

# Temperature-Dependent Development of Three Hymenopterous Parasitoids of Aphids (Homoptera: Aphididae) Attacking Citrus

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Environ. Entomol. 24(6): 1736-1740 (1995)

**ABSTRACT** Aphids attacking citrus can be serious pests when they transmit severe strains of citrus tristeza virus. As part of an environmental assessment of exotic parasitoids of aphids, we compared the thermal relationships of *Aphelinus spiraeocolae* Evans & Schauff and *Aphelinus gossypii* Timberlake with the indigenous parasitoid, *Lysiphlebus testaceipes* (Cresson), on the black citrus aphid, *Toxoptera aurantii* (Boyer de Fonscolombe), in growth chambers in Florida. Overall, *L. testaceipes* development time was shorter than that of the aphelinids ranging from 18.7 to 42.5% less throughout the range of temperatures tested (15–30°C). Development times between the aphelinids were similar except at 15°C where *A. spiraeocolae* required 3.5 d more than *A. gossypii*. Developmental thresholds and degree-day requirements for *L. testaceipes* were 7.5°C and 212.8 DD; for *A. spiraeocolae* were 7.9°C and 294.1 DD; and for *A. gossypii* were 6.7°C and 312.5 DD, respectively. Pupal mortality of *L. testaceipes* increased greatly at 27°C and above, ranging from 24.8 to 44%; whereas mortality of the aphelinids remained low, ranging from 9.1 to 10.2% for *A. spiraeocolae*, and 13.3 to 15.8% for *A. gossypii*. Based on these relationships, we anticipate that these parasitoids can coexist and that different thermal tolerances should broaden their potential as natural enemies of aphids attacking citrus.

**KEY WORDS** *Lysiphlebus*, *Aphelinus*, *Toxoptera*, biological control

WE INVESTIGATED PARASITIDS that attack aphids on citrus in response to the threat of introduction and establishment of the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Yokomi et al. 1993). This aphid is an efficient vector of citrus tristeza virus and has become recently established on citrus in much of the Caribbean Basin and the southern portion of Central America (Yokomi et al. 1994). Studies conducted in Puerto Rico for primary parasitoids of brown citrus aphid detected only *Lysiphlebus testaceipes* (Cresson) (Yokomi and Tang 1995b). In Florida, a gall midge, *Endaphis maculans* Gagné (Diptera: Cecidomyiidae), *L. testaceipes*, and *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae) were collected occasionally from populations of citrus aphids (Tang et al. 1994, Yokomi and Tang 1995a).

*Lysiphlebus testaceipes* is an important native parasitoid of aphids in the United States and has a wide host range (Mackauer and Stáry 1967, Stáry et al. 1988). *A. gossypii* was described in Hawaii (Timberlake 1924) and was introduced from India to Florida in 1969 (Denmark and Porter 1973). However, this parasitoid has been reported as recovered only recently in Florida (Yokomi and Tang

1995a). In 1992, the parasitoid, *Aphelinus spiraeocolae* Evans & Schauff (Hymenoptera: Aphelinidae) (Evans et al. 1995), was imported from Southern China into a quarantine facility in Florida. Here it was evaluated as a natural enemy of aphids including the spirea aphid, *Aphis spiraeicola* Patch, melon aphid, *Aphis gossypii* Glover, and black citrus aphid, *Toxoptera aurantii* (Boyer de Fonscolombe) (Yokomi et al. 1993).

A critical factor in evaluating the potential of a parasitoid as a natural enemy is tolerance to the physical environment (Tillman and Powell 1991). Because temperature is known to affect development of aphids and their parasitoids differentially (Campbell et al. 1974, Force and Messenger 1964), we conducted these experiments to compare temperature effects on development and mortality of the 3 parasitoids attacking the black citrus aphid. The results of these experiments were used to anticipate that the parasitoids can cohabit in the same parasitoid guild and that their different thermal tolerances should broaden their potential as natural enemies of aphids in different seasons.

## Materials and Methods

**Parasitoid and Host Aphid Culture.** *L. testaceipes* was collected from a parasitized black citrus

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**Table 1.** Development time (days) from oviposition to adult emergence of 3 parasitoids on the black citrus aphid at 6 constant temperatures

