

Development and predatory capacity of *Chrysoperla externa* (Neuroptera: Chrysopidae) larvae at different temperatures

Desarrollo y capacidad depredadora de *Chrysoperla externa* (Neuroptera: Chrysopidae) bajo diferentes temperaturas

ALYSSON R. FONSECA¹, CÉSAR F. CARVALHO², IVAN CRUZ³, BRÍGIDA SOUZA⁴ and CARLOS C. ECOLE⁵

Abstract: *Chrysoperla externa* (Neuroptera: Chrysopidae) is one of the natural enemies of the corn leaf aphid *Rhopalosiphum maidis* (Hemiptera: Aphididae) and has potential application in the biological control of that pest. The effect of temperature on the development and the predatory capacity of this chrysopid fed with *R. maidis* nymphs was investigated. Fresh eggs of *C. externa* were maintained at 15, 20, 25 or 30 °C under 70% relative humidity and 12 h photophase, and the duration of the embryonic stage, as well as the duration and viability of the larval (first, second and third instars), prepupal, pupal and adult forms of the predator were evaluated. The duration of each of these stages decreased with increasing temperature, whilst the viabilities of all forms attained 100% at 20 and 25 °C. The threshold temperature and the value of the thermal constant K obtained for the complete life cycle (egg to adult) were, respectively, 10.7 °C and 377.8 degree-days. Independent of temperature, the consumption of *R. maidis* nymphs by *C. externa* increased as the larvae reached maturity. At 20 and 25 °C the average number of aphids consumed during the complete larval stage was maximal at approximately 350 specimens. It is concluded, therefore, that the development of the immature forms of *C. externa* fed on *R. maidis* as well as its viability and predatory capacity, were favored at temperatures between 20 and 25 °C.

Key words: Biological control. Corn leaf aphid. Predator. Green lacewing. Thermal requirements.

Resumen: *Chrysoperla externa* (Neuroptera: Chrysopidae) es uno de los enemigos naturales del pulgón de la hoja del maíz *Rhopalosiphum maidis* (Hemiptera: Aphididae) y tiene potencial aplicación en el control biológico contra esta plaga. Fue investigado el efecto de la temperatura sobre el desarrollo y capacidad depredadora de *C. externa* alimentada con ninfas de *R. maidis*. Huevos frescos del crisópido fueron mantenidos a 15, 20, 25 ó 30 °C bajo una humedad relativa de 70% y 12 h fotofase. Fueron evaluadas la duración del período embrionario, la duración y viabilidad de la fase larvaria (primer, segundo y tercer estadio), fases de prepupa, pupa y adulta del depredador. La duración de todas las etapas del desarrollo se redujo al aumentar la temperatura, mientras que la viabilidad de todas las fases alcanzó el 100 % a los 20 y 25 °C. El umbral de temperatura y el valor de la constante térmica K obtenidos para el ciclo de vida completo (de huevo a adulto) fueron 10,7 °C y 377,8 grados-día, respectivamente. Independiente de la temperatura, el consumo de ninfas de *R. maidis* por *C. externa* aumentó con el desarrollo de las larvas. A los 20 y 25 °C, el número promedio de pulgones consumidos durante toda la fase larvaria alcanzó aproximadamente 350 especímenes. Se concluye que el desarrollo de las formas inmaduras de *C. externa* alimentada con *R. maidis*, así como la viabilidad y su capacidad depredadora, se favorecen a temperaturas entre 20 y 25 °C.

Palabras clave: Control biológico. Pulgón verde del maíz. Depredador. Crisópidos. Requerimientos térmicos.

