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## NEW RECORDS OF PREDATION ON EGGS OF *Bemisia tabaci* (Hemiptera: Aleyrodidae) BY *Chrysopodes* (*Chrysopodes*) *Lineafrons* (Neuroptera: Chrysopidae) IN NORTHWESTERN ARGENTINA

### NUEVOS REGISTROS SOBRE LA DEPREDACIÓN DE HUEVOS DE *Bemisia tabaci* (Hemiptera: Aleyrodidae) POR *Chrysopodes* (*Chrysopodes*) *Lineafrons* (Neuroptera: Chrysopidae) EN EL NOROESTE DE LA ARGENTINA

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#### ABSTRACT

*Bemisia tabaci* has become one of the major pests of economic importance that affects several crops worldwide. Among their natural enemies are found the Chrysopidae family, with larvae predators of different pests resulting very effective biological control agents. In order to contribute to possible use of this beneficial insect in *B. tabaci* biological control, the developmental time and survival of the immature stages of *Chrysopodes* (*Chrysopodes*) *lineafrons* was determined, as well as longevity and oviposition of the adults fed with eggs of *B. tabaci*. *C. (C.) lineafrons* adults were collected in tomato crops in Lules department, Tucumán province. To determine the developmental duration for each instars and survival of the larvae, 90 eggs of *C. (C.) lineafrons* were randomly selected, of which only 71 eggs hatched; of these, 34 larvae were fed with *B. tabaci* eggs and 37 with *Sitotroga cerealella* eggs, used as control. The oviposition and longevity of the adults were recorded, both fed with the two preys. *C. (C.) lineafrons* larvae consumed an average 127.04 ( $\pm$  40.2) eggs of *B. tabaci* and 44 ( $\pm$  19.04) eggs of *S. cerealella* per day. Mean developmental time of *C. (C.) lineafrons* fed with *B. tabaci* eggs was 45 ( $\pm$  3.54) days and 35 ( $\pm$  5.04) days with *S. cerealella* eggs. Survival of immature stages, number of eggs per adults and their longevity were higher when *C. (C.) lineafrons* were fed with *S. cerealella* eggs than with *B. tabaci* eggs. *Chrysopodes* (*C. (C.) lineafrons*) has proved to be an efficient predator which would represent an excellent tool for *B. tabaci* biological control in tomato crops.

**KEY WORDS:** green lacewing; whitefly; predation capacity

#### RESUMEN

*Bemisia tabaci* se ha convertido en una de las principales plaga de importancia económica, afectando a diversos cultivos en todo el mundo. Entre sus enemigos naturales se encuentran las especies de la familia Chrysopidae, con larvas depredadoras de diferentes plagas que resulta un agente de control biológico muy efectivo de diferentes plagas. Para contribuir al posible aprovechamiento de este insecto benéfico en el control biológico de *B. tabaci*, se determinó el tiempo de desarrollo y supervivencia de los estadios inmaduros de *Chrysopodes* (*Chrysopodes*) *lineafrons*, y la longevidad y oviposición de los adultos alimentados con huevos de *B. tabaci*. Los adultos de *C. (C.) lineafrons* fueron recolectados en cultivos de tomate en el departamento Lules, provincia de Tucumán. Para determinar la duración del desarrollo de cada estadio y la supervivencia de las larvas, se seleccionaron aleatoriamente 90 huevos de *C. (C.) lineafrons*, de los cuales sólo 71 huevos eclosionaron; de estas, 34 larvas fueron alimentadas con huevos de *B. tabaci* y 37 con huevos de *Sitotroga cerealella*, utilizados como control. Se registró la oviposición y la longevidad de los adultos alimentados con las dos presas. Las larvas de *C. (C.) lineafrons* consumieron un promedio de 127,04 ( $\pm$  40,2) huevos de *B. tabaci* y 44 ( $\pm$  19,04) huevos de *S. cerealella* por día. El tiempo promedio de desarrollo de *C. (C.) lineafrons* alimentados con huevos de *B. tabaci* fue de 45 días ( $\pm$  3,54); mientras que los alimentados con huevos de *S. cerealella* fue de 35 días ( $\pm$  5,04). La supervivencia de los estadios inmaduros, el número de huevos por adultos y la longevidad fue mayor cuando *C. (C.) lineafrons* fueron alimentados con huevos de *S. cerealella* que con los huevos de *B. tabaci*. *C. (C.) lineafrons* demostró ser un depredador eficiente, representando así una excelente herramienta para el control biológico de *B. tabaci* en cultivos de tomate.

