

## Predation and parasitism of *Cirrospilus neotropicus* (Hymenoptera: Eulophidae) on *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in laboratory

Predación y parasitismo de *Cirrospilus neotropicus* (Hymenoptera: Eulophidae) en *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) en laboratorio

ESTER FOELKEL<sup>1</sup>, LUIZA RODRIGUES REDAELLI<sup>2</sup>, SIMONE MUNDSTOCK JAHNKE<sup>3</sup>  
and PAULA BAERLE LOSEKANN<sup>4</sup>

**Abstract:** The predation and parasitism of *Cirrospilus neotropicus* (Hymenoptera: Eulophidae) was evaluated on third instar *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) larvae reared on *Citrus limonia* under controlled conditions ( $25 \pm 1^\circ\text{C}$ ; L:D 12:12 h). Two groups of parasitoid females were evaluated: one in which pupae were collected from citrus orchards (field females) at Eldorado do Sul, RS, Brazil ( $30^\circ 29' \text{S}$ ,  $51^\circ 06' \text{W}$ ), and the other from a laboratory colony (laboratory females). After mating, each female was maintained in an individual container and fed honey, pollen and 12 *P. citrella* every 48 hours. In both groups the behaviors of predation (host feeding), oviposition and superparasitism were observed. The average percent mortality caused by field females ( $48.71 \pm 3.29\%$ ) was significantly higher than that caused by laboratory females ( $35.12 \pm 3.45\%$ ) ( $H = 4.74$ ;  $P = 0.01$ ). Mortality due to predation was  $19.15 \pm 1.75\%$  in field females and  $25.11 \pm 3.82\%$  in laboratory females, with no significant difference ( $H = 1.57$ ;  $P = 0.21$ ). The average percent parasitism without predation did not differ significantly between field ( $49.15 \pm 2.89\%$ ) and laboratory females ( $42.33 \pm 4.07\%$ ) ( $H = 0.53$ ;  $P = 0.47$ ). Superparasitism represented  $6.92 \pm 1.21\%$  of the total hosts killed by field females, and  $3.51 \pm 0.99\%$  by laboratory females. The results show the influence of *P. citrella* in the death of *C. neotropicus* caused by parasitism and predation.

**Key words:** Citrus leafminer. Parasitoid. Mortality. Biological control. Superparasitism.

**Resumen:** Se evaluaron la depredación y parasitismo de *Cirrospilus neotropicus* (Hymenoptera: Eulophidae) sobre larvas del tercer instar de *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) alimentadas con hojas de *Citrus limonia* en condiciones controladas ( $25 \pm 1^\circ\text{C}$ ; L:D 12:12 h). Dos grupos de parasitoides hembras fueran evaluadas: uno en que las pupas se coleccionaron de pomar cítrico (hembras del campo) en Eldorado do Sul, RS, Brasil ( $30^\circ 29' \text{S}$ ,  $51^\circ 06' \text{W}$ ), y el otro de un cría laboratorio (hembras de laboratorio). Después del apareamiento, se mantuvo cada hembra en un contenedor individual, alimentada con miel, polen y 12 larvas de *P. citrella* cada 48 horas. En los dos grupos se observaron los comportamientos de depredación, oviposición y superparasitismo. La porcentaje de mortalidad promedio causado por las hembras del campo ( $48,71 \pm 3,29\%$ ) fue significativamente superior al de laboratorio ( $35,12 \pm 3,45\%$ ) ( $H = 4,74$ ;  $P = 0,01$ ). La mortalidad debido a la depredación fue  $19,15 \pm 1,75\%$  en las hembras de campo y  $25,11 \pm 3,82\%$  en las de laboratorio, sin diferencia significativa ( $H = 1,57$ ;  $P = 0,21$ ). El porcentaje promedio de hospederos parasitados sin depredación no se diferenció entre las hembras de campo ( $49,15 \pm 2,89\%$ ) y las de laboratorio ( $42,33 \pm 4,07\%$ ) ( $H = 1,57$ ;  $P = 0,21$ ). El superparasitismo representó  $6,92 \pm 1,21\%$  del total de los hospederos muertos por las hembras de campo, y  $3,51 \pm 0,99\%$  por las de laboratorio. Los resultados evidencian la influencia de *P. citrella* en la muerte causada por el parasitismo y predación de *C. neotropicus*.

