Compared nymphal development of *Tingis americana* (Hemiptera, Tingidae) in two *Handroanthus* species (Bignoniaceae) and reproductive parameters in seedlings of *Handroanthus heptaphyllus*

Daiane C. Moreira[1], Luiza R. Redaelli[1], Marcus Guidoti[1] & Aline Barcellos[2]

1. Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91501-970 Porto Alegre, RS, Brazil. (daiimoreira@gmail.com; luredael@ufrgs.br; marcus.guidoti@gmail.com)
2. Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Rua Dr. Salvador França, 1427, 90690-000 Porto Alegre, RS, Brazil. (alinebar@fzb.rs.gov.br)

ABSTRACT. Nymphs and adults of *Tingis americana* Drake, 1922 were found feeding on leaves of *Handroanthus heptaphyllus* (Vell.) Mattos and *Handroanthus chrysotrichus* (Mart. ex A. DC.) Mattos in the Botanic Garden, Porto Alegre, state of Rio Grande do Sul, Brazil. This is the first record of *T. americana* on these host plants and in the southern Brazil. We aimed to compare the nymphal development on both hosts and to analyze the reproductive parameters on *H. heptaphyllus* (25 ± 1°C; 60 ± 10% RH; 16 h photophase). The mean nymphal period (days) was shorter in individuals reared on *H. heptaphyllus* (12.69 ± 0.076) than on *H. chrysotrichus* (19.11 ± 0.208) (P < 0.0001), however, nymph viability was similar. On *H. heptaphyllus*, the mean embryonic period lasted 12.32 ± 0.274 days and the egg viability was 92%. The mean total and daily fecundity were 310.0 ± 19.40 eggs/female and 7.46 ± 0.302 eggs/female/day, respectively. Paired males and females showed similar longevity (P = 0.0691), while unpaired females lived longer than unpaired males (P = 0.0460).

KEYWORDS. Biology, life cycle, *Handroanthus chrysotrichus*, lace bug.

RESUMO. Desenvolvimento ninfal comparado de *Tingis americana* (Hemiptera, Tingidae) em duas espécies de *Handroanthus* (Bignoniaceae) e parâmetros reprodutivos em mudas de *Handroanthus heptaphyllus*. No Jardim Botânico de Porto Alegre, Rio Grande do Sul, Brasil, ninfas e adultos de *Tingis americana* Drake, 1922 foram encontrados alimentando-se de folhas de *Handroanthus heptaphyllus* (Vell.) Mattos e *Handroanthus chrysotrichus* (Mart. ex A. DC.) Mattos, causando morte de mudas. Este é o primeiro registro de *T. americana* nestas espécies hospedeiras e no sul do Brasil. O objetivo do trabalho foi avaliar o desenvolvimento ninfal nestes dois hospedeiros e registrar os parâmetros reprodutivos em *H. heptaphyllus* a 25 ± 1ºC; 60 ± 10% UR; fotofase de 16 horas. A duração média do período ninfal foi menor nos indivíduos mantidos em *H. heptaphyllus* (12,69 ± 0,076 dias) do que em *H. chrysotrichus* (19,11 ± 0,208 dias) (P < 0,0001), entretanto a viabilidade desta fase foi similar nos dois hospedeiros. Em *H. heptaphyllus*, o período embrionário médio foi de 12,32 ± 0,274 dias, e a viabilidade dos ovos de 92%. A fecundidade média total e diária foram de 310,0 ± 19,40 ovos/fêmea e 7,46 ± 0,302 ovos/fêmea/dia, respectivamente. Machos e fêmeas pareados mostraram longevidade semelhante (P = 0,0691), enquanto fêmeas não-pareadas foram mais longevas do que machos não-pareados (P = 0,0460).

PALAVRAS-CHAVE. Biologia, ciclo de vida, *Handroanthus chrysotrichus*, percevejo-de-renda.

Tingidae (Hemiptera, Heteroptera) are small insects (2–10 mm), most of them recognizable by the areolate pronotum and hemelytra (Miller, 2008). This group comprises three subfamilies: Cantacaderinae, Tinginae, and Vianaidinae, with about 2,600 species (Guilbert, 2004).

