### New records of predation on eggs of Bemisia tabaci (Hemiptera: Aleyrodidae) by Chrysopodes (Chrysopodes) lineafrons (Neuroptera: Chrysopidae) in Northwestern Argentina

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Bemisia tabaci has become a major economic importance pest, affecting several crops worldwide. Among their natural enemies, species of Chrysopidae family, with larvae predators of different pests, are a very effective biological control agent. The developmental time and survival of the immature stages of Chrysopodes (Chrysopodes) lineafrons, and the longevity and oviposition of adults fed with eggs of B. tabaci was determined. C. (C.) lineafrons adults were collected in tomato crops in Lules department, Tucumán province. To determine the developmental duration of each instar, and larvae survival, 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. Oviposition and longevity of adults fed with the two preys were recorded. C. (C.) lineafrons larvae consumed an average of 127.04 B. tabaci eggs and 44 S. cerealella eggs per day. Mean developmental time of C. (C.) lineafrons fed with B. tabaci eggs was 45 days; while for those fed with S. cerealella eggs it was 35 days. Immature stages survival, number of eggs per adults and longevity were higher when C. (C.) lineafrons were fed with S. cerealella eggs than with B. tabaci eggs. C. (C.) lineafrons proved to be an efficient predator, thus representing an excellent tool for the biological control of B. tabaci in tomato crops .

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### 24 ABSTRACT

Bemisia tabaci has become a major economic importance pest, affecting several crops 25 worldwide. Among their natural enemies, species of Chrysopidae family, with larvae predators 26 of different pests, are a very effective biological control agent. The developmental time and 27 survival of the immature stages of *Chrysopodes* (*Chrysopodes*) lineafrons, and the longevity and 28 29 oviposition of adults fed with eggs of B. tabaci was determined. C. (C.) lineafrons adults were collected in tomato crops in Lules department, Tucumán province. To determine the 30 developmental duration of each instar, and larvae survival, 90 eggs of C. (C.) lineafrons were 31 32 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. Oviposition and longevity of adults 33 fed with the two preys were recorded. C. (C.) lineafrons larvae consumed an average of 127.04 34 B. tabaci eggs and 44 S. cerealella eggs per day. Mean developmental time of C. (C.) lineafrons 35 fed with *B. tabaci* eggs was 45 days; while for those fed with *S. cerealella* eggs it was 35 days. 36 37 Immature stages survival, number of eggs per adults and longevity were higher when C. (C.)lineafrons were fed with S. cerealella eggs than with B. tabaci eggs. C. (C.) lineafrons proved to 38 be an efficient predator, thus representing an excellent tool for the biological control of *B. tabaci* 39 40 in tomato crops.

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42 Subjects Entomology, Ecology

43 Keywords *Chrysopodes*, Developmental time, Survival, Longevity, Oviposition, Ingest capacity.

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### 47 INTRODUCCIÓN

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49 annual, ornamental and industrial crops, fruit plantations and weeds worldwide (Byrne *et al.*,

50 1990; Brown *et al.*, 1995; Viscarret, 2000; López-Ávila, 2005). It causes direct damage through

The whitefly Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) is a serious pest of several

51 sucking sap and excreting sugary substances, which produces the growth of sooty mold, reducing

52 photosynthetic capacity of the plant; and indirect damage through viruses and bacteria

transmission (Berlinger, 1986; Viscarret, 2000). *Bemisia tabaci* has caused significant losses in

54 America since 1981, reducing crop productivity of tomato, sweet pepper, beans and textiles

55 (Brown, 1993). In Argentina, the first record of *B. tabaci* arises from specimens found in an

unspecified host plant in Tucumán province (Viscarret, 2000). Subsequently, its presence has

57 been reported in greenhouses and field crops such as cotton, tobacco, citrus, sugar cane, soybean,

58 forestry and horticultural crops of Solanaceae, Cucurvitaceae, Cruciferaceae and Compositae

59 families (Polack, 2005).