**PALABRAS CLAVE:** crisopa; mosca blanca; capacidad de depredación

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## INTRODUCCIÓN

The whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is a serious pest of several annual crops, ornamental, industrial, fruit and weeds worldwide (Byrne et al., 1990; Brown et al., 1995; Viscarret, 2000; López-Ávila, 2005). It causes direct damage through sucking sap and the excretion of sugary substances that produce the growth of sooty mold, which causes decrease of the photosynthetic capacity of plant; also causes indirect damage by transmitting viruses and bacteria (Berlinger, 1986; Viscarret, 2000). The species of *B. tabaci* has caused significant losses in America since 1981, reducing crop productivity of tomato, sweet pepper, beans and textiles (Brown, 1993). In Argentina, the first record of *B. tabaci* arises from specimens found on unspecified host plant in Tucumán province (Viscarret, 2000). Subsequently, its presence is reported in greenhouses and field crops such as cotton, tobacco, citrus, sugar cane, soybean, forestry and horticultural crops of the families Solanaceae, Cucurbitaceae, Brassicaceae and Asteraceae (Polack, 2005).

Currently, the most used control method against *B. tabaci* is chemical control; however, an alternative method is based on biological control using natural enemies of the pest in order to decrease its density (Reguilón et al., 2011; Flores et al., 2015), the environmental impact and to improve product quality (López et al., 1999). Among their natural enemies is found the genus *Chrysopodes* Navás (Neuroptera: Chrysopidae), of cosmopolitan distribution and with about 40 species distributed in two subgenera, *Chrysopodes* s. str. and *Neosuarius* Adams and Penny

(Adams and Penny, 1987). In Argentina, the species *Chrysopodes* (*Chrysopodes*) *lineafrons* Adams and Penny, *Chrysopodes* (*Chrysopodes*) *polygonicus* Adams and Penny, *Chrysopodes* (*Neosuarius*) *divisus* Walker and *Chrysopodes* (*Neosuarius*) *porterinus* Navás have been reported (Adams and Penny, 1987; González-Olazo et al., 1999; Monserrat and Freitas, 2005; González-Olazo and Reguilón, 2008; Ortega et al., 2014).

*Chrysopodes* (*C.*) *lineafrons* is considered as an effective predator in biological control, however it is necessary to investigate the preference for certain pest species or even pest stage to be controlled as well as a possible interaction with other natural enemies. As such knowledge is deficient in the literature, an assay to study life cycle of *C. (C.) lineafrons* fed with eggs of *B. tabaci* as prey was set up. Therefore, developmental time, survival, longevity and oviposition as biological parameters of *C. (C.) lineafrons* were studied, as well as their predation ability over *B. tabaci* eggs under laboratory conditions.

## MATERIALS AND METHODS

### Study area and collection of specimens

Entomological sampling was performed during the period 2009-2010 in two greenhouses and one field of tomato crop in Lules department (26° 55' 60" S – 65° 20' 60" W, 382 m.a.s.l), Tucumán province, northwestern Argentina (Figure 1).

