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Longevity of *Cleruchoides noackae* (Hymenoptera: Mymaridae), an egg parasitoid of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), with various honey concentrations and at several temperatures

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Abstract

Thaumastocoris peregrinus Carpintero and Dellapé (Hemiptera: Thaumastocoridae), damages eucalyptus plants by sucking their sap. This pest can be controlled by releases of the egg parasitoid *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae). Increasing the survival of this parasitoid is critically important for its mass rearing in order to release large numbers in integrated programs to manage *T. peregrinus*. The aim of this study was to evaluate the longevity of *C. noackae* adults fed various honey concentrations at 6 constant temperatures. The longevity of *C. noackae* was studied by keeping adults in a 1st experiment with 100, 50, or 10% honey solution, with distilled water, or without water and food in climate-controlled chambers at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod and—in a 2nd experiment—with 100% honey at constant temperatures of 15, 18, 21, 25, 28, or 31 °C in a climatic chamber at 70 ± 10% RH and a 12:12 h L:D photoperiod. Each adult parasitoid was held individually in a glass tube capped with plastic wrap under the conditions described, and the survival of adults was recorded daily. The longevity of *C. noackae* varied with food and temperature such that longevity was enhanced by all honey concentrations and temperatures of 25 °C and below. When fed honey, this parasitoid lived 2 to 3 fold longer when kept at 15, 18, 21, and 25 °C than at 28 and 31 °C. Thus, the parasitoid *C. noackae* should be mass reared with honey at temperatures from 15 to 25 °C for subsequent distribution of parasitoid adults in eucalyptus plantations for suppressing *T. peregrinus*.

Key Words: biological control; laboratory rearing; *Eucalyptus*

Resumen

Thaumastocoris peregrinus Carpintero y Dellapé (Hemiptera: Thaumastocoridae) causa daños a plantas de eucalypto succionando su savia. Este insecto plaga se puede ser controlado mediante liberaciones del parasitoide de huevos *Cleruchoides noackae* Lin y Huber (Hymenoptera: Mymaridae). El aumento de la supervivencia es esencial para producir y utilizar el parasitoide en programas integrado para manejo de *T. peregrinus*. El objetivo de este estudio fue evaluar la longevidad de adultos de *C. noackae* alimentados a diferentes concentraciones de miel en seis temperaturas constantes. La longevidad de *C. noackae* fue estudiada en un experimento por la alimentación con soluciones de miel de 100, 50, y 10%, agua destilada y sin comida, en cámara climatizada a 25 ± 2 °C, 70 ± 10% de humedad relativa y 12:12 h L: D de fotoperíodo y—en un segundo experimento—con el 100% de miel a temperaturas constantes de 15, 18, 21, 25, 28 y 31 °C en cámaras climatizadas con 70 ± 10% de humedad relativa y 12 h de fotoperíodo. Los adultos del parasitoide fueron individualizados en tubos de vidrio cubiertos con una envoltura de plástico en las condiciones descritas y su supervivencia se registró diariamente. La longevidad de *C. noackae* varió con el alimento y la temperatura, por lo que la longevidad fue realzada por todas las concentraciones de miel y temperaturas de 25 °C e inferior. El parasitoide alimentado con miel vivió 2-3 veces por más tiempo cuando se mantiene a 15, 18, 21 y 25 °C que a 28 y 31 °C. Por lo tanto, el parasitoide *C. noackae* debe ser criado con miel a temperaturas de 15 a 25 °C para la posterior distribución de parasitoides adultos en las plantaciones de eucalipto para la supresión de *T. peregrinus*.

Palabras Clave: control biológico; cría en laboratorio; *Eucalyptus*

Thaumastocoris peregrinus Carpintero and Dellapé (Hemiptera: Thaumastocoridae) is a pest of various *Eucalyptus* species and hybrids (Myrtales: Myrtaceae) in Argentina, Australia, Brazil, Chile, Kenya, South Africa, Uruguay, and Zimbabwe (Carpintero & Dellapé 2006; Nadel et al. 2010; Noack et al. 2011; Laudonia & Sasso 2012). The spread of *T. peregrinus*, outside its origin in the Southern Hemisphere, includes South Africa (Jacobs & Neser 2005; Nadel et al. 2010), Zimbabwe, Malawi, and Kenya (Noack et al. 2011). In South America, this pest has