Temp. °C		Avg development time, d								
		<i>A. gossypii</i>			<i>A. spiraeocolae</i>			<i>L. testaceipes</i>		
		♀	♂	♀ + ♂	♀	♂	♀ + ♂	♀	♂	♀ + ♂
15	Avg	39.9	39.5	39.7b	43.4	43.4	43.4a	24.9	25.0	25.0c
	SEM	1.2	0.8	0.8	1.1	1.0	0.8	1.4	2.0	1.1
	n	41	33	74	51	21	72	65	34	99
18	Avg	31.4	31.1	31.3a	32.1	32.1	32.1a	23.3	23.4	23.4b
	SEM	1.5	1.6	1.2	1.2	1.1	1.0	1.8	2.0	1.4
	n	50	26	76	50	27	77	60	35	95
21	Avg	20.2	19.5	20.0a	21.5	20.6	21.2a	15.3	15.2	15.3b
	SEM	1.4	1.9	1.1	2.0	2.3	2.0	0.8	1.6	0.7
	n	61	35	96	101	58	159	59	25	84
24	Avg	16.5	16.2	16.4a	17.3	17.0	17.2a	13.8	13.6	13.7b
	SEM	1.3	1.7	1.4	1.6	1.7	1.2	0.8	1.1	0.8
	n	102	55	157	49	31	80	73	56	129
27	Avg	15.9	15.4	15.7a	16.3	16.8	16.5a	10.5	10.5	10.5b
	SEM	1.0	1.3	0.8	2.0	2.1	1.6	0.8	1.4	0.7
	n	66	35	101	67	39	106	58	30	88
30	Avg	14.2	13.7	14.0a	13.4	13.1	13.3a	9.6	9.3	9.5b
	SEM	1.1	1.7	0.9	1.4	1.8	1.5	1.2	1.5	1.0
	n	50	32	82	124	55	179	40	27	67

Because no species  $\times$  temperature  $\times$  sex interaction was found ( $P > 0.45$ ), ♀ and ♂ data per temperature per species were combined. Pooled means followed by the same letter within a temperature are not significantly different by the Duncan multiple-range test ( $P < 0.05$ ). n, Number of individual parasitoids observed.

aphids in a citrus grove near Orlando, FL. *A. gossypii* was collected from the melon aphid on hibiscus, *Hibiscus rosa-sinensis* (Malvaceae), in a commercial nursery near Eustis, FL. *A. spiraeocolae* was collected from parasitized spirea aphids in Fusan City, Guangdong Province, People's Republic of China (Yokomi et al. 1993). The black citrus aphid was the aphid host used in these experiments because it is suitable host for all 3 parasitoids (Yokomi and Tang 1995a). This aphid was collected from citrus and maintained in an air-conditioned insectary on red tip photinia, *Photinia × fraserii* (Rosaceae), because its leaves support aphids longer than citrus under test conditions. Colonies of each parasitoid species were reared in the laboratory at  $24 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH, and a photoperiod of 14:10 (L:D) h using procedures previously described (Yokomi and Tang 1995a).

**Temperature Studies.** Thirty mated female parasitoid adults (2–4 d old) were introduced into a Plexiglas tube (15 cm diameter by 25 cm tall) (Yokomi and Tang 1995a) that contained a photinia plant infested with 250–300 2nd- and 3rd-instar aphid nymphs. Parasitoid adults were allowed an 8-h exposure period for oviposition after which time the parasitoids were removed. Plants and parasitized aphids were transferred to plant growth chambers, each chamber set to hold 1 of 6 constant temperatures (15, 18, 21, 24, 27, and  $30^\circ\text{C}$ ). All chambers maintained a photoperiod of 14:10 (L:D) h and  $70 \pm 10\%$  RH. Aphids were examined daily for mummy formation. All mummies were collected and transferred singly to gelatin capsules. Capsules with mummies were held in the growth chamber until adult parasitoid emergence. Adult

parasitoids were sexed under a stereoscope. Development time was defined as the period from oviposition to adult emergence, and this period was determined for each specimen.

**Data Analysis.** The rate of development was calculated as the reciprocal of days of development time for each gender. The data are expressed as the percentage of development per day. The relationship between temperature ( $T$ ) and the rate of development ( $y$ ) was described by regression analysis where  $y = a + bT$  following the method of Campbell et al. (1974). The thermal threshold ( $t$ ) and the thermal constant ( $K$ ) were estimated by the equation  $t = -a/b$  and  $K = 1/b$  where  $a$  and  $b$  are estimated parameters determined by least-squares regression and the data are expressed as degree-days (DD) (Campbell et al. 1974). Pupal mortality was analyzed by examining adult emergence at 6 constant temperatures (15, 18, 21, 24, 27, and  $30^\circ\text{C}$ ). Trends of mortality were then estimated for each species and compared. Data were analyzed by analyses of variance (ANOVA) and treatment differences were determined by the Duncan multiple-range test. Statistical tests were performed using PROC GLM (SAS Institute 1990).