Introduction

The corn leaf aphid *Rhopalosiphum maidis* (Hemiptera: Aphididae) is regarded as a specific pest of the Poaceae since it has been found to infest more than 30 genera belonging to that family (McColloch 1921; Robinson 1992; Kuo *et al.* 2006; Razmjou and Golizadeh 2010). Moreover, in Brazil, *R. maidis* is distributed mainly in regions where sorghum and maize ("safrinha" type) are cultivated (Fonseca *et al.* 2004). In maize, infestation begins in isolated plants but spreads to the whole crop during the vegetative phase, particularly through tassel emergence, at which stage the value of the culture may not be reduced significantly (Gassen 1996). In contrast, considerable economic loss can result when infestation occurs prior to inflorescence, especially if it is associated with water stress or with the period of fertilization and grain filling (Everly 1960; Brodbeck and Strong 1987; Honek 1991; Al-Eryan and El-Tabbakh 2004; Kuo *et al.* 2006). Additionally,

transmission of viruses by the aphid may inflict considerable indirect damage to the culture (Farrell and Stufkens 1992; Huggett *et al.* 1999; Almeida *et al.* 2001; SO *et al.* 2010).

The continuous use of broad spectrum insecticides in the control of aphids has intensified the occurrence of these insect pests and the emergence of others (Gahukar 1993; Oliveira *et al.* 2012). In order to minimize the environmental impact of such chemicals, alternative control measures have been employed including biological control. Amongst the natural enemies of *R. maidis* are larvae of the green lacewing *Chrysoperla externa* (Hagen, 1861) (Neuroptera Chrysopidae) (Albuquerque *et al.* 1994; Tauber *et al.* 2000; Fonseca *et al.* 2001; Maia *et al.* 2004; Barbosa *et al.* 2008; Oliveira *et al.* 2012). Although such larvae appear to be very effective, information regarding the population dynamics of the predator is very limited and this constitutes an impediment to its practical application in biological control. Indeed, many control programs crucially depend on an understanding

¹ Ph. D. Centro de Referência Técnica, Universidade do Estado de Minas Gerais (UEMG), 35501-170, Divinópolis, MG, Brazil. arodrigofonseca@hotmail.com. Corresponding author. ² Ph. D. Departamento de Entomologia, Universidade Federal de Lavras (UFLA), 37200-000, Lavras, MG, Brazil. cfcarvalho@den.ufla.br. ³ Ph. D. Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), 35701-970, Sete Lagoas, MG, Brazil. ivancruz@cnpmc.embrapa.br. ⁴ Ph. D. Departamento de Entomologia, Universidade Federal de Lavras (UFLA), 37200-000, Lavras, MG, Brazil. brgsouza@den.ufla.br. ⁵ Ph. D. Instituto de Investigação Agrária de Moçambique (IIAM), Mavalane, Maputo, Mozambique. ccecole@gmail.com.

of the biology and physical requirements (mainly temperature) of the predators (Cividanes 2000).

In view of the relevance of *C. externa* in the biological control of *R. maidis*, the objective of the present study was to determine the effect of different temperatures on the development and predatory rate of these chrysopids when fed with their natural prey.

Materials and methods

Laboratory-reared *C. externa* adults (generation F₄) were maintained in glass jars (20 cm high x 20 cm diameter) at 25 ± 2 °C, $70 \pm 10\%$ relative humidity and 12 h photophase, and fed with a mixture of beer yeast and honey (1x1 w/w). *R. maidis* aphids were reared on leaf segments obtained from sorghum plants (cultivar BRS303; 80 cm height) placed in plastic recipients (50 mL) containing 25 mL of water and fixed with acrylic.

The effect of temperature on the development of *C. externa* was studied by placing 15 freshly oviposited eggs into glass dishes (8.5 cm high x 2.5 cm diameter) and incubating at different temperatures (15, 20, 25 and 30 ± 1 °C) under the conditions previously described. The duration of the embryonic stage, as well as the durations and viabilities of the larval (first, second and third instars), prepupal, pupal and adult forms of the chrysopid were evaluated. The predatory capacity of *C. externa* was investigated by feeding first, second and third instars with an amount of the prey (in the form of 15, 30 and 120 third and fourth instars of aphid nymphs, respectively) that had been previously determined to be larger than the daily requirements of the individual lacewing instars. Each day, any aphid nymphs that remained in the dishes were removed and replaced with new individuals.

