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Development of *Cleruchoides noackae*, an egg-parasitoid of *Thaumastocoris peregrinus*, in eggs laid on different substrates, with different ages and post-cold storage

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Abstract *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae) is an egg parasitoid of *Thaumastocoris peregrinus* Carpintero and Dellapé (Hemiptera: Thaumastocoridae). The parasitism and development of *C. noackae* was studied in *T. peregrinus* eggs of different ages, laid on eucalyptus leaves or paper towel and stored at 5 °C. The emergence, sex ratio and development of *C. noackae* and hatched nymphs of *T. peregrinus* were evaluated. This parasitoid had an emergence rate higher than 60% from zero to one, one to two, and two to three-day old eggs and lower than 10% for those 3–4 and 4–5 days old. The female proportion was 78% and the egg-adult period for *C. noackae* was 19.5 days. The use of *T. peregrinus* eggs up to three days old, laid on paper towel and stored at 5 °C for 14 days did not affect the biological parameters of *C. nockae* and should be used for mass rearing of this parasitoid.

Keywords Biological control · *Eucalyptus* · Hemiptera · Hymenoptera · Mymaridae · Thaumastocoridae

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Introduction

Thaumastocoris peregrinus Carpintero and Dellapé (Hemiptera: Thaumastocoridae), native to Australia, has been introduced and caused damage to eucalyptus trees in several countries around the world (Jacobs and Neser 2005; Laudonia and Sasso 2012; Souza et al. 2016). The management of *T. peregrinus* in *Eucalyptus* spp. plantations includes classical biological control (Reis et al. 2012; Mutitu et al. 2013). This method is important to manage exotic pests in eucalyptus plantations with introduced natural enemies (Reis et al. 2012). The parasitoid *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae), found parasitizing eggs of the bronze bug *T. peregrinus* in Australia (Lin et al. 2007), is the main control agent for this insect (Souza et al. 2016). The Mymaridae family includes the smallest egg parasitoids amongst insects (Huber 1986). *C. noackae* was introduced into Brazil in 2012, and has been reared and released to manage *T. peregrinus*. The percentage of bronze bug eggs parasitized by *C. noackae* at release sites is close to 50% and the establishment of this natural enemy in the field was confirmed in Brazil (Barbosa et al. 2017).

Beneficial insect rearing depends on synchronization between the natural enemy, the host insect, and the plant or artificial diet, which must be studied to produce these organisms (Pratissoli et al. 2004; Silva et al. 2009; Parra 2010). The volume, surface odor, thickness, and chorion hardness, nutritional content, age, and distribution of host eggs and oviposition substrate, may affect the number, parasitism, quality, and sex ratio of natural enemies as recorded for *Trichogramma* spp. (Schmidt 1994; Liu et al. 1998).

Adequate egg age can improve parasitoid colonization and establishment, resulting in greater pest suppression as reported for *Lobesia botrana* Denis and Schiffermüller (Lepidoptera: Tortricidae) (Pizzol et al. 2012), *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) (Peverieri et al. 2013) and *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) (Zhang et al. 2014). The quality of the nutritive resources decreases with host embryo development. This reduces offspring and foraging of adult parasitoids (Zhou et al. 2014), as reported for *C. noackae* on *T. peregrinus* eggs on eucalypt leaves (Mutitu et al. 2013). This parasitoid is reared in the laboratory with *T. peregrinus* eggs on paper strips, i.e., different to natural conditions, where the eggs are grouped, preferably, on eucalyptus leaves. The possible effect of this substrate on the parasitism of *C. noackae* needs to be better studied (Barbosa et al. 2016).

Pest egg storage at low temperatures is important for integrated pest management and allows the mass rearing of parasitoids in the laboratory for field release. The efficiency of this method has been studied for parasitoids such as *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) (Chen and Leopold 2007), *Tetrastichus brontispae* Ferriere (Hymenoptera: Eulophidae) (Liu et al. 2014), *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) (Spínola-Filho et al. 2014) and *Gryon pennsylvanicum* Ashmead (Hymenoptera: Platygastridae) (Peverieri et al. 2015).

The introduction of new biological control agents can be inhibit due to inability to rear them in the laboratory. Thus, the objective was to evaluate the substrate effects on the *C. noackae* parasitism and the development and emergence of this parasitoid in *T. peregrinus* eggs of different ages and under cold-storage and post-storage conditions.