## Results

Development rates from oviposition to adult emergence of the 3 parasitoids were inversely correlated with the temperature (Table 1). Development time was significantly shorter for all temperatures tested for *L. testaceipes* than for either aphelinid ( $P < 0.05$ ). Development time was significantly longer for *A. spiraeocolae* than for *A. gos-*

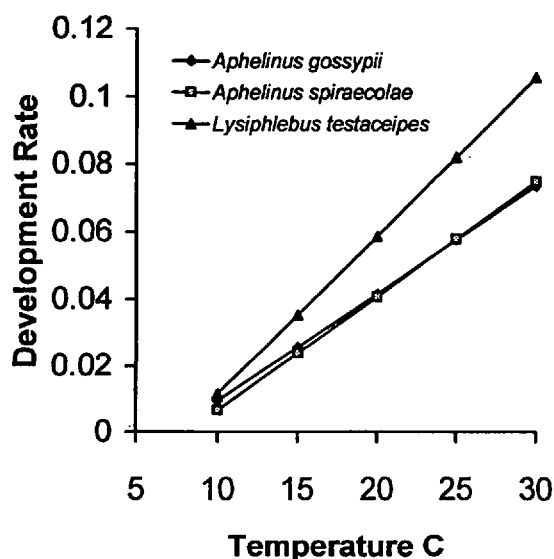
**Table 2.** ANOVA table (type III) for development time

Sources of variation	Mean development time, d		
	df	MS	P value
Species	2	8,788.1	< 0.0001
Sex	1	16.9	= 0.0007
Temperature	5	20,825.5	< 0.0001
Species × sex	2	4.5	= 0.0485
Species × temperature	10	718.3	< 0.0001
Temperature × sex	5	2.6	= 0.1228
Species × temperature × sex	10	1.5	= 0.4520

*sypii* at 15°C ( $P < 0.05$ ), but not at other temperatures (Table 1). In general, significant differences were obtained between species ( $P < 0.0001$ ), sex ( $P = 0.0007$ ), and temperatures ( $P < 0.0001$ ); species × sex ( $P = 0.049$ ) and species × temperature ( $P < 0.0001$ ); but not between temperature × sex ( $P = 0.12$ ) (Table 2).

Development times between sexes were pooled for each parasitoid species because interactions between species × temperature × sex and temperature × sex were not statistically different (Table 2). The relationship between temperature and development rate of *A. spiraeocolae* was  $y = -0.0272 + 0.0034 T$  ( $r^2 = 0.895$ ,  $P = 0.0001$ ); and for *A. gossypii* was  $y = -0.0224 + 0.0032 T$  ( $r^2 = 0.886$ ,  $P = 0.0001$ ); and for *L. testaceipes* was  $y = -0.0353 + 0.0047 T$  ( $r^2 = 0.929$ ,  $P = 0.0001$ ) (Fig. 1). By extrapolation, the lower temperature threshold for development was  $7.9 \pm 0.41$ ,  $6.7 \pm 0.33$ , and  $7.5 \pm 0.38^\circ\text{C}$  ( $\pm\text{SEM}$ ) for *A. spiraeocolae*, *A. gossypii*, and *L. testaceipes*, respectively. The thermal constant was  $294.1 \pm 8.7$ ,  $312.5 \pm 6.5$ , and  $212.8 \pm 7.3$  DD for *A. spiraeocolae*, *A. gossypii*, and *L. testaceipes*, respectively.

No significant correlation was found between pupal mortality and temperature for *A. spiraeocolae* and *A. gossypii* ( $P > 0.05$ ), but a significant quadratic effect existed for *L. testaceipes* ( $P = 0.004$ ). Pupal mortality of *L. testaceipes* increased with temperature from values of around 10% at the lower temperatures (15–24°C) to values 24.8% at 27°C and 44.6% at 30°C (Table 3) and was described as  $y = 1.242 - 0.012x + 0.003x^2$  (where  $y$  = pupal mortality and  $x$  = temperature,  $r^2 = 0.97$ ,  $P = 0.004$ ). In contrast, pupal mortality of the aphelinids remained low throughout the range of temperatures tested; 5.3–10.2% for *A. spiraeocolae* ( $y = 0.243 - 0.016x + 0.0003x^2$  ( $r^2 = 0.30$ ,  $P =$



**Fig. 1.** Temperature-dependent development rate of 3 parasitoids on the black citrus aphid. Thermal thresholds and degree-day requirements for *L. testaceipes* were  $7.5^\circ\text{C}$  and 212.8 DD ( $r^2 = 0.929$ ,  $P = 0.0001$ ,  $df = 5$ ); *A. spiraeocolae* were  $6.7^\circ\text{C}$  and 312.5 DD ( $r^2 = 0.886$ ,  $P = 0.0001$ ,  $df = 5$ ); and *A. gossypii* were  $7.9^\circ\text{C}$  and 294.1 DD ( $r^2 = 0.895$ ,  $P = 0.0001$ ,  $df = 5$ ).

