United States Department of Agriculture
Forest Service
Southern Forest Experiment Station

Research Note

SO-304
February, 1984

Temporal Cold Storage of Eggs of the Poplar Tent Maker, *Clostera inclusa*, Prior to Use in Rearing the Egg Parasite, *Ooencyrtus ennomophagus*

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SUMMARY

After being retained in cold storage at −10°C over a 24-month period, some *Clostera inclusa* (Hubner) eggs were still able to be successfully parasitized by *Ooencyrtus ennomophagus* Yoshimoto, an egg parasite. An equation was developed for predicting parasite yield over time from cold-stored eggs. Predicted parasitism was 25 percent or better for up to 8 months of storage, then dropped to below 16 percent for eggs stored 12 months, 8 percent after 18 months, declining to less than 1 percent after 24 months of storage.

Additional keywords: Parasite rearing, biological control, *Populus deltoides, Salix nigra.*

INTRODUCTION

Egg parasites offer considerable promise for biological control of forest defoliators, but technology must be developed to fully utilize this control technique (Fedde, et al. 1976). Drooz and Solomon (1980) reported that the egg parasite, *Ooencyrtus ennomophagus* Yoshimoto (Hymenoptera: Encyrtidae), a natural parasite of elm spanworm, *Ennomos subsignarius* (Hubner) eggs, could develop successfully in eggs of the poplar tent maker, *Clostera inclusa* (Hubner). Their studies demonstrated that the encyrtid parasite developed at least as well on *C. inclusa* eggs preserved at −10°C for one month as on fresh ones, which can therefore be stored pending availability of parasites, and that eggs of hosts such as *C. inclusa* are killed by low temperature treatment, thereby making them suitable for transport without fear of introducing the species outside the host's native habitat. We now report on studies made to determine how long *C. inclusa* eggs can be stored and still be suitable for rearing *O. ennomophagus*.

METHODS

Host eggs were obtained from a nursery colony of *C. inclusa* reared on foliage of cottonwood, *Populus deltoides* Bartr., and black willow, *Salix nigra* Marsh. After pupation and emergence the moths were allowed to mate in screen cages and then transferred to brown paper bags for oviposition. The egg masses deposited on the sides of the bags were collected promptly and processed for storage tests within 24 hours of deposition, because the parasite normally develops only in unembryonated eggs. The egg masses were placed in double plastic bags, the air was removed from each bag, and the necks of the bags were twisted and tied. The bags of eggs were placed in a freezer at −10°C for storage tests.

Samples of preserved eggs were removed from storage at monthly intervals of 1 to 24 months for exposure to parasites. The *O. ennomophagus* parasites were from a laboratory colony maintained at the Forestry Sciences Laboratory, Research Triangle Park, NC, at 24°C and 76 percent humidity.

Egg masses of *C. inclusa*, containing 29 to 342 eggs (table 1), were put in cages with six adult *O. ennomophagus* parasites less than one month of age and held at 24°C and 76 percent RH. The percent of adult parasites that emerged from the *C. inclusa* eggs (percent = number parasites emerged ÷ number *C. inclusa* eggs exposed) was used to gauge rearing success.

RESULTS

The preserved *C. inclusa* eggs remain in the liquid state at the storage temperature of −10°C, which is not cold enough to freeze them, and the parasites began ovipositing into the eggs taken directly from the freezer. The parasite developed from egg to adult in 21 to 24 days. The resulting adult parasites were relatively long-lived—most lived for 2 to 3 months. Both development

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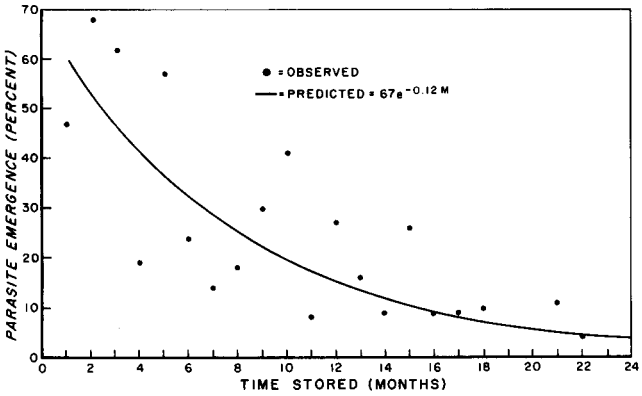

Figure 1.—*Observed and predicted emergence of* Ooencyrtus ennomophagus *from eggs of* Clostera inclusa *cold-stored at* $-10°C$ *up to 24 months prior to exposure to the parasite.*

time and longevity compared favorably with those reared from fresh eggs.

Development of the parasite on cold-stored eggs is summarized in table 1, and a prediction equation for parasite yield over time is presented in figure 1. Percent of parasite emergence (P) was fitted to the exponential equation $P = 67e^{-0.12m}$, where m = accumulative months in storage (in this case any whole number from 1 to 24) and e = 2.71828 (the base of the natural logarithm), by means of the SAS nonlinear package (SAS Institute 1979). The equation gives a residual error mean square of 151, resulting in a residual standard deviation of 12.28. If we compare data on the percent parasitism of fresh eggs (25 percent in the Drooz and Solomon 1980 study) with current results, equal or better results were obtained with cold-stored eggs for the first 8 months. The data from table 1 indicate that the quality of eggs was quite variable throughout the test. Predicted parasite yield (fig. 1) fell below 16 percent by year's end. After 18 months, predicted emergence dropped below 8 percent, and declined slowly thereafter. Part of the variation in parasite yield may be related to the number of infertile host eggs in the tests, some is related to damage due to length of storage, and some may have been caused by handling. The contents of infertile eggs dry up quickly at $-10°C$. After the 18th month the decline in parasite yield most likely signaled the loss in quality of the fertile host eggs stored at $-10°C$. Better packaging, possibly with impervious coatings, while host eggs are first held in evacuated plastic bags, might prevent dessication and forestall the decline.

Genduso (1978) and Gennadiev and Khlistovsky (1980) preserved eggs for parasitism for 3 and 5 years, respectively, using liquid nitrogen ($-196°C$). However, liquid nitrogen requires special handling techniques and is not readily available in most laboratories. The temperature we used has the advantage of being easily achieved even in the freezer unit of a household combination refrigerator/freezer. Therefore, our storage technique is readily adaptable to most field situations.

Table 1.—*Development of the parasite,* Ooencyrtus ennomophagus, *on eggs of* Clostera inclusa *held at* $-10°C$ *up to 24 months*

Clostera inclusa eggs		
Months in storage	Number per egg mass	No. parasites emerged
1	88	41
2	47	32
3	82	51
4	210	40
5	156	89
6	99	24
7	78	11
8	100	8
9	162	48
10	174	72
11	246	19
12	342	91
13	326	51
14	121	11
15	43	11
16	32	3
17	34	3
18	29	3
19	45	0
20	46	0
21	53	6
22	74	3
23	186	1
24	276	1

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