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ORIGINAL RESEARCH PAPER

Biological studies of *Agalliana ensigera* (Hemiptera: Cicadellidae), a leafhopper associated with several crop diseases in South America

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Abstract *Agalliana ensigera* Oman (Cicadellidae: Megophthalminae) occurs frequently in several crops and has been involved in the epidemiology, or at least mentioned, as a vector of several diseases. Its biology was unknown and its nymphs had not yet been described. Here, we provide information on behavioral and biological parameters including descriptions of its immature stages. *A. ensigera* was able to complete its entire life cycle on alfalfa. Eggs were laid individually beneath the epidermis, in the stem internodes, in either mature or young tissues. Females had a pre-ovipositional period of around 5 days, and the average duration of the egg stage was 11 days. The nymphal period ranged 14–31 days, with an average of 22 days. A period of high mortality was recognized from emergence to day 10. Adult longevity averaged 16.5 days, but some individuals survived up to 96 days. The sex ratio was about 1.2:1 females to males. Females lived longer than males. Data on behavior and new distributional records and host plants are also provided.

Keywords Megophthalminae · Vector · Phytoplasma · Life cycle · Nymph description · Alfalfa

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Introduction

The hemipteran infraorder Cicadomorpha comprises approximately 35,000 described species distributed worldwide (Dietrich 2005). Membracoidea is the most diverse and economically important superfamily of Cicadomorpha, comprising more than 25,000 known species with many pests to agriculture. They are sap-sucking insects, injuring plants either directly through feeding (Backus et al. 2005; Perez Grosso et al. 2016) or indirectly through transmission of plant pathogens (Maramorosch and Harris 1979; Nielson 1968; Weintraub and Beanland 2006). Within Membracoidea, Cicadellidae (leafhoppers) is the most species-rich group (Dietrich 2005). Relatively few leafhopper species are economically important, but some are major pests, such as the glassy-winged sharpshooter *Homalodisca vitripennis* (Germar), the potato leafhopper *Empoasca fabae* (Harris), and the beet leafhopper *Neoaliturus* (=*Circulifer*) *tenellus* (Baker) (Dietrich 2005).

Most of the Membracoidea species known to date as vectors belong to the Cicadellidae family (Dietrich 2005), being major vectors of phytoplasmas (Weintraub and Beanland 2006). Phytoplasmas are plant pathogenic prokaryotes associated with approximately 1000 plant diseases throughout the world (Hogenhout et al. 2008). In Argentina, phytoplasmas have been associated with more than 25 plant species and many of these pathogens cause economically significant crop diseases (Conci et al. 2014).

Alfalfa (*Medicago sativa* L.) is an important crop in Argentina because it is used as a source of silage for livestock, in addition to being important for soil conservation and recovery (Hijano and Navarro 1995). Many diseases have been reported to affect alfalfa crops in Argentina (Trucco et al. 2016), one of them being the "witches' broom" disease caused by the Argentinean alfalfa witches' broom phytoplasma (ArAWB;

16Sr VII-C). This phytoplasma is particularly prevalent in Argentinean seed production areas located mainly in the center and west of the country (Conci et al. 2005). The disease caused by this phytoplasma significantly reduces forage yield and seed production, and there are no resistant cultivars for this disease (Gieco et al. 2007).

Field studies on the fauna associated with alfalfa in Argentina demonstrated that one of the most abundant and frequent Auchenorrhyncha is the leafhopper *Agalliana ensigera* Oman (Cicadellidae: Megophthalminae). This insect species was reported as the vector of Argentine curly top virus of sugar beet and the solanacearum strain of Brazilian curly top of tomato (Bennett and Costa 1949; Nielson 1968), and it was also suggested as a potential vector of the ArAWB phytoplasma (L. R. Conci, unpublished data; N. Meneguzzi, personal communication). Also, GD-16SrIII-J phytoplasma was detected in *A. ensigera* individuals collected in Argentinean garlic fields affected by garlic decline disease (Catalano 2011). However, transmission tests have still not been performed to determine whether *A. ensigera* is a competent vector of either of these pathogens. The lack of biological and ecological information about pathogen vectors has been a limiting factor in the understanding and control of plant diseases.

Agalliana ensigera is abundant on many weeds and cultivated plants, mostly those in the Solanaceae family, as summarized by Paradell et al. (2014). The studies of geographic distribution of *A. ensigera* show that it is a Neotropical species present in Bolivia, Brazil and Argentina (20°–40° South Latitude) (Nielson 1968; Paradell et al. 2014). However, knowledge of the biology of this species is limited to the study of Bennett et al. (1946) who provided some information about its development on sugar beet (*Beta vulgaris* L.). One of the issues that takes place when carrying out population studies of *A. ensigera* is that their nymphs can be easily confused with another Agalliini sympatric species, since they are still not described.