Materials and methods

Parasitoid and host

Cleruchoides noackae rearing was initiated with individuals from a colony with specimens introduced from Australia in 2012. This parasitoid was reared on *T. peregrinus* eggs at 24 ± 2 °C, 70 ± 10% RH and L:D 12:12 photoperiod in polystyrene flasks (7.0 cm in length and 3.0 cm in diameter) with 50% honey solution on filter paper strips (0.5 × 5 cm). Parasitoids aged between 12 and 24 h post-emergence were used with *T. peregrinus* eggs. This insect was reared in the laboratory at 24 ± 2 °C, 60 ± 10% RH and L:D 12:12 photoperiod, from insects collected in the field. This stinkbug was kept in a bouquet of eucalyptus branches secured with a piece of foam, to prevent its being submerged (Millar et al. 2000) in a 500 ml Erlenmeyer flask filled with water. New bouquets were put in contact with the old ones, every two days, and the latter removed when the insects migrated to the new leaves. Paper towel strips, with depressions to stimulate egg laying due to preference of this insect to oviposit near the main vein, in the grooves and depressions of the eucalyptus leaves, were used among the bouquets as a substrate for *T. peregrinus* oviposition. The *T. peregrinus* eggs were obtained, preferably from the paper strips placed in the eucalyptus bouquets, as they dried and most insects began to search for fresh food. The paper strips with egg masses were removed daily. A portion was stored at 5 °C for rearing of the parasitoid and the remaining eggs were used in the experiments. Eggs on eucalyptus leaves were used to evaluate the effect of the host substrate on parasitism. *Eucalyptus benthamii* plants were cultivated in the field and branches of two-year-old plants were used in laboratory tests.

Effect of host egg substrate

Ten zero to 24-hour old *T. peregrinus* eggs obtained from eucalyptus leaves and from paper towel strips were exposed to 24-hour-old paired *C. noackae* females in either choice or non-choice tests. The female was placed at the center of a Petri dish (5 cm diameter) using a fine brush. Filter paper strips (0.5 × 1 cm), moistened in 50% honey solution, were used as food for the parasitoid and the dishes were closed and sealed with Parafilm®. After 24 h, the parasitoids were removed, the eggs transferred to a polystyrene vial (7.0 cm long and 3.0 cm in diameter) and kept in an air-conditioned room at 24 ± 2 °C and 70 ± 10% RH, photoperiod of L:D 12:12, until emergence of the parasitoid adults. *Thaumastocoris peregrinus* nymphs, hatched from non-parasitized eggs, were removed and counted. Sixteen replications with ten eggs each were used per oviposition substrate.

Host age effect

Five age classes of *T. peregrinus* eggs (0–1, 1–2, 2–3, 3–4, and 4–5 days), obtained on paper towel strips, were exposed to a 24-hour old parasitoid couple in a polystyrene vial (7.0 cm long and 3.0 cm in diameter) for 24 h. After this period, the parasitoids were removed and the eggs kept in an air-conditioned room at 24 ± 2 °C, 70 ± 10% RH, L:D 12:12 photoperiod. Filter paper strips (0.5 × 3 cm) moistened in 50% honey solution were used to feed the parasitoid. Twelve replications with ten eggs per egg age class were used.

Host egg storage at low temperature

Ten, zero to 24-hour old *T. peregrinus* eggs were collected and stored in polystyrene vials (7.0 cm long and 3.0 cm in diameter) for 14 days at 5 °C. After storage, a parasitoid couple (24 h old) was placed in the vial for 24 h. Filter paper strips (0.5 × 3 cm), moistened in 50% honey solution, were used to feed the parasitoid. The parasitoids were removed and the eggs kept in an air-conditioned room at 24 ± 2 °C, 70 ± 10% RH, L:D 12:12 photoperiod, under the same conditions as the control. Fifteen replications with ten eggs each were used.

Host eggs post-storage at low temperature effect on C. noackae parasitism

Twelve days after storage at 5 °C, ten *T. peregrinus* eggs were kept in a polystyrene vial (7.0 cm long and 3.0 cm in diameter) in an air-conditioned room at 24 ± 2 °C, 70 ± 10% RH, photoperiod of L:D 12:12) and zero, one, two, three, and four days after being removed from storage at low temperature, a *C. noackae* couple (24 h old) was placed per vial for 24 h. This process was repeated ten times per egg age.