**Palabras clave:** Minadora de cítricos. Parasitoide. Mortalidad. Control biológico. Superparasitismo.

**Resumo:** A predação e o parasitismo de *Cirrospilus neotropicus* (Hymenoptera: Eulophidae) em larvas de 3º instar de *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), utilizando como planta hospedeira *Citrus limonia*, foi observado sob condições controladas ( $25 \pm 1^\circ\text{C}$ ; L:D 12:12 h). Dois grupos de fêmeas foram acompanhados: um proveniente de câmaras pupais coletadas em pomares de citros (fêmeas do campo) em Eldorado do Sul ( $30^\circ 29' \text{S}$ ,  $51^\circ 06' \text{W}$ ), RS, Brasil, e o outro, de uma criação em laboratório (fêmeas do laboratório). Após o acasalamento, as fêmeas foram mantidas em caixas gerbox, recebendo, a cada 48h, 12 lagartas de *P. citrella*, mel e pólen. Os seguintes comportamentos foram observados em ambos os grupos: picadas alimentícias (predação) nos hospedeiros, oviposição e superparasitismo. A média do total da mortalidade dos hospedeiros pelas fêmeas do campo foi significativamente superior ( $48,71 \pm 3,29\%$ ) do que as de laboratório ( $35,12 \pm 3,45\%$ ) ( $H = 4,74$ ;  $P = 0,01$ ). Considerando apenas predação,  $19,15 \pm 1,75\%$  de hospedeiros foram mortos pelas fêmeas de campo, contra  $25,11 \pm 3,82\%$  de laboratório ( $H = 1,57$ ;  $P = 0,21$ ). A percentagem média de larvas mortas apenas por parasitismo não diferiu significativamente entre os dois grupos:  $49,15 \pm 2,89\%$  de mortos por fêmeas de campo e de  $42,33 \pm 4,07\%$  pelas de laboratório ( $H = 1,57$ ;  $P = 0,21$ ). O superparasitismo ocorreu em  $6,92 \pm 1,21\%$  do total de hospedeiros mortos pelo grupo do campo e  $3,51 \pm 0,99\%$  pelo de laboratório. Os resultados evidenciaram a morte de *P. citrella* por *C. neotropicus* através do parasitismo e da predação.

**Palavras-chave:** Minador-dos-citros. Parasitoide. Mortalidade. Controle biológico. Superparasitismo.

<sup>1</sup> M. Sc., Universidade Federal do Rio Grande do Sul. [ester.foelkel@via-rs.net](mailto:ester.foelkel@via-rs.net).

<sup>2</sup> Dra. Universidade Federal do São Carlos. [luredael@ufrgs.br](mailto:luredael@ufrgs.br).

<sup>3</sup> Dra. Universidade Federal do Rio Grande do Sul. [mundstock.jahnke@ufrgs.br](mailto:mundstock.jahnke@ufrgs.br). Autor para correspondencia.

<sup>4</sup> Agronomic Engineer Student. Universidade Federal do Rio Grande do Sul. [pblosekann@yahoo.com.br](mailto:pblosekann@yahoo.com.br).

<sup>1,2,3,4</sup> PPG em Fitotecnia, UFRGS, Av. Bento Gonçalves, 7712, 91540-000, Porto Alegre, RS, Brazil.

## Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae), is a microlepidopterous species, native from Southeast Asia (Clausen 1931). Since 1993, *P. citrella* has been rapidly colonizing citrus orchards in diverse regions worldwide (Hoy and Nguyen 1997). In Brazil, it was first recorded in 1996, in the region of Limeira of the state of São Paulo, and in a few months was able to spread to all other citrus growing areas of the country (Cônoli 2001). The citrus leafminer is considered a menace to citriculture due to the economical losses that it causes by mining the leaves. In addition to the reduction of the photosynthetic area, mining may facilitate the access of the citrus canker bacteria (Chagas and Parra 2000; Ujiye 2000; Chagas *et al.* 2001).

Among the alternatives to control the leafminer, the release of the exotic parasitoid *Ageniaspis citricola* Logvinoskaya, 1983 (Hymenoptera: Encyrtidae) has been carried out in Brazil and in various other citrus-growing countries around the world (Neale *et al.* 1995; Pomerinke and Stansly 1998; Paiva *et al.* 2000; Urbaneja *et al.* 2002). Nonetheless, the action of native parasitoids on the leafminer has been verified in diverse citrus growing regions (LaSalle and Peña 1997; Pentead-Dias *et al.* 1997; Paiva *et al.* 1998; Sá *et al.* 1999).