Data were submitted to analysis of variance and polynomial regression with the aid of SAEG 8.0 (Ribeiro Jr. 2001) and Windows Excel 98 software. The lowest temperature of development, or base temperature (bT), for the embryonic, larval and all off of the juvenile stages of the predator were calculated using the method of Haddad *et al.* (1999) based on the duration/time hyperbola and its reciprocal. The thermal requirements of *C. externa* were determined using the equation $K = D(T - T_b)$, in which K is the thermal constant (expressed in degree-days), D is the time taken (days) to complete development, and T is the temperature at which development occurred (Wigglesworth 1972).

Results and discussion

The duration of the embryonic stage was 15.1 days at 15 °C, whereas at higher temperatures this stadium was considerably reduced and lasted for only 3.0 days at 30 °C. The varia-

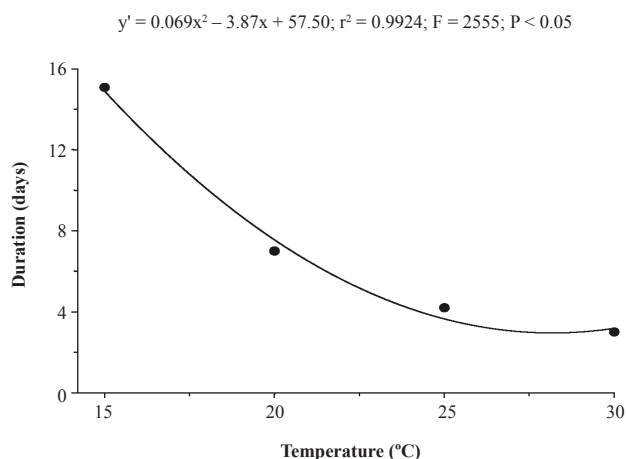


Figure 1. Duration of the embryonic stage of *Chrysoperla externa* shown as a function of temperature ($70 \pm 10\%$ relative humidity, 12 h photophase).

tion of the duration of the embryonic stage with increasing temperatures is shown in Figure 1 together with the fitted second-degree equation. Similar responses have been reported by other researchers (Maia *et al.* 2000; Fonseca *et al.* 2001; Figueira *et al.* 2002), who observed longer embryonic stages for *C. externa* at lower temperatures.

The rates of development of the individual instars and, consequently, the total larval stage of *C. externa* were also highly influenced by increasing temperatures (Figs. 2A - D). Thus, in the interval between 15 and 20 °C, the duration of the larval stage diminished by 18.4 days, whilst between 25 and 30 °C, the duration was reduced by 2.4 days (Fig. 2D). These results corroborated previous findings (Maia *et al.* 2000; Fonseca *et al.* 2001; Figueira *et al.* 2002; Oliveira *et al.* 2010; Lavagnini and Freitas 2012) concerning the sensitivity of *C. externa* instars to low temperatures. The viabilities of all instars were 100% at 20 and 25 °C (Table 1), but were reduced to approximately 93% at 15 and 30 °C. Fonseca *et al.* (2001) also observed higher mortalities of *C. externa* when immature stages were maintained at 15 and 30 °C. High temperatures may cause protein denaturation or metabolic imbalance due to the accumulation of toxins, whereas low temperatures can induce the formation of crystals in the body of the predator leading to difficulties in locomotion and, ultimately, death (Chapman 1998). However, according to Campbell *et al.* (1974), the deleterious effects of extreme temperatures only occur when such conditions are applied constantly.

The lengths of the prepupal and pupal stages of *C. externa* were also reduced as the temperature increased (Figs. 2E, F).

Table 1. Viabilities of individual instars, total larvae, prepupae and pupae of *Chrysoperla externa* fed on *Rhopalosiphum maidis* at different temperatures ($70 \pm 10\%$ relative humidity, 12 h photophase).

