LIFE-HISTORY OF *THAUMASTOCORIS PEREGRINUS* AND *THAUMASTOCORIS* SP. IN THE LABORATORY WITH SOME OBSERVATIONS ON BEHAVIOUR

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Summary

Thaumastocoris peregrinus Carpintero and Dellapé is a new pest of *Eucalyptus* in Sydney and southern South Africa and has recently arrived in Argentina. Recently another as yet undescribed species of this genus was discovered and is an emerging pest of *Corymbia* in Sydney. Laboratory studies were undertaken to elucidate life history parameters of both species such as instar duration, adult longevity and reproductive potential. At 17-22 °C the eggs of both species hatched in approximately six days. The duration of the five stadia were similar in both species: with *T. peregrinus* taking approximately 4.6, 3.5, 3.3, 3.7 and 5.3 days, while *T. sp.* 4.7, 3.4, 3.5, 4.0 and 5.2 days. The adult males and females of both *Thaumastocoris* spp. can live for approximately 40 days and females can produce at least 60 eggs during that time. The laying of virgin eggs is reported for this genus.

Keywords: *Thaumastocoris peregrinus, Thaumastocoris* sp., life-cycle, rearing methods, *Eucalyptus* pest

INTRODUCTION

Until now much of the interest in the family Thaumastocoridae (Heteroptera) has been generated by their "*novel morphology, austral distribution and relative scarcity in collections*" (Cassis *et al.* 1999). However, during the past eight years a thaumastocorid bug has been attacking a number of Sydney's eucalypts and the symptoms of infestation are now so widespread it has a common name - winter bronzing. Two species in particular, *Eucalyptus scoparia* Maiden and *E. nicholii* Maiden and Blakely, are being heavily infested. These trees are very commonly planted street and garden trees prized for their quick growth and compact habit (Noack and Coviella 2006). More recently, this bug has become a pest in South African plantations of *E. camaldulensis* Dehnh. (Jacobs and Neser 2005) and last year was collected on eucalypts in Buenos Aires, Argentina (Carpintero and Dellapé 2006, Noack and Coviella 2006).

Initially this species was thought to be *Thaumastocoris australicus* Kirkaldy (Jacobs and Neser 2005, Noack and Coviella 2006) however it has recently been identified and described as a new species, *Thaumastocoris peregrinus,* by Carpintero and Dellapé (2006).

Thaumastocoris belongs to the small family of phytophagous insects Thaumastocoridae which is comprised of three subfamilies: Thaumastocorinae Kirkaldy whose hosts are predominately dicotyledons; Xylastodorinae Barber which live exclusively on palms (Cassis *et al.* 1999); and Thaicorinae (Kormilev) whose hosts remain unknown. Thaicorinae were recently transferred from Piesmatidae by Heiss and Popov (2002). A host list for *T. peregrinus* has been tabulated (Noack 2002, Jacobs and Neser 2005) and supplemented (Noack and Coviella 2006).

During a survey to ascertain the host range and distribution of *T. peregrinus* within the Sydney basin a new species of *Thaumastocoris* was discovered on *Corymbia citriodora* Hook and *C. maculata* Hook. Over the past three or four years this insect has become evident on *Corymbia,* especially *C. maculata* which appear a washed out green when heavily infested (pers. obs.).

The biology of Thaumastocoridae has received little attention (Cassis *et al*. 1999) with most studies focused on the South American subfamily Xylastodorinae. Couturier *et al.* (2002) investigated the life-history, as well as the egg and nymphal morphology, of *Discocoris drakei* Slater and Ashlock on the Amazonian palm, *Oenocarpus mapora* Karsten. An earlier study by Baranowski (1958) examined the biology of *Xylastodoris luteolus* Barber an introduced pest of royal palms in Florida. Within the Australian subfamily Thaumastocorinae the information is less complete. Hill (1988) investigated the life-cycle and biology of *Baclozygum depressum* Bergroth infesting *E. globulus* Labill. in Tasmania. Kumar (1964) reared *T. australicus* in the laboratory and examined their reproductive morphology but did not present data on their biology.

Our studies are the first biological investigation of *Thaumastocoris*. Our objective is to obtain baseline information on the nymphal development, fertility and longevity of these insects as the knowledge of basic life-history traits is the foundation from which control methods may be derived.

MATERIALS AND METHODS

Our investigation of *Thaumastocoris* biology is the culmination of two studies. Our first examination focused on fecundity and was conducted during the spring of 2002. During the spring of 2006 we conducted an examination of the insect's life-stages. This latter study benefited from the technical experience of the earlier experiment.

