

Long term preservation technology for cross breed (PM x CSR2) eggs

Bivoltine silkworms of temperate origin lay diapause eggs which can be preserved safely for 20–300 days under suitable preservation schedule. But, multivoltine silkworms of tropical origin lay non-diapause eggs which cannot be preserved beyond 20 days. This leads to time-bound rearing of cross breeds with multivoltine race as mother parent, failing which the eggs go waste. The conditions prevailing in major parts of India especially the southern states are also congenial for rearing the cross breed, particularly PM x CSR2 throughout the year.

The demand for disease free layings (DFLs) of PM x CSR2 is cyclical. Slackness appears naturally after a high demand season. Unfortunately, shortage of quality seed cocoons of either multivoltine or bivoltine races or both, compounds the problem during period of high demand. This leads to a wider gap between demand and supply at times, and the seed cocoon and seed prices shoot up beyond manageable levels creating an unwelcome situation of the graineurs using even the below par seed cocoons for seed production. On the contrary, during the period of slackness, the price of seed and seed cocoons nosedives and even the best quality seed cocoons are not utilized for seed production.

In India, the slackness or higher demand for DFLs depend on the seasons and weather conditions. However, it is quite difficult to correctly predict the demand pattern due to irregular monsoon, etc. Thus, a systematic and planned production is always not possible. Added to this, large variations occur in the egg production efficiency / recovery in the cocoons processed during the unfavourable season i.e. March

– May. Interestingly, this season coincides with high demand for seed cocoons and price escalates exorbitantly affecting the whole economics of seed production.

All these problems could be solved to a great extent, if there is a system for postponing the hatching of cross breed layings. Silkworm Seed Technology Laboratory, Kodathi, Bangalore took this challenge head on, and developed a preservation technology so that the cross breed layings can be safely preserved for a period of 50 days. This technology can emerge as an effective tool in managing the demand / slackness situation of cross breed layings efficiently by preparing the DFLs utilizing good quality seed cocoons, preserving them under new schedule and utilizing during the demand season.

Even though non-diapause eggs are less tolerant to cold as compared to diapause eggs, the problem of supplying desired quantity of eggs to the farmers at right moment could be solved by following suitable method of refrigeration of non-diapause eggs. In practice, cross breed silkworm eggs are refrigerated for a maximum period of 20 days. The safe period of cold storage of silkworm eggs depends upon the stage of developing embryo, temperature of storage and characteristics of particular strain of silkworm. Normally, bivoltine silkworm eggs should be cold stored at different low temperatures, 2 or 3 phases in order to prolong the safe period of preservation. Silkworm Seed Technology Laboratory of Central Silk Board has evolved a new technology for longer duration preservation up to 50 days suitable for existing cross breed eggs with an intermediate care.

Methodology

After the egg laying under optimum conditions and centrifugal method of mother moth examination, the egg sheets are dipped in 2% formalin solution for 10–15 minutes for surface sterilization followed by drying in shade. The zero hour of egg laying is reckoned after 8 hours from the decoupling time when 85–90% eggs are laid. Based on the zero hour, the embryonic age of the eggs is calculated and 42–44 hour embryonic aged eggs (early appendages formation stage i.e., stage-17) are preserved at 5°C for 20 days followed by an intermediate temperature of 15°C for two days. Then the eggs are re-refrigerated at 2.5°C up to 30 days (double step refrigeration).

Both at the time of preservation and release, the eggs are compulsorily passed through 15°C for 2–3 hours to avoid cold injury and thermal shock. The released eggs have to be incubated at 25±1°C and 75±5% relative humidity for proper development of eggs/embryos (Fig. 1).

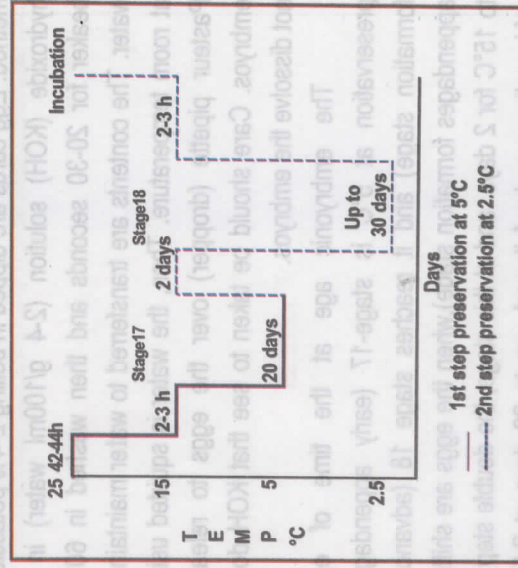


Fig.1: Double step preservation schedule for cross breed eggs

These eggs are to be black boxed at head pigmentation stage for 48 hours and expose them to 2 hours diffused light (450 lux) to obtain uniform hatching. For example,

Coupling time : 6.00 a.m. (07.06.2011)
Decoupling time : 9.00 a.m. (07.06.2011)
Laid on : 07.06.2011
Zero hour : 5 p.m. (07.06.2011)
Oviposition duration: 48 hours (up to 9 a.m. of 09.06.2011)

Embryonic age (stage-17 by 42-44h): 1.00 p.m. (09.06.2011).

The eggs, thus, should be preserved before 1.00 p.m. at 5°C on 09.06.2011. Eggs can be released on any day during first or second step refrigeration.

Embryo isolation technique

The embryos are isolated from the eggs and the stages are verified by adopting Potassium hydroxide method. Egg cards are dipped in boiling 2-4% potassium hydroxide (KOH) solution (2-4 g/100ml water) in a beaker for 20-30 seconds and then washed in 60°C water. The contents are transferred to water maintained at room temperature. Then, the water is squirted using Pasteur pipette (dropper) over the eggs to release embryos. Care should be taken to see that KOH does not dissolve the embryos.

The embryonic age at the time of egg preservation at 5°C is stage-17 (early appendages formation stage) and it reaches stage 18 (advanced appendages formation stage), when the eggs are shifted to 15°C for 2 days and at this stage, the double step of refrigeration can be followed up to 30 days at 2.5°C (Fig. 2).

1st step 2nd step

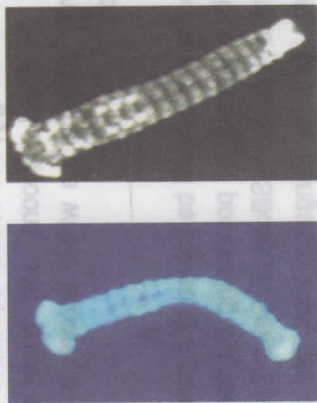


Fig.2: Embryonic stages during 1st and 2nd step preservation

Large scale field trials showed that there was no marked difference between 50 and 20 days (control) preserved eggs with respect to hatchability, cocoon yield and cocoon characters. Hence, this technology is recommended for the egg producers to meet the requirement of silkworm eggs during demand period and also to avoid wastage of cross breed eggs during slack season.

Adoption of technology by SSPCs of NSSO (DFLs in lakhs)

Cold storage Plants	2009-10	2010-11	2011-12 (upto July)	Total
SSTL	3.61	10.76	0.53	14.90
Hosur	0.11	5.53	9.83	15.47
Mysore	--	1.29	3.35	4.64
Total	3.72	17.58	13.71	35.01

Adoption of technology by NSSO units proved that the performance of long term preserved cross breed eggs was on par with the control batches pertaining to hatching and cocoon yield.

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