Temperature (°C)	Viability (%)					
	First instar	Second instar	Third instar	Larvae	Prepupae	Pupae
15	100	100	92.3	92.3	100	41.7
20	100	100	100	100	100	100
25	100	100	100	100	100	100
30	100	93.3	100	93.3	92.8	92.3

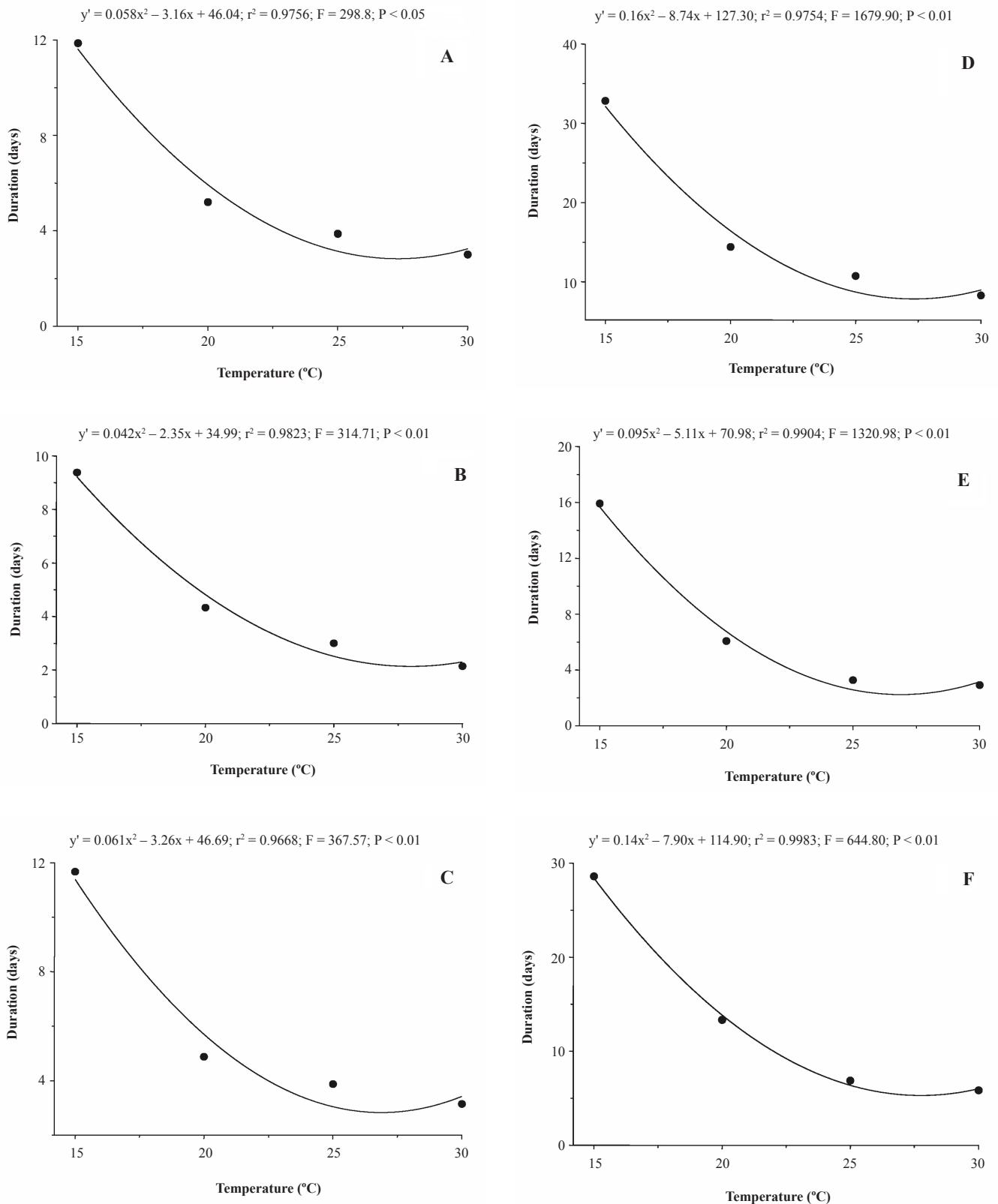


Figure 2. Duration on the first (A), second (B) and third (C) instar; larval (D), prepupal (E) and pupal (F) stages of *Chrysoperla externa* fed on *Rhopalosiphum maidis* shown as a function of temperature ($70 \pm 10\%$ relative humidity, 12 h photophase).

