

## RESEARCH

## Temperature-Dependent Development of Two Neotropical Parasitoids of *Liriomyza sativae* (Diptera: Agromyzidae)

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**ABSTRACT.** We studied two species of neotropical parasitoids that occur naturally in northeastern Brazil and are associated with *Liriomyza sativae* (Blanchard): *Opius (Gastrosema) scabriventris* Nixon (Hymenoptera: Braconidae) and *Chrysocharis vonones* (Walker) (Hymenoptera: Eulophidae). We evaluated the influence of seven temperatures on the duration of the egg–adult period and on the survivorship of the immature stages of the parasitoids. A temperature increase from 15 to 30°C shortened the egg–adult period of *O. scabriventris* and *C. vonones*. However, at 32°C, the developmental time for the braconid was prolonged, and no difference was observed for the eulophid, compared with 30°C. The highest temperature, 35°C, proved to be lethal for both species. At 15°C, *C. vonones* pupal survivorship was drastically reduced, whereas that of *O. scabriventris* was unaffected. At most temperatures, the eulophid had an egg–adult period shorter than or similar to the braconid, except at 15°C. The threshold temperature ( $T_t$ ) of the egg–adult period for *O. scabriventris* was 7.3°C with a thermal constant ( $K$ ) of 257.1 degree days (DD). For *C. vonones* the  $T_t$  was 7.4°C for the total cycle and 6.2°C for the pupal stage, with a thermal constant of 246.3 and 140.3 DD, respectively. These data allow an estimate of 29.4 annual generations for *O. scabriventris* and 30.5 for *C. vonones* in a melon production region in northeastern Brazil, values that are equivalent to 4.9 and 6.0 more generations than the host. These results demonstrate that both species have potential for application in biological control programs against the leafminer fly *L. sativae*.

**Key Words:** temperature threshold, thermal constant, degree day, Eulophidae, Braconidae

Leafminers in the genus *Liriomyza* (Diptera: Agromyzidae) cause yield losses in vegetable and ornamental crops worldwide (Murphy and LaSalle 1999). In Brazil these insects are important pests of melon (Araujo et al. 2007), bean (Costa-Lima et al. 2009), onion (Ramalho and Moreira 1979), potato (Bueno et al. 2007), lettuce, cucumber (Costa et al. 1961), and chrysanthemum (Polanczyk et al. 2008). Direct damage is caused by feeding punctures of adult females as well as larval feeding on the leaf mesophyll tissue, which in high densities can reduce yields and/or kill the plants (Spencer 1989). The greatest difficulty in controlling this pest is the rapid selection for resistance in many populations to different chemical products (Ferguson 2004, Nadagouda et al. 2010).

The most important species of *Liriomyza* in agriculture, *Liriomyza trifolii* (Burgess), *Liriomyza sativae* (Blanchard), and *Liriomyza huidobrensis* (Blanchard), are all native to the Americas (Spencer 1973). However, studies on the biology of their parasitoids from the New World are basically restricted to the United States, mostly on *Diglyphus begini* (Ashmead) (Heinz and Parrella 1989, 1990). The commercialization of *Diglyphus isaea* (Walker) and *Dacnusa sibirica* Telenga since the early 1980s (van Lenteren 2012) as successful biological control agents of leafminers in greenhouse crops has probably discouraged the search for other natural enemies.

Nonetheless, according to the feeding habit, leafminers are the guild with the greatest parasitoid richness (Connor and Taverner 1997). More than 150 species were already recorded attacking the genus *Liriomyza* (Liu et al. 2011), but the biology of only a few has been studied. In Brazil, no biological control companies are presently marketing leafminer parasitoids; however, there is growing interest in these agents, primarily for high-value crops such as melons in northeastern Brazil.

