

DEVELOPMENT OF *LYSIPHLEBIA JAPONICA*
(HYMENOPTERA: APHIDIIDAE), A PARASITOID OF
TOXOPTERA CITRICIDA (HOMOPTERA: APHIDIDAE) AT FIVE
TEMPERATURES

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ABSTRACT

The brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy), a newly introduced pest of citrus, has become established in Florida. BrCA has shown the capacity not only to inflict direct feeding damage but also to transmit various strains of citrus tristeza virus. As a component of integrated pest management (IPM) against BrCA, an aphidiid parasitoid, *Lysiphlebia japonica* (Ashmead) was imported from Japan by USDA. In this study, the development of *L. japonica* was measured at five constant temperatures (10, 15, 20, 25, and 30°C) using BrCA as a host. Development rate from oviposition to emergence of adult wasps increased linearly with increasing temperature between 10-25°C. The developmental periods from oviposition to adult wasp emergence ranged from 29.7 d at 10°C to 9.9 d at 25°C. Developmental threshold and degree day (DD) requirement for development from oviposition to adult eclosion were 2.9°C and 223.46 DD. The percentage of parasitism varied from 49.93-23.47% within the temperature range of 10-30°C. Pupal survivorship and sex ratio decreased as temperature increased between 10-30°C. Based on our data, this parasitoid is presumably more effective in control of BrCA in cooler months than in summer months.

Key Words: *Lysiphlebia japonica*; biological control, brown citrus aphid

RESUMEN

Se ha establecido en Florida el Afido Pardo de los Cítricos *Toxoptera citricida* (Kirkaldy) (BrCA, "Brown Citrus Aphid"), que es una plaga del cítrico recientemente introducida al estado. Este áfido ha demostrado tener la capacidad no sólo para causar daño directo al alimentarse pero también para transmitir varias líneas del virus de la Tristeza de los Cítricos. Como parte de un programa de manejo integrado de plagas (IPM) contra el áfido BrCA, el parasitoide *Lysiphlebia japonica* (Ashmead) (Hymenoptera: Aphidiidae) fue importado del Japón por el USDA. En este estudio, la duración del desarrollo de *L. japonica* fué medida bajo cinco temperaturas constantes (10, 15, 20, 25 y 30°C) utilizando el BrCA como hospedero. La tasa de desarrollo desde oviposición hasta la emergencia de la avispa adulta incrementó en forma lineal con el incremento de temperaturas entre 10-25°C. La gama de la duración del desarrollo desde oviposición hasta la emergencia de la avispa adulta varió de 29.7 d a 10°C a 9.9 d a 25°C. El umbral mínimo de desarrollo y los grados-días (DD) requeridos para el desarrollo desde oviposición hasta la eclosión del adulto resultaron ser de 2.9°C y 223.46 DD. El porcentaje de parasitismo estuvo en el rango de 49.93-23.47% dentro de la gama de temperaturas de 10-30°C. La supervivencia de las pupas y la proporción sexual (macho:hembra) disminuyeron cuando se aumentó la temperatura entre 10-30°C. En base a estos datos, este parasitoide es probablemente más efectivo para el control del áfido BrCA en meses fríos que en los meses de verano.

Citrus is the most important crop in Florida, encompasses 857,687 planted acres with a total of 107 million trees in the 33 counties. The annual earnings from citrus is estimated at \$1.1 billion in Florida. Citrus has many pest and disease problems, among them are the brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy) and citrus tristeza virus (CTV), which have combined as one of the most important problems for the last seven decades. Disastrous epidemics of CTV have occurred in Argentina, Brazil, Colombia, and Peru (Rocha-Pena et al. 1995). CTV was probably introduced to Florida in late 1980's from the Orient through the movement of citrus budwood plants in the quest for new citrus varieties (Roistacher & Moreno 1991, Roistacher et al. 1991).

