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Effect of temperature on the development of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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Abstract

The developmental responses of insects to temperature are important considerations in gaining a better understanding of their ecology and life histories. Temperature dependent models permit examination of the effect of temperature on the geographical distributions, population dynamics, and management of insects. The measurements of insect developmental and survival responses to temperature pose practical challenges that depend. The developmental characteristics of *A. diaperinus* were investigated at four temperature regimes (20, 25, 30 and 35°C), a relative humidity of 60%, and a light:dark photoperiod of 16:8h. The developmental time from larva to adult was 129.0, 49.8, 40.5 and 31.9 days at temperatures of 20, 25, 30 and 35°C, respectively. Pupal rate was 80.0%, 100%, 83.3% and 91.7% at temperatures of 20, 25, 30 and 35 respectively. There is an increasing need for a standardized manual for rearing this. Pupa had significantly lower weights at 35°C than at the other temperatures. Female pupae (20mg) were significantly heavier than male (17mg).

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Introduction

Alphitobius diaperinus is usually found infesting our, meal, and other grain products, especially in poorly maintained grain processing plants (Spilman, 1991). It has been connected with wheat, barley, rice, oatmeal, soybeans, cowpeas, and peanuts. It has also been reported from linseed, cottonseed, oilseed products, tobacco, skims, and drugs (Hosen *et al*., 2004). Because of its tropical origin, the lesser mealworm is well adapted to warm, humid conditions. It is a main inhabitant of poultry or brooder houses where it lives in poultry droppings and litter. Both adults and larvae are rich in manure from poultry farms (Francisco *et al*., 2001).

Insects are favorable animal feed components because they contain high levels of quality protein, have short life cycles, and are easy to produce and rearing, depending on the substrate used for their production.

Temperature is the most important factor influencing the development of insects. Effect of temperature on development and growth is determined an insect's life history strategy as

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growth rate and size at maturity are key traits in life-history evolution(Roff, 1992; Stearns, 1992). Temperature strongly influences the rate of development and growth, with higher temperatures leading to faster rates as long as temperature remains within the viable development range for that organism (Jarosik *et al*., 2004). Data on the effects of temperature on the development of other insect have been reported (Kim *et al*., 2015, Kim *et al*., 2016), but not provided in sufficient detail for the *A. diaperinus* in South Korea.

One common approach to model temperature effects on insect development is to convert the duration development to their reciprocals. This simple transformation is used to reveal the relationship type, as it will be shown later close to linear, between temperature and rate of development and permits the determination of two vital parameters of development namely, the thermal constant (K) and the base or lower temperature of development (T_{min}). The Thermal constant is expressed as the number of degree-days (in ^{o}C) and provides an alternative measure of the physiological time required for the completion of a process or a particular developmental event (Damos *et al*., 2008).

In general, within optimum ranges of development and as environmental temperature decreases, their rates of development slow and cease at the lowest temperature, while as temperature rises, developmental rates increase up to an optimum temperature, above which they again decrease and eventually cease at their temperature maximum.

The temperature-dependent developmental rate curve of an insect is an important feature of its life history (Taylor, 1981). Using degree-day accumulations to predict a wide variety of events such as egg hatch, adult emergence, or migratory flights may be feasible for insect rearing system. Degree day accumulations need knowledge of both insect developmental response to temperature and lower developmental threshold (Woodson *et al*., 1996).

Beetles are thought to be sensitive to changes in their environment and variations in the environment would lead to drastic changes in their numbers. This could prompt further study into what changes have occurred and the required remediation. Climate change is predicted to lead to an increased mean temperature and more frequent climatic extremes (Pollard, *et al*., 1997).

When planning the mass-rearing process, the relationship between temperature and development should be investigated to know how to regulate the reproduction of beetle. The present study was performed to clarify the development of *Alphitobius diaperinus*at several temperatures. The main goal of this study is to investigate the interaction of temperature on the larval development of *Alphitobius diaperinus*. The rate of insect development depends upon the temperature to which the insects are exposed. The amount of heat required over time for an insect to complete specified aspects of development is termed the thermal constant (Andrewata *et al*., 1954). We examined in detail the temperature effects on development of *Alphitobius diaperinus*.

Materials and methods

Insects

Alphitobius diaperinus colonies were established at NIAS (National Institute of Agricultural Sciences) that were collected from Sacheon, South Korea during 2016. Insects were reared at 25^{o}C, in constant temperature at a relative humidity of 60% and a daily LD 16:8h light cycle. In addition, wheat bran and fresh Chinese cabbage were provided every 3 days.

