Effect of temperature on development, survival, and adult body size of two green lacewings, *Mallada desjardinsi* and *Chrysoperla nipponensis* (Neuroptera: Chrysopidae)

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Abstract

To search for a useful biological control agent and to determine the optimal rearing conditions for the green lacewings *Mallada desjardinsi* and *Chrysoperla nipponensis*, thermal effects on development, survival, and adult body size were examined at seven constant temperatures (15.0, 17.5, 20.0, 22.5, 25.0, 27.5, and 30.0°C) with a photoperiod regime of 16L : 8D (h). The developmental zero of *M. desjardinsi* larvae was higher than that of *C. nipponensis* larvae. The developmental zeros of *C. carnea* and *C. nipponensis* in earlier reports were similar to that of *C. nipponensis* in the present study. On the other hand, that of *M. desjardinsi* in earlier studies was somewhat higher than that of *M. desjardinsi* in the present study. A lower survival rate of *C. nipponensis* was observed in the cocoon stage at 15.0°C. On the other hand, lower survival rates of *M. desjardinsi* were observed in the egg stage at 30.0°C and in the cocoon stage at 15.0, 27.5 and 30.0°C. The body size of both green lacewings was affected by temperature throughout the range tested. Smaller body sizes of *M. desjardinsi* and *C. nipponensis* adults were observed at 15.0, 27.5 and 30.0°C. These results indicate that *C. nipponensis* has a wider optimum range of temperature for immature development than *M. desjardinsi*.

Key words: Developmental zero; thermal constant; optimum range of temperature; biological control agent

INTRODUCTION

The common green lacewings *Mallada desjardinsi* and *Chrysoperla nipponensis* are known to be important predators of many serious pests in Japan (Tsukaguchi, 1995). They are considered to be prospective candidates for biological control agents of aphids because of their high predatory capability (Niijima, 1997). There have been many reports on the life history and rearing method of both green lacewings (Wei et al., 1986; Kabissa et al., 1995, 1996; Kubota and Shiga, 1995; Nakamura et al., 2000; Niijima and Tokuno, 2000). However, there is little information available regarding thermal effects on development and survival (Liu, 1989; Fujiwara and Nomura, 1999). Temperature and photoperiod are principal environmental factors affecting the development, survival and diapause of insects. Thus, determining the thermal and photoperiodic effects on insects is useful for selection of biological control agents and for determination of their rearing conditions.

In the present study, the thermal effects on developmental period, survival rate, and adult body size of *M. desjardinsi* and *C. nipponensis* were examined to determine their resistance to low- and high-temperature regimes.

MATERIALS AND METHODS

Insect materials and culture techniques. Laboratory cultures of *M. desjardinsi* and *C. nipponensis* were established by female adults collected in Nankoku City (33.5°N), Kochi Prefecture, Japan, in May 2003. The adults were individually reared in a plastic cup (9.0 cm in diameter, 9.0 cm in depth) with moistened cotton and an artificial diet that was a mixture of yeast autolysate AY-65 (Asahi Food & Healthcare Ltd.) and honey at a ratio of 1 : 1. The eggs laid were individually transferred to plastic petri dishes (5.5 cm in diameter, 1.5 cm in depth). Newly hatched larvae were reared with de-

frosted eggs of *Ephestia kuehniella* in the same plastic petri dish until adult emergence. All laboratory rearing was conducted under 16L:8D at 25.0±1.0°C. The eggs and larvae of both green lacewings in the second and third generations were used for experiments.

Experiment 1. Eggs of both lacewings laid within 24 h were used for the experiment. Eggs were individually transferred to a 24-well microplate (each well 1.6 cm in diameter, 1.7 cm in depth) with a lid, and maintained under seven different temperature regimes of 15.0, 17.5, 20.0, 22.5, 25.0, 27.5, and 30.0°C with 16L:8D. Eggs that had not hatched within 2 mo from the start of the experiment as well as pupae within the cocoon that had not emerged within 2 mo were regarded as dead.