Parameters evaluated

The parasitism percentage ((total number of parasitoids emerged + number of parasitoids retained in the egg)/ number of host eggs × 100), development period (egg-adult) and sex ratio (number of ♀/number of ♂ + ♀) of *C. noackae* and the percentage of *T. peregrinus* nymphs hatched were evaluated in the four tests. The sex ratio and the development period (egg-adult) of the parasitoid were not evaluated in the test conducted on three to four and four to five-day old host eggs and on the effect of the post egg storage period of the host parasitism by *C. noackae* on two, three and four-day old eggs, due to the low number of parasitoids emerged from the eggs (six and five individuals for three to four and four to five-day old and two, zero and six individuals for two, three and four day old post-storage eggs). The sex ratio was evaluated in the replicates in which adults emerged.

Data analysis

Parasitism, sex ratio of parasitoid and nymphs hatched were analyzed by generalized linear models (GLM) with Binomial (logit link function) distribution of error, accounting for over-dispersion (Hinde and Demétrio 1998). The parasitoid development time was analysed with Gaussian (identity link function) distribution. The fit quality of the model adjusted was evaluated based on the half-probability plot with a simulation envelope (Demétrio and Hinde 1997), using the *hnp* function from the *hnp* package in R (Demétrio et al. 2014). Tukey multiple comparison test ($P < 0.05$) were done by the *glht* function from the *multcomp* package in the case of differences between treatments (Hothorn et al. 2008). Statistical analysis was done using R language, version 3.3.2 (R Core Team 2016).

Results

Host substrate effect

The parasitism of *T. peregrinus* eggs with ($F_{1,30} = 0.0049$, $P = 0.9449$) or without ($F_{1,30} = 2.9081$, $P = 0.0984$) choice, the development period (egg-adult) (with: $F_{1,30} = 0.7515$, $P = 0.3929$ and without choice: $F_{1,30} = 0.2727$, $P = 0.6053$) and sex ratio (with: $F_{1,30} = 0.0041$, $P = 0.9493$ and without choice: $F_{1,30} = 1.1355$, $P = 0.2951$) of *C. noackae* were similar between the substrates, eucalyptus leaves, and paper towel (Tables 1, 2). The percentage of *T. peregrinus* nymphs on eucalyptus leaves and paper towel did not differ in the test with ($F_{1,30} = 0.2209$, $P = 0.6418$) or without ($F_{1,30} = 0.0468$, $P = 0.8301$) choice with eucalyptus leaves and paper towel (Table 2).

Host age effect

Parasitism by *C. noackae* ($F_{4,55} = 59.742$, $P < 0.001$) was higher in *T. peregrinus* eggs with 0–1, 1–2, and 2–3 days old than in three to four and four to five-day old eggs. The number of *T. peregrinus* eggs, parasitized by *C. noackae*, was inversely related to the host egg age (Fig. 1), with a higher value for those 0–1 (62.50 ± 3.92%), 1–2 (61.66 ± 3.65%) and 2–3 (62.50 ± 2.50%) days old, followed by those with 3–4 (5.0 ± 2.88%) and 4–5 (5.00 ± 1.94%) days (Fig. 1a). The development period (egg to adult) for *C. noackae* did not differ between zero and one, one and two, and two to three-day old eggs ($F_{2,33} = 1.5053$, $P = 0.2368$), with a mean of 15.97 ± 0.16; 15.71 ± 0.15 and 16.09 ± 0.15 days, respectively (Fig. 1b).

The sex ratio of *C. noackae* emerged from zero to one, one to two, and two to three-day old eggs was similar ($F_{2,33} = 0.0817$, $P = 0.9217$), with a mean proportion of 0.78% females in the three age classes (Fig. 1c). The percentage of *T. peregrinus* nymphs hatched from eggs with 0–1, 1–2, and 2–3 days (5.00 ± 1.94%, 7.50 ± 2.5% and 6.67 ± 3, 33%, respectively), was similar and lower than that from three to four, and four to five day old eggs ($F_{4,\ 55}$ = 65.575, $P < 0.001$), which were similar to each other, 71.66 ± 4.74% and 60.83 ± 4.68%, respectively (Fig. 1d).