For the larval stage the diet for the species is *Alphitobius diaperinus*. The insects were maintained at 25°C and 65% relative humidity under a photoperiod of LD 16:8h. The hatched larvae were individually reared in Petri dishes. The containers used for rearing included small Petri dishes (35×10 mm) for the all stages.

Developmental time and survival

Developmental characters of *Alphitobius diaperinus* were investigated in environmental chambers (HB 302-2s-4, Hanbaek Science, Korea) at four constant temperatures (20, 25, 30 and 35°C). Relative humidity and photoperiod were kept constant at 60% and LD 16:8h, respectively. Eggs were collected and transferred individually to small petri-dishes containing moist filter paper. Laval development was monitored daily. A number of newly ecdysed 1st instar larvae of *Alphitobius diaperinus* were collected from the laboratory colony. Individual larvae were isolated in a Petri-dish (35×10 cm) containing the wheat bran and placed in a randomized pattern within environmental chambers set at a selected temperature (20, 25, 30 and 35°C with a variation of 5°C at each temperature) and photoperiod (continuous light with LD 16:8h).

Developmental rate and Degree-day requirement

Developmental time at different temperatures was analyzed using the general linear model procedure for each instar separately as well as the total immature stages combined. Whenever significant ($P < 0.05$) F values were obtained, means were separated using the ANOVA test.

Linear Model

For larvae and total immature stage, the linear portion (20~35°C) of the developmental rate curve [$R(T) = a + bT$] was modeled using the linear regression, where T was temperature, and a and b were estimates of the intercept and slope, respectively. The base temperature threshold was estimated by the intersection of the regression line at $R(T) = 0$, $T_0 = -a/b$. Degree-day requirements for each stage were calculated using the inverse slope of the fitted linear regression line (Campbell *et al*., 1974). And adjustment of the data to second-degree polynomial(Hansen *et al*., 1981) equation was made using the computer program Excel.

Statistical Methods

Data were submitted to analysis of variance (ANOVA) at $\alpha = 0.05$. Means were separated by using the Tukey-Kramer honestly significant difference (HSD) test (Sokal *et al*., 1995, SPSS, 2013)

Results and Discussion

Incubation times of the beetle eggs were influenced by temperature. There was a significant decrease in the number of days required for egg development from 20°C to 35°C. Larval development times were influenced by temperature. These development times were significantly longest at 20°C and shortest at 35°C. Pupal development times also were influenced by temperature. These development times significantly decreased from 20°C to 35°C. Therefore, there was great factor of high temperature inhibition of development.

Alphitobius diaperinus larvae were reared at four different temperatures to investigate the effects of temperature variation on development. The survival rates of *Alphitobius diaperinus* at the four different temperatures are shown in Table. 1. Larval survival was lowest over all at 20°C (80%). Differences in pupa weight were not significant (female: $F_{3,54} = 1.649$, $p < 0.189$; male: $F_{3,51} = 0.689$, $p < 0.563$) (Fig. 1). And survival rates were 80.0, 100.0, 83.3 and 91.7% at 20, 25, 30 and 35°C respectively. The greater emergence percentages were investigated at 25°C, while the lowest at 20°C; the differences were significant. The optimal growth of the *A.diaperinus* occurred from 25 to 35°C.

The low temperature had detrimental effect on immature growth and caused high mortality and long larval duration. The mortality was caused by unable finishing larval stage, unable

Table 1. Mean proportion survival of eggs, larvae, pupae and total immatures of *Alphitobius diaperinus* at four constant temperatures.

Life stage	Temp. (°C)	Mean survival
Egg	20	100
	25	100
	30	100
	35	100
Larva	20	80.0
	25	100
	30	83.3
	35	91.7
Pupae	20	80.0
	25	100
	30	83.3
	35	91.7

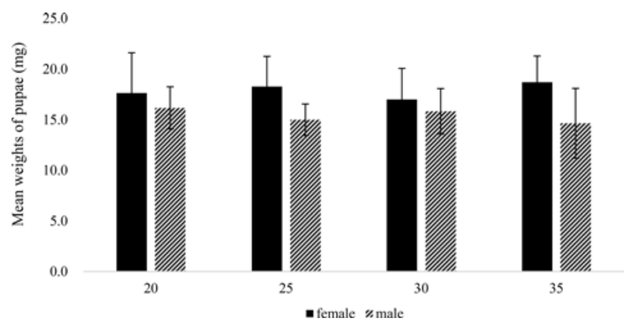

Fig. 1. Mean weights (mg) of pupae of Alphitobius diaperinus reared as larvae at four constant temperature (female: $F_{3,54} = 1.649$, $p < 0.189$; male: $F_{3,51} = 0.689$, $p < 0.563$).

pupating or by deformed adults with crippled wings.