Considering the abundance of *A. ensigera* in alfalfa crops in central west Argentina, the potential epidemiological relevance of this species as vector of virus or phytoplasma pathogens, and the lack of information on the development of *A. ensigera*, this work aimed to provide data on the behavioral and biological parameters, such as egg-laying behavior, duration of different developmental stages, sex ratio, and longevity on alfalfa, as well as to provide accurate descriptions of its immature stages.

Materials and methods

Origin of the colony and insect rearing

A colony of *A. ensigera* was established from 30 adults collected during the summer of 2013 from alfalfa fields located in Manfredi, Córdoba province (S31°25′–W64°11′, 390 m asl), Argentina. They were taken to the laboratory and placed in rearing cages in a controlled-environment chamber: 27 ± 2 °C, 60–80% RH and L16:D8 photoperiod.

Insects were reared in $50 \times 25 \times 50$ cm aluminum cages containing six healthy potted alfalfa plants (pots of 6.3 dm^3). Every side except the base was covered by a nylon mesh cloth for aeration. The cages had two doors, one at the front and another at the back.

Identification, immature stages and voucher specimens

The specimens were identified using the online keys of Gonçalves and Dietrich (2014). Eggs were described from those obtained by dissecting alfalfa stems.

The nymphs were identified through association with the adults reared in the cages. Specimens of each instar were preserved in 60% ethanol and mounted for study with a stereomicroscope (MZFL III, Leica, Switzerland) and a microscope (Axio Lab A1, Carl Zeiss, Germany) with a digital camera (Axio Cam ERc 5s, Carl Zeiss, Germany). We followed Dmitriev (2002) for morphological terminology. Morphological characteristics of the last (5th)-instar nymph were analyzed in detail, and only the observed changes were added to the descriptions of earlier instars. Color was described based on assessment of living specimens anesthetized with sulfuric ether.

Voucher specimens were deposited in the entomological collection at the Miguel Lillo Foundation, Tucumán, Argentina (IMLA).

Biological information

Lab-reared *A. ensigera* nymphs randomly taken from the colonies were confined separately until the adult stage, when they were sexed and cohorts were formed. Two cohorts consisting of five females and two males each were placed individually in oviposition cages made with two 10×10 cm transparent plastic sheets surrounded by adhesive weatherstrip borders (1 cm) and enclosing a stem containing at least one node and several leaves. Females were then allowed to oviposit on the potted alfalfa plants for 24 h. The cages containing leafhoppers were repositioned on different parts of the plants every day until the females died, with exposed stems labeled and monitored until hatching nymphs were observed. Males that died were replaced to ensure that all females had access to males throughout the study.

Nymphs obtained from the assay were isolated in PET (polyethylene terephthalate) cylindrical cages (20×6 cm diameter) containing an alfalfa stem. They were checked every day while the occurrence of molts was noted. Thus, the developmental time of each individual, from egg laying to adult death, was recorded. Differences between sexes were

analyzed (Sedlacek et al. 1986), and other aspects of nymph and adult behavior were also recorded.

Summarized life tables for cohorts were built by combining the observational data of two replicates, in order to calculate the survivorship (l_x) and the life expectancy (e_x) defined as:

$l_x = N_x/N_0$, where N_x is the number of survivors at age x, and N_0 is the number living individuals at age 0.

$e_x = T_x/l_x$, where T_x is the total number of remaining days of life for survivors who have reached the age x, and l_x the proportion of survivors at age x.

Survivorship and life expectancy curves were built following Rabinovich (1978).

Normality of data was tested using Shapiro–Wilks tests, and Student's t was used to test for differences with $P \leq 0.05$ considered statistically significant. Data were analyzed using InfoStat 2013 software (Di Rienzo 2013).

Results

Description of the immature stages (Fig. 1a–h)

Eggs (Fig. 1a)

Dimensions: length 0.4–1.0 mm, 0.65 ± 0.13 (mean ± SD, $n = 29$) mm; width 0.15–0.40 mm, 0.24 ± 0.06 (mean ± SD, $n = 29$) mm. The endophytic eggs are ellipsoidal with the anterior pole pointed and the posterior one rounded; the ventral surface is slightly concave and the dorsal side convex. The chorion lacks discernible sculpture. At oviposition, the *A. ensigera* eggs are translucent, 1 day later, they turn to creamy white and the eye spots become visible 5 to 7 days after oviposition.

