

Life-history analysis of *Thaumastocoris peregrinus* in a newly designed mass rearing strategy

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Abstract

The bronze bug, *Thaumastocoris peregrinus* Carpintero et Dellape (Heteroptera Thaumastocoridae), is one of the most important emerging pests of *Eucalyptus* LHeritier plantations worldwide. In the development of strategies to control this pest, establishing effective rearing protocols is fundamental to future research programs. We assessed life-history parameters of the bronze bug in a newly designed mass rearing strategy. Separated units were set up to contain different developmental stages. Egg production by females reared on commonly found *Eucalyptus grandis* Hill ex Maiden and *Eucalyptus tereticornis* Smith was evaluated in order to determine which plant species to use in rearing. Females laid more eggs on *E. tereticornis* than on *E. grandis*, so the former species was chosen for the rearing. A cohort of 207 eggs was followed in Petri dishes until the last individual died or reached the adult stage. We followed egg production by 15 adult couples from the original cohort. Preparation of 150-200 dishes with hatching eggs per week allows for an average production of 7,500 eggs per week. Under our rearing conditions, eggs started hatching on day six, and the first adults were obtained 23 days after oviposition. Almost half of the eggs did not hatch, and the highest nymphal mortality was recorded in the second instar, while the lowest mortality occurred in the last instar. We discuss the relevance of this mass rearing strategy, both within the context of basic behavioural studies of *T. peregrinus*, and as a tool for the mass rearing of the biological control agent, *Cleruchoidea noackae* Lin et Huber.

Key words: rearing protocols, *Eucalyptus*, forestry, *Cleruchoidea noackae*, biological control, bronze bug.

Introduction

The bronze bug *Thaumastocoris peregrinus* Carpintero et Dellape (Heteroptera Thaumastocoridae) is a major pest of *Eucalyptus* LHeritier plantations worldwide. This small true bug started to disperse from its Australian origin in the beginning of the 2000s invading South Africa (Jacobs and Naser, 2005), Argentina (Noack and Coviella, 2006), Brazil (Wilcken *et al.*, 2010), Uruguay (Martínez and Bianchi, 2010), Chile (Ide *et al.*, 2011), New Zealand (Sopow *et al.*, 2012), Italy (Laudonia and Sasso, 2012), and Portugal (García *et al.*, 2013). Management techniques proposed to control this insect include chemical control (Noack *et al.*, 2009), the use of entomopathogenic fungi (Mascarín *et al.*, 2012), and parasitoid wasps (Nadel and Noack, 2012) for biological control. The egg parasitoid *Cleruchoidea noackae* Lin et Huber (Hymenoptera Mymaridae) was identified in Australia from specimens collected in a rearing colony of *T. peregrinus* (Lin *et al.*, 2007) and has been proposed as a candidate for a biological control program (Nadel *et al.*, 2012; Nadel and Noack, 2012). This parasitoid has been released in Chile (SAG, 2010), Brazil (IPEF, 2012), Uruguay (INIA, 2013), and South Africa (Anonymous, 2013). In order to evaluate the parasitoid's biological characteristics and to support the large-scale implementation of biological control of *T. peregrinus* by *C. noackae*, a continuous supply of fresh eggs of the parasitoid's host is essential. However, developing a successful mass rearing methodology for *T. peregrinus* has proven to be difficult (Nadel and Noack, 2012).

The first rearing method described for this insect involved the use of leaf sections attached to entomological pins inside inverted polypropylene vials with caps half-filled with water, generating a 'fluid barrier' (Noack and Rose, 2007). Similarly, Soliman *et al.* (2012) used Petri dishes with leaf sections floating on water, a setup that allowed them to estimate the duration of different stages and the fecundity of females when reared on different *Eucalyptus* genotypes and hybrids. The water barrier was necessary to maintain the insects on the leaf section, because the juveniles are very mobile. However, the use of Petri dishes for mass production of eggs is costly in terms of labour, time and resources. Here, we describe a mixed method for mass rearing of *T. peregrinus* by using Petri dishes and mesh screen cages. In addition, we provide quantitative information on several life history parameters, obtained during the development of this rearing method.