Effect of host egg post-storage at low temperatures on *C. noackae* parasitism

The percentage of *T. peregrinus* eggs either fresh or following storage at 5 °C for 14 days parasitized by *C. noackae* was similar: 42.00 ± 6.26% and 30.67 ± 4.83%, respectively ($F_{1,28} = 2.0578$, $P = 0.1625$). The *C. noackae* sex ratio did not differ for parasitoids emerged from either fresh (0.69 ± 0.09%) or stored (0.53 ± 0.09%) *T. peregrinus* eggs ($F_{1,26} = 1.1994$, $P = 0.2835$). The percentage of *T. peregrinus* nymphs hatched from fresh eggs (32.67 ± 7.27%) was higher than for those cold stored for 14 days (10.00 ± 3.52%) ($F_{1,28} = 23.941$, $P = 0.001$).

Post-storage period at low temperature for host eggs on *C. noackae* parasitism

The percentage of *T. peregrinus* eggs parasitized by *C. noackae* was lower for those with two days post storage at 5 °C ($F_{4,45} = 21.011$, $P < 0.001$). *Cleruchoides nockae* parasitized the highest number of eggs with zero (50.00 ± 10.65%) and one (54.00 ± 9.21%) day old. The sex ratio ($F_{1,16} = 2.5258$, $P = 0.1316$) and the development period (egg-adult) ($F_{1,16} = 0.9537$, $P = 0.3433$) did not differ for these periods. The percentage of hatched *T. peregrinus* nymphs increased ($F_{4,45} = 40.937$, $P < 0.001$) in eggs at two, three and four days post storage (92.00 ± 3.27%, 92.00 ± 2.91% and 90.00 ± 2.98%, respectively) compared with eggs at zero and

Table 1 Parasitism and sex ratio (% female) (mean ± SE) of *Cleruchoides noackae* (Hymenoptera: Mymaridae) on *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) eggs offered on eucalyptus leaves or paper towel in choice and non-choice tests

	No choice		Choice	
	Parasitism (%)	Sex ratio	Parasitism (%)	Sex ratio
Leaves	61.25 ± 4.17	0.67 ± 0.04	46.87 ± 5.90	0.76 ± 0.04
Paper	70.00 ± 3.03	0.74 ± 0.05	46.25 ± 6.76	0.82 ± 0.03

Table 2 Development period (egg-adult) (Per.) of *Cleruchoides noackae* (Hymenoptera: Mymaridae) and percentage of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) eggs in which nymphs hatched (Nin.) (mean ± SE) after exposed to parasitoid on eucalyptus leaves and paper towel in choice and non-choice tests

	No choice		Choice	
	Per. (days)	Nin. (%)	Per. (days)	Nin. (%)
Leaves	17.50 ± 0.18	20.62 ± 3.92	17.38 ± 0.18	33.12 ± 5.61
Paper	17.63 ± 0.15	19.37 ± 4.23	17.69 ± 0.31	28.75 ± 7.35