Since the introduction of BrCA in Fall 1995 in Florida, BrCA presents a real and immediate threat to the Florida citrus industry especially to grapefruit and orange grafted on sour orange rootstock. Because it is the most efficient vector of CTV, especially for severe stem pitting strains (Costa & Grant 1951, Yokomi et al. 1994). Various control measures are being evaluated at the Institute of Food and Agricultural Sciences, University of Florida and Horticultural Research Laboratory, USDA/ARS in Orlando, FL.

One component of the IPM program against BrCA has been the importation of natural enemies. One of the parasitoids imported for control of BrCA is *Lysiphlebia japonica* (Ashmead). *L. japonica* has been recorded to parasitize several citrus aphids including *T. citricida*, *T. aurantii* (Boyer de Fonscolombe), *T. odinae* (Van Der Goot), *Aphis gossypii* Glover, and *A. spiraecola* Patch. in Japan and Taiwan (Stary & Schlinger 1967, Takada 1968, Kato 1970). No biological study on *L. japonica* has been conducted in the Western Hemisphere. The only report dealing with the biology of *L. japonica* was published in Japan by Takanashi (1990). Kato (1970) reported that *L. japonica* was quite effective in suppression of BrCA populations in citrus groves in Ja-

pan. We studied the effect of temperature on the development, the percentage of parasitism, pupal survivorship, and sex ratio of *L. japonica* at the five constant temperatures to evaluate the potential of *L. japonica* as a biocontrol agent of BrCA.

MATERIALS AND METHODS

Host Aphid Source

Toxoptera citricida used in this study were initiated by a single collection of wild aphids from a citrus tree on the campus of Broward Community College, Davie, Broward County, Florida. The colonies were maintained on potted trifoliolate citrus (*Poncirus trifoliata* (L.) Raf.) seedlings (40-50 cm tall) in an insect rearing room at $25 \pm 1^\circ\text{C}$, $80 \pm 5\%$ RH and a photoperiod of 14:10 (L:D) h. After a 3-month rearing period, the ensuing colonies were used for parasitoid experiments.

Parasitoid Source

Lysiphlebia japonica used were originally imported from Nagasaki, Japan in 1996 and maintained on *Aphis spiraecola* by R. K. Yokomi at the Horticultural Research Lab, USDA/ARS in Orlando, Florida. After 11 generations of selection and breeding of *L. japonica* reared on BrCA at the Fort Lauderdale Research and Education Center, Fort Lauderdale, Florida. *L. japonica* progenies with high survivorship and reproductive rate and short life cycle on BrCA host were used for the ensuing stock colonies in an insect rearing room with conditions described above. Parasitoid adults were obtained by isolating aphid mummies singly in a small glass vial (5 by 1.5 cm diameter). Upon adult emergence, the gender was determined under a stereomicroscope. Two male adults and one female were introduced into a glass tube (11 by 0.3 cm diameter) for at least a 4-h mating period. A small piece of tissue containing 15% sugar solution was placed in each glass tube for food. The glass tube ends were covered with a piece of stretched parafilm.

Temperature Studies

About 55-65 of 2nd instar BrCA were reared on potted Duncan grapefruit (*Citrus paradisi* Macfadyen) seedlings at 2-leaf stage. A mated parasitoid female was introduced into a cage (4.2 by 1.7 cm diameter) containing 55-65 BrCA nymphs for a 24-h oviposition period at $25 \pm 1^\circ\text{C}$. At least 14 potted seedlings each containing 55-65 2nd instar BrCA were used for each temperature. At the end of oviposition period, the plants with exposed nymphs were then placed in growth chambers (Percival, Boone, IA) at 10, 15, 20, 25, 30°C , $80 \pm 5\%$ RH, and a photoperiod 14:10 (L:D) h. Aphids at each temperature treatment were checked daily for presence of sedentary and bloated mummies. The mummies were collected in glass vials and returned to the same temperature treatment. All mummies were checked daily until all parasitoids emerged. The sex of adult parasitoids was determined under a stereomicroscope. Individual development time was recorded for the period from oviposition to adult emergence and from mummy formation to adult emergence.

