Biological Control 123 (2018) 97–104

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Rearing and releasing the egg parasitoid *Cleruchoides noackae*, a biological control agent for the *Eucalyptus* bronze bug

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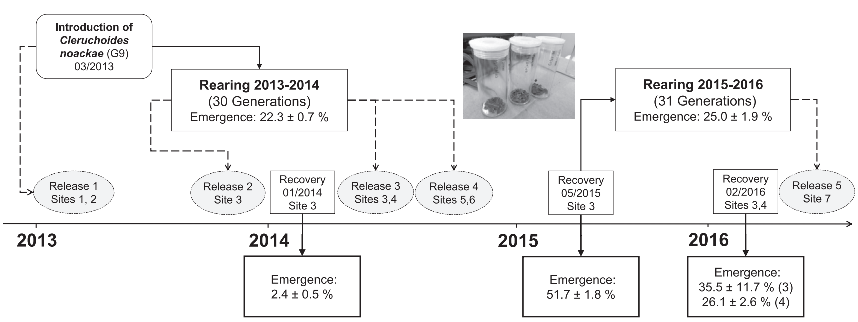
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Thaumastocoris peregrinus
Eucalyptus
Inoculative release
Plantation forestry
Uruguay
Rearing quality

ABSTRACT

Biological control is a major tool for forest insect pest management. We initiated a biological control program for the *Eucalyptus* bronze bug, *Thaumastocoris peregrinus*, with the egg parasitoid *Cleruchoides noackae*. Parasitized eggs were imported from a mass rearing in Brazil, and a rearing colony was set up. The wasp was reared in plastic tubes each containing ten individuals that were offered 100 eggs of the bronze bug and a solution of water and honey. During 30 generations, rearing quality parameters were compared against expected quality standards set *a priori*, based on information from other rearing colonies. We also assessed the effect of temperature on developmental time, measured as the first day of emergence of adult parasitoids, as well as the relation between host-egg abortion and parasitization by *C. noackae*, and the evolution of the sex ratio of the emergent wasps within a single generation. Field releases were made yearly during summers in commercial plantations, starting in March 2013. Survival and emergence rates were either stable or increased throughout 30 generations. The percentage of females was slightly above 50%. Rearing at 18 °C and 20 °C took longer than at 22 °C, but productivity was similar. Host-egg abortion was increased by parasitization. The proportion of females increased as the emergence period progressed. Field surveys in two sites revealed that the wasp survived for two years. Field-collected parasitoids exhibited better quality indices than individuals from the laboratory rearing. Results indicate that biological control of the bronze bug with *C. noackae* is feasible with this rearing and release protocol.

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https://doi.org/10.1016/j.biocontrol.2018.05.008
Received 21 July 2017; Received in revised form 4 May 2018; Accepted 16 May 2018
Available online 17 May 2018
1049-9644/

1. Introduction

Sustainability of global *Eucalyptus* plantation forestry has been increasingly threatened in the last decades by invasions of Australian insects into newly planted areas, as a consequence of global trade and climate change (Paine et al., 2011; Wingfield et al., 2008). This trend requires the strengthening of current quarantine and pest management strategies of forest pests at a global scale (Wingfield et al., 2015). However, any management strategy involving the use of chemical pesticides is strongly discouraged within the framework of the eco-certification schemes such as the Forest Stewardship Council (FSC), due to the economic costs and the risks that pesticides pose to people and the environment (Willoughby et al., 2009). Non-chemical alternatives extensively utilized in plantation forestry include silvicultural methods (Klapwijk et al., 2016), the use of infochemicals (Nadel et al., 2012b), and biological control. The latter represents the major contribution to pest management in forestry (Dittrich-Schröder et al., 2014; Garnas et al., 2012; Protasov et al., 2007; Slippers et al., 2015).

At the beginning of a biological control program, research efforts must focus on producing the biocontrol agent easily, effectively, efficiently, and in adequate quantities (Chambers, 1977; Van Lenteren, 2003). Developing reliable estimators of the quality of a biocontrol agent culture is important for monitoring the status of a rearing colony and for dealing with eventual problems such as inbreeding depression or adaptation to rearing conditions (Bigler, 1989; Chambers, 1977; Van Lenteren, 2003). For instance, it is essential to monitor the sex ratio of the colony to prevent undesirable male-biased colonies, which can affect the sustainability of the rearing in the future, and the efficacy of the control by the released agent (Heimpel and Lundgren, 2000). When the parasitized host (egg, larva or pupa) is the stage to be released in the field, as opposed to adult individuals of the natural enemy, it is crucial to estimate the number of actual natural enemies that will be delivered (Van Lenteren, 2003). Not only is it important to develop indicators that are easy to calculate, but also to monitor them throughout the development of the colony for an early detection of problems such as inbreeding depression.

One of the invasive pests that affects eucalypt plantations worldwide is the bronze bug *Thaumastocoris peregrinus* Carpintero et Dellape (Heteroptera: Thaumastocoridae). This is a small sucking insect that feeds on mature leaves within the genera *Eucalyptus* and *Corymbia*, causing leaf blight, increased stress and in severe cases defoliation or tree death (Nadel and Noack, 2012).

