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Biology, Parasitoids, and Damage of *Leptoglossus zonatus* and *Leptoglossus gonagra* (Heteroptera: Coreidae) on Citrus

Irwin Duarte S.[1], Ligia Nuñez B.[2], Jorge E. Peña[3]*, and Libardo Pinto[4]

[1]*Facultad de Ciencias, Escuela de Biología, Universidad Industrial de Santander, Bucaramanga–Colombia*

[2]*FUNDIC, Calle 40 No 28A-20, Bucaramanga–Colombia*

[3]*Department of Entomology and Nematology, Tropical Research and Education Center, University of Florida, Homestead, FL 33031*

[4]*Grupo de Prevención y Erradicación de Riesgos Fitosanitarios, Instituto Colombiano Agropecuario,Bucaramanga–Colombia*

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The appearance of watery lesions on citrus fruits in Santander–Colombia, coincided with high densities of *Leptoglossus zonatus* (Dallas) and *L. gonagra* (F.) (Heteroptera: Coreidae) adults present on citrus groves in the same area. The current information on the biology and natural enemies of these coreids in Colombia is scarce, therefore, we report the life cycle of both species, the role of parasitoids of both pests, and their alternative host plants. The first instar nymph of both species survived on *C. tangelo* fruits, but the second instar failed to survive on these hosts. The life cycle of *L. zonatus* and *L. gonagra* using *Phaseolus vulgaris* as a substrate for the first and second instar and *C. tangelo* Ingram & Moore (Sapindales: Rutaceae) for the third to fifth instar resulted on the development of *L. zonatus* in 69.11 ± 3.4 d and the development of *L. gonagra*, on 69.7 ± 3.6 d. Several parasitoids were observed: the encyrtid *Hexacladia* sp. was found parasitizing 1.6% adults of *L. gonagra*; the syrphid *Eristalis* sp. was observed laying-eggs in a male of the same coreid and the tachinid fly, *Trichopoda* sp parasited 11% adults of *L. zonatus*. The alternant host plants were *Solanum americanum* Mill. (Solanales: Solanaceae), *Melothria guadalupensis* (Spreng.) Cogn, *Momordica charantia* (L.) (Cucurbitales: Cucurbitaceae), *Zea mayz* (L.) (Poales: Poaceae), *Psidium guajava* (L.) (Myrtales: Myrtaceae), *C. reticulata* and *C. tangel*o. Statistical analysis of induced lesions to *C. tangelo* fruits, suggests that the presence of *L. zonatus* and *L. gonagra* are related to the presence of watery lesions on citrus fruits in the study area.

About 53 species are known within the genus *Leptoglossus* (Guérin-Menéville) (Heteroptera: Coreidae) (Brailovsky and Barrera, 2004). Among those, *Leptoglossus zonatus* (Dallas) and *Leptoglossus gonagra* (F.) (= *L. australis* F.; *L. membranaceus* F.) are highly polyphagous species with a distribution between southwestern USA and Argentina. *Leptoglossus gonagra* is also present in the eastern hemisphere (Allen, 1969; cited by Mitchell, 2000; Packauskas and Schaefer, 2001). Hosts for these coreids include *Gossypium* sp. (Malvaceae), *Psidium guajava* (L.) (Myrtaceae), *Cucumis* sp. (Cucurbitaceae), *Persea americana* Mill. (Lauraceae), *Passiflora* sp. (Passifloraceae), *Solanum* sp. (Solanaceae), *Zea mayz* (L.), *Sorghum* sp. (Poaceae), *Punica granatum* (L.) (Lythraceae) and *Citrus* spp. (Sapindales: Rutaceae) (Kubo and Batista, 1992; Matrangolo and Waquil, 1994; Mitchell, 2000; Panizzi, 1989; Schaefer and Mitchell, 1983). *Leptoglossus zonatus* and *L. gonagra* can carry *Phytomonas* sp. (Kinetoplastida: Trypanosomatidae) in their gut and salivary glands and are also consider potential vectors of flagellates (Sbravate et al., 1989) and fungi (Ballen et al., 1984; Dammer and Ravelo, 1990). Several encyrtids, eupelmids and eurytomids have been registered in Brazil and USA as natural enemies of *L. zonatus* (Jones, 1993; Marchiori et al., 2002). The tachinid *Trichopoda* sp. has been found parasitizing adults (Souza and Amaral Filho, 1999). *Leptoglossus gonagra* is parasitized by some scelionids and eupelmids (Masner, 1983; Yasuda and Tsurumachi, 1995; Mitchell, 2000). Among the predators, some reduviids, mantids, and arachnids are reported preying on *L. gonagra* (Yasuda and Tsurumachi, 1995). Panizzi (1989) and Mantrogolo and Waquil (1994) studied the life cycle of *L. zonatus* in Brasil and evaluated the nutritional quality of some plant species. Van Reenen (1973) and Amaral and Storti (1976) studied the life cycle of *L. gonagra* and observed that cucurbits are its most frequent host plants. To our knowledge there are no reports on the development of *L. gonagra* and *L. zonatus* on *Citrus* spp.