Materials and methods

Origin of the colony

Adults and 4th-5th instar nymphs of *T. peregrinus* were collected from natural populations in different plantations in Uruguay in the summer of 2010: a commercial plantation of *Eucalyptus grandis* Hill ex Maiden (31°53'56.40"S 55°48'29.80"W), a seed orchard of *Eucalyptus globulus* Labillardiere (34°39'49.62"S 56°20'23.23"W), and a shadow and shelter plantation of *Eucalyptus tereticornis* Smith (31°44'19.24"S

55°58'43.75"W). The specimens collected were placed in a mesh screen cage (founder cage) (0.5 × 0.5 × 1.0 m) containing a seedling of *Eucalyptus* spp. and were left to oviposit. The cages were located in a climate-controlled room at 20 ± 5 °C, 55 ± 10% RH (mean ± SEM throughout) and provided with natural daylight.

Egg production on different *Eucalyptus* species

The most abundant eucalypt species in the region are rose gum (*E. grandis*) and red gum (*E. tereticornis*; *Eucalyptus camaldulensis* Dehnhardt and hybrids). In order to decide which species to use in the rearing experiments, 60 males and 75 females of *T. peregrinus* were left to oviposit in cages containing either *E. grandis* or *E. tereticornis* branches, with six replications of each treatment. The total number of eggs from each cage was counted after three days and egg production rates were compared by a Wilcoxon rank sum test by using R statistical software (R Development Core Team, 2009).

Rearing scheme (figure 1)

After two generations in the founder cage, adult *T. peregrinus* were transferred to smaller mesh-screen cages (0.35 × 0.50 × 0.70 m), hereafter called oviposition cages (figure 2a). Each oviposition cage contained approximately 80 females and 60 males, in accordance with the sex ratio observed in field collections during summer, which was around 0.75 m/f (Gonzalo Martínez, unpublished). Fresh *E. tereticornis* branches were provided for food and oviposition. Branches were previously cleaned with 5% sodium hypochlorite solution and rinsed with distilled water to decrease the pathogen load, then placed in an Erlenmeyer flask with distilled water (figure 2b). Branches were replaced every two days and the insects were counted, sexed and relocated to the new

branches with the help of a paintbrush. Simultaneously, eggs laid in old leaves were collected and counted. Egg-carrying leaves were detached from the branch, and leaf sections containing eggs were cut out carefully. These leaf sections were placed in Petri dishes ('hatching dishes') (5.5 cm diameter) and incubated in a rearing chamber set at 25.0 ± 0.5 °C, 55.0 ± 1.5% RH, and 12:12 L:D. Each hatching dish contained up to 10 eggs placed onto a larger leaf disc, cut out from a mature *Eucalyptus* spp leaf, which was floating on water and covered most of the dish base (figure 2c). Eggs were checked each day for hatching. After molting, second instar nymphs were transferred with a paintbrush to a mesh screen cage (maturation cage) similar to that used for adults, and provided with fresh *E. tereticornis* branches. The maturation cages were checked daily for adult emergence. Recently molted adults, recognized by their whitish integument, were sexed and transferred to separate cages for virgin males and virgin females ('virgin cages'), with characteristics similar to the 'maturation' and 'oviposition cages'. If newly emerged adults had their cuticle already sclerotized, they were not considered virgins and they were transferred to oviposition cages with mixed sexes.

Life cycle at rearing conditions

A life table was constructed after following a cohort of 207 eggs from the hatching dishes in the rearing conditions described above. A life table represents quantitatively the main characteristics of the age-specific reproduction and mortality (Southwood, 1978). After hatching, each nymph was transferred individually to a Petri dish and observed each day at the same hour. All Petri dishes were incubated at 25.0 ± 0.5 °C, 55.0 ± 1.5% RH and 12:12 L:D. Survival and molting events were