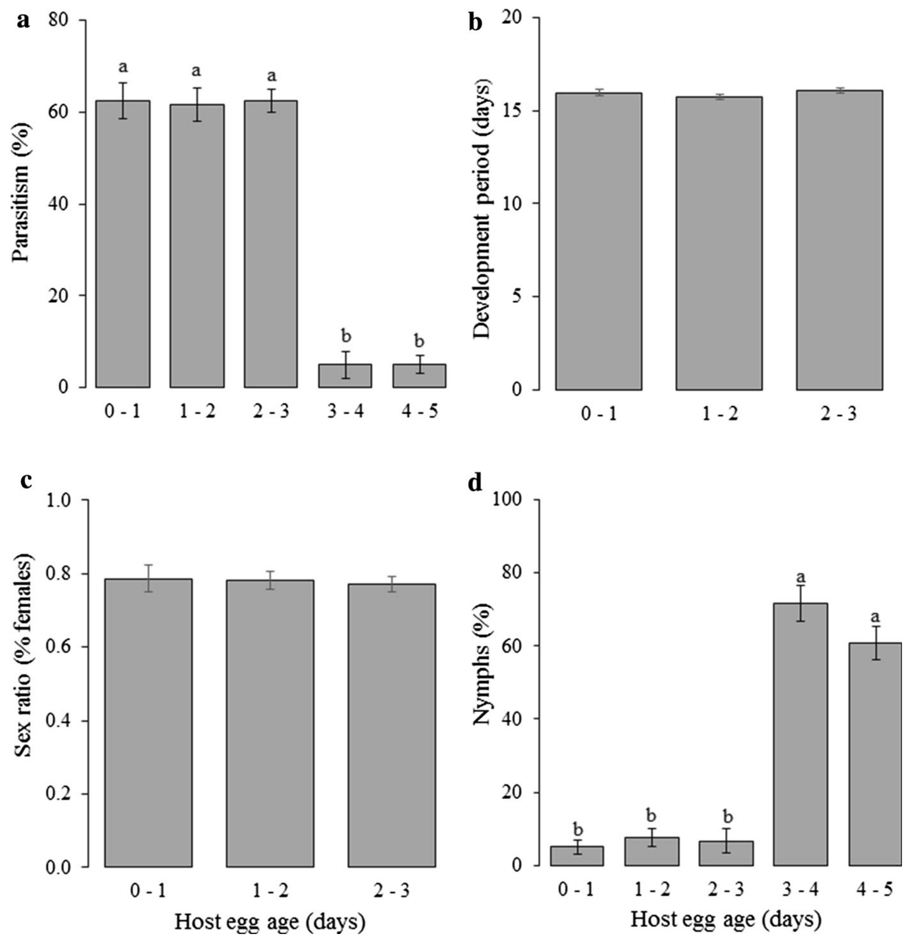

Fig. 1 Parasitism (**a**), development period (egg-adult) (**b**) and sex ratio (**c**) of *Cleruchoides noackae* (Hymenoptera: Mymaridae) and percentage of nymphs hatched (**d**) (mean ± SE) from eggs of different ages of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae). Bars with different letters, per parameter, differ by Tukey test ($P < 0.05$)

one day (41.00 ± 11.87% and 37.00 ± 11.16%, respectively) old (Table 3).

Discussion

The similar developmental period (egg-adult), sex ratio, and parasitism of *C. nockae* among *T. peregrinus* oviposition substrates suggests that the short-distance host location of this parasitoid is not related exclusively to chemical signals associated with the egg substrate as reported for other oophagous parasitoids (Colazza et al. 2014). However, this may be common for parasitoids such as *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) on *Ostrinia furnacalis* Guenée (Lepidoptera: Pyralidae) eggs on pieces of paper and *Zea mays* L. cultivar Si-Dan-Ba leaves (Liu et al. 1998). During the initial

Table 3 Parasitism, sex ratio (% female) and development period (egg-adult) (Per.) (mean ± SE) of *Cleruchoides noackae* (Hymenoptera: Mymaridae) and percentage of nymphs hatched (Nin.) on *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) eggs offered one, two, three and four days after 12 days storage at 5 °C (DPA)

DPA	Parasitism (%)	Sex ratio	Per. (days)	Nin. (%)
Control	50.00 ± 10.65[a]	0.74 ± 0.10[a]	16.78 ± 0.14[a]	41.00 ± 11.87[b]
1	54.00 ± 9.21[a]	0.59 ± 0.04[a]	16.59 ± 0.12[a]	37.00 ± 11.16[b]
2	2.00 ± 2.00[b]			92.00 ± 3.27[a]
3	0.00 ± 0.00[b]			92.00 ± 2.91[a]
4	5.00 ± 2.69[b]			90.00 ± 2.98[a]

Means per column and parameter, followed by the same letter, do not differ by Tukey test ($P < 0.05$)

stage of the host location process, many parasitoids respond to air-borne chemical cues (generally highly detectable but with low reliability). On the other hand, substrate-borne chemical cues (generally low detectability but highly reliable) become progressively more important after parasitoids have landed on a host-infested patch (Vet and Dicke 1992; Fatouros et al. 2008; Colazza et al. 2014). Both substrates exposed to *T. peregrinus* oviposition were kept in direct contact with the pest in the same conditions indicating that the chemical signals left by the hosts are more important than the plant tissue for *C. noackae* finding its host.

