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Influence of cold storage on pupal development and mortality during storage and on post-storage performance of *Encarsia formosa* and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae)

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Abstract

Pupae of *Encarsia formosa* were cold stored to determine the influence of low temperature and the duration of exposure on their development and mortality during storage, and on the pattern of eclosion following storage. Eclosion, the process of extraction of a pharate adult from the host scale, was prevented at temperatures below 10 °C although the pupae continued to develop even at 4 °C. The middle stages of *E. formosa* pupae appeared to suffer lower mortality due to chilling compared to early or late pupal stages. Detrimental effects of cold, expressed as pupal mortality, accumulated and increased as temperature was lowered and duration of storage extended. When compared to parasitoids that had not been stored, eclosion of *E. formosa* was advanced following storage at 10 °C as pupae continued to develop during storage, and delayed following storage at 4 and 7 °C, possibly due to high mortality of late pupal stages. To determine the influence of different storage regimes on eclosion, flight and parasitism, pupae of *E. formosa* and *Eretmocerus eremicus* were exposed to a continuous (7 °C) and an acclimation (12 °C for 7 days, followed by 7 °C) storage regime. All three fitness parameters declined with increased duration of cold storage and its harmful effects accumulated and increased over time. The patterns of decline for eclosion and flight relative to storage duration were different between the two storage regimes; acclimated pupae tolerated cold better. Unlike for eclosion and flight, the pattern of decline for parasitism was similar between storage regimes. Enhanced tolerance to cold could be attributed to acclimation and/or pupal development to a stage more tolerant to low temperatures. To assess the cumulative impact of cold storage on the biological control potential of the two parasitoids, we constructed a composite quality index model by combining results from eclosion, flight, and parasitism. The pattern of decline in the index relative to storage duration resembled the decline observed for parasitism. The composite model predicts fewer, but more realistic gains from the use of the acclimation regime than those predicted by individual models.

Keywords: Cold storage; *Encarsia formosa*; *Eretmocerus eremicus*; Pupae; Development; Cold tolerance; Eclosion; Mortality; Flight; Parasitism; Composite quality index

1. Introduction

The parasitic wasps *Encarsia formosa* Gahan and *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) are two commercially available parasitoids used for the control of the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) on a wide range of greenhouse ornamental and vegetable crops. *Encarsia formosa* has been applied since 1926 and is presently used with outstanding success in tomatoes and in several other greenhouse crops (van Lenteren and Wotes, 1988; Hoddle et al., 1998a). More recently, *E. eremicus* has been identified as an effective natural enemy of both *T. vaporariorum* and *Bemisia agrentifolii* Bellows and Pering (Greenberg et al., 2002; Simmons and Minkenberg, 1994). The success of the aphelinid parasitoids in controlling *T. vaporariorum* has been attributed largely to their effectiveness in locating host

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patches and their high parasitism rates (van Lenteren et al., 1996).

The two parasitic wasps are mass-produced on tobacco (*Nicotiana tabaccum*) plants infested with the greenhouse whitefly (Scopes and Biggerstaff, 1971). Commercial insectaries use different methods for introducing the parasitoid onto the infested tobacco plants, but they all harvest the pupae when the oldest parasitoids begin to eclose. At harvest, host scales contain pupae at a range of phenological stages. To accommodate sorting and processing and to address a fluctuating market demand, pupae of *E. formosa* and *E. eremicus* are cold stored at a few degrees below their developmental threshold. They are also shipped in cooled containers to prevent premature emergence. Although the pupae of the two parasitoids are routinely exposed to low temperatures, relatively little is known about their ability to tolerate a short-term (<1 month) exposure to low temperatures (0–12 °C).

The native range of *E. formosa* is uncertain while *E. eremicus* is indigenous to the south–western deserts of California and Arizona in the USA (Hunter et al., 1996). Neither of the two parasitoids has demonstrated diapause nor are they adapted to prolonged chilling. Past studies have demonstrated that pupae stored for several days at sub-threshold temperatures had reduced survival (López and Botto, 2005; Lacey et al., 1999; Scopes et al., 1973; Ganteaume et al., 1995a) and the adults were less able to parasitize hosts (Ganteaume et al., 1995b).