In Brazil, a noteworthy number of indigenous parasitoids were recorded associated with *P. citrella*. Among these, are the eulophids of the genera *Cirrospilus*, *Elasmus*, *Sympiesis* and *Chrysocharis* (Jahnke *et al.* 2005; Efrom 2006). Jahnke *et al.* (2006) and Efrom (2006), in studies carried out in Montenegro County, Rio Grande do Sul, in a four-year survey, found *Cirrospilus neotropicus* Diez & Fidalgo, 2003 (Hymenoptera: Eulophidae) as the most frequent species among the native parasitoids. Only the exotic *A. citricola*, previously released in a nearby area to the study orchard, was more frequent. The same phenomenon was observed in Argentina, where *C. neotropicus* kept a parasitism percentage of 19% on *P. citrella*, only lower than that of *A. citricola* (54%) (Diez *et al.* 2000).

The ectoparasitoid *C. neotropicus* was recorded parasitizing *P. citrella* practically all over Latin America. However, there are no records of the native hosts of this parasitoid. The first records of this species were done in Cuitlahuac and in Colima, Mexico in 1996 (Bautista-Martinez *et al.* 1996; Perales-Gutiérrez *et al.* 1996), but the species was only described in 2003 by Diez and Fidalgo (2003).

Information regarding bionomic aspects of *C. neotropicus* and its contribution to *P. citrella* mortality is very scarce. Desirable characteristics of a biocontrol agent are described by Jervis and Kidd (1996) as synchronism with the host and seeking efficiency. According to Garcia (1991), the viability of parasitoid progeny may vary depending on the suitability both of the host of the immature female and of the plant upon the host had developed. Thus, females that developed in orchard plants in the field may present offspring with higher survival rates than those that were grown in artificial conditions. Therefore, this work endeavored to study the behavior of predation and parasitism of *C. neotropicus* females aiming to gather data to evaluate the potential of this parasitoid as a candidate to biological control agent of *P. citrella*.

## Material and Methods

Individuals of *C. neotropicus* emerged from pupae and collected from citrus orchards ("field females") located in Eldorado do Sul County (30°29'S, 51°06'W), Rio Grande do Sul State, Brazil, and individuals obtained from the first generation reared in laboratory (laboratory females) were utilized. Soon after emergence each female was transferred to a glass test tube (diameter = 3 cm; length = 10 cm) and kept with five males in a climatic chamber (25 ± 1°C, L:D 12:12 h) for five days to promote mating. Adults were fed with honey and pollen, and water was provided with a wetted cotton placed in the bottom of the glass tube (Urbaneja 2000). Posteriorly, in the same climatic chamber, females were individualized in Plexiglas boxes (12 cm x 12 cm x 3cm) with leaves of *Citrus limonia* Osb. attached to water-agar (WA) culture medium, to keep them fresh enough during two days. Each leaf had 12 unparasitized third instar larvae of *P. citrella*, originated from a laboratory culture. At two-day intervals, citrus leaves were replaced with fresh leaves containing 12 third instar larvae. This procedure was repeated until the death of the females. We considered third instar larvae those measuring between 3 to 4 mm in length according to Cônoli *et al.* (1996) and Lim and Hoy (2005).

The leaves bearing *P. citrella* larvae were removed from the Plexiglas box, checked using a stereomicroscope, and the condition of each host larva recorded after exposition to parasitoids. Leaves with parasitized larvae were kept singly in Petri dishes (diameter = 10 cm; height = 1.5 cm) containing 2% WA medium and maintained in the same climatic chamber. Host larvae without parasitism were removed. The number of larvae killed by host feeding, oviposition or superparasitism were recorded, and the mean percentage at each sampling occasion, was estimated. Parasitism and predation (host feeding) were examined on 23 females of *C. neotropicus* from the field group, and on 22 females from the laboratory group. The females from the field group were exposed to 2.304 larvae of *P. citrella* and those from of the laboratory group to 1.752 larvae.

The statistical analysis was performed on the percentage arcsin-transformed data and tested to normality using the Shapiro-Wilk test. The percentage values of the simple parasitism, superparasitism, and feeding stings were compared by one-way analysis of variance (ANOVA) or by the Kruskal-Wallis test. The association between variables was calculated using Pearson correlation coefficient. The significance level adopted was 0.05. All calculations were performed using the computer programs BioEstat® 4.0 and Microsoft Office Excel® 2000.