Most lace bugs feed on a single or on closely related host plant species, usually on the abaxial side of Angiospermae leaves (Schuh & Slater, 1995). Their feeding habits may result in puncture marks, white spots, and/or leaf abscission (Stonedahl *et al*., 1992; Neal & Schaefer, 2000). The oviposition site is reported as highly species-specific and determines the mode of oviposition (Livingstone & Yacoob, 1987). Eggs can be laid singly or in clusters, in any part or position relative to the hostplant, as horizontally to the leaf surface ("lamina oviposition"), or perpendicularly along midrib and veins, and also on different parts of flowers and stems (Livingstone & Yacoob, 1987).

Tingis Fabricius, 1803 includes about fifty extant species, distributed worldwide. In Brazil, nine species have been recorded: *T. americana* Drake, 1922; *T. beieri* Drake, 1935; *T. colombiana* Drake, 1929; *T. corumbiana* Drake, 1926; *T. neotropicalis* Monte, 1940; *T. oliveirai* Drake & Hambleton, 1938; *T. saueri* Drake & Hambleton, 1939; *T. silvacata* Drake, 1926, and *T. tecomae* Monte, 1940 (Drake & Ruhoff, 1965). Their host plants include Bignoniaceae, Dileniaceae and Malpighiaceae (Drake & Ruhoff, 1965).

Recent cladistic analyses on suprageneric taxa within Tingidae have suggested that *Tingis* is non-monophyletic (Guilbert, 2001; 2004), hypothesis that still needs testing. Up to now, we have biological information only for North Hemisphere species - *T. ampliata* (Herrich-Schaeffer, 1838) (Eguagie, 1972, 1975; Peschken, 1977), *T. beesoni* Drake, 1928 (Mathur, 1979), *T. cardui* (Linnaeus, 1803) (Eguagie, 1974), and brief comments for *T. stachydis* (Fieber, 1844) (Stusák, 1968).

Trumpet trees (*Handroanthus* spp. and *Tabebuia* spp.) comprise about a hundred species, mainly found in Neotropics (Olmstead & Grose, 2007) where are used as ornamental elements in urban landscapes (Lorenzi, 2002). In studies carried out in a seasonal dry forest, in southeastern Brazil, *Tingis stecoma* sic (= *T. tecomae*) was the most abundant herbivorous insect on *Handroanthus spongiosus* (Rizzini) S. O. Grose (Oliveira *et al*., 2012; Silva *et al*., 2012).

Tingis americana is recorded in Brazil, Paraguay and Argentina and so far its only host plant record is

Tecoma sp. (Bignoniaceae) (DRAKE & RUHOFF, 1965). Nymphs and adults of this species were found feeding on two Bignoniaceae, *Handroanthus heptaphyllus* (Vell.) Mattos (purple trumpet tree) and *H. chrysotrichus* (Mart. ex DC) Mattos (yellow trumpet tree) in the Porto Alegre Botanic Garden, causing seedling death. Biological data of *T. americana* are still lacking. In this paper we compared the nymphal development of this lace bug on both *H. heptaphyllus* and *H. chrysotrichus*, analysing its reproductive parameters on *H. heptaphyllus*. Additionally, this is the first record of *T. americana* on these host plants and in southern Brazil.

MATERIAL AND METHODS

***Tingis americana* colony rearing.** Individuals were collected in the Botanic Garden in March 2011, from seedlings of *H. heptaphyllus*. Adults were kept at 25 ± 1°C; 60 ± 10% RH; 16 h photophase, in seedlings obtained from the same place. Adults were maintained on the same seedlings for egg obtaining. Seedlings were replaced by fresh ones whenever damaged.

Nymphal development. Newly emerged nymphs were individualized in Petri dishes with a leaflet of *H. heptaphyllus* (n = 117 individuals) or *H. chrysotrichus* (n = 110) inserted in agar and water, plus antifungical (nipagin) and antimicrobian (streptomycin sulfate) (adapted from FOELCKEL *et al.*, 2009). The dishes were kept under the same conditions as the colony. Previously, the leaflets were cleaned in a 1–2% sodium hipochlorid solution during one to two minutes, and then dried with paper towel. Leaflets were replaced every three days. Exuviae and dead individuals were daily recorded under stereomicroscope. Sex ratio was calculated based on the emerged insects.