A previous study of the *L. sativae* parasitoid assemblage was conducted in melon and cowpea plants in the State of Rio Grande do Norte, Brazil. We identified four species (T.C.C.L., unpublished data), and selected two for this study. The criteria used for the chosen species were: 1) abundant in the region, consequently, more adapted to the studied area; 2) could be maintained in laboratory culture; and 3) with different effects on host development (koinobiont and idio-biont). According to the points, one species was the braconid *Opius (Gastrosema) scabriventris* Nixon (Hymenoptera: Braconidae), a koinobiont larval–pupal parasitoid; and the other was the eulophid *Chrysocharis vonones* (Walker) (Hymenoptera: Eulophidae), an idio-biont larval parasitoid. Both species were the most abundant and were easily multiplied in laboratory. The other parasitoids collected and not included in this work were the koinobiont *Zaeucoila* sp. (Figitidae) and the idio-biont *Neochrysocharis* sp. (Eulophidae). Besides being less abundant, the first one had a longer egg–adult period compared with *O. scabriventris* and the second showed a male-biased population (over 90%).

The leafminers parasitoids, as other insects, are influenced by temperature as one of the main factors delimitating survival (Hallman and Denlinger 1998). This information is important for rearing technique (Etzel and Legner 1999) and also to know the relationship between the development rate of the natural enemy and its host for biological control purpose (Bernal and Gonzalez 1996).

Therefore, we studied the influence of temperature on the immature development and the respective thermal requirements of the parasitoids *O. scabriventris* and *C. vonones* associated with *L. sativae* in cowpea plants.

## Materials and Methods

**Initial Population and Rearing Procedures.** The initial population of the leafminer *L. sativae* was obtained in the larval stage, in melon (*Cucumis melo*) (L.) leaves in Mossoró (5° 11'15" S, 37° 20'39" W), Rio Grande do Norte. The parasitoid species *O. scabriventris* and *C. vonones* were collected in the same state from *L. sativae* larvae infesting melon and cowpea plants [*Vigna unguiculata* (L.) Walp.], respectively in Baraúna (5° 04'44" S, 37° 37'26" W) and Parnamirim (5° 55'45" S, 35° 11'21" W).

Voucher specimens of *C. vonones* and *O. scabriventris* were deposited in the "Oscar Monte" Entomophagous Insect Collection, in the Instituto Biológico in Campinas, Brazil, and in the Vienna Museum of Natural History, Vienna, Austria, respectively. The voucher specimen of *L. sativae* was deposited at the Museum of Entomology and Acarology of ESALQ/USP in Piracicaba, Brazil.

Cowpea plants were used for the maintenance of *L. sativae*, which served as the host for multiplying the parasitoids. Cowpeas were chosen because this species is also a natural host for *L. sativae* in Rio Grande do Norte (Costa-Lima et al. 2010) and is easier to grow than melon plants. Inside insect cages (40 by 40 by 50 cm, width by length by height), cowpea plants infested predominantly with second-instar leafminer larvae were exposed to the parasitoids for 24 h. After removing the plants from the cages, leaves exposed to the koinobiont *O. scabriventris* were harvested and placed inside a plastic container (30 by 20 by 11 cm, width by length by height). After 2 d, the pupae were collected with a fine brush and placed in a Petri dish (6.5 cm in diameter). Leaves on plants exposed to the idiobiont *C. vonones* were harvested only 5 d after plant removal from the cages and were then placed inside a plastic container (12 by 8 cm, diameter by height) with a voile mesh lid. After emergence, adult parasitoids were placed in each species rearing cage.

**Temperature Influence.** We studied the influence of seven temperatures (15, 18, 20, 25, 30, 32, and 35 ± 1°C) on the immature development and survivorship of *O. scabriventris* and *C. vonones*. Second-instar larvae of *L. sativae* in cowpea leaves were offered to each parasitoid species, for 2 h in each rearing cage. In each treatment, 10 *L. sativae* larvae were analyzed to certify the instar according to Petit (1990).

The leaves with *L. sativae* larvae that were exposed to *O. scabriventris* parasitism were placed in individual Petri dishes (9 by 1.5 cm, diameter by height) and kept in climate-controlled chambers at each designated temperature, with 70 ± 10% RH and a photoperiod of 12:12 (L:D) h. In the first 48 h, six daily evaluations were made (06:00, 09:00, 12:00, 15:00, 18:00, and 21:00 h), and prepupae that exited the leaves were placed in individual test tubes (1 by 6.5 cm, diameter by height). After the first 48 h, adult emergence was observed daily.