## Results

*C. neotropicus* females insert its ovipositor into the body of *P. citrella* larvae paralyzing it before laying eggs outside its body. Regarding the distance from the host body, it was recorded that 2.56% of the total eggs deposited by females in the field group and 4.47% laid by females in the laboratory group, were located 5 mm further from the body of the host. Females in the field group were responsible for a significantly higher mean percentage of total mortality (48.7 ± 3.3%) than the females from the laboratory group (35.1 ± 3.4%) (H = 4.74; df = 1; P = 0.01). The total mean number of individuals

**Table 1.** Mean percentage  $\pm$  SE of different conditions of third instar larvae of *Phyllocnistis citrella* (Lep.: Gracillariidae) killed by females of *Cirrospilus neotropicus* (Hym.: Eulophidae) from field (n= 23) and laboratory (n=22) ( $25 \pm 1$  °C; L:D 12:12 h).

Host condition	% killed larvae (mean $\pm$ SE)	
	field	laboratory
Host feeding	19.51 $\pm$ 1.75 A <sup>1</sup>	25.11 $\pm$ 3.82 A
Host feeding and 1 egg	28.21 $\pm$ 2.15 B	30.9 $\pm$ 3.33 A
Host feeding and 2 eggs	2.72 $\pm$ 0.525 A	1.31 $\pm$ 0.58 B
Host feeding and 3 eggs	0.62 $\pm$ 0.258 A	0.12 $\pm$ 0.12 A
Host feeding and 4 eggs	0.08 $\pm$ 0.053 A	0.22 $\pm$ 0.22 A
Host feeding and 5 eggs	0.09 $\pm$ 0.059	—
1 egg	45.75 $\pm$ 2.846 A	40.47 $\pm$ 3.95 A
2 eggs	2.16 $\pm$ 0.62 A	1.47 $\pm$ 0.61 A
3 eggs	0.77 $\pm$ 0.3467A	0.32 $\pm$ 0.21 A
4 eggs	—	0.08 $\pm$ 0.06
5 eggs	0.09 $\pm$ 0.059	—

<sup>1</sup> Data within the same row followed by the same letter means that are not significantly different (P = 0.02).

killed by field females was  $51.13 \pm 7.5$ , varying from 11 to 120, meanwhile for the laboratory females the average was  $26.5 \pm 9.4$ , fluctuating from five to 82, being the former significantly higher (F = 10.45; df = 1; P < 0.01).

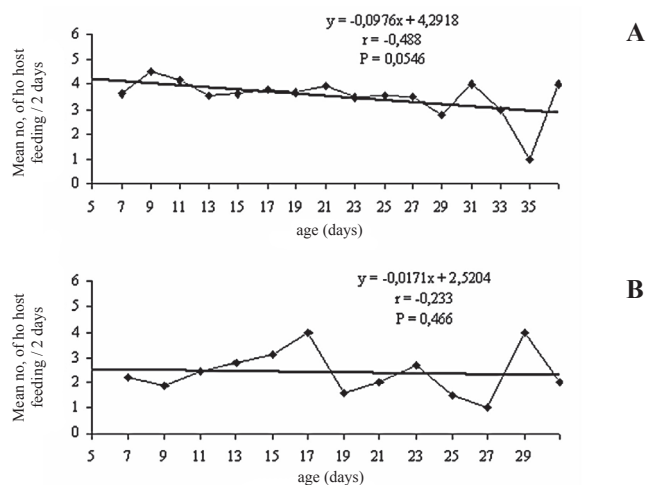
In both groups of parasitoids, host feeding, host feeding + oviposition, only oviposition, and superparasitism were observed. Forty eight hours after the first exposure to the host, 96% of the field females and 100% of the laboratory females had already oviposited. Considering the 1.176 larvae of *P. citrella* killed by field females, 258 of them suffer host feeding, 397 host feeding + oviposition and 521, only oviposition. In relation with the 583 killed larvae by the laboratory group, 130 suffered host feeding, 191 host feeding + oviposition and 262 only oviposition. There were no significant differences between the field ( $31.7 \pm 2.2\%$ ) and laboratory female groups ( $32.6 \pm 3.4\%$ ) regarding the host feeding and parasitism rates (H = 0.08, df = 1, P = 0.77). The mean percentage of hosts killed by host feeding only, also did not differ significantly between field ( $19.5 \pm 1.7\%$ ), and laboratory females ( $25.1 \pm 3.8\%$ ) (H = 1.57; df = 1; P = 0.21) (Table 1).