Nymphal fifth instar (Fig. 1f)

Coloration: pale yellow with two dark spots on crown. Body length 2.1–2.3 mm, 2.2 ± 0.12 (mean ± SD, $n = 10$) mm, head width 0.9 ± 0.03 (mean ± SD, $n = 10$) mm. Body robust, not depressed dorsoventrally. Crown about as long as pronotum, 2 times as wide as long, medially as long as eye; anterior margin rounded, posterior margin concave. Face slightly wider than long, anteclypeus with slightly convex lateral margin, with constriction at base, about twice as long as wide; postclypeus slightly longer than wide; lorum half as wide as anteclypeus, 2/3 of its inner margin bordered by postclypeus. Antennae of moderate length, reaching apices of forewing pads. Ocelli on anterior margin of head, mid-way between eyes and molting suture. Width of pronotum 3 times its length. Wing pads 1.5–1.8 times length of

pterothorax. Setal formula of hind femur 2 + 1, hind tibia with 8 macrochaetae in dorsal rows with 1 or 2 intercalary setae. Abdominal tergites with 6 macrochaetae at posterior margin. Pygofer about 1.5 times the length of preceding abdominal tergites. Sexes are distinguishable in nymphs of later instars (Fig. 1g, h). In females, the suture between the pygofer lobes extends as far as the pregenital segment, in males it does not extend this far. Gonapophyses I triangular; length significantly exceeding basal width; apices rounded; apices of gonapophyses II tapered, slightly projecting from under gonapophyses I.

Early instars (Fig. 1b–e)

Early instars are similar to the fifth instar in coloration and chaetotaxy but may be readily identified by the development of the wing pads, which are progressively longer in later instars.

Biological information

Egg laying and egg distribution in the plant

Females laid their eggs immediately beneath the epidermis (endophytic) in two sites of the stems of alfalfa plants: 75% in the internodes, where the eggs were entirely inserted, and 25% in the stem node where the eggs were inserted in the axil, hidden between the buds and petioles and partially covered by the plant pubescence ($n = 278$). No eggs were located on leaves. Females laid their eggs in either mature or young tissues. They were generally visible without much effort. The eggs were laid individually, although occasionally two eggs were laid close to each other; but each egg always had its own oviposition incision.

Life cycle

Individuals of *A. ensigera* are solitary. Females had a preoviposition period of 5.0 ± 2.0 (mean ± SD, $n = 10$) days and subsequently laid eggs for the rest of their lives. The duration of the egg stage lasted 8–16 days, 11.1 ± 1.34 (mean ± SD, $n = 278$) days.

Agalliana ensigera has 5 nymphal instars (Fig. 1), and the duration of the entire nymphal stage was 14–31 days, 22.3 ± 3.01 (mean ± SD, $n = 114$) days (Table 1). Wide variation in development time was observed among individuals, with some reaching the adult stage more than a week earlier than others. In the nymphs that developed into females, the whole nymphal stage lasted 17–31 days, 22.7 ± 3.26 (mean ± SD, $n = 63$) days, while the nymphal development time in males was 14–30 days, 21.9 ± 2.65 (mean ± SD, $n = 51$) days. Significant differences were observed in the duration of the fourth instar, with females lasting almost a
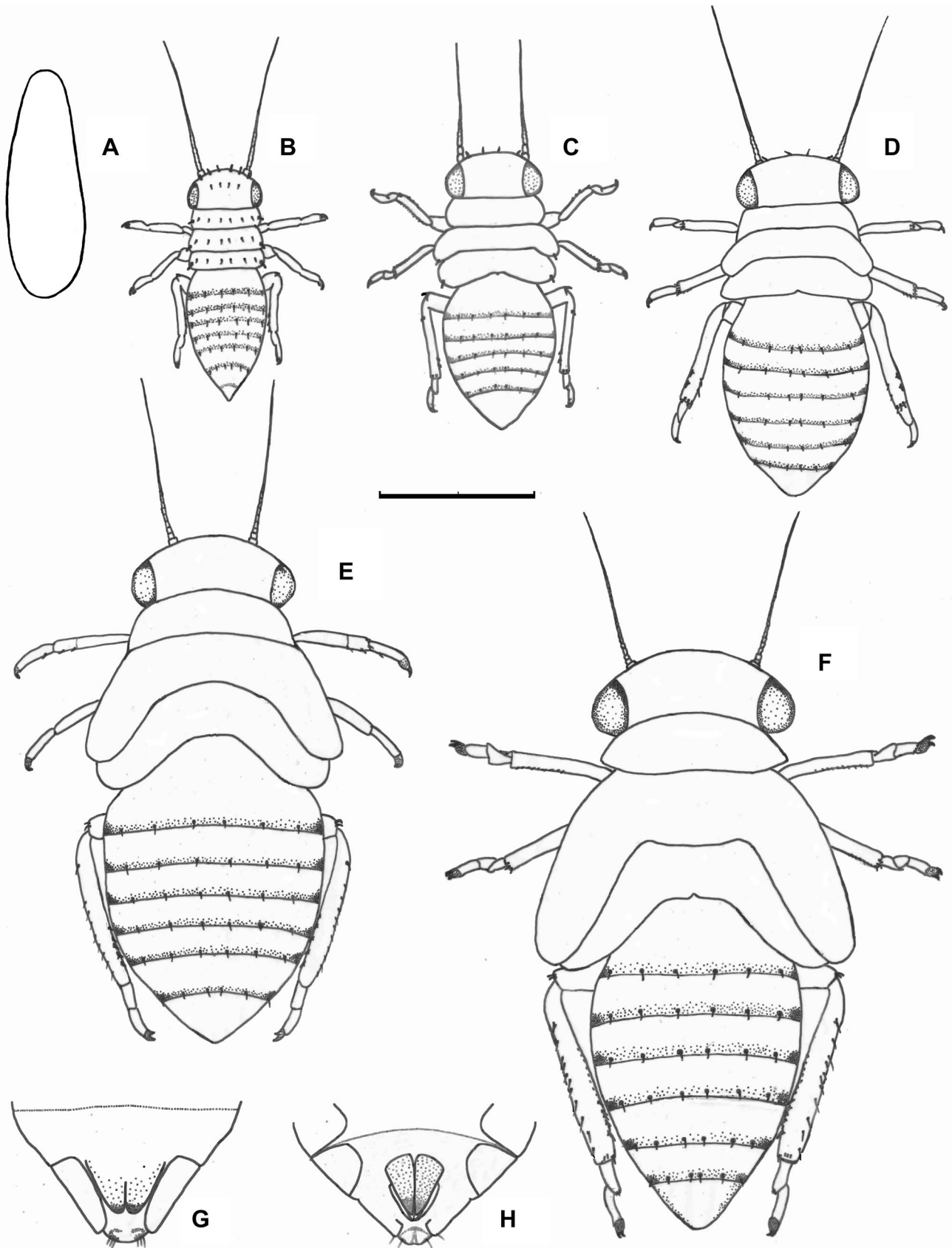
Fig. 1 *Agalliana ensigera* Oman preimaginal stages. **a** Egg, **b** first instar, **c** second instar, **d** third instar, **e** fourth instar, **f** fifth instar, **g** ventral view of male abdomen tip. **h** female abdomen tip. *Scale bar* 0.44 mm for **a**, 0.56 mm for **b–f**, 0.35 mm for **g–h**