The original populations of *Thaumastocoris* for both studies were collected from street and amenity trees growing in inner western suburbs of Sydney, New South Wales. Leaves were clipped from the lower branches of *E. scoparia* and *C. maculata* infested with *T. peregrinus* and an undescribed species of *Thaumastocoris* (henceforth referred to as *T.* sp.) respectively, bagged and taken back to the laboratory.

Examination of fecundity (conducted spring 2002)

In the laboratory 4th and 5th instar nymphs of both species of *Thaumastocoris* were removed from the leaves of the street trees and placed individually into 8.5 cm x 2.5 cm plastic vials (Bacto Laboratories) along with two or three pieces, approximately 2 cm^2, of eucalypt leaf. *T. peregrinus* received leaf material taken from a mature *E. scoparia* and *T.* sp. was provisioned from a *C. maculata* which was at least ten years old. Both trees were growing on the main campus of the University of Sydney. A small piece of moistened tissue paper was added to the vial, it was then sealed with Parafilm® (Pechiney Plastic Packaging). The nymphs were checked daily for adult emergence and fed new leaf material every second day. Newly moulted adults were transferred to a 10.5 cm x 4.5 cm vial (Bacto Laboratories) containing two or three leaves from the above trees. Damp tissue was added and the vial sealed with Parafilm. The date of emergence and sex of the adult were recorded. Adults were placed on the laboratory bench (17-20 °C; photoperiod 12L:12D) and given fresh leaves every two days. Vials were changed approximately weekly or when excreta accumulated.

Approximately half the females and all the males were held individually until death while the remaining females were held with two field-caught males. The field-caught males were taken from the same eucalypts used to provision the laboratory *Thaumastocoris* and were introduced to the female's vial two days after emergence. The insects were checked daily during which time the leaves were searched for eggs. Eggs were cut from the leaves and placed into an 8.5 cm x 2.5 cm vial along with moistened tissue and sealed with Parafilm. The date, identity code of the female (producer) and the number of eggs produced were recorded. From these data the pre-oviposition period (the time between adult emergence and production of the first egg) was attained. The vial was coded and placed on the laboratory bench as above.

Eggs were checked daily. Newly hatched nymphs were removed, placed on a piece of leaf (2 cm^2) and held individually in a vial as above. The date of emergence was recorded and the vial coded. Nymphs were checked daily for exuviae and given fresh leaf pieces every two days. Unhatched eggs were discarded after three weeks.

Examination of life-stages (conducted spring 2006*)*

Egg collection

In the laboratory adults from each species were removed from the leaves of the street trees, separated into sexes under a microscope and placed in approximately even numbers into 10.5 cm x 4.5 cm plastic screw top vials with perforated caps. Adults were provisioned with the fully expanded leaves taken from four year old potted *E. scoparia* (for the *T. peregrinus*) and *C. maculata* (for the *T.* sp.) trees. These leaves were washed to remove any insects or eggs and checked under a microscope before being placed in the vials with the adults. The eggs of *Thaumastocoris* were harvested in two ways. In the first method a circular disc of roughened (scratched with a pin in a cross hatched pattern) blotting paper the diameter of the vial was pushed into the cap of each vial. These insects, especially when agitated, preferentially lay eggs on roughened non-leaf surfaces (Hill 1988). This disc was replaced daily and the eggs were carefully cut from the paper. This method was devised to simplify egg collection and to reduce fungal growth on the eggs during incubation. During the earlier experiment (2002) incubating eggs on the leaf surface readily became covered with fungus. In the second method for collecting eggs the leaves were searched under a microscope for eggs. These eggs were cut from the leaf and as much leaf material around the egg was removed to reduce the quantity of fungal spores near the egg (Hill 1988). Eggs were counted and placed in a 10.5 cm x 4.5 cm screw top vial with a moistened 1cm square piece of blotting paper. The vials were labeled with the date the eggs were laid and placed on the bench top (17-20 °C; photoperiod 12L:12D). Adults were given fresh leaves every second day.

Harvested eggs were checked daily to ensure the blotting paper was moist. At three days of age the eggs were placed on a leaf section (from potted trees) to provide sustenance to newly hatched nymphs. These leaf sections were replaced every second day. Hatching date was recorded and newly hatched nymphs were removed and placed individually in vials. Some eggs

did not hatch completely but for the purpose of the experiment were recorded as hatched if any visible section of the operculum had disengaged from the rim of the egg.