During 2003, several citrus fruits started showing watery lesions, brown staining, gumming followed by severe fruit drop in the state of Santander, Colombia (ICA, 2003). This damage coincided with unusual high populations of *L. zonatus* and *L. gonagra* in the same area. The objective of this study was to 1) study the life cycle of both *L. zonatus* and *L. gonagra* on tan-

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*Corresponding author; phone: (305) 246-7001, ext. 223; email: jepe@ifas.ufl.edu

gelo, *C. tangelo* and *Phaseolus vulgaris*; 2) identify parasitoids as well as host plants; and 3) evaluate coreid feeding injury on citrus fruits.

Materials and Methods

Life cycle. During Aug. and Sept. 2004, adults of the leaf footed bugs *L. zonatus* and *L. gonagra* were collected on citrus fruits in Girón, Santander, Colombia (873 m.a.s.l. 1295.25 N 1098 E 06). Life cycle studies were conducted inside a screenhouse at the Universidad Industrial de Santander located at 990 m.a.s.l at 29±1 – 21±6 °C, 70% R.H. Adults of both species were placed separately inside 20 × 20 × 30 cm glass containers covered on the top with muslin. Each container had small stalk pieces of corn and guava as oviposition substrates, following the methodology of Amaral and Storti (1976) and Panizzi (1989). Once oviposition was obtained, adults were removed. Initially, emerging nymphs were individually transferred to 8 × 8 cm plastic containers and deposited on *C. tangelo* and *C. reticulata* fruits; containers were then covered with muslin. Survival of both species was recorded. Because both species reached 100% mortality at the end of the second instar (Table 1), rearing of the first instars on citrus fruits was discontinued. Then, a new set of eggs was collected for both species, following the same methodology as expressed above. Emerging nymphs were placed on beans *Phaseolus vulgaris* (L.) (Fabales: Fabaceae) placed in containers as explained above and nymphs emerging under these condictions, kept on a bean diet until they reached the third instar. Once the third instar was reached, nymphs were placed individually in 8 × 8 cm plastic containers covered with muslin and *C. tangelo* fruits placed as a feeding substrate, and changed when necessary. Observations were made daily until adulthood and longevity was determined.

Parasitoids and alternative host plants. The frequency of adults and nymphs were observed from 16:00 to 22:00 hr, once per month, between Aug. 2004 through June 2005, in a citrus grove located at Girón-Santander. Observations also included activity of nymphs and adults on weeds and other potential host plants present in the grove. Those plants where we could only find nymphs were called alternative host plants. Plant samples were collected and identified at the Herbario Nacional del Instituto de Ciencias Naturales (ICN), Universidad Nacional de Colombia (UNAL). Each species of insects were collected, identified using the key of Van Reenen (1973) and Fernandes and Grazia (1992). Both nymphs and adults were held in containers until emergence of parasitoids. Emerged parasitoids were identified by taxonomists at the Universidad de Antioquia, Medellin (UDEA) and the ICN.

