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Effects of Temperature and Host on the Immature Development of the Parasitoid *Neochrysocharis okazakii* (Hymenoptera: Eulophidae)

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The development of *Neochrysocharis okazakii*, an eulophid parasitoid attacking pest *Liriomyza* leafminers, was studied under laboratory conditions at seven constant temperatures (15°, 17.5°, 20°, 22.5°, 25°, 27.5° and 30°C) on the hosts *L. chinensis* and *L. trifolii*. *Neochrysocharis okazakii* completed development on both host species at all temperatures examined. The total development time from egg to adult emergence was similar on the two host species at 25–30°C. Male parasitoids developed faster than females did. The developmental time was inversely proportional to temperature, and decreased from 41 to 9 days for temperatures from 15° to 30°C, with pupae requiring shorter time for development than the earlier stages. The lower developmental temperature thresholds and degree-days were estimated from linear regression equations. For egg to adult emergence, male *N. okazakii* required 166.7 degree-days (DD) above a lower developmental threshold of 11.5°C on *L. chinensis* and 166.7 DD above 11.6°C on *L. trifolii*; females required 172.4 DD above 11.3°C on *L. chinensis* and 178.6 DD above 11.3°C on *L. trifolii*. Although the two host species were equally suitable as host for *N. okazakii*, our findings suggested that *L. trifolii* is an ideal host for *N. okazakii* mass-rearing.

Key words: biological control, IPM, leafminer, onionpests

INTRODUCTION

Agromyzid leafminers are known to have many natural enemies, particularly insect parasitoids, in both their native and invaded ranges. Over 40 species of parasitoids have been recorded worldwide from *Liriomyza* spp. (Waterhouse and Norris, 1987). The communities of these parasitoids have been recognized for their potential contribution to the integrated pest management (IPM) of leafminers in both glasshouses and open fields (Waterhouse and Norris, 1987; Minkenberg, 1990).

Neochrysocharis okazakii Kamijo (Hymenoptera: Eulophidae) is well known as a parasitoid of *Liriomyza* leafminers, widely common and dominant in warm regions of many Asian countries including China, Japan and Vietnam (Murphy and LaSalle, 1999; Tran *et al.*, 2006). This endoparasitoid is capable of developing on several *Liriomyza* leafminer species, including *L. trifolii* (Burgess), *L. sativae* Blanchard, *L. brassicae* (Riley) and *L. chinensis* (Kato) (Saito *et al.*, 1996; Arakaki and Kinjo, 1998; Konishi, 2004; Bjorksten *et al.*, 2005; Tran *et al.*, 2006). This wasp species is also predominant among the parasitoids attacking *L. chinensis* in onion crops, and is likely to be useful for the leafminer control in Vietnam (Tran *et al.*, 2006).

Neochrysocharis okazakii can complete its development on both *L. chinensis* and *L. trifolii*, with rapid development, giving minimum life cycles of 11–12 days at 25°C (Tran and Takagi 2006; Tran *et al.*, 2007). Like other insects, the rate of development of the parasitoid is temperature dependent. The knowledge of thermal constants and lower developmental thresholds provides essential information to determine the development rate of a particular species of arthropod (Jarošík *et al.*, 2002). Developmental rates and threshold temperatures are frequently used to create predictive models of insect development (Lactin *et al.*, 1995). The objectives of our study were to determine the effect of selected constant temperatures and host species *L. chinensis* and *L. trifolii* on the development rate of immature stages of *N. okazakii*, and to estimate lower developmental thresholds and thermal constant (degree-day) for each stage.

MATERIALS AND METHODS

Insect rearing

Colonies of the two leafminers, *L. chinensis* and *L. trifolii*, were maintained separately in MIR-253 Sanyo incubator chambers at 25±0.5°C, 60–70% relative humidity and a photoperiod of 16:8 hours light:dark. *Liriomyza trifolii* had been reared on kidney bean, *Phaseolus vulgaris* L. (Tran *et al.*, 2004), and *L. chinensis* was maintained on Welsh onion, *Allium fistulosum* L. (Tran and Takagi, 2005).