Experiment 2. First instar larvae of both green lacewings within 24 h of hatching were individually transferred to petri dishes (5.5 cm in diameter, 1.5 cm in depth), and maintained under seven different temperature regimes of 15.0, 17.5, 20.0, 22.5, 25.0, 27.5, and 30.0°C at 16L:8D until adult emergence. Adequate *Ephestia kuehniella* eggs to sustain development of both green lacewings' larvae were supplied as its prey. The dishes were inspected daily for the presence of the larval exuviae. The body weight and forewing length of adult green lacewings were measured within 24 h after emergence.

Data analyses. Developmental zero (T_0) for egg, larva and cocoon stages were estimated from the simple regressions between velocities of development ($1/D$) and temperatures, and the thermal constant (K) was similarly calculated from T_0 and the developmental period (D). The effect of temperature on the developmental periods of egg, larva and cocoon stages of *M. desjardinsi* and *C. nipponensis* were analyzed using ANOVA (SPSS Inc., 2002). The effect of temperature on the forewing length and body weight of *M. desjardinsi* and *C. nipponensis* were also analyzed using ANOVA (SPSS Inc., 2002). The significance of differences in the forewing length and body weight of *M. desjardinsi* and *C. nipponensis* at each temperature were analyzed among the seven constant temperatures based on the 5% level of the Bonferroni's multiple comparison test (SPSS Inc., 2002). The significance of differences in the arcsin-root transformed survival rates of egg, larva, and cocoon stages of *M. des-*

Table 1. Developmental period of egg stages of *M. desjardinsi* and *C. nipponensis* at seven constant temperatures with 16L:8D

Temperature (°C)	Developmental period of egg stage (d)			
	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]
15.0	30	12.3±0.6	23	13.6±0.7
17.5	31	8.6±0.6	22	10.3±0.5
20.0	31	7.0±0.4	26	7.6±0.6
22.5	33	5.4±0.5	26	6.2±0.5
25.0	29	5.0±0.2	26	5.0±0.0
27.5	28	4.0±0.2	24	4.6±0.5
30.0	11	3.8±0.4	18	4.1±0.2
F^c		1,243.418		1,192.851
df^c		6		6
p^c		<0.001		<0.001

[a] Number of eggs hatched.
[b] Data indicate the mean±SD.
[c] Results of ANOVA.

jardinsi and *C. nipponensis* at each temperature were analyzed among the seven constant temperatures based on the 5% level of the Tukey-type multiple comparison test after the 5% level of the Chi-square test (Zar, 1999).

RESULTS AND DISCUSSION

The developmental periods of egg, larvae and cocoon stages of *M. desjardinsi* and *C. nipponensis* were significantly affected by temperatures from 15.0 to 30.0°C (Tables 1, 2, and 3; $p < 0.05$, ANOVA). The same developmental period was observed in the cocoon stage of *M. desjardinsi* at 15.0 and 17.5°C (Table 3). For this reason, the data at 15.0°C for this species were excluded from the calculation of developmental zeros and thermal constants. Regression equations between the velocities of development and temperatures, developmental zeros and thermal constants for *M. desjardinsi* and *C. nipponensis* are shown in Table 4.

Determining the developmental zero of potential biological control agents is useful for the estimation of their available seasons, as it shows the minimum temperature required for development (Kiritani, 1997). The larval developmental zeros of *M. desjardinsi* and *C. nipponensis* are especially useful in comparing their performance as biological control agents, as they prey on pests at the larval stage only (Niijima, 1997). Because of the higher

Table 2.   Developmental period of the larval stage of *M. desjardinsi* and *C. nipponensis*
at seven constant temperatures with 16L : 8D