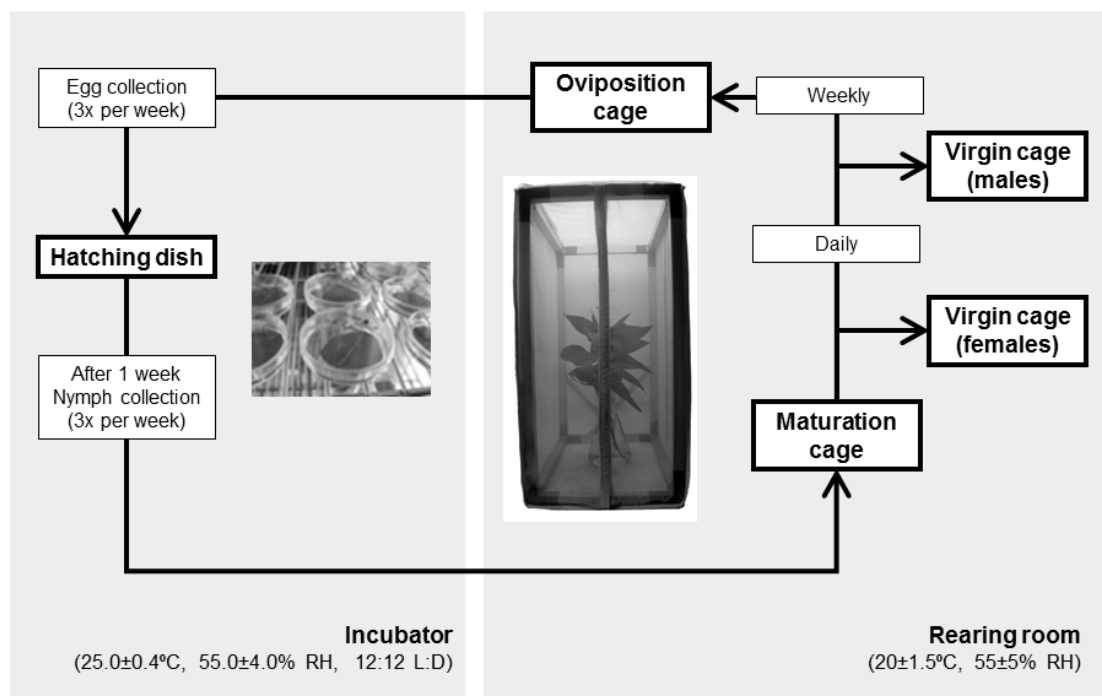


Figure 1. Rearing scheme developed for *T. peregrinus*.

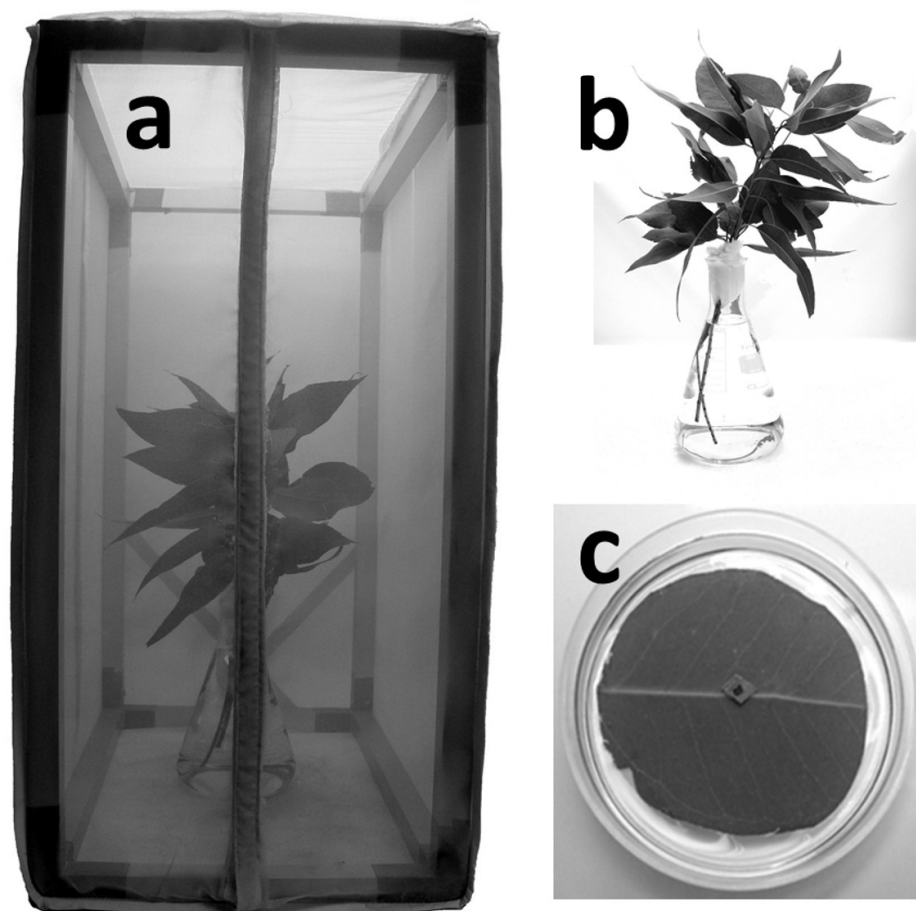


Figure 2. Different units used for the rearing of *T. peregrinus*: **a.** Mesh screen cage; **b.** Branches used within the cages; **c.** Hatching dish with a patch of eggs of *T. peregrinus* in the centre.