However, the prepupal stage was less sensitive to temperature variation in comparison with the late instar form, since viability remained at 100% between 15 and 25 °C and only diminished to 98.2% at 30 °C (Table 1). With respect to pu-

pae, viability was in the range 92.3 and 100% between 20 and 30 °C, but decreased remarkably to 41.7% at 15 °C. The sensitivity of pupae to low temperatures may be associated with the physiological problems described by Chapman (1998).

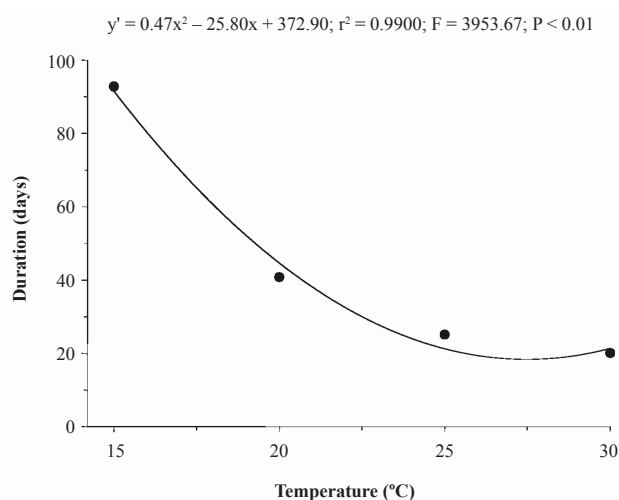


Figure 3. Duration of the biological cycle (egg to adult) of *Chrysoperla externa* fed on *Rhopalosiphum maidis* shown as a function of temperature ($70 \pm 10\%$ relative humidity, 12 h photophase).

The complete biological cycle of *C. externa* from egg to adult ranged from 92.8 days at 15 °C to 20.1 days at 30 °C, representing an overall reduction of almost 73 days (Fig. 3).

It is clear that the lacewing is particularly sensitive to temperatures between 15 and 20 °C, whilst between 25 and 30 °C the rate of development tended to be more stable (Fig. 3). The overall viabilities of *C. externa* during the biological cycle (egg to adult) were 41.7% at 15 °C, 100% at 20 and 25 °C, and 85.7% at 30 °C. The poor viability observed at the lowest temperature may be attributed to the high mortality observed during the pupal stage and to the reduced capacity for wing growth during the pharate stage). Indeed, the viability of *C. externa* was 50% higher at 30 °C compared with 15 °C, although the most appropriate conditions for the development of this predator would be 20 or 25 °C.

The predatory capacity of *C. externa* depended on the phase of development and the temperature (Fig. 4). At 25 °C, the daily consumption of *R. maidis* nymphs by third instar predators (78.4) was 18-fold higher than that of their first instar counterparts (4.4). This finding is in agreement with that of Fonseca *et al.* (2001) who observed that as *C. externa* developed (at 24 °C) the consumption of *Schizaphis graminum* increased 22 fold. Figure 4 also reveals that the daily consumption of nymphs typically increased with increasing temperature. Thus, in the interval 15-30 °C, there was a progressive increase in the number of prey consumed by *C. externa* with respect to the total larval stage, such that, the daily consumption at 30 °C was 4 times greater than at 15 °C. First

instar predators, however, did not follow the general trend but rather showed a tendency to consume fewer nymphs at 30 °C than at 25 °C.

With respect to the goodness of fit of the regression lines shown in Fig. 4, Fonseca *et al.* (2001) also observed that the variation in the determination coefficient (r^2) increased progressively from the first to third instar as a function of development (Figs. 4A-C). Although the value of r^2 for the first instar was only 0.74, when the three instars were analyzed in conjunction (as larval stage) the value of r^2 was 0.99 (Fig. 4D). On this basis it is predicted that, under field conditions, the predatory capacity of *C. externa* might be favored by higher temperatures. Knowledge regarding the effect of temperature on the consumption of prey by *C. externa* can also contribute to the management of laboratory-reared predators and therefore, to the increased effectiveness of biological control programs, particularly when the availability of prey is limited.