Nymph rearing

Difficulties were encountered culturing nymphs in the 2002 experiment, so an alternative methodology was used. To confine the nymph on a leaf section a fluid barrier system was devised. A stainless steel entomological pin (Austerlitz Insect Pin®, size 3) was inserted through the cap of a 8 cm x 2.5 cm polypropylene vial (Bacto Laboratories) and a piece of leaf, approximately 1 cm x 3 cm, was threaded onto its point. The cap was inverted and half-filled with water. The nymph was placed on the leaf section by carefully sliding a micropin under its abdomen and lifting it. Care was taken to slightly agitate the insect first to ensure it had removed its stylets from the leaf material before being lifted. The transference of 1st–3rd instar nymphs was completed in this way but older nymphs were collected onto the fresh leaf section before it was threaded onto the pin. All nymph transferences were conducted under a microscope. The inverted cap with pin, insect and water would then have the body of the vial screwed on to it. The base of the vial was perforated with large holes to reduce relative humidity within the vial. The nymphs were checked each day for exuviae and given fresh leaf sections every second day.

Number of nymphs and stadia were recorded. Data from drowned nymphs was recorded from its preceding instar only.

Adult rearing

Newly moulted adults were moved off the pins and maintained in a 8 cm x 2.5 cm screw top vial with a perforated cap along with two or three leaf pieces (4 x 5 cm) and moistened blotting paper. All males and approximately half the females were held individually until death. Field-caught males (two per female) were held with the remaining females. These males were introduced when the female was two days old. The insects were checked daily and the leaves from the females' vials were checked under a microscope for eggs which were removed, counted and processed as above. Eggs laid on the surface of the vial were removed with a moistened micropin. Insects were given fresh leaf sections every second day and vials were replaced every four days or when excreta accumulated.

RESULTS

Examination of fecundity

Fifteen 4th and 5th instar nymphs from each *Thaumastocoris* species were taken from the street trees (in 2002) and from this stock 11 adult (7♀:4♂) *T. peregrinus* and 14 adult (6♀:8♂) *T.* sp. emerged. Longevity in males was similar to females in both species and there was little difference between species. *T. peregrinus* males lived for an average of 16 ± 16 days (range 2–38 days) and *T.* sp. for 16 ± 11 days

Figure 1. Wild-caught female *Thaumastocoris peregrinus*. Photograph by Celia Symonds, Australian Museum.

Table 1. Laboratory observations of fecundity parameters (longevity, pre-oviposition and production rate in days, total eggs produced and percentage hatched) in *Thaumastocoris peregrinus* and *T.* sp. during spring 2002.

Species	Mating status	Longevity			Pre-oviposition[§]			Total eggs			Production rate[*]			% hatched		
		Mean±SD	Range	*n*	Mean±SD	Range	*n*	Mean±SD	Range	*n*	Mean±SD	Range	*n*	Mean±SD	Range	*n*
T.peregrinus	Virgin	9.5±2.7	6-12	4	8.0±1.4	7-9	2	6.0±4.2	3-9	2	1.6±0.2	1.5-1.8	2	0	0	0
	Mated	23±18.1	4-40	3	8.5±2.1	7-10	2	45.5±20.5	31-60	2	1.9±0.2	1.8-2.1	2	19.0±23.5	10-28	2
T. sp.	Virgin	23±11.7	14-32	3	9.0±3.5	8-11	3	21.0±14.6	2-46	3	1.4±0.9	0.6-1.9	3	0	0	0
	Mated	22.7±17.1	3-34	3	8.0±1.4	7-9	2	50.5±10.6	43-58	2	2.1±0.1	2.0-2.1	2	21.0±0.0	21-21	2

[§] Pre-oviposition is time between maturation and production of first egg.
[*] Production rate is Total eggs divided by (Longevity – Pre-oviposition).

The fecundity parameters of female *Thaumastocoris* species held with males (presumed mated) and without males (virgin) are summarized in Table 1. Longevity of female *T. peregrinus* ranged from 4 to 40 days (mean 15 ± 12 days) and for *T.* sp. the range was between 3 to 34 days averaging 23 ± 12 days. Two of the four virgin *T. peregrinus* females died before they produced their first egg as did one of the mated females. While all the virgin females of *T.* sp. lived to produce at least one egg, one member of the mated group died. The pre-oviposition period was similar between all groups. The egg production rate is the average number of eggs the females produced per day, over their life time (excluding pre-oviposition period). In both species the egg production rate of virgin females was lower than for mated females but as our sample size was small no statistical analysis was performed.