Damage to *Citrus tangelo*. During July 2005, 24 trees of *C. tangelo* in the same citrus grove were chosen. One branch located in the middle part of the canopy with two fruits of approximately 225 d old were covered with a 20 × 35 cm muslin bag per tree. Following the methodology of Bolkan et al. (1984), six of these branches were infested each with 2 males and 1 female *L. zonatus*. The same methodology was applied to *L. gonagra*. To compare lesions caused by the leaf footed bugs with those caused by mechanical injury, six other branches were selected on different trees, and fruits were punctured with a sterilized needle to simulate the feeding puncture and bagged. Similar numbers of bagged branches were used as untreated control. During 1 month, each cage was checked every week to observe any insect mortality and dead insects were replaced. At the end of the experiment the fruits were placed in plastic bags, labeled and transported to the laboratory. Numbers of feeding lesions, excluding needle punctures, were analyzed by Kruskal–Wallis and U Mann–Witney tests. Data from diameter of lesions, including needle punctures, previous transformation to log, were analyzed by ANOVA and differences among means were found using Tukey's test. The presence and identification of fungi inhabiting the fruits' peel enclosed in the lesions was done at the Diagnostico Vegetal del Instituto Colombiano Agropecuario (ICA).

Table 1. Development of *Leptoglossus zonatus* and *L. gonagra* feed exclusively with *Citrus tangelo* fruits.

	Eggs	Nymph instar		
Species	N	(N) 1st	(N) 2nd	(N) 3rd
L. zonatus	125	120	102	0
L. gonagra	88	80	76	0

Results and Discussion

Mortality on *Citrus* fruit. Eggs of *L. zonatus* (125) and of *L. gonagra* (88) obtained from the corn and guava stalks were 96% and 91% fertile, respectively, and 85% and 95% of the nymphs of first instar from both species, respectively, survived on tangelo fruits. However, 100% of the nymphs that reached the second instar died (Table 1). According with Panizzi (1989), the first instar do not usually feed because its stylet is very weak and depend during this period on the nutrients from the egg to survive. The mortality of the second instar might be due to because the stylet cannot penetrate the citrus fruit epidermis. During our field observations, we only observed fourth and fifth instars as well as adults feeding on the *Citrus* fruits, but not earlier instars . The first to third instars of both species are usually found on fruits other host plants such as cucurbits.

Life cycle. *Leptoglossus zonatus* deposited a total of 324 eggs in 16 egg masses and *L. gonagra* deposited 214 eggs in 11 egg masses. The highest percentage of eggs hatching of *L. zonatus* (37%) was observed 14 d after oviposition (X ± SD) 12.72 ± 1.15 and the highest number of eggs (X ± SD; 10.18 ± 1.56) hatching for *L. gonagra* (32.5%) was observed 10 d after oviposition (Fig. 1). These data agree with those studies conducted in Brazil by Matrangolo and Waquil (1994) with *L. zonatus* and by Amaral

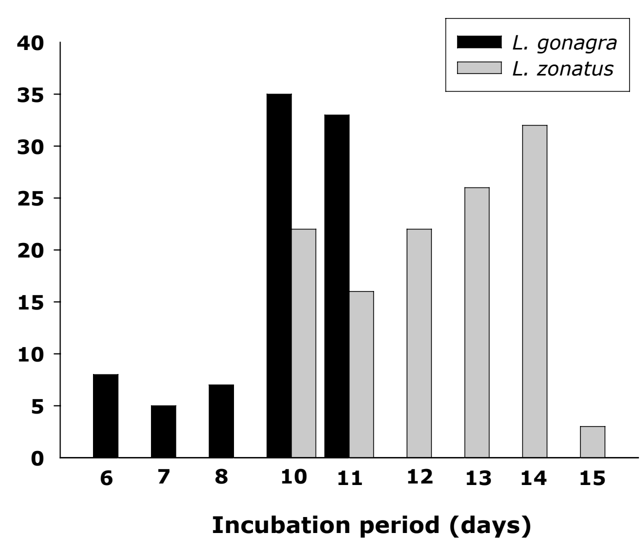

Fig. 1. Frecuency of egg hatch for *Leptoglossus zonatus* and *L. gonagra*, during incubation period (days).