The mean percentage of killed hosts by parasitization only did not differ between the field ( $49.2 \pm 2.9\%$ ) and the laboratory groups ( $42.3 \pm 4.1\%$ ) (H = 0.53; df = 1; P = 0.47). A difference was found between the percentage of host larvae showing host feeding and a single egg (H = 7.36; df = 1; P < 0.01) and the percentage of those with host feeding and two eggs (H = 5.79; df = 1; P < 0.01) (Table 1).

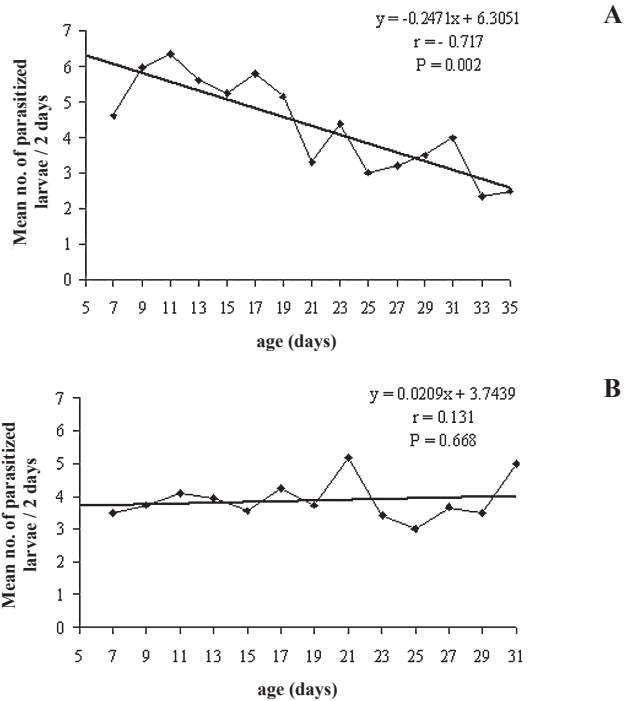
There was no significant difference in the mortality of host larvae, (H = 0.52; df = 1; P = 0.47) with feeding stings, between the field ( $50.9 \pm 2.9\%$ ) and the laboratory groups ( $57.7 \pm 4.1\%$ ). The feeding stings occurred with or without parasitism. The feeding stings, caused by a host feeding, resulted in a darkened coloration in the body of the host at the lesion site, and showed a turgid aspect with a spot and haemolymph exudation. A decreasing number of host feeding associated with female age was recorded for both groups (Fig. 1). There was no difference in the percentage of total number of larvae killed by parasitization between field females (80.01%) and laboratory females (79.9%) (H = 1.42; df = 1; P = 0.23).

All females of the laboratory generation made the first oviposition up the second day of host exposition, and only one from the field generation oviposited after the fourth day. For field females, the number of parasitized hosts decreased as their age increased (r = -0.72; P < 0.01). This decrease was not significant for laboratory females (r = 0.13; P = 0.67) (Fig. 2). The mean percentage of larvae with oviposition only was higher than the superparasitism in both female groups, being  $90.6 \pm 1.6\%$  for the field females and  $85.9 \pm 6.4\%$  for the laboratory females (H = 1.79; df = 1; P = 0.18) (Table 1).

When superparasitism occurred, more than one larva hatched. Even though, the emergence of a single parasitoid per host was verified. The mean percentage of superparasitism found for field females ( $6.9 \pm 1.2\%$ ) was higher (H = 5.2; df = 1; P = 0.02) than of the observed for laboratory females ( $3.5 \pm 0.9\%$ ). Total percentage of superparasitism in the field



**Figure 1.** Mean number of *Phyllocnistis citrella* (Lep.: Gracillariidae) larvae with host feeding performed by females of *Cirrospilus neotropicus* (Hym.: Eulophidae), after the 5<sup>th</sup> day of life, recorded at 48 h intervals, (A) field females and (B) laboratory females ( $25 \pm 1$  °C; L:D 12:12 h).