The percentage of *T. peregrinus* nymphs on eucalyptus leaves and paper towel was similar showing that the substrate did not influence this biological parameter and eggs offered to *C. noackae* had good quality. Suitable oviposition substrates are also important to produce predatory bugs in the laboratory (Lundgren 2011) and were studied for *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) (Constant et al. 1996; Castañé and Zapata 2005), *Orius laevigatus* (Hemiptera: Anthocoridae) (Bonte and De Clercq 2010; De Puysseleyr et al. 2014) and *Nesidiocoris tenuis* (Heteroptera: Miridae) (De Puysseleyr et al. 2013). The paper towel did not reduce *C. noackae* parasitism and can be used as a substrate to obtain *T. peregrinus* eggs, facilitating mass rearing of this parasitoid for field release.

The preference for younger *T. peregrinus* eggs shows that this parameter can affect parasitism, developmental time, emergence and sex ratio of parasitoids (Vinson 1998; Brodeur and Boivin 2004). The host egg age is important to provide nutritional resources of good quality for parasitoid offspring success (Zhou et al. 2014). The drastic reduction of the *C. noackae* parasitism rate with increasing *T. peregrinus* egg age is a common pattern for *Trichogramma* spp. (Liu et al. 1998; Pak et al. 1986) and Mymaridae species such as *Gonatocerus triguttatus* Girault parasitizing eggs of *Homalodisca vitripennis* Germar (syn. Coagulata) (Hemiptera: Cicadellidae) (Irvin and Hoddle 2005). The low emergence of parasitoids from older eggs may be related to the advanced stage of host embryonic development, which hampers feeding (Strand 1986). However, preference for host eggs at a given age may limit parasitoid efficiency in the field by reducing parasitism (Liu et al. 1998; Pak et al. 1986). Therefore, parasitoids that can develop in hosts of different ages may be more efficient in the field (Song et al. 2015).

Parasitism of *T. peregrinus* eggs up to three days old (62.50 ± 2.50%) by *C. noackae* was higher than with zero to one-day old eggs of this host (34.1 ± 8.6%) for this natural enemy without choice in South Africa (Mutitu et al. 2013) These differences can be attributed to the methodology, materials and processes used as well as to the population and/or strain origin as this has been shown for *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), *T. ostriniae*, and *T. japonicum* with *Chilo suppressalis* Walker (Lepidoptera: Crambidae) (Ko et al. 2014). The host density may influence the parasitism rate as this has been shown for *Diaeretiella rapae* (Hymenoptera: Braconidae) at higher densities of the host *Brevicoryne brassicae* L. (Hemiptera: Aphididae) (Kant and Minor 2017). The higher parasitoid density may also influence the parasitism rate (Pereira et al. 2010). Parasitoids foraging together increase the frequency of encounters between females, which can affect foraging efficiency (Lynch et al. 1998; Hassell 2000; Kristoffersen et al. 2001; Elliott 2003), thus reducing parasitism. The highest percentage of parasitism and emergence of *C. noackae* from *T. peregrinus* eggs with a maximum age of three days shows the importance of their use to produce this natural enemy for biological control programs.

The development of *C. noackae* in zero to one, one to two, and two to three-day old *T. peregrinus* eggs was similar to that reported for this parasitoid with 0–1, 2–3, and 4–5 days old eggs of this host in South Africa (Mutitu et al. 2013). Younger eggs appear to be more suitable for progeny development, as demonstrated for egg parasitoids such as *Trichogramma japonicum* Ashmead (Hymenoptera: Trichogrammatidae) on eggs of *Chilo suppressalis* Walker (Lepidoptera: Crambidae) (Zhang et al. 2014) and *T. leucaniae* Pang and Chen and *T. chilonis* Ishii on those of *Leguminivora glycinivorella* Matsumura (Lepidoptera: Tortricidae) (Song et al. 2015). The lack of development of *C. noackae* in 3–4 and 4–5 day old *T. peregrinus* eggs shows a reduction in the quality and availability of food due to the transformation of nutrients into more complex substrates (Vinson 1998).

The parasitoid offspring sex ratio, usually, decreases as host quality increases (King 1987). Fewer female eggs being laid indicates a decrease in acceptance levels of older host eggs resulting in an increase in offspring sex ratio (Sousa and Spence 2001). The similar sex ratio of *C. noackae* from *T. peregrinus* eggs of different ages agrees with that reported for *Homalodisca vitripenis* Say (Hemiptera: Cicadellidae) eggs parasitized by *Gonatocerus triguttatus* Girault (Hymenoptera: Mymaridae) (Irvin and Hoddle 2005) and *G. deleoni* Triapsitsyn, Logarzo and Virla (Hymenoptera: Mymaridae) (Lytle et al. 2012). No change in sex allocation between host age classes was observed, presumably due to acceptance levels being similar between treatments. Host egg age may be a relatively unimportant determinant of sex allocation for *C. noackae* in relation to other factors.