Cleruchoides noackae Lin Huber et La Salle (Hymenoptera: Mymaridae) is an egg parasitoid of *T. peregrinus* (Lin et al., 2007). This wasp is able to parasitize eggs of the bronze bug up to three-days-old (Mutitu et al., 2013). Adult lifespan of *C. noackae* extends between two and three days if fed on a honey solution (Mutitu et al., 2013; de Souza et al., 2016). Given the potential of *C. noackae* as a biological control agent for the bronze bug, rearing colonies were set up in South Africa (Mutitu et al., 2013) and Brazil (de Souza et al., 2016). Researchers in Chile also imported the parasitoid, reared it for two generations and released it in the field, this being the first release of *C. noackae* outside its natural range (Jaques, 2010).

Information on monitoring the quality parameters in long term rearing of *C. noackae* is not available. Rearing of *C. noackae* has been done at 24 ± 2 °C in South Africa (Mutitu et al., 2013) and at 22 °C in Brazil (Leonardo Barbosa pers. comm.). At the latter temperature, the first emergence occurs on day 19 after exposing the eggs to the parasitoids. That would facilitate the harvest of a new generation two times per month, which would not be necessary in winter, when no parasitoids need to be released in Uruguay. Given that the optimal thermal range for development of *C. noackae* lies between 15 °C and 25 °C (Mutitu et al., 2013; de Souza et al., 2016), decreasing rearing temperature to increase developmental time and reduce the frequency of parasitoid harvest would improve rearing efficiency at times when no parasitoids are needed. To date, the developmental time of *C. noackae* at different temperatures has not been reported.

There are no Thaumastocorinae species native to South America, and no native parasitoids attacking *T. peregrinus* have yet been reported in the continent. In order to start a biological control program for the bronze bug in Uruguay, a rearing colony of *C. noackae* was set up and the wasp was periodically released in the field. Here, we report the results, including the temporal assessment of the quality of the wasps in the rearing, during 4 years of rearing of the parasitoid in two consecutive rearing colonies, the second of which was started with individuals recovered in the field. We also report for the first time on the occurrence of parasitism in the field in Uruguay.

2. Material and methods

2.1. First introduction and rearing setup

Cleruchoides noackae was introduced for the first time in Uruguay on February 22nd, 2013, from an *in vitro* rearing colony at EMBRAPA (Curitiba, Brazil). We imported a total of 24 tubes, each containing 100 bronze bug eggs exposed to ten *C. noackae* wasps belonging to the 8th generation emerged in Brazil after their introduction from Australia. We followed the official importation procedures, and obtained the rearing and release permits required by ISPM 3 and regional COSAVE standards (COSAVE, n.d.; FAO, 2005). Half of the tubes were immediately released in two commercial eucalypt plantations (Sites 1 and 2, Table 1, Fig. 1). The extant 12 tubes produced 180 females and 87 males of *C. noackae* (9th generation), which were the founders of the rearing colony. The parasitoid was reared on its natural host, bronze bug eggs, which were supplied from a permanent mass rearing at INIA Tacuarembó, Uruguay (Martínez et al., 2014). We selected eggs up to 48-h-old following information from previous studies on age-dependent parasitization (Mutitu et al., 2013).

This rearing colony of *C. noackae* (hereafter referred to as Colony 1) was maintained from March 2013 to November 2014. It comprised generations 10 to 40, following the counting started in Brazil. The wasps multiplied inside plastic tubes (3 cm diameter by 7 cm high, hereafter referred to as "rearing tubes") closed with a perforated plastic cap covered by voile mesh (Fig. 2A). Each rearing tube was filled with 100 eggs of *T. peregrinus* and 10 adult individuals of *C. noackae*. A paper strip soaked with 50% honey diluted in water was provided as a food source. The rearing tubes were placed in a rearing chamber (Daihan Wisd SWGC-450) at 22 ± 1 °C, 65 ± 1% relative humidity and 12:12 light: dark photoperiod). The tubes were opened 8 days later, nymphs were counted, and the wasps were sexed under a dissection microscope (Olympus SZH) under a magnification of 500×. After day 18, we inspected each rearing tube daily for wasp emergence until the occurrence of three consecutive days without emergence. Emerged wasps were counted and sexed, and assigned to new-generation rearing tubes.

A new rearing colony was established in 2015 with 547 female and 83 male founder wasps that emerged in the laboratory from egg samples collected in the field (see below). We named the first generation of wasps that emerged from these founders generation zero. Here, we analyzed data from generations 0 to 31 of this rearing colony, hereafter referred to as Colony 2.

2.2. Temperature

Emerged wasps, not more than 24-h-old, were separated into groups of five couples and placed into new rearing tubes. The tubes were then randomly allocated to rearing chambers at 18 ± 1 °C, 20 ± 1 °C or 22 ± 1 °C, at 65 ± 1% r.h. and 12:12 L:D. On day 10, the tubes were opened and dead nymphs and wasps were removed. The tubes were then inspected daily for wasp emergence until three consecutive days were recorded without new emergent wasps. A total of 13,500 *T. peregrinus* eggs (4,500 eggs per treatment in 45 tubes) were used.