The leaves that were exposed to *C. vonones* parasitism were isolated in plastic containers, 12 cm in diameter in the lower part, with a fine mesh on the upper surface (9.5 cm in diameter). The base was composed of moistened florist's sponge in which the leaves containing the larvae were inserted. The containers were kept in climate controlled chambers at each designated temperature with 70 ± 10% RH and a photoperiod of 12:12 (L:D) h. The presence of *C. vonones* larvae was observed daily, using a stereomicroscope (40× magnification) with transmitted light. When a pupa of the parasitoid was found, the leaf area containing the insect was cut out and placed in an individual test tube (1 cm in diameter and 6.5 in height). Eulophid adult emergence was observed daily. For *C. vonones*, in addition to the egg–adult period, it was possible to record the duration of the pupal stage because the larvae and pupae could be distinguished.

**Statistical Analyses.** The effect of temperature on the developmental rate of both parasitoids was fitted within the linear regression model. The equation applied was  $Y = a + bT$ , where  $Y$  is the rate of development at temperature  $T$ ,  $a$  is the intercept, and  $b$  the slope. The lower developmental threshold temperature ( $T_l$ ) and thermal constant ( $K$ ) were estimated with the parameters:  $T_l = -a/b$  and  $K = 1/b$ , respectively (Campbell et al. 1974). For the linear regression model, the developmental rate is an

increasing function of temperature, therefore, the temperatures used in the model ranged from 15 to 30°C; the developmental times at 32°C were not used, because they were longer than at 30°C.

Because of the importance of *L. sativae* for melon crops, the climatological norm of an important melon-producing region (city of Mossoró) was used to estimate the number of generations annually and during the melon season for *O. scabriventris* and *C. vonones*. To obtain these values, we used the previously calculated parameters  $K$  and  $T_l$  with the equation  $K = D(T - T_l)$ , where  $D$  was the duration of one generation and  $T$  is the mean temperature in the city of Mossoró, Rio Grande do Norte state, Brazil. Dividing 365 and 272 d (melon season) by the length of one generation ( $D$ ), the predicted number of annual generations and during the growing season for both parasitoids was obtained for this city.

The experiment in this study used a randomized design, with each insect corresponding to one replicate, which ranged from 26 to 53 replicates. The means and SEs for the pupal (*C. vonones*) and egg–adult periods (*O. scabriventris* and *C. vonones*) were computed from the Kaplan–Meier estimator (Kaplan and Meier 1958). Pairwise tests with different treatments were performed with the log-rank test, and an overall significance of 5% was maintained using a modified Bonferroni procedure.

The larval (*C. vonones*) and pupal survivorship (*O. scabriventris* and *C. vonones*) was analyzed by means of a logistic regression model: a generalized linear model specifically designed for modeling binomial data using a logit link function. Pairwise tests were performed by choosing appropriate contrasts, and an overall significance of 5% was maintained using a modified Bonferroni procedure. All statistical analyses were performed using R statistical software (R Development Core Team 2010).

## Results

The parasitoids, *O. scabriventris* and *C. vonones*, completed their cycle in the temperature range from 15 to 30°C. At 35°C no emergence was observed for either parasitoid species.

For *O. scabriventris*, in the range between 15 and 30°C the temperature increase reduced the egg–adult period from 29.8 to 11.2 d, respectively. However, at 32°C the 13.3-d duration was longer than that observed at 30°C, i.e., above 30°C the temperature was unsuitable (Table 1). The *O. scabriventris* pupal survivorship was similar between 15 and 30°C (86.1–92.9%) and showed a sharp reduction at 32°C (6.6%) (Table 2).