Table 1 Summary of the duration of each nymphal instar, all nymphal stages and adult longevity of *A. ensigera*

Nymphal and adult stage	Sex	n	Duration (days)		Range
			Average ± SD[a]		
N-I	Male	51	5.75 ± 1.52	ns	2–8
	Female	63	5.35 ± 1.35		3–8
	Total	114	5.53 ± 1.43		3–8
N-II	Male	51	3.33 ± 1.24	ns	1–6
	Female	63	3.67 ± 1.40		1–7
	Total	114	3.51 ± 1.34		1–7
N-III	Male	51	3.51 ± 1.27	ns	1–6
	Female	63	3.60 ± 1.54		1–7
	Total	114	3.56 ± 1.42		1–7
N-IV	Male	51	3.51 ± 1.45	*	2–9
	Female	63	4.38 ± 1.97		2–12
	Total	114	3.99 ± 1.80		2–12
N-V	Male	51	5.82 ± 2.12	ns	2–10
	Female	63	5.71 ± 2.29		2–13
	Total	114	5.76 ± 2.21		2–13
All nymphal instars	Male	51	21.92 ± 2.65	ns	14–30
	Female	63	22.70 ± 3.26		14–31
	Total	114	22.35 ± 3.01		14–31
Adult longevity	Male	50	11.36 ± 11.39	*	1–49
	Female	62	20.58 ± 18.19		1–96
	Total	112	16.46 ± 16.13		1–96

[a] Asterisk indicates significant difference between males and females ($P < 0.05$; t-test) *ns* not significantly different ($P > 0.05$; t-test)

day more than males (Table 1). The time required for development from egg laying to adult emergence was nearly 33.5 days.

After the eggs hatched, the newly emerged nymphs were very active and moved to the tender portions of the leaves and petioles. Most exuviae resulting from molts between instars were found on the abaxial part of the leaves on the midrib. The progeny had a sex ratio of about 1.25:1 female:male.

In the trials carried out to determine the durations of different developmental stages, adult longevity was found to be variable with a mean of 16.5 ± 16.1 (mean ± SD, $n = 112$) days (Table 1), and 49.1% of the individuals died before day 10 after reaching the adult stage, with females tending to live longer than males.

Elevated mortality occurred from egg hatch to day 10, a period in which the e_x values remain high (Fig. 2). Those nymphs that survived this period had a high probability of reaching the adult stage (nearly day 23). The survivorship curve (l_x) showed that the mortality rate was approximately constant throughout the lifespan until day 70, when only a few individuals were still alive (Fig. 2).