recorded until the last individual had died or reached the adult stage. This methodology was chosen to compare the results with other studies (Soliman *et al.*, 2012). A horizontal life table (i.e.: grouping individuals into the same instar) was built by calculating the following variables:

a_0 : total number of living individuals at the beginning of the experiment;

a_x : total number of living individuals observed at the beginning of each instar;

$l_x = (a_x/a_0)$: proportion of the individuals of the original cohort that remained alive at the beginning of each instar;

$d_x = (l_x - l_{x+1})$: proportion of the original cohort that died during each instar;

$q_x = d_x/l_x$: stage-specific mortality rate;

$K_x = \log_{10}(a_x) - \log_{10}(a_{x+1})$: killing force.

Once the adult stage had been reached, individuals from the original cohort were placed as male-female couples into new Petri dishes, and egg production was recorded daily until the last female had died.

Simultaneously, the productivity of the rearing strategy was measured by computing the total number of eggs produced per week and per cage. The number of eggs laid by individual females was estimated by dividing the total number of eggs by the number of females alive during that week.

Results

A total of 516 adults and 407 nymphs were collected in the field and used for rearing the first generations in three founder cages. The rearing system consisted of six oviposition cages, three maturation cages, two virgin cages (one for each sex), and an average number of 250 egg-hatching dishes in two incubator chambers. Three technicians spent an average of six hours per day performing rearing tasks; including counting and sexing individuals, mounting hatching dishes, preparing and changing branches, and processing data.

Egg production on different *Eucalyptus* species

Females laid significantly more eggs on *E. tereticornis* than on *E. grandis* (Wilcoxon rank sum test; $W = 0$, $p = 0.002$, $n = 6$, figure 3), so we decided to use this species in all rearing activities.

Life cycle at rearing conditions

A total of 113 nymphs hatched from the 207 eggs, most of them during the sixth day after egg laying. The total duration of the nymphal stages of *T. peregrinus* was 17.2 ± 1.1 days (table 1), which were divided in five instars as recorded in previous studies (Noack and Rose, 2007; Soliman *et al.*, 2012) (figure 4). Higher nymph mortality rates were recorded in the early instars, with

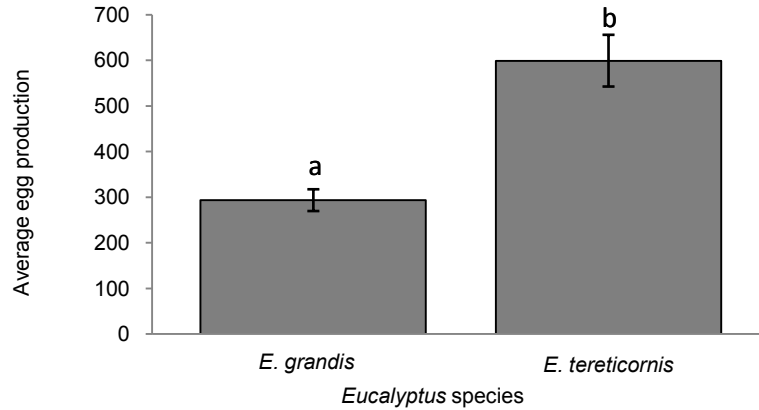


Figure 3. *T. peregrinus* egg production after three days in rearing cages containing 75 females and 50 males. Branches of two *Eucalyptus* species were used as feeding and oviposition substrates. Different letters indicate significant differences (Wilcoxon rank sum test; $p = 0.002$, $n = 6$).

Table 1. Duration of the stages of *T. peregrinus* in the rearing conditions. N represents number of individuals.

State	Instar	Duration (days)	N
Egg	-	6.0 ± 0.9	207
Nymph	Nymph I	3.7 ± 0.8	80
	Nymph II	2.8 ± 1.0	41
	Nymph III	2.5 ± 0.9	26
	Nymph IV	3.5 ± 1.0	21
	Nymph V	4.7 ± 0.7	20
Total nymph	-	17.2 ± 1.1	80
Adult	Pre-oviposition	6.9 ± 0.6	15
	Oviposition	15.5 ± 2.3	15
	Post-oviposition	0.5 ± 0.2	15
Total adult	-	23.0 ± 2.3	15

the second instar showing the highest stage-specific mortality rate ($q_x = 0.488$) (table 2). The last nymphal instar lasted longest (4.7 ± 0.7 days) (table 1) and exhibited the lowest mortality rate ($q_x = 0.05$) (table 2).