For the eulophid *C. vonones*, the temperature increase reduced the egg–adult period over the temperature range from 15 to 30°C, with no difference detected between 30 and 32°C. A temperature increase reduced the pupal-stage period up to 25°C, and the development rate stabilized starting from 30°C (Table 1). The pupal stage occupied 48.9 to 57.9% of the egg–adult period of *C. vonones* at the temperatures

**Table 1. Developmental time (day ± SE) of pupal stage and egg–adult period of *C. vonones* and egg–adult period of *O. scabriventris*, at different temperatures [RH 70 ± 10% and 12:12 (L:D) h photoperiod] on *L. sativae* in cowpea leaves**

Temp (°C)	Pupal stage	Egg–adult period	
		<i>O. scabriventris</i>	<i>C. vonones</i>
15	24.00 ± 0.31 a	29.84 ± 0.34 aA	42.00 ± 0.31 aB
18	13.53 ± 0.13 b	24.60 ± 0.19 bA	25.00 ± 0.21 bA
20	9.40 ± 0.17 c	22.54 ± 0.27 cA	19.20 ± 0.14 cB
25	7.00 ± 0.17 d	14.27 ± 0.11 dA	13.10 ± 0.16 dB
30	6.12 ± 0.22 d	11.21 ± 0.19 eA	11.29 ± 0.20 eA
32	7.23 ± 0.16 d	13.33 ± 0.19 fA	12.18 ± 0.18 eB

Means and SEs were computed from the Kaplan–Meier estimator of the corresponding survival function. The upper- and lowercase letters correspond to the lines and columns, respectively; values with the same letters are not significantly different at  $P < 0.05$  (log-rank test).

**Table 2. Larval and pupal survivorship of *C. vonones* and pupal survivorship of *O. scabriventris* on *L. sativae* in cowpea plants at different temperatures [70 ± 10% RH and 12:12 (L:D) h photoperiod]**

Temp (°C)	Larval survivorship (%)		Pupal survivorship (%)	
	<i>C. vonones</i>		<i>O. scabriventris</i>	<i>C. vonones</i>
15	96.6 ± 1.71 a		86.1 ± 4.15 a	6.0 ± 3.05 a
18	97.1 ± 2.14 a		89.4 ± 3.33 a	81.2 ± 4.56 b
20	100.0 a		92.3 ± 3.71 a	88.0 ± 4.45 b
25	100.0 a		92.9 ± 2.76 a	96.4 ± 3.51 b
30	93.3 ± 2.53 a		92.3 ± 3.12 a	96.6 ± 2.20 b
32	16.6 ± 1.85 b		6.6 ± 3.21 b	7.2 ± 3.10 a
35	0		0	–

Columns with the same letters are not significantly different at  $P < 0.05$  (logistic regression).

**Table 3. Lower temperature threshold ( $T_t$ ), thermal constant ( $K$ ), regression equation and coefficient of determination ( $r^2$ ) estimated by linear regression for *O. scabriventris* (egg–adult period) and *C. vonones* (pupal stage and egg–adult period) on *L. sativae* in cowpea plants [70 ± 10% RH and a photoperiod of 12:12 (L:D) h].**

Stage	$T_t$ (°C)	$K$ (DD)	Regression equation	$r^2$
<i>O. scabriventris</i>				
Egg–adult	7.3	257.1	$y = -0.0284 + 0.0039x$	0.98
<i>C. vonones</i>				
Pupae	6.2	140.3	$y = -0.044 + 0.0071x$	0.94
Egg–adult	7.4	246.3	$y = -0.0301 + 0.0041x$	0.97

studied. The eulophid larval survivorship was similar between 15 and 30°C (93.3 to 100.0%), with a reduction at 32°C (16.6%). Extreme temperatures reduced pupal survivorship, with 6.0% adult emergence at 15°C and 7.2% at 32°C, while at 35°C had no pupal formation (Table 2).

At the lower temperatures studied, *O. scabriventris* showed a shorter egg–adult period compared with *C. vonones*. However, at 20, 25, and 32°C, this period was shorter for *C. vonones*, with no differences at 20 and 30°C (Table 1). The lower developmental-threshold temperature of the egg–adult period for *O. scabriventris* was 7.3°C with a thermal constant of 257.1 degree days (DD). For *C. vonones*,  $T_t$  was 7.4°C for the egg–adult period and 6.2°C for the pupal stage, with a thermal constant of 246.3 and 140.3 DD, respectively (Table 3). In the melon-producing region of Mossoró in northeastern Brazil, *O. scabriventris* and *C. vonones* were predicted to have 29.4 and 30.5 generations per year, respectively.