Other observations

Agalliana ensigera has a broad distribution in Argentina. It was previously cited for 15 provinces (Jujuy, Salta, Tucumán, La Rioja, Catamarca, Santiago del Estero, Chaco, Misiones, Entre Ríos, San Juan, Mendoza, Santa Fe, La Pampa, Buenos Aires, and Río Negro) (Paradell et al. 2014). In this contribution we cite for the first time the occurrence of *A. ensigera* in alfalfa crops in Córdoba province (Manfredi and Coronel Olmedo locations), and a new host-plant, feral white clover (*Trifolium repens* L.), in San Miguel de Tucumán (Tucumán province) (T.P.G., unpublished data).
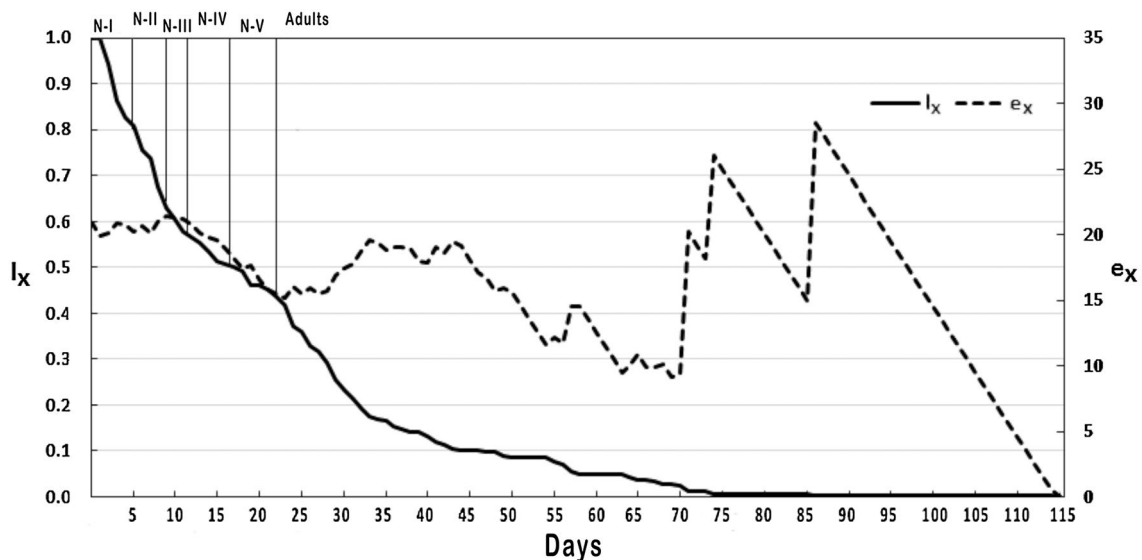
Fig. 2 Survivorship (l_x) and life expectancy (e_x) curves of *A. ensigera* reared on alfalfa plants at 27 ± 2 °C, 60–80% RH and L16:D8 photoperiod. In addition, the mean duration of each stage is shown (NI–NV: nymphal instars)

Discussion

Little information is available on the biology of Megophthalminae leafhoppers, particularly on oviposition sites on host plants. In contrast, biology and oviposition behavior are better known for some leafhopper species belonging to other subfamilies. The eggs of *A. ensigera* are endophytic, as in most other Cicadellidae, being inserted just beneath the epidermis. In other leafhoppers eggs may be laid in clusters as in the Cicadellinae subfamily (tribes Proconiini and most of Cicadellini) (Rakitov 2004; Rossi and Strong 1990), or individually as in Typhlocybinae (Carlson and Hibbs 1962) and Deltocephalinae (Heady et al. 1985; Meyerdirk and Moratorio 1987) subfamilies. Females of *A. ensigera* observed on alfalfa laid their eggs individually mostly in the stem or in the axil of petioles, but never in the leaves. However, considering that this species has a wide range of host plants, including monocots (Paradell et al. 2014), its oviposition behavior may be different on other plant hosts. Other leafhopper species specializing on grasses (monocots), such as *Dalbulus maidis* (De Long), *Psammotettix alienus* (Dahlbom) and *Draeculacephala floridana* Ball, lay eggs mostly in the leaf sheath or blade (Guglielmino and Virla 1997; Heady et al. 1985; Rossi and Strong 1990).

Guglielmino and Virla (1997) observed that *P. alienus* females lay eggs mostly in older oat (*Avena sativa* L.) leaves. In our study, females of *A. ensigera* laid eggs in the epidermis of any portion of the stem regardless of the tissue age. In *A. ensigera* it is possible to distinguish eggs on the stem clearly, in contrast to published observations of other Cicadellidae in which eggs are not obvious unless the plants are dissected (Carlson and Hibbs 1962; Curtis 1942; Rossi and Strong 1990) or, as in some treehoppers, such as *Ceresa nigripectus* Remes Lenicov (Membracidae), where eggs are inserted between the epidermis and the cortical tissues (Perez Grosso et al. 2014). Our observations of *A. ensigera* appear to be the first observation of oviposition sites for a Megophthalminae leafhopper species.

