Rural Development Administration, Wanju-gun Jellabuk-do 565-851, Republic of Korea.

Abstract

In the present study, we investigated the effects of temperature and photoperiod on oviposition of *Protaetia brevitarsis*. The effects of long- and short-day cycles on oviposition and egg hatching of *P. brevitarsis* were investigated at different temperatures. Three male–female pairs were confined to oviposition chambers maintained at 20°C, 25°C, 30°C, and 35°C, with 16L:8D and 8L:16D photoperiod. Oviposition was observed at all temperatures. The total number of eggs laid per female was between 46.8 and 110.8, and the optimal temperature for oviposition and fertility was between 20°C and 30°C. Furthermore, it was difficult for the eggs to hatch at 35°C. Fewer eggs were laid under short photoperiod than under long photoperiod at all temperatures. Hatching success was 93.5% at 20°C, 90.9% at 25°C, 71.5% at 30°C and 37.3% at 35°C under long-day(16L:8D) condition and . Temperature had a strong effect on the time to hatching. Neither oviposition nor subsequent egg hatching was influenced by photoperiod and temperature. The information obtained will be useful for mass rearing *P. brevitarsis*.

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Introduction

Reproductive success is one of the most important measures of fitness of an insect, and its study is a prerequisite for understanding insect evolutionary biology. The development and reproduction of insects occur within a specific temperature range, with best performance at an optimum temperature. Climate affects the distribution and abundance of most insect species, with temperature and photoperiod being the main abiotic factors affecting the seasonal development of arthropods. For many insects, photoperiod is the principal cue to season (Tauber, 1986), and the timing of life history events often changes in response to photoperiod (Ishihara, 2000; Tanaka, 1993; Miles, 1998; Nakao,

1998). However, data on the oviposition behavior of insects are scarce among the environmental factors; temperature probably has the greatest influence on the geographic distribution and abundance of insects.

White-spotted flower chafer (*Protaetia brevitarsis*) is a beetle belonging to the subfamily Cetoniinae, and it is distributed in Japan, Taiwan, Korea, China, and parts of Europe. *Protaetia brevitarsis* is reared and distributed commercially in South Korea (Cho, 1969). The adults are observed from late June through July in Korea. The adults can be collected from late June through July in Korea (Kim, 2005). The larvae of *P. brevitarsis* have been approved as food by the Ministry of Food and Drug Safety of Korea (MFDS, 2016).

*Corresponding author.

Seonghyun Kim

Department of Agricultural Biology, National Academy of Agricultural Science, RDA, Jellabuk-do 565-851, Korea.

Tel: +82-63-238-2936 / FAX: +

E-mail: ichibbang@korea.kr

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The development of a rearing method to selectively mass-produce heavy and average individuals in laboratory would help increase *P. brevitarsis* quality and population. Therefore, the main goal of this study is to investigate the relation between various temperature and photoperiod for *P. brevitarsis*.

Materials and Methods

Experimental insect

A laboratory culture of *P. brevitarsis* was maintained at $25 \pm 2^\circ\text{C}$ and $50 \pm 10\%$ relative humidity (RH), with a photoperiod of 16:8 h (light dark, LD), and reared on saw dust that was changed once a week. Immature individuals were exposed to 16L:8D h and 25°C .

Effects of temperature and photoperiod on fecundity

Newly-mated females were subjected to photoperiods of 16L:8D and 8L:16D h, at seven temperature regimens 20°C , 25°C , 30°C , and 35°C . Each pair was kept in a plastic oviposition container ($207 \times 140 \times 124$ (h) mm, Korea). At the beginning of experiments for each environments, newly emerged virgin female and male ($3\sigma \times 3\phi$) were taken from those reared at different temperature. The number of eggs deposited each week was recorded, and then the eggs were collected. Insect jelly (Choongwoo, Korea) was supplied to adults in small plastic containers with a hole in the cover. Realized fecundity was determined by counting the number of eggs produced per female per day until emergence. All experiments were conducted in incubators with controlled illumination, temperature, and humidity (Multi-room chamber, HB-302S-4H, Suwon, Korea). A comparative study of the influence of day length on oviposition and subsequent egg hatching was conducted under two photoperiodic regimes (16L:8D h and 8L:16D h) in the laboratory. Newly emerged *P. brevitarsis* were used in further analysis.