Currently, the most used control method against *B. tabaci* is chemical control; however, 60 alternative methods based on biological control with natural enemies of the pest can reduce its 61 density (Reguilón *et al.*, 2011), as well as environmental impact, and improve product quality 62 (López et al., 1999). Among the natural enemies of this species, the genus Chrysopodes Navás 63 (Neuroptera: Chrysopidae) has been mentioned, with a cosmopolitan distribution and with about 64 40 species distributed in two subgenera: Chrysopodes s. str. and Neosuarius Adams & Penny 65 66 (Adams & Penny, 1987). In Argentina, Chrysopodes (Chrysopodes) lineafrons Adams & Penny, Chrysopodes (Chrysopodes) polygonicus Adams & Penny, Chrysopodes (Neosuarius) divisus 67 68 Walker and *Chrysopodes* (*Neosuarius*) porterinus Navás have been cited (Adams & Penny,

69 1987; Gonzalez Olazo et al., 1999; Monserrat & Freitas, 2005; Gonzalez Olazo & Reguilón,

70 2008; Ortega *et al.*, 2014).

*Chrysopodes (C.) lineafrons* is considered an effective predator for biological control. Thus, research focusing on its preference for certain pest species or even for certain pest stages, and its possible interaction with other natural enemies is of great need. Since such knowledge is scarce in literature, a study of the life cycle of *C. (C.) lineafrons* fed with eggs of *B. tabaci* as prey was set up in this paper. Developmental time, survival, longevity and oviposition were analyzed as biological parameters of *C. (C.) lineafrons*, and their predation ability over *B. tabaci* eggs under laboratory conditions was assessed.

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#### 79 MATERIALS AND METHODS

#### 80 Study area and specimens collection

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

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

89

90 Laboratory assay

91 Rearing of *C*. (*C*.) *lineafrons* took 12 months. Adults were placed in 5 liter plastic containers 92 covered with voile, secured with an elastic band and properly labeled with collection date and 93 number of individuals. A circular paper was placed inside the container, for the females to lay 94 the eggs. Specimens were fed daily with a mixture of yeast, pollen, honey and water in a 10:1:5:/ 95 proportion and provided of water with moistened cotton.

To evaluate the ingestion of *C. (C.) lineafrons*, eggs breeded in the laboratory were selected
randomly and placed in 2 cm diameter individual plastic containers with an hermetic seal,

98 maintained at 27°C, 65% humidity and a photoperiod of 12:12 (L:D) until hatching. Once

99 lacewing larvae emerged, they were separated in two groups. A known number of *B.tabaci* eggs

100 were offered to one of the groups while *S. cerealella* eggs were fed to the other group, used as

control. After 24 hours, the number of predated eggs and *C. (C.) lineafrons* larvae survival were
recorded.

When the larvae of *C. (C.) lineafrons* achieved the pupal stage, they were placed in 1 liter plastic containers covered with voile. The number of males and females which emerged was recorded in each repetition. Emerging individuals were fed and maintained under the same breeding conditions of the adults. Number of eggs laid and adults longevity was registered every 24 hours (Fig.2).

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#### 109 Data analysis

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

113 Mean, average and percentage of eggs predated by *C. (C.) lineafrons* were calculated. In 114 addition, the different life stages survival (egg, larva and pupa) of lacewing was determined, as 115 well as adults longevity and number of eggs laid per female per day fed both with *B. tabaci* eggs 116 and *S. cerealella* eggs.

117

#### 118 **RESULTS**

119 Of the 90 eggs of *C*. (*C*.) *lineafrons* selected at the beginning of this study, only 71 hatched, of

120 which 34 were fed with *B. tabaci* eggs and 37 with *S. cerealella* eggs. During the ingest assay, it

121 was observed that a total of 34 individuals reached the adult state; 28 of these having been fed

with *S. cerealella* eggs, and 6 with *B. tabaci* eggs.

123

#### 124 Ingestion assay

In general, larvae of C. (C.) lineafrons consumed an average of 127.04 B.tabaci eggs and 44 S. 125 cerealella eggs per day. When each larval stage was evaluated separately, it was observed that 126 larvae I consumed a maximum of B. tabaci 556 eggs and 189 S. cerealella eggs, while larvae II 127 consumed 531 B. tabaci eggs and 240 S. cerealella eggs, and larvae III consumed 619 B. tabaci 128 129 eggs and 150 S. cerealella eggs per day (Table 1). On the other hand, the developmental time of the different larval stages was similar between larvae I and II, whereas it was shorter for larvae 130 III. Regarding C. (C.) lineafrons developmental time, an average of 45 days was recorded when 131 132 the larvae fed with *B. tabaci* eggs and 35 days when they fed with *S. cerealella* eggs (Table 2). 133