The colony of *N. okazakii* originated from Hue City, Vietnam. This parasitoid was reared on larvae of *L. chinensis* in the MIR-253 Sanyo incubator chambers at

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25±0.5°C, 60–70% relative humidity and 16:8 h light:dark photoperiod at the Laboratory of Entomology, Faculty of Agronomy, Hue University of Agriculture and Forestry, Vietnam in the same manner as described by Tran *et al.* (2007). Each leaf of the infested onion plants (30–40 cm height, 2–3 leaves per plant) had 20–40 second- and third-instar *L. chinensis*. For parasitization, four host-infested potted plants were placed in a plastic cage (45×30×32 cm) covered with a fine nylon mesh. A piece of tissue paper (2×2 cm) saturated with a honey solution was placed in the cage to give food for the parasitoids. About 100–200 parasitoids were introduced into the cage. After an exposure for 24 h, these plants were transferred into a vented plastic container (60×50×40 cm) until pupation of the parasitoids (approximately 6 days after parasitization). The onion leaves with parasitoid pupae were removed from the plant stems and transferred into a polyethylene terephthalate (PET) bottle (1.5 litres). Emergence of parasitoids was checked daily. The parasitoids collected from the bottle were placed in grass vials (28×60 mm diameter) and provided with honey immediately after emergence.

Immature development

The effects of seven constant temperatures (15°, 17.5°, 20°, 22.5°, 25°, 27.5° and 30°C) on the development of *N. okazakii* reared on *L. chinensis* and *L. trifolii* were investigated. Experiments were conducted in parallel. Four potted onion plants at 2–3 leaf stage, 30–40 cm high or four potted kidney bean plants with well-developed first post-cotyledon leaves were placed in a plastic cage (45×30×25 cm) covered with a fine nylon mesh. Fifty mixed sex *L. chinensis* or *L. trifolii* adults were released in the cage for 2–4 h to allow oviposition. Then, the potted plants were removed from the cage and held in environmental chambers at a constant temperature of 25°C and a photoperiod of 16:8 h light:dark until all leaf-miner larvae reached the final instar.

Either host plant infested with final-instar leafminers was placed in a plastic cage (45×30×32 cm) covered with a fine nylon mesh and then mixed sex 2-day-old *N. okazakii* adults were introduced into the cage for parasitism. Female parasitoids were allowed to attack and parasitize leafminer larvae for 6 h. After the parasitization period, the plants were removed. The leaves of onion plants were then dissected under a microscope to check for paralyzed larvae. The paralyzed larvae were removed and placed into Petri dishes (6 cm diameter). A piece of cotton wool saturated with distilled water was laid on each dish, and then a piece of filter paper (5.5 cm diameter) was placed on the cotton wool. The paralyzed larvae were placed on the paper and then covered with another piece of the filter paper. The leaves of kidney bean plants were cut off and then placed in Petri dishes (9 cm diameter) lined with a piece of water-saturated cotton wool and filter paper. The dishes with paralyzed larvae were maintained in each of eight environmental chambers set at 15°, 17.5°, 20°, 22.5°, 25°, 27.5° and 30°C, and a 16:8 h light:dark photoperiod until pupation of par-

asitoids. Parasitoid pupae were collected once per day in the afternoon. The development time of combined egg-larva stages was defined as the time from oviposition until pupa collection. The pupae were individually placed in Petri dishes (6 cm diameter) lined with filter paper. These dishes were placed in the same experimental conditions and supplied daily with some drops of water for maintaining appropriate humidity in the Petri dishes. The day of adult parasitoid emergence and the sex of parasitoids were recorded daily to determine mean development time.

Statistical analysis

The effect of temperature on development time of *N. okazakii* on *L. chinensis* and *L. trifolii* was analyzed with one way analysis of variance (ANOVA). The means were separated by Tukey's HSD test. The combined effects of temperature and host species on development time were tested using two-way ANOVA (SAS Institute, 1998).

The effect of temperature on the developmental rate of various stages (i.e., egg-larva, pupa and total development) was examined by linear regressions using the model: $Y = bX + a$ where Y is the developmental rate ($1/[\text{developmental time}]$), X is temperature, and a and b are the regression parameters obtained from the regression. The lower developmental thresholds (T_0) and the degree-day (DD) requirement were estimated using the parameters: $T_0 = -a/b$; $DD = 1/b$ (Campbell *et al.*, 1974).

RESULTS

Neochrysocharis okazakii completed development on both host species at all temperatures examined. Development time for *N. okazakii* on the two hosts at different temperatures is summarized in Table 1. The total development time was similar on both host species at 25–30°C, whereas parasitoid development was significantly slower on *L. trifolii* at 17.5–20°C ($P < 0.0001$) and 22.5°C ($P < 0.05$), and faster at 15°C ($P < 0.0001$). Male parasitoids developed faster than females at all tested temperatures.