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been detected in Argentina (Noack & Coviella 2006), Chile, Uruguay (Martínez & Bianchi 2010; Ide et al. 2011), and Brazil (Wilcken et al. 2010; Soliman et al. 2012). Furthermore, *T. peregrinus* was observed in Italy (Laudonia & Sasso 2012), Portugal (Garcia et al. 2013), and New Zealand (Sopow et al. 2012). *Thaumastocoris peregrinus* causes silvering, tanning, and drying of eucalyptus leaves. These symptoms result from the insect's feeding habits of piercing leaves and branches to suck sap, leading to chlorosis and reduction of tree growth and productivity (Soliman et al. 2012). Owing to the extension of plantations, the height of the eucalyptus trees, and the behavior of this insect pest, the efficiency of pesticide use in forest crops is reduced (Zanuncio et al. 2010).

Biological control is one of the strategies for the management of insect pests in forest plantations (Pereira et al. 2008; Garnas et al. 2012). The endoparasitoid *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae), the main biological control agent of *T. peregrinus* (Nadel & Noack 2012), was found in the eggs of *T. peregrinus* on *Eucalyptus scoparia* Maiden in Australia (Lin et al. 2007; Nadel et al. 2012).

Successful introduction of a biological control agent such as *C. noackae* requires knowledge of its lifecycle and interactions with the host *T. peregrinus* (Mutitu et al. 2013). Programs of biological control with egg parasitoids depend on producing large numbers of these natural enemies in the mass rearing facilities and releasing them when this insect pest is present in the eucalyptus plantation (de Carvalho Spínola-Filho et al. 2014).

Mass rearing of *C. noackae* requires analysis of its feeding habits, because in nature, insects seek different nutritional sources such as sugars and other carbohydrates (Harvey et al. 2012) than in the laboratory, where they depend on the food provided. Generally, the feeding of parasitoids in the laboratory positively affects their longevity and fecundity as demonstrated for *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) (Heimpel et al. 1997), *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) (Wäckers 2001), *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) (Chen et al. 2005), and *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae) (Jamont et al. 2013). Consumption of food, especially sugars, provides energy to maintain metabolism, longevity, fecundity, and flight activity of insects (Tenhumberg et al. 2006; Winkler et al. 2009).

The complexity of the interaction between various factors can affect the success of classical biological control programs (Vorsino et al. 2012). Temperature is one of the most important abiotic factors affecting survival, behavior, distribution and colonization (Pratissoli et al. 2004, 2005; Colinet & Boivin 2011), duration of development and parasitism (Zago et al. 2007; Soares et al. 2012), reproduction and sex ratio (Frazer & McGregor 1992), and longevity (Burgi & Mills 2013) of natural enemies. Enhanced longevity of parasitoids allows them to search for longer periods and therefore increases the probability of parasitism (Evans et al. 2010; Vollhardt et al. 2010).

Suboptimal temperatures can negatively affect the biological characteristics of adult insects, which must be evaluated for parasitoids (Lessard & Boivin 2013). Low temperatures can cause physiological dysfunctions (Colinet & Boivin 2011), reduce fertility (Pandey & Johnson 2005), and decrease the parasitoid's mobility (Tezze & Botto 2004; Ayvaz et al. 2008). Thus, knowledge on development and longevity is important for maintaining and releasing the parasitoid *C. noackae* in integrated pest management programs against *T. peregrinus*. The objective of this study was to evaluate the longevity of *C. noackae* fed with several honey concentrations and reared at 6 constant temperatures.

Materials and Methods

The experiments were conducted in the laboratory of Biological Control of Forest Pests of the Faculty of Agricultural Sciences, São Paulo State University (UNESP) in Botucatu, São Paulo State, Brazil. Individuals of *C. noackae* were obtained from a rearing facility of Embrapa Forestry (Brazilian Agricultural Research Corporation) in Colombo, Parana State, Brazil, with *T. peregrinus* as the host. These parasitoids were obtained from *T. peregrinus* eggs originally collected in urban trees of *Eucalyptus camaldulensis* Dehnh. and *E. scoparia* in Sydney, New South Wales, Australia. The newly emerged adults of this parasitoid were held individually in glass tubes (8.5 cm high × 2.5 cm wide) capped with plastic film.