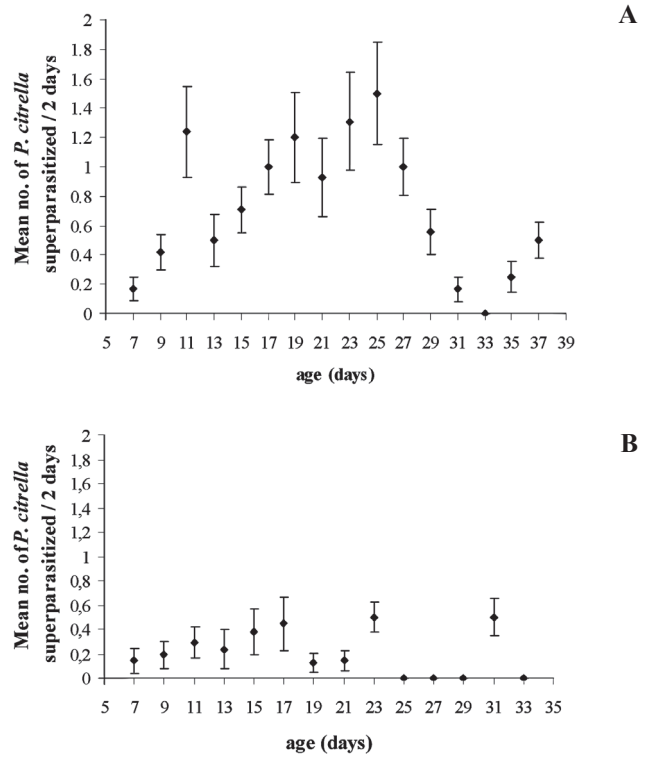


**Figure 2.** Mean number of *Phyllocnistis citrella* (Lep.: Gracillariidae) larvae parasitized by females of *Cirrospilus neotropicus* (Hym.: Eulophidae), after the 5<sup>th</sup> day of life, recorded at 48 h intervals. (A) field females and, (B) laboratory females (25 ± 1°C; L:D 12:12h).

generation was 11.7% and 7.1% in the laboratory colony. Superparasitism was observed in both groups since the first observation, carried out two days after the initial exposure to the hosts. Three days after the first oviposition, the total superparasitism average numbers increased especially in the field group (Fig. 3). After the 25<sup>th</sup> day of observation, a reduction in both groups was observed. The maximum mean percentage recorded at two days interval represented 20% and 5% of the host total offered, in the field and in the laboratory groups respectively.

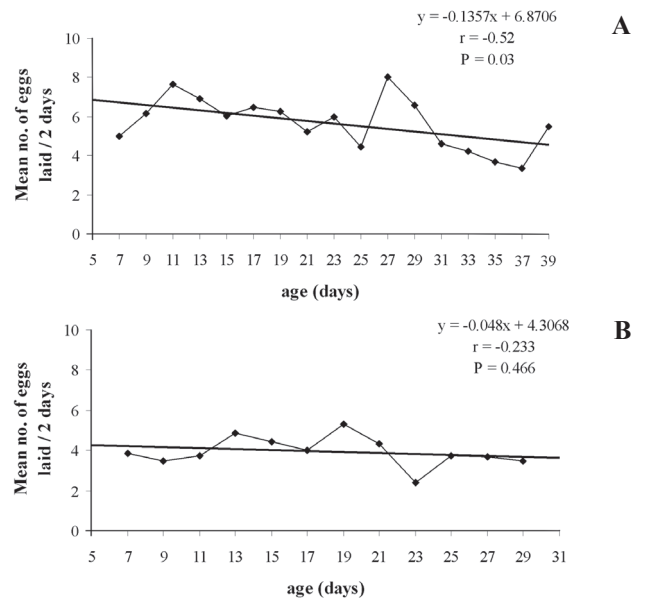
Female host feeding behavior was more frequent than superparasitism. All females from both groups showed host feeding, while only ten females from the laboratory generation and 20 from the field showed superparasitism, laying a minimum of two and a maximum of five eggs per host (Table 1).