Longevity and reproductive parameters. Unpaired, newly emerged adults (60 females and 41 males) obtained from the colony were individualized in Petri dishes with a leaflet of *H. heptaphyllus*, which was replaced every three or four days. These individuals were observed until their death. Paired specimens (16 couples) were maintained under the same conditions in order to register longevity, fecundity, fertility and, pre-, post- and oviposition periods. *Handroanthus heptaphyllus* leaflets were used as food and oviposition substrate as well. Couples were daily observed and transferred to a new leaflet, until their death. Fecundity was evaluated by daily computing the number of eggs laid in each leaflet, observed under a stereomicroscope provided with transmitted light. Fertility was measured through the number of hatched insects.

Statistical analyses. Data were tested for normality by D'Agostino test and, according to their distribution, compared by t-Student, Mann-Whitney, ANOVA or Kruskal-Wallis tests. Sex ratio (sr) was calculated by [sr = number of females/(number of females + males)], and nymphal viability (%) was compared by Qui-square test (heterogeneity and adherence). The relation between age of females and number of laid eggs was measured by Pearson correlation. We adopted the significance level of 0.05 in all the tests performed. For these analyses, we used the softwares Microsoft® Excel 2010 and Bioestat® 5.0 (AYRES *et al.*, 2007).

The lace bug specimens were identified by Dr. Luiz Costa (Museu Nacional – MNRJ, Rio de Janeiro, Brazil). Vouchers are deposited in the entomological collection of the Museu de Ciências Naturais – MCNZ, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil (40 specimens, catalog numbers 181.841 to 181.880).

RESULTS

Compared nymphal development on *H. heptaphyllus* and *H. chrysotrichus*. The mean nymphal period was shorter on insects reared on *H. heptaphyllus* (12.69 ± 0.076 days) than on *H. chrysotrichus* (19.11 ± 0.208 days) (U = 0.50; P < 0.0001). When analyzed separately by sex, this pattern was repeated, respectively on *H. heptaphyllus* and *H. chrysotrichus*, either for males (12.71 ± 0.094; 18.89 ± 0.313 days) (U = 0; P < 0.0001); or for females (12.68 ± 0.110; 19.35 ± 0.110 days) (U = 0; P < 0.0001). The second and third instars were the shortest and the fifth the longest, on both hosts (Tab. I). Nymphal viability on individuals reared on *H. heptaphyllus* (86.3%) and on *H. chrysotrichus* (77.3%) was similar (χ^2 = 3.139; P = 0.0764; Tab. I) .

From 101 nymphs that became adults on *H. heptaphyllus*, 60 were females and 41 males, resulting in a sex ratio of 0.59, which did not differ from the expected (χ^2 = 3.74; P = 0.0587). On *H. chrysotrichus*, 40 females and 45 males were obtained (sr = 0.46), the

Tab. I. Developmental time [mean period ± SE and variation interval (VI), in days] and viability (%) of nymphs of *Tingis americana* Drake, 1922 maintained on *Handroanthus heptaphyllus* and *H. chrysotrichus* (n = number of observations) (25 ± 1°C; 60 ± 10% RH; 16 h photophase) [means followed by the same letter, lowercase in the column and uppercase in the line, do not differ significantly (α = 0.05)].

Plant species/ Instar	*H. heptaphyllus*			*H. chrysotrichus*		
	Period (days)	VI (days)	Viability (%)	Period (days)	VI (days)	Viability (%)
1st	2.84 ± 0.037 bB (n = 109)	2 – 4	93.2	3.93 ± 0.077 bA (n = 99)	3 – 6	90.0
2nd	1.89 ± 0.036 dB (n = 108)	1 – 3	99.1	3.09 ± 0.069 cA (n = 93)	2 – 6	94.0
3rd	1.94 ± 0.040 dB (n = 105)	1 – 3	97.2	3.05 ± 0.070 cA (n = 88)	2 – 5	94.6
4th	2.25 ± 0.051 cB (n = 103)	1 – 4	98.0	3.6 ± 0.080 bA (n = 86)	2 – 5	97.7
5th	3.74 ± 0.052 aB (n = 101)	3 – 5	98.1	5.5 ± 0.097 aA (n = 85)	3 – 7	98.8

sex ratio was also similar to expected ($\chi^2 = 0.294$; $P = 0.876$).

Longevity and reproductive parameters on *H. heptaphyllus*. *Tingis americana* oviposited anywhere on the leaves, but usually near primary or secondary veins, on abaxial side. Eggs, laid singly or in small groups, were partially or deeply inserted into vegetal tissue, characterizing an endophytic oviposition. However, the operculum was always apparent just above foliar epidermis.