In this study, we exposed pupae of *E. formosa* to different regimes and durations of storage to determine the influence of cold on pupal development during storage, the survival of pupae, and the pattern of eclosion. We also examined the ability, following storage, of *E. formosa* and *E. eremicus* to fly and parasitize hosts. The storage regimes consisted of either a fixed temperature or exposure to an acclimation temperature followed by continued storage at a lower temperature. Acclimation is a physiological response to an environmental trigger that results in increased tolerance to cold. To determine the flight capability of parasitoids we modified a previously described flight chamber (van Lenteren et al., 2003) and determined the best temperatures for the flight test (unpublished results). The results from cold storage experiments were used to construct a composite quality index, a synthetic measure of post-storage quality of parasitoids. Our work advances the knowledge of *E. formosa* and *E. eremicus* responses to cold, and has the potential to improve shelf life, post-storage quality, and efficacy of biological control programs.

2. Materials and methods

To measure the influence of cold storage on the quality, from a biological control perspective, of the two parasitoid species, we conducted two types of experiments. The first group of experiments examined development of pupae during storage while the second group of experiments measured post-storage changes in adult eclosion, flight capability and parasitism. We used results from these experiments to construct a composite index of parasitoid quality.

Experiments were conducted using newly harvested (<24 h) *E. formosa* pupae supplied by Applied Bionomics, BC, Canada and 4-day-old *E. eremicus* pupae supplied by Koppert B.V., The Netherlands. Both parasitoids were produced using *T. vaporariorum* and tobacco plants as the insect and plant host, respectively. The strain of *E. formosa* originated from the Cheshunt Experimental Station in England and has been mass-produced since 1972, while the strain of *E. eremicus* originated from the USDA collection in Phoenix, AZ. and has been mass-produced since 1992. For easier dissection, we mounted the host scales containing *E. formosa* onto cardboard cards by cutting a hole in the center of the cards, placing a piece of adhesive tape over the hole, and dipping the exposed sticky tape into the bulk scales. Unlike parasitoids prepared for commercial distribution, the parasitized hosts used in all experiment were not sorted at the insectaries and contained an unknown proportion of hosts and parasitoids damaged during harvest.

Upon delivery, portions of hosts containing the two parasitoid species were immediately tested and results from these tests are henceforth referred to as the control. The remaining hosts were stored under cold conditions using either a constant temperature or, by first acclimating the pupae through exposure to 12 °C for 7 (*E. formosa*) or 5 (*E. eremicus*) days followed by a lower temperature (e.g., 7 °C) (Fig. 1).

2.1. Development and eclosion of E. formosa and E. eremicus pupae during and following storage

Host scales with *E. formosa* pupae were stored using three constant temperatures and three acclimation regimes. The constant temperature regimes were 4, 7, and 10 °C each stored for 7, 14, and 21 days. The acclimation regimes consisted of storage for 7 days at 12 °C followed by storage at 4, 7, or 10 °C for an additional 7 and 14 days. To determine whether *E. formosa* pupae developed under these storage

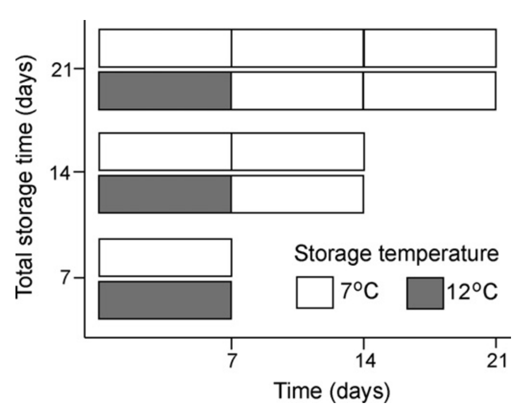

Fig. 1. Schematic of acclimation (12 + 7 °C) and constant (7 °C) temperature storage regimes. The *x*-axis depicts the storage duration in 7 day increments and the *y*-axis the total storage time.