

BIOLOGICAL CONTROL**Effect of Temperature on the Development and Thermal Requirements of *Wolbachia*-Infected and Antibiotically Cured *Trichogramma kaykai* Pinto and Stouthamer (Hymenoptera: Trichogrammatidae)**CELSO L. HOHMANN¹ AND ROBERT F. LUCK²¹Instituto Agronômico do Paraná, Caixa postal 481,
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Efeito da Temperatura no Desenvolvimento e Exigências Térmicas de *Trichogramma kaykai* Pinto e Stouthamer Infectados e Não Infectados com *Wolbachia* (Hymenoptera: Trichogrammatidae)

RESUMO – Com o objetivo de entender a influência da temperatura em alguns parâmetros biológicos de *Trichogramma kaykai* Pinto e Stouthamer (Hymenoptera: Trichogrammatidae) infectado e não infectado com *Wolbachia* (tratado com antibiótico), determinaram-se a duração do desenvolvimento e as exigências térmicas da progênie dos mesmos sob quatro temperaturas constantes (15, 20, 25 e 30°C), UR 70 ± 5% e fotofase de 14 h, usando como hospedeiro ovos de *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae). O conhecimento dos efeitos da temperatura é fundamental para ajudar a entender a dinâmica de organismos habitantes do deserto como *T. kaykai* que estão sujeitos a extremos de temperatura ao longo das estações. Parasitóides não infectados tiveram um desenvolvimento mais rápido a temperaturas mais elevadas (20, 25 e 30°C) do que aqueles infectados. Contudo, parasitóides infectados apresentaram desenvolvimento mais rápido do que parasitóides não infectados a temperatura mais baixa (15°C). A duração do desenvolvimento da progênie de parasitóides não infectados e infectados aumentou de 7,0 e 7,1 dias a 30°C, respectivamente para 37,3 e 36,7 dias a 15°C, respectivamente. Os machos desenvolveram-se mais rápido que as fêmeas em todas as temperaturas avaliadas. A temperatura base de desenvolvimento da progênie de parasitóides não infectados e infectados foi 12,0°C e as exigências térmicas 127,71 e 132,10 graus dias, respectivamente.

PALAVRAS-CHAVE: Insecta, parasitóides, temperatura, paternogênese induzida por bactéria.

ABSTRACT - The development times and the thermal requirements of *Wolbachia*-infected and antibiotically cured *Trichogramma kaykai* Pinto and Stouthamer (Hymenoptera: Trichogrammatidae), using *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) eggs, were assessed at four constant temperatures (15, 20, 25, and 30°C), 70 ± 5% RH, photophase 14 h. The knowledge of tempera-

ture effects are very important in helping understand the dynamics of desert organisms like *T. kaykai* that face extreme temperatures during the season. Cured wasps developed faster at higher temperatures (20, 25, and 30°C) than infected wasps. However, infected wasps developed faster at the lowest temperature (15°C) than cured wasps. Developmental times of cured and infected wasps increased from 7.0 and 7.1 days at 30°C, respectively to 37.3 and 36.7 days at 15°C, respectively. Males developed sooner than females at all temperatures. The developmental thresholds for the progeny of uninfected and infected *T. kaykai* were 12.0°C and the thermal requirement for development was 127.71 and 132.10 degree-days, respectively.

KEY WORDS: Insecta, parasitoids, temperature, parthenogenetic induced bacteria.

Arthropod development and survival is affected by a wide range of biotic and abiotic factors. One of the most critical is temperature. Temperature direct effects on developmental rate and other life history parameters, or in association with other environment factors, is considered one of the most critical factors (Andrewartha & Birch, 1954). Each species or strain is adapted to grow best within certain temperature ranges and its growth rate depends to a large extent upon the amount of heat units it accumulates within this range over a period of time (degree-days).

The effects of temperature on various components in fitness of entomophagous insects have long been emphasized (DeBach & Hagen 1964, Force & Messenger 1968, Messenger 1970). The knowledge of the thermal requirements and of temperature's influence on the interaction between the entomophage and its host is crucial in all phases of the biological control processes. It is a key component in developing adequate mass-rearing facilities (Cônoli & Parra 1995), in the selection of natural enemies (Harrison *et al.* 1985, van Lenteren 1986), in predicting an insect's distribution and dynamics (Andrewartha & Birch 1954, Messenger 1970, Flint 1980) as well as its efficiency in biological control (Butler & Lopez 1980, Chihrane *et al.* 1993, Bernal 1995). Numerous studies have concentrated on the effects of temperature on the biologi-

cal attributes of *Trichogramma*, however, no references were found that relate the ecology of these wasps to temperature in the field.