Data Analysis

Effect of temperature on time periods from parasitoid oviposition to mummy formation, and from mummy formation to adult emergence, was analyzed by one way analysis of variance (ANOVA) and means were separated using Student-Newman-Keuls (SNK) multiple range test (GLM Procedures, SAS Institute 1985). Survival

data and the percentage of parasitism were arcsine-square-root transformed before one way ANOVA and SNK multiple comparisons. A t-test was run to compare the difference of development time on male versus female within each temperature treatment. Linear regression was applied to compute the lower developmental thresholds of aphid mummies and parasitoids, using developmental rate data (1/days) as dependent variables (y-axis) and constant temperature treatments of 10-25°C as independent variables (x-axis). Development above 25°C was outside of the linear growth curve and therefore not included in the linear regression. The lower developmental threshold was determined as x-intercept of the linear equation. The degree-day (DD) required was determined as the value of the inverse of equation slope (Campbell et al. 1974). The nonlinear logistic model of Stinner et al. (1974) was used to describe the temperature-dependent development of aphid mummy and parasitoid immature: $R_t = C / (1 + \exp(k_1 + k_2 t'))$ where R_t = rate of development at temperature t , C = asymptote of the curve, k_1 ; k_2 = empirical constants, $t' = t$ for $t \leq \text{topt}$, $t' = 2 * \text{topt} - t$ for $t > \text{topt}$, and topt = temperature at which the maximum developmental rate occurs.

RESULTS

Development of Aphid Mummies

The developmental times for the mummy formation at 5 temperatures are presented in Table 1. The developmental time of mummy formation linearly decreased as temperature increased in the range of 10-25°C. However, the average developmental period at 30°C was not significantly different from the time of development at 25°C ($p < 0.05$, Table 1). A linear regression analysis comparing temperature with mummy developmental rate (10-25°C) resulted in the equation $R = 0.006314 * t - 0.007122$ ($r^2 = 0.9986$, $p = 0.0007$). Therefore, the mummy development of BrCA required 158.38 DD above a lower developmental threshold of 1.13°C. The nonlinear logistic model gave a good fit to the data within the range of 10-30°C. (Fig. 1) resulting in the equation: $R_t = 0.1669 / (1 + \exp(2.4130 - 0.1695 * t))$. When aphid host density was in the range of 55-65, the percentage of parasitism was significantly affected by temperature ($p < 0.05$, Table 1). The lowest percentage of parasitism, 23.4%, occurred at 30°C, indicating high temperatures had a detrimental effect on the parasitization rate.

TABLE 1. MEAN \pm SE DEVELOPMENTAL TIME OF *T. CITRICIDA* MUMMY AND PARASITIZATION RATE AT 5 CONSTANT TEMPERATURES.

Temp. (°C)	# of mummies	Development, d	Percentage of Parasitism
10	374	17.4 \pm 1.1a	49.93 \pm 8.30b
15	323	11.7 \pm 1.0b	47.64 \pm 7.92b
20	277	8.4 \pm 0.7c	49.29 \pm 13.33b
25	144	6.6 \pm 0.5d	61.84 \pm 15.00a
30	165	6.6 \pm 0.6d	23.47 \pm 12.29c

Within the columns means followed by the same letters are not significantly different $p < 0.05$ (Student-Newman-Keuls multiple comparison). Percentage of parasitism data were transformed arcsine square root before Student-Newman-Keuls multiple comparison; untransformed data are presented. ANOVA statistics were: development day, $F = 10660.30$, $df = 4, 1578$, $p < 0.001$; percentage of parasitism, $F = 20.68$, $df = 4, 70$, $p < 0.001$.

