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Development of Myanmar strain of *Cotesia vestalis* (Hymenoptera: Braconidae) on Its Host *Plutella xylostella* (Lepidoptera: Plutellae) at Different Temperatures

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The effect of seven constant temperatures on the development of the Myanmar strain of *Cotesia vestalis*, (Haliday), a larval parasitoid of diamondback moth *Plutella xylostella* (L.) was studied in the laboratory. Developmental times for immature stages were inversely proportional to temperature between 15 and 33°C but increased at 35 °C. Total developmental times from egg to adult emergence decreased from 63.7 to 9.4 d for temperatures from 15 to 33 °C, with pupae requiring less time for development than the combined egg and larva stage. Both linear and nonlinear (Logan equation VI) models provided a reliable fit of development rates versus temperature for all immature stages. The lower developmental thresholds that were estimated from linear regression equations for the egg to pupa, pupa to adult, and total combined immature stages were 8.9, 10.1, and 9.4 °C, respectively. The degree–day accumulation was calculated as 200 DD for development from egg to adult emergence. By fitting the nonlinear models to the data, the upper and optimal temperatures for egg to pupa, pupa to adult and total development stages were calculated as 38.2 and 35.0, 35.0 and 31.8, 30.5 and 31.3 °C, respectively. These data are useful for predicting population dynamics of *C. vestalis* under field conditions.

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), is a serious pest of economically important cruciferous crops worldwide (Abro *et al*., 1992, Talekar and Shelton, 1993; Reddy *et al*., 2004 and Shelton, 2004). It is a major pest of cabbage, cauliflower and other cultivated crucifers and severe infestations are found in the main vegetable growing areas in Myanmar (Morris and Waterhouse, 2001; Maung and Morris 2001; Maung N, 2002). Although insecticides have been often used to control DBM, this pest has been developing resistance to many different insecticides (Shelton *et al*., 1993 and Sarfraz and Keddie, 2005). Biological control using parasitic wasps has also been attempted because biological control is more environmentally sound pest management approach (Talekar and Shelton, 1993). Although over 130 species of parasitoids have been recorded worldwide attacking various developmental stages of DBM, of these, most control worldwide is achieved by relatively few hymenopteran species belonging to the ichneumonid genera *Diadegma* and *Diadromus*, the braconid genera *Microplitis* and *Cotesia*, and the eulophid genus *Oomyzus* (Sarfraz *et al*., 2005).

Cotesia vestalis (Haliday) (Hymenoptera: Braconidae), commonly called Cotesia (or formerly *Apanteles*) *plutellae* (Kurdjumov), but now should be referred to as *C. vestalis* (Shaw, 2003) is a solitary endolarval parasitoid of DBM (Talekar and Yang, 1993; Noda *et al*., 1996; Kawaguchi and Tanaka, 1999). This parasitoid is distributed worldwide (Lim, 1986; Waterhouse and Norris, 1987; Talekar and Shelton, 1993; Talekar and Yang, 1993). *Cotesia vestalis* has been used in biological control programs of DBM in several countries because of its resistance against insecticides (Waterhouse and Norris, 1987; Fitton and Walker 1992). In Southeast Asia, *C. vestalis* is the only parasitoid known to survive the hot and humid climate of the lowlands (Verkerk and Wright, 1997).

Biological control, whether using the introduction, conservation or augmentation approaches, is facilitated when the climatic responses of biocontrol agents are known, especially responses to temperature (Royl *et al*., 2002). The development rate of an insect under natural conditions is largely determined by temperature (Liu *et al*., 2002) and the temperature is a critical abiotic factor influencing the dynamics of insects (Roy *et al*., 2002). Complementary studies on development rates at different temperatures and the estimation of thermal requirements are useful for enhancing the efficiencies of biological control strategies. In biological control, details concerning such responses and estimation of the temperature thresholds and thermal constant for development of natural enemies can contribute substantially to the selection of the most suitable natural enemy to be used at different environmental conditions (Obrycki and Kring, 1998; Roy *et al*., 2002).

In Myanmar, native species of *C. vestalis* has been found to be a promising bio–control agent for diamondback moth and the effective release percentage is 50 to 70 percent (Thwin, 2001). But no information is available for temperature effect on development of *C. vestalis* in Myanmar. The study was conducted to observe the effect of different temperature, and to determine the optimal temperature rang for development of Myanmar strain of *C. vestalis*.

