Post Refrigeration Viability of *Pteromalus puparum* (Hymenoptera: Pteromalidae) Prepupae within Host Chrysalids[1]

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ABSTRACT *Pteromalus puparum* (L.) prepupae within host *Pieris rapae* (L.) chrysalids could be stored at 10°C and 80% R. H. for periods of up to 15 months and still produce viable progenies. The mean number of *P. puparum* adults emerging per chrysalis was not different between four months of cold storage and the non-refrigerated control, but declined progressively with duration of cold storage thereafter.

KEY WORDS Parasitism, Cold storage, *Pteromalus puparum.*

Pteromalus puparum (L.) (Hymenoptera: Pteromalidae) is a gregarious pupal endoparasite of the imported cabbageworm, *Pieris (=Artogeia) rapae* (L.) (Lepidoptera: Pieridae). It was accidentally introduced into the United States in the late 1800's (Oatman 1966). Since 1981, studies conducted on cabbage in southwestern Virginia have shown parasitization of *P. rapae* chrysalids by *P. puparum* to be consistently high, exceeding 70%, during the latter part of the season (Chamberlin & Kok 1986, Lasota & Kok 1986a). This high rate of parasitization by *P. puparum* late in the season indicates the potential for augmentative releases of the parasite for early season control of the imported cabbageworm.

To produce large numbers of *P. puparum* for augmentative field releases, manipulation of the parasite in the laboratory may be necessary. Lasota & Kok (1986b) investigated refrigeration of *P. puparum* adults at different temperatures as a method of maintaining and manipulating laboratory colonies. They found that survival of adult *P. puparum* caged in groups and supplied with a 10% sugar solution exceeded 80% after 30 days at both 15° and 23°C. To have more flexibility in the rearing of large numbers of *P. puparum,* successful storage of the prepupal stage without loss of subsequent reproductive viability would enhance the process. We believe that the prepupa would be the most suitable stage for prolonged cold storage because larval feeding is completed, and the parasite still enjoys some protection by being inside the host chrysalis.

Objectives of this study were to develop a method for long term storage of *P. puparum* prepupae and to determine the maximum storage time that would produce viable *P. puparum* adults.

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Materials and Methods

Colonies of *P. puparum* (obtained from field collected hosts) and *P. rapae* (obtained from Paula Peters of the USDA-Biological Control of Insects Research Laboratory in Columbia, MO) were maintained at the Virginia Polytechnic Institute and State University Entomology Department Insectary. Forty 0-24 hour old *P. rapae* chrysalids were placed in a covered petri dish with 10 mated week-old *P. puparum* females. This was replicated five times. *P. puparum* adults were given a 10% sucrose solution which was lightly misted on the lid of the petri dish before the parasites were added. After 48 h, the *P. puparum* females were removed, and the parasitized *P. rapae* chrysalids were held in an environmental chamber at 25° C, and a photoperiod of 16:8 (L:D), until parasite prepupae (motionless oval shaped mature larvae) were observed through the translucent chrysalid exoskeleton. The chrysalids containing *P. puparum* prepupae were stored in an environmental chamber at 10°C and 80% R. H. in total darkness. The high level of humidity was maintained by having a tub of water within the growth chamber with humidity control.

Ten randomly selected chrysalids, two from each replicate, were removed from cold storage at specific monthly intervals and individually placed in 100 ml cylindrical plexiglas containers having an organdy cloth end to facilitate air circulation. The plexiglas cylinders were held at 30° C, 16:8 (L:D), and 50% R. H. in an environmental growth chamber with humidity control, and monitored daily for parasite emergence. Upon emergence, the number of *P. puparum* adults and sex ratios per chrysalis were recorded. Results obtained from the various durations of 10°C storage treatments were compared with a control which was not refrigerated, but was maintained at 25°C. Exponential plots of the total number of wasps, numbers of female and male wasps, emerging after different storage times at 10° C were carried out and the respective regression equations derived using a Cricket Graph graphics program.

To test for parasite viability after cold storage, all *P. puparum* adults from a single storage period were placed in a 40 cm^3 plexiglas cage at room temperature. A 10% sucrose solution was lightly misted on the walls of the cage daily. Parasites were allowed to feed and mate for 48 h and were then offered groups of twenty 0-24 old *P. rapae* chrysalids to parasitize. Parasitized chrysalids were placed in a cage in the same room and observed daily for emergence of *P. puparum* adults.

Results and Discussion

Pteromalus puparum adults emerged after storage periods of up to 15 months at 10°C (Fig. 1), but there was no emergence of adults from the 17 month treatment. The total number of parasites emerging from the various durations of cold storage progressively declined with time, except for the four month treatment which had a slightly higher number of emerged parasites than the control. The peak in parasite emergence at four months was probably the result of the small sample size, and differences in numbers of *P. puparum* adults emerging during the first four months were most likely due to chance selection of parasitized *P. rapae* chrysalid samples containing large numbers of *P. puparum* prepupae. More females emerged in the control and at the four month treatment interval than the other