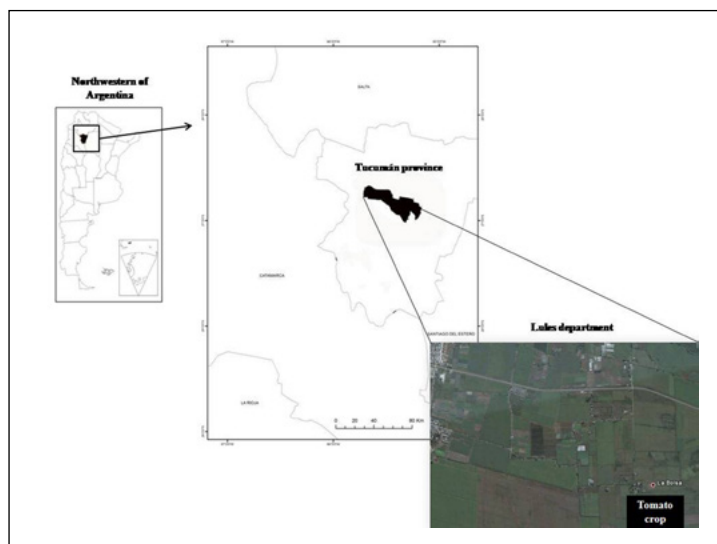


Figure 1. Geographical location of the tomato crops in Tucumán Province, northwestern Argentina.

*Chrysopodes (C.) lineafrons* specimens were collected in tomato crops and the surrounding vegetation using manual aspirators for the adults and manually with a brush for the immature stages. Subsequently, adults were placed in plastic containers of 500 cm<sup>3</sup> covered with voile and larvae in petri dishes with paper accordions, used to avoid cannibalism, and feeding with *Sitotroga cerealella* Oliver eggs.

### Assay in laboratory

Rearing of *C. (C.) lineafrons* was maintained for 12 months. The adults were placed in 5 L plastic containers covered with voile, secured with an elastic band and properly labeled with the date of the collect and the number of individuals. A circular paper was placed inside the container for the females to lay eggs. They were fed daily with a mixture of yeast, pollen, honey and water in a proportion 10-1-5-7 and provided water with moistened cotton (Nuñez, 1988; Holguin et al., 2004; Reguilón et al., 2006).

To evaluate the ingestion of *C. (C.) lineafrons*, eggs breeding in the laboratory were selected randomly and placed in individual plastic containers of 2 cm diameter with hermetic seal, maintained at 27 °C, 65 % humidity and a photoperiod of 12: 12 (L: D) until hatching (Díaz-Aranda and Monserrat, 1990; Giffoni et al., 2007) Once lacewing larvae emerged, they were separated in two groups. A known number of eggs of *B. tabaci* ( $190.3 \pm 113.2$ ) were offered to one of the groups and *S. cerealella* eggs ( $122.9 \pm 45.0$ ) to the other, the latter used as a control, because it is the most used prey for the mass rearing of species of green lacewing. After 24 hours, the number of eggs predated and the *C. (C.) lineafrons* larvae survival were recorded (Legaspi et al., 1994; Hagler et al., 2004).

When the larvae of *C. (C.) lineafrons* achieved the pupal stage, they were placed in 1 L plastic containers covered with voile. The number of adults that emerged was recorded in each repetition and it were fed and maintained under the same conditions that the adults of breeding. The number of eggs laid and longevity of adults was registered every 24 hours (Figure 2).

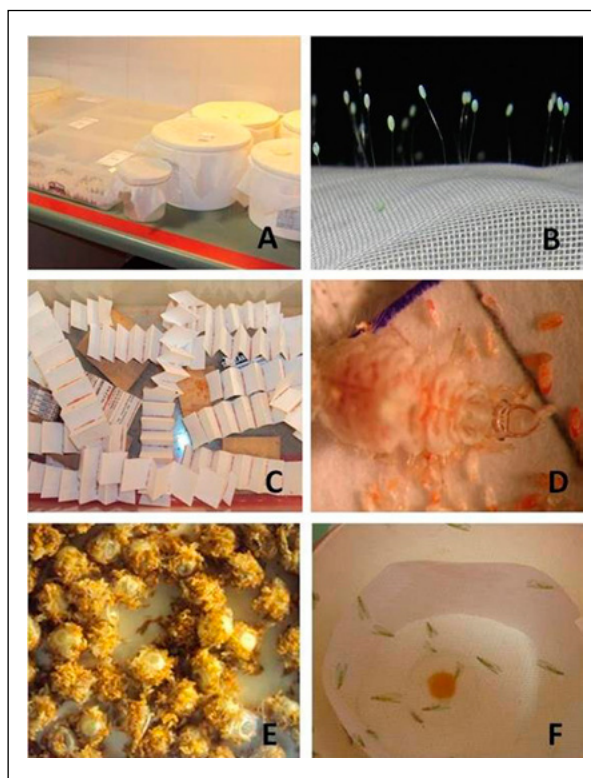


Figure 2. a. Rearing of *C. (C.) lineafrons*; b. Eggs; c. Larval feeding box; d. Larvas; e. Pupal stage; F. Adults.