The mean total fecundity was 310.0 ± 19.40 eggs/female, while the mean daily fecundity was 7.46 ± 0.302. The maximum number of eggs was 21/female during a 24h period, but the most frequent pattern (85 of 718 occasions) was one egg a day, followed by clusters of eight, nine and seven eggs, respectively. The oviposition period represented about 84.5% of the female total longevity (Tab. II). As the female age increased, the mean daily fecundity decreased ($r = -0.7171$; $P < 0.0001$) (Fig. 1).

Considering the total of 4,960 eggs laid, the mean number of fertile eggs per female was 286.25 ± 18.920. The egg viability was 92% and the mean embryonic period was 12.32 ± 0.274 days.

Paired males and females showed similar longevity ($t = -1.9575$; $P = 0.0691$) (Tab. II). In average, unpaired females lived longer (51.88 ± 1.679 days) than males (45.21 ± 2.910 days) ($U = 941.50$; $P = 0.0460$).

Tab. II. Longevity of paired males and females, pre-oviposition, oviposition and pos-oviposition periods (days) (± SE) and respective variation intervals (VI) of *Tingis americana* Drake, 1922 maintained on *Handroanthus heptaphyllus* leaflets (25 ± 1°C; 60 ± 10% RH; 16 h photophase) (* = non significant).

Biological parameter	Mean duration (± SE)	VI
Longevity (days)*		
Females	53.13 ± 3.999	28 – 79
Males	60.81 ± 4.070	35 – 89
Period (days)		
Pre-oviposition	3.1 ± 0.14	2 – 4
Oviposition	44.9 ± 3.77	23 – 70
Post-oviposition	3.3 ± 0.80	0 – 11

DISCUSSION

Despite the longer developmental period on *H. chrysotrichus*, the nymphal viability of *T. americana* was similar on both host plants. The higher amount of tricomes on the leaflets of *H. chrysotrichus* could explain the difference. Tricomes are usuallly efficient barriers to immature or small insects, hampering their movements or their feeding activities (Schoonhoven *et al.*, 2005). This may result in a quantitative or qualitative nutritional deficit, reflecting on development, fecundity, and/or longevity (Parra, 1991). However, Ribeiro *et al.* (1994) demonstrated that the congeneric *T. tecomae* did not show preference by leaves of *Tabebuia ochracea* [= *Handroanthus ochraceous* (Cham.) Mattos] with less density of tricomes. Instead, that species preferred larger leaves. Other hypotheses for our results include differences on chemical composition and nutritional value among the host plant species. The influence of these factors should be also investigated for the *T. americana* – *H. chrysotrichus* and *H. heptaphyllus* interaction.

The shorter second and third instars and the longest fifth was also observed for congeners of *T. americana*, such as *T. ampliata* (Southwood & Scudder, 1956), *T. cardui* (Eguagie, 1974), and *T. beesoni* (Mathur, 1979). The longer fifth instar was obtained for other Tingini, as *Gargaphia torresi* Lima, 1922 (Silva, 2004), *Leptopharsa heveae* Drake & Poor, 1935 (Cividanes *et al.*, 2004), *Stephanitis pyrioides* (Scott, 1874) (Braman