Temperature (°C)	Developmental period of larval stage (d)							
	Male				Female			
	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]
15.0	9	24.5±2.3	12	24.7±1.8	13	24.2±0.8	16	25.0±1.9
17.5	13	17.5±1.3	22	16.5±1.0	19	17.0±0.9	18	18.1±1.9
20.0	6	12.5±1.6	17	12.9±1.0	24	12.9±1.5	21	12.8±0.8
22.5	12	9.8±0.7	18	10.2±0.6	22	9.4±0.6	22	10.4±0.8
25.0	14	7.6±0.8	17	9.3±1.0	16	7.8±0.9	15	9.6±1.0
27.5	8	6.4±0.5	17	7.2±0.8	21	7.0±0.6	23	7.4±0.7
30.0	7	5.9±0.4	19	6.4±0.6	9	5.8±0.4	14	6.9±0.6
F^c		287.530		692.447		728.609		588.967
df^c		6		6		6		6
p^c		<0.001		<0.001		<0.001		<0.001

[a] Number of larvae that spun a cocoon.
[b] Data indicate the mean±SD.
[c] Results of ANOVA.

Table 3.   Developmental period of the cocoon stage of *M. desjardinsi* and *C. nipponensis*
at seven constant temperatures with 16L : 8D

Temperature (°C)	Developmental periods of cocoon stage (d)							
	Male				Female			
	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]
15.0	9	23.9±2.5	12	27.8±1.2	13	23.5±1.2	16	28.7±1.9
17.5	13	23.9±1.9	22	18.9±1.3	19	24.3±1.4	18	18.9±1.3
20.0	6	17.7±1.0	17	15.0±1.8	24	18.8±1.7	21	15.0±1.8
22.5	12	14.3±1.2	18	11.4±0.8	22	14.6±1.0	22	11.4±0.8
25.0	14	12.1±0.5	17	10.1±0.7	16	12.6±0.9	15	10.1±0.7
27.5	8	10.8±0.5	17	9.7±1.3	21	10.9±0.9	23	9.7±1.3
30.0	7	10.0±0.0	19	7.9±0.8	9	10.2±0.4	14	7.9±0.8
F^c		184.022		449.138		375.526		442.847
df^c		6		6		6		6
p^c		<0.001		<0.001		<0.001		<0.001

[a] Number of cocoons emerged.
[b] Data indicate the mean±SD.
[c] Results of ANOVA.

larval developmental zero of *M. desjardinsi* (Table 4), one would expect that its active period in the winter season is shorter than that of *C. nipponensis*.

Although *C. nipponensis* was treated as synonymous with *C. carnea* by Tsukaguchi (1985), the Japanese *C. carnea* was later revised to *C. nipponensis* because the colors of the adult wing grate cross veins are green in *C. carnea* but black in *C. nipponensis* (Brooks, 1994). There is a wealth of developmental zero information for *C. carnea* but very little for *C. nipponensis*. The developmental zero of *C. carnea* was 9.5°C in a New York population, 9.8°C in an Alaskan population, 10.5°C in a Mexican population, and about 10°C in a Russian population (Tauber and Tauber, 1982; Volkovich,

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Table 4.   Regression equation between the velocity of development and temperature, developmental zero, and thermal constant for *M. desjardinsi* and *C. nipponensis*

Stage	Species	Sex	Regression equation[a][b]	R^2	Developmental zero (°C)[b]	Thermal constant (K) (degree-d)[b]
Egg	*M. desjardinsi*		$Y=0.0123X-0.0994$	0.9841	8.1	80.5
	C. nipponensis		$Y=0.0119X-0.1067$	0.9953	9.0	83.8
Larva	*M. desjardinsi*	♂	$Y=0.0095X-0.1084$	0.9930	11.4	106.3
		♀	$Y=0.0091X-0.1009$	0.9938	11.1	110.7
	C. nipponensis	♂	$Y=0.0077X-0.0755$	0.9919	9.8	130.7
		♀	$Y=0.0072X-0.0674$	0.9885	9.4	140.5
Cocoon	*M. desjardinsi*	♂	$Y=0.0047X-0.0376$	0.9859	8.0	213.5
		♀	$Y=0.0047X-0.0392$	0.9872	8.3	214.0
	C. nipponensis	♂	$Y=0.0059X-0.0499$	0.9864	8.5	171.8
		♀	$Y=0.0056X-0.0452$	0.9854	8.1	180.1

[a] X and Y indicate the temperature and developmental rate $(1/D)$, respectively.