134 Survival, longevity and oviposition

135	In general, C. (C.) lineafrons eggs survival was 81.1%. The immature stages of C. (C.) lineafrons
136	fed with <i>B. tabaci</i> eggs exhibited lower survival (8.2%) than those fed with <i>S. cerealella</i> eggs
137	(30.1%). Lacewings survival significantly decreased from the larval to the adult stage, for
138	individuals fed both with <i>B. tabaci</i> eggs and <i>S. cerealella</i> eggs (Fig. 3).
139	In relation to C. (C.) lineafrons adults longevity, both a greater number of specimens and a
140	greater longevity (41 days approximately) was observed from larvae fed with S. cerealella eggs,
141	while for those fed with B. tabaci eggs longevity was 20 days (Fig. 4a). Lastly, C. (C.) lineafrons
142	oviposition was also higher in females fed with S. cerealella eggs (Fig. 4b).

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#### 144 DISCUSSION

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

In relation to ingest capacity, C. (C.) lineafrons larvae consumed a higher number of B. 149 tabaci eggs than S. cerealella eggs per day, with larvae II standing out. These results are 150 151 comparable to those reported by Legaspi et al. (1994), who determined that a greater number of B. tabaci eggs were necessary for the development of Chrysoperla rufilabris Bumeister larvae 152 III. Other studies reported that Chrysoperla carnea Stephens was able to eat up to 200.5 B. 153 154 tabaci nymphs and 171.8 Amrasca devastans Distant nymphs (Nisar Syed et al., 2005); and that the larvae could consume about 8000 S. cerealella eggs and 510 B. tabaci pupae throughout its 155 development (Gallardo et al., 2005). Also, Legaspi et al. (1994) determined that C. rufilabris 156 157 larvae consumed an average of 531.55 B. tabaci eggs per day, whereas Avila et al. (2009)

reported that Chrysoperla argentina Steimann larvae ingested an average of 275 B. tabaci eggs 158 per day. This behavior might be attributed to the hypothesis suggested by Nisar Syed et al. 159 (2005), who pointed out that prev density exhibits a strong influence on predation potential; i.e. 160 the increase in egg consumption could be due to increases in larvae density. 161 Furthermore, the developmental time of C. (C.) lineafrons was 45 days when they were fed 162 163 with *B. tabaci* eggs and 35 days when they were fed *S. cerealella* eggs. In relation to this, studies performed by Ramirez-Delgado et al. (2007) with Ceraeochrysa sp. nr. cincta determined that 164 the total developmental time, from egg to adult emergence, was 29 days when the species was 165 fed with S. cerealella eggs. Moreover, Legaspi et al. (1994) recorded a longer duration of larval 166 developmental time in C. rufilabris fed with B. tabaci eggs compared to those fed with S. 167 cerealella eggs. However, Nisar Syed et al. (2005) recorded a shorter developmental time on C. 168 cornea fed with B. tabaci eggs compared to those fed with A. devastans eggs. 169 C. (C.) lineafrons survival decreased as the life cycle progressed; both for individuals fed 170 with B. tabaci eggs, and for those fed with S. cerealella eggs. These results are similar to those 171 obtained by Ramirez-Delgado et al. (2007), who determined a steady decrease of C. sp. nr. 172 cincta longevity when it was fed with S. cerealella eggs. The longevity of C. (C.) lineafrons 173 174 adults fed with S. cerealella eggs was 41 days; while for those fed with B. tabaci eggs it was 20 days, results that agree with those reported by Ramírez-Delgado et al. (2007), who determined a 175 176 greater longevity for C. sp. nr. *cincta* adults fed with S. *cerealella* eggs. 177 In general, C. (C.) lineafrons survival, longevity and number of eggs laid per female were greater when they were fed with S. cerealella eggs than with B. tabaci eggs. In relation to this, 178 179 Giffoni et al. (2007) determined that C. externa completed its life cycle only when being fed