There was a significant difference in the total mean number of eggs laid every two days by the field females (5.1) and by the laboratory females (4.0) ( $H = 11.77$ ,  $df = 1$ ,  $P = 0.04$ ). The maximum mean number of eggs deposited by the field females, at each sampling occasion, was 7.7, at the 27<sup>th</sup> day of life, and 5.3, at the 19<sup>th</sup>, by the laboratory females (Fig. 4). The number of eggs laid every two days decrease significantly with the ageing of the field females ( $r = -0.52$ ;  $P = 0.03$ ), meanwhile, this correlation was not significant for the laboratory females ( $r = -0.23$ ;  $P = 0.47$ ) (Fig. 4). The majority of females in both groups oviposited until the last day of their lives, suggesting that there is not a post-oviposition period in this species. The mean longevity was 21.8 days for the field generation, and 16.8 days for the laboratory generation. In the first half of their lives, the field females laid 49.3% of their total egg load. Meanwhile, the laboratory females laid



**Figure 3.** Mean number and standard error of *Phyllocnistis citrella* (Lep.: Gracillariidae) larvae superparasitized by females of *Cirrospilus neotropicus* (Hym.: Eulophidae), after the 5<sup>th</sup> day of life, recorded at 48 h intervals. (A) field females and, (B) laboratory females (25 ± 1°C; L:D 12:12h).

28.3% in this period, indicating a higher oviposition activity in the second half of their lives.



**Figure 4.** Mean number of eggs laid on *Phyllocnistis citrella* (Lep.: Gracillariidae) larvae by females of *Cirrospilus neotropicus* (Hym.: Eulophidae), after the 5<sup>th</sup> day of life, recorded at 48 h intervals. (A) field females and, (B) laboratory females (25 ± 1°C; L:D 12:12h).



## Discussion

Host paralyzation before oviposition, a characteristic of idiobiontic parasitoids, is also listed for other eulophids such as *Cirrospilus pictus* (Ness, 1834), *Cirrospilus diallus* (Walker, 1838), *Cirrospilus vittatus* (Walker, 1838) and *Cirrospilus* near *lyncus* (Urbaneja 2000; Urbaneja *et al.* 2002; Lo Pinto *et al.* 2005). A percentage of 21.55% eggs of *C. pictus* and *C. diallus*, deposited at a distance of 6 mm from the host (*P. citrella*) was observed by Lo Pinto *et al.* (2005). Total mortalities similar to these observed for *C. neotropicus* were found, in laboratory conditions, by Lo Pinto *et al.* (2005) for *C. pictus* (30%) and *C. diallus* (35%) at similar host densities.

Host feeding as a factor of host mortality is a very common behavior in eulophids, as well as in other groups because the body of the host represents a rich protein source, which gives the females energy for oviposition (Christie and Parrella 1987; Ridgway and Mahr 1990; Jervis and Kidd 1996; Urbaneja 2000; Urbaneja *et al.* 2002; Lim and Hoy 2005; Lo Pinto *et al.* 2005). The behavior of host feeding may be related to the reproductive strategy of synovigenic, where, at emergence, the eggs are not mature and need proteins from the females to reach a mature stage (Van Driesche and Belows Jr. 1996).

Ridgway and Mahr (1990) observed that the eulophid *Sympiesis marylandensis* Girault, 1917, kills its host, *Phylonorhynchus blancardella* (F., 1761) (Lepidoptera: Gracillariidae), by inserting its ovipositor in an exploratory behavior without necessarily laying eggs, but which consequently lead to host paralyzation. According to these authors, the paralyzed larva has a different appearance when compared to that which suffered host feeding: the former showing a yellowish coloration and the latter, a darkened coloration, similar to that observed for *P. citrella* exposed to *C. neotropicus*. Thus, it is possible that in the present study, some hosts that were considered killed by host feeding were indeed killed by paralyzation, considering that no difference was observed between these conditions. Lim and Hoy (2005) mentioned that in experiments where *Semiolacher petiolatus* Girault, 1917 parasitized *P. citrella*, some figures attributed to host feeding were, possibly, due to inserting its ovipositor which might lead to overestimate of the former figures.