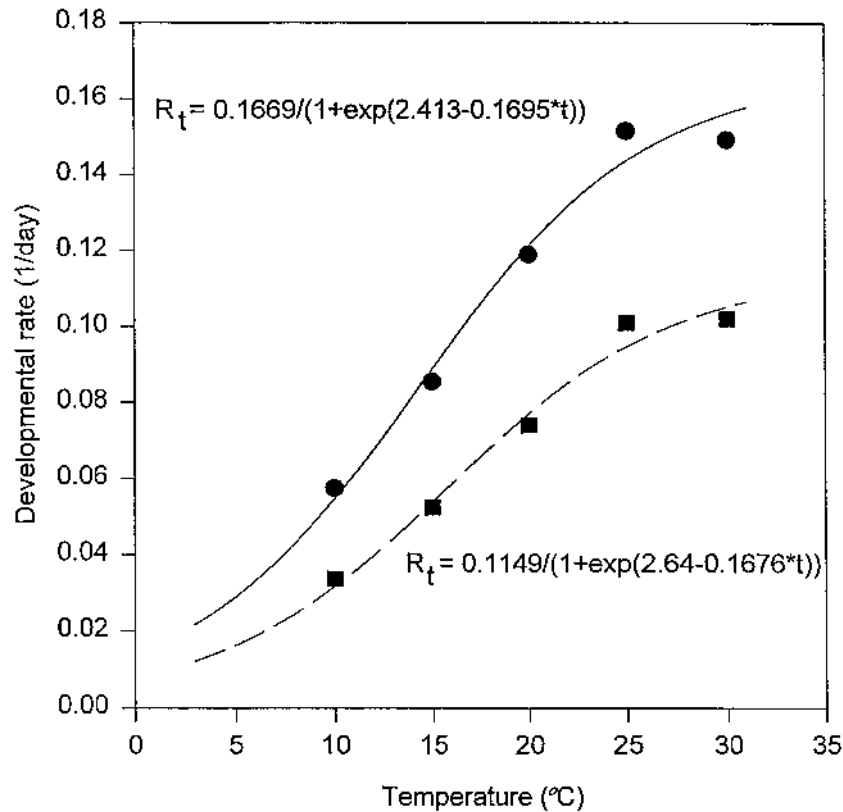


Fig. 1. Development rate (R_t) of *T. citricida* mummies (circle and solid line) and *L. japonica* (square and dotted line) at 5 constant temperatures ($^{\circ}\text{C}$). Circles and squares are observed rates.

Development of Parasitoid

The time from oviposition to emergence of *L. japonica* was inversely correlated with the temperature in the range of 10-25 $^{\circ}\text{C}$ (Table 2). An average of 29.9 \pm 1.6 d and 10.1 \pm 1.0 d was required for female development from oviposition to adult eclosion at 10 and 25 $^{\circ}\text{C}$, respectively. The average female developmental period at 30 $^{\circ}\text{C}$, 9.8 \pm 0.6 d, was not significantly different ($p < 0.05$, Table 2) from the time of development at 25 $^{\circ}\text{C}$. The developmental times on male versus female within each temperature treatment were not significantly different (t-test, $P = 0.05$). The pooled developmental times for both sexes were also not significantly different ($p < 0.05$, Table 2) when reared on BrCA. A linear regression analysis was applied to the developmental data within the 10-25 $^{\circ}\text{C}$ range. Developmental rate increased linearly with temperature, resulting in the equation $R = 0.004475 * t - 0.01303$ ($r^2 = 0.9932$, $P = 0.0034$). The theoretical developmental threshold (i.e. the point where developmental rate presumably equal 0) was estimated at 2.9 $^{\circ}\text{C}$ for the male and female parasitoids. Thus, it required 223.46 DD for the parasitoid to become an adult based on this threshold. The

TABLE 2. AVERAGE NUMBER OF DAYS \pm SE FROM OVIPOSITION TO ADULT EMERGENCE OF *L. JAPONICA* AT 5 CONSTANT TEMPERATURES.