The objective of this study is to evaluate the influence of temperature on life history components of *Wolbachia*-infected and a cured *Trichogramma kaykai* Pinto and Stouthamer strain in order to help to understand their dynamics. *T. kaykai* have sympatric populations of infected and uninfected females that occur naturally over an extensive area of the Mojave Desert of southern California parasitizing the mormo metalmark butterfly, *Apodemia mormo deserti* eggs (C. and R. Felder) (Lepidoptera: Riodinidae) (Pinto *et al.* 1997). *Wolbachia* is a cytoplasmatically transmitted bacteria that alters the reproductive mode of their hosts and causes virgin female wasps to produce only daughters (Stouthamer *et al.* 1990, Stouthamer & Werren 1993, Stouthamer & Kazmer 1994).

Material and Methods

Host Culture. Eggs of the cabbage looper, *Trichoplusia ni* Hübner (Lepidoptera: Noctuide) were used as hosts for *T. kaykai*. They were obtained from a colony maintained at the University of California, Riverside, on artificial diet developed by Shorey & Hale (1965) as modified by Pak & Oatman (1982). Female *T. ni* laid their eggs on paper toweling

that lined the inside of an oviposition unit (Knott *et al.* 1966). The eggs were collected daily and irradiated with a cobalt 60 source to kill the *T. ni* embryos within the eggs. Sheets were stored for up to 3 h in a refrigerator at $6 \pm 1^\circ\text{C}$ before use.

Parasitoid Culture. Two lines of *T. kaykai* were established from *Wolbachia* infected females collected from an *A. mormo deserti* egg laid on *E. inflatum* in Last Chance Canyon, El Paso Mountains, Kern County, California, spring 1997. The initial culture was established with offspring from the thelytokous female by R. Stouthamer, The Agricultural University Wageningen, The Netherlands. A second culture was initiated by R. Stouthamer from thelytokous females of the laboratory population treated with 0.5% w/v tetracycline to eliminate the *Wolbachia* infection (van Meer 1999). These two cultures were maintained as separate lines on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs for about six months and, they were in January 1998 sent to Riverside where they remained as separate cultures, reared on *T. ni* eggs. The rearing unit consisted of a 9.5 x 2.5cm glass shell vial, the open end of which was sealed with a filter paper disk held in place by a polyethylene lid with a 1.8cm diam. hole cut in its center. When the parasitoids emerged, strips of toweling containing several hundred *T. ni* eggs were exposed to the parasitoids inside the glass shell vial. Seven cultures, which were each started on a different day of the week, provided wasps whenever they were needed to initiate experiments. All cultures were maintained at $28 \pm 1^\circ\text{C}$, RH $50 \pm 10\%$, and photophase of 16 h.

Experimental Conditions. To determine the developmental rate of *T. kaykai* as a function of temperature, *T. ni* eggs parasitized by cured and infected *T. kaykai* were exposed to one of four constant temperatures 15, 20, 25, and $30^\circ\text{C} \pm 1^\circ\text{C}$. *Trichogramma kaykai* females, mated and < 6 h old were randomly selected and placed individually in rearing units [cured wasps: 15°C (n = 15), 20°C (n = 13), 25°C (n

= 14), 30°C (n = 17); infected wasps: 15°C (n = 21), 20°C (n = 18), 25°C (n = 18), 30°C (n = 17) containing more than 50 *T. ni* eggs < 30 h old for 3 h. The parental females were then removed and killed and the vials containing both the eggs parasitized by the cured or the *Wolbachia*-infected females in each temperature treatment were placed in the same plastic container (61.5 x 36.5 x 15.2cm) containing a saturated NaCl solution to maintain the humidity within the containers at $70 \pm 5\%$ RH (Winston & Bates 1960). Each of the four plastic containers were then transferred to the appropriate temperature cabinets (Percival Scientific Inc., Model: E-30, 2.6 sq. ft. of shelf area) at a photoperiod of 14L: 10D.

The number and sex of the wasps and their time of emergence were recorded twice daily (early morning and late afternoon) during their period of emergence. In addition, the number of parasitized (black) eggs and the brood size was also recorded.

Statistical Analysis. Single treatment mean of the developmental times for each temperature group was compared using a t-test (Zar, 1984). The number of parasitoids per host, and sex ratio between the treatment groups were compared using a one-way analysis of variance (ANOVA) (SAS Institute Inc. 1988). Data on sex ratios were arcsin transformed before subjecting them to an ANOVA. Relationships between temperature and development rates were assessed using regression analysis (MINITAB Inc. 1985) and the slopes for developmental rates of cured and infected *T. kaykai* females were compared using a Covariance Analysis (Zar 1984). The development thresholds (T) and thermal constants (degree-days) were calculated using least squares regressions of temperature ($^\circ\text{C}$) versus developmental rate (1/D) (Campbell *et al.* 1974).