180	with S. cerealella eggs; while Legaspi et al. (1994) recorded that C. rufilabris larvae showed a
181	greater preference for S. cerealella eggs than for B. tabaci eggs.
182	It has been observed that C. (C.) lineafrons exhibits a similar behavior to that of other
183	lacewing species; with the peculiarity that their predation efficiency is much higher, thus turning
184	this species into an excellent tool for efficient biological control of <i>B. tabaci</i> in tomato crops.
185	Therefore, we conclude that the larval stages of C. (C.) lineafrons require a greater number of B.
186	tabaci eggs than of S. cerealella eggs to complete their life cycle; larvae II and III of C. (C.)
187	lineafrons consume more B. tabaci eggs; larval I stage lasts longer than the two other larval
188	stages; survival and longevity of C. (C.) lineafrons adults was greater in individuals fed with S.
189	cerealella eggs, and the number of eggs laid per female was also higher.
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206	The authors declare there are no competing interests.
207	
208	Author Contributions
209	• Eugenia S. Ortega performed collecting and determination of specimens, analyzed the data,
210	contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables,
211	reviewed drafts of the paper.
212	• Cecilia A. Veggiani Aybar performed analyzed the data, contributed wrote the paper, prepared
213	figures and/or tables, reviewed drafts of the paper.
214	• Ana L. Ávila contributed wrote the paper, prepared figures and/or tables, reviewed drafts of the
215	paper.
216	• Carmen Reguilón contributed wrote the paper, prepared figures and/or tables, reviewed drafts
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218	
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283	FIGURE CAPTIONS
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285	Figure 1 Geographical location of the tomato crops in Tucumán Province, northwestern
286	Argentina.
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288	Figure 2 A. Rearing of C. (C.) lineafrons; B. Eggs; C.and D. Larvas; E. Pupal stage; F. Adults.
289	
290	Figure 3 Viability of different developmental stages of C. (C.) lineafrons fed with B. tabaci eggs
291	and S. cerealella eggs.
292	

- **Figure 4** (a) Longevity and (b) Oviposition of *C. (C.) lineafrons* adult fed with *B. tabaci* and *S.*
- 294 *cerealella* eggs.
- 295
- 296 TABLE CAPTIONS
- 297
- **Table 1.** Daily egg consumption of *C*. *(C.) lineafrons* larvae fed with *B. tabaci* eggs and *S.*
- 299 *cerealella* eggs.
- 300
- **Table 2.** Developmental time of different stages of *C*. *(C.) lineafrons* fed with *B. tabaci* eggs and
- 302 *S. cerealella* eggs.
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- 306

### Table 1(on next page)

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

- 1 Table 1 Daily eggs consumption of C. (C.) lineafrons larvae fed with B. tabaci eggs and S.
- 2 *cerealella* eggs.
- 3

	Number of		Eggs c	onsumption	
Stage	individuals evaluated	Total	Average/individuals	Average/individuals/day	Rank
B. tabaci					
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
S. cerealella					
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

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### Table 2(on next page)

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

- 1 Table 2 Developmental time of different stages of C. (C.) lineafrons fed with B. tabaci eggs and
- 2 *S. cerealella* eggs.
- 3

Stage	Mean $\pm$ SD	Life cycle duration
B. tabaci		
L1	$11.23 \pm 0.56$	45.2
L2	$11.16 \pm 1.03$	
L3	$7.09 \pm 0.74$	
Pupa	$15.75 \pm 0.51$	
S. cerealella		
L1	$9.97 \pm 0.69$	34.9
L2	$6,06 \pm 0,7$	
L3	$3,66 \pm 0,4$	
Pupa	$15,21 \pm 0,57$	

4 Means  $\pm$  SE followed by P < 0.05

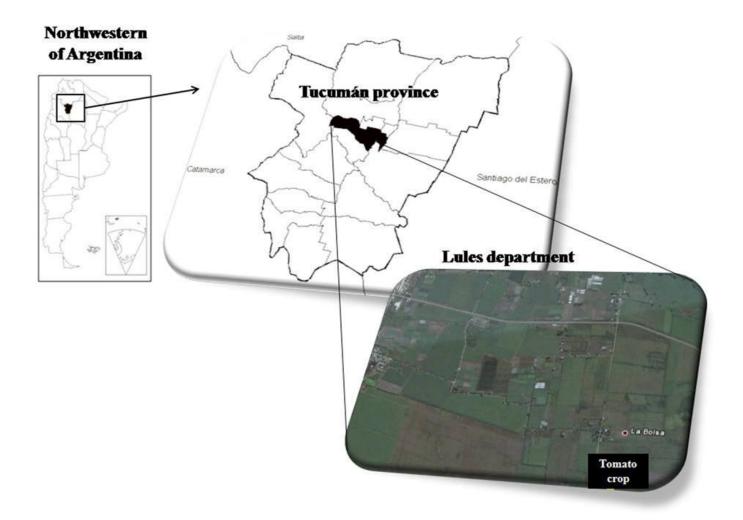
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## Figure 1