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MATERIALS AND METHODS

Plutella xylostella larvae were obtained from a stock culture maintained at Kyushu University. The laboratory colony of DBM was originated from field collected larvae and pupae and maintained on artificial diet (Htwe *et al*., in preparation). The culture of *C. vestalis* was established from cocoons and parasitized DBM larvae collected from cabbage fields in TadarOo City, Mandalay Township, Myanmar. The parasitoid was maintained on DBM larvae fed on artificial diet. Male and female *C. vestalis* was allowed to mate in a plexiglass cage (30×30×30 cm), one side of which had slide door (13 cm×13 cm) at the center, and two other sides of which were covered with fine nylon mesh, at 25 °C and 16L; 8D. One to two day old mated female were used in this experiment. Individual *C. vestalis* female were first allowed to antennate frass of DBM larvae for 10s to stimulate their oviposition behavior. Second instar DBM larvae were exposed individually to each female. Parasitization was done in 25 °C climatic condition room. After hosts were stung by *C. vestalis*, the stung hosts were kept in 10 larvae/petri dish (9 cm in diameter) containing artificial diet at different temperatures of 13, 15, 20, 25, 30, 33, 35±0.5 °C (at 16L: 8D). Total of six replicate and sixty larvae were done for each tested temperature. Parasitized larvae were checked everyday and the diets were renewed everyday. The parasitized larvae were maintained at the test temperature until emergence of adult parasitoids or host moths. After parasitoid larvae emerge and span cocoons, cocoons were collected and placed individually in small vial until adult emergence. Emergence of the parasitoid adults was checked everyday. The development time from egg to pupa and from pupa to adult emergence, and total development time were recorded.

Statistical Analysis

The effect of temperature (13–33 °C) on development time was analyzed by one–way analysis of variance (ANOVA). The means were separated by Tukey's honestly significant difference (HSD) test (SAS Institute 1998). The effect of temperature on the development rate of various stages (i.e., egg + larva, pupa and total development) was tested by linear regression using the model Y=bX+a, where Y is developmental rate (1/developmental time), X is temperature, and a and b are the regression parameters obtained from the regression. The lower developmental thresholds (T_0) and the thermal constant (DD) requirement were estimated using the parameters: T_0= – a/b and DD=1/b (Campbell *et al*., 1974). Estimation of constants was based only on data obtained at 13–33 °C. The relationships between temperature (13–35 °C) and rate of development were also fitted by the nonlinear Logan model VI (Logan *et al*., 1976).

$$Y= P_1\times \{\exp [P_2\times(X–T_0)] – \exp [P_2\times (T_{max}–T_0) – P_3\times (T_{max}–X)]\}$$

where X is the temperature, T_0 is the lower developmental threshold, T_{max} is the upper (lethal) temperature threshold, P_1, P_2 and P_3 are coefficients. The optimum temperature for development was calculated after setting the first derivative of the Logan equation equal to zero. T_{max} was estimated with Logan equation by Quasi–Newton curve fit using MacCurveFit version 1.5 (Kevin Raner Software, Mt. Waverley, Australia).

RESULTS

Developmental time for *C. vestalis* at different temperatures is summarized in Table 1. *Cotesia vestalis* completed its development on *P. xylostella* at all tested temperatures except 35 °C. Although some larvae reached to pupa stage, no pupae succeeded in completing development at 35 °C. The duration of egg + larva stage decreased significantly as the temperature increased from 13 to 33 °C ($P<0.0001$). The duration of egg + larva stage varied from 37.3d at 13 °C to 5.9d at 30 and 33 °C (Table 1). The duration of pupa stage and total duration from egg to adult were also decreased significantly as the temperature increased from 13 to 33 °C ($P<0.0001$). Pupa developmental time varied from 26.4d at 13 °C to 3.5d at 33 °C. Total developmental time from egg to adult emergence declined from 63.7d at 13 °C to 9.4d at 33 °C. There was no significant difference in duration of egg + larva stage and pupa stage as well as total development time from egg to adult emergence at 30 and 33 °C ($P>0.05$). The shortest total developmental time was 9.4d at 33 °C and it was not significantly difference from

Table 1. Developmental times (days) of *C.vestalis* at different constant temperatures

Temperature °C	No. of insect observed	Stage		
		Egg + larva	Pupa	Total Development
13	25	37.3±0.45a	26.4±0.34a	63.7±0.46a
15	41	23.6±0.19b	17.4±0.21b	41.0±0.32b
20	45	11.87±0.14c	7.8±0.11c	19.6±0.16c
25	50	7.7±0.13d	4.8±0.08d	12.5±0.12d
30	46	5.9±0.06e	3.6±0.08e	9.5±0.10e
33	35	5.9±0.10e	3.5±0.09e	9.4±0.11e
35	22	6.7±0.14		

Means with the small letters within the same stage and different temperatures (13 to 33 °C) are not significantly different by Tukey's HSD test after one way ANOVA, $p<0.05$.