The total numbers of prey consumed by the individual instars of *C. externa* were highly influenced by temperature (Table 2). For example, the maximum consumption of the first instar (18.1 nymphs in total) was observed at 15 °C, and this was probably due to the lengthy duration of this stage under such conditions, i.e. the larvae were fed for a longer period. Such a hypothesis does not explain the unexpectedly large reduction in consumption observed at 20 °C, however. With respect to the second instar, highest consumption (42 - 44 nymphs) occurred at 15 and 30 °C. Whilst the explanation given previously for the first instar at 15 °C applies here, the high consumption of nymphs by the second instar at 30 °C can be justified only by the intensification of metabolism and predatory activity, since the duration of this stage was much shorter at the higher temperature (Table 2). The highest number of prey (301 - 302 nymphs) consumed by the third instar of *C. externa* was at 20 and 25 °C.

The overall consumption of prey by the larval stage as a whole was essentially determined by the predatory capacity of the third instar, which was typically 20 times greater than that of the first and approximately seven times greater than that of the second instar. Whilst the total consumption of prey by the first instar at 20 °C was half of that observed at 25 °C, the difference was compensated by the increased consumption of the second instar at 20 °C. Additionally, there were no differences in the numbers of prey consumed by the third instar at 20 and 25 °C. Thus, the highest consumption of prey by the larval stage (348 - 351 nymphs) occurred at 20 and 25 °C, demonstrating that such conditions would be the most favorable for biological control of *R. maidis* by the predator.

The thermal requirements of *C. externa*, as represented by Tb and K values determined in the present study, varied

Table 2. Total numbers of *Rhopalosiphum maidis* consumed by individual instars and during the whole larval stage of *Chrysoperla externa* at different temperatures ($70 \pm 10\%$ relative humidity, 12 h photophase).

Temperature (°C)	Mean number of <i>R. maidis</i> ($\pm$ standard error) consumed by predator		
	First instar	Second instar	Third instar
15	18.1 $\pm$ 0.8	42.3 $\pm$ 1.9	256.4 $\pm$ 4.9
20	9.5 $\pm$ 0.5	40.3 $\pm$ 1.4	301.1 $\pm$ 6.8
25	17.1 $\pm$ 0.9	28.5 $\pm$ 1.6	301.8 $\pm$ 5.7
30	11.1 $\pm$ 0.3	44.4 $\pm$ 3.1	245.7 $\pm$ 7.3
CV% ²	17.5	20.4	8.6