The development time of each parasitoid stage was inversely related to temperature (Table 1). On *L. chinensis*, the duration of egg-larva, pupa and total development of females, and pupal stage of males decreased significantly as the temperature increased ($P < 0.0001$), whereas egg-larva development time of males decreased significantly when temperature was increased up to 25°C ($P < 0.0001$). There was no significant difference between total development duration of males at 27.5° and 30°C ($P > 0.05$). On *L. trifolii*, the durations of egg-larva and total development of males, and pupa and total development of females decreased significantly as the temperature increased ($P < 0.0001$). There was no significant difference in pupal development duration of males between 25° and 27.5°C ($P > 0.05$), and egg-larva development duration of females between 25° and 27.5°C ($P > 0.05$).

At 15°C, *N. okazakii* took about 40 and 41 days to complete its development on *L. trifolii* and *L. chinensis*.

Table 1. Developmental times (days) of *N. okazakii* reared on *L. chinensis* and *L. trifolii* at different constant temperatures

Temp.	Stage	<i>L. chinensis</i>		<i>L. trifolii</i>		Sources of variation (<i>P</i>)		
		Male	Female	Male	Female	Sex	Species	Sex× species
15°C	Egg + larva	19.6 ± 0.36a	20.7 ± 0.24a	19.5 ± 0.19a	20.0 ± 0.18a	<0.05	NS	NS
	Pupa	20.4 ± 0.25a	21.3 ± 0.23a	19.9 ± 0.13a	20.5 ± 0.13a	NS	<0.0001	NS
	Total	41.0 ± 0.35a	41.9 ± 0.19a	39.5 ± 0.22a	40.5 ± 0.22a	<0.001	<0.0001	NS
	N	24	26	48	61			
17.5°C	Egg + larva	14.0 ± 0.24b	14.3 ± 0.17b	15 ± 0.26b	15.6 ± 0.25b	NS	<0.0001	NS
	Pupa	14.5 ± 0.17b	15.0 ± 0.17b	14.5 ± 0.19b	14.9 ± 0.19b	<0.05	NS	NS
	Total	28.6 ± 0.33b	29.3 ± 0.23b	29.6 ± 0.22b	30.6 ± 0.29b	<0.05	<0.001	NS
	N	28	46	36	45			
20°C	Egg + larva	10.2 ± 0.18c	10.2 ± 0.16c	10.7 ± 0.26c	11.4 ± 0.32c	NS	<0.001	NS
	Pupa	10.1 ± 0.12c	10.2 ± 0.08c	10.3 ± 0.19c	10.5 ± 0.14c	NS	NS	NS
	Total	20.3 ± 0.17c	20.4 ± 0.11c	21 ± 0.26c	21.9 ± 0.3c	<0.05	<0.0001	NS
	N	22	29	29	35			
22.5°C	Egg + larva	7.7 ± 0.15d	8.0 ± 0.08d	8.2 ± 0.14d	8.6 ± 0.14d	<0.05	<0.0001	NS
	Pupa	7.7 ± 0.08d	7.6 ± 0.06d	7.4 ± 0.08d	7.6 ± 0.11d	NS	NS	NS
	Total	15.4 ± 0.12d	15.6 ± 0.09d	15.7 ± 0.14d	16.1 ± 0.13d	<0.05	<0.05	NS
	N	35	83	55	38			
25°C	Egg + larva	6.1 ± 0.07e	6.3 ± 0.08e	6.4 ± 0.12e	6.3 ± 0.1e	NS	NS	NS
	Pupa	5.9 ± 0.05e	5.9 ± 0.08e	5.5 ± 0.11e	5.9 ± 0.13e	NS	<0.05	NS
	Total	12.1 ± 0.5e	12.2 ± 0.09e	11.7 ± 0.16e	12.2 ± 0.16e	<0.05	NS	NS
	N	19	42	33	55			
27.5°C	Egg + larva	5.2 ± 0.09e	5.6 ± 0.13f	5.4 ± 0.13f	5.7 ± 0.15ef	NS	NS	NS
	Pupa	5.0 ± 0.11f	5.1 ± 0.09f	5.3 ± 0.09e	5.4 ± 0.11f	NS	<0.05	NS
	Total	10.3 ± 0.14f	10.7 ± 0.15f	10.5 ± 0.09f	11.1 ± 0.15f	<0.05	NS	NS
	N	24	25	68	77			
30°C	Egg + larva	4.9 ± 0.23e	4.9 ± 0.07g	4.6 ± 0.09g	4.9 ± 0.14f	NS	NS	NS
	Pupa	4.0 ± 0.17g	4.3 ± 0.06g	4.4 ± 0.08f	4.5 ± 0.09g	<0.05	<0.05	NS
	Total	8.9 ± 0.9f	9.2 ± 0.06g	8.9 ± 0.09g	9.5 ± 0.1g	<0.001	NS	NS
	N	17	94	54	40			