The increase in the percentage of hatched *T. peregrinus* nymphs as the parasitism rate on older eggs decreased is similar to that reported for *Homalodisca vitripennis* Germar (syn. Coagulata) (Hemiptera: Cicadellidae) nymphs hatched from ten day old eggs compared to younger ones after parasitism by *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) (Irvin and Hoddle 2005). This result is expected because older eggs have a lower parasitism rate by *C. noackae*.

The similar parasitism of *T. peregrinus* eggs by *C. noackae* after storage at 5 °C for 14 days shows that the egg quality of this host can be maintained post-storage at this temperature. *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) eggs can be stored for longer periods at ultra-low temperatures (− 80 and − 140 °C) and for shorter periods at 4 °C without affecting the fitness of the parasitoid *Gryon pennsylvanicum* Ashmead (Hymenoptera: Platygastridae) (Peverieri et al. 2015). Eggs of *Homalodisca coagulata* Say (Hemiptera: Cicadellidae), *Nezara viridula* L. (Hemiptera: Pentatomidae), and *Sitoraga cerealella* Olivier (Lepidoptera: Gelechiidae) stored up to one month at + 8 or + 10 °C, remained suitable for rearing *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae), *Trissolcus basalis* Wollaston (Hymenoptera: Platygastridae) and *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), respectively (Corrêa-Ferreira and Moscardi 1993; Greco and Stilinovic 1998; Chen and Leopold 2007). This strategy can be used to obtain a large number of high quality parasitoids, when required. However, storage may reduce the natural enemy ability as reported for *Trichogramma ostriniae* Pang and Chen (Hymenoptera: Trichogrammatidae) (St-Onge et al. 2016) and *G. ashmeadi* (Chen and Leopold 2007). Therefore, the success of storing host eggs by cooling or freezing appears to be dependent upon the host/parasite combination.

Thaumatocoris peregrinus eggs stored at low temperatures did not affect the sex ratio of *C. noackae* agreeing with that observed for *Tetrastichus brontispae* (Ferriere) (Hymenoptera: Eulophidae) (Liu et al. 2014), but this varies with parasitoid species (Ayvaz et al. 2008). Eggs of *Riptortus pedestris* (= *clavatus*) Fabricius (Hemiptera: Alydidae) can be stored at 2 °C for up to two months without affecting the sex ratio of the egg parasitoid *Ooencyrtus nezarae* Ishii (Hymenoptera, Encyrtidae) (Alim and Lim 2009). Further, the parasitoid *Trissolcus nigripedius* Nakagawa (Hymenoptera: Platygastridae) reared on *Dolycoris baccarum* L. (Hemiptera: Pentatomidae) egg storage at 2 °C did not affect host suitability after up to two months of storage, again with no effects on sex ratio for any storage period tested (Mahmoud and Lim 2007).

The reduction of the percentage of hatched *T. peregrinus* nymphs from host eggs stored for 14 days at 5 °C agrees with that reported for *Dolycoris baccarum* L. (Hemiptera: Pentatomidae) eggs stored for 15 days at 2.1 ± 0.7 °C (Mahmoud and Lim 2007) and *Riptortus Pedestris* (Fabricius) (Hemiptera: Alydidae) stored for 30 days at 10 °C (Mainali and Lim 2013). Similar parasitism rates on eggs after storage at 5 °C demonstrates that *T. peregrinus* egg quality is maintained in one-day old eggs after 12 days of storage at 5 °C. Mass rearing of natural enemies is a complex and important process in biological control programs. The use of up to three-day old *T. peregrinus* eggs, on paper towel stored at 5 °C for 14 days did not affect the biological parameters of *C. nockae* and should be used for mass rearing of this parasitoid. Therefore, oviposition substrate, age, and low temperature storage of *T. peregrinus* eggs are important for rearing and in strategies making use of *C. noackae* for the biological control of *T. peregrinus*.

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