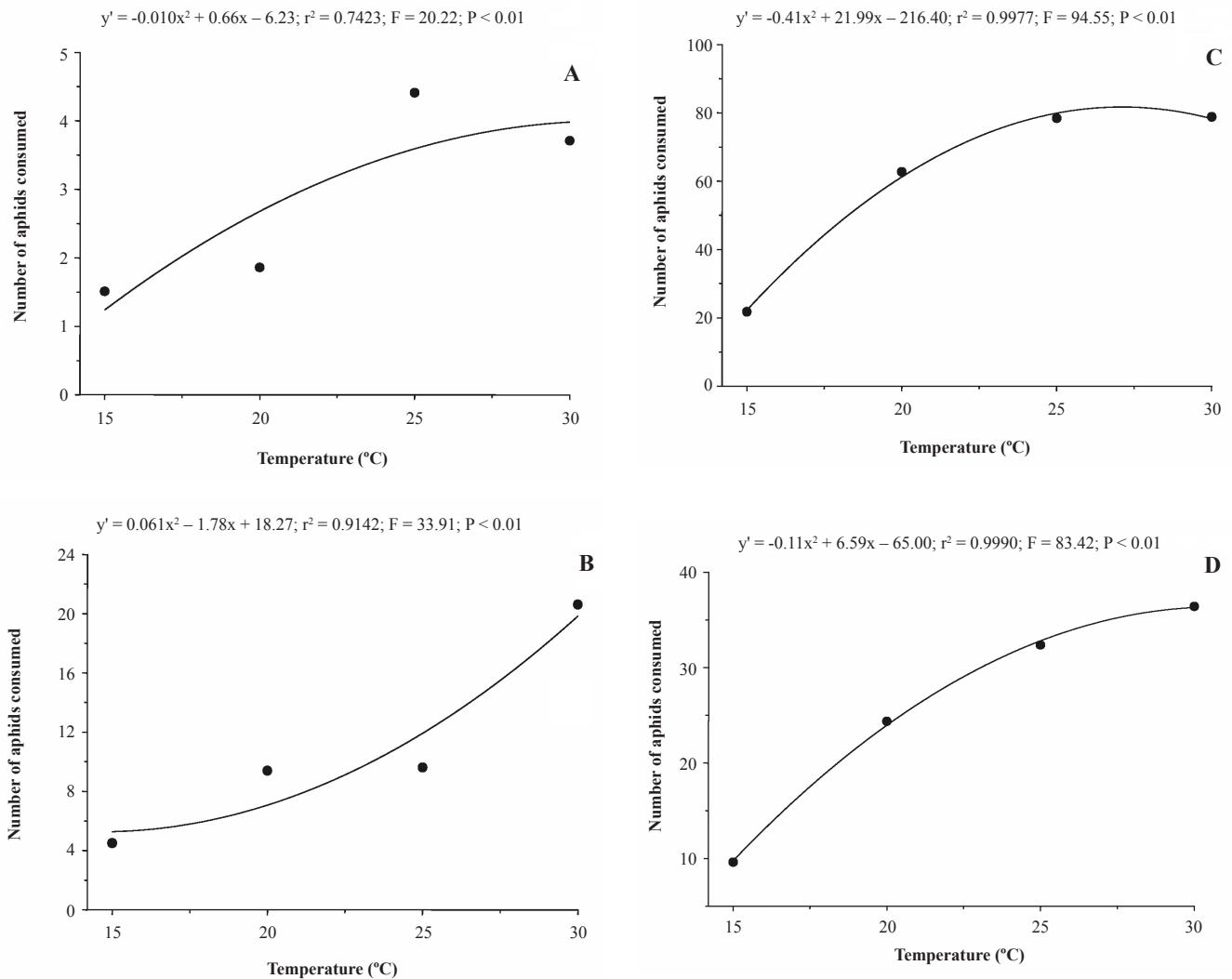


Figure 4. Daily consumption of *Rhopalosiphum maidis* by the first (A), second (B) and third (C) instars, and larval stage (D) of *Chrysoperla externa* shown as a function of temperature ($70 \pm 10\%$ relative humidity, 12 h photophase).

according to the developmental stage of the predator (Table 3; Fig. 5), and were similar to those reported by Maia *et al.* (2000) for *C. externa* fed on *S. graminum*. The rate of development of *C. externa* was affected by temperature, with the duration of the immature stages being significantly reduced at increasing temperatures (Fig. 5). *C. externa* was particularly sensitive to temperature changes between 15-20 °C, since an incremental temperature increase in this range had a much greater effect on the duration of the immature stages than a similar variation at 25 °C or above. The determination coefficients of the regression equations were large at all stages, and

varied from 98.8 to 99.7% demonstrating that the duration of development of these chrysopids is intimately correlated with the variation in temperature (Fig. 5).

In conclusion, this study has demonstrated that the most favorable temperatures for the development of the immature forms of *C. externa* are 20 and 25 °C, conditions in which the consumption of *R. maidis* is also at a maximum. The beneficial effect of higher temperatures on immature forms of *C. externa* was confirmed by the bT and K values, which varied according to the development stages of the predator.

Table 3. Threshold temperature (Tb), and thermal constant (k) of *Chrysoperla externa* fed on *Rhopalosiphum maidis* ($70 \pm 10\%$ relative humidity, 12 h photophase).