Temp. (°C)	♂♂		♀♀		Pooled (♂ + ♀)	
	n	d	n	d	n	d
10	150	29.3 \pm 1.5a	205	29.9 \pm 1.6a	351	29.7 \pm 1.5a
15	148	18.6 \pm 0.9b	166	19.5 \pm 1.1b	314	19.1 \pm 1.1b
20	107	13.3 \pm 0.6c	125	13.7 \pm 0.4c	230	13.5 \pm 0.8c
25	204	9.7 \pm 0.6d	118	10.1 \pm 1.0d	315	9.9 \pm 0.5d
30	31	9.7 \pm 0.5d	12	9.8 \pm 0.6d	43	9.8 \pm 0.5d

Within columns means followed by the same letters are not significantly different at $p > 0.05$ (Student-Newman-Keuls multiple comparison). ANOVA statistics were: ♂♂, $F = 9796.46$, $df = 4,636$, $p < 0.001$; ♀♀, $F = 7326.41$, $df = 4,621$, $p < 0.001$; Pooled (♂ + ♀), $F = 16132.00$, $df = 4,1262$, $p < 0.001$.

nonlinear logistic model gave a good fit to the data within the range of 10-30°C (Fig. 1), resulting in the logistic equation: $R_t = 0.1149 / (1 + \exp(2.64 - 0.1676 * t))$.

Pupal Survivorship and Adult Sex Ratio

The pupal survivorship decreased significantly as temperature increased between 20-30°C ($p < 0.05$, Table 4). The average pupal survivorship was 94.91 and 26.67% at 10 and 30°C, respectively. The average survivorship at 10°C was not significantly different from that at 15°C ($p < 0.05$, Table 4). The sex ratio was also affected by temperature, and became more male-biased as temperature increased. The values of sex ratio essentially remained the same as temperature increased within the range of 10-20°C, but change was more evident from 20 to 25 than from 25 to 30°C (Table 4).

DISCUSSION

The genus *Lysiphlebia* Stary and Schlinger is morphologically similar to the genus *Lysiphlebus* Forster (Stary & Schlinger 1967). As a result, *L. japonica* has been erro-

TABLE 3. AVERAGE NUMBER OF DAYS \pm SE FROM MUMMY FORMATION TO ADULT EMERGENCE OF *L. JAPONICA* AT 5 CONSTANT TEMPERATURES.

Temp. (°C)	♂♂		♀♀		Pooled (♂ + ♀)	
	n	Days	n	Days	n	Days
10	150	11.9 \pm 1.7a	205	12.5 \pm 1.3a	355	12.3 \pm 1.4
15	148	7.0 \pm 1.0b	166	7.8 \pm 1.2b	314	7.4 \pm 1.2
20	107	4.9 \pm 0.8c	125	5.3 \pm 0.8c	232	5.1 \pm 0.8
25	204	3.1 \pm 0.6d	118	3.5 \pm 0.4d	322	3.3 \pm 0.5
30	32	3.1 \pm 0.5d	12	3.2 \pm 0.6d	44	3.2 \pm 0.5

Within columns means followed by the same letters are not significantly different at $p > 0.05$ (Student-Newman-Keuls multiple comparison). ANOVA statistics were: ♂♂, $F = 1794.38$, $df = 4,636$, $p < 0.001$; ♀♀, $F = 1662.31$, $df = 4,621$, $p < 0.001$; Pooled (♂ + ♀), $F = 3834.49$, $df = 4,1262$, $p < 0.001$.

TABLE 4. PUPAL SURVIVORSHIP AND SEX RATIO OF *L. JAPONICA* AT 5 CONSTANT TEMPERATURES.