As for other ectotherms, insect physiological functions such as development and performance are strongly influenced by environmental temperature (Inward et al. 2012; Sedlacek et al. 1990). In *A. ensigera* the duration of the egg stage lasted 11.1 days (at 27 °C). This is similar to that reported for several Deltocephalinae leafhoppers including *P. alienus*, 10.6 days at 27 °C (Guglielmino and Virla 1997); the corn leafhopper *Dalbulus maidis*, 14.6 days at 25 °C and 9.9 days at 30 °C, respectively (Van Nieuwenhove et al. 2015); the black-faced leafhopper, *Graminella nigrifrons* (Forbes), 7.5 days at 27 °C (Sedlacek et al. 1990); the rice green leafhopper, *Nephotettix virescens* (Distant), 6 days at 29.7 °C (Salim 2002); as well as in the Cicadellinae *Draeculacephala floridana,* 10–12 days at 23 °C (Rossi and Strong 1990).

As in all other leafhoppers for which life-history data is available, *A. ensigera* has five instars. The developmental time from first instar to adult (22.35 days) is quite similar to that of *Graminella nigrifrons*, 20.8 days at 24 °C (Stoner and Gustin 1967) and *Dalbulus maidis*, 25 days at 23.3 °C (Van Nieuwenhove et al. 2015), but longer than that of *P. alienus*, 16 days at 27 °C (Guglielmino and Virla 1997). The duration of the nymphal period of *Nephotettix virescens* is 25.9 days at 25 °C, and 17.4 days when the rearing temperature is 29 °C (Salim 2002). We did not find statistically significant differences in the development times of nymphs of different sexes. Sedlacek et al. (1990) observed that male and female nymphs of the black-faced leafhopper, *Graminella nigrifrons*, reach adulthood in 15 and 17 days, respectively, at 27 °C.

The five nymphal instars differ in size and proportions, and in the degree of development of wing pads and external genitalia. The general morphology of the nymph confirms the characters that Oman (1934) considered typical for this species. The nymphs present typical characteristics which can be used to classify them as Cicadellidae or Agalliini (Zenner et al. 2005). Nymphs are morphologically similar to adults; the same sclerites are present in the head, and their location is the same; and also the chaetotaxy of the legs is also the same. This pattern of similarity between nymphs and adults was previously noted by Vilbaste (1982) for Cicadellidae in general and Dmitriev (2002) and Dmitriev and Dietrich (2009) for Deltocephalinae.

Adult females of *A. ensigera* live substantially longer than males (20.58 vs 11.36 days). Such differences in longevity between sexes are common among cicadellids. Female longevity of *P. alienus* averages 19.8 days while males live 12.7 days (Guglielmino and Virla 1997). Remarkable differences were recorded for the corn leafhopper, *D. maidis*, in which females have a mean longevity of 42.1 days and males of only 16.3 days (Van Nieuwenhove et al. 2015). In *Nephotettix virescens*, longevity differences between females and males are not only present but maintained at different rearing temperatures: 19.5 and 17.5 days at 29 °C for females and males, respectively, and 22.9 and 22.0 days, respectively, at 25 °C (Salim 2002).

The pre-oviposition period in *A. ensigera* females lasted 5.0 ± 2.0 days, which is similar to that observed by Guglielmino and Virla (1997) for *P. alienus* (5.35 ± 2.00 days), but shorter than that observed by Van Nieuwenhove et al. (2015) for *D. maidis* (8.5 days at 23.3 °C).

In our rearing experiments, the progeny of *A. ensigera* had a slightly female-biased sex ratio of 1.25:1. Sex ratio in leafhopper species is poorly documented; data available for a few species indicate that the usual sex ratio is almost 1:1 (Beanland et al. 1999; Guglielmino and Virla 1997) or with females only slightly outnumbering males (Nielson 1968). Some studies demonstrated that adult sex ratios in the

field are highly variable at different stages in the host plant growth cycle (Flinn et al. 1990; Marais 1988). Additionally, in *D. maidis* the sex ratio seems to be influenced by rearing temperature, i.e., 1.13:1 female: male at 20 °C; 1:1 at 25 °C and 0.85:1 at 30 °C (Van Nieuwenhove et al. 2015).