Values given are mean ± SE. Means with the same letters within the same stage and column are not significantly different by Tukey's HSD test after one-way ANOVA, $P < 0.05$. NS = not significant.

sis, respectively. In comparison, at 20°C developmental time dropped to about half on both species (20–21 days). Parasitoid development was completed after about 12 days at 25°C and 9 days at 30°C. At all tested temperatures and on both hosts, the parasitoid pupal stage was slightly shorter than the egg–larva period except at 15°C for males (t test; $t = 4.0$, $n = 48$, $P < 0.001$) and females ($t = 1.97$, $n = 52$, $P < 0.05$) on *L. chinensis*, and for females ($t = 2.19$, $n = 122$, $P < 0.05$) on *L. trifolii*, and at 17.5°C for females on *L. chinensis* ($t = 3.26$, $n = 92$, $P < 0.001$), where the parasitoid showed a longer egg–larva period.

Development rate of *N. okazakii* from oviposition to completion of egg–larva, pupal and total immature stages increased with temperature over the range tested. Significant linear relationships were indicated for the regressions of mean development rate on temperature

for each lifecycle stage (Table 2). From these equations, lower developmental thresholds (LDT) were estimated, which ranged from 10.7° to 12.2°C for the egg–larva, pupal and total immature stages. Thermal constants (DD) of 166.7, 172.4 and 178.6 degree–days were estimated as the effective temperature sums for completing development of males on both hosts and of females on *L. chinensis* and *L. trifolii*, respectively.

DISCUSSION

Some species of the genus *Neochrysocharis* have been reared on leafminers in the laboratory (Maryana, 2000; Tran *et al.*, 2004; Hondo *et al.*, 2006). However, few data on the development of *N. okazakii* on *Liriomyza* are available (Tran and Takagi, 2006). Our

Table 1. Linear regression equations of development rate versus temperature, and estimated lower developmental threshold (LDT) and thermal constant (DD) for the immature stages of *N. okazakii* reared on *L. chinensis* and *L. trifolii* at different constant temperatures

Sex	Stage	Slope $\pm$ SE	Intercept $\pm$ SE	ANOVA parameters			R^2	LDT	DD
				F	df	P			
<i>L. chinensis</i>									
Male	Egg + larva	0.0109 $\pm$ 0.0005	-0.1164 $\pm$ 0.0113	499.3	1, 5	<0.0001	0.990	10.7	91.7
	Pupa	0.0135 $\pm$ 0.0006	-0.1652 $\pm$ 0.0145	458.8	1, 5	<0.0001	0.989	12.2	74.1
	Egg to adult	0.0060 $\pm$ 0.0002	-0.0689 $\pm$ 0.0035	1549.5	1, 5	<0.0001	0.997	11.5	166.7
Female	Egg + larva	0.0106 $\pm$ 0.0003	-0.1135 $\pm$ 0.0059	1758.9	1, 5	<0.0001	0.997	10.7	94.3
	Pupa	0.0127 $\pm$ 0.0004	-0.1507 $\pm$ 0.0089	1075.0	1, 5	<0.0001	0.995	11.9	78.7
	Egg to adult	0.0058 $\pm$ 0.0001	-0.0655 $\pm$ 0.0034	1591.3	1, 5	<0.0001	0.997	11.3	172.4
<i>L. trifolii</i>									
Male	Egg + larva	0.0114 $\pm$ 0.0005	-0.1291 $\pm$ 0.0105	624.5	1, 5	<0.0001	0.992	11.3	87.7
	Pupa	0.0122 $\pm$ 0.0007	-0.1393 $\pm$ 0.0164	294.3	1, 5	<0.0001	0.983	11.4	81.9
	Egg to adult	0.0060 $\pm$ 0.0003	-0.0694 $\pm$ 0.0061	510.5	1, 5	<0.0001	0.990	11.6	166.7
Female	Egg + larva	0.0108 $\pm$ 0.0006	-0.1206 $\pm$ 0.0129	367.9	1, 5	<0.0001	0.987	11.2	92.6
	Pupa	0.0119 $\pm$ 0.0005	-0.1356 $\pm$ 0.0114	579.8	1, 5	<0.0001	0.991	11.4	84.0
	Egg to adult	0.0056 $\pm$ 0.0003	-0.0631 $\pm$ 0.0058	490.5	1, 5	<0.0001	0.989	11.3	178.6