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

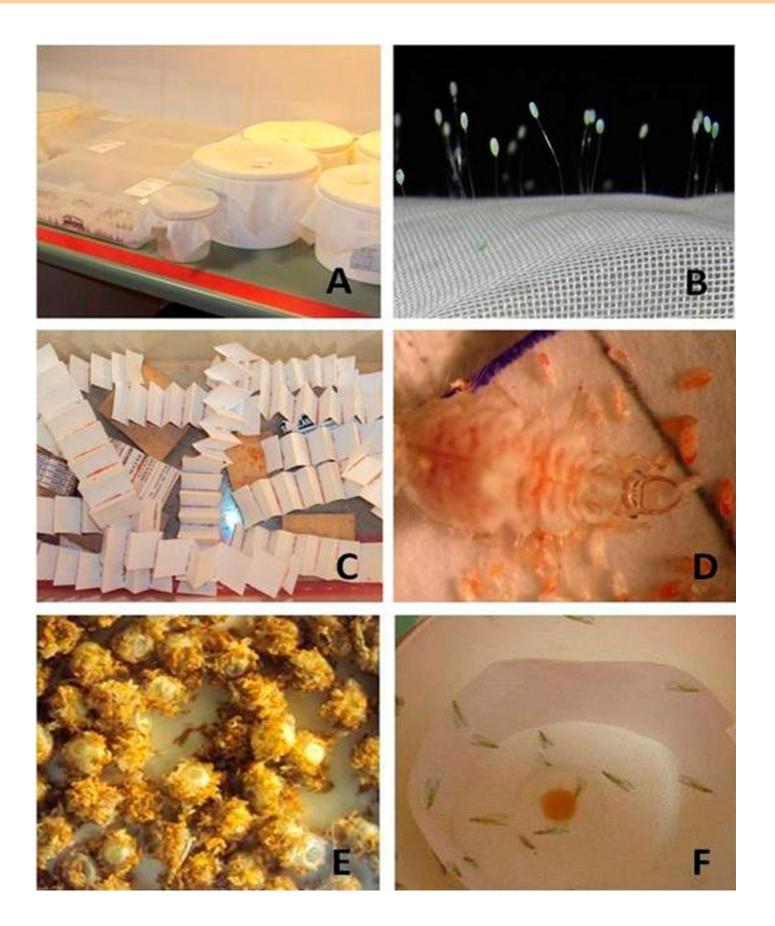


## Figure 2

A. Rearing of C. (C.) lineafrons, B. Eggs, C.and D. Larvas, E. Pupal stage, F. Adults.

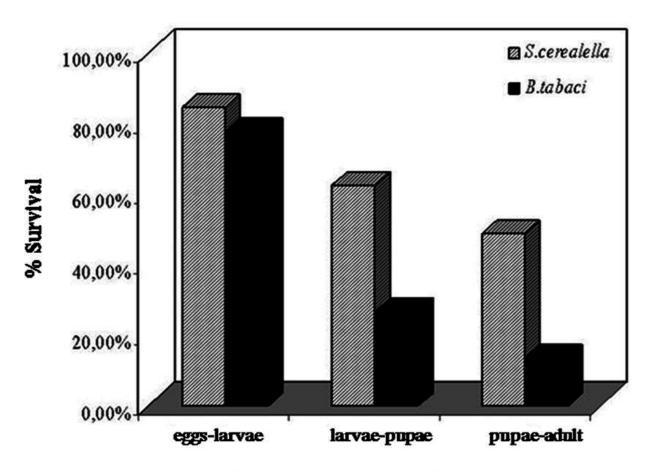
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## Figure 3

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



Chrysopodes (C.) lineafrons stage

## Figure 4

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