Table 2. Life cycle of *Leptoglossus zonatus* (days), fed on *Phaseolus vulgaris* (first–second instars) and *Citrus tangelo* (third–fifth instars).

Instar	Avg	SD	CV
1st	4.5	± 0.7	15.72
2nd	17.9	± 5.2	29.05
3rd	10.7	± 3.5	33.19
4th	13.2	± 2.9	22.22
5th	22.8	± 4.7	20.66
	69.1	**3.4**	**4.9**

Table 3. Life cycle of *Leptoglossus gonagra* (days), fed on *Phaseolus vulgaris* (first–second instars) and *Citrus tangelo* (third–fifth instars).

Instar	Avg	SD	CV
1st	3.7	± 0.6	16.6
2nd	19.5	± 4.9	25.1
3rd	11.0	± 4.5	40.9
4th	15.2	± 4.8	31.6
5th	20.3	± 2.9	14.5
	69.7	**± 3.6**	**5.1**

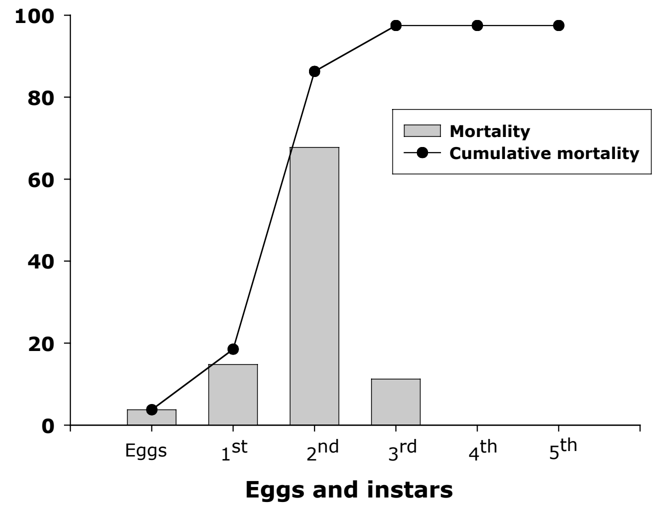

Fig. 2. Relative mortality of eggs and instars of *Leptoglossus zonatus*.

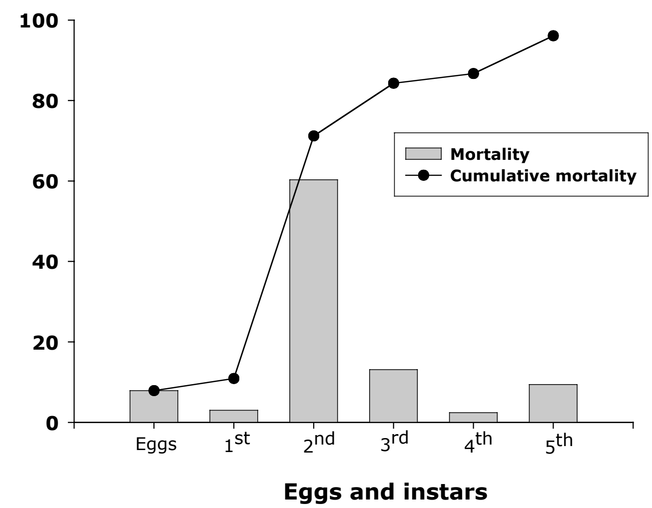

Fig. 3. Relative mortality of eggs and instars of *Leptoglossus gonagra*.

and Storti (1976) with *L. gonagra*. Nymphal development for both species (Tables 2–3) was twice longer than that recorded by Van Reenen (1973) and by Panizzi (1989). These researchers used Poaceae and Curcubitaceae plants as food source. Panizzi (1989) concluded that developmental time was related to the food quality consumed by the nymphs. Therefore, according with the results of this research, citrus could be considered as a poor quality host for these insects. The first nymphal instar lasted 4.5 d average for *L. zonatus* and 3.7 d for *L. gonagra*. These results are in agreement with results of other species of *Leptoglossus* and other coreids (Wheeler Jr. and Miller 1990; Caldas et al. 2000). The fifth instar of *L. zonatus* and the fifth and second of *L. gonagra* lasted longer than all other instars. Similar trend had been found on other species of *Leptoglossus* (Wheeler Jr. and Miller 1990). According to Caldas et al. (2000), the longest duration of the fifth instar is due both to the development of wings and the reproductive organs during this instar.