## Discussion

One of the desirable requirements for a biological control agent is that its life cycle must be shorter than that of its host. The parasitoids studied, *O. scabriventris* and *C. vonones*, showed a shorter egg–adult period compared with *L. sativae* on cowpea plants (16.5 days), i.e., 2.2 and 3.4 fewer days, respectively at 25°C (Costa-Lima et al. 2009). Comparing the egg–adult period (25°C) of *O. scabriventris* and *C. vonones* with other leafminer parasitoid species already commercialized, their developmental times are ≈ 0.8-fold longer than the eulophid, *D. isaea* (Bazzocchi et al. 2003; Haghani et al. 2007) and similar to *O. dissitus* Muesebeck (Braconidae) (Petit and Wietlisbach 1993).

Above 30°C, survivorship decreased for the immature stages of *O. scabriventris* and *C. vonones*; and at 32°C the egg–adult period increased for the braconid. This longer developmental period with increased temperatures shows that the higher temperature is unsuitable, similarly to observations for *Hemiptarsenus varicornis* (Girault) and *C. pentheus* (Walker) on *L. trifolii*, when the temperature was increased from 30 to 33°C (Hondo et al. 2006). The higher temperatures proved

to be harmful for *O. scabriventris* and *C. vonones*, since neither species completed its cycle at 35°C. The natural occurrence of *O. scabriventris* and *C. vonones* in northeastern Brazil, where temperatures can exceed 40°C in the warmest hours of the day, may indicate a discrepancy in the results. However, this study was carried out with constant temperatures, without the thermal fluctuation that may help the insect to recover from heat stress. With respect to *O. scabriventris*, the longest developmental period of this species occurs in the leafminer pupal stage. Because of the negative phototropic behavior of *Liriomyza* prepupae, the pupal stage is usually passed in shaded areas of the soil, with lower temperatures (Parrella 1987). Consequently, the parasitoid develops in a microclimate with a lower temperature than the overall ambient temperature.

The lower developmental thresholds temperatures estimated for *O. scabriventris* (7.3°C) and *C. vonones* (7.4°C) were similar to the 7.3 found for the host, *L. sativae* (Costa-Lima et al. 2009). When compared with the  $T_t$  of other leafminer parasitoids, the values observed in this study were higher than *C. pentheus* (6.8°C) (Hondo et al. 2006), and lower than those found for *D. isaea* (8.8 and 12.8°C) (Cheah 1987, Minkenberg 1989). The parasitoids *O. scabriventris* and *C. vonones* are not restricted to regions of high temperatures such as northeastern Brazil; both species are abundant in temperate central Argentina, which has lower temperatures than the original collection site of the present study population (Salvo and Valladares 1998). This could explain the low  $T_t$  obtained in this study. However, it is important to remember that these  $T_t$  values are based in a prediction model and further field studies are required to validate these results.

According to the thermal constant, *C. vonones* required 10.8 fewer DD than *O. scabriventris*. For the eulophid, the pupal stage was responsible for 57% of the total cycle, similar to the proportion observed for *D. isaea* on *L. trifolii* (Minkenberg 1989). This result is important for the rearing procedure of leafminers idiobiont parasitoids, such as *C. vonones*, which after pupating inside the *Liriomyza* mine emerges normally, even during leaf desiccation. The annual number of generations predicted for the studied parasitoids, for the melon producing region in northeastern Brazil, represented 4.9 (*O. scabriventris*) and 6.0 (*C. vonones*) more generations than their host *L. sativae* (Costa-Lima et al. 2009). For both species, this indicates good potential for application in biological control programs for this pest.

The knowledge obtained with these two parasitoids and their host (Costa-Lima et al. 2009, 2010) represents a great advance in the construction of a biological control program for *L. sativae* in Brazil. Essential information is available for optimization of mass-rearing protocols, as well as for the timing of occurrence of these species in the field and for the parasitoids field release.

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