The longevity of *C. noackae* adults was evaluated in the following treatments: T1, 100% honey; T2, 50% honey; T3, 10% honey; T4, distilled water; and T5, without honey or water in chambers conditioned at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod. The honey concentrations were obtained by diluting the honey with distilled water. These foods were provided on paper towel every 24 h to *C. noackae* adults during the evaluation of their survival.

In the 2nd experiment, the longevity of *C. noackae* adults was evaluated at 15 °C (T1), 18 °C (T2), 21 °C (T3), 25 °C (T4), 28 °C (T5), and 31 °C (T6) in a climatic chamber at 70 ± 10% RH and a 12:12 h L:D photoperiod, and adults were fed 100% honey. The longevity of these parasitoids was determined every 24 h until all adults died.

The data of *C. noackae* longevity were subjected to the Shapiro–Wilk normality residues test and analysis of variance. The means were compared by using the nonparametric Kruskal–Wallis test.

Results

Cleruchoides noackae adults lived 3.4, 3.3, and 3.7 d (Table 1) when fed 100, 50, and 10% honey, respectively, and these 3 values were not significantly different. However, these longevities were significantly longer than those of adults fed only distilled water or nothing. The latter 2 longevities were 1.7 and 1.2 d, respectively, and they did not differ significantly from one another (Table 1).

At temperatures of 15, 18, and 25 °C, *C. noackae* adults showed the greatest longevities, which were 2.9, 3.3, and 3.5 d, respectively (Table 2). However, these 3 longevities did not differ significantly from the longevity of 2.2 d at 21 °C. At 28 and 31 °C, the longevities were 1.1 and 1.1 d, respectively, and these values were significantly shorter than those at 15, 18, and 25 °C, but not different from that at 21 °C (Table 2). The longevity of *C. noackae* adults was 2 to 3 fold greater at 15, 18, and 25 °C than at 28 and 31 °C.

Discussion

The increased longevity of *C. noackae* adults fed honey agrees with observations of other parasitoid species in the laboratory, such as *Ibalia leucospoides* (Hochenwarth) (Hymenoptera: Ibaliidae), in which longevity was increased by 10 d when adults were fed (Fischbein et

Table 1. Adult longevity of *Cleruchoides noackae* (Hymenoptera: Mymaridae) (mean ± SE and variation interval) with several concentrations of honey in chambers at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod (n = 10).

Food	Mean longevity ± SE (d)	Variation interval (d)
Honey 100%	3.4 ± 0.51 a	3–4
Honey 50%	3.3 ± 0.94 a	2–5
Honey 10%	3.7 ± 1.70 a	2–7
Water	1.7 ± 0.48 b	1–2
Without food	1.2 ± 0.42 b	1–2

Means followed by the same letter do not differ by the Kruskal–Wallis test.

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Table 2. Adult longevity of *Cleruchoides noackae* (Hymenoptera: Mymaridae) (mean ± SE and variation interval) fed 100% honey at 6 constant temperatures in climatic chambers at 70 ± 10% RH and a 12:12 h L:D photoperiod (*n* = 10).

Temperature (°C)	Mean longevity ± SE (d)	Variation interval (d)
15	2.9 ± 0.3 a	2–4
18	3.3 ± 0.2 a	3–4
21	2.2 ± 0.3 ab	1–4
25	3.5 ± 0.6 a	1–6
28	1.1 ± 0.1 b	1–2
31	1.1 ± 0.1 b	1–2

Means followed by the same letter do not differ by the Kruskal–Wallis test.

al. 2013). Furthermore, *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae) showed 2 to 3 fold greater longevity when fed honey and water than water alone (Almeida et al. 2002). The parasitoid *D. rapae* (Jamont et al. 2013) and the hyperparasitoid *Gelis agilis* (F.) (Hymenoptera: Ichneumonidae) (Harvey et al. 2012) also lived longer with honey than with sugar alone. This can be explained by the fact that honey is a highly concentrated solution of at least 181 substances such as sugars, proteins, enzymes, amino acids, minerals, and vitamins (Alvarez-Suarez et al. 2009).

