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FEASIBILITY OF USING CRYOSTORED COLORADO POTATO BEETLE  
(COLEOPTERA: CHRYSOMELIDAE) EGGS FOR REARING *EDOVUM*  
*PUTTLERI* (HYMENOPTERA: EULOPHIDAE)

R.F.W. Schroder<sup>1</sup>

ABSTRACT

Cryostored eggs of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) eggs were suitable hosts for *Edovum puttleri*. Parasitism rates were low, however this study was first to show that rearing the parasite on eggs stored at ultra low temperatures ( $-70^{\circ}\text{C}$ ) is feasible. This method of storage will facilitate mass production of *E. puttleri* as well as prevent loss of the parasite in the event of a crash in the Colorado potato beetle colony.

The potential of *Edovum puttleri* (EP) as a biocontrol agent of the Colorado potato beetle (CPB) and its utilization in IPM programs on potatoes and eggplant were demonstrated by Schroder and Athanas (1989) and Lashomb et al. (1987), respectively. The limiting factors for implementing EP release program on a broad scale are the inability to provide adequate numbers on demand, the expense of mass production of CPB eggs. There are presently no effective artificial diets for rearing the CPB, and the costs of providing healthy potato foliage is expensive. If a method could be developed to store and recall host eggs on demand, egg production facilities could be continuously and efficiently operated at specific levels. A stockpile of eggs could be created and used to mass produce EP on demand. The quantities produced could far exceed seasonal production capabilities. Therefore, the cost of production may be significantly reduced as well as make the entire biocontrol program cost effective.

Cryogenic storage is a possible means of stockpiling CPB eggs. For the purpose of this study, cryostorage is the storage of Colorado potato beetle eggs at ultra low temperatures. Morrison (pers. comm.) showed the cryostorage of *Sitotroga cerealella* (Olivier) eggs were acceptable hosts for *Trichogramma pretiosum* Riley parasites. Fedde et al. (1979) chilled host eggs to keep them in a susceptible state for the egg parasite *Telenomus alsophilae* Vierck. Drooz and Solomon (1980) reared the encyrtid egg parasite *Ooencyrtus ennomophagus* Yoshimoto on eggs of *Clostera inclusa* (Hubner) kept below freezing. Lopez et al. (1987) showed that previously frozen *S. cerealella* eggs are suitable hosts for rearing the predator *Geocoris punctipes*. Maini and Nicoli (1990) observed that it was possible for EP to parasitize and develop in CPB eggs stored at  $18^{\circ}\text{C}$  for 1–7 days. The purpose of our study was to show the feasibility of using cryostored Colorado potato beetle eggs as a host for rearing EP.

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## MATERIALS AND METHODS

Colorado potato beetle eggs used in these studies were obtained from the colony maintained in the laboratory. Eggs used in the individual tests ranged in age from 24 to 96 h old. Eggs at a given age were collected and the remaining foliage attached to the egg mass was trimmed to the edge of the mass. Three to four egg masses were then glued with Elmer's® glue on a filter paper tab or strip. Two tabs were placed back to back and suspended (by the configuration of the vial) in the center of 1.8 ml NUNC star foot cryotube vials which were placed in NUNC cryostore canes and preconditioned in a refrigerator at 5° C or placed directly in liquid nitrogen (-70° C). After preconditioning, the eggs were placed in a liquid nitrogen cylinder. Following a designated time in liquid nitrogen the vials were removed and allowed to reach room temperature (20° C). The egg mass tabs/strips were then removed from the vials and placed on filter paper in a petri dish and exposed to EP. A control was set up for each test using 0-24 h or 24-72 h old eggs and exposing them in a parasite stinging jar for 24 h. The lids of petri dishes containing the cryostored eggs were removed and placed in a 1 gal clear glass wide-mouth jar containing approximately 50 EP (1m: 1f). The open end of the jar was closed with a cotton sleeve to prevent escape of the parasites. The eggs were exposed to the parasites for a prescribed time in a growth chamber set at 20° C., 60 % RH and 16L : 8D photoperiod. The dishes containing the control and cryogenic eggs were removed and held in the same growth chamber for hatching of CPB larvae and emergence of parasites. The number of eggs in each test varied according to the availability of eggs harvested from the laboratory colony. The mean % parasitism, % hatch of CPB larvae and % parasitism in the control were determined for these data.

## RESULTS AND DISCUSSION

Tests 1 to 9 include a wide range of: preconditioning times from 0 to 144 hours; cryostorage from 11 to 26 days; parasite ages from 3 to 42 days; parasite exposure times from 20-120 hours and number eggs exposed (Table 1). The resulting percent parasitism expressed in the various tests ranged from 5.8 to 17.8%. In one of the cryovials in Test 9, 30.0% of the eggs exposed to EP were parasitized. Although low parasitism rates were obtained compared to the parasitism rate in the controls we were encouraged by the results, since prior to these studies no Colorado potato beetle eggs were reportedly placed in cryostorage for use in rearing *E. puttleri* or any other parasites or predators.

This was a study to determine the feasibility of using cryostored eggs for rearing *E. puttleri*, therefore no detailed biological studies were conducted to determine sex ratios and fecundity of the parasites produced from cryostored eggs. However this study is the first to show that cryostorage/storage at very low temperatures of Colorado potato beetle eggs for use in rearing the parasite is feasible.

Much additional work is required to fully understand the optimal preconditioning temperature/time, cryostorage/ conventional freezer storage time and what effect all these variables have on the sex ratio and fecundity of the emerging adult parasites.

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Table 1. Differences in % parasitism of cryostored Colorado potato beetle eggs by *Edovum puttleri*.

Test #	Treated eggs <sup>2</sup>	Conditioning to,e (hrs) <sup>b</sup>	Cryo time (days)	Parasitoid age (days)	Parasitoid exposure (hrs)	Untreated eggs <sup>c</sup>	% Parasitism of untreated eggs	% Control parasitism
1	225	0	15	4-12	72	197	5.8	53.0
2	358	4	12	6-19	46	71	6.1	69.0
3	691	8	11	4-7	65	22	7.2	72.1
4	176	144	19	16-40	20	138	7.4	75.4
5	649	24	26	23	21	94	8.3	30.0
6	501	24	12	6-19	46	61	9.0	54.0
7	637	24	11	3	24	26	9.4	53.3
8	162	96	21	18-42	96	19	10.5	73.0
9	321	24	11	11	120	197	17.8	53.0

\*Note: All Colorado potato beetle eggs were 0-24 hrs old at beginning of test, except tests 3, 6, & 8 which were 24-72 hrs old. <sup>a</sup>Treated = cryostored eggs. <sup>b</sup>Conditioning time = # of hours eggs were held at 5°C. <sup>c</sup>Untreated = eggs not exposed to ultra low temperatures.

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