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Assessment of frozen larvae of *Callosobruchus maculatus* as hosts for rearing *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae)

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ABSTRACT

The suitability of frozen host larvae for rearing *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae), an ectoparasitoid of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) and other stored-product insects was investigated. The reproductive potential (number and sex ratio of progeny) of female *P. cerealellae* was compared on live (fresh) *C. maculatus* larvae (concealed within cowpea seeds) versus frozen larvae (obtained by freezing infested cowpea seeds at −20 °C for 48 h) which were subsequently thawed and held at ambient conditions (~25 ± 1 °C, 50 ± 5% RH) for 4, 24, 48, 72, 96, and 120 h before exposure to female parasitoids. No significant differences were recorded in the numbers and sex ratios of the progeny produced by female *P. cerealellae* on live larvae compared to frozen host larvae that were thawed and held at ambient conditions for up to 96 h, suggesting that live and frozen larvae of *C. maculatus* are equally suitable for rearing *P. cerealellae*. However, the data showed that progeny production on frozen hosts gradually declined with thawing duration and was significantly reduced at the thawing duration of 120 h. When live and frozen host larvae were simultaneously presented together to female *P. cerealellae* at different exposure periods, relatively greater progeny production was recorded on live hosts than on frozen hosts at 12, 24, and 48 h of exposure. This may suggest preference of female *P. cerealellae* for live versus frozen host larvae. These results are discussed in relation to the life history strategy and host location behavior of *P. cerealellae*, and may have practical implications in the development of efficient mass rearing systems for the parasitoid.

1. Introduction

Parasitoids are potentially important regulators of host insect populations and some are commercially produced as biological control agents of various pests (Mills, 1994; Cranshaw et al., 1996; Donnelly and Phillips, 2001; Floate and Spooner, 2002). Mass rearing and release of parasitoids and other natural enemies are critical components of any biological control program to suppress pest populations (Rueda and Axtell, 1987; Petersen and Cawthra, 1995; Kaufman et al., 2001; Geden and Hogsette, 2006; Geden and Kaufman, 2007). Typically, parasitoids are reared on living immature hosts and this strategy has many limitations. Use of live (fresh) larvae or pupae as rearing hosts for parasitoids may reduce the window of opportunity for parasitism due to rapid development of the hosts from potentially suitable stages for parasitism to unsuitable stages. Furthermore, rearing of parasitoids on live hosts has the inherent risk of accidental releases of non-parasitized pests in the field (Geden and Kaufman, 2007).

The use of killed (frozen or irradiated) hosts to rear parasitoids could potentially mitigate these limitations and may even have some advantages over the use of live hosts in certain applications, such as in foreign exploration efforts to establish colonies of exotic parasitoids in locales where live hosts are not available (Pickens and Miller, 1978; Geden et al., 2006). Use of frozen (freeze-killed) hosts for maintenance of parasitoid colonies can increase the efficiency of rearing programs. For instance, when hosts are reared in excess of needs, they can be frozen and used when normal supplies are low or can be stockpiled and used later in mass release programs (Klunker and Fabritius, 1992; Geden and Kaufman, 2007). In addition, rearing parasitoids on frozen hosts may reduce the risk of contamination of one population with another because any potential contamination which may occur during host maturation can be eliminated by freeze-killing (Geden and Kaufman, 2007).

Several studies have reported on the ability of some pupal parasitoids to successfully develop on frozen pupal hosts (Richerson and Borden, 1972; Petersen and Matthews, 1984; Rueda and Axtell, 1987; Rivers and Delinger, 1995; Floate and Spooner, 2002). For instance, Pickens and Miller (1978) successfully reared a fly pupal parasitoid, *Pachycrepoideus vindemiae* (Rondani) Hymenoptera: Pteromalidae) on frozen pupae of the housefly, *Musca domestica* Linnaeus (Diptera: Muscidae) and concluded that continued, peri-

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odic additions of frozen house fly pupae could increase the effectiveness of the parasitoid in chicken houses. Rueda and Axtell (1987) also reported that frozen pupae of *M. domestica* were as suitable as fresh pupae for mass rearing of three pteromalid parasitoids (Hymenoptera: Pteromalidae): *Muscidifurax raptor* Girault and Sanders, *P. vindemiae*, and *Spalangia cameroni* Perkins. The majority of the available literature on the ability of parasitoids to develop on frozen hosts has however, focused on pupal parasitoids of flies; not much is known about the development of larval parasitoids on frozen hosts (Kaschef, 1959).

Pteromalus cerealellae (Ashmead) (Hymeoptera: Pteromalidae) is a solitary larval ectoparasitoid of several stored-product pests including *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae), *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), *Lasioderma serricorne* (Fab.) (Coleoptera: Anobiidae), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), and *Sitophilus* spp. (Coleoptera: Curculionidae) (Ashmead, 1902; Brower, 1991; Howard, 2001; Onagbola et al., 2007). Females of *P. cerealellae* lay eggs in the larvae of these insects, which typically are concealed within grain seeds. This parasitoid is considered as a promising candidate for utilization in the biological control of stored grain pests (Brower, 1991; Mbata et al., 2005; Onagbola et al., 2007). The ability of *P. cerealellae* or other parasitoids of stored-product insects to successfully develop on frozen host larvae has not been previously investigated. This study was therefore conducted to determine if *P. cerealellae* could be reared successfully on frozen larvae of *C. maculatus*, one of its principal hosts. First, the reproductive potential (number and sex ratio of progeny) of female *P. cerealellae* when presented frozen fourth-instar larvae of *C. maculatus* versus live (fresh) larvae of the same instar stage was compared. To determine the effect of thawing duration on the viability of frozen larvae as hosts, reproductive potential of female *P. cerealellae* was compared on frozen larval hosts which were subsequently thawed and held at ambient conditions for various lengths of time (4, 24, 48, 72, 96, and 120 h) prior to exposure to female *P. cerealellae*. Finally, the relative suitability of frozen versus live larvae was compared by presenting both host types simultaneously to female *P. cerealellae* in the same arena.