Results

Cured wasps developed significantly faster than infected wasps at 20 ($t = 11.61$, $P < 0.0001$, d.f. = 227), 25 ($t = 13.59$,

$P < 0.0001$, d.f. = 361), and 30°C ($t = 5.98$, $P < 0.0001$, d.f. = 267). Infected wasps developed significantly faster than cured wasps at 15°C ($t = 5.29$, $P < 0.0001$, d.f. = 342) (Table 1). The mean time to develop from an egg to an adult took from 7.0 days at 30°C to 37.3 days at 15°C for a cured *T. kaykai*, whereas it took an infected wasp from 7.1 days at 30°C to 36.7 days at 15°C . The corresponding developmental rates increased from 2.7 to 14.2 days, and from 2.7 to 14.0 days for the cured

broods per host egg than infected wasps over the entire temperature range ($F_{[7, 132]} = 44.03$, $P < 0.0001$) (Table 2). However, the brood size did not differ significantly with temperature within a line, either cured ($F_{[3, 56]} = 0.15$, $P > 0.05$) or infected ($F_{[3, 72]} = 2.32$, $P > 0.05$).

The lower temperature for development of uninfected and *Wolbachia*-infected *T. kaykai* was estimated to be 12.0°C , whereas the number of degree-days required for either line to complete their development was

Table 1. Developmental times in days from egg to adult ($x \pm \text{s.e.}$) and development rates (1/days $\times 100$) ($x \pm \text{s.e.}$) for cured and *Wolbachia*-infected *T. kaykai* from Last Chance Canyon, Kern County, California, reared on *T. ni* eggs at four constant temperatures ($\pm 1^{\circ}\text{C}$).

Parasitoid strain	Temperature ($^{\circ}\text{C}$)			
	15	20	25	30
Cured				
Developmental time	37.3(0.09) ^a	17.6(0.02) ^a	9.8(0.02) ^a	7.0(0.01) ^a
Development rate	2.7(0.011)	5.7(0.008)	10.2(0.028)	14.2(0.023)
Sample size	195	147	184	215
Infected				
Developmental time	36.7(0.07) ^b	18.2(0.04) ^b	10.3(0.01) ^b	7.1(0.02) ^b
Development rate	2.7(0.002)	5.5(0.013)	9.7(0.025)	14.0(0.035)
Sample size	164	142	179	154

^aMeans followed by the same letter within a column were not significantly different at the 0.0001 level (*t*-test).

and infected strains, respectively (Table 1). The developmental times for sons was significantly shorter than that for daughters at 15°C ($t = 2.92$, $P < 0.005$, $n = 195$), 25°C ($t = 3.02$, $P < 0.005$, $n = 208$), and 30°C ($t = 2.95$, $P < 0.005$, $n = 215$).

Temperature did not affect the production of female offspring within the cured or infected lines. As expected, the infected wasps produced no male offspring, hence, the sex ratio of the two groups differed ($F_{[7, 127]} = 68.50$, $P < 0.0001$) (Table 2).

Cured wasps laid significantly larger

127.71 and 132.10, respectively (Table 3). The slopes for developmental rates of cured and infected *T. kaykai* females differed significantly ($F = 2.62$, $P < 0.01$, $n = 1,376$).

Discussion

On average, offspring of cured wasps developed faster at the higher temperatures (20, 25, and 30°C) than those of infected wasps; however, they developed more slowly than those of infected wasps at the lowest temperature (15°C). The reason why *Wolbachia*-in-

Table 2. Percentage of female and clutch size ($\bar{x} \pm s.e.$) of cured and *Wolbachia*-infected *T. kaykai* from Last Chance Canyon, Kern County, California, on *T. ni* eggs at four constant temperatures ($\pm 1^\circ\text{C}$).

Parasitoid strain	Temperature ($^\circ\text{C}$)			
	15	20	25	30
Cured				
% of females	82a ¹	89a	83a	83a
Brood size	1.79(0.11)a	2.09(0.15)a	2.04(0.12)a	1.98(0.08)a
Sample size	14	13	14	17
Infected				
% of females	100b	100b	100b	100b
Brood size	1.38(0.06)b	1.55(0.07)b	1.70(0.12)b	1.60(0.10)b
Sample size	21	17	17	15

¹Means followed by the same case letter either within rows or within columns were not significantly different at the 0.001 (% of females) or 0.05 level (clutch size) (Duncan's Multiple Range Test).

ected females developed faster than their thelytokous counterparts at the lowest temperature is unknown. It could be a character-

1993, Cônsoli & Parra 1995).

In *T. kaykai*, male offspring developed slightly faster than female offspring at 15, 25,

Table 3. Developmental threshold (T) and thermal constants (K) of cured and *Wolbachia*-infected *T. kaykai* from Last Chance Canyon, Kern County, California reared on *T. ni* eggs.