Longevity of adult females

Newly emerged adult females obtained were subsequently used in this experiment. Females were individually transferred

to the above-mentioned insect cages with insect jelly, and the cages were placed in incubators maintained at four difference conditions. The insect jelly was exchanged every three days. The survival rate of adult female was calculated by Kaplan–Meier analysis.

Statistical analyses

The number of females and eggs hatched was analyzed by two-way factorial analysis of variance (ANOVA) with SPSS 25.0.0 software (IBM Corporation, Somers, NY, USA) for photoperiod and temperature. Means were separated by Tukey–Kramer HSD test ($\alpha = 0.05$). Linear regression was used to correlate mean oviposition with pupal weight.

Results

The oviposition behavior of insects cultured at various temperatures is shown in Fig. 1. The total fecundity period was 10–14 wk (Fig. 1 and 2). Therefore, deposition of fertilized eggs on the substrate starts at wk 1 of the adult phase and ends at wk 10–14. The maximum weekly fecundity uniformly occurred at wk 1–2 of the active reproduction phase (Figs. 1 and 2). The total number of eggs laid was significantly different among females raised at different temperatures, and it varied positively with temperature. We observed a short phase with rapid increase in weekly oviposition (wk 1–2) followed by a long phase with continuous exponential decrease in daily reproductive activity (wk 3–10).

There was a significant difference in fecundity among the

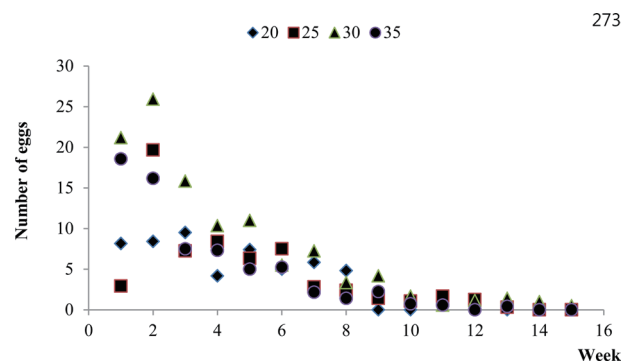


Fig. 1. Weekly oviposition profile of *P. brevitarsis* maintained at different temperatures (LD 16:8 h). Week 1 indicates first week of oviposition, and it was usually the day after mating.

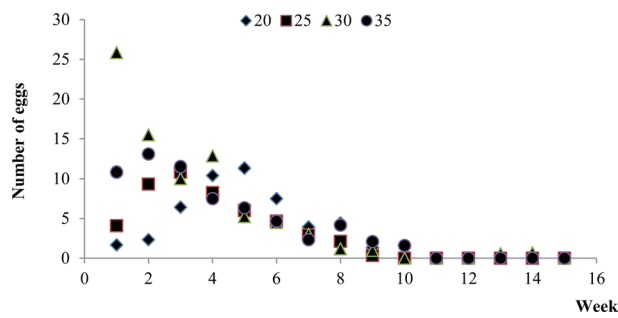


Fig. 2. Weekly oviposition profile of *P. brevitarsis* maintained at different temperature (LD 8:16 h). Week 1 indicates first week of oviposition, and it was usually the day after mating.

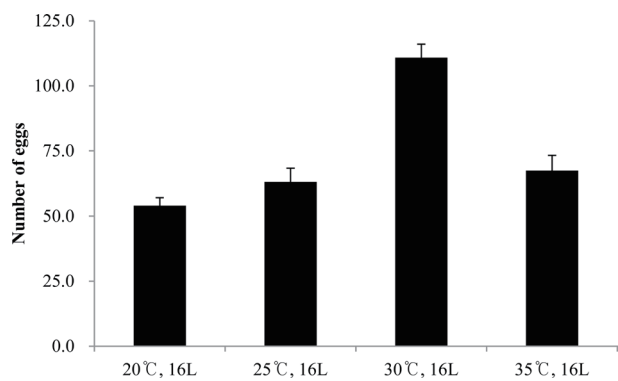


Fig. 3. Number of eggs laid by *P. brevitarsis* at different temperatures (LD 16:8 h).