Cumulative nymphal mortality was high and similar for both species (Figs. 2–3). Only 3.7% *L. zonatus* eggs and 2.8 % *L. gonagra* eggs reached adulthood. Apparently, high mortality is common when coreids are reared in individual containers in the laboratory (Amaral and Storti, 1976; Fernándes and Grazia, 1992; Panizzi 1989). It is possible that gregariousness is important for nymphal survival; however, food quality might also influence survivorship. Highest mortality was registered during the second instar for both species (Figs. 2–3). Sex ratio (M:F) for *L. zonatus* was 0.7:1 and 1:1 for *L. gonagra*. *Leptoglossus zonatus* male and female longevity (Table 4) was higher than the one recorded previously for the same species and for other coreids (Panizzi 1989; Matrangolo and Waquil 1994; Caldas et al. 2002). Fecundity, the number of eggs per egg mass and total eggs per *L. zonatus* female (Table 4), was higher than the numbers recorded in the same species by Panizzi (1989) and by Matrangolo and Waquil (1994). The difference in longevity and fecundity observed during this study could be due that *L. zonatus* adults were obtained from nymphs that were feeding on their natural hosts in the field. The longevity of *L. gonagra* (Table 4) was shorter than the one reported by Amaral and Storti (1976). *Leptoglossus gonagra* fecundity was lower than that reported by Van Reenen (1973) and by Amaral and Storti (1976). Only 25% of *L. gonagra* females oviposited at least once, whereas 91.6% *L. zonatus* oviposited.

Parasitism. Parasitism was observed on 72 adults and 121 nymphs of *L. zonatus* and on 62 adults and 132 nymphs of *L. gonagra*. *Hexacladia* sp. (Hymenoptera: Encyrtidae) parasitized 1.6% adults of *L. gonagra*. *Hexacladia* sp. emerged from the terga in the abdomen. These are known parasitoids of Scutelleridae and Pentatomidae (Nunes and Correa-Ferreira 2002; Noyes 1980). This is the first report of *Hexacladia* parasitizing adults of *Leptoglossus*. Jones (1993) and Mitchell (2000) did not included *Hexacladia* as parasitoids of *Leptoglossus* in their reviews. *Eristalis* sp. (Diptera: Syrphidae) oviposited on one *L. gonagra* male in the laboratory. Adults of *Eristalis* were also observed in close proximity to a colony of adults of *L. gonagra* in the field. According with Feener Jr. (1997) syrphids are not known as parasitoids. Therefore, this is the first report of *Eristalis* sp. parasitizing insects. In the laboratory, *Trichopoda* sp. (Diptera: Tachinidae) and other unidentified tachinid parasitized 11% adults of *L. zonatus*. These tachinids oviposited on the occipital region and on the pronotum of their hosts. Two or more parasitoid eggs were laid on each coreid, but only 1 larva developed per host.

Table 4. Longevity and reproductive parameters of *Leptoglossus zonatus* and *L. gonagra*.

	L. zonatus			*L. gonagra*		
Parameters	x	SD	Range	X	SD	Range
Longevity ♀[z]	134.5	± 54	57–230	31.5	± 21.21	6–82
Longevity ♂[z]	151.6	± 41.2	49–200	56.6	± 21.18	33–88
Pre-oviposition[z]	36	± 16	15–60	25	± 6.55	19–32
No. egg mass/♀[y]	7.5	± 2.9	3–14	2	± 1	0–3
Eggs/egg mass[y]	20.7	± 9.1	3–40	14.6	± 8.5	5–27
Eggs/♀[y]	143	± 47.2	66–248	29.3	± 0	0–37

[z](days).
[y](unit).