Twenty of the 91 eggs laid by mated *T. peregrinus* hatched. Similarly 21 of the 101 eggs produced by mated *T.* sp. hatched. No eggs laid by virgin females from either group hatched (Table 1). The eggs of both *Thaumastocoris* species are large (0.60 x 30 mm) compared to the adult female body size (abdomen 1.5 x 1 mm) (Figure 1.) and under a light microscope, at 100x magnification, the eggs laid by virgin *Thaumastocoris* are not discernibly different from those produced by mated conspecifics. None of the nymphs hatched from either species progressed beyond the 2nd instar with the vast majority found dead on the surface of the vial.

Examination of life-stages

From the field-caught population of *T. peregrinus* 173 eggs were harvested. Of these, 70 (40%) hatched. A similar result was recorded for *T.* sp. (109 eggs harvested and 54 (49%) hatching). It is impossible to interpret these results in terms of fertility as virgin females of both species lay eggs. Mortality of 1st instar nymphs was higher in *T. peregrinus* (72%), while for *T.* sp. 57% did not live to the next stage. Deaths in the subsequent life-stages were less frequent and consistent for both species (Table 2).

Of the *T. peregrinus* that reached maturity, seven were male and three were female. Only one of the three females laid eggs. This female was not held with a male. The pre-oviposition period was nine days and she lived for 16 days producing five eggs. Of the remaining two females, one died the day after maturing and the other lived for 14 days and did not produce an egg. The longest surviving male lived for 20 days.

The sex ratio of the eight *T.* sp. that matured to adult was equal. Males lived for up to 29 days while the females survived for up to 26 days. Two females were

Table 2. Laboratory observations (February and April 2006) of life-history parameters (duration in days) of *Thaumastocoris peregrinus* and *T.* sp.

Life-stage	Duration in days					
	T. peregrinus			*T. sp.*		
	Mean±SD	Range	n	Mean±SD	Range	n
Egg	6.1±0.9	4-8	70	7.3±1.1	5-10	54
1st instar	4.6±0.8	4-7	19	4.7±1.2	2-6	23
2nd instar	3.5±1.1	3-6	15	3.4±1.0	3-5	20
3rd instar	3.3±1.6	2-5	12	3.5±0.7	2-4	15
4th instar	3.7±0.5	3-4	11	4.0±0.6	3-5	12
5th instar	5.3±0.5	5-6	10	5.2±0.5	5-6	8
Total nymph time	20.0±2.7	17-25		21.3±2.4	19-25	

each held with two field-caught males and both produced eggs after a pre-oviposition period of 13 days. One female lived for 23 days and laid nine eggs (of which three hatched and subsequently died), the other produced two eggs (neither hatched) and lived for 16 days. Eggs laid by virgin females were also recorded from this species with one female being held individually until death (26 days). During this time she produced eight eggs after a pre-oviposition period of 12 days. The remaining female died two days after maturation.

DISCUSSION

Thaumastocoridae are difficult to culture in the laboratory environment as evidenced by high mortality and the limited literature. Baranowski (1958) attempted to raise *Xylastodoris luteolus* on palm pieces housed within a variety of vials with poor results. Introduced nymphs died within four days and newly-hatched nymphs survived for two days. Consequently, he attempted to culture these insects on small potted palms brought into the laboratory but it netted the same result. His data was obtained from caged populations on mature palms and no nymphal stadia or individual data was obtained. Couturier *et al.* (2002), likewise, obtained their data from observing populations on mature palms. Hill (1988) worked with *Baclozygum depressum* in the laboratory and reported greater than 50% mortality during the first instar.

We were able to refine our methods in the 2006 study to increase the survivorship of *Thaumastocoris* at the egg and latter nymph stage. For example, having the females oviposit on paper and increasing the size of the vial used to incubate eggs reduced the incidence of fungus, and the water barrier imposed on the nymphs increased their survival as they could not move off their food source. Overall, however the survivorship was low. This may have been caused by a myriad of reasons, the most intrinsically obvious perhaps being the relative humidity within the vials during the cultivation of immature stages. Even though the adults were not cultivated over water on leaf pieces, and latter instar mortality was reduced, there could have been a cumulative deleterious effect on the nymphs that manifested in the adults. Also quality of the potted eucalypt leaves may have impacted on our insects as the leaves fed in the 2002 culture were taken from much older street trees. Further work is required to improve longevity and fecundity in the laboratory.