The benefits of feeding *C. noackae* with honey at 3 concentrations may not be a standard for parasitoids. Honey fed at 50% concentration increased the longevity of *Apanteles metesae* (Nixon) (Hymenoptera: Braconidae), but honey fed at higher concentrations decreased the longevity of this natural enemy (Salmah et al. 2012). This effect may be explained by honey, at low concentration, containing more water than carbohydrates, whereas high concentrations of honey contain more energy than water. Thus, excess energy or low water content can reduce the parasitoid's longevity (Salmah et al. 2012).

The shorter longevity of *C. noackae* without sugar is similar to that observed for *A. melinus*, whose females lived 30.5 d with honey and 17 d without food (Heimpel et al. 1997). Moreover, the longevity of *A. metesae* was longer when adults were fed 50% honey and 20% sucrose than when fed 50% sucrose or only distilled water (Salmah et al. 2012). Females of *D. rapae* showed greater longevity when fed with extrafloral nectar plus water, 20% honey, and honeydew plus water than those held without food (Jamont et al. 2013). These food sources may allow the acquisition of nutrients such as sugars, proteins, enzymes, amino acids, minerals, and vitamins from honey (Alvarez-Suarez et al. 2009) and sugars and lipids from the extrafloral nectar. Providing food to adult parasitoids is important because these natural enemies have only the energy accumulated during their immature stage, which may restrict longevity, fecundity, parasitism rate, ability to locate hosts, and flight (Lewis et al. 1998; Fadamiro & Heimpel 2001; Winkler et al. 2009).

Longevity of *C. noackae* in response to temperature was similar to that of *Meteorus ictericus* (Nees) (Hymenoptera: Braconidae) (Burgi & Mills 2013). The adaptation of the parasitoid to climatic conditions is important for its efficiency in biological control (Yu & Byers 1994). Temperature is one of the most important factors in acclimation of introduced parasitoids (Loni 1997), making it important to define the responses of these natural enemies to this factor (Ables et al. 1976).

Increased longevity of *C. noackae* at low temperature agrees with data for egg parasitoids such as *Trissolcus simoni* (Mayr) (Hymenoptera: Scelionidae) between 20 and 32 °C (Kivan & Kilic 2005) and *Trichogramma pratissolii* Querino & Zucchi (Hymenoptera: Trichogrammatidae) from 15 to 33 °C (Zago et al. 2007). Increased longevity may be associated with reduced activity and metabolism at lower temperatures (Gerling 1972; Bleicher & Parra 1990). Suboptimal temperatures can shorten the lifespan (Jalali & Singh 1992; Torres et al. 2002), reduce body size (Rundle et al. 2004) and energy reserves (Renault et al. 2003), increase the development period (Pratissoli et al. 2004), and reduce emergence and fecundity of parasitoids (Colinet & Boivin 2011). However, the intensity of the response varies with natural enemy species (Lessard & Boivin 2013); for example, the parasitoid *Bracon vulgaris* Ashmead (Hymenoptera: Braconidae) showed 2-fold greater longevity at 20 °C than that at 25 and 30 °C (Wanderley et al. 2007), and *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) showed longevity of 34.4 and 1.5 d at 16 and 28 °C, respectively (Rodrigues et al. 2013).

The reduction in the longevity of *C. noackae* adults at higher temperatures is similar to that reported for the parasitoids *Trichogramma pretiosum* Riley and *Trichogrammatoidea annulata* De Santis (Hymenoptera: Trichogrammatidae) (Maceda et al. 2003), *T. diatraeae* (Rodrigues et al. 2013), and *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) (Pereira et al. 2011) between 10 and 31 °C. The lowest survival may have resulted from increased metabolic activity and destruction or denaturation of enzymes in the insects (Mohan et al. 1992). The low survival of *C. noackae* at high temperatures indicates that this parasitoid has few possibilities of field adaptation to warm temperatures. Therefore, one option for its use in management programs should be to release large numbers of *C. noackae* adults in these warm regions.

The parasitoid *C. noackae* showed greatest longevity when fed with honey at concentrations of 100, 50, and 10% and reared at 15 and 18 °C. The provision of food at appropriate temperature is important for the production of *C. noackae* in the laboratory. The findings of this study can help in defining strategies for the rearing to release this parasitoid in biological control programs against *T. peregrinus*.

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