Temp. (°C)	# of mummies	# of wasps emerged	Pupal survivorship	Sex ratio (♀:♂)
10	374	355	94.9 ± 6.1a	1:0.73
15	323	314	97.2 ± 2.3a	1:0.89
20	277	232	83.8 ± 12.9b	1:0.86
25	444	322	72.5 ± 10.8c	1:1.73
30	165	44	26.7 ± 6.1d	1:2.38

Within columns means followed by the same letters are not significantly different at $p < 0.05$ (Student-Newman-Keuls multiple comparison). Survivorship data were transformed arcsine square root before Student-Newman-Keuls multiple comparison; untransformed data are presented. Pupal survivorship ANOVA: $F = 64.06$; $df = 4, 70$; $p < 0.001$.

neously synonymized as *Lysiphlebus japonica* (Takanashi 1990). Overall, the developmental times from oviposition to adult emergence of *L. japonica* were shorter than those reported for *Lysiphlebus testaceipes* (Cresson) reared on *T. aurantii* at 15, 18, 21, 24, and 27°C (Tang & Yokomi 1995). Developmental times ranging 0.8-3.6 d for both males and females reared at 15, 20, and 25°C (Table 2) were shorter than those reported for both sexes at identical temperatures (Takanashi 1990). Takanashi (1990) reported that the developmental times of *L. japonica* were 21.9, 16.9, and 10.5 d for males and 22.8, 17.3, and 11.0 d for females at 15, 20, and 25°C, respectively. Although the test temperatures and host aphid species in these 2 studies were the same, the resultant developmental times were different. These differences could be attributed to differences in biotypes of host aphid and/or parasitoid. Temperature is known to differentially affect the development of host aphids and parasitoids (Campbell et al. 1974, Force & Messenger 1964). The low temperature threshold (2.9°C) of *L. japonica* was much lower than that (7.5°C) of *L. testaceipes* (Tang & Yokomi 1995). Developmental time (9.1 d at 21°C) for mummy formation of *L. testaceipes* reared on *Schizaphis graminum* (Rondani) (Hight et al. 1972) was longer than 8.4 d for *L. japonica* (Table 1).

High temperature (>25°C) had a marked negative effect on pupal survivorship of *L. japonica* (Table 4). This is in agreement with the report by Tang and Yokomi (1995) that pupal mortality of *L. testaceipes* increased greatly at 27°C and above, ranging from 24.8 to 44%. High pupal mortality of *L. japonica* at elevated temperature suggests that this parasitoid cannot tolerate the extreme temperatures in south Florida during the summer months and the seasonal diapause during hot periods as described for other aphidiid parasitoids (Stary 1988) does not occur.

One of the factors potentially affecting success or failure of released parasitoid is the offspring sex ratio (Waage & Hassell 1982). The sex ratio of parasitoids is affected by such factors as host quality, temperature, and light. The usual type of reproduction and development in parasitic Hymenoptera is for females to mate soon after emergence and to store sperm from the males in spermatheca and then, depending on external stimuli, either release sperm as eggs are being laid and produce female offspring, or to retain sperm in spermatheca so that the eggs remain unfertilized and produce male progeny (DeBach 1974). Our study showed that low temperatures (at 10 and 15°C) were a favorable external factor for *L. japonica* to produce more female progeny as compared to higher temperatures (at 25 and 30°C).

The characteristics of temperature-dependent development can be useful to conduct and evaluate biological control potential (Miller & Gerth 1994). Based on developmental periods of BrCA mummies and *L. japonica*, lower temperature threshold, pupal survival, sex ratio and parasitization rate, we suggest parasitoid release in the cooler months in south Florida. The initial recovery of *L. japonica* was only successful in the cool months (January and March) from releases during 1996-1997 reasons in the citrus groves in Davie and Ft. Pierce, Florida (Tsai unpublished) which is in agreement with the laboratory test.

ACKNOWLEDGMENTS

Y. X. Deng is a visiting scholar from the Department of Plant Protection, Southwest Agricultural University, Chongqing, The People's Republic of China. Appreciation is extended to R. K. Yokomi for providing the initial colony of *L. japonica*, and to Ru Nguyen and R. H. Scheffrahn for reviewing the manuscript. This research is supported in part by grant funds from Florida Citrus Production Research Advisory Council and University of Florida, IFAS Faculty Enhancement Award. This is Florida Experiment Station Journal Series No. R-06057.

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