2. Materials and methods

2.1. Insects: cowpea bruchids

Callosobruchus maculatus was utilized as the host for *P. cerealellae* in this study. The starting culture of *C. maculatus* was obtained from Fort Valley State University, Fort Valley, GA, USA (contact: Dr. George Mbata), where it had been reared continuously on cowpea seeds (*Vigna unguiculata* Walp.) for several years. *Callosobruchus maculatus* was reared in our laboratory on cowpea seeds (California Black Eyed variety) in 1-L wide-mouthed Mason glass jars. A fresh culture was started every 5 days by placing ~25 pairs of 3-day-old mated *C. maculatus* in a glass jar containing ~100 g of cowpea seeds held at 30 ± 1 °C, 70 ± 5% RH, and L12:D12 h (Mbata et al., 2005; Onagbola et al., 2007). The beetles were allowed to lay eggs on the seeds for 24 h after which they were removed with an aspirator. The infested seeds were incubated at the conditions specified above until the larvae had reached the fourth-instar stage, which were then provided to *P. cerealellae* for parasitization.

2.2. The parasitoid

The original culture of *P. cerealellae* was obtained from Fort Valley State University, Fort Valley, GA, USA where the parasitoid had been reared continuously for several years. The *Pteromalus cerealellae* culture was maintained in our laboratory by transferring about 30 adult pairs into a glass jar containing *C. maculatus*-infested cowpea seeds at a stage when most of the bruchid larvae were at the fourth larval instar. This was determined to occur, in a preliminary experiment approximately 15 days after infestation of cowpea seeds under our rearing conditions. The jars were held at the environmental conditions stated above for *C. maculatus*. Adult *P. cerealellae* were removed from the jars after 5 days of oviposition and the attacked *C. maculatus* larvae were incubated in a growth chamber at the above environmental conditions until the emergence of adult parasitoids.

2.3. Reproductive potential of P. cerealellae on live (fresh) and frozen C. maculatus larvae

The development of *P. cerealellae* was compared on live (<1-day-old) and frozen (<1-day-old) fourth-instar larvae of *C. maculatus* (concealed within cowpea seeds). Frozen larvae were obtained by freezing infested cowpea seeds containing a fourth-instar larva of *C. maculatus* at −20 °C for 48 h (Johnson and Valero, 2003) and thereafter exposing (thawing) it to ambient laboratory conditions (~25 ± 1 °C, 60 ± 5% RH) for ~4 h prior to exposure to a female parasitoid (frozen larvae were confirmed dead by dissection). Eighty infested cowpea seeds containing live or frozen larvae (1 larva per seed) were placed in a 10-cm diameter plastic Petri dish. A mated 2-day-old female *P. cerealellae* was then placed in each Petri dish and allowed to parasitize the larval hosts for 120 h (5 d). At the end of the 120 h exposure period, the female parasitoid was removed and the Petri dish containing host larvae was incubated at 30 ± 1 °C, 70 ± 5% RH, and L12:D12 h until the completion of emergence of parasitoid F_1 progeny (~10 days after the start of parasitoid emergence). Each treatment was replicated 20 times (i.e. 20 female parasitoids each exposed to 80 host larvae). The number and sex of offspring produced by each female parasitoid was recorded daily and summed at the end of the incubation period. Data were analyzed by using the Student's *t*-test (JMPIN Version 5.1, SAS Institute, 1998) to determine significant differences in reproductive potential of female *P. cerealellae* on live versus frozen host larvae.

2.4. Effects of duration of thawing of frozen host larvae on parasitoid reproductive potential

Having demonstrated the ability of *P. cerealellae* to develop on frozen host larvae in the preceding experiment, a second experiment was conducted to test for possible effects of duration of thawing of frozen host larvae on reproductive potential of female *P. cerealellae*. Infested cowpea seeds containing fourth-instar (<1-day-old) *C. maculatus* larvae were placed in a 10-cm diameter plastic Petri dish (80 seeds per dish each containing 1 larva) and frozen at −20 °C for 48 h as described in the preceding experiment. The Petri dishes were then removed from the freezer (thawed) and subsequently held at ambient laboratory conditions for various lengths of time: 4, 24, 48, 72, 96, and 120 h before exposure to female parasitoids. The control treatment consisted of infested cowpea seeds containing live fourth-instar (<1-day-old) *C. maculatus* larvae (80 seeds per dish each containing 1 larva). A mated (2-day-old) female *P. cerealellae* was then placed in each Petri dish and allowed to parasitize the larval hosts for 24 h as described in the preceding experiment. At the end of the 5 day exposure period, the female parasitoid was removed and the Petri dish containing host larvae was incubated as described in the preceding experiment. Each treatment was replicated 20 times (i.e. 20 female parasitoids each exposed to 80 host larvae). The number and sex of off-