results indicated that *N. okazakii* could complete its development on both *L. chinensis* and *L. trifolii* at the tested temperature range of 15–30°C. At an intermediate range of 25–30°C, total development time was similar on the two host species. The result has demonstrated that the host species are almost equal in quality for *N. okazakii* development, at least, in terms of developmental time. Also, the result has shown that the development time of immature stages of *N. okazakii* on both host species is shorter than that of *N. formosa* on *L. trifolii* (i.e., 52.5, 14 and 11.9 days at 15°, 25° and 30°C, respectively) (Hondo *et al.*, 2006). Short developmental time is a crucial to biological control because developmental time can determine how quick a biocontrol agent can follow an increase of pest populations.

At all tested temperatures and on both hosts, male parasitoids developed faster than females did. This result is in agreement with Maryana (2000) for *N. formosa* on *L. trifolii*, and other eulophid parasitoids reared on *L. trifolii* (Minkenberg, 1990; Bazzocchi *et al.*, 2003; Hondo *et al.*, 2006).

No substantial deviations from linearity in the various stages and total development rate were observed with *N. okazakii* males and females reared on *L. chinensis* and *L. trifolii* over the temperature range tested. This result is consistent with previous studies, indicating that, at an intermediate range of temperatures (e.g., 15–30°C), the developmental rate of most eulophid parasitoids of leafminers is linearly related to ambient temperatures (Minkenberg, 1990; Saito *et al.*, 1997; Maryana, 2000; Bazzocchi *et al.*, 2003; Hondo *et al.*, 2006).

Estimated lower threshold temperatures of *N. okazakii* were similar regardless of the developmental stage, the sex and host species (range 10.7–12.2°C). Similar values are reported by Maryana (2000) and

Hondo *et al.* (2006) for *N. formosa* (10.6° and 10.4°C for male and female, respectively) and *Hemiptarsenus varicornis* (11.8°C) reared on *L. trifolii*. Hondo *et al.* (2006) also report lower values for five other eulophid parasitoids reared on *L. trifolii* (*Pnigalio katonis*, *Diglyphus isaea*, *D. minoensis*, *D. pusztensis* and *Chrysocharis pentheus*).

Bazzocchi *et al.* (2003) report a significant difference between estimated DD values for *D. isaea* between host species, i.e., *L. trifolii* and *L. huidobrensis*. However, our findings showed that male *N. okazakii* reared on both *L. trifolii* and *L. chinensis* required a similar DD to complete the development, while DD values for females reared on *L. trifolii* were slightly higher than those on *L. chinensis*. The result again suggests both host *Liriomyza* is almost equal in host quality for *N. okazakii*. Given the wide range of linearity in the developmental rate curves, the DD concept may be useful in predicting number of generations of the eulophid parasitoids in the field (Pereira *et al.*, 2011).

Several factors affect the cost-competitiveness of biological control agents as an option for pest control. For many natural systems, the agents are mass-produced using the natural host or prey, which itself has been reared on one of its normal food plants. Therefore, those systems usually require intensive labor to rear plants and hosts (or prey organisms), which will make the production cost of biocontrol agents expensive, and consequently the use of natural enemies is a unfavored option. The production costs can normally be lowered only by reducing the costs of raw materials, largely by finding cheaper substitutes at either the plant or herbivore trophic level in a rearing system (van Driesche and Bellows, 1996). The host plants of *L. chinensis* are *Allium* spp. (Spencer, 1973), and ideally Welsh onion

should be used as normal host food in a natural rearing system for *N. okazakii* to ensure that the parasitoids are well adapted to *L. chinensis*. Because onion plants are slow-growing, their use in a mass-rearing system is not conducive to producing large numbers of parasitoids at an economical price. In addition, to keep parasitoid survival high, removal of parasitized leafminers from onion leaves is required when onion-*L. chinensis* system is used to rear the parasitoid because onion leaves deteriorate during the final rearing process of parasitoids developing on *L. chinensis* (i.e., parasitoid emergence stage). Our findings have shown that *L. trifolii* is suitable for development of *N. okazakii* as with *L. chinensis*. Thus, *L. trifolii* and kidney bean could be used to improve the mass-rearing system of *N. okazakii* because mass-rearing of *L. trifolii* with kidney bean is easier and more cost-effective.

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