9.5d at 30 °C. At all tested temperatures, pupal period was significantly shorter than the egg + larva stage period (Table 1).

Development rate of *C. vestalis* such as egg + larva, pupa and total development rate from egg to adult increased with temperature within temperature range of 13–33 °C but declined at 35 °C (Fig. 1). Significant linear relationships were indicated for the regression of individual development rate on temperature for each life stage period (Table 2; Fig. 1). From these equations, a lower developmental threshold (T_0) was estimated at 8.9, 10.1 and 9.4 °C for egg + larvae, pupa and total immature stages, respectively. A thermal constant (DD) of 125.0,71.4 and 200.0 DD was estimated as the effective temperature sums for the development of the egg + larva, pupa and total development periods, respectively.

The non linear Logan model gave a good fit (R2 between 0.9162 and 0.9670) to the data sets of developmental rate for the range of temperature used (Fig. 1; Table 3). The optimal developmental temperatures estimated for egg + larva, pupa, egg to adult stages were 38.1,30.5and 31.3 °C, respectively. The upper limit temperatures for immature development of *C. vestalis* ranged between 35.0 and 38.2 °C (Table 3).

DISCUSSION

Our result is the first report on the effect different

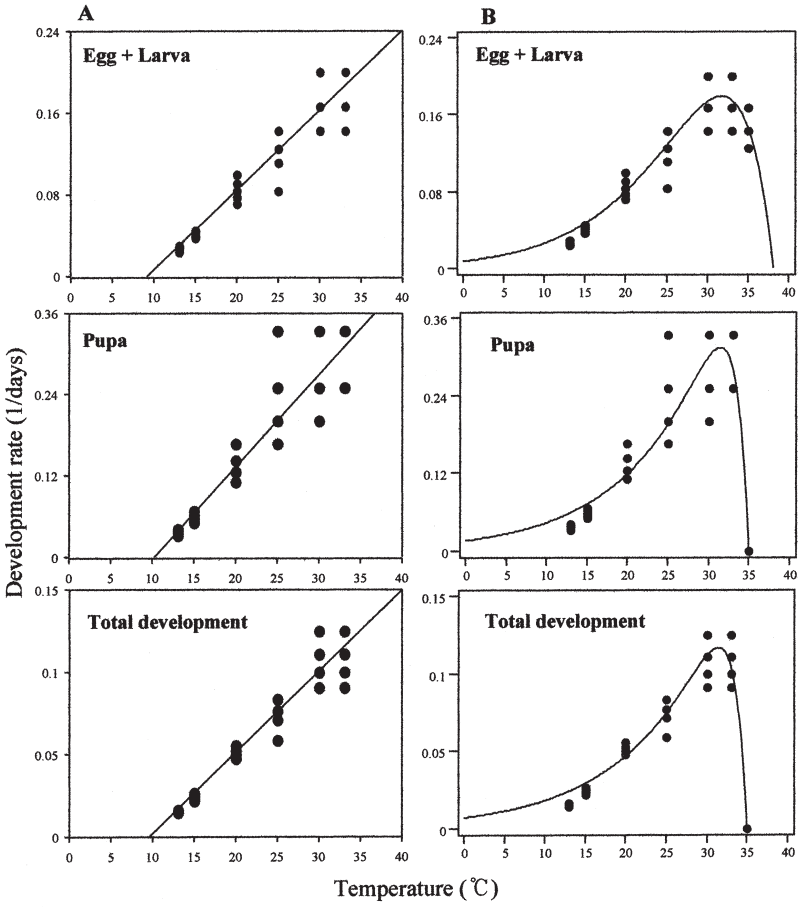

Fig. 1. Fitting of equations (A) Linear and (B) Logan–6 for the total biological cycle of *C. vestalis* on *P. xylostella* as a function of temperature.