### Data analysis

Data obtained in the laboratory such as number of prey offered and consumed, date of emergence of *C. (C.) lineafrons* larvae, oviposition, number of individuals adults placed in each container and numbers of eggs laid daily were registered into spreadsheets.

The mean, average and percentage of eggs predated by *C. (C.) lineafrons* were calculated. In addition, the survival of the different life stages (egg, larva and pupa) of lacewing was determined, as well as, the adult longevity and number of eggs laid per female per day fed with *B. tabaci* eggs and *S. cerealella* eggs. The analysis was carried out using the Statistica 6.0 (StatSoft, 2001) software.

### RESULTS

Of the total of 90 eggs of *Chrysopodes (C.) lineafrons* chosen at the beginning of the assay, only 71 hatched, and of these 34 were fed with *B. tabaci* eggs and 37

with *Sitotroga cerealella* eggs. During the ingest assay it was observed that a total of 34 individuals reached the adult state, 28 fed with *S. cerealella* eggs and 6 with *Bemisia tabaci* eggs.

### Ingestion assay

In general, the larvae of *C. (C.) lineafrons* consumed an average of 127.04 ( $\pm$  40.2) eggs of *B. tabaci* and 44 ( $\pm$  19.04) eggs of *S. cerealella* per day. When each larval stage was evaluated separately, it was observed that

the larva I of *C. (C.) lineafrons* consumed a maximum of 556 eggs of *B. tabaci* and 189 eggs of *S. cerealella*, the larva II consumed 531 eggs of *B. tabaci* and 240 eggs *S. cerealella*, while larva III consumed 619 eggs of *B. tabaci* and 150 eggs of *S. cerealella* per day (Table 1). On the other hand, the developmental time of the different larval stages were similar between larvae I and II, whereas for larvae III was shorter. With regard to developmental time of *C. (C.) lineafrons* an average of 45 days was recorded when the larvae were fed with of *B. tabaci* eggs and 35 days with *S. cerealella* eggs (Table 2).

Table 1. Daily eggs consumption of *C. (C.) lineafrons* larvae fed with *B. tabaci* eggs and *S. cerealella* eggs.

Stage	Number of individuals evaluated	Eggs consumption			
		Total	Average/individuals	Average/individuals/day	Rank
<i>B. tabaci</i>					
L1	34	33607	988.4	88.01	0-556
L2	31	43188	1393.2	124.8	0-531
L3	23	27446	1193.3	168.3	0-619
<i>S. cerealella</i>					
L1	37	8125	219.6	22.02	0-189
L2	31	10367	334.4	55.2	0-240
L3	30	6020	200.6	54.8	0-150

Table 2. Developmental time of different stages of *C. (C.) lineafrons* fed with *B. tabaci* eggs and *S. cerealella* eggs.

Stage	Mean $\pm$ SD	Life cycle duration
<i>B. tabaci</i>		
L1	11.23 $\pm$ 3.28	45.2 $\pm$ 3.54
L2	11.16 $\pm$ 6.02	
L3	7.09 $\pm$ 4.37	
Pupa	15.75 $\pm$ 2.99	
<i>S. cerealella</i>		
L1	9.97 $\pm$ 4.21	34.9 $\pm$ 5.04
L2	6.06 $\pm$ 4.27	
L3	3.66 $\pm$ 2.47	
Pupa	15.21 $\pm$ 3.51	

Means  $\pm$  SD followed by P < 0.05

### Survival, longevity and oviposition

In general, survival of eggs of *C. (C.) lineafrons* was 81.1 %. The immature stages of *C. (C.) lineafrons* fed with *B. tabaci* eggs showed lower survival (8.2 %) than those fed with *S. cerealella* eggs (30.1 %). Lacewings survival decreased significantly from the larval stage toward the adult stage, for both individuals fed with *B. tabaci* eggs as *S. cerealella* eggs (Figure 3).