The developmental period at four constant temperatures are presented in Table 1. The rate of development was positively correlated with temperature until the upper limit of 35°C for *A. diaperinus*. At higher temperatures, of *Alphitobius diaperinus* completed development faster. The Value of r^2 varied between 0.8569 and 0.9785 for *A. diaperinus*. The means of developmental periods for each developmental stage decreased with increases of temperature (Table 2).

The reciprocal of days required to develop each stage at different temperature became the developmental rate (1/days for development). The regression formula is found in the relationship between the developmental rate (Y) and the rearing temperature (X). The thermal constant is 1/a in Y = aX-b, and X is the developmental zero in Y = 0. In the mass rearing of any insect, a well-regulated work sequence must be planned, for example adult rearing, oviposition, and rearing from egg to emergence (Koyama *et al*., 2004).

Table 2. Developmental Duration (mean ± SE) of *Alphitobius diaperinus* from egg to adult emergence at four constant temperatures.

Life stage	Temp. (°C)	n	3rd
Egg	20	30	8.00±0.0a
	25	37	2.0±0.0c
	30	24	3.0±0.0b
	35	36	3.1±0.5b
Larva	20	24	107.3±9.8a
	25	37	40.0±2.9b
	30	20	35.0±.3.7c
	35	36	24.8±2.9d
Pupae	20	23	14.0±3.4a
	25	37	7.9±1.3b
	30	20	6.1±1.0c
	35	33	3.8±1.3d

*Means within the same column followed by a different letter are significantly different at P < 0.05 (ANOVA followed by Duncan's new multiple range test). (Egg: $F_{3,123}$= 3081.815, p < 0.0001; Larva: $F_{3,110}$= 1290.834, p < 0.0001; Pupae: $F_{3,110}$= 143.084, p < 0.0001).

The influence of temperature throughout development and its effect on biological stage and quality of *Alphitobius diaperinus* were studied. As expected, temperature indicated a notable effect on developmental time. It appears that the main advantage for keeping pupae at 25°C, is the drastic reduction in pupal developmental time, which is important in reducing infections and rearing costs(Table 1,2).

Body size is also flexible: It can modify in response to different environmental conditions(Stern, 2001). For example, insects that develop at higher temperatures are generally smaller than those that develop at lower temperatures(Atkinson, 1994), and well-fed organisms are typically larger than those fed a poor-quality diet(Chapman, 1998). Given a particular growth rate, final size of an insect should be proportional to the duration of growth. This means that an individual may either shorten its development at a cost of being small, or may grow large at a cost of long development.

In insect, female life span reproductive success is more closely correspond to body size (Honek, 1993) than the major part of male reproductive success, in precise his mating ability. Thus, in terms of fitness females gain more than males from being large.

As a larva grows, it usually progresses through a fixed instar number. During its final larval instar, the larva reaches a critical size that sets in motion the endocrine events that trigger metamorphosis. With few exceptions, insect males are therefore smaller and hence should develop faster than females. Protandry, i.e. the faster development of males, can also increase male mating success, thus further enhancing his fitness. Developmental thresholds and degree-day requirements will provide information about life stage events and may aid in developing rearing systems.

Table 3. Mean developmental time of thresholds (T_0) in °C, thermal constants (K) in day-degrees above base, regression equations and coefficients of determination (r^2) for total stage of the *Alphitobius diaperinus.*

stage	Linear equation	R^2	Second-degree equation	R^2
Egg	Y=-0.247x+11.56	0.4303	Y=0.061x2-3.629x+55.785	0.8569
Larva	Y=-5.051x+190.67	0.7535	Y=0.515x2-36.484x+605	0.9464
Pupa	Y=-0.647x+25.755	0.9169	Y=0.0375x2-2.7095+52.943	0.9785
Total	Y=-5.972x+227.98	0.7554	Y=0.67x2-42.822x+713.33	0.9455

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