Table 2. Linear regression equations of development rate vs temperature and estimated lower developmental threshold (T_0; °C) and thermal constant (DD) for the immature stages of *C. vestalis* Myanmar strain[a]

Stage	Slope ± SE	Intercept ± SE	ANOVA parameters			r^2	T_0	DD
			F	df	P			
Egg–Pupa	0.008±0.0001	–0.071±0.003	3960.6	1,241	<0.0001	0.943	8.9	125
Pupa–Adult	0.014±0.00003	–0.141±0.007	2188.8	1,241	<0.0001	0.901	10.1	71.4
Total	0.005±0.00006	–0.047±0.002	6232.9	1,241	<0.0001	0.963	9.4	200

[a] Data were obtained from experiments conducted at six constant temperatures (13, 15, 20, 25, 30 and 33 °C)

Table 3. Parameters of Logan equation, upper temperature thresholds, and optimum development temperature for immature stages of *C. vestalis* Myanmar strain[a]

Stage	P1	P2	P3	R^2	Upper threshold (°C)	Optimum temperature (°C)
Egg–Pupa	0.1638	0.1555	0.1574	0.9505	38.2	31.8
Pupa–Adult	0.2153	0.1000	0.6132	0.9162	35.0	30.5
Total	0.0172	0.0944	0.0621	0.9670	35.0	31.3

[a] Data were obtained from experiments conducted at seven constant temperatures (13, 15, 20, 25, 30, 33 and 35°C)

temperatures on development of Myanmar strain of *C. vestalis* on *P. xylostella*. No data on development of Myanmar strain of *C. vestalis* on *P. xylostella* were previously available. The lower threshold temperature for total development of *C. vestalis* in this study (9.4 °C) is slightly higher than that of Nofemela (2004) (8.14 °C) for the South African strain, but is lower than that calculated by Lim (in Nofemela 2004) (13.8 °C) for the Malaysian strain of *C. vestalis* and (11.0 °C) for the Chinese strain (Shi and Liu, 1999). The thermal constant estimated from our data for total development (200.0 DD) was lower than estimated by Nofemela (2004) (217.39 DD) but higher than by Shi and Liu (1999) (169.28 DD). These differences could be difference of civestalis populations or difference of host reared on different food source, because thermal characteristics may vary between populations (Campbell *et al*., 1974; Lee and Elliott, 1998), and developmental stages (Honek and Kocourek, 1988), and with other ecological factors such as food source (Gilbert and Raworth, 1996). At all tested temperatures pupal period was shorter than the egg + larva period (Table 1). This finding is in agreement with (Kawaguchi and Tanaka, 1998; Shi *et al*., 2002; Nofemela, 2004) and for *C. vestalis* on DBM reared on cabbage (Htwe, unpublished).

Development rate was positively correlated with temperature at 13–33 °C. Some *C. vestalis* larvae reach to pupa stage but no pupae succeeded in completing development at 35 °C, indicating that this temperature falls out of development temperature range. Our findings on the effect of constant temperatures on development of *P. xylostella* as a host of *C. vestalis* showed that *P. xylostella* could not developed until adult at 35 °C, and this could be an evolutionary relationship between them. The linear model is widely used for calculating low temperature threshold and thermal constant in numerous studies (Dent and Walton, 1997; Roy *et al*., 2002; Kontodimas *et al*., 2004). But the weakness of the linear model are; the relationship holds only for a medium range of temperatures (Campbell *et al*., 1974; Gilbert *et al*., 1976) and the estimated threshold is an extrapolation of the linear portion of the relationship into a region where the relationship is unlikely to be linear (Jervis and Copland, 1996) thus lower developmental threshold and the thermal constant may be underestimated at temperatures close to the lower threshold (Howe, 1967). Although it has disadvantages the linear model has been used widely because it requires minimal data for formulation to calculate and apply easily and is the simplest method for estimation of the thermal constant (Worner, 1992).

We applied the nonlinear Logan model to study the adverse effect of extreme temperatures on developmental rate. It is one of the most commonly used models for description of temperature–dependent development of insects and other arthropods (Kontodimas *et al*., 2004). The biological interpretation of all fitted coefficients of the equation 6 is remarkable and should be regarded as a major advantage when evaluating this model (Logan *et al*., 1976). The model fit the data well and predicted the values for the upper temperature ranged between 35.0 and 38.2 °C, and the optimum temperature ranged between 30.5 to 31.8 °C. The Logan model has certain advantages over linear regression, including good estimates of upper threshold and optimum temperature and a description of the nonlinear response to temperature.

Our study has provided the necessary information and mathematical description of the development rate of *C. vestalis*. Liu *et al*. (1995) suggested that rate functions derived from constant temperature can be used effectively to predict population development in the field. Information on thermal requirement of *C. vestalis* could be useful in selection of appropriate candidate for release under given climatic conditions for biological control of diamondback moth. Furthermore our findings may provide necessary information for biological control by augmentation and introduction program. For augmentative biological control timing is also important because the potential use of parasitoids or entomopathogenic nematode would require precise timing for success (Petit and Wietlisbach, 1993; Head *et al*., 2000). It will be valuable when this information used in association with other biological and ecological data for development and implementation of DBM management program.

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