The *Thaumastocoris* species in our study (2006) hatched sooner and progressed through their five instars quicker than *B. depressum*. Hill (1988) cultured *B. depressum* eggs at three temperatures: room temperature (16-20 °C), 20 °C and 30 °C and reported a decrease of incubation time with increased temperature, 13-20, 11-14 and 7-9 days respectively. Incubation time of both *T. peregrinus* (4-8 days) and *T.* sp. (5-10 days) were well under these ranges. At a similar temperature, 17-20 °C, both *Thaumastocoris* species hatched in approximately half the time of *Baclozygum*, their incubation period being closer to that of *Baclozygum* at a constant 30 °C. Although Hill (1988) does not report nymphal stadia at room temperature, at a constant 20 °C they take from 29-33 days in total to reach maturity. This period is longer than *T. peregrinus* (17-25 days) and *T.* sp. (15-25 days). Mortality of 1st instars of both *T. peregrinus* (79%) and *T.* sp. (57%) was high and possibly

comparable to those accounted by Hill (1988) who reports greater than 50% mortality. High mortality of 1st instars has been documented in many other Hemiptera (Duan and Messing 2000, Zanuncio *et al*. 2004, Cárcamo *et al*. 2006).

Although the longevity and fecundity of our laboratory-reared adults was disappointing, our results are comparable to the results achieved by Hill (1988). He was able to maintain six laboratory-reared females for up to 45 days and during this time the oldest female produced 78 eggs. The most fecund female in our research produced 60 eggs in 40 days and another female almost attained the same result. The average daily production rate of eggs from the mated females of both *Thaumastocoris* species were similar and ranged from 1.8–2.1 eggs per day. This result is higher than the production rate achieved by *Baclozygum* in the laboratory (0.93–1.73 per day) (Hill 1988).

Our work reports, for the first time, the production of eggs by virgin *Thaumastocoris*. Although the production of eggs by unmated females has been reported from other families (Hinton 1981, He and Wang 2000, Kudo *et al*. 2006) this is the first record for the Thaumastocoridae. The average daily production rate of eggs from the virgin females of both *Thaumastocoris* species was less than the rate achieved by the mated conspecifics. Mating, in *Thaumastocoris*, may stimulate an increase in egg production and oviposition as it has been reported in many other groups (Davey 1985).

The Thaumastocoridae egg is large relative to their body size and has been commented on by others (Drake and Slater 1957, Couturier *et al.* 2002). The eggs of *Discocoris drakei*, for example, are ovoid (0.53 x 0.23 mm) and its abdomen is 1 mm x 1 mm (Couturier *et al.* 2002). *T. australicus* eggs are similar (Kumar 1964). The eggs produced by virgin *T. peregrinus* and *T.* sp. are the same size as the eggs produced by mated conspecifics. They are large compared to the size of the insect.

For virgin females to lay such large eggs is a waste of reproductive effort and resources. One explanation is that the laboratory environment has stressed the insects and their eggs were expelled prematurely. This explanation has some merit as when these insects are agitated they tend to lay eggs on unnatural surfaces, for example, the side of a vial. This may be a sign of stress but as they continued to produce and lay eggs, more or less regularly, until death it is difficult to suggest it does not happen under natural conditions.

The close nature of our work with *Thaumastocoris* afforded opportunities to observe some aspects of their behaviour. Both adults and nymphs exhibit erratic behaviour when disturbed. Adults can run fast and unpredictably, often flipping from one side of a leaf or object when they sense disturbance. Collecting adults is difficult as they will readily move onto any non-leaf surface when disturbed. Placing a barrier in front of them is often ineffectual in getting them to change direction as they will simply run onto it. Nymphs also readily move onto non-leaf surfaces when disturbed. During experimentation confining nymphs to the pieces of leaf was a major challenge.

Hill and Schaefer (2000) suggested Thaumastocoridae will remain an innocuous pest except under special circumstances because of their limited mobility, low fecundity and host range. Our research indicates that *Thaumastocoris* is very mobile. Its behaviour suggests it could easily disperse on many vectors, especially birds. Although our fecundity and fertility data are limited our data on the immature life-cycle suggests that *Thaumastocoris* completes its life-cycle quite quickly. The host lists compiled by Jacobs and Neser (2005) and Noack and Coviella (2006) indicate *T. peregrinus* can survive on a variety of eucalypts. One of these, *E. globulus*, is being planted in increasing numbers in many parts of Australia and represents the largest component of new hardwood plantations (Strauss 2001). We suggest that *Thaumastocoris* is now a persistent pest of some eucalypt species growing in Sydney, and given its rapid expansion to South Africa and Argentina, has the potential to infest plantation eucalypts in other parts of Australia and the temperate world.

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