The recorded decrease in the number of host feeding and in the number of laid eggs, which occurred with female ageing, could be related to the reduction in their energy consumption. Also, it is possible that this reduction was not noticeable in the laboratory females due to their lower mean longevity (16.8 days), when compared to the field females (21.8 days). Therefore, the former would not have demonstrated all their fecundity potential. The solitary parasitism, recorded in a greater percentage relative to other behaviors, may be beneficial to idiobiontic and solitary parasitoids such as *C. neotropicus*. According to Quicke (1997), Jervis and Kidd (1996) and Godfray (1994) the solitary parasitism is advantageous to this group because usually either the female parasitoid kills the host when ovipositing or the parasitoid larva does it soon after eclosion. Therefore, the immature individual has a limited resource to its development, which may not allow for the successful development of another individual. This does not occur in koinobiont parasitoids, because the host remains alive and growing. Solitary parasitoids demonstrate a remarkable intra-specific competition, eliminating the resource competitors in the same host (Jervis and Kidd 1996). In the present

study, the emergence of a single individual, from larvae superparasitized, supports the data of Urbaneja (2000) that did not verify the survival of a second parasitoid in the same host, in the case of *C. near lyncus*. In both cases, probably occurred competition for resources and cannibalism. Cannibalism is a very common behavior in solitary parasitoids (Viggiani 1964; Urbaneja 2000; Urbaneja *et al.* 2002; Goubault *et al.* 2003; Lim and Hoy 2005; Lo Pinto *et al.* 2005). On the other hand, Urbaneja *et al.* (2002) observed, for *C. vittatus*, that in conditions of superparasitism, up to four eggs per *P. citrella* larva, there was the emergence of more than one parasitoid individual per host.

For the eulophid *S. petiolatus* superparasitism in *P. citrella* larvae was observed in citrus orchards in Sicily, Italy (Rizzo and Mineo 2000). Lim and Hoy (2005) verified this behavior for this same species, in laboratory conditions, when 30 third instar larvae of *P. citrella* per day were offered to each female. According to these authors, the confinement in laboratory conditions would have stimulated superparasitism in *S. petiolatus*. Superparasitism, when carried out by a single female, is advantageous in assuring its progeny survival and avoiding the parasitization of the same host larva by other female parasitoids (Godfray 1994). Nevertheless, this author points out that this behavior may be a consequence of female inability to distinguish already parasitized larvae. Percentages of parasitism higher than the recorded in the present study were found by different authors: Urbaneja (2000), in laboratory conditions (25°C; 70% RH; L:D 16:8 h), recorded 34.3% of total parasitism of *C. near lyncus* on *P. citrella* larvae; Urbaneja *et al.* (2002), 42.8% for *C. vittatus*; and Lo Pinto *et al.* (2005), 27% for *C. diallus* and 37.2% for *C. pictus*.

Host feeding had also been recorded by other authors more frequently than superparasitism. Lo Pinto *et al.* (2005) in controlled conditions (26 ± 1°C; 65 ± 5% RH; L:D 16:8 h) recorded for *C. diallus* and *C. pictus* that about 70% of the mortality was caused by stings. Urbaneja (2000), in studies on *C. near lyncus*, at 25°C, observed the following mean percentages: 47.78 ± 7.87 of total parasitism, 21.0 ± 5.7 of host feeding and 68.1 ± 9.3 of total mortality (host feeding % + parasitism %), with a high frequency of parasitism behavior. The eulophid ectoparasitoid, *Diglyphus begini* (Ashmead, 1904), caused mortality of *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae), either by parasitism or by predation, in commercial cultures of ornamental plants and in green houses, in Colombia, totalizing 40% of host predation (Cure and Cantor 2003). The predation activity of various eulophids, in laboratory conditions, has been frequently recorded (Urbaneja 2000; Urbaneja *et al.* 2002; Lim and Hoy 2005; Lo Pinto *et al.* 2005). However, in field conditions, this behavior had not been recorded for Eulophidae in the literature.

The field females, which went through their larval phase in hosts that have developed in adult plants, probably larger than plants kept in laboratory, might have originated more vigorous individuals, which would explain the higher mean number of eggs recorded for this group. Urbaneja (2000) observed in *C. near lyncus* females, at 25°C, that the number of eggs laid increased until the 18<sup>th</sup> day, similar to that recorded for *C. neotropicus* in the laboratory generation. Nevertheless, the author found a higher mean value (10.5 eggs/day) and a steep decrease after this period. Urbaneja *et al.* (2002), studying *C. vittatus*, observed that the oviposition pattern was de-

pendent on female age thus, supporting what was observed in the field females of *C. neotropicus*. Urbaneja *et al.* (2002) verified that all females began oviposition until the second day of life. From the sixth until the 16<sup>th</sup> day, females produced a mean oviposition rate of 5 eggs/day, and they kept laying eggs until the last day of their lives. The present study allowed us to demonstrate that females of *C. neotropicus* of both groups were able to kill *P. citrella* larvae, either by parasitism or predation.

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