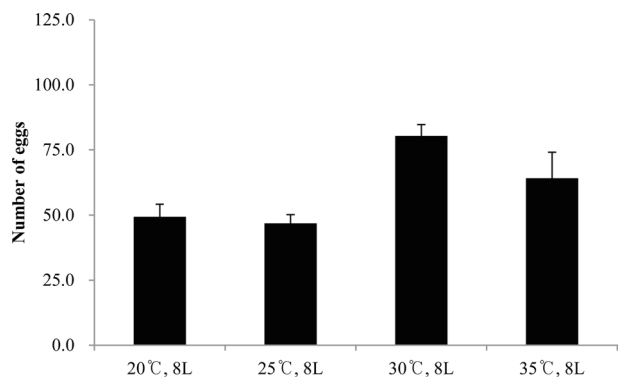


Fig. 4. Number of eggs laid by *P. brevitarsis* at different temperatures (LD 8:16 h).

temperature treatment groups, with the highest number of eggs (110.8 eggs) at 30°C (LD 16:8 h), followed by 54.1 at 20°C, 63.1 at 25°C, and 67.4 at 35°C ($F_{3,12} = 26.181$, $p < 0.001$) (Fig. 3). And number of oviposition also showed significant differences among the short-day length (LD 8:16h) ($F_{3,12}=6.239$, $p<0.001$) (Fig. 4). The ANOVA indicated that there was an overall effect of photoperiod, and the length of photoperiod affected the percent

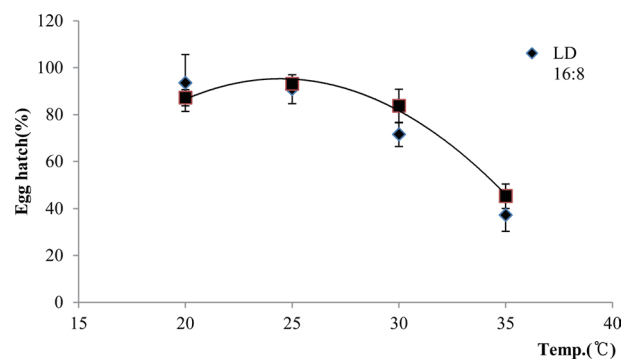


Fig. 5. Effects of temperature on *P. brevitarsis* egg hatching.

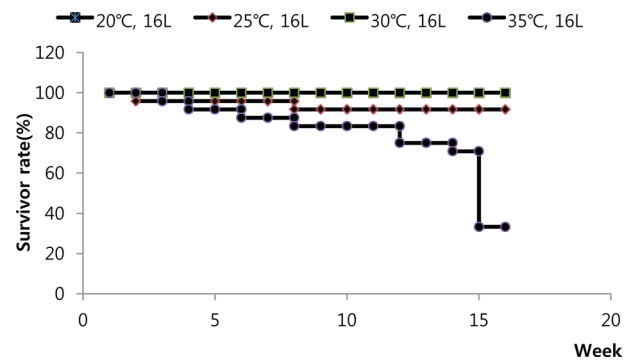


Fig. 6. Survival rate of *P. brevitarsis* at different temperatures (LD 16:8 h).

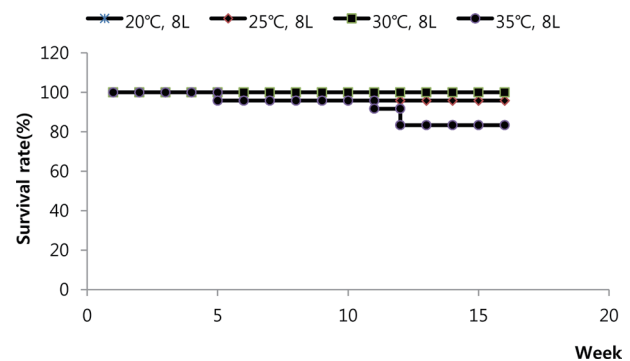


Fig. 7. Survival rate of *P. brevitarsis* at different temperatures (LD 8:16 h).

of ovipositing females.

More than 93% of eggs hatched at 20°C (8L:16D), and lower temperature levels led to higher survival rate of egg in the laboratory. Egg hatchability at 25°C, 30°C, and 35°C was higher under the long-day photoperiod (93.1%, 83.7%, and 45.2%) than that under the short-day photoperiod (90.9%, 71.5%, and 37.3%). Photoperiod had a significant effect on oviposition and total fecundity at 20°C, 25°C, 30°C, and 35°C (Fig. 5), and

the hatching rate of insects was for a very short period at high temperature (35°C) (Figs. 6 and 7). Significant differences in the hatching rate were observed among the temperature treatment groups ($F_{3,12} = 41.390$, $p < 0.001$). These data suggest that increasing temperature has a positive effect on the number of eggs laid and the proportion of female ovipositing, except at 35°C. Infertility was significantly high among eggs oviposited at high temperatures. It is possible that the increase in the percent of infertile eggs is because it is difficult to manage humidity.