[b] Regression equation or values for *M. desjardinsi* and *C. nipponensis* at each stage were calculated based on the data from 17.5 to 30.0°C and from 15.0 to 30.0°C, respectively.

1998). The developmental zero of *C. nipponensis* (Chiba population) was 10.2°C (Fujiwara and Nomura, 1999). These developmental zeros of *C. carnea* and *C. nipponensis* were similar to that of *C. nipponensis* in the present study (Table 4). The developmental zero of Chinese *M. desjardinsi* (Guandongs population) was 12.4°C for egg stage and 13.7°C for larval stage (Liu, 1989). These values are somewhat higher than that of *M. desjardinsi* in the present study (Table 4). Therefore, the population of *M. desjardinsi* in this study can generally develop under lower thermal conditions than the Guandongs population of *M. desjardinsi*.

In the present study, a low egg survival rate (20.8–68.8%) was observed in second and third generations of *M. desjardinsi* and *C. nipponensis* at all temperature examined (Table 5). Kubota and Shiga (1995) reported that the rearing for successive generations of the green lacewing led to lower egg survival rates in the later generations. Improvement of the rearing method for adult green lacewings is necessary for establishing a more efficient rearing system.

A lower survival rate of *C. nipponensis* was observed in the cocoon stage at 15.0°C (Tables 5, 6, and 7; $p<0.05$, Tukey-type multiple comparison test). On the other hand, lower survival rates of *M. desjardinsi* were observed in the egg stage at 30.0°C and in the cocoon stage at 15.0, 27.5 and 30.0°C (Tables 5, 6, and 7; $p<0.05$, Tukey-type multiple comparison test). The body size of both green lacewings was affected by temperatures of

Table 5.   Survival rate of egg stage of *M. desjardinsi* and *C. nipponensis* at seven constant temperatures with 16L : 8D

Temperature (°C)	Survival rate of egg stage (%)	
	M. desjardinsi[a]	*C. nipponensis*[a]
15.0	62.5 a	47.9 a
17.5	64.6 a	45.8 a
20.0	64.6 a	54.2 a
22.5	68.8 a	54.2 a
25.0	60.4 a	54.2 a
27.5	58.3 a	50.0 a
30.0	20.8 b	37.5 a

[a] Number of eggs tested was 48 at each temperature. Percentages in a given column followed by the same letter are not significantly different at the 5% level (Tukey-type multiple comparison test).

15.0°C to 30.0°C (Tables 8 and 9; $p<0.05$, ANOVA). Smaller adult body sizes were observed in *M. desjardinsi* and *C. nipponensis* at 15.0, 27.5 and 30.0°C. (Tables 8 and 9; $p<0.05$, Bonferroni's multiple comparison test). The lower survival rate of immature development and smaller adult body size indicate the possible growth inhibition in high- and/or low-temperature regimes during the immature stages of *M. desjardinsi* and *C. nipponensis*. Therefore, the growth inhibition of immature development was severe in *M. desjardinsi* at 15.0, 27.5 and 30.0°C, and in *C. nipponensis* at 30.0°C, but it is slight in *C. nipponensis* at 15.0 and 27.5°C.

In this study, the thermal effects were examined

Table 6. Survival rate of the larval stage of *M. desjardinsi* and *C. nipponensis* at seven constant temperatures with 16L : 8D

Temperature (°C)	Survival rate of larval stage (%)			
	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]
15.0	42	69.1 a	48	72.9 a
17.5	43	79.1 a	50	88.0 a
20.0	45	73.3 a	50	82.0 a
22.5	45	82.2 a	50	84.0 a
25.0	45	68.9 a	50	80.0 a
27.5	45	80.0 a	50	84.0 a
30.0	45	66.7 a	50	90.0 a

[a] Number of larvae examined.
[b] Percentages in a given column followed by the same letter are not significantly different at the 5% level (Tukey-type multiple comparison test).