The survivorship curve of *A. ensigera* resembles a theoretical type III, characterized by a high initial mortality followed by an approximately constant mortality throughout the life span. However, the life expectancy curve shows a critical period of high mortality during the first 10 days after the eggs hatch. This period includes the two episodes of ecdysis between the first and third instars. Guglielmino and Virla (1997) also observed an initial period of high mortality for *P. alienus* during the ecdysis from the second to the third instar. In contrast, in *Graminella nigrifrons* (Sedlacek et al. 1986) and *Empoasca fabae* (Simonet and Pienkowski 1980), the highest mortality was observed in the fifth instar and during molting to the adult stage.

There are currently more phytoplasma diseases known than vectors identified. As more vector species are confirmed and as more phytoplasma diseases are characterized, the relationships between vector, disease and host plant will be better understood. Transmission experiments can provide evidence of the ability of candidate species to transmit phytoplasmas to healthy plants. Different contributions from investigations will provide elucidation of phytoplasma–vector relationships, and a fundamental step is to know the life cycle and characteristics of potential vectors of each disease. In the case of *Agalliana ensigera*, the lack of knowledge about the management of its populations in the laboratory delayed the possibility of carrying out transmission tests, and thus to advance the knowledge of the diseases transmitted by it as support for its rational management.

References

Backus EA, Serrano MS, Ranger CM (2005) Mechanism of hopperburn: an overview of insect taxonomy, behavior and physiology. Annu Rev Entomol 50:125–151

Beanland L, Hoy C, Miller S, Nault L (1999) Leafhopper (Homoptera: Cicadellidae) transmission of aster yellows phytoplasma: does gender matter? Ann Entomol Soc Am 28:1101–1106

Bennett CW, Costa AS (1949) The Brazilian curly top of tomato and tobacco resembling North American and Argentine curly top of sugar beet. J Agric Res 78:675–693

Bennett CW, Garsner E, Goons G, Brandes E (1946) The Argentine curly top of sugar beet. J Agric Res 72:19–48

Carlson O, Hibbs E (1962) Direct counts of potato leafhopper *Empoasca fabae* eggs in *Solanum* leaves. Ann Entomol Soc Am 55:512–515

Catalano MI (2011) Cicadélidos vectores de fitoplasmas a cultivos de importancia económica en la Argentina. Sistemática y bioecología (Insecta: Auchenorrhyncha: Cicadellidae). Ph.D Thesis, Fac. Cs. Nat. y Museo, La Plata, Argentina

Conci L, Meneguzzi N, Galdeano E, Torres L, Nome C, Nome S (2005) Detection and molecular characterization of an alfalfa phytoplasma in Argentina that represents a new subgroup in the 16S rDNA ash yellows group ('Candidatus *Phytoplasma fraxini*'). Eur J Plant Pathol 113:255–265

Conci L, Saavedra Pons A, Guzman F, Fernandez F, Galdeano E, Perez Grosso T, Torres L, Meneguzzi N (2014) Advances in knowledge about phytoplasma diseases in Argentina. In: Bertaccini A (ed) Phytoplasmas and phytoplasma diseases management: how to reduce their economic impact. IPWG, Bologna, pp 82–89

Curtis W (1942) A method of locating insect eggs in plant tissues. J Econ Entomol 35:286

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2013) Infostat versión 2013. Grupo Infostat, Fca, Universidad Nacional De Córdoba, Argentina

Dietrich CH (2005) Keys to the families of Cicadomorpha and subfamilies and tribes of Cicadellidae (Hemiptera: Auchenorrhyncha). Fla Entomol 88:502–517

Dmitriev DA (2002) General morphology of leafhopper nymphs of the subfamily Deltocephalinae (Hemiptera: Cicadellidae). Acta Entomol Slov 10:65–82

Dmitriev DA, Dietrich CH (2009) Review of the species of new world Erythroneurini (Hemiptera: Cicadellidae: Typhlocybinae) III. Genus Erythridula. Ill Nat Hist Surv Bull 38:215–334

Flinn P, Hower A, Taylor R (1990) Immigration, sex ratio, and local movement of the potato leafhopper (Homoptera: Cicadellidae) in a Pennsylvania alfalfa field. J Econ Entomol 83:1858–1862

Gieco J, Moreno M, Basigalup D (2007) Enfermedades de la alfalfa y abordaje molecular de la selección por resistencia. In: Basigalup D (ed) El cultivo de la alfalfa en la Argentina. INTA, Buenos Aires, pp 449–476

Gonçalves A, Dietrich C (2014) An interactive key to Megophthalminae genera. In: List of 3I interactive keys and taxonomic databases. http://dmitriev.speciesfile.org/3i_keys.asp. Accessed 03/VII/2016

Guglielmino A, Virla EG (1997) Postembryonic development and biology of *Psammotettix alienus* (Dahlbom) (Homoptera: Cicadellidae) under laboratory conditions. Boll Zool Agrar Bachic 29:65–80