Egg production

For the reproductive period we followed egg production of 15 adult couples (table 1). As we obtained less than 30 individuals from the original cohort, couples were completed by using individuals from the general rearing, that had emerged on the same day. The conditions during this part of the study were not the same as in the general rearing, because the couples were placed in Petri dishes instead of mesh screen cages, in order to follow individual couples. Nonetheless, the results provide a rough estimate of life span and egg production

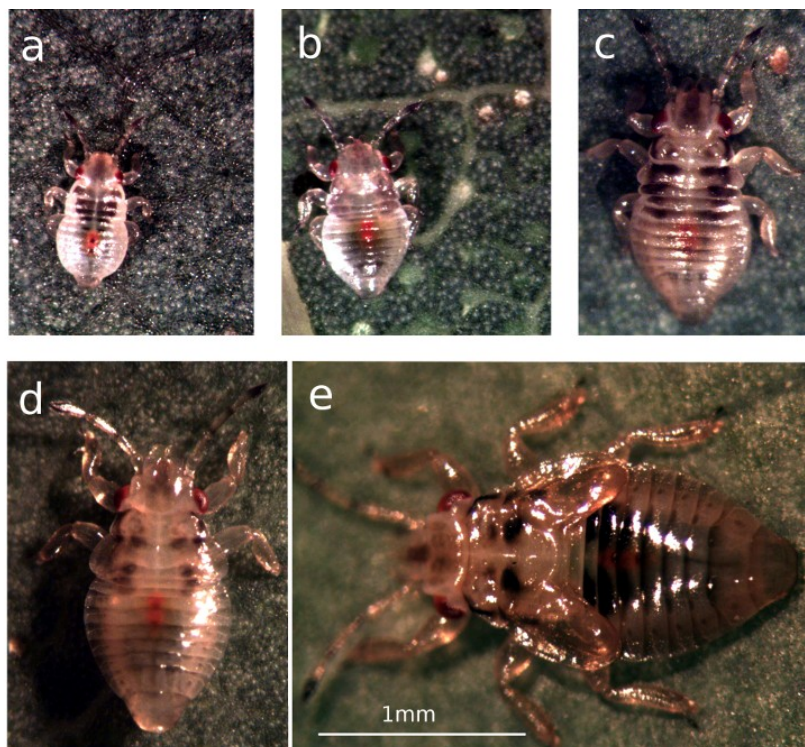


Figure 4. Nymphal stages of *T. peregrinus*. a. Nymph I; b. Nymph II; c. Nymph III; d. Nymph IV; e. Nymph V.

Table 2. Life table for *T. peregrinus* in the rearing conditions (n = 207).

Instar	a_x	l_x	d_x	q_x	k_x
Egg	207	1.000	0.454	0.454	0.263
Nymph I	113	0.546	0.159	0.292	0.150
Nymph II	80	0.387	0.188	0.488	0.290
Nymph III	41	0.198	0.073	0.366	0.198
Nymph IV	26	0.126	0.024	0.192	0.093
Nymph V	21	0.101	0.005	0.048	0.021
Adult	20	0.097	0.097	-	-

a_x : total number of living individuals observed at the beginning of each instar; l_x : proportion of the individuals of the original cohort that remained alive at the beginning of each instar; d_x : proportion of the original cohort that died during each instar; q_x : stage-specific mortality rate; k_x : killing force.

rates that can be expected in the rearing conditions. The average total duration of the adult stage was 23.0 ± 2.3 days, with a pre-oviposition period of 6.9 ± 0.6 days.

The females laid eggs for 15.5 ± 2.3 days, and died soon after egg laying ended (post-oviposition period: 0.5 ± 0.2 days). Daily egg production during the reproductive period was 2.5 ± 0.1 . After day 29 only one couple was alive, and hence data on its egg production were not included (figure 5).