Regarding to *C. (C.) lineafrons* adults longevity, a greater number of specimens was observed from larvae fed with *S. cerealella* eggs, as well as greater longevity of 41 days approximately, while the larvae fed with *B. tabaci* eggs it was 20 days (Figure 4a). Oviposition of *C. (C.) lineafrons* was also higher in females fed with *S. cerealella* eggs (Figure 4b).

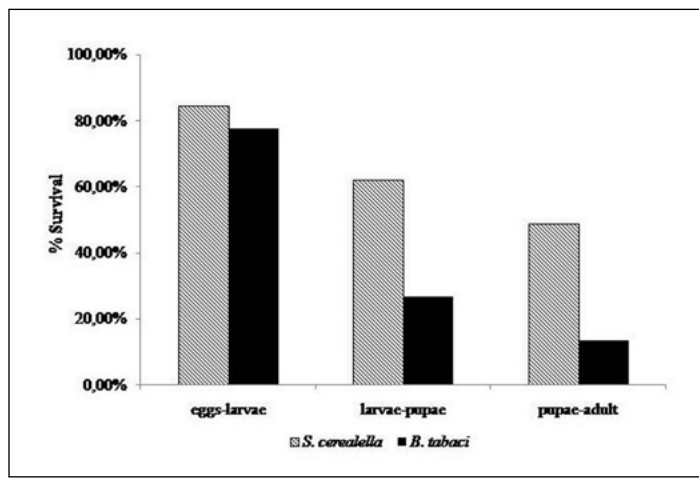


Figure 3. Viability of different developmental stages of *C. (C.) lineafrons* fed with *B. tabaci* eggs and *S. cerealella* eggs.

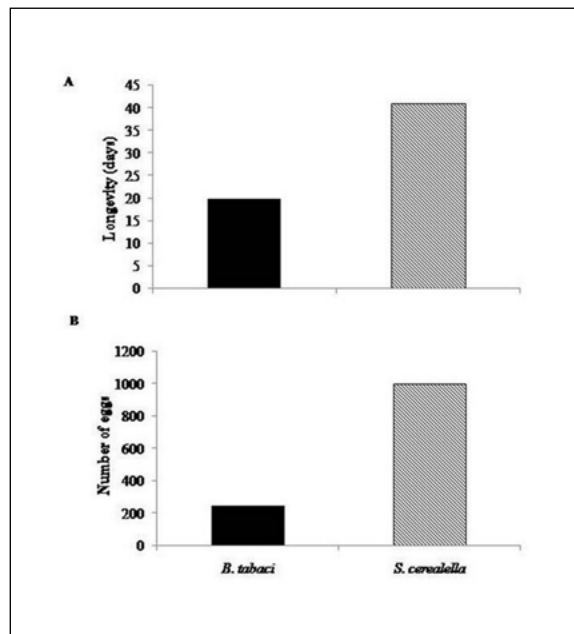


Figure 4. (a) Longevity and (b) Oviposition of *C. (C.) lineafrons* adult fed with *B. tabaci* and *S. cerealella* eggs.

## DISCUSSION

This study represents the first record of *C. (C.) lineafrons* predation capacity over *B. tabaci* eggs in Argentina, pointing at this specie as a potential biological control agent. It should be noted that this lacewing specie was recently cited in the province of Tucumán, northwestern Argentina (Ortega et al., 2014).