Heady S, Madden L, Nault L (1985) Oviposition behavior of *Dalbulus* leafhoppers (Homoptera: Cicadellidae). Ann Entomol Soc Am 78(7):23–727

Hijano E, Navarro A (1995) La alfalfa en Argentina. Enciclop Agro Cuyo Manuales 11:13–149

Hogenhout S, Oshima K, Ammar E, Kakizawa S, Kingdom H, Namba S (2008) Phytoplasmas: bacteria that manipulate plants and insects. Mol Plant Pathol 9:403–423

Inward D, Wainhouse D, Peace A (2012) The effect of temperature on the development and life cycle regulation of the pine weevil *Hylobius abietis* and the potential impacts of climate change. Agric For Entomol 14:348–357

Marais E (1988) Biology of the leafhopper *Aciali natrifrons* (Naudé) (Homoptera: Cicadellidae), on grapevines in the Western Cape. S Afr J Enol Vitic 10:3–7

Maramorosch K, Harris KF (1979) Leafhopper vectors and plant disease agents. Academic Press, New York

Meyerdirk D, Moratorio M (1987) *Circulifer tenellus* (Baker), the beet leafhopper. Laboratory studies on fecundity and longevity. Can Entomol 119:443–447

Nielson MB (1968) The leafhoppers vectors of phytopathogenic viruses (Homoptera: Cicadellidae): taxonomy, biology and virus transmission. USDA Tech Bull 1382:1–386

Oman PN (1934) South American leafhoppers of the genus *Agalliana*. Rev Entomol 4:333–340

Paradell S, Defea B, Dughetti A, Zárate A, Remes Lenicov AM (2014) Diversity of Auchenorrhyncha (Hemiptera: Cicadellidae: Delphacidae) associated with *Vicia villosa* in southern Buenos Aires province, Argentina. Fla Entomol 97:674–684

Perez Grosso T, Conci L, Saavedra Pons A, Remes Lenicov AM, Virla EG (2014) First biological data on *Ceresa nigripectus* (Hemiptera: Membracidae), a common treehopper on alfalfa crops in Argentina. Fla Entomol 97:176–1773

Perez Grosso T, Mercado I, Ponessa G, Conci L, Virla EG (2016) Characterization of feeding injuries caused by *Ceresa nigripectus* Remes Lenicov (Hemiptera: Membracidae) on alfalfa stems (*Medicago sativa* L., Fabaceae). Neotrop Entomol 45:211–216

Rabinovich J (1978) Ecología de poblaciones animales. Programa regional des. Cient, 21st edn. Y-Tec, OEA

Rakitov R (2004) Powdering of egg nests with brochosomes and related sexual dimorphism in leafhoppers (Hemiptera: Cicadellidae). Zool J Linn Soc 140:353–381

Rossi AM, Strong JR (1990) Natural history of the leafhopper *Carneocephala floridana* (Homoptera: Cicadellidae) in a North Florida salt marsh. Fla Entomol 73:147–153

Salim M (2002) Biology of rice green leafhopper, *Nephotetix virescens* (Distant) under laboratory conditions. Pak J Agric Res 17:49–54

Sedlacek J, Kenneth Y, Freytag P (1986) Laboratory life table studies on the blackfaced leafhopper (Homoptera: Cicadellidae) on Johnsongrass and corn. Environ Entomol 15:1119–1123

Sedlacek J, Kenneth Y, Freytag P (1990) Effect of temperature on the development of the blackfaced leafhopper (Homoptera: Cicadellidae). Environ Entomol 19:209–214

Simonet D, Pienkowski R (1980) Temperature effect on development and morphometrics of the potato leafhopper. Environ Entomol 9:798–800

Stoner W, Gustin R (1967) Biology of *Graminella nigrifrons* (Homoptera: Cicadellidae), a vector of corn (maize) stunt virus. Ann Entomol Soc Am 60:496–505

Trucco V, De Breuil S, Bejerman N, Lenardon S, Giolitti F (2016) Beanleafroll virus (BLRV) in Argentina: molecular characterization and detection in alfalfa fields. Eur J Plant Pathol 146:207–212

Van Nieuwenhove G, Frias E, Virla EG (2015) Effects of temperature on the development, performance and fitness of the corn leafhopper *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae): implications on its distribution under climate change. Agric For Entomol 18:1–10

Vilbaste J (1982) Preliminary key for the identification of the nymphs of North European Homoptera Cicadinea. II. Cicadelloidea. Ann Zool Fenn 19:1–20

Weintraub P, Beanland L (2006) Insect vectors of phytoplasmas. Annu Rev Entomol 51:91–111

Zenner G, Stöckmann M, Niedringhaus R (2005) Preliminary key to the nymphs of the families and subfamilies of the German Auchenorrhyncha fauna (Hemiptera, Fulgoromorpha et Cicadomorpha). Beit Zikaden 8:59–78