Figure 6 shows the weekly egg production in the rearing colony of *T. peregrinus* during 2012, when the rearing scheme presented here was fully implemented. The first weeks showed the lowest production, which coincided with moving to a new building with better thermal insulation. After week 10, egg production stabilized between 1000 and 1500 eggs per cage per week, showing sporadic peaks such as those observed during weeks 10, 17 and 46. Not only a higher total production of eggs but also a higher number of eggs laid per female was recorded during these peaks, which in turn coincided with longer periods without manipulation because of holidays, suggesting that stress due to manipulation (changing of branches, counting of individuals) may have an effect on egg productivity.

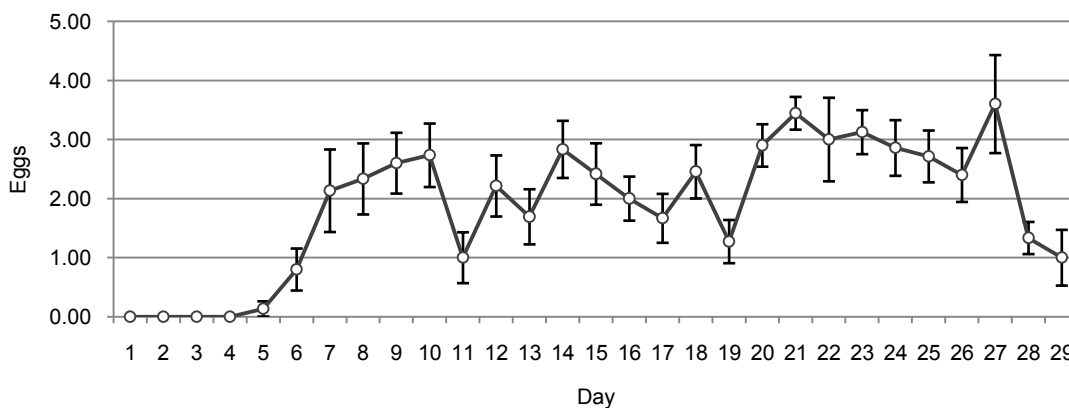


Figure 5. Average daily egg production by individual *T. peregrinus* couples in Petri dishes (initial n = 15).

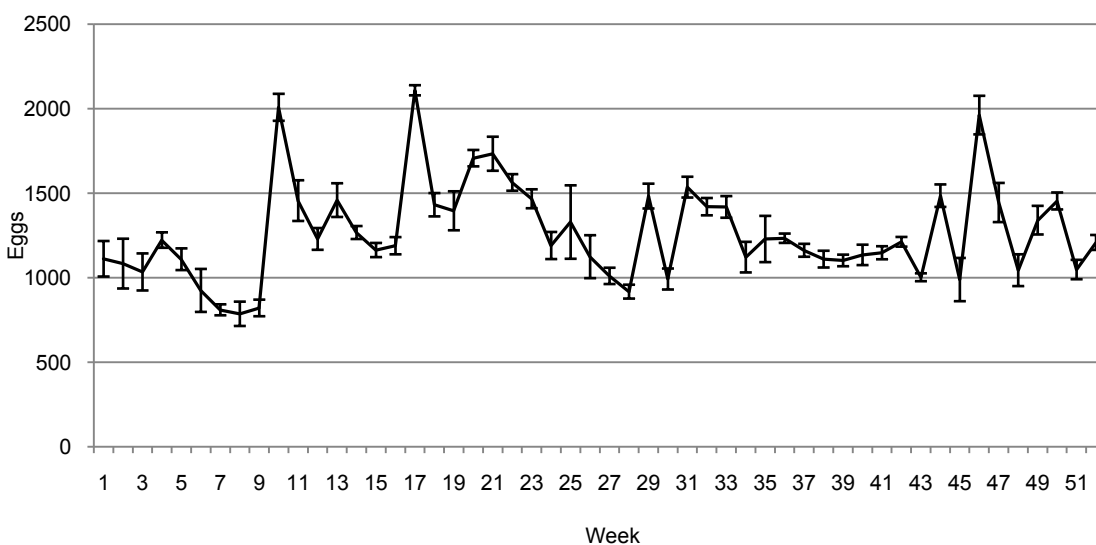


Figure 6. Average weekly egg production by *T. peregrinus* per cage, once the rearing scheme had been fully implemented (n = 6).