Regarding to the ingest capacity, the *C. (C.) lineafrons* larvae consumed a greater amount of *B. tabaci* eggs per day than *S. cerealella* eggs, standing out the larva II, respectively. These results are comparable with those reported by Legaspi et al. (1994), who determined that a greater number of *B. tabaci* eggs were necessary for the development of larvae III of *Chrysoperla rufilabris* Bumeister. Other studies cite that *Chrysoperla carnea* Stephens was able to eat up 200.5 nymphs of *B. tabaci* and 171.8 nymphs of *Amrasca devastans* Distant (Nisar-Syed et al., 2005) and that the larvae can consume about 8 000 eggs of *S. cerealella* and 510 pupae of *B. tabaci* throughout its development (Gallardo et al., 2005). As well, Legaspi et al. (1994) determined that *C. rufilabris* larvae consume an average of 531.55 eggs of *B. tabaci* per day, whereas Avila et al. (2009) reported that *Chrysoperla argentina* Steimann larvae ingested an average of 275 eggs of *B. tabaci* per day. This behavior can be attributed to that reported by Nisar-Syed et al. (2005), who determined that the prey density has a strong influence on the predation potential, i.e. the consumption of number of egg increase as the prey density was increased.

Furthermore, it was observed that *C. (C.) lineafrons* presented a developmental time of 45 days when they were fed with *B. tabaci* eggs and 35 days with *S. cerealella* eggs. Regarding this, investigations performed by Ramirez-Delgado et al. (2007) with *Ceraeochrysa* sp. nr. *cincta* determined that total developmental time, from egg until adult emergence was 29 days when they were fed with *S. cerealella* eggs. Moreover, Legaspi et al. (1994) recorded an increased duration of larval developmental time in *C. rufilabris* fed with *B. tabaci* eggs compared with those fed with *S. cerealella* eggs; however, Nisar-Syed et al. (2005) recorded that *C. carnea* present a shorter developmental time when they were fed with *B. tabaci* eggs compared that with *A. devastans* eggs.

Survival of *C. (C.) lineafrons* as the life cycle progresses decreased for both individuals those fed with *B. tabaci*

eggs and those fed with *S. cerealella* eggs. These results agree with those obtained by Ramirez-Delgado et al. (2007), who determined a steadily decreased over time of *C. sp. nr. cincta* survival fed with *S. cerealella* eggs. Longevity of *C. (C.) lineafrons* adults fed with *S. cerealella* eggs was 41 days and 20 days with *B. tabaci* eggs, results that agree with those reported by Ramirez-Delgado et al. (2007), who determined a greater longevity for adults of *C. sp. nr. cincta* fed with *S. cerealella* eggs.

Generally, survival, longevity and number of eggs laid per female of *C. (C.) lineafrons* was greater when they were fed with *S. cerealella* eggs than with *B. tabaci* eggs. In this regard, Giffoni et al. (2007) determined that *C. externa* only completed its life cycle when being fed with *S. cerealella*; while Legaspi et al. (1994) recorded that *C. rufilabris* larvae showed a greater preference for *S. cerealella* eggs than for *B. tabaci* eggs.

It has been observed that *C. (C.) lineafrons* present a similar behavior to other lacewing species but with the peculiarity that the predation efficiency is much higher, which would represent an excellent tool to an efficient biological control of *B. tabaci* in tomato crops. Therefore, we can conclude that the larval stages of *C. (C.) lineafrons* require a greater number of *B. tabaci* eggs than of *S. cerealella* eggs to complete its life cycle; larvae II and III of *C. (C.) lineafrons* consume more *B. tabaci* eggs; larval I stage is longer than the other two larval stages; survival and longevity of *C. (C.) lineafrons* adults was greater in individuals fed with *S. cerealella* eggs, as well as the number of eggs laid per female was higher.

In general, we can say that *C. (C.) lineafrons* feed with *S. cerealella* reached a complete development in a shorter time, whereas individuals feeding with *B. tabaci* required a higher density of the prey to complete the development since the number of eggs offered failed to meet nutritional needs. These results would indicate that the greater efficiency of the predator would be given when the availability and number of whitefly are greater than those offered in the present study. Therefore, survival, longevity and oviposition are determined by the nutritional value of the prey, that is, *C. (C.) lineafrons* require a higher number of whitefly eggs to increase these values and indicate their potential as a control agent.

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