The larva exits the host in the posterior part of the abdomen. Four hours after emerging from the abdomen, the larva turns brown and pupariates. The tachinid adult emerges 13 days after pupariation approximately. Souza & Amaral Filho (1999) has also reported *Trichopoda* sp. parasitizing adults of *L. zonatus* in Brazil.

Host plants. Four and six plant species were determined as feeding hosts of *L. gonagra* and *L. zonatus* respectively. Forty-eight percent of the collected *L. gonagra* nymphs were found on *Melothria guadalupensis* (Spreng.) Cogn (Cucurbitales: Cucurbitaceae), 26.5% on *P. guajava*, 14.4% on *Momordica charantia* (L.) (Cucurbitales: Cucurbitaceae) and 10.6% on *Solanum americanum* Mill. (Solanales: Solanaceae). All nymphs were observed feeding on fruits and not on leaves. No nymphs of *L. gonagra* were observed feeding on *Citrus* spp. Several host plants have been recorded for *L. gonagra*, however, according with Mitchell (2000) this species appears to prefer cucurbit leaves. During the course of this study, nymphs were observed feeding on fruits of their cucurbit hosts. This might indicate that *L. gonagra* nymphs have variable feeding behavior.

Forty-nine percent nymphs of *L. zonatus* were found on *Z. mayz*, 17.3% on *S. americanum*, 14% on *M. charantia*, 9.1% on *P. guajava*, 5.7% on *C. reticulata* and 4.1% on *C. tangelo*. The preferred host appears to be *Z. mays* whereas *C. tangelo* and *C. reticulata* were the least preferred hosts. According to Panizzi (1989) nymphs of *L. zonatus* adapt very well to corn as a food source.

Damage to *Citrus tangelo*. All treatments, except the untreated control, presented at least one injured fruit. *L. zonatus* injured 58.3% of the observed fruits while *L. gonagra* injured 50% of the fruits. There were not significant differences in the number of lesions per fruit between both species of leaf footed bugs but there were differences between these treatments and the untreated control (Table 5). In Brazil, Kubo and Batista (1992) found that *L. zonatus* caused a similar number of lesions, but the percent of attacked fruits was close to 90%. The diameter of the lesions caused by the coreids was significantly higher than the lesions caused by the puncture with a needle. ANOVA (F = 183,13; df = 65; $P<0,05$). There were no differences on the lesions diameter between the two coreids (Table 5). The lesions caused by the needle look like a small scar, while those caused by *L. zonatus* and *L. gonagra* were watery with a defined diameter. The fungi, *Fusarium* sp. (Hypocreales: Hypocreaceae), *Colletotrichum* sp. (Melanconiales: Melanconiaceae) and *Thielaviopsis* sp. (Moniliales: Dematicaceae) were isolated from the lesions caused by the coreids. There were no fungi from the lesions caused by puncturing with a needle. Fruits that were infected had a ripened prematurely, changing from green to an intense orange, and then fell prematurely and few remained attached to the tree (ICA 2003). These results are in agreement with the results of Albrigo and Bullock (1977), Ballen et al. (1984) and Dammer and Ravelo (1990) who determined that coreids can help pathogen entry, i.e., *Colletotrichum* sp., which causes fruit necrosis and premature fruit fall (Kupper et al., 2003).

Table 5. Injury to *Citrus tangelo* infested with *Leptoglossus zonatus,* and *L. gonagra* (X ± SD).

Treatments	Lessions/fruit	Lession diameter (mm)
L. zonatus	3 ± 1.8 *a*	15.6 ± 5 *a*
L. gonagra	1.16 ± 0.4 *a*	11.8 ± 4.5 *a*
Needle punctures	---	1.14 ± 0.7 *b*
Control	0.0 ± 0.0 *b*	---

Values with the same letter in the same column did not differed according to Mann-Whitney U test, 5% probability.
Values with the same letter in the same column did not differed according to Tukey test, 5% probability.

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