Parasitoid strain	T($^\circ\text{C}$)	K (degree-days)	Regression ($y = a + bT$)	R ²
Cured	12.0	127.71	-0.09367 + 0.00783T	0.99
Infected	12.0	132.10	-0.09055 + 0.00757T	0.98

(F(slope) = 2.62, $P < 0.01$, $n = 1,376$).

istic of this line or result of an experimental artifact. Comparisons of the developmental periods for *T. kaykai* with those for other *Trichogramma* species revealed a great degree of variability among species (Russo & Voegelè 1982, Harrison *et al.* 1985, Maceda

and 30°C . Other studies, however, reported that the developmental times of males and females of *T. minutum* Riley (Lund 1934), *T. pretiosum* Riley (Butler & Lopez 1980), and *Trichogrammatoidea bractea* Nagaraja, (a genus related to *Trichogramma*) (Hutchinson

et al. 1990) were similar over a wide range of temperatures.

Offspring sex ratios within infected and uninfected lines did not differ significantly with temperature. This agrees with the results reported for other species of *Trichogramma* (Lund 1934, Calvin *et al.* 1984, Bleicher & Parra, 1989). Differences in the sex ratio with temperature, however, have been documented for species of *Trichogramma* (Harrison *et al.* 1985, Maceda 1993, Cònsoli & Parra 1995), and *Trichogrammatoidea* (Hutchinson *et al.* 1990, Maceda 1993). Offspring emergence in *T. kaykai* remained high throughout the temperature range used in this study. This contrasts with reports for several *Trichogramma* species where offspring emergence at the lower (15–18°C) and higher temperatures ($\geq 30^\circ\text{C}$) was less than that for intermediate temperatures (Lund 1934, Goodenough *et al.* 1983, Harrison *et al.* 1985, Cònsoli & Parra 1995). The contrasting effects at the extreme temperatures between these species and that of *T. kaykai* suggest that *T. kaykai* is more tolerant over a greater temperature range. This tolerance may be one of the consequences of its occurrence in the desert where temperatures range from freezing to values above 38°C.

Smaller brood sizes emerged from hosts parasitized by infected compared with those parasitized by cured females at all temperatures. These smaller brood sizes in infected wasps could result either from lower clutch sizes being laid by infected females or differential immature mortality of immatures in the infected form. This has been consistently the case, whether they involved comparisons between antibioticly cured *T. kaykai* with infected females of the same genetic background or field collected thelytokous (*Wolbachia*-infected) compared with arrhenotokous females lacking a known history of *Wolbachia* infection (Hohmann, unpub. data, van Meer 1999).

The developmental thresholds for the progeny of uninfected and infected *T. kaykai* ($T = 12.0^\circ\text{C}$) were similar to that observed for *T. pretiosum*. *Trichogramma pretiosum* reared on *Sitotroga cerealella* (Olivier) (Lepi-

doptera: Gelechiidae) eggs had a threshold of 11.2°C; on *Helitohis virescens* (F.) (Lepidoptera: Noctuidae), a threshold of 11.9°C (Goodenough *et al.* 1983), and on *T. ni*, a threshold of 11.3°C (Butler & Lopez 1980).

The number of heat units required for development in *T. kaykai* (127.7 for uninfected wasps and 132.1 for infected wasps) was similar to those reported for *T. pretiosum* on *S. cerealella* (128.7) and *T. ni* (131.5). However, other workers reported a wide range of heat units for the development of *Trichogramma*, both within and between species. These estimates varied from a low of 119.7 degree-days for *T. pretiosum* developing in *S. cerealella* (Goodenough *et al.* 1983) to 143.1 degree-days for *Trichogrammatoidea bactrea* reared on *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Hutchinson *et al.* 1990). The differences in these estimates probably reflect a combination of differences in experimental protocols and genetic variability within and among species.

Significant differences in developmental times and rates were found between the two reproductive forms, i.e. cured wasps developed faster than thelytokous wasps when temperatures ranged from 20 to 30°C. Their delay in development reached 5% of that for uninfected wasps when the wasps were reared at 25°C. Thus, potentially, infected wasps would have fewer generations than their cured counterparts. Slower emergence rates of *Wolbachia*-infected wasps compared with their cured counterparts has also been observed consistently in colonies reared in the laboratory at $28 \pm 1^\circ\text{C}$ (Hohmann, pers. obs.). Although the analysis has shown that the two reproductive forms differ in development times and rates, the magnitude of the differences observed in the experiments is small and it is derived from only one line of cured and infected *T. kaykai* under constant temperature conditions. Other lines of cured and infected wasps should be tested. However, the differences obtained between the two lines when coupled with the reduced fecundity and immature mortality of *Wolbachia*-infected wasps (Hohmann unpub. data, van Meer 1999) all

point to a substantial cost in fitness of infected wasps, suggesting their inferiority when competing with uninfected *T. kaykai*.

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