0.72); and 9.4–15.8% for *A. gossypii* ( $y = 0.083 - 0.002x + 0.0003x^2$  ( $r^2 = 0.39$ ,  $P = 0.14$ ).

### Discussion

Development time from oviposition to adult emergence of *L. testaceipes* reared on banana aphid, *Pentalonia nigronervosa* Coquerel, was 9.4 and 9.8 d at  $21^\circ\text{C}$  for males and females, respectively, and 8.9 and 9.5 d at  $24^\circ\text{C}$  for males and females, respectively, but these differences between sexes were not statistically significant (Volkl et al. 1990). Development times for *L. testaceipes* in our study were  $\approx 5$  d longer than those estimated for development on the banana aphid. This difference may be the result of high genetic variability observed within this parasitoid (Stáry et al. 1988). Differences may also be caused by the different aphid host. There are no previous reports of development times of *A. gossypii* and *A. spiraeocolae*.

**Table 3.** Pupal mortality of 3 hymenopterous parasitoids on the black citrus aphid at 6 temperatures

Temp, °C	<i>A. gossypii</i>		<i>A. spiraeocolae</i>		<i>L. testaceipes</i>	
	No. mummies	% mortality	No. mummies	% mortality	No. mummies	% mortality
15	86	12.8	79	8.9	110	9.5
18	85	9.4	85	9.4	105	9.5
21	110	12.7	169	5.3	93	9.7
24	189	16.9	90	7.8	142	9.9
27	120	13.3	118	10.2	117	24.8
30	95	15.8	197	9.1	121	44.6

Performance of parasitoids could be influenced by cultural conditions of the experiments. Although *A. spiraeocolae* develops better when the host is the spirea aphid and *A. gossypii* prefers the melon aphid as its host, both parasitoids oviposit in the black citrus aphid. Consequently, this aphid was determined to be a good host of both parasitoids based on a high percentage of emergence and biomass of adult parasitoids (Yokomi and Tang 1995a).

The observed shorter development time of *L. testaceipes* suggests that parasitism by this parasitoid would increase faster than that either aphelinid. For example, our data indicates that at 27°C, the time that the aphelinids require to develop 2 generations, *L. testaceipes* could develop 3 generations. Lower pupal mortality for the aphelinids at higher temperatures suggests that they could better tolerate the high temperatures in Florida than the indigenous *L. testaceipes*. However, *A. gossypii* released in 1969 (Denmark and Porter 1973) has not become widely established and does not appear promising as natural control agent for citrus aphids in Florida (Yokomi and Tang 1995a).

Because there are few effective parasitoids of the spirea aphid in the New World (Cole 1925, Miller 1929, Stáry and Schlinger 1967, Tang et al. 1994), establishment of additional parasitoids has merit for this aphid. Our data suggest that *A. spiraeocolae* is compatible with parasitoids associated with aphids on citrus in the United States. In addition, the only parasitoid found attacking the brown citrus aphid in the New World is *L. testaceipes* (Murakami et al. 1984, Stáry and Cermeli 1989, Yokomi and Tang 1995b). Furthermore, we previously showed that each of these parasitoids had a different aphid host preference and suitability (Yokomi and Tang 1995a). Because *A. spiraeocolae* can also attack the brown citrus aphid (Y.Q.T. and R.K.Y., unpublished data), establishment of *A. spiraeocolae* in the New World could be beneficial as another natural enemy of the brown citrus aphid.

### Acknowledgments

We are indebted to Limhout Nong (Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville) for originally maintaining the parasitoid colony in a containment facility; Bill Bodnaruk (Jon's Nursery, Eustis, FL) for providing some of the plants used in laboratory studies; V. Chew (USDA-ARS, Gainesville, FL) for assisting in the statistical analysis; C. Y. Zheng (Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville) and Cortina Lewis (U.S. Horticultural Research Laboratory, USDA-ARS, Orlando, FL) for technical assistance. We also thank Jorge Peña, Jim Tsai, and Phil Stansly for critically reviewing earlier drafts of the manuscript. We gratefully acknowledge USDA, Foreign Agricultural Service, ICD-RSED, Washington, DC, for its kind support and cooperation. Funds for this project were made available from the Citrus Production Research Marketing Or-

der by the Division of Marketing and Development (Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Tallahassee); and a grant from the USDA, APHIS-PPQ, Southeastern Region to Larry G. Brown (Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville).

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*Received for publication 11 April 1995; accepted 29 August 1995.*