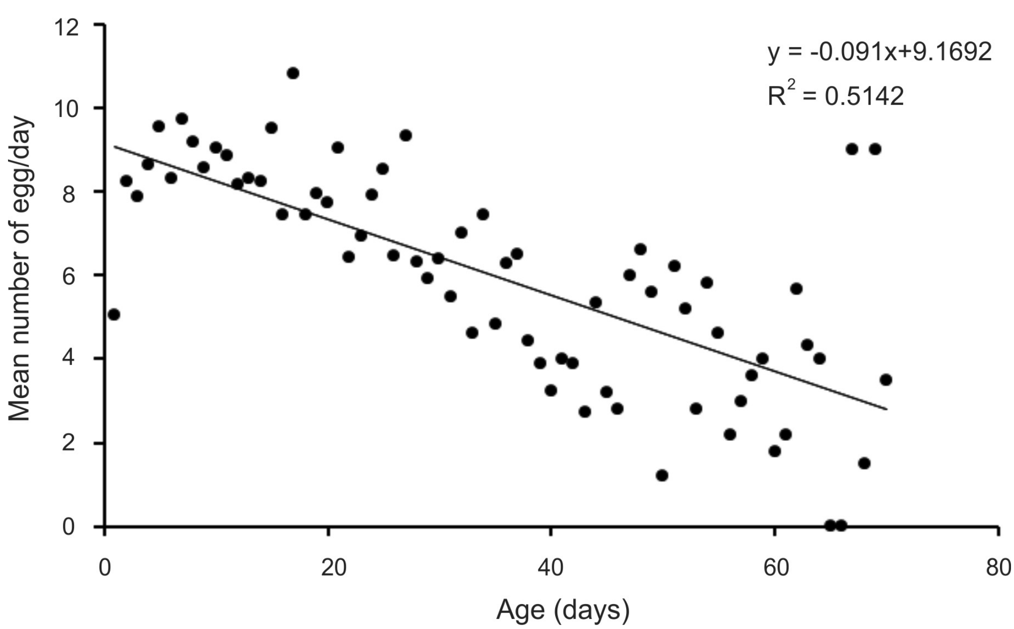

Fig. 1. Mean number of eggs/day through the female lifespan of *Tingis americana* Drake, 1922 on *Handroanthus heptaphyllus* leaflets (25 ± 1°C; 60 ± 10% RH; 16 h photophase).

et al., 1992), and *Leptoypha hospita* Drake & Poor, 1937 (ZHANG *et al.*, 2011). According to PARRA (1991), the maturation of reproductive organs and the full development of the wings occur during this phase. The mean periods of the different instars in *T. americana* were similar to the observed for *T. beesoni* in field conditions, in India (MATHUR, 1979). For *T. ampliata* (SOUTHWOOD & SCUDDER, 1956) and *T. cardui* (EGUAGIE, 1974), also under natural conditions, these periods were longer. These differences among *Tingis* species may be related, besides different rearing methods, to their distinct host plants. *Tingis beesoni* is reported on *Gmelina arborea* Roxb. (Verbenaceae), *T. ampliata* on *Cirsium arvense* (L.) Scop. (Asteraceae) and *T. cardui* on several Asteraceae - *Carduus* spp., *Cirsium* spp., *Galactites tomentosa* (L.) Moench., besides *Pinus* spp. (Pinaceae), and others (DRAKE & RUHOFF, 1965).

Oviposition pattern in *T. americana* shows common traits with *T. cardui*, which oviposits singly on the abaxial side of leaves, usually at either side of veins (EGUAGIE, 1974), and also to *T. beesoni*, whose eggs are laid in small groups, spread on foliar surface (MATHUR, 1979). The mean embryonic period varies among *Tingis* species. In *T. beesoni*, this period lasts for two to six days (MATHUR, 1979). For *T. cardui*, EGUAGIE (1974) reported a mean of 18 days, longer than *T. ampliata* (8.4 days) (EGUAGIE, 1972). However, comparisons should be done cautiously, as many of these studies were carried out under uncontrolled conditions and based on few specimens. The high egg viability showed by *T. americana* was similar to the obtained for *T. ampliata* at 25°C (93.7%) (EGUAGIE, 1972), while the mean total fecundity was higher to that recorded for *T. cardui* (65 eggs/female) (EGUAGIE, 1974). The oviposition period in *T. americana* represented about 84.5% of the total female lifespan.

The sex ratio in *T. americana*, on both hosts, was similar to the observed for *T. cardui* (EGUAGIE, 1974). The shortest longevity showed by unpaired individuals could be explained by the distinct treatment, because these lace bugs were provided with leaflets replaced every three or four days. Conversely, paired individuals were fed on fresh, daily replaced leaflets in order to obtain eggs. Further experiments standardizing this procedure could be carried out to test this hypothesis.

Based on pre-oviposition, egg and nymphal periods, under these conditions, we could obtain a new generation of *T. americana* in about every 28 days. *Handroanthus heptaphyllus*, in the state of Rio Grande do Sul, retains its foliage from October to April (LONGHI, 1995). Supposedly, it would allow the development of about seven or eight generations of this lace bug. Further studies about *T. americana* on adult trees, under natural conditions, are needed to have a clearer picture of its biology, including the possible occurrence of diapause at this latitude, when leaves are not available. Another aspect we should take into account is that *Tingis* may be a paraphyletic genus, composed by unrelated species. This could explain the differences found regarding their biology.

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