Discussion

The rearing method presented here allowed for a successful production of *T. peregrinus* eggs at a large scale; which can be both used as a source for new colonies (if needed for additional studies) or as hosts for biological control agents. By preparing 150-200 hatching dishes per week, which can be accomplished by two people, part time, an average production of 7,500 eggs can be obtained. The eggs were collected within 48 h after oviposition, which coincides with the time window for parasitization by the egg parasitoid *C. noackae* (Mutitu *et al.*, 2013).

An efficient rearing method requires plant material that not only optimizes egg production, but is also easy to obtain and manipulate. In our study, a larger number of eggs were produced when *T. peregrinus* females were offered *E. tereticornis* instead of *E. grandis*. Although to our knowledge no reports are available on the fecundity of the bugs when feeding on *E. tereticornis*, a previous study showed higher oviposition rates on *E. grandis* than on *E. camaldulensis* (Soliman *et al.*, 2012), a species closely related to *E. tereticornis*, with which hybrids are usually formed in Uruguay and Argentina. Hence, the eucalypt species has an impact on *T. peregrinus* oviposition rates, and therefore is an important factor for rearing optimization.

Under the rearing conditions used in this study, it took 23 days for eggs to develop into adults, including an average of 6 days for egg eclosion. Since first-instar nymphs are highly susceptible to fungal attack, which in turn may be favoured by water condensation inside the Petri dish, the frequency of checking hatching dishes for newly emerged nymphs was important for improving survival. The duration of the immature stages in our study was somewhat shorter than that previously reported for *T. peregrinus* in the field (Jacobs and Naser, 2005; Bouvet and Vaccaro, 2007). The relative duration of the immature instars was similar to that reported by Soliman *et al.* (2012) for a laboratory study: the first, fourth and fifth instars lasting longer than the second and third. While mortality varied among stages and instars, highest mortality occurred during the egg stage, since almost half of the eggs did not hatch. This can be due to several causes such as desiccation of the leaf on which they had been laid, drowning in condensation water, or the fact that *T. peregrinus* can lay unfertilized eggs just as recorded for other heteropterans (Schuh and Slater, 1995). Previous studies have reported variable hatching rates for eggs obtained *in vitro*, from 22% (Noack and Rose, 2007) to 80% (Soliman, 2012) and even 95% (Mutitu *et al.*, 2013). It is hence evident that factors affecting egg viability should be studied and eventually improved, for example by dissecting unhatched eggs and preventing desiccation or excessive water. Early nymphs showed greater susceptibility to manipulation, which led us to wait for at least one molt in the dish before touching the individuals. Later nymphal instars showed significantly less mortality, and were hence easily manipulated. In our study in Petri dishes, pre-oviposition time lasted about a week, which is shorter than the ten days reported on *E. camaldulensis* (Soliman *et al.*, 2012).

The total duration of the adult stage was 23.0 ± 2.3 days, slightly more than half of what has been previously reported (Bouvet and Vaccaro, 2007; Martínez and Bianchi, 2010; Wilcken *et al.*, 2010; Soliman *et al.*, 2012). Such reduction in adult lifespan could be a consequence of the experimental setup, including the choice of the *Eucalyptus* species, which could have reduced the post-ovipositional period in our experiment. The total egg production per female in individual Petri dishes was 39.5 ± 6.1 , which falls between the productivity reported for females feeding on *E. camaldulensis* and *E. grandis* (23 and 75 eggs/female, respectively) (Soliman *et al.*, 2012).

Egg production has been maintained within the range of 1000 to 1500 per cage per week, or ranging from 11 to 24 per female per week. These values correspond to a daily oviposition rate between 1.6 and 3.4 eggs per female, which matches the daily egg production we observed for individual *T. peregrinus* in Petri dishes (2.5 ± 0.1), as well as previous reports (Noack and Rose, 2007; Soliman *et al.*, 2012). The egg production in the rearing increased during several periods when the cages remained un-inspected, suggesting that manipulation of the individuals due to manual transfer from old branches to new ones negatively affected reproductive parameters. An alternative way of transferring the insects to new feeding leaves is currently under investigation.

The importance of developing a reliable rearing scheme for the bronze bug is evident given the rapid spread of this pest in the world, and the need for a continuous egg supply to develop a successful biological control program with the egg parasitoid *C. noackae*. Furthermore, a constant supply of individuals is particularly required in order to continue basic biological studies of both the pest and the parasitoid. Finally, the weekly counting of the products obtained from each rearing unit (whether eggs, juveniles or adults) paves the way for assessing the health of the colony, and for identifying the sources of eventual rearing problems.

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