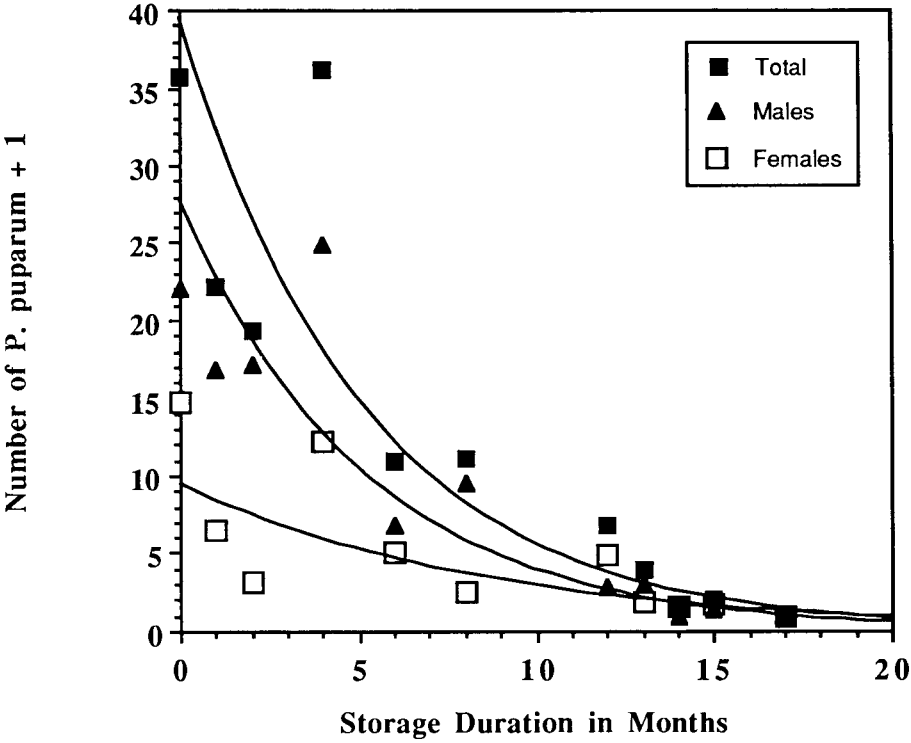

Fig. 1. Exponential plots of mean number of *P. puparum* total adults, males and females emerging per chrysalis after different periods of storage at 10°C. Some observations are hidden where points are overlaid; regression equations are as follows:

Total number of adults ..... $Y = 39.46*10^{-0.0846x}$, r = 0.90
Number of males ..... $Y = 27.78*10^{-0.0845x}$, r = 0.91
Number of females ..... $Y = 9.49*10^{-0.0582x}$, r = 0.69

treatment times, but there was no significant difference in the number of males emerging between treatment intervals during the first four months of storage.

The decrease in emergence of the number of *P. puparum* adults per host chrysalis dropped sharply beginning with the six month treatment. Part of the decrease in total number of parasites emerging from each chrysalis at the longer storage treatments might be attributed to temperature stress and possibly desiccation.

Adult *P. puparum* females emerging from parasitized hosts at each treatment interval were viable, as *P. puparum* colonies could be started from adults at each storage interval. No male *P. puparum* emerged from chrysalids at the fourteen month storage treatment; however, the arrhenotokous females were fertilized by their male progeny to produce a viable colony. There were no apparent differences in fecundity and longevity of the emerged parasites from the various storage

durations. Females from each treatment readily attacked host pupae, and progeny numbers per chrysalis fell within the range reported for the species, from a mean of 23 (Muggeridge 1943) to 52 (Lasota & Kok 1986a). Adult longevity varied from 2 - 4 wk. Since developmental time from egg to adult takes only about three weeks, and each *P. puparum* female can deposit up to 697 eggs (Clausen 1962), it would not be difficult to produce large numbers of the parasite within a relatively short period if hosts are not limiting. The main problem is to be able to stockpile sufficient viable parasites for use when needed. Hence long term storage of viable prepupa *P. puparum* in host chrysalids would negate the labor and time required for constantly maintaining a large colony of the parasite. Lasota & Kok's (1986b) previous findings that refrigeration did not inhibit viability of adult *P. puparum* females kept at 10, 15, and 23°C for 30 days can thus be extended to refrigeration of *P. puparum* prepupae in the hosts for periods of up to 15 months without loss of reproductive viability.

These data suggest that *P. puparum* exhibits a facultative dormancy induced by temperatures of 10°C or less, which slows its rate of development. Developmental retardation of some members of the same brood of *P. puparum* due to diapause is known to occur (Moss 1933, Bouletreau & David 1967). Nealis et al. (1984) reported a distinct interaction between temperature and parasite density on parasite weight of *P. puparum* within the host chrysalids. In southwestern Virginia, *P. puparum* may be subjected to near freezing temperatures for periods of up to six months, and nutrient reserves in *P. puparum* prepupae would have to be adequate for successful overwintering. Natural overwintering sites of the host pupae include sides of buildings, tree trunks, and fence posts surrounding cabbage fields (Lasota & Kok 1986a), and *P. puparum* overwintering within host chrysalids would be subjected to the same environmental conditions as the hosts. Based on the viability of prepupae subjected to prolonged refrigeration in this study, parsitized *P. rapae* chrysalids which contain *P. puparum* prepupae and pupae at the onset of cold temperatures in the field should successfully overwinter. Although our study did not involve storage of larvae, the results suggest that parasitized chrysalids which contain *P. puparum* larvae that have not yet reached the prepupal stage, but continue to develop during warmer periods above 10°C without prematurely killing the host or causing desiccation of the *P. rapae* chrysalis, may also successfully overwinter.

In conclusion, *P. puparum* prepupae within *P. rapae* chrysalids can be successfully stored at 10°C and 80% R. H. for periods of up to four months without significant decrease in number of parasites emerging from each host chrysalis. Viable colonies of *P. puparum* can be obtained from chrysalids which have been in cold storage for up to 15 months. Thus, in addition to the previously reported cold storage of *P. puparum* adults, *P. rapae* chrysalids can be sequentially parasitized and refrigerated to stockpile large numbers of *P. puparum* for laboratory studies or augmentative field releases.

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