Table 7. Survival rate of the cocoon stage of *M. desjardinsi* and *C. nipponensis* at seven constant temperatures with 16L : 8D

Temperature (°C)	Survival rate of cocoon stage (%)			
	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]
15.0	29	75.9 b	35	80.0 ab
17.5	34	94.1 a	44	90.9 ab
20.0	33	90.9 a	41	92.7 ab
22.5	37	91.9 a	42	95.2 a
25.0	31	96.8 a	40	80.0 ab
27.5	36	80.6 b	42	95.2 a
30.0	30	53.3 b	45	68.9 b

[a] Number of cocoons examined.
[b] Percentages in a given column followed by the same letter are not significantly different at the 5% level (Tukey-type multiple comparison test).

Table 8. Forewing length of emerged *M. desjardinsi* and *C. nipponensis* at seven constant temperatures with 16L : 8D

Temperature (°C)	Forewing length (mm)							
	Male				Female			
	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]
15.0	9	12.3±0.6 ab	12	12.0±0.4 ab	13	13.2±0.6 bc	16	12.5±0.5 abc
17.5	13	12.5±0.4 ab	22	12.1±0.6 a	19	13.5±0.4 ab	18	13.1±0.3 a
20.0	6	12.6±0.2 ab	17	12.3±0.4 a	24	13.6±0.3 a	21	13.0±0.4 ab
22.5	12	12.7±0.2 a	18	12.0±0.3 ab	22	13.7±0.5 a	22	12.6±0.7 ab
25.0	14	12.6±0.4 ab	17	11.6±0.5 bc	16	13.7±0.4 ab	15	12.8±0.6 ab
27.5	8	12.3±0.2 ab	17	11.5±0.6 bc	21	13.3±0.3 ac	23	12.5±0.5 bc
30.0	7	12.1±0.5 b	19	11.2±0.6 c	9	12.8±0.4 c	14	12.1±0.6 c
F^c		2.741		11.489		7.938		8.007
df^c		6		6		6		6
p^c		0.020		<0.001		<0.001		<0.001

[a] Number of adults examined.
[b] Data indicate the mean±SD. Values in a given column followed by the same letter are not significantly different at the 5% level (Bonferroni's multiple comparison test).
[c] Results of ANOVA.

in *M. desjardinsi* and *C. nipponensis* to search for useful biological control agents and to determine their optimal rearing conditions. *C. nipponensis* had a wider optimum range of temperature for immature development than *M. desjardinsi*. Photoperiodic response of *M. desjardinsi* and *C. nipponensis* is also useful to evaluate their performance as biological control agents. The effect of the photoperiod on the two green lacewings should be investigated before determining their performance in greenhouses and fields.

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Table 9. Body weight of emerged *M. desjardinsi* and *C. nipponensis* at seven constant temperatures with 16L : 8D

Temperature (°C)	Body weight (mg)							
	Male				Female			
	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]	n^a	*M. desjardinsi*[b]	n^a	*C. nipponensis*[b]
15.0	9	6.1±1.2 bc	12	5.4±0.5 abc	13	8.0±1.1 abc	16	6.6±0.7 bc
17.5	13	6.9±0.7 ac	22	5.8±0.8 a	19	8.2±0.6 ab	18	7.2±0.6 ab
20.0	6	7.2±0.3 ab	17	5.8±0.5 a	24	8.2±0.8 ab	21	7.0±0.4 ab
22.5	12	7.1±0.8 ab	18	5.7±0.7 ab	22	8.4±0.6 a	22	7.2±0.7 ab
25.0	14	7.3±0.7 a	17	5.6±0.9 ab	16	8.3±0.3 ab	15	7.7±1.4 a
27.5	8	6.4±0.5 ac	17	5.0±0.5 bc	21	7.7±0.6 bd	23	6.6±0.6 bc
30.0	7	5.8±0.9 c	19	4.6±0.7 c	9	7.1±1.0 cd	14	6.1±0.8 c
F^c		4.609		10.325		5.163		7.176
df^c		6		6		6		6
p^c		0.001		<0.001		<0.001		<0.001

[a] Number of adults examined.

[b] Data indicate the mean±SD. Values in a given column followed by the same letter are not significantly different at the 5% level (Bonferroni's multiple comparison test).

[c] Results of ANOVA.

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