Discussion

Survival, development, and fecundity of insects are significantly affected by temperature and photoperiod (Huang, 2008; Aksit, 2007). Temperature has long been recognized as an important environmental factor that influences the behavior of insect. In the present study, the egg hatching success rate decreased with increase in temperature. The number of eggs deposited was the highest on the first and second weeks of egg laying, and then decreased logarithmically. This pattern is similar to that in other insects such as *Helicoverpa armigera*, *Apium graveolens*, and *Spodoptera pectinicornis*. (Jallow, 1998; Robert, 1998; Wheeler, 1998). Temperature affects the development of immature individuals, emergence of adults, reproduction, and longevity of insects. Since insects are constantly exposed to temperature fluctuations in their natural environment, they might show differences in tolerance to extreme temperatures and in their acclimation responses.

In the present study, we aimed at evaluate the effects of temperature on biological parameters of *P. brevitarsis* and confirmed its role. Several studies have reported that 35°C is too low survival rate of insects (Regniere, 2012). Our results suggest that gravid females prefer to oviposit in areas with adequate temperature and avoid or delay oviposition under high temperature conditions. Temperature has been proved a vital factor in the development and survival of insects.

In the present study, *P. brevitarsis* females laid the most number of eggs during the early ovipositional periods and showed a significant peak. The fecundity of insects decreased sharply with age. In the present study, we reared the insects at 20°C, 25°C, 30°C, and 35°C to examine the effects of temperature on the development of *P. brevitarsis*. The temperature of 35°C was apparently lethal to *P. brevitarsis*, as

the hatching rate was 37% (16L:8D) and 45% (8L:16D). Our results verified the sensitivity of *P. brevitarsis* adults to high temperatures. The female adults were capable of reproducing at all other temperatures (20°C–30°C), even when the fecundity of individuals was significantly low at 35°C compared to that at other three temperatures.

Further, the temperature of 35°C had an overall negative effect (Fig. 5), as the eggs hatched at a considerably low rate compared to that of the control eggs, maintained at 25°C. Further research is needed to investigate the effects of fluctuating temperatures, a situation more likely to occur under field conditions.

The developmental and metabolic rates apparently increased as the temperature increased; however, as the temperature reached the upper lethal limit, the metabolic rate decreased. The fecundity of *P. brevitarsis* is a vital parameter that was directly related to variation in rearing temperature. This phenomenon has been recorded in various other insect species (*Hyalopterus pruni*, *Lycaena dispar*), where increase in culture temperature often results in noticeable decrease in female productivity (Atlihan, 2008; Kim, 2014).

The relatively higher fecundity at 30°C might indicate that the temperature is suitable for *P. brevitarsis*. The extremely low hatching rate and high mortality rate of *P. brevitarsis* at 35°C demonstrated that the temperature is not suitable for development and that it was close to the insect's lethal temperature limits. Some researchers have reported an increase in fecundity with increase in temperature for some insects (Ahmed, 1989; Mori, 2005; Piesik, 2006; Yigit, 1986). The results of the present study showed that an increase in temperature increased the mortality rate. Moreover, a decrease in photoperiod increased the mortality of *P. brevitarsis*.

To the best of our knowledge, the present study is the first to consider the interactive effects of photoperiod and temperature on oviposition performance of *P. brevitarsis*. Moreover, we represented conditions likely to be experienced by different species in nature.

The fact that temperature affect the optimal and fecundity supports the argument that evolutionary responses to photoperiod will be critical to understand how different taxa will respond to future warming (Bradshaw, 2001; Bradshaw, 2006). The study establishes a baseline constant temperature life table for the oviposition of *P. brevitarsis*, which can used to model the development of the species in the wild and estimate potential distribution limits. Knowledge of temperature-dependently

growth and oviposition of *P. brevitarsis* can be used to farm edible insects. Key bioclimatic parameters such as reproductive growth under different temperatures can be used to optimize the production method in the mass rearing of edible insect.

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