Stages	Tb ^a (°C)	K (degree-days)	Regression equation ^b	r ² (%)
Eggs	11.6	55.8	$Y = 0.0179x - 0.2081$	99.7
Larvae	9.2	169.9	$Y = 0.0059x - 0.0540$	98.8
Biological cycle	10.7	377.8	$Y = 0.0026x - 0.0283$	99.3

^a Calculated using the Haddad *et al.* (1999) method.

^b $y' = 1/\text{duration}$.

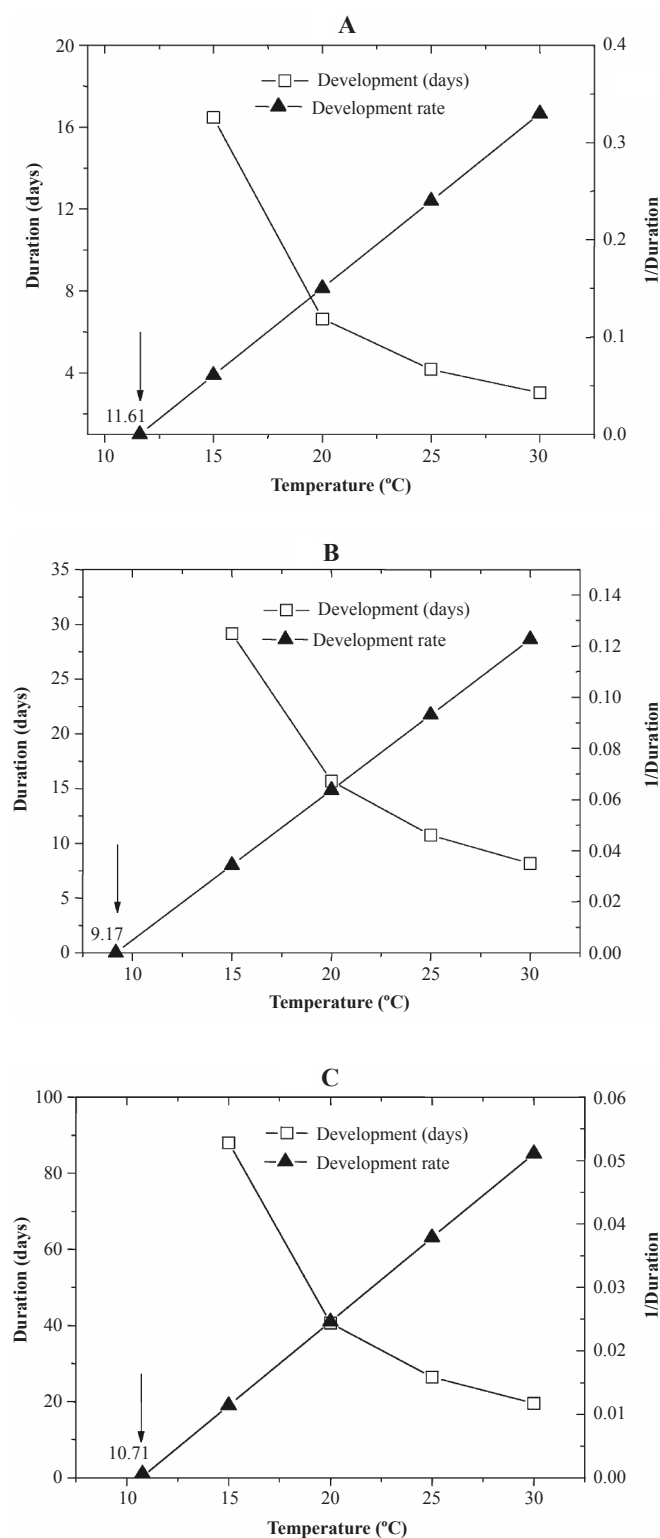


Figure 5. Relation between temperature, duration of development (days) and development rate (1/duration) for the embryonic (A), larval (B) and juvenile (C) stages of *Chrysoperla externa* fed on *Rhopalosiphum maidis* shown as a function of temperature (70 ± 10% relative humidity, 12 h photophase).

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